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- ✓ **BIOLOGY**
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**SWEET CHERRY BREEDING PROGRAMME AT ISTRITA FARM,  
USAMV BUCHAREST**

Asănică A.<sup>1\*</sup>, Tudor Valerica<sup>1</sup>, Badea Adriana<sup>1</sup>, Micu M.M.<sup>2</sup>

**Keywords:** *breeding, cross pollinated, hybrids, genitor, P. avium*

**ABSTRACT**

*Breeding sweet cherry (Prunus avium L.) takes long time and in order to obtain new cultivars, there are mandatory specially steps to follow. The big number of sweet cherry cultivars established in the 12 ha orchard of the Istrita Farm become a good opportunity and challenge for hybridization and new cultivar creation. The main traits taken into consideration were: ripening time, size of the fruits, fruit appearance, organoleptic quality, resistance or tolerance to specific pests and diseases, self-fertility, blossoming time, vigor, type of the fruiting branches and the productivity. In this paper are presented the results obtained during the last two years since the Breeding Programme at USAMV Bucharest started. Now we have two series of sweet cherry hybrids (414 hybrids from 12 combinations/2013 and 447 hybrid stones from 24 combinations/2014 and 1000 stones from open pollination).*

**INTRODUCTION**

In the last decade 120 new varieties have been released by breeders from Europe and Asia. Most of these varieties have one or more outstanding traits that make them noteworthy (Sansavini S., 2005).

The main goal of breeders is to create varieties resistant to unfavorable environmental factors, but possess a greater capacity to exploit the optimal climate conditions, soil and culture that lead to increased production (Botu I., 1994; Cociu et al., 1999).

Aims to improve the cherry worldwide concern: self fertility, fruit size, compactness trees (spur), extend the season of consumption by staggering the time of ripening fruit, early ripening, quality of fruit, flesh firmness, mechanical harvesting possibility, disease resistance, late flowering. The achievement of these objectives dealing large research centers, such as Davis - California and Geneva - New York in USA, Summerland and Vineland in Canada, Agen and Bordeaux in France, Rome and Verona in Italy, Gembloux in Belgium, Gissen and Oppenheim in Germany, Holovousy in Czech, Skiernewice in Poland, Kiev in Ukraine, Chisinau in Moldova, Pitesti, Bistrita and Iasi in Romania, etc.

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Genetic cherry improvement Canadian program on auto restore fertility species ended with approval of autofertile first commercially valuable cultivars such as 'Stella' (1970), 'Lapins' & 'Sunburst' (1984), 'Newstar' (1988). These cultivars have opened a new perspective of the cherry culture, representing cultural and technical advantages: reducing the height of the trunk, proper summer or winter cutting, treatment with growth inhibitory substances, etc. Enhancers were obtained by mutagenesis "compact" form cultivars of "standard" ones such as 'Lambert Compact', 'Compact Stella', 'Early Compact', 'Van Compact' in Canada, 'Van Compact' and 'Compact Napoleon' in Czech, 'Big Burlat', 'Duron Compact' and 'Compact' in Italy (Lapins, 1976; Fideghelli, 1988; Vondracek J., 1986, cited by Budan S., 2000). USA program to improve the cherry in 1995 were released potentially high agrobiologic cultivars (earliness, yield, fruit size and quality): 'Early Red', 'Giant Red' and 'Firm Red'.

Currently, cherry genetic improvement program in Romania, is one of the highest rated programs compared to other European countries. Ideal cultivar that satisfy to the interests of growers, technologists, traders and consumers alike and the whole, it was created and continues to be the desire of all genetic improvement programs (Zwintscher, 1973; Cociu V., 1990, cited by Budan S., 1992; Tudor V. et al, 2010; Asănică A., 2013). Achieving in short term of any goal depends largely on the existence and proper use of sources of genes. And in this respect, there is sufficient information available to breeders. Given to this, the current program envisages improvement Romanian complex objectives that can be achieved in successive stages and refers to both trees and fruit. The trees must meet the following conditions:

- have low vigor and many spur formations (type spur or semispur);
- precocity of fruiting and high productivity each year regular;
- resistance to frost, white frost and drought or a better ability to adapt into new ecological conditions of culture;
- resistance to virus diseases, hard managed by conventional means.

## **MATERIAL AND METHODS**

Fruit Growing Istrita Farm and Nursery is located on the parallel 43° 29' north latitude and the meridian 25° 34' east longitude, at an altitude of 134 m, in the municipality Sahateni, Buzau. Distances that separate the major urban centers are 105 km from Bucharest and 25 km from the city of Buzău. Nursery area falls within the plain area starts at the foot of Carpathian hills (Dealul Mare), just below the hill Istrita and connects this area Bărăgan Plain. The climate is semi-humid, very warm. The average annual temperature is around 11,3°C summer and winter around 23°C around 0°C, average monthly temperatures recorded negative values only in the months of January to March. Average annual rainfall amounts is about 500 mm. Groundwater is a continuous canvas to a depth of about 6 m. Climatic conditions were favourable for hybridization works in the spring of 2013, in exchange spring of 2014 was difficult, registering over two consecutive frost mornings (31.03 and 1.04), -4°C and -2°C, causing serious damage material to be worked up to that time. And in the immediate future minimum temperatures were within 2 to 7°C, which persisted until noon, accompanied by strong winds, which thus affecting the pollination of flowers and fruits binding. Area planted with cherry is over 12 ha, with young orchards established since 2008, intensive, with a density of 1000 trees/ha. The cultivars grown are: 'Van', 'Celeste', 'Lapins', 'Kordia', 'Giant Red', 'Ferrovia', 'Early Red', 'Firm Red', Skeena, 'New Star', 'Regina', 'Durona', 'Sweetheart' grafted on different rootstocks: PHLC, CAB6P, CAB11E, COLT and Gisela 6.

Breeding sweet cherry takes long time and in order to obtain new cultivars, there are mandatory especially steps to follow (Table 1). Standard breeding techniques (i.e. self pollination, open pollination, crossing by emasculation and hand pollination) have been employed (Cociu V., Oprea Șt., 1989). Selection of suitable parents from geographically distant groups (USA and Canada) were chosen in association with from the European Group cultivars.

Table 1

Scheme of obtaining new cherry cultivars

Year I	Observations specification. The choice of the parents. Establish the work plan and supply with the necessary materials. Diallel hybridization and self pollination as planned. Obtaining hybrid seeds.
Year II	Sowing hybrid seeds in pots. Planting the hybrids in the selection orchard.
Year III	Grafting on low vigour rootstocks.
Years IV–VI	Observations in selection orchard for resistance to frost, drought, disease, plant habitus, type of branching. Selective fruit quality and other criteria established by the program. Grafting on rootstocks with low vigour of the most valuable hybrids (20 plants each), in order to organize the competition microculture.
Year VII	Establishment of the competition microculture with hybrids grafted last year (series of 10 trees) and whose value was confirmed.
Year IX	First observations on yield and fruit quality in microculture grafted hybrids.
Years XII–XV	Submission documentation for preomologation hybrids tested during 3-5 years of fruiting. Establish the competition culture in two or three different areas with the preomologated hybrids.

The main characters of interest in sweet cherry breeding program are: age of maturation, fruit size, fruit appearance, taste quality, resistance or tolerance to diseases and pests, self fertility, the flowering time, growth vigour, fruit type formations and productivity. In this sense for the period 2013-2014 we have chosen sources of genes for the characteristics set forth in Table 2.

Table 2

Sources of genes for goal achievements

Earliness	'B. Burlat', 'Celeste', 'Early Red', 'Spectral', 'Sublim', 'Ponoare'
Lateness	'Skeena', 'Regina', 'Amar Galata', 'Amar Maxut'
Productivity	'New Star', 'Early Red', 'Giant Red'
High quality of fruits (size, taste)	'Giant Red', 'Firm Red', 'Regina', 'Celeste', 'Van', 'Sublim', 'Kordia'
Bitter fruit	'Amar Galata', 'Amar Maxut', 'Margo'

## RESULTS AND DISCUSSIONS

In order to execute controlled hybridization performed in the spring of 2013, it were selected maternal genitors ♀: 'Early Red', 'New Star', 'Van', 'Giant Red' and 'Firm Red' and as paternal genitors ♂ were selected: 'B. Burlat', 'New Star', 'Giant Red', 'Kordia', 'Early Red' and 'Van'.

Cherry breeding program at the Istrita Farm and Nursery started in 2013 with a favourable development conditions for hybridization, obtaining 414 hybrid plants of 12 hybrid combinations (Figure 1); in 2014 resulting hybrids from 24 combinations: 447 hybrid stones and 1000 stones from open-pollination.



Figure 1. Results, 2013

(left – image of orchard, right – hybrid population (♀N.Star x ♂E.Red))

Using 'Early Red' cultivar as maternal genitor in combination with: 'B. Burlat', 'New Star', 'Kordia', 'Giant Red' and 'Van' results fruit hybrid percentages between 1.4 to 5.9%, absorbable fruit occur because that they did not reach harvest maturity (Figure 2). The same cultivar used as the paternal genitor, performs well in combination with the good compatibility of the 'Giant Red', resulting 77 hybrid fruits (43.5%), which were matured 49 hybrid fruits and stones (Figure 5). From the interbreeding with 'New Star', it resulted 98 hybrid fruits (20.8%) at fruit set time, and a number of 50 hybrid stones finally (Figure 3).

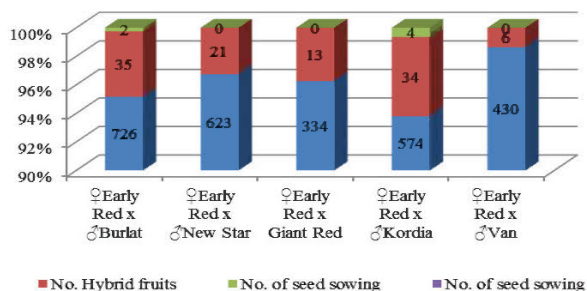


Figure 2. Behaviour of 'Early Red' cultivar as a maternal genitor (♀), 2013

From hybrid combination ♀'New Star' x ♂'B. Burlat' resulted the largest number of hybrid seeds (64) from a number of 456 self-pollinated flowers and 94 hybrid fruits recorded after fruits sett (Figure 3). 'New Star' used as the maternal genital (♀) showed very good compatibility with all cultivars were crossed yielding the percentage of fruits with 'Early Red' of 27.6%, 26.4%

with 'Kordia', 20.8% with 'B. Burlat', 19.5% with 'Van' and 13.5% with 'Giant Red'. Significant hybrid progeny resulting from a combination with 'B. Burlat' – 64, with 'Early Red' 50, 36 of the cross with 'Van', 27 with 'Kordia' and 16 with 'Giant Red' (Figure 3).

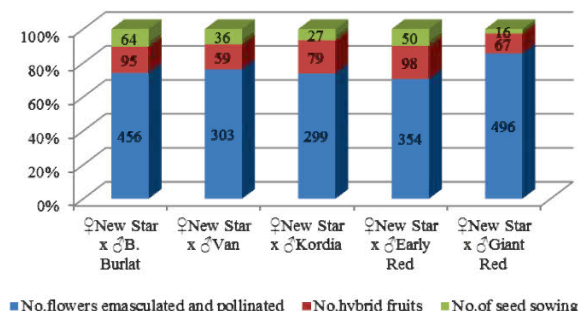


Figure 3. Behaviour of 'New Star' as a maternal plant (♀), 2013

'Van' maternal genitor performed well in cross with 'Early Red', setting 75 hybrid fruits, but they have stopped growing and fell (Figure 4). Good compatibility has recorded 'New Star', about 55 (50.9%) hybrid fruit were obtained and 52 hybrid seeds. Also, 'B. Burlat' sett 27 hybrid fruits and finally 24 hybrid stones from the fruits carried by the harvest maturity time (Figure 4).

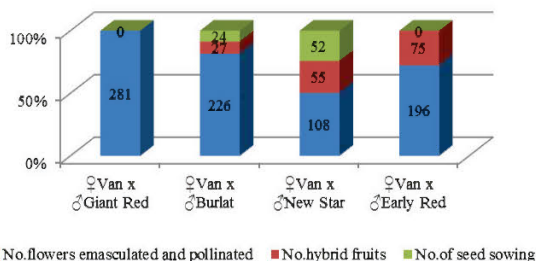


Figure 4. Behaviour of 'Van' as a maternal plant (♀), 2013

From combination ♀ 'Giant Red' x ♂ 'Early Red' resulted in total 49 hybrid stones and lower hybrid populations from crosses ♀ 'Giant Red' x ♂ 'New Star' with only 7 stones (Figure 5).

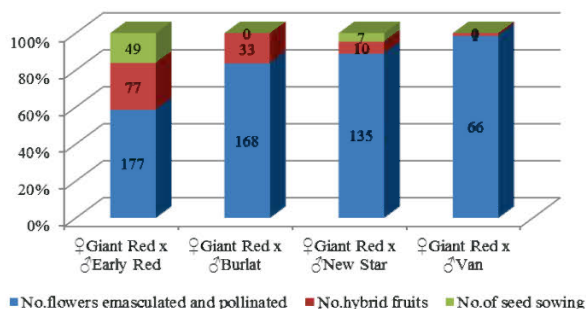


Figure 5. Behaviour of 'Giant Red' as a maternal plant (♀), 2013

'Firm Red' (Large Red x Garnet) crossed with 'Giant Red' (Large Red x Ruby) had encounter poor results, possibly due to the existence in their pedigree of the 'Large Red' cultivar as a maternal genitor (Figure 6).

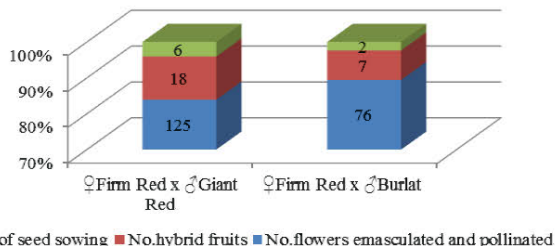


Figure 6. Behaviour of 'Firm Red' as a maternal plant (♀), 2013

Self fertile ability of New Star and 2013 satisfactory results prompted us to use it in the next hybridization year.

In 2014, difficult hybridization climatic conditions registered between 31.03-7.04, have reduced the number of flowers (Figure 7), this could be observed from the number of initial castrated flowers and the pollinated flower number with percentages ranging from 56.3 to 79.6% (Table 3). The lowest percentage of viable pistils pollinated associated with a lower resistance at low temperatures is observed at 'Giant Red' cultivar (from 14.6 to 29.3%) compared with 'Skeena' (60.7 to 73.9%) and 'Celeste' with high resistance (61.0 to 90.0%) (Asănică et al, 2012). Controlled hybridization works were performed and the Regina cultivar too, with somewhat late flowering pbenophase (11.04) but the bags were destroyed due to strong winds (Figure 7).



Figure 7. Damage to biological material, 2014 (left-frozen pistils, right-bags destroyed by wind)



Table 3

## Resistance of cherry flowers during the controlled hybridization operation, 2014

Hybrid combination	No. flowers emasculated	No. flowers pollinated	%
♀Giant Red x ♂Burlat	988	259	26.2
♀Giant Red x ♂Amar Maxut	1160	361	31
♀Giant Red x ♂Spectral	630	185	29.3
♀Giant Red x ♂Ponoare	1066	156	14,6
Total	3844	961	14.6 – 29.3
♀New Star x ♂Giant red	558	364	65.2
♀New Star x ♂Burlat	500	355	71
♀New Star x ♂Early red	885	657	74.2
♀New Star x ♂Skeena	428	337	78
♀New Star x ♂Celeste	310	212	68
♀New Star x ♂Amar Galata	569	453	79.6
♀New Star x ♂Amar Maxut	701	520	74.1
♀New Star x ♂Spectral	548	382	69.7
♀New Star x ♂Sublim	797	449	56.3
♀New Star x ♂Ponoare	987	702	71.1
Total	6283	4431	56.3 -79.6
♀Celeste x ♂Skeena	456	412	90.0
♀Celeste x ♂New Star	376	230	61.0
♀Celeste x ♂Giant Red	317	200	63.0
♀Celeste x ♂Amar Galata	331	245	74.0
♀Celeste x ♂Sublim	374	242	64.7
♀Celeste x ♂Spectral	295	225	76.2
♀Celeste x ♂Ponoare	525	349	66.4
Total	2674	1903	61.0-90.0
♀Skeena x ♂New Star	312	224	71.7
♀Skeena x ♂Early Red	353	261	73.9
♀Skeena x ♂Giant Red	415	252	60.7
Total	1080	737	60.7-73.9

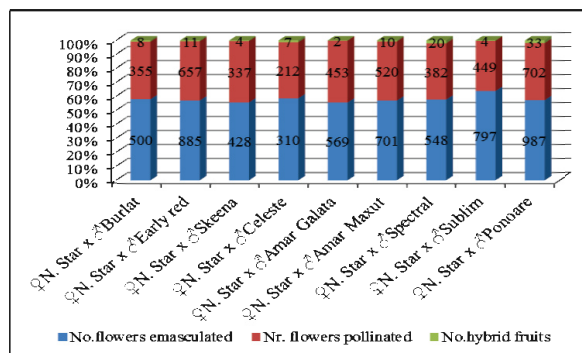


Figure 8. Behaviour of 'New Star' cultivar as a maternal genitor (♀), 2014

'Ponoare' cultivar used as paternal genitor (♂) in all crosses made, showed very good compatibility, resulting in the largest hybrid progeny: 64 stones with 'Celeste' (Figure

10), 45 with 'Giant Red' (Figure 9) and 33 with 'New Star' (Figure 8). A population of 20 stones resulted from crossing 'New Star' (♀) x 'Spectral' (♂), the other crossings resulting in lower hybrid progenies.

Although large losses of floral organs ready for pollination were registered, 'Giant Red' proved pretty good compatibility both in crossing with 'Ponoare' (45 hybrid stones) and 'Spectral' (27 stones) even with 'B. Burlat' (26 stones) (Figure 9).

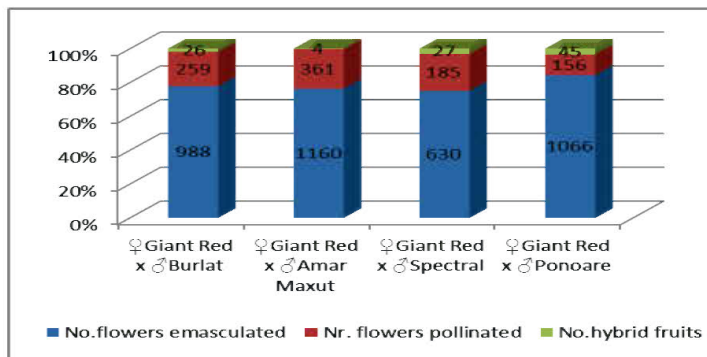


Figure 9. Behaviour of 'Giant Red' cultivar as a maternal genitor (♀), 2014

'Celeste' had good cross compatibility with 'Ponoare' (64 stones) and 'Amar Galata' (31 stones) and moderate to 'New Star' (15 stones) and 'Spectral' (11 stones) (Figure 10).

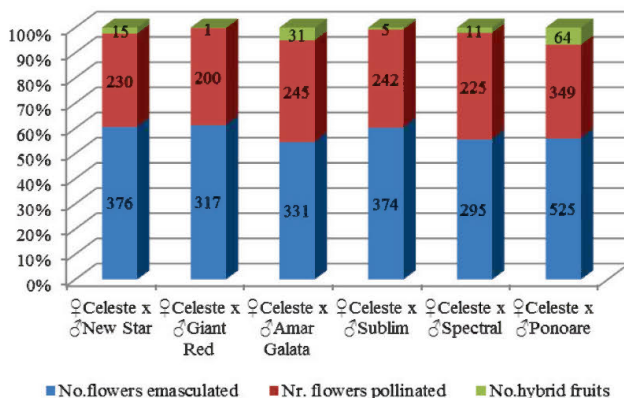


Figure 10. Behaviour of 'Celeste' cultivar as a maternal genitor (♀), 2014

From early cultivars crossings with 'Early Red' and 'Giant Red', late one 'Skeena' had a reduced compatibility, obtaining only 9, respectively 10 stones (2.8-3.5%) (Figure 11).



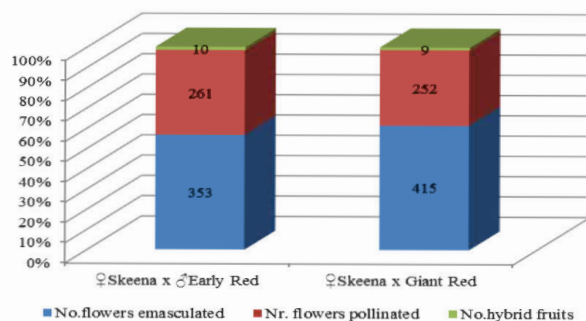


Figure 11. Behaviour of 'Skeena' cultivar as a maternal genitor (♀), 2014

The data presented in Figure 12 confirms that 'New Star' is a self fertile cultivar, being the only one who set up to 12.8%, resulting in 23 hybrid stones (Figure 12) and in 2014 resulted from 'Skeena' a surprising number of 34 fruits. This requires to be repeated to become concluding by initiated studies (Figure 13).

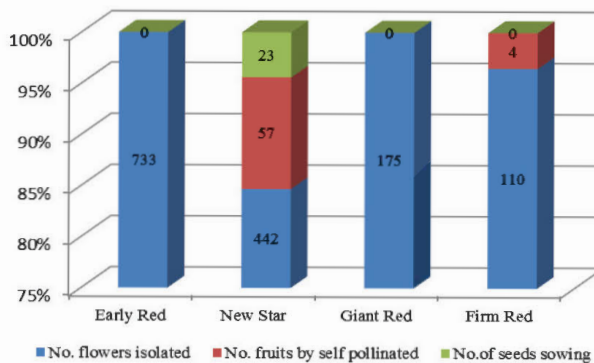


Figure 12. Self pollination made in 2013

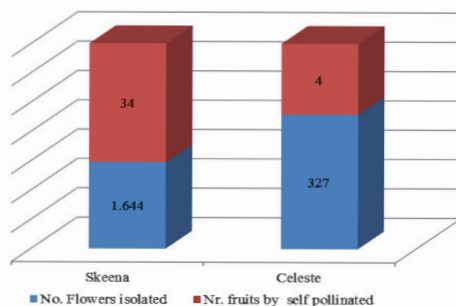


Figure 13. Self pollination made in 2014

## CONCLUSIONS

The climate is of major importance in the development of successful hybridization operations.

For getting significant hybrid populations have to be pollinated around 1000 flowers/combination, in order to eliminate any possible damages occurred by climatic accidents.

During the two years after the initiation of cherry breeding program at Istrita Farm and Nursery (UASVM Bucharest) were already obtained two hybrid cherry series (414 hybrid plants of 12 combinations / 2013, of these 6 combinations with higher hybrid progeny than 25 individuals/population and 447 hybrid stones of 24 combinations/2014 and 1000 stones from open-pollination).

During the past two years of experience, significant results had emphasized by 'New Star' used both as maternal and paternal genitor.

'Ponoare', 'Early Red', 'Spectral' and 'Amar of Galata' can be used as paternal genitors (♂).

## ACKNOWLEDGMENT

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**AGROTECHNICS APPLIED AT NEW VARIETIES OF TABLE GRAPES  
OBTAINED AND CULTIVATED IN VINEYARD ȘTEFĂNEȘTI-ARGES**

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*Keywords: variety, resistance, table grapes, agricultural technique, quality*

**ABSTRACT**

*The research in the present paper, started from the assumption that pruning represents the most important agricultural technique link in the culture technology of grapevine. The execution of correct working, in accordance with the variety and the culture conditions of the grapevine ensures the achievement of large, constant, high quality production every year. At INCDBH Ștefănești-Argeș, located in the vineyard Ștefănești-Argeș, researches on the aspects regarding the level of the crop load for the table grapes varieties have not been carried on before. To this effect, we have considered useful to undertake a study on this agricultural technique link for a better promotion of these varieties in production.*

**INTRODUCTION**

In recent years there has been a significant increase in consumption of fresh grapes, due to the general trend to move towards a healthy diet, rich in plant resources more (Dejeu L., 2010, 2011, Necula Cezarina et al., 2010).

Romanian viticulture confrontation with the international market and foremost with the European is one of the most actual problems requiring the development of quality products (Messegue M., 1998). Because the Romanian market are very few varieties of table grapes ripening early and enjoyed commercial aspect should promote new varieties and clones grapes which meet these requirements and are already grown in plantations in the Ștefănești Arges. These are newly approved varieties such as Argessis, Auriu de Ștefănești, clones Muscat Adda 10 Șt. and Perlette 22 Șt.. These varieties are added-known variety grown in the area: Victoria.

**MATERIAL AND METHODS**

Newly created varieties approved in recent years, but are only known in the units where they were obtained, are placed in culture for replacement of older varieties that no longer meet quality requirements changing consumer (Popa Camelia et al., 2003).

Placing such experiences two-way 4 x 3 for each experimental year (2012-2013) was identical, taking into study two factors, namely:

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- *Factor A* - Variety, which included graduations: Argessis, Auriu de Ștefănești, Muscat Adda 10 Șt., Perlette 22 Șt., Victoria .

- *Factor B* - fruit load applying differentiated cuts bearing vines that covered graduations:  $b_1$  fruit load of 15 eyes per vine, the drill-cutting;  $b_2$  fruit load of 20 eyes per vine, cutting to the heart;  $b_3$  fruit load of 25 eyes per vine, cutting the string;

Observations and examinations on the influence of load bearing on production, have been performed by various methods:

- amount of wood removed at slaughter; by cutting the dry eliminates three quarters of annual wood that forms the hub; to determine the amount of timber removed from wood cutting to separate weighed annual and multiannual when dry cutting in at the beginning of March, from a total of five hubs on repeat for each of the varieties studied;

- vegetative growth of the vine were measured at entry into the first fruits, when shoot growth stops, determining the average and total length of shoots; for the sprouts were measured every second block in each repetition by the repetition, and the average was calculated average of the experimental variant;

The experimental group located in the viticultural area representative Muntenia (Arges Ștefănești) have made observations and measurements on the quality of table grapes in the climatic conditions of the area Arges. Plantation of grape-vine has distance 2.2/1 m, type Guyot pruning is applied on stalk.

Table grapes were harvested when their maturity provides superior and efficient recovery (Nicolaescu Gh. at al., 2007). This was determined according to the variety in general, when the grapes have reached full maturity. The moment of full maturity for table grape varieties has been established with a good approximation, by making the following determinations: grain weight, sugar content and acidity titrable wort. These measurements were performed at intervals of 3-5 days from the entry grapes ripen.

## RESULTS AND DISCUSSIONS

Brief description of the variety:

*Argessis* (photo 1) - sort approved in 2002 SCDVV Ștefănești. The first kind of vine grapes obtained in specific climatic conditions Ștefănești-Argeș vineyard. Commercial aspect pleasant large grain (7.5-8.0 g), ovoid, black and blue; sea grape (450-480 g), uniaxial; good tolerance to fungal diseases (blight, mildew, rot); hub of great force, suitable for growing in the sky and average production reached 15 t/ha (Camelia Popa at al., 2003, 2009).

*Auriu de Ștefănești* (photo 2) - the first variety of grape vines white table with green early obtained in specific climatic conditions vineyard Ștefănești-Argeș, approved in 2007 to INCDBH Ștefănești. Commercial aspect pleasant large grain (5.5 to 6.8 g); spherical, yellow-gold; sea grape (400-450 g), wing; good tolerance to fungal diseases (blight, mildew, rot); ripening grapes in the first period (28.07-05.08) and suitable for organic crops. Expanding the variety in culture provides an average of 14 t/ha (Camelia Popa at al., 2009).

*Muscat d'Adda 10 Șt.* (photo 3) - was obtained by clonal selection Muscat d'Adda in INCDBH Ștefănești and approved in 2008; variety for fresh consumption; leaf is green; grapes are medium-sized to large (270-340 g); beans are placed uniformly cluster size is large-sized; flesh is crisp, specific taste and aroma; skin is thick, black-violet, strong bloom; force hub is medium to large; has good resistance to drought and diseases (mildew, powdery mildew and gray mold); grape maturation takes place in September; production is 4.9 to 5.3 kg/vine; accumulating 187 g/l of sugar and 3.9 g/l acid; have increased resistance to handling, transport and storage (Costescu Adriana at al., 2010).



Photo 1. Argessis



Photo 2. Auriu de Stefanesti



Photo 3. Muscat d'Adda 10 St.

*Perlette 22 Șt.* (photo 4) - was obtained by clonal selection from the variety Perlette to INCDBH Ștefănești and approved in 2008. Morphological characters: adult leaf is large, pentagonal, glabrous on both sides; grape is high (330-400g), conical, uniaxial, Fenders, semi-compact; grain is medium (1.8-2.2g), spherical, thin skin yellow-green; core is meaty, crunchy seeds and rudiments.

Characterization technological and agrobiological clone has a great effect; has high tolerance to drought and less frost; show medium tolerance to mildew, powdery mildew and gray mold due to the compactness of the cluster beans. Coefficients of fertility: absolute = 1.10-1.30 and ratio = 0.70 to 1.00. Productivity indices were 350-400 (ipa) and 200-230 (ipr). Grapes aged at the beginning of the decade Take August (epoch II). The average concentration of sugars in the mash is raised to maturation, reaching up to 220 g / l in years with favorable climatic conditions and 2,0- acidity 3.0 g/l H<sub>2</sub>SO<sub>4</sub>.

Qualities: used for fresh consumption and for jam, grapes large variety early (Catalog clones certified biological material, 2010).



Photo 4. Perlette 22 St.



Photo 5. Victoria

*Victoria* (photo 5) - is a hybrid obtained by crossing varieties: Cardinal x -Afuz Ali White. Hybridization was performed in the I.C.H.V. Bucharest by Victoria Lepădatu. Selection work continued at the Vineyard Research Station Dragasani variety was approved in 1978 is one of the most valuable creations Romanian varieties for table grapes. Required by earliness, but especially the beautiful appearance of the grapes and productivity. Grapes are very large, conical or cylindrical-conical, with grains arranged compact cluster. Large and very large grain, ovoid yellow-amber (amber); crunchy pulp, balanced taste,

unflavoured. Agro biological attributes: it is a variety with medium to large effect, good fertility (63-73% fertile shoots) and high productivity due to the size of grapes.

The research in this paper have assumed that cutting is the most important link agronomic technology culture of the vine vines. Proper execution of works in accordance with the variety and growing conditions, ensuring the achievement of high yields, consistent and quality from one year to another. At INCDBH Ștefănești- Arges, located in the vineyard Ștefănești-Arges, research on issues related to determining the level load bearing table grape varieties created here were not taken. In this respect it was considered useful to study the agro links for better promotion of these varieties in production later.

The production of grapes, in addition to climatic conditions and variety cultivated a decisive influence has applied agrotechnics plantation. Achieve high yields of quality and affordable low cost require the application of agrotechnical differentiated varieties.

Shoot growth was recorded by variety, load assigned to each block separately. The largest increases in the shoots, regardless of year and fruit load were recorded variety Argessis averaging 169.6 cm, followed by Auriu de Stefanesti (163.6 cm) and Muscat Adda 22 St.(162.0 cm) and the smallest increases were recorded at 22 St. Perlette clone. 160.5 cm. Variety Victoria annual increases averaged 165.5 cm (Table 1).

Table 1

The average length of shoots studied varieties 2011-2013 (cm)

Variety	2012			2013			Average on variety
	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	
Argessis	186	181	173	167	160	151	169.6
Auriu de Ștefănești	177	175	170	168	149	143	163.6
Muscat d'Adda 22 Șt.	176	170	165	162	150	149	162.0
Perlette 22 Șt.	174	170	168	165	146	140	160.5
Victoria	173	170	168	162	160	160	165.5

Analyzing the three variants and increases depending on the type of cut shoots was found that the highest increases were registered in the drill-cutting versions of fruit, compared to cutting the string, so the variety Argessis version 15 eyes/stump sprouts increases were 169.6 cm, and the clone Perlette 22 St. the variant with 25 buds / vine, shoots increases were only 160.5 cm.

The vigor of the vine-growing vine was assessed by the amount of wood removed from the cut. This amount of wood is made of wood and multi-year. Data from the two years of experimentation (2012-2013) reveal major differences between varieties, the number of strings left to cut.

Table 2

Pruning weight (g/block vine), depending on bud load, mean 2012-2013

Variety	Version	The amount of wood removed (g / vine)		
		Total Wood	Annual Wood	Multiannual wood
Argessis	V <sub>1</sub>	1425	1150	275
	V <sub>2</sub>	1375	1075	300
	V <sub>3</sub>	1430	1050	380
	The average	1410	1092	318
Auriu de Ștefănești	V <sub>1</sub>	1380	1200	180
	V <sub>2</sub>	1250	1000	250
	V <sub>3</sub>	1170	850	320
	The average	1266	1016	250
Muscat Adda 22 Șt.	V <sub>1</sub>	1560	1210	350
	V <sub>2</sub>	1550	1170	380
	V <sub>3</sub>	1550	1150	400
	The average	1552	1176	376
Perlette 22 Șt.	V <sub>1</sub>	1640	1270	370
	V <sub>2</sub>	1635	1250	385
	V <sub>3</sub>	1605	1200	405
	The average	1627	1240	387
Victoria	V <sub>1</sub>	1120	820	300
	V <sub>2</sub>	1150	800	350
	V <sub>3</sub>	1150	760	390
	The average	1139	793	346

Total amount of wood removed from cutting the highest value at Perlette 22 St. clone (1627 g) and the lowest value was recorded in the variety Victoria (1139 g). The amount of annual timber values between 793 g and 1240 g variety Victoria to Perlette 22 St..

Multiannual wood values of 250 g at Auriu de Ștefănești variety and 376 g at Muscat d'Adda 10 St., Table 2.

### CONCLUSIONS

The highest increases were registered in the drill-cutting versions of fruit, compared to cutting the string, so the variety Argessis version 15 buds/vine shoots increases were 169.6 cm, and the clone Perlette 22 St. the variant with 25 buds /vine, shoots increases were only 160.5 cm.

Shoot growth was recorded by variety, load assigned to each block separately. The largest increases in the shoots, regardless of year and fruit load were recorded variety Argessis averaging 169.6 cm, followed by Auriu de Stefanesti (163.6 cm) and Muscat Adda 22 St. (162.0 cm) and the smallest increases were recorded at Perlette 22 St. clone (160.5 cm).

With vineyard Ștefănești Arges table grape varieties grown obtained can compete in terms of quality with many other varieties produced worldwide.

Variety Auriu de Stefanesti is considered as a variety early for Ștefănești vineyard, varietal successfully completes conveyor varietal (maturing in the first period).

By promoting the production of table grapes with high biological resistance to attack by pests and diseases will protect the environment in terms of the viticultural area Ștefănești Argessis and Auriu de Stefanesti varieties of clones with Muscat d'Adda and successfully completes conveyor varietal Perlette area, varieties for fresh consumption.

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**CERTIFIED ORGANIC OF VEGETABLE PRODUCTS OBTAINED AT  
INCDBH ȘTEFĂNEȘTI**

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**Key words:** *spontaneous flora, organic agriculture, tomatoes*

**ABSTRACT**

*Organic farming, over many years has demonstrated technological capabilities, the beneficial and high economic efficiency. With organic farming, increasing fruit and vegetable recorded occurrence of additional nutritional value. Due to additional nutrition organic crops, especially those used fresh, though less appealing by size and colour, are more palatable after organoleptic qualities. Besides diversification of crops, organic farming contributes to the preservation of wild flora species. So, for example, in organic farms are 2-3 times more plant species of wild flora. This type of agriculture is vital, our proper conditions, it should be a clear basis for undertaking concrete actions to develop the legal, institutional and technological stepping towards a better life, they deserve every one of us.*

**INTRODUCTION**

Though the vegetable production in the traditional system has characteristics of organic farming yields obtained are quantitatively insignificant and are not officially certified (Barrett Diane M. at al., 2006). Scientific Organic farming is in its infancy in Romania both areas officially registered and certified products are insufficient to supply a growing market. Romanian farmer currently not offered an alternative cost-effective and competitive, which leads away than the level required in EU countries, protecting ecological environment and increase quality of life by offering products that gives food safety (Chilom Pelaghia, 2002, Ciofu Ruxandra at al., 2003; Pieper J.R. at al., 2008). Maintaining soil fertility, environmental protection and respect for the consumer are not only principles of organic farming, but also to be met in the future.

**MATERIAL AND METHODS**

The researches in this paper were performed at INCDBH Ștefănești Arges, on a plot of 250 m<sup>2</sup> in the protected space (cold glass) during 2010-2013.

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Environmental certification of land, crops and horticultural products is based on records that prove both inspectors certification bodies and beneficiaries. Obtaining certificate ecological travelled three mandatory stages:

- decision-making and record producer national ecological system;
- the conversion technology application specific
- evaluation and certification of crops carried out by an accredited certifying organic production.

For INCDBH Stefanesti this body was Austria Bio guaranteed. Each phase has a specific verifiable documentation both by organic certification organization and inspectors official organization. The research was completed Certification of organic.

Prevention of infestation by pests and pathogens specific to tomato crops and weeds by cultivating land issue resolved with other vegetable species resistant to cold, since organic greenhouse is unheated and not common pathogens and pests. Thus, lettuce and onions grown in two culture systems: interlaced and simple (Figure 1).



Figure 1 - Appearance of greenhouse *Eco* clean by keeping land culture onion and lettuce

The greenhouse designed demonstration batch weed establishment was performed manually and repeatedly since reducing pathogen populations wintering in soil and wild plants can be achieved by removing intermediate support plants and soil creating improper development conditions pathogens regardless of stage where they overwinter (Măniuțiu D., 2006, 2008).

## RESULTS AND DISCUSSIONS

Steps parked in the period of three years of research were: upgrading organic greenhouse to prevent primary infection to reduce pest infestation and allow diversification experiment consisted of:

- Choosing those design elements in order to maintain thermal comfort for vegetable plants as long a period of the year is to achieve double culture without resorting to heated in winter, either to maximize the productivity of genotypes with indeterminate growth;
- Finding the most efficient ventilation system and natural ventilation (non-energy) but at the same time, prevent pathogenic infections and infestations vectors of pathogenic organisms or pest infestation;
- Use as few resources (irrigation water, fuel, electricity, organic and mineral fertilizers, etc.);
- To protect genetic material against natural disasters (hail, frost, mist late etc.

Getting the most effective plant extracts from wild local populations to contribute both to the maintenance of harmful organisms below the economic damage and to boost growth and fruiting of crop plants by applying complex mixtures during the most susceptible to pathogens or pests the most dangerous (Ciofu Ruxandra at al., 2003). Replacing chemical fertilizers and pesticides banned in organic culture with plant extracts containing organic compounds repellent effects, bacteriostatic, fungicide, insecticide or stimulating the growth and fructification of the crop has a major effect on the profitability of organic crops especially during greening, where production often decreases by half. Recovery of spontaneous local plants is essential to effectively combating pathogens or maintaining populations below the minimum threshold of pest and, in addition, offers hope to address brave beginners ecological culture given that there is currently a market biopesticides or bioherbicides (Cărbunaru M. C. Domuța, 2006, Tița I., 2009).

Were harvested from wild local plants: leaf sumac (*Rhus typhina*) alcoholic extract insecticide and repellent effects, Horsetail (*Equisetum arvense*) used as a soak and infusion against aphids and mites, nettle (*Urtica dioica*) used as a soak fermented the effect of strengthening the repellent against insects and plants. For the first time we purchased *Clematitis aristolochia* plants and has been used for the control of pathogenic fungi family (Indrea Dumitru at al., 2004).

Selection of genotypes with resistance or tolerance to specific pathogens and pests resulting from their experiments is necessary since the existing valuable crop varieties are very sensitive to specific pathogens and pests, weed competition and applied technology. Often no varietal adaptability to extreme conditions in areas devoted to vegetables is not widely known, many gardeners having trouble with some imported varieties. The existence of ecological culture biotypes tolerant or resistant to specific pests and pathogens is essential to the success of economic and financial, with the prevention of infection or infestation by pests or their vectors and local preparation biopesticides necessary to control pest populations below the economic threshold pest.



Figure 2 - Tomato culture in Greenhouse Eco

Prepare documentation for certification of organic products and the approval of modernized greenhouse tomato biotypes in various stages of testing.

Certified organic greenhouse vegetable products obtained cross can only be achieved if the minimum requirements imposed by regulations (completion of the conversion of the land, obtaining organic plant material application technology officially approved organic fertilizer use and accept biopesticides official organic crops). Organic certification was conducted by a prestigious company approved by the Ministry of Agriculture and Rural Development (Austria Bio Garanty).

Representative biotypes were tested tomato resulted from the improvement (both fixed and indeterminate growth) in order official registration at least two new varieties for obtaining organic products (Figure 2).

Testing was conducted in two different locations: the greenhouse breeder (INCDBH Stefanesti and test fields of ISTIS (Targoviste).

The conversion period (3 years), were tested two varieties indeterminate growth (Costate 21, Ștefănești 22) and two varieties determined (Arges 11, Arges 20) for organic production.

Brief description of the variety: *Tomato variety Arges 11* (Figure 3) Morphological characters: anthocyanin coloration of hypocotyl seedlings stage is present and is the average intensity. Type of plant growth is determined. Leaf is medium length, narrow to medium width is and division language is pinnate. Pubescens style and color of the flower is absent flower is yellow. The abscissa of the stem is absent. The fruit is very large and the height/diameter is medium. Fruit shape in longitudinal section is rectangular.

Table 1

Variety denomination – Argeș 11

No. crt.	No. CPVO*	Characteristics	States of Expression	Note
1.	2	Plant: growth type	Determinate	1
2.	10	Leaf: division of blade	Pinnate	1
3.	23	Fruit: size	very large	9
4.	25	Fruit: shape in longitudinal section	Rectangular	4
5.	34	Fruit: number of locules	three or four	3
6.	35	Fruit: green shoulder (before maturity)	Absent	1
7.	39	Fruit: colour at maturity	Red	5
8.	45	Resistance to <i>Melodogyne incognita</i>	Resistant	3
9.	46	Resistance to <i>Verticillium</i> sp. (Va and Vd) Race 0	Present	9
10.	47	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i>	Present	9

\* The official Catalog of varieties of crop plants in Romania

Preparation for sowing seed treatment consisted in performing infusion of chamomile and basil, both species having disinfectant and bacteriostatic (Figure 4).



Figure 3  
Variety Arges 11



Figure 4  
Tomato seedlings before planting



Figure 5  
Variety Arges 20

*Tomato variety Argeş 20* (Figure 5) Morphological characters: anthocyanin coloration of hypocotyl seedlings stage is present and is the average intensity. Type of plant growth is determined. The leaf is long and wide. Division leaf lamina is bipinnated. Pubescens style and color of the flower is absent flower is yellow. The abscissa of the stem is absent. The fruit is very large and the height / diameter is medium. Fruit shape in longitudinal section is rectangular (The official Catalog of varieties of crop plants in Romania - CPVO, 2013).

Table 2

Variety denomination – Argeş 20

No. crt.	No. CPVO*	Characteristics	States of Expression	Note
1.	2	Plant: growth type	Determinate	1
2.	10	Leaf: division of blade	Bipinnate	1
3.	23	Fruit: size	very large	9
4.	25	Fruit: shape in longitudinal section	Rectangular	4
5.	34	Fruit: number of locules	three or four	3
6.	35	Fruit: green shoulder (before maturity)	Absent	1
7.	39	Fruit: colour at maturity	Red	5
8.	45	Resistance to <i>Melodogyne incognita</i>	Resistant	3
9.	46	Resistance to <i>Verticillium</i> sp. (Va and Vd) Race 0	-	-
10.	47	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	-	-

\* The official Catalog of varieties of crop plants in Romania

Table 3

Measurements on new varieties of tomatoes

Variety	Type of growth	Number fruit the inflorescence	The average fruit weight (g)	The average diameter of the fruit median (mm)	The average length of fruit (mm)
Argeş 11	determined	8-10	176,6	70,9	76,3
Argeş 20	determined	4-5	242,6	70,5	75,9
Stefanesti 22	indeterminate	5-6	218,3	76,5	66,8
Costate 21	indeterminate	5-6	294,3	112,4	72,6

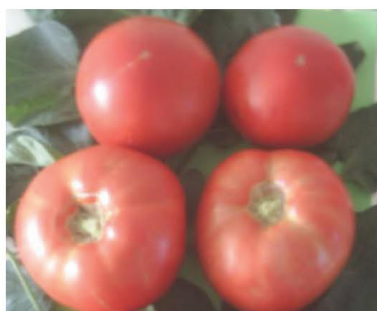


Figure 6-Variety Stefanesti 22

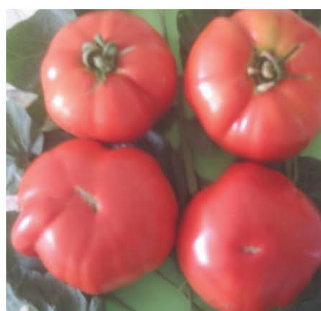


Figure 7-Variety Costate 21

In this respect, were used: seeds of its own production to produce seedlings, plant extracts from wild local prevention and control of harmful organisms were recycled waste some previous culture.

During the experiments were grown tomatoes efectauat biometric measurements. Biometric measurements consisted of determining the average height of plant, number of inflorescences per plant, average number of fruits per inflorescence, average size and average weight of fruit.

Varieties grown in greenhouse crops destined green were harvested at physiological maturity, their seeds being used to establish the following crops by obtaining seedlings or marketing. Measurements made during the experiment are shown in Table 3.

### CONCLUSIONS

For efficient organic crops required the intelligent use of local resources in an integrated knowledge-production-marketing skills resulted in innovative technologies.

Tomato varieties obtained from INCDBH Stefanesti are recommended and can be expanded in culture and other areas of the country.

New tomato varieties suitable for both fresh consumption and for processing.

At INCDBH Ștefănești producing tomato seed varieties approved in the institution is maintained even Bio crops.

At INCDBH Stefanesti resumed activity of creating new varieties of vegetables, organic certification requirements, which was discontinued in this institution for over 20 years.

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## **FOLIAR FERTILIZATION INFLUENCE ON PEPPER SEEDLINGS**

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*Keywords: pepper, root, fertilization, photosynthesis, perspiration*

### **ABSTRACT**

*Pepper is known for its weak root system. Therefore, is extremely important to obtain seedlings with balanced growth and development, but especially with a strong root system.*

*This paper presents the results of some researches related to foliar fertilization, single or combined with Cropmax 0.05% and 0.1% Razormin of pepper seedlings.*

*Analysis of the results showed that 0.1% Razormin fertilization allowed the obtaining of some pepper seedlings of better quality and with balanced growth and development, but more important with strong roots. These results are supported by physiological and biochemical processes, which were intensely expressed at the plants in this experimental variant.*

### **INTRODUCTION**

Cultivation of vegetables is an important source of income for production units and households. Obtaining of high yields of quality with maximum economic efficiency in terms of environmental protection is due to the use of best quality seedlings for vegetable crop establishment.

In recent years there has been a revolution on the vegetable products market regarding on the fertilizers used due to a powerfully entry of some large corporations in this area. The new produced fertilizers are characterized by complexity both in terms of their chemical composition, but also regarding on their possibilities of application and combination so that allow obtaining of varied recipes. Depending on the phase of plants growth requirements, one can use different foliar fertilizers with very good results. For example, a fertility stimulating product named Cropmax is used in at least two treatments, especially at *Sollanaceae* plants (Chilom P. et al., 2000).

### **MATERIAL AND METHODS**

The experiences were established in 2014 in a private sector of seedlings production located in the village of Palanca village Florești-Stoenești, Giurgiu County, at 20 km south-west of Bucharest. The main objective was testing of simple and combined effects of Razormin and Cropmax fertilizers on the pepper seedlings in order to develop some recommendation for seedlings producers in Romania regarding on their use as supportive treatment for the seedlings growth by improving of plants metabolism.

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**Razormin** is an environmentally friendly bio-stimulating product, which determines a rooting effect. Their chemical composition is complex and balanced, so that induces mainly a root system development, than the development of vegetative part through cell division. It contains free amino acids and polysaccharides, which stimulate the nutrients absorption, leading to the further development of plant.

**Cropmax** is a complex superconcentrated nutrient used for foliar fertilization, being 100% natural organic product certificated by BCS–KO Germany, according to EU.

We established a monofactorial experiment with 4 variants, considering application of simple and combined fertilizers Razormin 0.1% and 0.05% Cropmax on pepper seedlings (Vedrana F1) in two distinct stages: three, respectively five weeks after the seedlings mass emergence (7 March and 17 March).

The experimental variants were: V1–unfertilized seedlings; V2–seedlings fertilized with 0.05% Cropmax (March 7 and March 17); V3–seedlings fertilized with 0.1% Razormin (March 7 and March 17); V4–seedlings fertilized with combined products: 0.1% Razormin (March 7) and 0.05% Cropmax (March 17).

The experiment was installed using the block method in linear alignment with 4 repetitions. The total number of plants in the experiment was 240, each variant containing 60 plants, with 15 plants per repetition.

Sowing was made directly into alveolar pallets (alveolar  $\varnothing = 8.5$  cm) on February. Because heat and water were optimal provided, mass emergence of seedlings occurred after 16 days. During the growth period specific agrotechnics for seedling production was applied: daily ventilation, watering, weeding weeds. The temperature was kept at 16–17 °C to 28 °C at day and 14–17 °C at night. A treatment with CE Bravo 0.2% was made in order to prevent seedlings fall and also to avoid a *downy mildew* attack.

Particular attention was given to hardening off the seedlings since March 20, by lowering the temperature of the space to 12–14 °C at night and about 18–22 °C at day, as well as reducing the watering. This procedure is absolutely necessary in order to accomodate the seedlings with unlethal variable stress condition which they will face in the cultivation place so that allow the formation of "stress memory" (Agnieszka Sękara et al., 2012; Mangrich and Saltveit, 2000; Knight et al. 1996; Jennings and Saltveit, 1994).

During the development of experiments observations and measurements of plant growth were made in two different stages: before the second fertilization (March 17, one month after emergence); at the end of the experiment, when the seedlings have reached the optimum for a succesfull planting (March 25).

Observations and measurements were made on seedlings growth, as follow:

- **biometric parameters of seedlings:** plant height; the number of true leaves; weight of aerial vegetative unit; seedlings total weight; root weight and volume;
- **measurements of the main physiological processes intensity** (photosynthesis, perspiration, stomatal conductance) at the end of the experiment. We used the LC pro+ photosynthesis system. The measurements were performed on the active leaves located in the middle third part of the of the plant;
- **determinations of the assimilatory pigments content** in the active leaves: *chlorophyll* and *carotenoid pigments* were extracted in 80% acetone and determined spectrophotometrically (wavelengths 663 nm, 647 nm and 480 nm) using the extinction coefficients and equations described by Schopfer (1989). The results were expressed in mg/100 g fresh weight.

## RESULTS AND DISCUSSIONS

The results of the analysis of the first stage (March 17: one month after the emergence, respectively 10 days after the first fertilization) are shown in Table 1.



The pepper seedlings showed an uniform behavior regarding on the growth and development at this analysis stage. A small change of amplitude (0.2 cm) was registered by plants height, ranging from 8.6 cm at V3 to 8.4 cm at the other variants. The number of formed leaves varied between 4.6 at V1 and 5 at the other variants. Although the frequency of leaves showed no large variation, there are still nuances that designates the V1 variant as the one with the lowest frequency.

Table 1

Growth and development of pepper seedlings 10 days after the first fertilization

Variant	Plants height HPA (cm)	No. of leaves	Leaves frequency (nr/ cm HPA)
V <sub>1</sub>	8,4	4,6	0,55
V <sub>2</sub>	8,4	5,0	0,59
V <sub>3</sub>	8,6	5,0	0,58
V <sub>4</sub>	8,4	5,0	0,59

To determine the overall effect of the fertilization program applied on the pepper seedlings were made observations and measurements also one week after application of the second fertilization, respectively on March 25. The results obtained are shown in Tables 2 and 3, respective in Figures 1 and 2.

Table 2

Growth of pepper seedlings one week after the second fertilization (25.03.2014)

Variant	No. of leaves	Plants height HPA (cm)	Roots length HR (cm)	Total lenght of plants HT (cm)	Leaves frequency (nr/ cm. HPA)
V <sub>1</sub>	9,2	17,6	10,6	28,2	0,52
V <sub>2</sub>	8,4	19,4	18,4	37,8	0,43
V <sub>3</sub>	9,8	20,8	13,8	34,6	0,48
V <sub>4</sub>	9,6	18,8	14,5	33,3	0,51

Applied fertilizer program determined differences regarding on the growth of pepper seedlings.

So, the number of formed leaves showed an amplitude of 1.4 leaves, given by variants V3 (9.8 leaves formed) and V2 (8.4 leaves formed). The second place as performance is occupied by V4 variant with 9.6 leaves formed.

Plants height varied between 17.6 cm (V1) and 20.8 cm (V3). The other variants registered intermediary values (19.4 cm at V2 and 18.4 cm at V4).

The length of the roots have a large variation (7.8 cm) registered between V4 (18.4 cm) and V1 (only 10.6 cm). An overview of this indicator values allow us observing that applying fertilizer Razormin 0.1% has as results a decreasing of the roots length (13.8 cm at V3; 14.5 cm at V4), while 0.05% Cropmax fertilization caused an increase of the root length at pepper seedlings.

The total length of seedlings show large variations: from 37.8 cm at V2 variant (fertilized with Cropmax) to 28.2 cm at V1 variant (unfertilized) and is strongly influenced by the length of roots ( $R^2 = 0.9123$ , Figure 1).

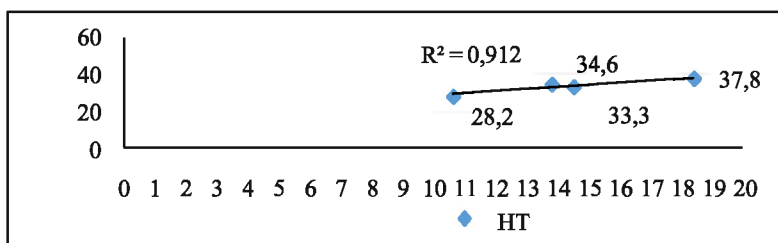


Figure 1. The relationship between root length and plant length at pepper seedlings

Leaves frequency ranged from 0.43 at V2 to 0.52 at V1, other variants registered higher intermediate values.

Taken together the results obtained by pepper seedlings morphometry, we estimate that the most balanced variant is V3 (fertilized with 0.1% Razormin) regarding on plants growth.



Figure 2. Growth of pepper seedling at the end of experiment

Developing of pepper seedlings one week after second fertilization (25/03/2014) was quantified by various indicators of weight and volume and by diameter of collet. The results obtained are shown in Table 3 and Figure 3.

Weight of the aerial part of seedlings registered a small variation, reaching at 5.5 g for most of the variants, except the V2 variant (only 5 g).

Roots weight registered also insignificant variation (3 g at V1, V2 and V4). Only V3 variant reached a higher value of roots weight (3.5 g).

Total weight of the plants differentiates the variants from each other. The highest total weight was measured at V3 variant (9g), while V2 variant registered the lowest weight (8 g). Variants V1 and V4 achieved a total weight of 8.5g.

Significant changes registered the roots volume: fertilization with Razormin leading to achievement of a higher roots volume (5.5 cm<sup>3</sup> at V3 and V4) comparing to variants V1 and V2, which registered a lower value (3.3 cm<sup>3</sup>).

The diameter of the collet is clearly expressed at each variant. The best value from this point of view was registered by the V3 variant (4.5 mm), followed by V4 (4 mm) and V1 (3.5 mm). The lowest diameter of the collet (3 mm) was determined at variant V2, fertilized with Cropmax.

Table 3  
Developing of pepper seedlings one week after the second fertilization (25.03.2014)

Variant	Weight of aerial part (g)	Roots weight (g)	Total weight (g)	Roots volume (cm <sup>3</sup> )	Ø collet (mm)
V <sub>1</sub>	5,5	3,0	8,5	3,3	3,5
V <sub>2</sub>	5,0	3,0	8,0	3,3	3,0
V <sub>3</sub>	5,5	3,5	9	5,5	4,5
V <sub>4</sub>	5,5	3,0	8,5	5,5	4,0

Analysis of the results of measurements performed on the pepper seedlings allow establishment of a hierarchy of the experimental variants: V3 registered the highest values of the biometric parameters, so was designated as the best variant of fertilization, comparing with variant V2, which registered the most reduced development. It appears that the applying of a certain fertilization program exert a significant influence on the developing of the roots (figure 3) as respects their height and volume.

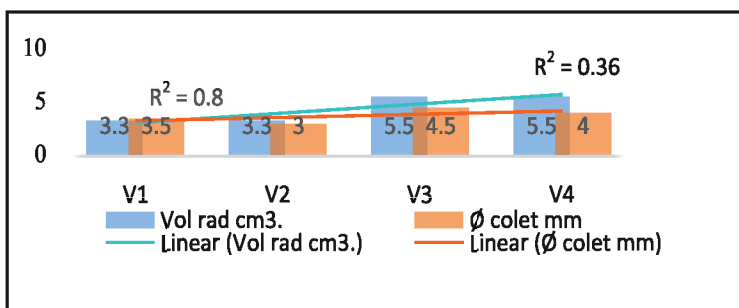


Figure 3. Influence of fertilization program on the roots volume and the collet diameter

The results of the physiological measurements performed on the experimental variants are shown in Table 4. As can be seen, the leaf temperature was relatively constant (25.2–26.3 °C) and light intensity registered the value of 890-900 mmol/m<sup>2</sup>/s.

Table 4

Physiology of the pepper seedlings one week after the second fertilization

Variant	Photosynthesis rate - A - [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]	Perspiration rate - E - [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]	F/T	Stomatal conductance - GS - [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]	Leaf temperature - T - [°C]	Light intensity - Q - [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]
V <sub>1</sub>	11,31	1,41	8,02	0,055	26,3	900
V <sub>2</sub>	14,15	1,17	12,39	0,04	26,3	900
V <sub>3</sub>	<b>21,7</b>	<b>2,59</b>	8,38	<b>0,20</b>	25,8	900
V <sub>4</sub>	14,79	3,13	4,72	0,135	26,3	900

Variant V<sub>3</sub> registered the highest values of photosynthesis rate (21.7  $\mu\text{mol}/\text{m}^2/\text{s}$ ), perspiration rate (2.59  $\mu\text{mol}/\text{m}^2/\text{s}$ ) and stomatal conductance (0.2  $\mu\text{mol}/\text{m}^2/\text{s}$ ). Also, this variant occupies the second place regarding the photosynthetic efficiency F/T (8.38). The lowest physiological activity was performed by variant V<sub>1</sub>, which registered the most reduced values of photosynthesis (11.31  $\mu\text{mol}/\text{m}^2/\text{s}$ ) and perspiration (1.41  $\mu\text{mol}/\text{m}^2/\text{s}$ ) and also a low value of stomatal conductance (0.055  $\mu\text{mol}/\text{m}^2/\text{s}$ ). Under these conditions, photosynthetic efficiency is relatively good and due of this adaptations plants manage to survive and to have also accumulations.

Variant V<sub>4</sub> registered a very reduced photosynthetic efficiency (4.72), which was for almost 4 times lower than the best variant (V<sub>2</sub> with value of 12.39). This situation can be attributed to high evapotranspiration, pepper being quoted in many papers as example in this point of view (Ciofu R., 2003; Popescu V. et al., 2000).

Table 5

Assimilatory pigments of the seedlings leaves one week after the second fertilization

Variant	Assimilatory pigments (mg/100 g)		
	Chlorophyll a	Chlorophyll b	Carotenoids
V <sub>1</sub>	96,50	36,10	5,07
V <sub>2</sub>	194,30	68,03	6,28
V <sub>3</sub>	118,54	45,61	13,51
V <sub>4</sub>	75,02	27,45	3,73

The measurement of some biochemical parameters (Table 5) completes picture of the results achieved under physiological aspect.

The highest amount of assimilatory pigments was registered at variant V2 (194 mg/100 g chlorophyll a; 68 mg/100 g chlorophyll b), but growth and development of seedlings are not fully supported due to the low stomatal conductance.

The second place is occupied by the variant V3, containing the highest amount of carotenoids (13.51 mg/100 g), which indicates a high photoprotective condition. V4 is the variant with the lowest content of assimilatory pigments, in correlation with their very low photosynthetic efficiency (water use efficiency F/T).

### CONCLUSIONS

In the first stage of analysis the pepper seedlings showed an uniform behavior regarding on the growth and development: no large variation was registered by the plants height, by the number of formed leaves and also by the frequency of leaves, although there are still nuances that designates the V1 variant as the one with the lowest frequency.

In the second analysis stage we noticed that applied fertilization program determined differences regarding on the growth, development and physiology of pepper seedlings: the most balanced variant from the point of view of plant growth is V3 (fertilized with 0.1% Razormin), while variant V2 (fertilized with 0.05% Cropmax) registered the lowest results.

The obtained data indicated that the applying of the fertilization program exert a significant influence also on the developing of the roots by increasing their height and volume.

Variant V3 registered the highest values of photosynthesis rate, perspiration rate and stomatal conductance. Also, this variant occupies the second place regarding the photosynthetic efficiency F/T, while the lowest physiological activity was performed by variant V1.

The results achieved under physiological aspect are confirmed also by the values of the analyzed biochemical parameters: the highest amount of assimilatory pigments was registered at variant V2, while the variant V4 reached the lowest value, in correlation with their very low photosynthetic efficiency. Variant V3 contains the highest amount of carotenoids, which indicates a high level of photoprotection.

In conclusion, the analysis of the accumulated data allow to remark that fertilization with Razormin led to obtaining of pepper seedlings of high quality and with strong roots.

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## THE BIOPREZERDARDACZ 060 SL INFLUENCE ON THE PRODUCTION OF FRUITS AND FRUIT BUD DEPOSITS

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**Key words:** *thinning, rootstock, variety, yield.*

### ABSTRACT

*Investigations were carried out in 2013-2014 in apple orchard in SA Zubresti planted in spring 2003 planting scheme 4x2 m. Trees are led by improved thin spindle-shaped crown. Apple fruit thinning was studied by chemical thinning, manual and mixed varieties Golden Delicious, Idared and Florina, grafted on rootstock M26. Was determined number of flowers, number of fruits. Norming application load the rod leads to obtain a stable quantity of high quality fruit and the submission of a sufficient number of fruit buds for next year. Inflorescence with a fruit variant 2 with chemical thinning fruit and mixed variant 3 with thinning (thinning chemical + hand thinning fruit) is the same for 164 pcs / tree and 166 pcs / tree. In variant 4 by manual fruit thinning inflorescence number is 174 pcs / tree.*

### INTRODUCTION

The chemical and manual fruit thinning a contribution to significant to maintain physiological balance between growth and fruiting and at increasing the quantity and quality of fruit (Balan V., 2009).

Thinning trees sprinkling flowers is during or after flowering with chemicals that destroy pollen, unfertilized flowers, stigmas and embryo of seeds from the fruit barely related.

Fruit thinning is the removal of a number of apples than the optimum number of fruits per tree. Se the optimal number of apples per tree allows obtaining high yields necessary commercial aspect, colour characteristic of the variety, taste and flavour appropriate and also ensures favourable conditions for the formation and differentiation of flowering buds. (Cimpoiș Gh, 2012).

The number of apples on a fruit tree is optimal if assigned well-developed leaves 30-50 cm<sup>2</sup> or a cross-sectional area of the trunk 5-7 fruits (BabucV., Rusu T., 1997).

Thinning apples is achieved by chemical and manual thinning. In Hungary, from substances using chemical thinning effect is auxin and ethylene. The most effective are alpha-and alpha-naphthylacetamide naphthylacetic (Babuc V., 2012).

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Chemical thinning greatly reduces the need for expensive labour force, but also the products used are polluting chemical thinning. Another disadvantage is that the crown fruit thinning is uniform.

Hand thinning of fruit is the safest and most effective method. It provides a uniform thinning of fruits can let the tree exactly the optimal number of apples and not polluting method, but this method is very thorough, requiring 20-40 days / ha man for a short time (Cimpoieş Gh, 2012).

Formation in apple harvest takes place over two growing seasons separated by a period of rest. In the second growing season linking occurs, growth and fruit maturation. Linking fruit going through the reserve substances accumulated in the tissues of the previous year because the trees bloom before the leaves form aces or when they are poorly developed (Balan V. et al.2001).

To obtain a constant and qualitative production in apple plantations super intensive expected load control cutting trees with buds flowering crop calculated as planned according to the biological characteristics of varieties and supplemented by manual fruit thinning after June drop (Sumudrea P& Sumudrea M. 2005).

### MATERIALS AND METHODS

We studied the interaction of foliar fertilization and fruit thinning, as key determinants of the quantity and quality of apple fruit.

Study of the influence of fruit load thinning processes of growth and fruiting in apple orchard covered the period of 2011-2012 in the experiment organized in a apple orchard in SA "Zubresti" Străşeni district. We studied Idared apple variety, grafted on rootstock M 26, planted in 2003, at a distance of 4 m between rows and 2 m between trees in a row. Chemical fruit thinning was achieved when the central fruit have 10-12 mm in diameter (Table 1) the preparation Bioprzerzedzacz 060SL is consuming 0.075% by 7.5 ml per 10 litres of water for 12 trees and 1000 l solution per hectare respectively.

Hand thinning of fruit is usually made after the fall physiological June until July 5 to 10 that is no later than 30 days after the bound. This thinning has a role in improving fruit size and quality. Thinning is leaving one fruit inflorescence 10-15 cm from each other. Remove the first small fruit, deformities, diseases and then to attack normal.

Table 1

Thinning fruit methods

Variant	Fruit thinning methods
V <sub>1</sub> (control)	Untreated.
V <sub>2</sub> (chemical thinning).	Managing chemicals when fruit diameter central inflorescence is 10-12 mm.
V <sub>3</sub> (chemical thinning + manual thinning).	Managing chemicals when fruit diameter central inflorescence is 10-12 mm. Fruit thinning is performed manually by physiological fall when fruits reach 16-18 mm in diameter.
V <sub>4</sub> (manual thinning).	Fruit thinning is performed manually by physiological fall when fruits reach 16-18 mm in diameter.

The set for each tree harvest is separate from the production weighing 24 trees and making the arithmetic mean. The average weight of the fruit is determined by weighing with a balance of the 100 apples, which are both formed and the degree of maturity.

## RESULTS AND DISCUSSIONS

In the apple orchards are considered the best tree fruit load, whether obtained one kilogram of fruit back 12-15 flowering buds located on branches of different ages depending on the particular biological variety. In the favourable weather conditions, the trees form a large number of fruit and there is need for thinning flowers and fruit.

In the Golden Delicious variety the inflorescence number in variant control is much smaller (Figure 1) than the variations of thinning due to weaker deposits fruit buds of the previous year.

Cleaning variants increase the amount of many flowers 241 pcs 285 pcs V4 variant with manual fruit thinning.

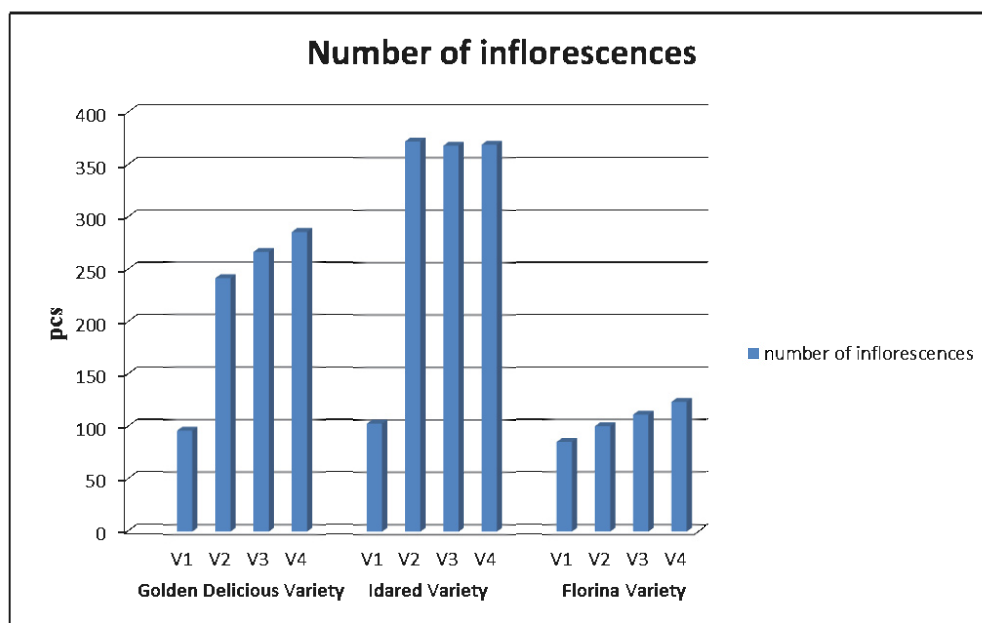


Figure 1. Number of inflorescence, pcs.

(Rootstock M 26 distance 4x2m planting, tree age 11 years, SA "Zubresti")

The Idared variety V1 difference between variant control and variants with thinning is over 200% and has been V2 variant with chemical thinning fruits 372 inflorescence which peak at this variety. The thinning mixed variants V3 inflorescence number was 368 units and 369 units respectively in manual fruit thinning variant V4.

The Florina cultivar difference between variants is the smallest of the three varieties studied and constituted from 85 pcs to 123 pcs in variant control variant with manual fruit thinning.

Location fruit crown this year is influenced by biological peculiarities of the variety (Figure 2).

The Golden Delicious by thinning method applied to biological peculiarities of the variety. The significant difference is in the placement percentage of fruit 2-3 m where the highest percentage of fruit is V2 variant of chemical fruit thinning (22 %).

Location Idared variety of fruits in different parts of the crown is a small percentage difference between variants of fruit thinning, but big difference is in variant

control where the percentage of fruit in the 2 to 3 m is 20 % compared to variants with normalization of fruit load where the percentage of fruit is from 12% to V2 and V4 solution, and the solution mixed fruit thinning has been 15 %.

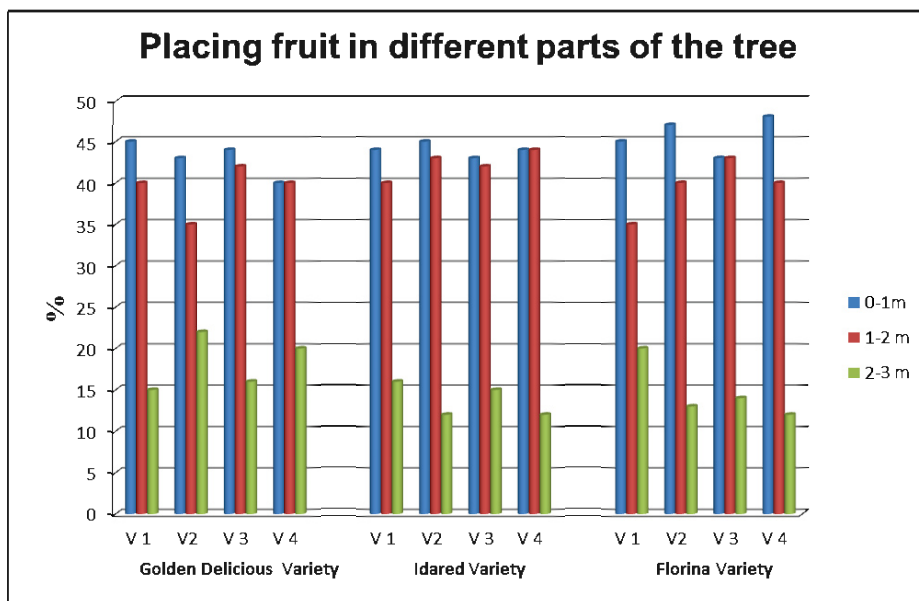


Figure 2. Placing fruit in different parts of the tree, %.  
(Rootstock M 26 distance 4x2m planting, tree age 11 years, SA "Zubresti")

Florina variety depending on the particular variety of biological highest percentage of fruit in the 0-1 m was recorded in 65% V1 variant. Variants of fruit thinning highest percentage of fruit in the same area (0-1 m) was recorded in 48% V4 variant, and V2 by 47% and the lowest percentage in the variant mixed fruit thinning V3 by 43%.

The applying all methods of thinning the fruit, chemical thinning and manually mixed fruit formations number are different. Thus the variety Golden Delicious fruit thinning variant with a number fruits formations each fruit is only 14 pc, but fruits are formations 2 - 43 pc worth. V2 variant where chemical thinning Bioprzerzedzacz 060SL apical concentration of 0.075% the number of formations floriferous single fruit grew to 181 units or 95% of total fruit. Formations with two fruits are in number of 10.

In the variants with mixed fruit thinning and hand thinning fruit formations percentage of single fruit reaches 96-98% of total fruit formations.

Idared variety fruit with a fruit formation in variant control V1R are only 80 units or 36% of total fruit. The Fruit thinning variants that percentage increases to 98 percent until V2R variant where the total number of configurations with a single fruit is 190buc. V3r variant with mixed fruit thinning fruit formations number 2 fruit is 12 pcs (Figure 3).



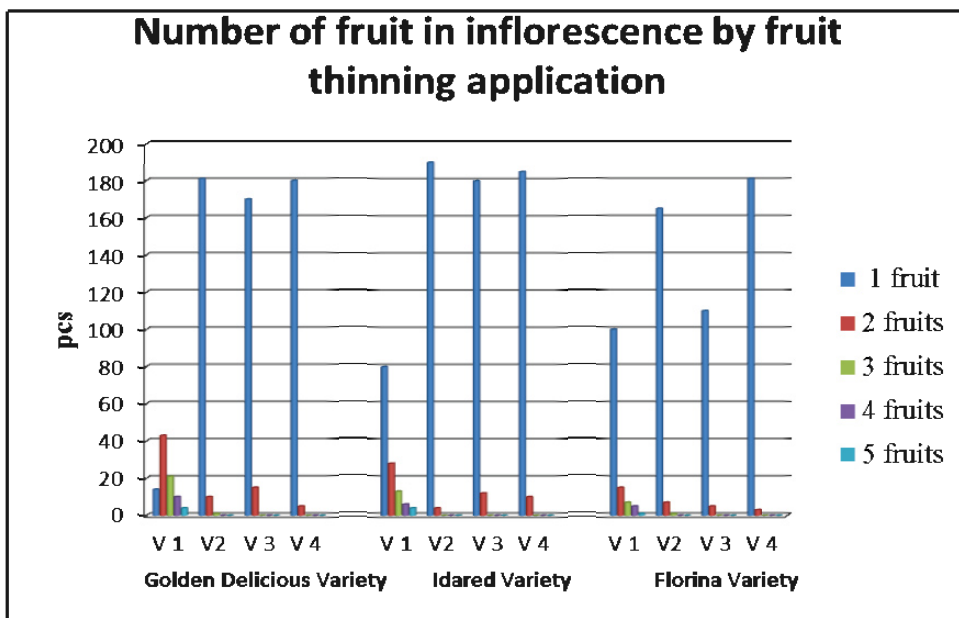


Figure 3. Number of fruit in inflorescence by fruit thinning application. (Rootstock M 26 distance 4x2m planting, tree age 11 years, SA "Zubresti")

The Florina variety of biological peculiarity adjustable single variety as number of parties with a berry fruit is very high clear and variant control V1R of 100 but the number of configurations with 2 and more fruit is much higher than in variants of fruit thinning and is 28% of the total.

### CONCLUSIONS

Application norming load the rod is an important link in achieving a stable harvest fruit. Golden Delicious and Idared showed the same intensity regardless of the method of thinning applied. Thus the number of inflorescence with a fruit variant 2 with chemical thinning fruit and mixed variant 3 with thinning (thinning chemical + hand thinning fruit) is the same for 164 pcs / tree and 166 pcs / tree. In variant 4 by manual fruit thinning inflorescence number is 174 pcs / tree.

Depending on the variety of biological thinning should be applied that method to ensure high quality fruit harvest.

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**QUALITY EVALUATION OF THE G<sub>0</sub> INITIAL PROPAGATION MATERIAL SAFELY KEPT IN THE DEPOSITORY GREENHOUSE**

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**Keywords:** *grapevine propagating material, maturation, soluble carbohydrates, starch*

**ABSTRACT**

*The initial G<sub>0</sub> viticulture propagation material is the first step in the multiplication of valuable genotypes and is of growing interest in the Romanian viticulture, with strategic and perspective importance in improving grape-vines. As a result, the only depository of the G<sub>0</sub> initial propagating grape-vine material in the country, at NRDIBH in Ștefănești, protects and promotes biological material with good status as of health and physiological terms. This study aimed to assess the adaptability of some grape-vine genotypes in the growing conditions of the depository greenhouse through quantitative and qualitative evaluation of the biological wood material obtained over five years, from 2009 to 2013. It was conducted on 6 genotypes (Golden of Ștefănești, Perlette 10 Șt., White Fetească 97 Șt, 16 Muscat Ottonel 16 Șt, Merlot 202 Șt and Large Burgund 86 Șt.), varieties and clones for which NRDIBH Ștefănești is the official maintainer.*

**INTRODUCTION**

Keeping the initial viticulture propagation material (G<sub>0</sub>) in the depository greenhouse helps solve two current issues in the Romanian viticulture: first, it avoids natural or accidental disappearance of indigenous grape-vine varieties and, secondly, it protects and promotes a biological propagating material of higher quality.

To obtain this material, under depository greenhouse conditions, particular attention was paid to a balanced supply of nutrients and water throughout the growing season in order to help the grape-vine plants adapt to the growing system in pots. It has at the same time been decided, since the establishment of the depository greenhouse (in 2008), the monitoring of all the biological processes specific to the growing phenophases according to the climatic conditions during the year.

**MATERIAL AND METHODS**

Compared to the grape-vine plants grown in the field, the higher values of solar radiation in the depository greenhouse conditions favour the earlier evolution of buds, flowers and maturation but at the same time, they extend the period of vegetation and

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temperatures, on the other hand. The total water content of the material originating from the analyzed genotypes in the depository greenhouse registered multiannual average values between 47 and 50% (Fig. 2 d) with optimal humidity conditions provided in the depository greenhouse (with drip irrigation watering schedule). The material originated from clones for the production of white wines showed average total water content of 47.9 - 48.6% and in the red wine grape clones it was of 47.2% (Figures 2 b and c). The multiannual averages ranged around 50% (Fig. 2) for the genotypes of table grape-vines.

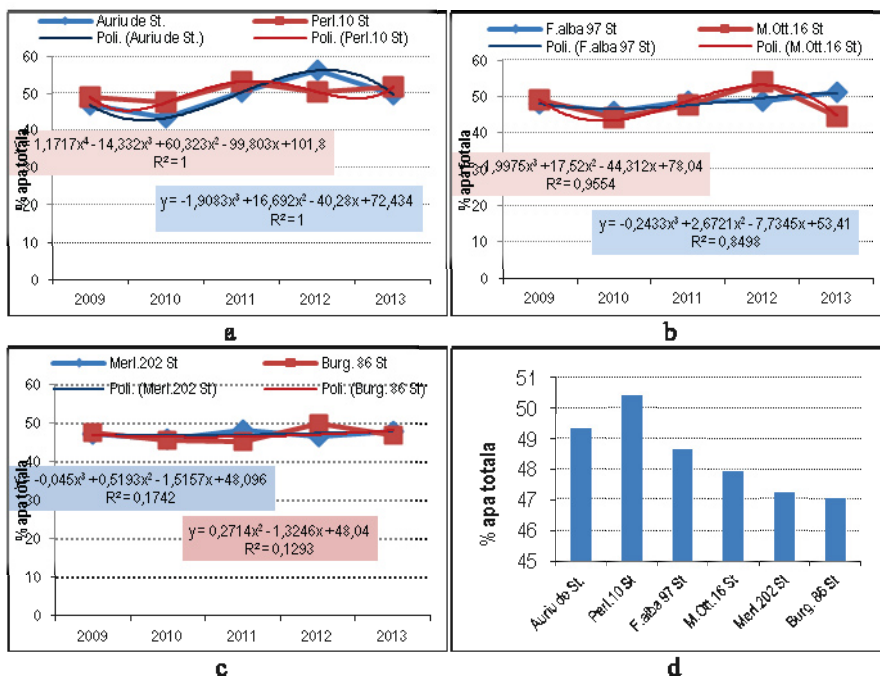


Figure 2. The total water content of wood material originating from genotypes and clones of grape-vines tested between 2009-2013 (a, b, c) and the multiannual average (d)

Bound water content is an index of the tolerance ability of the organ under test to a biotic stress factors (frost, drought). In the studied genotypes, the bound water content in shoots recorded close annual average values (2.3 - 2.9%), observing a decrease in this index for all the genotypes tested in 2010 and 2012 (Fig. 3 a, b, c). As quantity, the bound water showed higher values to the clone of Perlette 10 St. (Fig. 3).

The quantification of carbohydrates showed a good maturation of the wood in all the tested genotypes, both the soluble sugars and the starch reaching higher values. In the table varieties, the soluble carbohydrate content ranged from 10.3% dm, for the variety Golden of Ștefănești, to 12.7%, for the clone of Perlette 10 St. (multiannual average - Fig. 4 d).

In the year 2012 - 2013, both genotypes accumulated significant amounts of carbohydrates in wood (12.3% for Golden variety and 13.9% in the clone of Perlette 10 St. respectively).

In the genotypes of grape-vine varieties for obtaining quality red and white wines, the accumulation of soluble sugars were within the range of 10.1% - Burgund 86 St. and 11.8% - Muscat Ottonel 16 St., which are acceptable limits for a biological material during

the dormancy period of the months of December and January, when the soluble carbohydrate content reaches a maximum of 10-12% carbohydrates (Fig. 4 a, b, c).

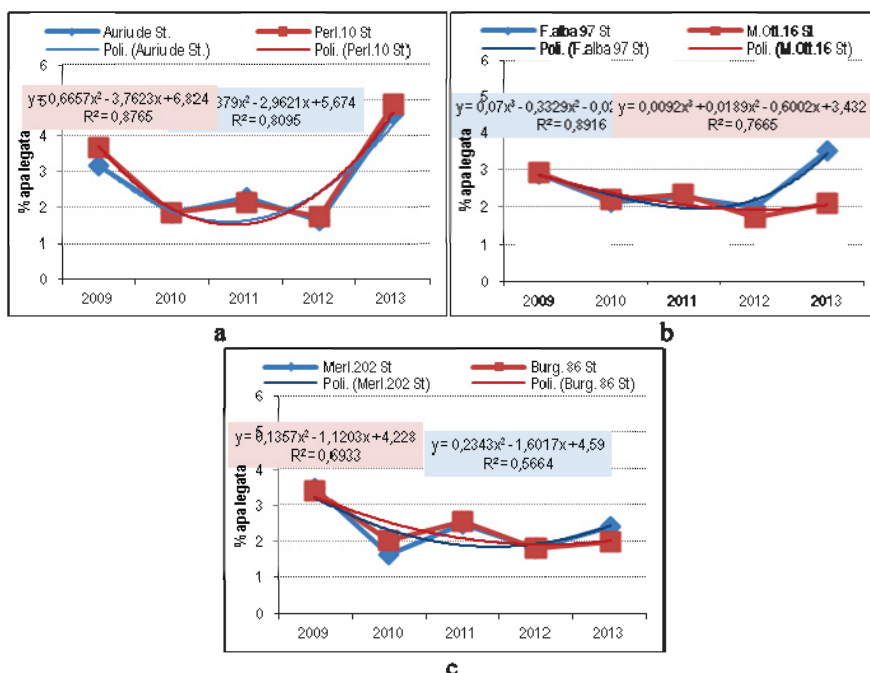


Figure 3. The bound water content of the wood material in the grape-vine genotypes and clones tested in the period 2009-2013 (a, b, c)

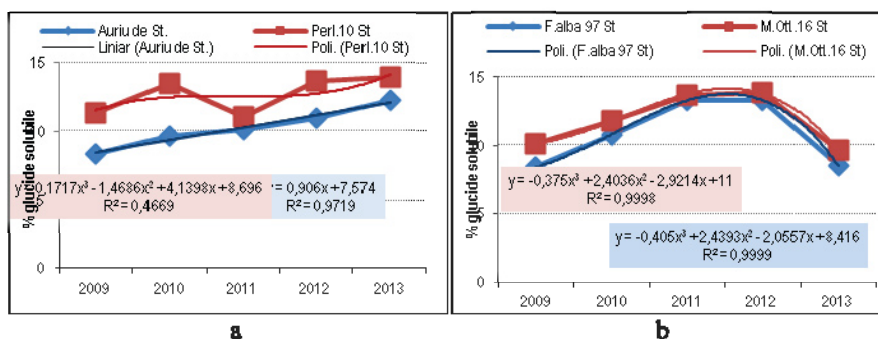


Figure 4. The starch content of shoots showed values close to those normal for January-February, falling between 3.8 - 6.1% (multiannual averages).

Higher accumulation of starch has been reported throughout the viticulture year of 2012 - 2013 in the clones of the varieties for quality red wines (Merlot 202 St. - 7.3%, Burgund 86 St. - 7.4%). The clone of the seedless variety of Perlette 10 St. showed minimum values of the starch in all the studied years (Fig. 5 a, b, c).

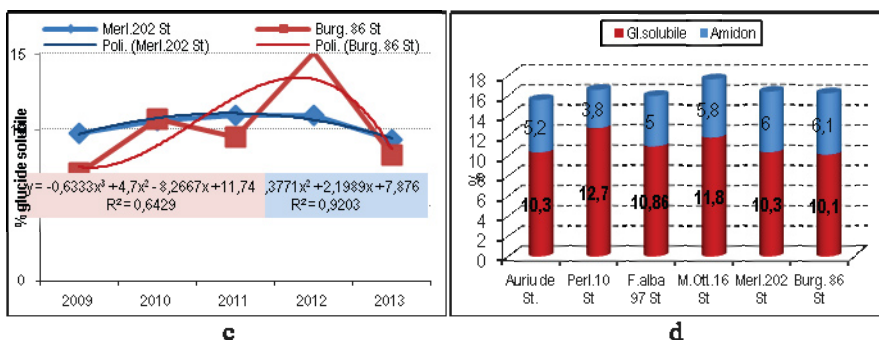


Figure 5. The soluble carbohydrate content of wood originating from genotypes and clones of grape-vines tested in 2009-2013 (a, b, c) and the multiannual average (d)

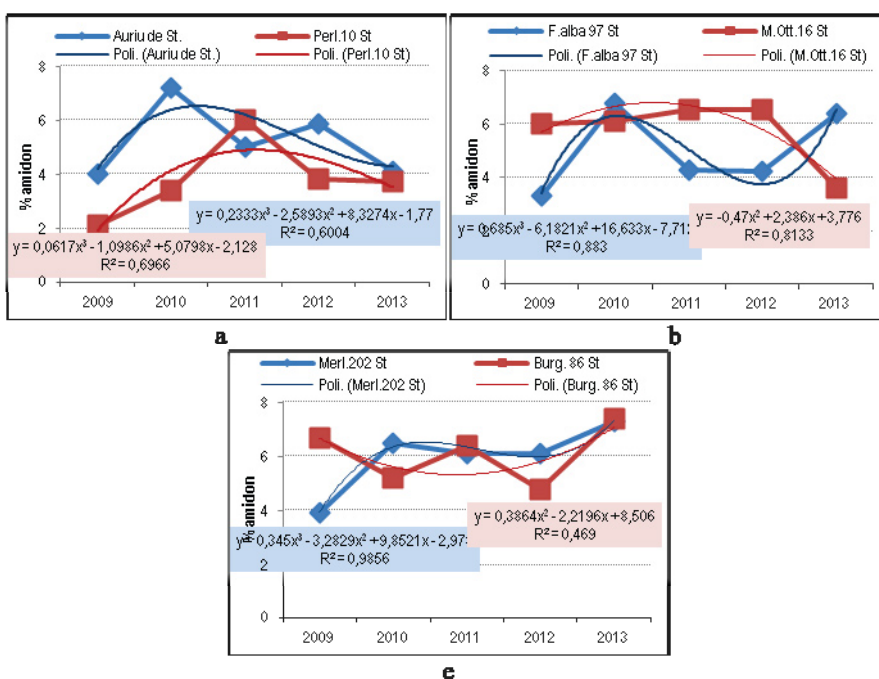


Figure 6. The starch content of wood originating from genotypes and clones of grape-vines tested in 2009-2013 (a, b, c)

For the overall carbohydrates, the studied genotypes showed significant amounts of sugar, due to the optimum status of nutrition and health of the stocks as well as to the mild conditions in the depositary greenhouse. The evolution of the content in total carbohydrates in the wood material belonging to the clones and varieties studied in 2009-2013 is shown in Fig. 6 a, b, c.



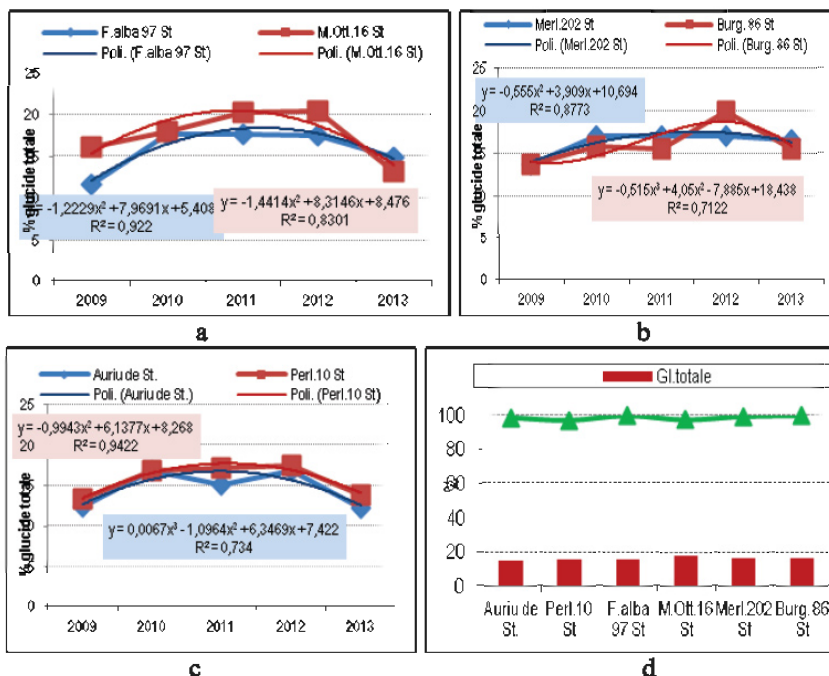


Figure 6. The content of total carbohydrates in the wood originating from genotypes and clones of grape-vines tested in 2009-2013 (a, b, c) and the multiannual average (d)

The accumulation of total carbohydrates between 14.7 - 17.6% (multiannual averages) allowed obtaining higher primary buds viability i.e., 97.2 - 99.6% (Fig. 6 d).

### CONCLUSIONS

Overall, the results of biochemical analyzes performed on biological material in the form of shoots, show that the accumulation of soluble sugars and starch in the wood tissue was correlated with the total length of the shoots and the matured shoot part in the studied genotypes.

The results show a very good adaptation capacity of the studied genotypes in the growing conditions of the depository greenhouse, due to the plasticity of the grape vines under a balanced supply of nutrients and water, and a good health status throughout the growing period.

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## RESEARCH REGARDING THE LANDSCAPE PLANNING OF A LEISURE AREA – THE GHIOROC LAKE, ARAD DEPARTMENT

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*Keywords: landscape planning, lake, fishing, Ghioroc*

### ABSTRACT

*The present project presents the research regarding the landscape planning for the entertaining area of Ghioroc lake from the Arad department. The lake is ideal for practicing aquatic sports and in this area one can find entertaining and accomodation. Being also a destination for fishing, this sight doesn't attract only tourists.*

*The social and natural circumstances of the area have been examined and a landscape planning proposal has been made. After presenting the current situation, we justified the general solution of landscape planning. In the study, the make-up principles used, the style, circulation and functionality, choosing vegetation, the utilitarian and ornamental endowments are described. The project was drawn-up in ArchiCAD programme and the rendering in ARTlantis programme.*

### INTRODUCTION

It is a well known fact that tourism is one of the least exploited economic sectors in Romania. It can only be developed if we are to invest in infrastructure and the development of area with potential. Such an example is the lake Ghioroc, which, in time, has become one of the most wanted places to spend their leisure time for the people in Arad county.

With a surface of over 15 ha and a laid out beach, the lake attracts more and more tourists each year. Thus it has earned the title of „the home beach” among people. The lake is ideal for water sports, and one can find recreation spaces as well as accommodation in the area. The resort does not only attract tourists, it is also a fishing destination.

The best known recreation area is placed on the Western lake shore, more precise, a laid out beach, close to a terraces and restaurants chain. Although the aforementioned beach is one the most beloved recreation areas among Arad city dwellers, and more, it does not have specific facilities for this type of resort.

### MATERIAL AND METHODS

After analysing the situation plan (Figure 1), the current functions and the high potential of the chosen recreation area, we have decided upon dividing the terrain in two

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large subareas: a beach laid out with adjacent spaces up to the terraces and a fishing area (Figure 2). Between the two mentioned spaces we proposed the development of two parking lots, which should accommodate the inhabitants' number.

The amplification of urban agglomerations and their neighbourhood, the prolongation of spare time at weekends, as well as modern transport means facilitating excursions to the neighbouring areas of big cities even during weekdays, determine the organization and confer greater importance to preurban touristic areas (Iliescu A.F., 2003). Situated in the suburban area of the Arad city, the Ghioroc commune is part of the renowned Arad vineyard, which has a special touristic potential, unfortunately insufficiently capitalized.

The examples offered by a series of approaches from preurban areas in western Europe show that around localities with touristic potential a series of functions complementary to tourism have been developed, especially regarding sports and recreation. These complementary functions support tourism and make it possible due to the fact that leisure time is spent in a pleasant, diversified way, so that visitors may wish to spend a longer period of time in the place that offers them such facilities. The conclusion drawn from these examples is that tourism cannot exist without developing complementary functions. This type of approaches brings great benefit to tourists, their families and everybody in the touristic locality circuit.

The recreation area of the Ghioroc lake is such a resort. The Ghioroc lake with an over 10 ha surface and a laid out beach, is one of the local tourist attraction centres. Fishing and swimming in this lake are the main activities of the visitors. Although this part of the lake is one of the most beloved recreation areas by Arad city inhabitants, and more, it does not have a series of facilities specific to such resorts. The best example is the beach with a surface of 0.5 ha which does not have modern public toilets and dressing rooms. The access road to the beach and the adjacent area is not asphalt, and the other access ways to the fishing areas which have developed in time are actually earth alleys.

In time, a number of terraces and restaurants were opened near the beach. On the right side of the access road, there is a non-asphalt parking lot, which in summer, because of the intense traffic, is a real source of dust and noise.

## **RESULTS AND DISCUSSIONS**

Due to the fact that, at the time being, the beach does not present a series of facilities, we proposed that there should be open air showers, public toilets and dressing rooms nearby. The beach will be equipped with an open bar, and a beach volley field. Also, there will be canopies, wooden chaize lounges and reed umbrellas.

The space between terraces and beach will be transformed into a promenade area rest point with meeting and standing points, with urban furnishing and facilities including simple benches, modernly designed benches, garbage cans, public spring fountains and photovoltaic lamps. The vegetation was chosen so that it would create a cheerful summer atmosphere with a diversified colour panel in harmonious composition. This area is extended along the terraces, facilitating their access. Here, we have also proposed to lay out a playing ground for children. It shall be equipped with swings, seesaws, and a playing complex with slides and a climbing frame.

Due to the fact that in the perimeter adjacent to the beach, more precisely, to the north, there are already earths paths which lead to the fishing places on the lake's shore (Figure 3), and many people prefer to camp overnight in their tents, we proposed that this subarea



Figure 1 Ghioroc Lake – the situation plan of the area

should be dedicated exclusively to camping and fishing. As facilities, we have suggested to create two parking lots which should accommodate the visitor numbers, camping cabins, ecologic toilets and a shower room. The alley number was restricted to the necessary minimum.

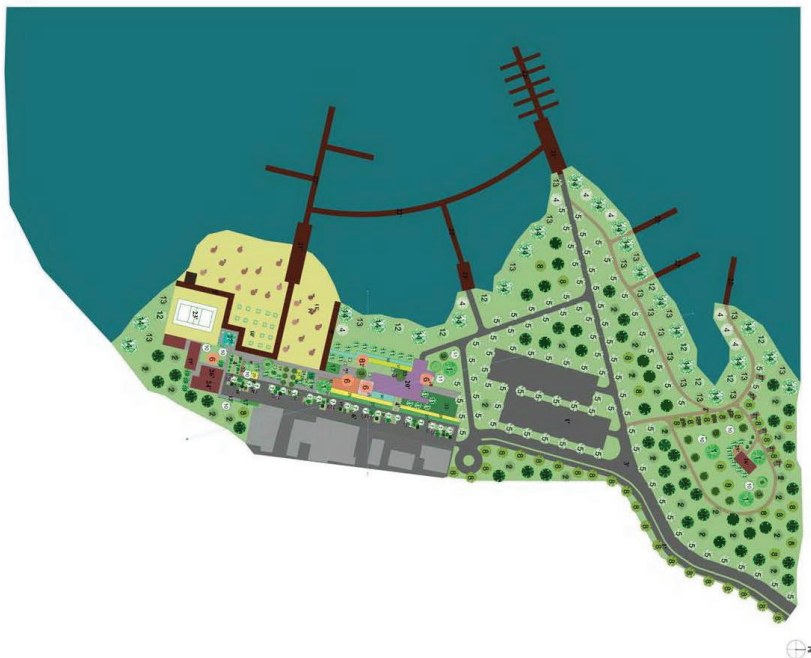


Figure 2 Ghioroc Lake – general landscaping plan

The development proposition for the recreation area is made in a mixed style. By combining the natural style with the geometric one, the mixed style has been created, which supposes treating some composition parts in a geometric style, and others in a landscaping style, so that an organic unity should be achieved.

The development is carried out taking into account certain composition principles valid for all styles:

- The unity principle;

- The functionality principle;
- The compatibility principle;
- The proportionality principle.

The vegetation influence on health may be direct or indirect. In this sense, trees in groups and alignments reduce temperatures in high temperature days, offer protection against wind, participate in atmosphere cleaning and reduce noise (Rosemary A., 2009).

Landscaping confers a territory a certain ornamental value, appreciated through the satisfaction given to man by a vegetation whose components (body, branches, scapes, flowers, fruit, seeds), make people think of a well organized thing, a space where parts combine harmoniously in order to highlight the inherent measure of each composition, which means beauty (Simonds J.O.,Starke B.W., 2006).

The laid out alleys follows routes which link the main interest points (entrances, objectives). Circulation is easy on straight alleys which open a wide perspective. The main alleys' dimensions are of 2m, from these leading secondary subordinated alleys. less circulated areas will measure 1.5m. The alley sides will be connected in order to facilitate circulation and will be laid out in such a way as to insure access to the entire garden surface.

The material used for the main alleys, the access road and the parking lot will be asphalt, and for the camping alleys we chose pebbles, because it does not implicate high costs. For pedestrian areas we opted for two types of paving: concrete slabs of the same dimension and of various dimensions.

The access between the two areas can be achieved by bridges, as an alternative to get to the desired place.



Figure 3 – Lake shore perspective – proposition

Wooden species as well as flowers used in the landscape planning proposition where chosen according to the areas and their ecologic demands, so that they can develop harmoniously in this area with mostly sand grounds.

The surface proposed for development will include resiniferous and deciduous trees, resiniferous and deciduous shrubs and flower plants. The placing of trees and shrubs will be done so as to combine colours, leave, flower and fruit nuances, according to the vegetation season, so that the green space is varied from the viewpoint of the colour panel.

In elaborating the project we took into account the leafing out, the blooming and the fruit period in order to obtain their permanent succession from spring to autumn (Iliescu A.F, 2002).

Deciduous trees: *Acer negundo*, *Acer platanoides* „Crimson King”, *Acer platanoides* „Globosum”, *Betula pendula*, *Elaeagnus angustifolia*, *Koelreuteria paniculata*, *Rhus typhina*, *Salix alba*, *Salix matsudana*, *Salix babilonica*, *Sophora japonica*.

Resiniferous trees: *Cedrus atlantica*, *Pinus nigra*, *Pinus strobus*, *Taxodium distichum*.

Deciduous shrubs: *Berberis x media*, *Cotinus coggygria* „Royal purple”, *Euonymus alatus* „Compactus”, *Euonymus fortunei* „Emerald Gold”, *Prunus laurocerasus*, *Salix capraea* Kilmanrock, *Salix integrifolia*.

Resiniferous shrubs: *Juniperus horizontalis* „Blue Chip”, *Juniperus sabina tamariscifolia*.

Perennial flowers: *Armeria maritima*, *Cerastium tomentosum*, *Euphorbia enyrsinthes*, *Perovskia atriplicifolia*, *Rudbeckia fulgida*, *Salvia officinalis* „Tricolor”, *Santolina rosmarinifolia*, *Saponaria ocymoides*.

Facilities and furnishing used in landscaping are represented by benches, garbage cans, lamp posts, spring fountains, bicycle stands.

The children play ground will be equipped with one, two or three seat swings, a play complex with slides, seesaws with two seats, climbing frame and circular bench. For protection and safety the space will be enclosed with a wire fence, and the pavement will be tartan.

Tartan is the premium solution for playground paving. The polyurethane surfaces are made up of a controlled mix of EPDM granules and polyurethane resin, the mixture being laid, according to the placement, in one, two or three layers. The guarantee for this material varies between 3 and 8 years. After a long usage it can be regenerated at low costs and does not need maintenance.



Figure 4 – Promenade area perspective – proposition

For the promenade area (Figure 4), we proposed a sprinkler watering system which should insure the water necessary in hot summer days. Due to the fact, that this area is situated near the lake, the costs of using a watering system will be reduced, the water being extracted from the lake with the help of a house water supply plant.

## CONCLUSIONS

We believe that research carried out have proven that the Ghioroc Lake in the Arad county has a special touristic potential, which can be capitalized very well through proper landscape planning. In this sense, the project we proposed can be carried out by involving local decision factors the green space is easily maintained, and the species used in landscaping are not particular to natural conditions.

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**URBAN SQUARE – LANDSCAPE PLANNING IN MODERN DISTRICT,  
TIMISOARA CITY**

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*Keywords: landscape planning, urban square, plants*

**ABSTRACT**

*The present project presents the research regarding the idea of urban square implemented in an area from the district of Modern, the city of Timisoara. The corresponding area of the site has experienced in recent years a development characteristic for the entire city and specific to the market requirement: trade, services, living.*

*The social and natural circumstances of the area have been examined and a landscape planning proposal has been made. After presenting the current situation, we justified the general solution of landscape planning. In the study, the make-up principles used, the style, circulation and functionality, choosing vegetation, the utilitarian and ornamental endowments are described. The project was drawn-up in ArchiCAD programme and the rendering in SketchUp programme.*

**INTRODUCTION**

Throughout the entire world, urbanization takes place at an unprecedented level, and the concept of "urban" keeps extending especially in Asia, where the urbanization process and town, territory and even innovatory settlement forms development is happening at an unparalleled rhythm. Urban landscape planning is an integrated part of modern urban construction and is developing in accordance with the change of style, lines and architectural evolution.

Modern urban squares are based on conceptual landscape planning, the starting point being the idea and not the vegetation or the materials. Creating these spaces sets out from a concept or visual motif, which can be noticed in the entire composition of the landscape planning. If in the case of gardens and parks, the conceptual process shows a human dimension, permanent proportion maintenance, this, however, does not apply in the case of conceptual urban squares. Still, the design will take into account the functional and social aspects of the space (Simonds J.O.,Starke B.W., 2006).

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## **MATERIAL AND METHODS**

In carrying out the landscape planning proposal, we took into account the multiple functionality of the space, as well as its aesthetics.

Firstly, a functional space is desired, with the predominant characteristics of an urban square: large circulation surface, colour spots brought by vegetation and the stripes where resting places are accommodated for the moving population. Secondly, the intention was to achieve a modern design, with a preference for minimalism, which should confer the space the atmosphere and characteristics of the 21<sup>st</sup> century.

The carrying out concept introduced the following aspects:

- achieving certain composition principles at a high qualitative level;
- systemising component elements from the point of view of proportion as well as functionality;
- introducing elements of visual impact;
- introducing high quality urban furniture (wooden pergolas, modern benches, art objects);
- enlarging the functionality sphere of the urban square;
- installing lighting.

## **RESULTS AND DISCUSSIONS**

The area proposed (Figure 1) for landscaping occupies a total surface of 32,050 m<sup>2</sup>, has a irregular pentagon shape and is bordered as follows:

- to the North-West: Calea Dorobanților;
- to the North: private property, activating in commerce and services;
- to the North-East: Palmierilor street;
- to the South-East: Mătăsarilor street;
- to the South: dead end Calea Dorobanților and private properties, functioning in services and as accommodation;
- to the West: Calea Dorobanților.

In the last years, the area pertaining to the studied site has experienced a development characteristic to the entire city and specific for market requirements: commerce, services and accommodation.

The placement studied in the present paper belonged to the former "Filty" shoe factory, where dismantling activities of the former industrial site buildings where undertaken.

The functional reconversion of an industrial site is part of the general urban modernization context, especially manifested in former industrial areas, of great dimensions, in the city of Timisoara.

Recent urban planning projects have analyzed the area limitrophe to the studied site, towards the town exit on Calea Lugojului, the residential district in the southern part, between Calea Dorobanților and Ștefan cel Mare street and the mixed area, services, commerce and residential, situated in the western part of the site, on the Simion Bărnuțiu Boulevard, all with no touristic impact on the studied ground.

There are no buildings on the grounds, the land occupation percentage (POT) is of 0% at the time being, and the land occupation factor (CUT) is 0.

The studied placement is bordered on two sides by major traffic arteries: Calea Dorobanților and Calea Lugojului, and on the north-western side by the Simion Bărnuțiu Boulevard - Calea Dorobanților crossroads, thus generating an intens car traffic area.

Car traffic bordering the site on the north-eastern and eastern sides: Palmierilor street, respectively Mătăsarilor street, are secondary arteries with reduced traffic.



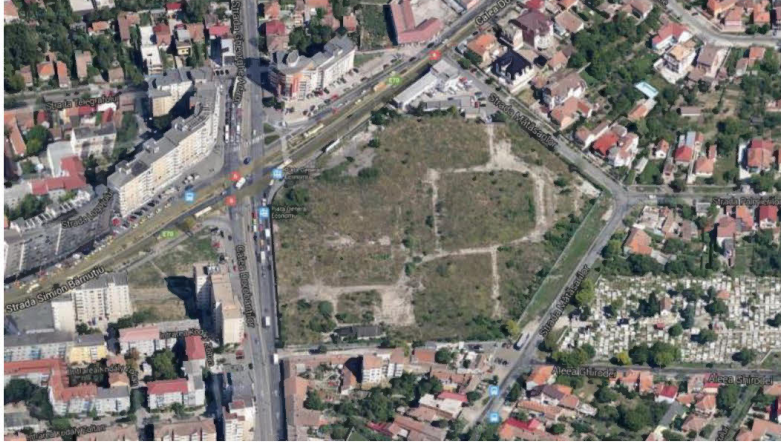


Figure 1 Studied site – the situation plan of the area



Figure 2 – Urban square – general landscaping plan

Pedestrian traffic is happening on sidewalks pertaining to the aforementioned streets.

The landscape planning ensemble concept (Figure 2) is based on the linear intersection of materials, textures and nuances. The resulting elements are geometric shapes, mostly rectangular and triangular. The materials used are meant to create contrast, shape outline, rhythmicity and also elegance and harmony. The level difference in lawned areas destined to recreation, the height of the steel concrete window boxes, of the fountains and the pergola arrangement, the alignment stripes enclose or prolong perspectives, depending on the view angle of the visitor (Rosemary A., 2009).



Figure 3 – Urban square perspective – proposition

The composition was created with the help of the conceptual landscape planning principle, where pattern is a preset point. The two steel concrete window box constructions shadowed by wooden pergolas transversally divide the space in three parts, and the stripes created by the *Catalpa bignonioides* alignment and *Liquidambar styraciflua* alignment longitudinally divide the space in three parts. According to these divisions, the spaces and element geometry were outlined, applying symmetry and parallelism principles.

Destined for a large number of visitors, the access to the *urban square* can be achieved from all sides of the site, the grounds being elevated as an immense pedestal with +0.20 m compared to  $\pm 0.00$  m quota of sidewalks pertaining to the surrounding car traffic. This difference in level, alongside with material changes, is the only characteristic delimiting the square visually and physically. The landscape can be looked at from a distance, their perception being mostly contemplative, but, more often, they are explored thoroughly by covering their various areas; unlike natural landscapes, the ones designed for direct use by the population, must allow access and pedestrian traffic and with various transport means inside them.

The current landscape planning is dominated by traffic routes which answer firstly to functional requirements: they insure visitor access, lead to various areas organized for diverse functions, connect the objects included and all parts of the landscape planning, insure circulation comfort.

The urban square being made up of a series of spaces and landscape scenery implies movement, through which successive images merge into a gradual visual perception. The alley aspect is given by the cover materials, which confer a certain texture. This influences the pedestrian traffic speed.

The material chosen for the traffic is stamped concrete and, in some places, decking and pebbles.

In areas especially created, destined to be admired, the alleys will be combined with decking stripes, vegetation and pebbles which determine a slower walk, thus raising the interest for the composition details.

The lawn spaces, destined for rest, relaxation and sunbathing, display slopes and various heights, meant to achieve plane shape disruptions.

Wooden decks with benches are placed rhythmically, creating a strong order sensation, next to texture disruptions achieved by the vegetation and ornamental pebble stripes. The promenade spaces are wide, opening generous perspectives, their dimensions

encouraging the visit of people on bicycles, rollerblades, skateboards and open air movement (jogging).

In the aired spaces of the square, but also on the wooden decks, the possibility of exhibit large art objects, which are not easily exhibited in small closed spaces, is foreseen. This aspect offers the urban square a cultural characteristic, enlarging not just the number of visitors, but also the cultural contribution.

In the north and north-eastern area of the space the building of two ultra-modern restaurants was suggested, in minimalistic style, in order to be elegantly framed by the general landscape of the urban square planning. The restaurants and pertaining terraces are situated at both ends of the north-eastern side of the square, alongside which a parking lot was developed.

In developing the terraces, the same materials were used as in the landscape planning.

A mixed style was used to carry out the landscape planning project, with a special predilection for the geometric style.

The mixed style, also known as composite style, is characterized by designing some areas of the park or garden in a geometric style, and others in natural style, with harmonious connections between them, constituting a unitary ensemble. The mixed park may be more attractive because of the variety in scenery, where elements of geometric aesthetic order can merge with picturesque or romantic aspects. The mixed style allows a greater solution freedom, it adapts better to requirements of public, multifunctional, modern park landscape planning, including various equipment and secondary systemising, especially in the case of larger surfaces, with the capacity to accommodate a high number of visitors.

Within the framework of these projects, the geometric style elements will be used near buildings, composition centres, cultural and sport objectives.

For the execution of the project, the following composition principles were considered:

- functionality principle;
- duality principle;
- rhythmicity principle;
- harmony principle;
- balance principle (Iliescu A.F., 2003).

Being destined for a large number of visitors, the access to the square can be achieved from all sides of the site, the grounds being elevated as an immense pedestal with +0.20 m compared to  $\pm 0.00$  m quota of sidewalks pertaining to the surrounding car traffic. This difference in level, alongside with material changes, is the only characteristic delimiting the square visually and physically. The landscape can be looked at from a distance, their perception being mostly contemplative, but, more often, they are explored thoroughly by covering their various areas; unlike natural landscapes, the ones designed for direct use by the population, must allow access and pedestrian traffic and with various transport means inside them.

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The material chosen for the traffic is stamped concrete and, in some places, decking and pebbles.

In areas especially created, destined to be admired, the alleys will be combined with decking stripes, vegetation and pebbles which determine a slower walk, thus raising the interest for the composition details.

The pergolas placed along the steel concrete window boxes have a functional shadowing role, but also an aesthetic one, so that the proper material choice is defining for the global colour harmony of the proposed urban square. The urban furniture elements were designed in a modern stylistic manner, according to the style opted for the entire landscape planning.

Benches destined for resting have a modern design and the materials used are corroded metal and wood. Being placed on the decking stripes, they will present the same finish nuance as the decking and the pergolas.

Garbage cans, especially designed for modern urban spaces, will have a minimalist aspect, from materials and nuances similar to the lamps, since their placement will be executed according to the placement of the lamp posts.



Figure 4 – Urban Square perspective – proposition

The lighting of the main and secondary alleys of the urban square will be achieved by choosing lamps whose aspect should blend in with the landscape planning stylistic manner. They insure an illumination level under the tree corona. The lamp posts placement is optimal for an area with abundant vegetation creating a pleasant atmosphere at night. The lamp model we chose comes to complete the range of park furniture, being especially created for urban green spaces.

Woody vegetation represents the “main material” of the landscaping of a green space, a material which changes its volume, colour, texture and shape in the course of a year or during a longer period of time. This material creates shapes, volumes, compositions, simultaneously constituting the harmonizing link for all anthropic elements which will form a unity in the end.



Since the landscape planning of an urban square commands it, the woody vegetation was mostly placed under the shape of alignments, along the promenade and resting areas, and, in some places, in combination with tree and shrub groups.

We opted for contrasting vegetation, by shape, colour and texture, certain areas of the landscape planning being strongly dominated by the vegetation shape and colour (Figure 4).

The planted surface will include resiniferous and deciduous trees and shrubs, ornamental grasses and lawn.

Thus, the intention was to combine colours, silhouettes and heights, to shade pedestrian, resting and reading areas. The growth rate in trees and their height were taken into account. Solitary specimens belong to spectacular tree and shrub species, with a strong visual impact: *Albizzia julibrissin*, *Paulownia tomentosa*, *Aesculus*, *Chaenomeles japonica*.

The entire landscape planning is divided by main alignments, one with *Catalpa bignonioides*, and the other with *Liquidambar styraciflua*. Close to the waters, species with predominantly pendent build were placed (*Salix*, *Betula*).

The groups include from 2 - 7 specimens with irregular spatial placement. Homogenous groups were designed – with a single species, as well as heterogeneous groups (groups resulting from the association of *Juniperus horizontalis*, *Juniperus communis* and *Thuja occidentalis* species) (Iliescu A.F, 2002).

List of species used in the project:

- deciduous trees: *Acer saccharinum*, *Aesculus hippocastanum*, *Albizzia julibrissin*, *Betula pendula*, *Betula pendula* “Youngii”, *Catalpa bignonioides* “Nana”, *Liquidambar styraciflua*, *Magnolia kobus*, *Malus floribunda*, *Paulownia tomentosa*, *Platanus X acerifolia*, *Prunus cerasifera* “Nigra”, *Robinia pseudoacacia* “Umbraculifera”, *Salix alba*, *Salix matsudana*, *Tilia argentea*, *Quercus robur*.

- resiniferous trees: *Cupressus sempervirens*, *Picea abies*, *Pinus sylvestris*, *Thuja occidentalis*.

- deciduous shrubs: *Acer palmatum* “Dissectum”, *Berberis thunbergii* “Red Rocket”, *Buddleja davidii*, *Chaenomeles japonica*, *Hibiscus syriacus*, *Philadelphus coronarius*, *Pyracantha coccinea* “Red Column”.

- resiniferous shrubs: *Chamaecyparis lawsoniana* “Columnaris”, *Juniperus horizontalis* “Blue Chip”, *Juniperus sabina* “Tamariscifolia”, *Juniperus sabina* “Variegata”.

- undershrubs: *Lavandula angustifolia*, *Rosmarinus officinalis*.

- ornamental gramineae: *Cotaderia selloana*, *Festuca glauca* “Elijah Blue”, *Miscanthus zebrinus*, *Ophiopogon planiscapus* “Nigrescens”, *Pennisetum setaceum*.

## CONCLUSIONS

We believe that the research carried out have proven that capitalizing the studied area would mean a considerable gain for the city of Timisoara. The current maintenance works can be carried out satisfactory by a team established for this objective. Taking into account the necessary wages, the materials used, and the equipment depreciation, compared to the analyses carried out for other parks, the annual maintenance cost is estimated to 5.000-6.000 Eur/ha.

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## GALAȚI 2030: A TOURISM DEVELOPMENT STRATEGY FOCUSED ON LANDSCAPE VALORISATION AND PROTECTION

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*Keywords:* Galați, Green Infrastructure, Landscape Planning and Assessment, Sustainability, Tourism

### ABSTRACT

*Sustainable tourism development has become a priority in urban planning in the last two decades. Thus, approaching issues regarding valorization and protection of the landscape within development strategies represents a key condition for creating a sustainable urban environment.*

*The aim of the approach is to analyze the relation between landscape and tourism, in order to generate an integrated development strategy for the city of Galați, focused on local resources.*

*The study comprises a first step analysis for classification and assessment of the landscape potential, an analysis of touristic resources and types of tourism. The second stage includes the proposed concept and the landscape development strategy. The resulting strategic vision is based on the concept entitled "Enclosing the Green-Blue Ring", which contributes to the enhancement of Galați within sightseeing cruise circuits on the Danube, by creating a navigable green-blue network.*

### INTRODUCTION

In recent decades, due to the increasing competition between cities, the municipalities started to give more importance to urban planning, including green infrastructure problems. The main issues covered in urban development projects are: the spirit of place, the local history and the environment (Kotler, Haider, Rein, 2001). Improving green infrastructure is essential to highlight the attractive elements of the city (Bran, Simon, Nistoreanu, 2000).

Assuming that the attractiveness of a city varies depending on the diversity and multiplicity of touristic interest points, creating new attractions and related infrastructure to support the interest for existing objectives is one of the main tasks for municipalities. To enhance the quality of the natural frame, there may be proposed projects and programs on urban green systematization and improvement of the physical environment. The most attractive urban areas are: the places where historical events occurred, commercial areas, cultural and educational places, recreational and entertainment facilities, sports arenas, events and special events, iconic buildings and monuments. (Kotler, Haider, Rein, 2001)

The study is focused on the relationship between green infrastructure and tourism development and on the importance of an integrated approach in landscape planning in order to increase the attractiveness of the tourism resources exploitation.

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## MATERIAL AND METHODS

The studied site, the city of Galați, offers a relatively wide range of landscapes with a diverse touristic potential, poorly exploited at present. The approach is focused on integrating the attraction elements within the green network development strategies and policies. The relationship between green infrastructure and tourism is highlighted both through development directions and through local interventions.

The first phase of the study includes the analysis of landscape typologies, existing tourism resources and the types of tourism practiced in Galați. The second phase comprises the concept and a tourism development strategy focused on landscape valorization, containing directions and intervention proposals. In addition, we propose a set of measures for the recovery and protection of the landscape in the city of Galați.

## RESULTS AND DISCUSSIONS

**Landscape typologies** (Figure 1). The main types of landscapes found in Galați neighborhoods can be classified as:

- a) Mostly natural landscapes:
  - Riparian landscape – consisting of streams and characterized by a high biodiversity;
  - Lacustrine landscape - with large areas of water, spontaneous vegetation and valuable natural environment;
  - Riverscape - dominated by water and boats, providing a wide field of view;
  - Floodplain landscape – located across the river, mostly used for recreational activities.
- b) Mostly anthropogenic landscapes:
  - Infrastructure landscape – the main visually elements are the compositional lines of railways and bridges;
  - Industrial landscape – high density of built areas and visual landmarks (industrial buildings), having a negative impact on the environment;
  - Late communist urban landscape - high buildings with uniform architectural style and high density of built areas;
  - Early communist landscape – medium height buildings with large green spaces between them, offering an open appearance of the landscape;
  - Old urban landscape – low height buildings, patriarchal ambiance;
- c) Suburban landscapes:
  - Agricultural landscape – wide views, without notable elements;
  - Fallow landscape – wide views, damaged aspect;

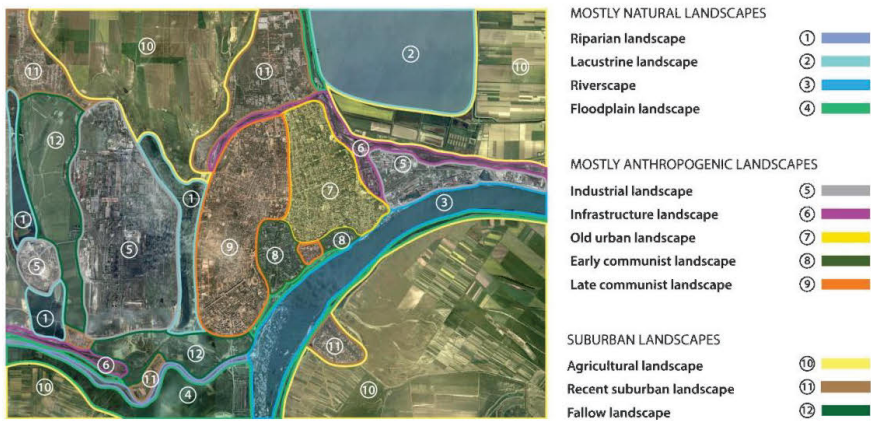


Figure 1. Landscape Typologies Plan



- Recent suburban landscapes – various buildings without stylistic unity, colour and density, irregular green areas character.

Of these, the mostly natural landscapes (riparian, lacustrine, riverscape, floodplain) and fallow lands, near which is considerable territorial development availability, have the highest potential for tourism development.

Threatened landscapes that require protection are located close to the Siret River and Danube riverbank. These landscapes are threatened and require special attention, according to the tourism development strategy which aims to valorise the "blue" areas in the surroundings of Galați.

**Tourism resources.** Tourism resources located in or near Galați include four major categories:

a. Cultural resources - places of worship, historical buildings, theaters, museums.

b. Natural resources - Gârboavele Nature Reserve, "Siret Valley" Nature Reserve, Prut's Ostrov, Trighina Bărboși fossils place, Brateș Lake, Vânători Lake, Seromgal Marsh, Cătușa Lake and the Danube River.

c. Sports and leisure resources - "Waves of the Danube" Beach, Brateș Lake Beach "Briza Dunării" Complex, "Viva" Complex, "Danube" Rink.

d. Recreation resources - Danube Promenade, Mihai Eminescu Park, Zoological Garden, Botanical Garden, Cloșca Park, Rizer Park, Liberty Park.

Thus, Galați has four categories of tourist resources: cultural - concentrated in the central area; Natural - located around the city; leisure and sports - present especially near the Danube river and in the city center; recreation resources - located on the Danube shore and near Brateș Lake.

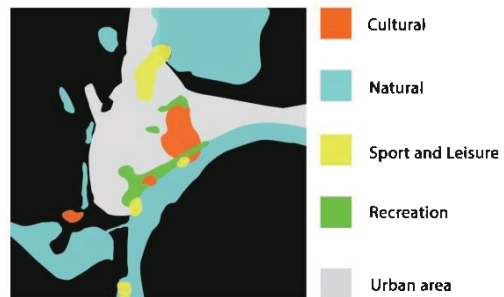
**Types of tourism** (Figure 2). As shown in resources classification, the main types of tourism in Galați are cultural, natural, leisure-sports and recreation. In terms of accommodation units, we can notice a strong concentration of hotels in the city center, while in the rest of the city there is a lack of tourism structures.

**Tourism development strategy.** The concept, entitled "Enclosing the Blue Ring" (Figure 3) proposes to develop Galați as a touristic attraction in Danube cruise circuits by creating navigable "blue ring", located around the city, to facilitate access to the major points of interest.

The main directions regarding tourism development are (Figure 4):

- Naval and nautical tourism development;

#### TYPES OF TOURISM



#### EXISTING TOURISM STRUCTURES



Figure 2. Types of tourism and existing tourism structures - Diagrams

- Create a "green-blue" network;
  - Environmental awareness among residents and tourists;
  - Promoting and strengthening the international image of the city;
  - Enhancing the local identity by using "blue" infrastructure;
  - Increasing the attractiveness of urban infrastructure;
  - Rehabilitation and improvement of the quality of green areas;
  - Increasing the dendrological fund;
  - A stronger relationship with other cities across Danube River and the Delta;
- Specific proposals:**
- Establish a continuous peripheral water circuit by creating waterways to connect water surfaces around the city;
  - Redevelopment and expansion of Danube promenade;
  - Creating a network of tourist information points;
  - Establishment of jetties for water sports;
  - Creating of city tours;
  - Construction of new accommodation units on the waterfront areas, located in the proximity proposed "blue ring";
  - Introducing a public transport ship between Galați and nearby cities (Brăila, Tulcea, Sulina, Cernavodă);
  - Proposing a planted embankment with different types of vegetation, arranged gradually to combat the effects of floods (Danube);
  - Creation of new parking spaces in outlying areas;
- Extending green areas in order to create a green belt.

### CONCEPT: ENCLOSING THE "BLUE RING"

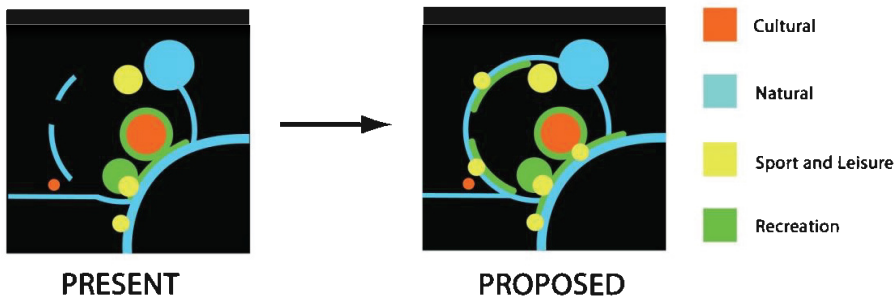


Figure 3. The Concept of the Tourism Development Strategy

### **Protection and valorisation measures related to the landscape tourism development strategy in 2030.**

Policies related to landscape tourism development strategy aim to integrate and protect the suburban landscapes of Galați. Specific measures have been proposed in order to improve the relation between the suburban areas and the rest of the city, through several main directions (Figure 5):

1. Reducing environmental degradation:
  - Encouraging alternative energy - use of agricultural land in the proximity of the city in order to obtain biomass crops;

- Promoting "industrial symbiosis", a process by which waste products are turned into industry resources for one or more types of industries;
- Conservation and enhancement of biodiversity by planting trees;
- Waste management;
- Wastewater treatment by phyto-treatment plants;
- Limiting and preventing infiltration of pollutants in the soil;
- 2. Recovery natural suburban landscapes and unused areas:
  - Redeveloping abandoned or degraded areas - conversion of former industrial areas, greening the landfill located in the south of the city;
  - Programs for greening the affected areas, especially those adjacent industrial areas;
  - Protecting and preserving the natural environment;
  - Consolidation of banks subject to erosion – in the case of Danube River and Brateş Lake;
  - Draft study and analysis regarding various panoramic points and their arrangement;
  - Guiding spatial expansion of the city and urban dispersal limitation in areas with natural character;
  - Reshaping the landscape of Danube promenade;
- 3. Improving the quality of life in the city:
  - Creating a system of suburban greenbelt;
  - Increasing access to green spaces by creating pedestrian and biking trails closely with urban public transport stations;
  - Creating physical connections the suburban green spaces by creating and filling greenways leading to peripheral landscaped areas;
- 4. Awareness of existing landscape values;
  - Increasing the public interest for ecological tourism in natural areas;
  - Involving urban actors in the implementation of various programs and projects;
  - Organising information campaigns and environmental education and aesthetics;
- Introducing sports and recreational activities on the river banks

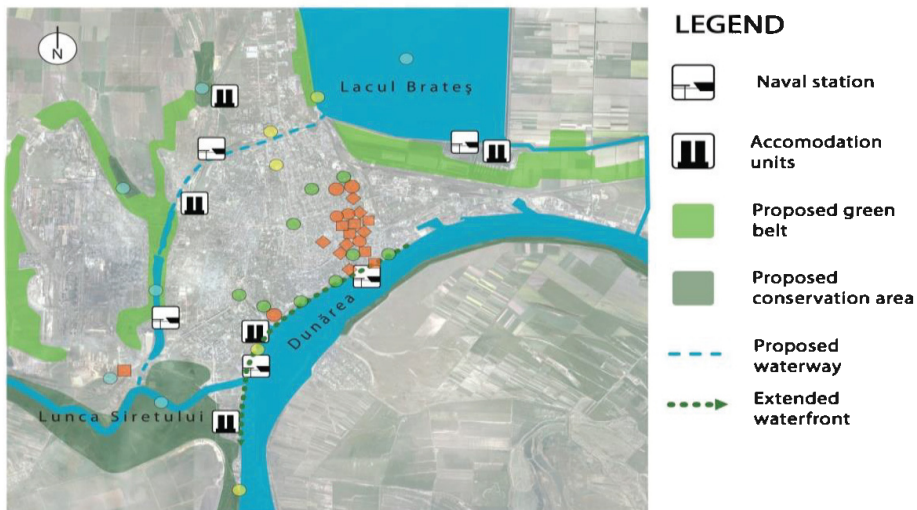
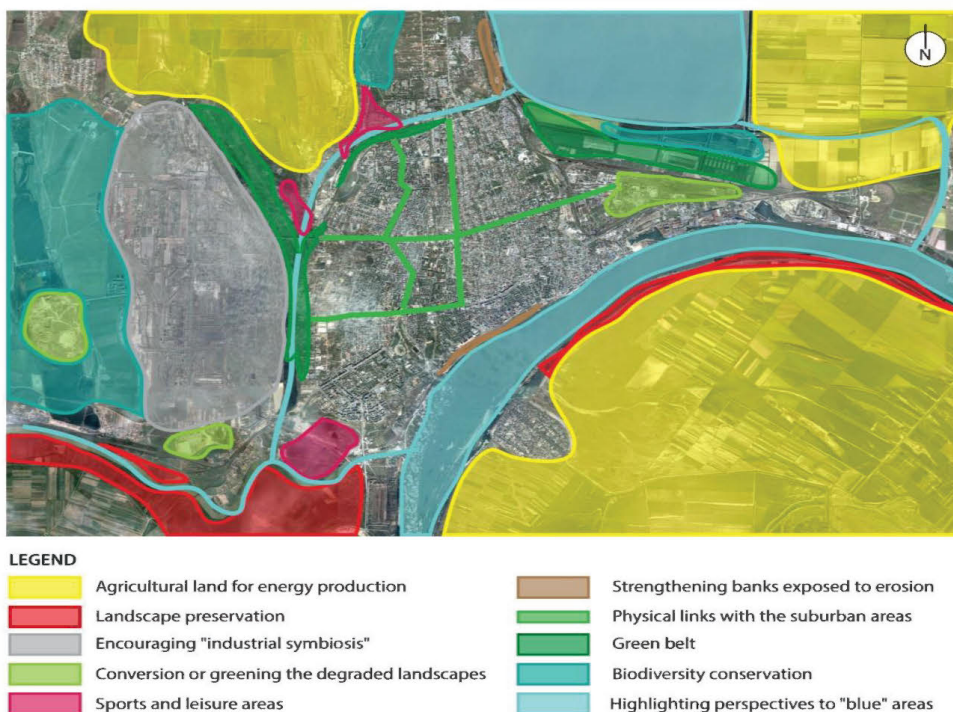


Figure 4. Tourism Development Strategy Plan



**Figure 5. Landscape Protection and Valorization Measures**

### **CONCLUSIONS**

Green infrastructure development through strategies associated with tourism development measures may contribute significantly to the sustainable growing of cities. Such actions generate benefits both economically, environmentally and socially. In economic terms, it can reduce planning and development costs, by increasing investment attraction. At ecological level, it reduces negative environmental impacts and natural resources are exploited for tourism. In social terms, local communities benefit from new facilities and take advantage at the same time upon the arrival of tourists.

Few places in the world have all the elements of attraction, but each economic sector can provide local development in certain directions. An essential way to increase the level of local attractiveness is to enhance the landscape values through urban regeneration measures and thus, to improve the image of the city.

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## CIȘMIGIU GARDEN, BUCHAREST: AN INVENTORY METHOD REGARDING VALUABLE HERITAGE ELEMENTS

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*Keywords: Cișmigiu Garden, Cultural landscape, Heritage inventory, Historical garden restoration, Landscape heritage.*

### ABSTRACT

*Management and restoration of historical parks and gardens are conditioned by researching and evaluating heritage objects and ensembles, whether they are natural or constructed. Currently, numerous historical landscape ensembles are in an advanced stage of decay, the identity of the place being affected by recent interventions and by the ignorance of authorities.*

*The aim of the approach is based on promoting historical awareness by using a method of landscape heritage inventory of valuable items from Cișmigiu Garden, applied in the "Mound-Waterfall-Lake" Area. The study includes the following steps: identification of historical objects and ensembles, surveying, historical research and assessment, organizing presentation sheets. Study's results can be a starting point for developing a management plan and future restoration projects for Cișmigiu Garden.*

### INTRODUCTION

Historic gardens are testimonies of the past, which give identity to cities and to local communities. Inadequate management of these sites endangers the visual identity of those heritage landscapes in many cities across Romania. In the last decade it can be observed an insertion of irregular elements phenomenon, which alters the composition and ambience designed by renowned landscape architects as Karl Friedrich Mayer, Friedrich Rebhuhn and Edouard Redont.

The present project is focused on the identification and analysis of ensembles and objects of historical value in Cișmigiu Garden, one of oldest public gardens in Wallachya. Cișmigiu is also one of the oldest and most representative Romanian public gardens influenced by French Landscape Architecture. The „somptuous rectangular green lawn bordered by two lateral rectilinear promenades as well as its small devious alleys which sometimes follow the winding line of pond's shore strikingly remind of the Jardin des Plantes in Paris” (Harhoiu Dana, 2001).

The study was conducted in the central area of the park called "Mound-Waterfall-Lake". (Figure 1)

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## MATERIAL AND METHODS

"Mound-Waterfall-Lake" area, located in the center of Cișmigiu Garden, includes a number of ensembles with distinct characteristics. The area concentrates various historic valuable elements and a number of well known places frequented by many visitors make it one of the most emblematic zones in the garden. Since the late nineteenth century until today, Cișmigiu Lake views and postcards became and still remains a prominent landmark of the garden.

The research method consists of three major steps: directly documenting, indirectly documenting, organizing material and making presentation sheets.

Directly documentation comprised the inventory of valuable historic elements such as sculptures, urban furniture, valuable trees, water features and landforms. Inventory stage involved: object survey, taking photos and finding each location on the situation plan.

Indirect documentation included researching numerous written documents (books, journals, articles) and visual (postcards, photos, movies) in order to identify dating, historical value and original appearance of every object and ensemble.

In the final stage of the study, were drawn sheets for each item or group of historical and environmental. The presentation sheets follow the model proposed by Violeta Răducan, including: dating, description (materials, textures, color and size), landscape value, plant specimens, mapping, photographs of (Răducan Violeta, 2009). Sheets are presented in two categories. The first includes items of historical value, such as sculptures, benches, trellises, fences and other built elements. The second one comprises ensembles of elements such as the successive water features, the Monument of French Heroes, the Mound, the Alignment of yews and lindens.



Figure 1. The studied area in Cișmigiu Garden

## RESULTS AND DISCUSSIONS

„Buturuga” („The Stump”). Made around 1900, the building is made of concrete and the exterior texture imitates a giant clump of linden. "The stump" was surrounded by a summer garden, famous in Bucharest for the beer and the tasty goat loin served there. Currently this facility is closed. (Figure 2)

The aesthetic value of the structure lies in its uniqueness, visible by shape and texture. Former character of the "Buturuga" area is affected by ecological toilets situated nearby. Reopening "The Stump" could substitute some restaurants near the lake, located in unfavourable places aesthetically and functionally.



Figure 2. „The Stump”

**The Lake Fountain.** Built in the early twentieth century, the fountain is located in the central area of the southern Cișmigiu Lake and the water jet has a maximum height of approximately 5 m. The construction is made of natural stone and partly covered with moss. The structure is similar to a pyramid, with the base (on the bottom) of four sides of equal length of 3.20 m and a height of 3.20 m all. Fountain height from the water level is from 2.4 to 2.5 m.



Figure 3. The Lake Fountain

The water jet dominates the south of the lake, being a landmark and a symbol of Cișmigiu Garden. It could be seen in many postcards from different historical periods. A negative aspect is the recent placement of a large fixture in the center of the lake, near the fountain, affecting the overall image of the mirror of water. (Figure 3)

**Statue of Gheorghe Pannu** (historical monument - code LMI 2004: B-III-mB-20049). The bust of publicist Gheorghe Pannu, dating from 1912, is made of bronze and the concrete pedestal base is plated with white marble. The statue is made by the sculptor Gheorghe Horvath and was raised by the newspaper "Adevărul" in the memory of the journalist. The height of the statue from the pedestal base is approx. 3 m.

The aesthetic value of the statue results from its profiling on the contrasting evergreen vegetation of *Buxus* sp. which highlights the sculpture. It is located near two other statues: The French Heroes Monument and the "Negresa". The location of the sculpture is a favourable one: at the crossroads of three major paths, providing a perspective on the end of the Great Bridge.

#### **The Great Bridge.**

Initially a wooded structure, it was rebuilt by concrete during the lake regularization interventions in the 1880s. The bridge is made in an eclectic style, following the fashion trends found in Europe during that period. (Figure 4)

The maximum height of the bridge arch at the water level is 2.10 m. The concrete balustrades have a height of 80 cm. At the sides of the bridge there are two pillars, approx. 3.50 m height above the water level.



Figure 4. The Great Bridge

The bridge offers a broad perspective to the southern part of the lake, one of the most popular points of interest in the park, for skating in winter and for boating during the summer time. The bridge fits harmoniously into the landscape with sloping silhouettes of trees and the water surface by volumes, colours and textures. Currently, the bridge requires restoration.



**The alignment of yews and lindens.** The alignment, called "The Promenade Axis" was conceived in 1912 by landscape architect Friedrich Rebhunn. The alleys are located along the "Central ground floor", linking Queen Elizabeth Boulevard at South and. Ştirbei Vodă Street at North.

The visual value of the promenade axis consists of the rich ligneous vegetation that creates a green tunnel ambiance. The landscape of the alley could be improved by introducing elements of furniture (such as lighting or bins) that fit harmoniously together. (Figure 5)



Figure 5. The Promenade Axis

The bilateral alignment is composed of *Tilia* sp. and *Taxus baccata*. The yews date from the early twentieth century being among the oldest and most spectacular examples of conifers in the park. The lindens are planted recently, approx. 30-40 years ago, instead the original alignment.

**The Mound and the Waterfall.** The mound is depicted on a plan of Cîsmigiu for the first time on Cerkez Survey in 1883. On the same plan it can be observed the extension of the southern lake. Comparing with the previous Borroczyń plan it is likely that the mound was created with the expansion and regularization of the southern lake. The Mound has a relatively circular shape with a diameter of approx. 50 m and a maximum height of circa. 10 m. (Figure 6)

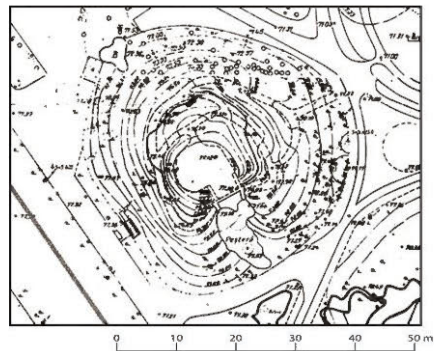


Figure 6. The Mound Plan

The waterfall, located on the southern side of the mound, was performed by landscape architect Friedrich Rebhunn in the 1920s, and originally bore the name of the Mayor Pache Protopopescu. Then it was created a cave in the mound. While in Cîsmigiu worked a mini-zoo, the cave housed wild animals (bears, wolves). (Figure 7)

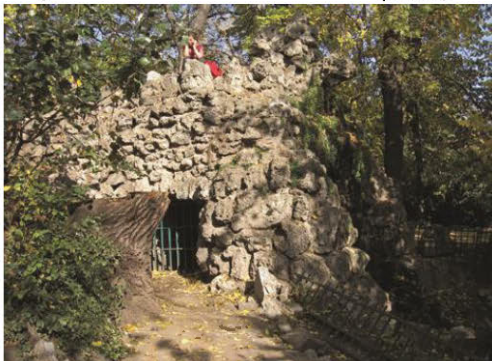


Figure 7. The Mound's Cave

Today, the mound largely maintains its original character and is one of the most preserved areas of the park. Cobblestone alleys bordered with rocks, waterfalls and natural stone basin, old fences, vegetation and the location in the center spot of Cîsmigiu gives a special ambience to the whole garden.

Currently, the ensemble should be restored by completing a number of fences and trees, shrubs and undergrowths needs to be enriched by new plantings. On top of the mound, a place frequented by visitors, there is a lack of seats.



On the mound there are a number of trees and shrubs, including large secular plants, such as: a white poplar (*Populus alba*), a plane (*Platanus hybrida*), several groups of boxwood (*Buxus semperivrens*) and yew (*Taxus baccata*).

**The Successive Water Features.**

The ensemble was made after World War I by the German landscape architect Friedrich Rebhunn. A water trail starts on the mound through the waterfall basin. An adjacent basin continues with "Negresa" Statue and finally arrives at the pools located near the lake, where it intersects with a stream of water that flows out of a small cave. The pools tanks are constructed of concrete, natural stone and pears. The basins have an average water depth of approx. 40 cm. (Figure 8)

The combination of successive water features creates a link between the mound and the lake through water and rock elements, offering a relaxing ambience for the visitors. In the adjacent area to the lake there are a number of specific romantic garden pots and sculptures. Currently, the ornamental elements of the area are not enhanced, and the vegetation is poorly maintained. (Figure 9)

**The French Heroes Monument** (historical monument - code LMI 2004: B-III-mB-20045). The French Monument, created by sculptor Ion Jalea in 1920, is dedicated to the fallen French soldiers in Romania during World War I. The statue, made of white marble, is surrounded by a gravel path and dark evergreen vegetation. The monument has a height of approx. 4 m (including its base).

The contrast between vegetal and mineral highlights the statue and its location at the intersection of several main paths, making the monument one of the most evidenced in the park. (Figure 10)

Recently, there has been a restoration intervention of the ensemble, but it did not follow the visual testimonies from the 1920s. The most negative striking aspects are currently two vases of flowers placed in front of the monument and the improper way of trimming shrub vegetation. As can be seen in view of the initial planning, the fountain in the center of the lake was end perspective, looking at the lake.

In the ensemble there are a number of coniferous trees, almost unique

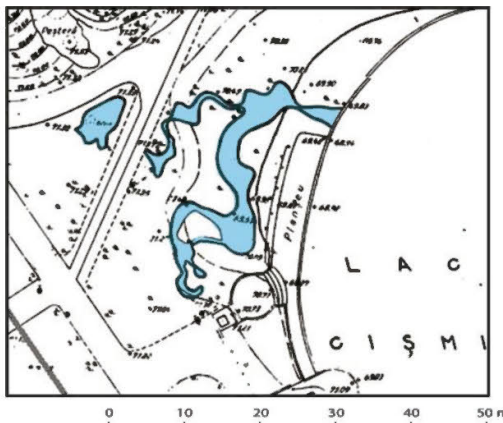


Figure 8. The Water Features Plan



Figure 9. The Successive Water Features

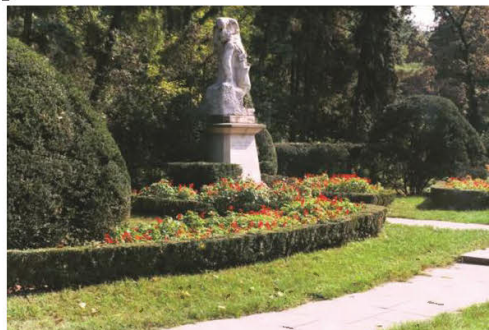


Figure 10. The French Monument Ensemble

in the park, such as *Pinus strobus* and *Picea pungens* 'Argentaea'. The shrubs vegetation is composed of *Buxus sempervirens*.

In recent years, at the monument various celebrations organized by the French Embassy together with the Romanian State have been hosted.

### **CONCLUSIONS**

The importance of landscape heritage conservation in Romania is highlighted since the first half of the twentieth century, when landscape architect Friedrich Rebhuhn stated that "landscape management means more than protecting nature" and that "monuments of the past" are a "precious heritage that we have a duty to keep" (Rebhuhn F., 1942). He also believes that "protection means preservation and management means development and creation", which gives the landscape a "new value" (Rebhuhn F., 1942).

Achieving a stage of knowledge and awareness regarding landscape heritage values, composed of ambiental elements, both mineral and vegetal, could be a first step in order to recreate the authentic ambiance of historic parks and gardens.

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## FIRST RESULTS ON TESTING PECAN (*CARYA ILLINOIENSIS*) AS A POSSIBLE NUT CROP FOR ROMANIA

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**Keywords:** horticulture, pecan, selections

### ABSTRACT

The pecan [*Carya illinoensis* (Wagenh.) K. Koch] is a deciduous nut tree native to North America. Present paper is presenting the results of introducing in Romania in 2005 of pecan hybrid families from USDA-ARS-NCGR in Brownwood, Texas (USA). The hybrid families consisted in open pollinated nuts of 18 hardy pecan cultivars. 179 seedlings from the hybrid families were planted into a selection plot in SW of Romania at University of Craiova – SCDP Vâlcea in Bujoreni. The variability of trunk cross sectional area (TCSA), tree height, crown diameter, number of leaflets per compound leaf weew determined. A comparison between pecan favorable climates from U.S.A. and potential favorable ones from Romania have been taken into account. The study requires to be continued to select the most adapted genotypes for the ecological conditions and to analyze if limited commercial growing of pecan is possible in Romania.

### INTRODUCTION

The pecan is a deciduous nut tree native to North America known and used for its fruits since ancient times by the American Indian tribes.

Europeans travelers have seen pecan fruits for the first time in 1533 near Guadalupe River, in present day state of Texas (U.S.A.). Later, Jean Penicaut, one of the Frenchmen who escaped from the massacre at Natchez in Mississippi in 1729, wrote that the natives (American Indians) have three different kinds of walnut trees, one of which produce excellent, edible nuts as small as man's thumb. These nuts were called „pacanes" (Rosengarten, 2004).

In 1758, Marshall classified pecan as *Juglans pecan*. Later, pecan was considered part of a separate genus in the *Juglandaceae* Family (Madden, 1979).

The pecan trees are native in a large area of the United States (Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Kansas, Illinois, Indiana, Tennessee, Iowa, Georgia, Nebraska, etc), Mexico and even southern Canada (Volk et al., 2009; Hogan et al, 2004; Frith, 2004; Hall, 2000).

Pecans were introduced in the states of New Mexico, Arizona, Alabama, South and North Carolina, Florida, Hawaii (USA) and in Australia, Brazil, South Africa, China,

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Israel, Turkey, etc. Trees are usually planted at 9 to 10 m by 9 m, which correspond to densities of 111 to 123 trees per hectare, bear fruits in the 7<sup>th</sup> to 9<sup>th</sup> leaf and produce fruit yields of 1500 to 2000 kg /ha (Madden, 1979; McEachern, 1978 and 1995).

The scientific name of pecan is *Carya illinoensis* (Wagenh.) K. Koch (Grauke, 1991). The pecan is a deciduous tree of large vigor, which reach heights of 20-40 m, monoecious (with male and female flowers on same plant). Self-pollination is very rare due to dichogamy (maturation of male and female flowers at different times) and the pecan trees are divided into two categories: Type I or protandrous and Type II or protogynous (Goff et al, 2003; Conner, 2010).

Pecan leaves are compound, alternate and odd pinnate, long (30-45 cm) and consist of between 9 and 17 leaflets. The trunk wood is very valuable for furniture and flooring.

The fruit is a round to oval shaped drupe or nut enclosed in a thick, green husk that splits into four parts at maturity. The kernel is usually liquid until September, when it solidifies (Andersen and Crocker, 2012). Pecan fruits are particularly valuable in the human diet and can be used fresh, roasted or cooked. Pecan kernels are rich in fat (72.0%, over 40.8% of them unsaturated), proteins (9.2%), carbohydrates (13.8%), vitamins (A, B1, B2, B5, K), minerals (K, P, Mg, Ca, etc.) (USDA Nutrient Database, 2014).

Because pecan trees are very demanding regarding the climatic conditions (requiring long, hot and humid summers and moderately cool winters without strong frosts) and soil (sandy loam or a loamy sand, well drained, deep) it is difficult to grow this nut crop in many countries.

In the present paper we intend to present several aspects of pecan growing regarding the cultivar assortment and growing conditions in order to be promoted in favorable areas (micro-regions) of Romania.

## MATERIAL AND METHODS

University of Craiova - SCDP Vâlcea received in 2005 from USDA-ARS-NCGR in Brownwood, Texas (USA), a number of 19 samples of pecan fruits.

Each sample was represented by 60 open pollinated fruits (nuts) of different pecan varieties known to be more hardy. The nuts were stratified, then sown in pots and seedlings were obtained (Table 3). Seedlings were planted into the nursery and in 2007 the hybrid families were planted in SW of Romania, in a selection plot located in Bujoreni, near the town of Râmnicu Vâlcea (Vâlcea County), situated on a deep alluvial soil of sandy loam type and medium fertility, with a pH of 6.8. Planting distances were 4.0 m by 4.0 m and the trial was set up without irrigation. Area is characterized by temperate climate, temperatures lower than -20°C are rare. During 2006-2013 period the annual average temperature was 12.4°C, rainfall of 745 mm and relative humidity of 72.53%.

In the 7<sup>th</sup> leaf after planting, the number of hybrids obtained per family, variability of trunk cross sectional area (TCSA), tree height, crown diameter, number of leaflets per compound leaf was determined.

A comparison between pecan favorable climates from U.S.A. and potential favorable ones from Romania have been taken into account.

## RESULTS AND DISCUSSIONS

The study was carried out during 2005-2014 period at the University of Craiova – SCDP Vâlcea on 18 hybrid families resulted from open pollination of 18 pecan cultivars (Table 1).

Pecan culture is heavily influenced by climatic conditions, absolute minimum temperatures during winter, temperatures during blooming time and length of vegetation period playing important roles.

In the United States, pecans are grown in areas corresponding with the hardiness zone 5 (where temperatures drop during winter from  $-23^{\circ}\text{C}$  to  $-29^{\circ}\text{C}$ ), zone 6 ( $-18^{\circ}\text{C}$ ;  $-23^{\circ}\text{C}$ ), zone 7 ( $-12^{\circ}\text{C}$ ;  $-18^{\circ}\text{C}$ ), zone 8 and zone 9 ( $1^{\circ}\text{C}$ ;  $-12^{\circ}\text{C}$ ) (Table 2). Hardiness zones 6 to 9 are considered most favorable for growing pecans and such climatic conditions can be found in Texas, Virginia, Florida, New Mexico. Pecan plantations have expanded in areas from hardiness zones 6 and even 5. In most cases of pecan grown in hardiness zone 5 the trees does not produce fruits or kernels are not formed inside the nuts (Madden, 1979).

The climate from several areas of Romania corresponds to some extent to the climate from USDA hardiness zone 6 ( $-23^{\circ}\text{C}$ ;  $-18^{\circ}\text{C}$ ) and even zone 7 ( $-12^{\circ}\text{C}$ ;  $-18^{\circ}\text{C}$ ). It is the case of areas from Northern Oltenia, Banat, Dobrogea and Bihor County. Besides the absolute minimum temperatures during winter, other factors such as soil quality, number of days without frost and amount of rainfall have important influences. Therefore, establishing the suitable zones for pecan planting will be done in certain micro-areas benefiting of adequate microclimates. Râmnicu Vâlcea micro-area (which includes Bujoreni, the location of the trial) have similar climatic conditions like USDA hardiness zone 6 ( $-23^{\circ}\text{C}$ ;  $-18^{\circ}\text{C}$ ), which is considered acceptable for pecan growing.

Out of the total number of 1080 open pollinated pecan nuts from 19 cultivars received from USDA-ARS-NCGR, courtesy of Dr. L.J. Grauke, 990 nuts were stratified and 228 seedlings were obtained (emergence rate of 21.1%). In case of 18 hybrid families, the number of seedlings per family varied from 3 to 22 (Table 3). After planting into the selection plot, 179 seedlings survived (78.5% from those planted). Most of the losses were caused by mechanical and animal destruction. The most important number of offspring was obtained in case of Dumbell Lake Large x o.p. family (22 hybrids), Fritz x o.p. (21 hybrids), Flack x o.p. (20 hybrids) and Kanza x o.p. (17 hybrids).

Important variability was observed in the 7<sup>th</sup> leaf in case of measurement of trunk cross sectional area (TCSA) between hybrid families, but also between offspring of same cultivar. The mean TCSA values (Table 4) varied from  $32.7\text{ cm}^2$  (Shoals West x o.p.) to  $58.6\text{ cm}^2$  (Dumbell Lake Large x o.p.). TCSA values varied from  $33.4$  to  $22.0\text{ cm}^2$  for Shoals West x o.p. offspring and from  $116.8$  to  $58.6\text{ cm}^2$  for Hodge x o.p. (Table 3). Amplitude of TCSA variability (Table 4) oscillate from  $11.4\text{ cm}^2$  (Shoals West x o.p.) to  $95.4\text{ cm}^2$  (Kanza x o.p.).

High variability was recorded also on pecan tree height, which varies between 2.0 m up to 7.5 m. The amplitude of tree height oscillates between 1.0 m (Warsaw North x o.p. and Shoals West x o.p.) and 5.5 m (Flack x o.p.).

Crown diameters were measured too. The lowest values of crown diameter were recorded in case of trees from Bolten's S-24 x o.p. hybrid family (3.17 to 1.50 m) and the largest values were observed for Ralph Upton x o.p. family (4.61 to 2.50 m). Amplitude of crown diameter was only 0.36 m for the Canton offspring and 2.88 m for Hodge offspring. Annual growth length ranged between 40 to 80 cm for most of the offspring.

Pecan compound leaf has variable length and different number of leaflets (Table 4). The number of leaflets varies between families and offspring. The largest number of leaflets were recorded in cases of Fritz x o.p. (21.2), Fisher x o.p. (19.8), Warsaw West x o.p. (19.6), Flack x o.p. (19.2), etc., and the smallest for Dumbell Lake Large x o.p. and Bolten's S-24 x o.p. (12.6). Compound leaf length varies from 20 to 50 cm in case of pecan offspring.

In the 7<sup>th</sup> leaf after planting in the selection plot, only one hybrid from Kanza x o.p. family produced the first fruits. Ripening of fruits of this hybrid can be estimated to take place at the end of October, beginning of November. This hybrid confirms that pecan trees on own roots start to produce first nuts in the 7<sup>th</sup> to 9<sup>th</sup> leaf.

Table 1

Geographic and genetic origin of pecan genotypes tested at University of Craiova – SCDP Vâlcea

No.	Cultivar	Original area of culture in U.S.A.	Fruit weight (g)		Kernel ratio (%)	Fruit shape	Fruit ripening period	Origin
			Average cultivar fruit weight in U.S.A.*	Fruit weight determined on samples in Romania				
1	Canton	Missouri	5.92	5.23	50.0	oblong elliptic	medium	natural hybrid
2	Steuck	Montana	6.20	4.90	49.0	oblong	-	natural hybrid
3	Pawnee	Texas	10.22	9.06	58.0	elliptic	early	Mohawk x Stark Hardy Giant
4	Bolten's S-24	Indiana	3.30	5.33	49.4	elliptic	medium	natural hybrid
5	Fisher	Illinois	6.08	6.30	48.0	oblong elliptic	medium	natural hybrid
6	Hodge	Illinois	7.62	5.43	59.0	oblong	-	natural hybrid
7	Posey	Indiana	6.80	6.53	54.0	oval-elliptic	early	natural hybrid
8	Busseron	Indiana	7.25	5.43	47.0	elliptic	-	natural hybrid
9	Kanza	Texas	6.84	7.10	54.0	ovate	-	Major x Shoshoni
10	Osage	Texas	5.55	6.06	55.0	elliptic	early	Major x Evers
11	Dumbell Lake Large	New Jersey	5.22	5.03	47.0	oblong elliptic	early	natural hybrid
12	Frisbie	Texas	4.95	3.26	-	ovate	-	natural hybrid
13	Fritz	Illinois	5.56	5.33	-	elliptic	early	natural hybrid
14	Shoals West	Montana	4.45	4.66	44.0	elliptic	-	natural hybrid
15	Warsaw North	Missouri	4.76	4.20	-	elliptic	-	natural hybrid
16	Ralph Upton	Iowa	4.15	4.76	54.0	elliptic	-	natural hybrid
17	Green Island Beaver	Kentucky	3.25	3.86	49.8	elliptic	-	natural hybrid
18	Flack	Texas	8.21	7.90	48.6	oblong elliptic	-	natural hybrid

\*(Sources: Reid and Hunt, 2000; Carroll and Smith, 2014; Conner, 2014)

Table 2

The favorable climatic factors for pecan growing in U.S.A. comparatively with potential of several areas from Romania

No.	Favorable climatic zones for pecan in the U.S.A. (USDA Hardiness Zones)			Climatic zones from Romania which might respond to the pecan demands				
	Areal (Favorability)	Average absolute minimal temperatures	Type of climate	Geographical areal	Areal (Favorability)	Average absolute minimal temperatures	Type of climate	Geographical areal
1	Zone 5 (low)	-29°C; -23°C	Temperate continental	Northern part of U.S.A. and partially South of Canada (Great Lakes region)	North East Area (low)	-23°C; -30°C	Temperate continental	North of Moldova, Eastern Intra-Carpathian areas
2	Zone 6 (acceptable)	-23°C; -18°C	Temperate with slight maritime influences	From East to West of U.S.A. (N 45° to N 50° latitudes)	South and Western areas (acceptable)	-22°C; -18°C	Temperate with slight Mediterranean influences	Northern Oltenia (hilly area), Banat, Bihor and Dobrogea
3	Zone 7 (good)	-18°C; -12°C	Medium temperate climate	Area between Southern States (Virginia and Texas)	South areas with warmer microclimates (good)	-18°C; -12°C	Mediterranean or Black Sea influences	Drobeta Turnu Severin, Rim. Vâlcea, Horezu, Constanța, Medgidia, etc.
4	Zones 8 and 9 (very good)	-12°C; -7°C	Warm climate	Texas, New Mexico, Florida (in U.S.A.) and Mexico	Areas not present	-12°C; -7°C	-	-

Table 3

Behavior of pecan hybrids in the first 7 years (2008-2014) in the selection plot at  
University of Craiova - SCDP Vâlcea

No.	Hybrid combination	No. of pecan hybrid seeds	No. of pecan hybrid seeds stratified	No. of pecan seedlings obtained	No. of pecan seedlings planted in the selection plot	Range of T.C.S.A. (cm <sup>2</sup> )	Range of tree height (m)	Range of crown diameter (m)	Range of average no. of leaflets
1	Canton x o.p.	60	55	7	4	53.3 – 35.0	6.5 – 4.5	2.95 – 2.59	16.4 – 14.2
2	Steuck x o.p.	60	55	15	11	83.9 – 25.0	7.0 – 4.0	4.03 – 2.45	17.4 – 14.0
3	Pawnee x o.p.	60	55	10	7	75.4 – 29.8	7.0 – 4.0	3.45 – 2.05	17.0 – 13.0
4	Bolten's S-24 x o.p.	60	55	10	7	62.1 – 27.5	6.0 – 2.5	3.17 – 1.50	17.8 – 12.6
5	Fisher x o.p.	60	55	8	4	41.8 – 31.1	5.5 – 4.0	3.25 – 2.35	19.8 – 15.4
6	Hodge x o.p.	60	55	10	8	116.8 – 52.9	7.0 – 4.0	4.58 – 1.70	17.4 – 12.6
7	Posey x o.p.	60	55	22	15	62.8 – 38.9	6.0 – 4.5	3.84 – 2.65	17.0 – 13.6
8	Busseron x o.p.	60	55	8	7	69.4 – 28.2	7.5 – 4.5	4.00 – 1.75	16.2 – 13.0
9	Kanza x o.p.	60	55	22	17	113.0 – 17.6	7.0 – 3.0	4.18 – 1.89	17.4 – 14.2
10	Osage x o.p.	60	55	12	10	67.9 – 39.3	6.5 – 4.5	3.95 – 2.85	17.4 – 14.2
11	Dumbell Lake Large x o.p.	60	55	22	22	76.6 – 27.5	6.5 – 4.0	4.15 – 2.25	17.4 – 12.6
12	Frisbie x o.p.	60	55	12	9	78.5 – 14.8	6.0 – 2.5	3.48 – 1.85	17.0 – 14.6
13	Fritz x o.p.	60	55	22	21	82.9 – 13.6	6.0 – 2.0	3.48 – 1.75	21.2 – 14.8
14	Shoals West x o.p.	60	55	5	3	33.4 – 22.0	5.0 – 4.0	2.75 – 2.18	16.8 – 14.0
15	Warsaw North x o.p.	60	55	7	4	69.9 – 43.2	6.0 – 5.0	4.05 – 3.16	19.6 – 15.4
16	Ralph Upton x o.p.	60	55	7	5	70.5 – 44.1	7.0 – 5.5	4.61 – 2.50	16.2 – 14.0
17	Green Island Beaver x o.p.	60	55	7	5	61.6 – 22.0	7.5 – 4.0	3.47 – 1.95	17.4 – 15.6
18	Flack x o.p.	60	55	22	20	69.3 – 9.07	7.5 – 2.0	3.52 – 1.56	19.2 – 14.4
	<i>Total</i>	1080	990	228	179	-	-	-	-



Table 4

Variability of some growth elements for pecan hybrids in the 7th leaf in the selection plot

No.	Hybrid combination	Values of T.C.S.A. for hybrid combinations		Amplitude of tree height (m)	Amplitude of crown diameter (m)
		Average T.C.S.A. (cm <sup>2</sup> )	Amplitude of T.C.S.A. variability (cm <sup>2</sup> )		
1	Canton x o.p.	40.9	18.3	2.0	0.36
2	Steuck x o.p.	50.7	58.9	3.0	1.58
3	Pawnee x o.p.	47.5	45.6	3.0	1.40
4	Bolten's S-24 x o.p.	46.8	34.6	3.5	1.67
5	Fisher x o.p.	35.7	10.7	1.5	0.90
6	Hodge x o.p.	46.8	63.9	3.0	2.88
7	Posey x o.p.	41.6	23.9	1.5	1.19
8	Busseron x o.p.	47.0	41.2	3.0	2.25
9	Kanza x o.p.	48.5	95.4	4.0	2.29
10	Osage x o.p.	51.5	28.6	2.0	1.10
11	Dumbell Lake Large x o.p.	58.6	49.1	2.5	1.90
12	Frisbie x o.p.	38.0	63.7	3.5	1.63
13	Fritz x o.p.	40.9	69.3	4.0	1.73
14	Shoals West x o.p.	32.7	11.4	1.0	0.57
15	Warsaw North x o.p.	57.3	26.7	1.0	0.89
16	Ralph Upton x o.p.	52.5	26.4	1.5	2.11
17	Green Island Beaver x o.p.	46.8	39.6	3.5	1.52
18	Flack x o.p.	39.3	60.2	5.5	1.96
	<i>Mean</i>	<i>43.1</i>	<i>42.6</i>	<i>2.7</i>	<i>1.55</i>

This research work on pecan behavior will be continued in order to select the most adapted genotypes to the ecological conditions and to check if limited commercial growing of pecan is possible in Romania.

### CONCLUSIONS

Pecan is a valuable tree that might be used after further studies in Romania as a nut crop or in forest plantations, with the condition of choosing the most favorable climate zone and soils. Several micro zones from North of Oltenia region, Dobrogea, Banat and Bihor correspond to the criteria of Hardiness Zone 6 from U.S.A. regarding pecan favorability.

High variability of growth elements of the pecan hybrids allows in the future selecting most valuable genotypes adapted to the specific conditions of Romania.

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**MONITORING THE INFLUENCE OF THE ABUNDANT  
PRECIPITATION RELATIVE TO THE WHEAT CONTAMINATION  
WITH MYCOTOXINS - PRODUCTION 2014**

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*Keywords: fusarium, wheat, mycotoxins*

**ABSTRACT**

*Fusariosis is a cereals'disease which does reduce the cereals'production and their quality. It usually appears during the years with excessive precipitations. The present study aims to point out the influence exerted by precipitations upon the contamination with mycotoxins of the 2014 wheat harvests from the zone of Oltenia. The determinings of the existing amounts of mycotoxins' residues were performed through the ELISA method. 30 wheat samples were taken into consideration, brought from five Oltenian departments. The residues of aflatoxins, of the ochratoxin A and of the T<sub>2</sub> toxin did present levels situated below the respective methods detection limits; at 9 samples, zearalenone residues were detected, standing between 2,55 and 25,2 µg/kg; at 11 samples, deoxynivalenol residues were detected, with concentrations standing between 52 and 513 µg/kg. These results do lead us to the conclusion that the abundant precipitations did indeed favour the appearance of mycotoxins generated by fungi from the kind *Fusarium* sp. , but that their respective concentrations had never exceeded the admitted limits; therefore we are entitled to speak of a moderate contamination.*

**INTRODUCTION**

Wheat harvests might be affected by fusariosis, a disease which, at least judging by what currently seems to occur, will bear a significative impact upon the quality of the harvests to come. Fusariosis is a disease caused by fungi from the *Fusarium* kind, known as being producers of mycotoxins (deoxynivalenol, zearalenone, etc.). The most frequently met *Fusarium* species for the cereals within our country are *Fusarium graminearum* and *Fusarium culmorum*, both producing deoxynivalenol (Alexa E., 2011).

The *Fusarium* attack was associated in specialized literature (Scudamore, K.A., 2005), suiting the level of its intensity: with the reducing of the grain's specific weight; with minor decreases of the fall figure and with a slight increase of the protheolithical activity. Yet the dangerous problem is constituted by the level of mycotoxins within the grain itself, which might determine the impossibility of making use of the concerned harvest for consumption purposes, either human or fodder ones.

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There are extensive studies in literature (Valcheva, A., 2003) which do tightly correlate, for a given quantity of wheat, the percentage of grains attacked by *Fusarium sp.* with the respective existing level of deoxynivalenol. However, the species from the *Fusarium* kind also produce other mycotoxins, like zearalenone, the amount of which are not necessarily correlated with the percentage of grains attacked by *Fusarium* (an adequate marker for identifying zearalenone seems rather to be the grains' reddish colour, determined by the red pigment named golden fusarine). Most of the mycotoxins are endowed with chemical stability, therefore they are resistant towards temperature, storage or other biotechnological procedures made use of in the alimentary industry. Consequently, they may be found in wheat flour, in bread, in breakfast-shaped cereals, in beer etc. Still, we may notice a somewhat reduced amount of them within processed cereals. For example, once the pollard is removed, the wheat flour also loses with it the zearalenone or the deoxynivalenol (SpanicValentina, 2010).

Zearalenone (ZON) is a lactonic mycotoxin produced by *Fusarium sp.* which owns phytoestrogenic abilities. Its toxicity resides into the fact that it does tie itself up to the cellular estrogenic receivers and, by that, it does become able to intervene upon the endocrine settlement of the reproductive function at females and upon the analogous process of spermatogenesis at males. Within a humid and warm environment, it is known to appear upon all graminaceae and upon all leguminous plants (Beev G. et. & , 2013, 2011).

As for deoxynivalenol (DON), it does cause the organisms' general weakening as well as necroses (gangrenes) within various tissues (such as the gastrointestinal precinct, the bones' marrow or the lymphatic tissue). It also does modify the blood's intrinsic indices and it does attack the immunity system. The European Commission's Science Committee upon Alimentation Problems does suggest the fact that the deoxynivalenol's toxicity could be, ultimately, due to its general effect upon all organisms and especially to its lesion-like effect on the immunity system, as well as to the deoxynivalenol's synergical effect when combined with other trychothycenes, from which, practically speaking, it could never be separated, because their syntheses are accomplished simultaneously (Council for Agricultural Science and Technology, 2003).

## MATERIAL AND METHODS

It is a known fact that wheat-shaped and bread-like products are basic components of the usual alimentary behaviour of most of the people from our country. Consequently, we have chosen as our goal to investigate the contamination degree concerning the mycotoxins to which was submitted the wheat harvest produced during the year 2014, an year when precipitations had fallen more than their usual annual amount.

After the wheat's harvesting in 2014, samples were prelevated from the departments of Dolj, Olt, Teleorman, Vâlcea and Carash-Severin. In view of determining their respective amounts of mycotoxins, the samples were analyzed at the Sanitary Veterinary and Food Safety Laboratory from Dolj. The determining of the mycotoxins' residues was performed through the ELISA immunoenzymatic method, by making use of R-Biopharm kits. The concerned samples were rendered homogeneous, grinded and extracted through the use made of solvents which are specific for each of the studied mycotoxins (that is to say: for determining the amounts of aflatoxins, of zearalenone and of the T<sub>2</sub> toxin, it was methanol 70%; for the case of deoxynivalenol, it was water; for the ochratoxin A, it was sodium bicarbonate 0,13 M). After their extraction, the samples were filtered, diluted according to the respective protocols of each among the ELISA kits and pipetted within wells, in order to determine the respective amounts of mycotoxins' residues. The respective optical densities of standards and of samples have

been read through a STAT FAX 3200 spectral photometer, at 450 nm, then introduced within the RIDAWIN software, which is designed in order to draw the calibrating graph and does automatically calculate the respective concentrations of mycotoxins.

30 wheat samples were analyzed, among which: 12 samples from the department of Dolj; 11 samples from the department of Teleorman; 4 from the department of Olt; 2 samples from Carash-Severin and 1 sample from the department of Vâlcea.

### RESULTS AND DISCUSSIONS

The results obtained after performing the laboratory analyses do state of a relatively low contamination degree with mycotoxins for the 2014 wheat harvest, though the respectively correlative amounts of precipitations had been pretty high and had also rather succeeded in decreasing the efficiency of the administrated phytosanitary treatments. Residues of aflatoxins, of ochratoxins A and of T<sub>2</sub> toxin were not detected at all among the analyzed samples. Deoxynivalenol was detected at:

- 3 samples from the department of Dolj, with values standing between 259 and 513 µg/kg;
- 7 samples from the department of Teleorman, with values from 49 to 457 µg/kg;
- 3 samples from the department of Olt, going from 96 to 138 µg/kg;
- 1 sample with 68 µg/kg and 1 situated under the measuring limit in the department of Carash-Severin;
- 1 sample situated under the detection limit in the department of Vâlcea.

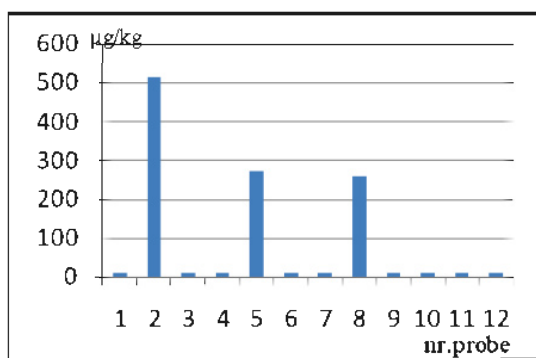


Figure 1. Distribution of the DON concentrations (µg/kg) at the samples from the department of Dolj

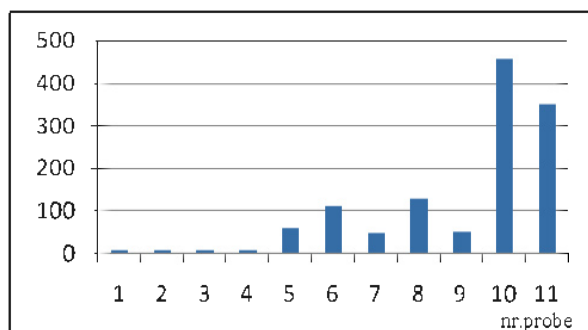


Figure 2. Distribution of the DON concentrations (µg/kg) at the samples from the department of Teleorman

Zearalenone was detected at:

- 4 samples in the department of Dolj, with values from 5 to 25,2 C;
- 2 samples from the department of Teleorman with values of 2,5 and of 35  $\mu\text{g}/\text{kg}$  and one sample under the measuring limit;
- 1 sample with 3,87  $\mu\text{g}/\text{kg}$  and 1 under the measuring limit in the department of Carash-Severin;
- 2 samples in the department of Olt, with values of 24 and 26  $\mu\text{g}/\text{kg}$ .

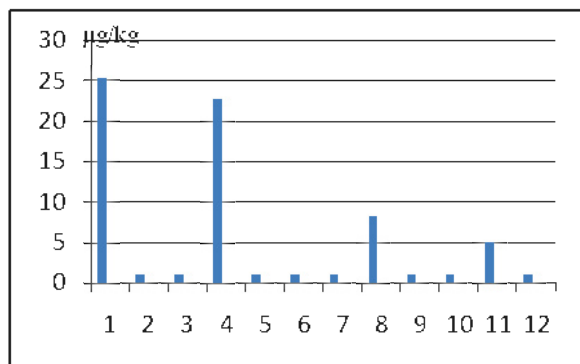


Figure 3. Distribution of the ZON concentrations ( $\mu\text{g}/\text{kg}$ ) at the samples from the department of Dolj

From the total of 30 wheat samples analyzed for DON, at 14 samples (46,66%) it was not detectable, at 3 samples it was under the measuring limit (10%) and at 13 samples (43,33%) it had values situated under the highest admitted limit (MRL DON 1250  $\mu\text{g}/\text{kg}$ , Commission Recommendation UE, 2006).

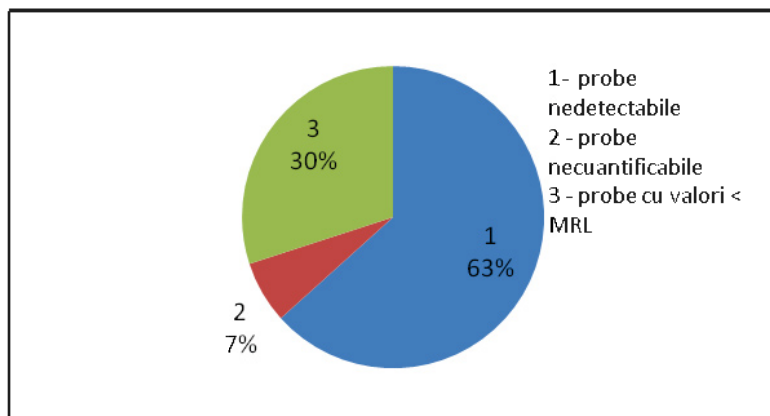


Figure 4. DON contamination degree for the analyzed samples

From the total of 30 wheat samples analyzed for ZON, at 19 samples (63,33%) it was not detectable, at 2 samples it was under the measuring limit (6,66%) and at 9 samples (30%) it had values situated under the highest admitted limit (MRL ZON 100  $\mu\text{g}/\text{kg}$ , Commission Recommendation UE, 2006).

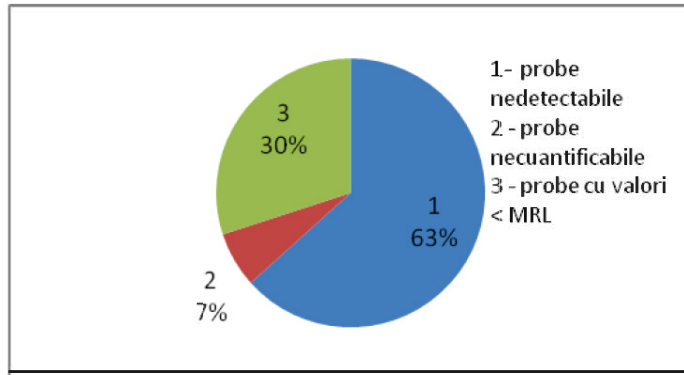


Figure 5. ZON contamination degree for the analyzed samples

The wheat sample harvested from the department of Teleorman which had the concentrations of DON 513  $\mu\text{g}/\text{kg}$  and, respectively, of ZON 22,58  $\mu\text{g}/\text{kg}$  was microbiologically analyzed and fungi from the *Fusarium sp.* kind were identified. At microscope's examining the presence of *Fusarium sp.* fungi upon the thin plate was confirmed. (Photo nr. 1)



Photo 1. Microscopic view of the fungi from the *Fusarium sp.* kind

### CONCLUSIONS

The laboratory analyses we have performed for this year's wheat harvest have demonstrated a low degree of mycotoxins contamination. For aflatoxins, for the ochratoxin A and for the  $T_2$  toxin, the respective amounts of residues were below the detection limit of the methods made use of.

Among the mycotoxins bearing detectable values, deoxynivalenol and zearalenone did present values framed between the limits admitted by legislation. Most of the wheat samples at which DON and ZON residues have been detected were harvested from the department of Teleorman, where, indeed, the recorded quantities of precipitations have been way beyond the normal ones.

For the 2014 harvest, the number of samples at which mycotoxins' residues were detected is averagely comparable to the ones of the samples harvested from the zone of Oltenia in the previous years. However, in the drought-shaped years, the predominant contaminations were the ones with fungi from the kinds of *Aspergillus* and *Penicillium sp.*

The harvested wheat ought to be continuously monitored, even after having been stored and processed in order to be used into products destined for human or animal consumption, so that we could be sure of the fact that no contaminations might occur along the undergoing of the wheat through the technological chain composed of storage, transportation and processing.

The cereals' processors might in no way blame the meteorological conditions only, should contaminations appear at the final products destined for consumption. Such contaminations might as well be due to the improper circumstances of the storage or of the exerted manipulation.

The actions of monitoring in search of mycotoxins ought to continue as well upon the products destined for consumption that are processed from the initial wheat grains; it is only this way that consumers might be effectively protected from the toxins noxious effects.

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## **RESEARCH ON THE BEHAVIOR OF TOMATO CULTIVARS OBTAINED UNCONVENTIONAL SYSTEM IN SOLARIUM**

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**Keywords:** *tomatoes culture, fertilization, yield, quality*

### **ABSTRACT**

*Growing tomatoes in cold greenhouses on unconventional substrates is a basic problem for crop technologies should provide clear information about the schemes and the range recommended planting densities, the amount of substrate provided each plant and arrangement thereof in pots, buckets or bags. Were tested cultivars of recent sourcing created in Europe in the soil culture and / or culture substrate. Experimental cultures were fertirigate with a nutrient solution to major macro and micronutrients were provided by solubilizing needs of their complex fertilizers Universol type. There were production results and quality of fruit for fresh consumption.*

### **INTRODUCTION**

As shown in the literature, the vegetable, the sector is constantly evolving assortment of positive performance, initiate and conduct an experimental program invariably rely on a prior study of the behavior of new cultivars in the conditions of the research undertaken.

Assortment of tomato crops in greenhouses and solariums made in recent years only F1 hybrids evolve in the following areas: increased productivity, increased earliness, improved commercial quality production, increase nutritional and therapeutic importance of fruits, expanding and strengthening resistance and tolerances attacks pathogens that can damage even irreversible plant health plant adaptation to unfavorable vegetation factors that may cause physiological decrease production and lower quality.

Choosing and using newly created cultivars is the most important option for improving crop production results achieved technological system practiced by grower. (Hoban Adriana & all, 2008; Gosselin, A. & all, 1988)

After 1990, the liberalization of the movement of biological material has led to a market in Romania has over 150 new tomato cultivars created in traditionally vegetable culture countries such as Holland, France, Italy, Israel, USA and others.

No new cultivar introduced in production by producers in Romania, give the best results in all vegetable basins in our country.

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Before the introduction of hybrid tomato crop new test to determine production performance in a particular ecological zone is a common practice, both seed traders and producers of tomatoes.

Given these considerations, it was proposed to make a comparative study of the production performance of F1 hybrids recently established protected crops destined cold greenhouses or solarium. (Marschner, H & all, 1995; Mengel, K and Kirkby, 1979)

Because the main problem of the research is the growing unconventional technologies considered that it must be studied the range of cultivars combined with research on the effect of organic substrate culture (peat) and soil, on yield and quality of tomatoes grown in solarium it.(Tattini,M and Traversi, 1992; Whipker and Hammer, 1994)

### MATERIAL AND METHODS

In order to achieve experience variants which were studied are presented in the following table.

Table 1

Experimental variants

Var.	Location	Substrat	Cultivar	Origine
1	Tărtășești	Soil	Lady Rosa F1	Zeraim Gedera Israel
2			Precos F1	Bulgaria
3		Peat	Lady Rosa F1	Zeraim Gedera Israel
4			Precos F1	Bulgaria
5	Brezoaiele	Soil	Lady Rosa F1	Zeraim Gedera Israel
6			Precos F1	Bulgaria
7		Peat	Lady Rosa F1	Zeraim Gedera Israel
8			Precos F1	Bulgaria

In order to achieve the eight embodiments, three experimental factors were combined - culture substrate (A) and the range (B) cultivars used and the location of the experiment (C).

Combining three experimental factors graduations resulted three phactorial experience type 2 x 2 x 2 with eight variants.

Research purposes- Performance evaluation of production of new cultivars of tomatoes in solarium, biodegradable organic substrates peat respectively, compared to those made on the soil, in order to develop specific hybrids technological measures that lend themselves to this unconventional modern culture.

The analyzed research objectives were:

- Establishment of the total production of F1 hybrids, new tomatoes to their recommendation for production;
- Determination and / or evaluation of commercial production and biochemical qualities;
- Develop technical advice on cultivation on organic substrates newly create the best performing hybrids in some countries with highly advanced agricultural technology. Experience has been placed in two individual tunnels located in the municipalities Tartasesti and Brezoaiele, Dâmbovița County. These tunnels functioning in good ventilation installations, shading, water from a source of electrical energy and continuous operation.

Soil solar was disinfected with formalin and its interior (roof, side walls) by spraying with fungicides.

The conditions for specific climatic areas where the crops have been placed, the micro solar affect only from the point of view of temperatures, which decreases the standard values that are not tolerated by plants and in summer rise to harmful values for binding and fructification.

The 8 experimental variants were installed in three repetitions, without randomization, with bands of protection imposed by the experimental technique.

Peat used for the experiment was placed in bags 10 liters in which have been placed 2 tomatoes transplants. The biological material used in the experiment consisted of two cultivars, Lady Rosa F1 and Precos F1.

Lady Rosa F1-Tomato Hybrid is designed for greenhouses and solariums with indeterminate growth. Lady Rosa F1 is a plant with short internodes, good foliar coverage specifically designed for cultivation in protected environment. It performs very well both in terms of high temperature and low temperature conditions. Fruits have a good uniformity, each 4-6 fruits / floor 180-200g, strongly built and very attractive color, dark carmine red, no green collar. It has resistance to transport and storage over 20 days (shows gene LSL) is an excellent product for sale in supermarkets. Lady Rosa F1 shows resistance: V; F-1; F-2; ToMV; N\* (N\* - nematode tolerant up to 28 ° C, soil temperature).

Precos F1- Hybrid fruit is a large, spherical, bright red with 4 boxes, with an average weight of 120-130g are grouped in inflorescences 5-6. Tolerates to low temperatures. Commercial quality and firmness of ripe fruit are very good. Resistance to *Fusarium*, tobacco mosaic virus, root system and *Verticilium fusariosis*. It is resistant to nematodes, this attribute is very important for crops on the soil.

Other materials and equipment used- Fertilizers needed to prepare the solutions were nutrition Universol blue, Universol Violet, calcium nitrate, magnesium sulfate.

Equipment for the preparation and distribution of nutrient solution composed of a head of the plant fertilizer tank and provided with the network of distribution pipes and transport. Solution components were dosed manually by stability consumption at intervals of 4-7 days. Leaching and sending the plant was carried out by groups of fertilizers and alkaline reaction with acid to avoid precipitation and clogging nozzles fertigation equipment. Terminal spaghetti tubes (one for each plant) used for distribution of fertilizers had a rate of 2 l / hour. In parallel, control nutrient solution concentration collected weekly basis peat container under the drainage holes. The concentration of plant nutrient solution delivered from the nozzle was 2.6-2.8 mS / cm<sup>2</sup>, and the bottom of the bags or pots, 3 – 3.2mS / cm<sup>2</sup>. To overcome this concentration, was applied only watering water by subtracting the electroconductibility 2.8-2.9mS / cm<sup>2</sup>.

In this way, while maintaining optimal value of electroconductibility were made fertilizer and consumption was savings.

Specific culture technology applied experience- The experiment was designed and made great recommendations respecting the tomato crop technology in buildings (greenhouses cold and / or tunnels) extended-cycle.

Paving and greenhouse were made by: soil disinfection with formalin and greenhouse skeleton with a concentrated blend of fungicides (Mospilan and Champion).

Paving the variants grown on soil made by leveling and loosening deep, 30cm deep.

Production was done by planting seedlings often followed transplanters in tapered side 7cm pots and hardened well before planting. Land mulching was conducted for both versions on the soil and those substrates.

Experiences planting scheme was for variants on the soil 80 / 40cm and for variations on substrates sacks with 2 plants at 160cm between rows and 40cm in turn, substrate volume 10 liter / bag.

Both embodiments ensure that the density of 3.12plante / m<sup>2</sup>, corresponding to the majority of the tomato cultivars, the extended cycle in solarium.

After the establishment of culture were performed following work care: filling gaps, trellising plants - vertical variants planted in rows spaced at 80cm and slant V plants in bags substrate volume 10 liter, defoliation by just removing leaves that have hit the ground mulch bags that were installed, maintaining the plant foliar apparatus intact, contributed to increased production.

Meat 8 inflorescences with 1-2 leaves after the last inflorescence designed to assure the plant height, 0.05% Mospilan treatments to combat whitefly attacks. Note that plants experiences were not attacked by diseases, plant health very good experimental culture contribute directly and effectively to achieve a high level of total production.

Fertigation, fertilizer tank by feeding separately, at different times, with fertilizers acid reaction with alkaline reaction. Immediately after sowing, the use of very low water 50-100ml / plant / day of the 3-4 days required to repeat fertigation. Timing of new fertigation was determined by measuring the moisture substrate sample with a portable.

Maximum consumption during the months of June and July, daytime consumption of 1.9-2.0 liter / day / plant was provided by four watering administered from 7, 11, 15 and 18. Momentele and duration were splashing performed correctly mounted programmer circuit water supply installation and management of temperature vents fertigation. The control increasingly stronger, complete with shading measures of interior space greenhouse culture and repeated spraying with water.

Fertigation nutrient solutions used for the experiments were prepared with the main ingredient two soluble complex fertilizers - Universol Blue for early plant growth and fruiting period Universol Violet. Universol Blue, richer in nitrogen maintains good vegetative growth and Violet Universol supports fruition because of the high content in potassium.

In order to complete solution of all the necessary nutrients have been used: calcium nitrate nitrogen-like source of calcium, magnesium sulfate as a source of magnesium and sulfur, and small amounts of potassium nitrate and urea nitrate ammonium.

Harvesting was done by hand, spread, giving for each plant harvested fruit growing number and weight for all collections from their debut until the abolition of culture.

Observations and measurements- As shown, at each harvest were recorded total quantities of fruit harvested to calculate experimental data. Primary data production were aggregated and reported in kg / plant and kg / m<sup>2</sup>.

In specialized laboratories at the University of Agronomic Sciences and Veterinary Medicine Bucharest, were performed for all variants, chemical and biochemical determinations necessary to establish the content of the components that support and highlight the nutritional content of nitrates, which in higher concentrations could polluting production harvested from the experimental field.

## **RESULTS AND DISCUSSIONS**

Quantities of fruits harvested installments were pooled for each plot repetition, and then for each variant. Harvested fruit were counted and weighed to determine later, the exact number of fruit harvested their average weight and production per plant and per unit area (m<sup>2</sup>). The results of these determinations are shown in Table 2.

Looking at the data in Table 2 shows that all variants the number of fruits produced by the culture as compared to the peat is at the ground crops. Thus most fruits harvested were obtained from variant 7 (Brezoaiele, peat, cultivar Lady Rosa) respectively 29.2 pc / plant and 28.1 units / plant in variant 3 (Tartasesti, peat, cultivar Lady Rosa) and the lowest number of 21.5 pc fruit / plant was obtained from variant 6 (Brezoiele, soil, cultivar Precos) .If examine only in terms of cultivar best results were obtained compared with Precos with Lady Rosa.

Also average fruit weight varied depending on the culture substrate, thus providing fruit culture peat heavier. Comparing location experiments and heavier fruit were obtained from peat substrate Tartasesti that 117,72g Lady Rosa F1 and F1 Precos 100,80g. Harvests kg / m<sup>2</sup> are higher in peat variants that 10,32kg / m<sup>2</sup> Tartasesti - Lady Rosa and 8.08 kg / m<sup>2</sup> followed Tartasesti -Precos 8,47kg / m<sup>2</sup> Brezoiele - Lady Rosa.

Table 2  
Results regarding the number of fruits, medium weight and total yield

Var.	Place	Substrat	Cultivar	No of fruits	Medium weight of fruits, g	Yield, kg/plantă	Yield, kg/m <sup>2</sup>
1	Tărtășești	Soil	Lady Rosa F1	25.3	95.40	2.20	7.11
2			Precos F1	24.2	95.80	2.32	7.24
3		Peat	Lady Rosa F1	28.1	117.72	3.308	10.32
4			Precos F1	25.7	100.80	2.590	8.08
5	Brezoaiele	Soil	Lady Rosa F1	26.4	90.89	2.399	7.48
6			Precos F1	21.5	92.550	1.989	6.21
7		Peat	Lady Rosa F1	29.2	92.99	2.715	8.47
8			Precos F1	23.4	93.848	2.196	6.85

Table 3  
The quality of tomatoes cultivated in solarium

Var.	Place	Substrat	Cultivar	Content in ppm		
				NO <sub>3</sub>	P-PO <sub>4</sub>	K
1	Tărtășești	Soil	Lady Rosa F1	142	200.7	1320
2			Precos F1	124	187.7	1120
3		Peat	Lady Rosa F1	148	304.48	2001
4			Precos F1	140	276.12	1960
5	Brezoaiele	Soil	Lady Rosa F1	148	127.3	1250
6			Precos F1	142	110.2	1154
7		Peat	Lady Rosa F1	149	138.4	2241
8			Precos F1	145	117.6	2503

Quality analyzes (Table 3) followed the contents of nitrates, phosphates and potassium. If tomatoes analyzed nitrate content ranging from 124ppmNO<sub>3</sub> and 149ppm, which provides content in all experimental variants compliance with the maximum permissible limit for this compound 150ppmNO<sub>3</sub>. It is known that a high content of nitrates in tomatoes may lead to exclusion from the market because of a lot of tomato that this compound hazard phenomenon known as blue baby to children or duodenal and gastric cancer in adults.

The phosphorus content was between 117,6ppm and 304,48ppm a good content in this element that ensures firmness and good quality food tomato. Potassium varied widely, between 1120ppm and 2503ppm respectively, determines a good quality content that tomato consumption and for storage and transport.

### CONCLUSIONS

Experiments conducted on tomatoes in greenhouses in compliance with recommended technology in our country showed that:

- Peat culture achieves a greater number of tomatoes per plant, the average weight of the tomatoes is greater than the peat soil substrate as the final output kg / plant kg / m<sup>2</sup> is higher than in the culture the culture substrate soil;

- Quality of tomatoes obtained both growing media and soil was adequate legislation in Romania and has no limitation on consumer issues.

- It recommends continuing experiences of a long time and show varying factors.

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## RESEARCH REGARDING THE QUALITY AND QUANTITY OF SOME CARROT CULTIVARS

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**Keywords:** cultivars, carrot's, technology

### ABSTRACT

*Carrot is a vegetable consumed than in Romania. In recent years the market in Romania was invaded by products from different countries of the European Community thus largely replaced production based on traditional range. Local producers have been forced to introduce new technologies such as vegetable production and new cultivars lead to obtaining high yields and quality. The research aimed to test carrot comparative behavior of five cultivars of carrot: Nantes (Control), Morelia RZ F1, Fidra Rz F1, Nantes Rz 29350, Chantenay Red Core 29510 in climatic conditions in southern Romania in compliance with advanced technology. The results obtained showed high yields qualitatively and quantitatively compared with the control selected.*

### INTRODUCTION

The carrot is a root vegetable group vegetable species, of prime importance for its use in human food, so fresh after a short storage period, medium and long term and industrialized (sterilized canned carrots in salted water, juices, purees for infants, dehydrated or frozen carrots as such or in mixtures with other vegetable species). (Ciofu et al, 2003; da Silva et al, 2007; Gajewski et al, 2007)

Being a well known vegetable species and used by consumers in Romania, carrots technology evolved very quickly. The emergence of a diverse assortment cultivars characterized by us (hybrids and varieties) with different growing seasons, rooted bold, intensely pigmented, high in beta carotene biochemical content and carbohydrate specialized in own field or successive fresh consumption and use as raw material for processing, provision of equity and European modern equipment for precision seeding, thinning work entailed compulsory classical culture technology is used the universal seed random distribution . However the emergence of a diverse range of herbicides and other pesticides has eliminated the need for mandatory and weeding technologic works. (Grassmann et al, 2007; Neamtu et al, 1993)

On the other hand eating fresh carrots and cooked increased so that a closer scrutiny is needed in quality both in terms of toxic chemical compounds and the binding characteristics of sensory held consume carrots. (Olymbios, 1973; Rubatzky et al, 1999; Surlles et al, 2004)

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Given these reasons research conducted in 2013 attempted to elucidate quantitative and qualitative characteristics of cultivars with high yield potential recently introduced in our country.

Some of the objectives are: establish vegetation period of new cultivars to use for achieving a varietal conveyor must cover as well use the spring, summer and autumn in Dobrogea region; establish production potential of new cultivars in the adjacent area of Constanta; determination of biochemical quality new assortment of edible parts.

### MATERIAL AND METHODS

The area chosen for research is the Dobrogea region in a large firm that has the ability to apply the results obtained from research conducted (SC Big Land Company Ltd. - Clearing, Constanta) .Experimental variants included four new cultivars introduced and control Nantes control cultivar, the characteristics of which are listed below.

Table 1

Experimental variants - New carrot cultivars S.C. Big Land Company S.R.L. – Constanța

Var. no.	Cultivar	Group of earliness	Intended for consumption
1 (ct)	Nantes (Control)	Semi-early	Fresh for immediate consumption or storage
2	Chantenay Red Core 29510	Semi-early	Fresh for immediate consumption or storage
3	Fidra Rz F1	Early	Storage, industrial, fresh consumption
4	Nantes 29350Rz	Early	Storage, industrial, fresh consumption
5	Morelia Rz F1	Semi-early	Fresh for immediate consumption or storage

The experience made the production fund was placed in an own field. In the cultivated carrot field as in other similar experiences made under production conditions, the five experimental variants were fitted on experimental polygon for research in four repetitions, the storey blocks without randomization. Due to the planting scheme, each plot-variant had 4 m width (two passes of aggregate) with 8 lanes, with a distance of 0.5 m between the centers of two adjacent bands. Plots rehearsal for all variants has length 5 m and 20 m<sup>2</sup> area. In order to ensure protection bands, experimental area was located within a parcel planted all carrot production. Seeding density is provided by the establishment of 100 plants / m<sup>2</sup>. On the surface of a parcel repetition, number of seed distribution was scheduled for 2000. In this way, the parameters sketch location experimental technique to requirements specific rules vegetable crops with high and very high densities.

Establishment of cultures was made on March 22, 2013, three days after loosening topsoil. Harrowing and loose soil was considered as the optimal degree of grinding and compaction after rollers. On vegetation period was performed maintenance work necessary to implement modern technology.

During the preparation and running of the experience were conducted observations and determinations regarding: registration of all calendar data important cultural life, in



order to determine the time of appearance and main phenological phases of vegetation of all cultivars with which to work, determination tuberous root size (length, diameter) and harvested root mass experience, determination and production recording and reporting on variations and repetitions of that tons / ha, laboratory determination of key biochemical components of the cultivars used in the experiment.

Sampling was conducted during the period 10 July to 6 September period of quality cultivars: early, semi.

## RESULTS AND DISCUSSIONS

### 1 Results on measurements of carrot cultivars

Harvesting experimental variants were measured by 10 randomly selected carrots and the results are presented in Table 2.

External diameter ranged from 2.6 cm to 3.6 cm, the smallest diameter was recorded in cultivar Chantenay Red Core 29510 (2.6 cm) and the largest average diameter of Morelia Rz F1 was 3.6cm.

The internal diameter varied between cultivar Nantes 29350 Rz 1.25cm and 1.5cm at Fidra RZ F1 cultivar.

Carrots length of experimental variations ranged between cultivar Nantes 29350 of 17,60cm to cultivar Morelia RZ F1 20,5cm.

Size fits experimental variations recorded in the data presented by the manufacturer on the appearance of carrots.

Table 2

Biometric measurements of carrot cultivars from experience

No.	Cultivar	The external diameter, cm	The internal diameter, cm	Medium length, cm
1.	Nantes (Control)	2.70	1.30	18.70
2.	Chantenay Red Core 29510	2.60	1.40	17.80
3.	Fidra Rz F1	3.00	1.50	18.20
4.	Nantes 29350Rz	3.30	1.25	17.60
5.	Morelia Rz F1	3.60	1.35	20.50

### 2. Results regarding the production of carrot cultivars

Results regarding the production of cultivars (Table 3) shows that the highest one is at Fidra Rz F1 with 71.43t/ha.

Control variant Nantes had the smallest production of 39.64t/ha.

Statistical interpretation of results for production shows that all variants from V2 to V5 obtained high production which was very significant assured.

The results of productions confirms data catalog of the company.

Table 3

Summary of experimental results- Total production - t / ha. Poiana, Constanta, 2013

Variant no.	Cultivar	Total production (t/ha)	Differences of production (t/ha)	Total production (%)	Differences of production (%)	Sign.
V <sub>1</sub> (ct)	Nantes (Control)	39.64	–	100.00	–	–
V <sub>2</sub>	Chantenay Red Core 29510	68.15	+28.51	171.92	+71.92	***
V <sub>3</sub>	Fidra Rz F1	71.43	+31.79	180.19	+80.19	***
V <sub>4</sub>	Nantes 29350Rz	62.57	+22.93	157.84	+57.84	***
V <sub>5</sub>	Morelia Rz F1	68.24	+28.60	172.15	+72.15	***

DL – 5% - 6.05 t/ha

DL – 1% - 8.84 t/ha

DL – 0.1% - 12.48 t/ha

### 3.Results on chemical and biochemical compounds of carrot cultivars

Nitrate content (Table 4) in the samples examined ranged from 220ppm to Chantenay Red Core 29510 cultivar to 396ppm at cultivar Nantes 29350Rz. The values recorded are good showing where nitrogen fertilizers were applied according to the needs of plants.

If it is examined the nitrate content of cultivars in terms of maximum permitted concentration of carrot nitrates specified by the law No. 5 from 2002 of Romanian Health Ministry it can be say that carrots are good for the obtained consumer experience without putting human health problem, all values being below the Maximum Admitted Concentration of 400ppmN-NO<sub>3</sub> (Figure1).

Table 4

Quality characteristics for the production of carrots. Choice of carrot, Poiana, Constanta, 2013

Var. nr.	Cultivar	N-NO <sub>3</sub> <sup>-</sup> ppm	P-PO <sub>4</sub> <sup>3-</sup> ppm	K ppm
V <sub>1</sub> (mt)	Nantes (Martor)	250	215,3	2180
V <sub>2</sub>	Chantenay Red Core 29510	220	254,3	2007
V <sub>3</sub>	Fidra Rz F1	335	225,6	2165
V <sub>4</sub>	Nantes 29350Rz	396	220,7	2250
V <sub>5</sub>	Morelia Rz F1	387	221,3	2158

Phosphorus is an element of providing quality for commercial aspect of carrots fluctuated between Nantes 215,3ppm P-PO<sub>4</sub> and 254,3ppmP-PO<sub>4</sub> for Chantenay Red Core 29510 cultivar F1. In our case the phosphorus content is good which shows good crop fertilization and ensure a good quality of carrots for storage.

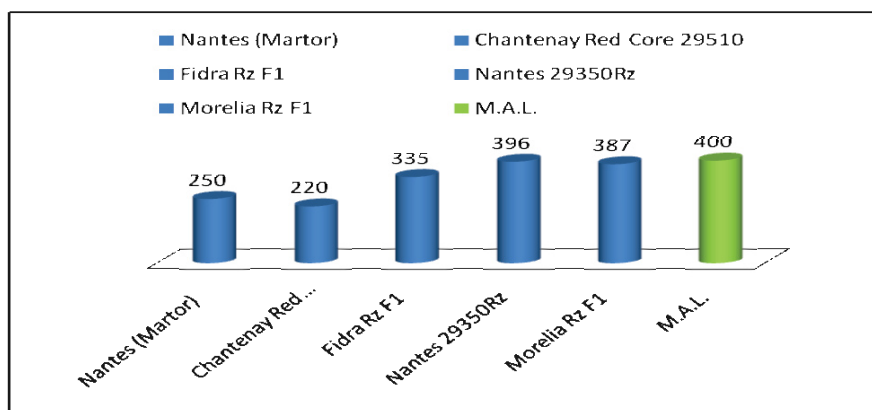


Figure 1. Comparison between the nitrate contents of cultivars and Maximum Admitted Limit (400ppm)

Potassium considered a final element that determines the storage capacity of carrots, was insured under culture, oscillating between 2007ppm at Chantenay Red Core K F1 and 2250ppm K at Nantes 29350 Rz.

### CONCLUSIONS

The results obtained at new cultivars studied in this scientific research, can be grown with good and very good in the Constanta:

- The total production stands Fidra RZ F1 (early, 71.43 t / ha);
- The use of cultivars that worked, cover a period of remarkable harvest, from mid-June to autumn;
- Accumulation of nitrate is influenced by fertilization. If excessive nitrogen fertilization that 300kg /ha or N200P200K200 combined fertilization can overcome the maximum permitted levels of 400ppm (Ministry of Health Order No. 1/2002) which may lead to a downgrading of carrots though their appearance is not affected;
- Absorption of nitrates is higher at the beginning of the harvest which means that before the final harvest is necessary to analyze this dangerous element to achieve optimal loading time;
- We must insist with research on nitrate uptake carrots to see which the best fertilizer technology is.

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## GETTING ESSENTIAL OILS TO AROMATIZE CERTAIN FOOD

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**Keywords:** *aromatic oil, aromatizing elements, herbs*

### ABSTRACT

*As the number of natural aromatic ingredients has increased and the numberless individual components are isolated and synthetized, it has been possible to study and imitate the spices flavor.*

*Based on these considerations, in these studies it is analyzed the aromatization power of two herbs: coriander and basil, known both because of their use in the Romanian cuisine but also because of their active principles, benefic to the human health. In this respect, the studies have aimed at flavoring two food products (butter and fresh cow cheese) so that they could be used as appetizers.*

*To make these brands, it has been studied the way the aromatizing element can be used (in the form of essential oils or by adding the herbs as such directly into the butter or cheese: dried basil leaves taken from the top of the plant).*

### INTRODUCTION

The cultivated medical and aromatic plants represent inexhaustible sources of raw materials for industries such as: pharmaceutical, food industry, aromatic, cosmetic, soap, colouring, paint industry, etc. They are also appreciated for their lack of toxicity in case of prolonged use (L. S. Muntean și col. - 2008).

Considering their quality and quantity, Romania is ranked among the first five countries in the world, being able to meet the national demands and accomplishing a high volume of exports in over 20 countries (Crăciun Mihaela -2010).

Nowadays, people tend to turn back to healthy food and natural remedies (Andreea Rausch, 2008, R. Ravi and all. - 2007).

The essential oils are volatile liquids with specific smell, etc.. They exist in a large number of plants and are produced in special tissues (egg: *Ocimum basilicum* flower and *Coriandrum sativum* L). Essential oils have disinfectant, bactericidal and expectorant effect (Gh. V. Roman – 2008).

The essential oil resulted from the young leaves, flowers and branches of basil is as much as 0,10 – 0,20% and is made of linalool, metilcavicol or estragole, cineol, camphor,  $\alpha$ -pinene, methyl cinnamate, eugenol, oleanolic acid,  $\beta$ -sitosterol, anethole, triterpenoid saponin, tannic acids, etc. The seeds contain mucilages (according to C. Pârnu – 2006, S. Norashikin and all. - 2008).

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## MATERIAL AND METHODS

Until flavoring the new obtained products (butter and fresh cow cheese), it was conducted a thorough organoleptic analysis of the studied herbs, so that to determine the water content at the moment of harvesting and when getting the essential oils by using different methods (pressing followed by centrifugation for the fresh fruit of coriander and maceration in vegetable oils for basil).

Determination of moisture content by means of oven drying consists of drying the freshly harvested sample (leaves and flowers) of coriander and basil. The method was performed for both plants at temperatures of  $95 \pm 2^\circ\text{C}$ , in the oven, in certain conditions.

### *Maceration (hot extraction) of basil flowers to obtain the essential oil*

The essential oils are spread through the entire plant or, more frequently, are gathered in one of its parts. In the case of coriander, the accumulation of flavors is in the fruit while in basil, the essential oils are concentrated in the aerial part of the plant, especially the flower.

Maceration is a method used to obtain essential oils by immersing the plant material in grease or oils heated at  $50 - 70^\circ\text{C}$ . The grease used during this study contains 60% sunflower oil, 30% rapeseed oil and 10% soya oil.

In order to obtain essential oils from fresh basil flowers, they were harvested in the morning, after the dew. The sampling was done using special scissors, so that to avoid damaging the material subjected to analysis (in order to mitigate the loss of oil).

In the lab, they were automatically chopped by means of a kitchen chopper.

Basil flowers chopped as such were kept in oil mixture for 12 hours and after that they were replaced (after a prior filtering of the macerated mixture of grease and initial flowers) by fresh flowers. This operation was repeated 10 times. At this stage, the grease mixture was saturated with odorant. The resulted mixture is then spun out for 5 minutes at 2000 rotations/minute. It occurs the separation between the supernatant (essential oil mixed up with a small amount of grease) of grease.

### *Extraction of coriander essential oil by pressing*

This method of obtaining essential oils is used in those plants or parts of the plant in which the essential oil is found in a large quantity, gathered in the secreting glands located superficially. It is used especially for aromatic plants such as the lemon, orange and bergamot trees, etc.), but also in case of aromatic fruits such as coriander.

The coriander fruit are washed, dried and pressed mechanically, by means of a press in the lab. The liquid resulted is transferred in a dark recipient fitted with a ground stopper. It is left to settle down for about 10 minutes and then it is filtered.

The filter material is spun out for 10 minutes at 2000 rotations/minute. The supernatant is separated by filtration, obtaining the essential oil needed for the analysis of the present study.

## RESULTS AND DISCUSSIONS

The content of the essential oils made of aromatic herbs such as coriander and basil, the preservation and the science of using these to flavor the taste of different dishes is an art.

The organoleptic aspects (general appearance of the plant, colour, smell, taste, degree of infestation, percentage of yellow leaves, percentage of damaged leaves, flowers and fruit, as well as the percentage of undeveloped leaves and fruit) are complying with the regulations in force.

The first analysis performed was to determine the moisture content, as it is well known that a high content of water in the aromatic herbs helps dragging along the essential oil at the moment of extraction in the laboratory according to the indicated method (Figure 1).

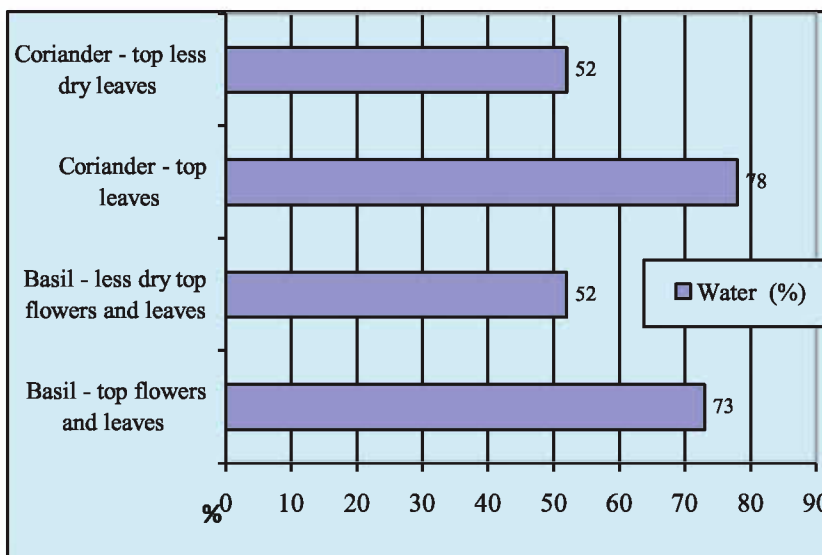


Figure 1. Determination of water content depending on the drying stage

Regarding the determination of the quantity of essential oil, from 1 kilo of plant material taken for analysis, there were obtained different values, both among different plants and within the range of the same plant compared to the analyzed portion of the plant (flowers, top leaves and flowers or fruits in the case of the coriander) – figure 2.

In figure 2, we can also notice that the best constituents of basil in terms of essential oils are found in the flowers, being followed by the top leaves cut out together with the flowers. From the top leaves, analyzed separately, it resulted a reasonable quantity of essential oil.

The quantity difference between the essential oil resulted from the basil flowers and the one obtained from the coriander fruit is obvious (2,6% in the case of basil leaves and 5,2% in the case of coriander fruit).

If we make a comparative analysis of the essential oils resulted from the top leaves of different species subjected to analysis, we can notice that there is a larger quantity in the case of coriander (2,8% compared to 2,1%).

Following the lab tests, it was noticed that for aromatizing the butter it is indicated to use grinded dry fruit to the detriment of the essential oil from various considerations:

- for 100 g of butter, adding 1 ml of coriander essential oil led to obtaining an appetizer with a proper smell and taste, but the persistence of the oil for the next 24 hours was reduced;

- for 100 g butter, adding 2 ml of coriander essential oil led to obtaining an appetizer with persistent flavor even after 24 hours since storing, but the taste was too strong (slightly hot).

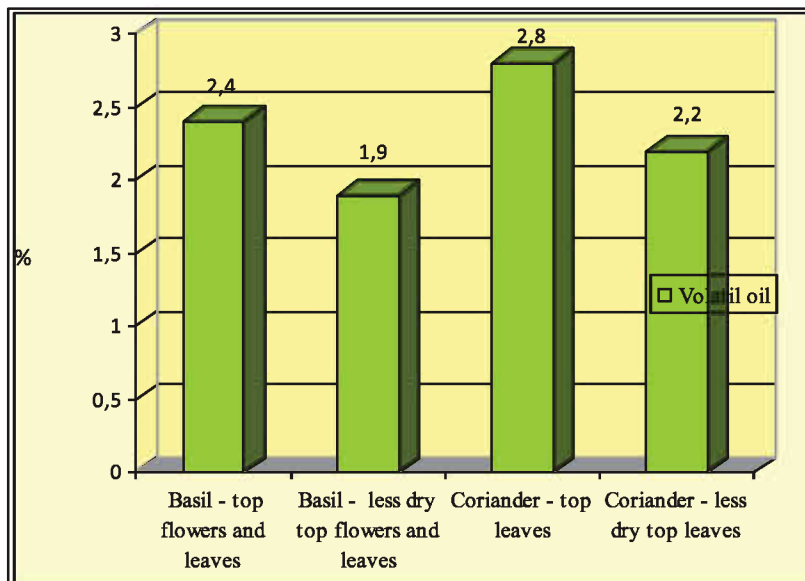


Figure 2. Quantity determination of essential oil depending on humidity level

Dry coriander fruit, grinded with a lab grinder, were added to 100 g of butter, starting from mixing 1 g up to adding 8 g of finely chopped coriander fruit and the conclusions were the following:

- by adding 1g of grinded dry coriander fruit to 100 g of butter it resulted a slightly aromatized appetizer;
- the experiment continued by adding each time 1 g more of grinded dry coriander fruit;
- when adding 6 g of grinded dry coriander fruit, the flavor of the butter was well perceived having a proper taste too (sweetish, nicely aromatized, specific). After storing it for 24 hours at 3°C, it was noticed that the butter has lost its flavor and the taste was slightly perceived.

- Furthermore, step by step it was added 1 more g each time, concluding that after adding 8 g of grinded dry coriander fruit, it resulted a buttery appetizer perfectly aromatized which maintained its specific flavor and special taste after 24 hours from storing.

Consequently, if we desire to aromatize the butter with grinded dry coriander fruit for an immediate consumption, it is indicated to use 6 g of aromatizing material or 1 ml of aromatic oil resulted from pressed coriander fruit.

If we desire to keep the obtained product for the next day, we should add 8 g of grinded dry coriander fruit.

Having 8 g of grinded dry coriander fruit in a mass of 100 g butter has as result a product served as appetizer or used to aromatize different dressings, salads, stakes etc. with certain aroma, highly appreciated by the consumers.

In order to aromatize 100 g of cow cheese with basil, just like when aromatizing the butter, it was used a basil oil got from fresh flowers and leaves from the top of the plant.

Following the lab analysis, it was noticed that, in term of percentage, the largest quantity of essential oil was got from the flowers, followed by the one got from the top leaves + flowers and top leaves.



In the case of the second alternative (cheese aromatized with basil) the herbaceous and aromatic nuances of basil combined very well with the stylish taste of cheese, resulting in a pleasantly aromatized product.

We must mention that, in order to aromatize the cheese with fresh herbs, there were used the leaves taken from the top of the plant. The flowers were used only for getting essential oil.

A good organoleptic combination was also got by adding the resulted essential oil to the cheese. It was followed the same procedure as for aromatizing the butter.

It was taken an amount of 100 g of cheese to which it was added 1 ml of basil essential oil. It was noticed that after 24 ore from preparation, its flavor was not as intense as at the moment of the addition (following the tasting); still the smell was perceivable.

As a result, it was concluded that, in order to get specific products (butter with coriander and cheese with basil) it is recommended to use dry coriander fruit after being prior grinded and fresh basil leaves from the top of the plant.

### CONCLUSIONS

The top of the plant (basil leaves and flowers and coriander fruits) contains an important quantity of essential oils compared to the semi-dry leaves where it is noted a less quantity.

With regard to the oil got from the coriander fruit, it resulted definitely more in terms of quantity compared to the one resulted from the basil leaves and flowers and it was stronger in terms of organoleptic perception.

Consequently, in order to have a good efficiency, both the basil leaves and flowers and the coriander fruit used to aromatize some food products as part of this project must be subjected to the process of essential oil extraction while being fresh.

After analyzing the moisture content of the vegetable material, one can say that, in order to get essential oil they must be fresh during extraction.

The vegetable material subjected to the classical drying process can be used successfully just in the case of preserving the analyzed vegetable material for a long period of time (for storing).

This involves a direct correlation between the turgidity degree of the plant and the quantity of the resulted essential oil, namely: the more turgid the more essential oil results from the vacuolar cells consequently to an absorption (for basil) and mechanic process (for coriander fruit).

In case of using top leaves, it was preferred to use them fresh, due to their aromatized properties well perceived compared to the semi-dry leaves. Before adding the fresh basil leaves to the cheese, the leaves were finely chopped (by knife). It was noticed that for a good aromatization of the cheese and basil appetizer, it was needed a quantity of approximately 15 g of chopped fresh basil leaves.

To get the butter aromatized with coriander and the cheese aromatized with basil for the purpose of storing, it is recommended to use fresh parts of the plant, while the basil and coriander essential oil can be successfully used in aromatizing both the butter and the cheese at the moment of consuming them fresh.

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## ANALYSIS OF BIOTECHNOLOGICAL FAULTS OF CERTAIN TYPES OF BEE HONEY

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**Keywords:** honey, sensorial and chemical characteristics

### ABSTRACT

*During honey extraction and preservation, treatment is essential for the quality and especially for the enzyme content of commercial honey. Sometimes, the types of honey with a high enzymatic content degrades due to the improper action of light and temperature, essentially losing its nutritional and therapeutic value.*

*This essay would like to make noticeable the biotechnical changes in the substance of certain types of honey (acacia honey, sunflower honey and rape honey – chosen for their different crystallization) kept in inadequate preservation conditions (direct light, high humidity, inadequate temperatures – too low or too high).*

*It will also carry out a comparative framework with a honey batch of each analyzed type, properly preserved in terms of temperature and humidity.*

### INTRODUCTION

For centuries, honey represented a main source for sweetening the food for our predecessors, knowing its nutritional and energetic value. The beekeeping has been possible due to the fact that our country is located on geographic areas with rich melliferous resources (Nic N. Mihăilescu – 1997, D. R. Paini, 2004).

Bee honey also contains a set of important compound, such as: protein substances (on average, 0,5% for the floral honey and slightly higher doses for the honeydew honey); a large spectrum of microelements (beryllium, gallium, vanadium, zirconium, nickel, silver, etc.); vitamins (B1, B2, B6, C, K, PP, H, etc.); small amounts of organic acids (malic, citric, lactic, oxalic, succinic etc.), vegetable gums, improperly called dextrin, colouring and odorous substances. (Bulancea, M., 2002, Molan P., 2001). These properties can be lost if the process of getting and preserving the honey is faulty.

The overheated honey immediately leads to the increase of the concentration of hydroximetilfurfural which may also determine the degradation of the honey quality. The optimum temperature should be between 10° and 20°C (Diaconescu Ion, 1998).

In time, the initial colour of the honey is fading; it usually becomes darker, but during crystallization the colour becomes lighter (Eugen Mârza at all – 1990).

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## MATERIAL AND METHODS

The nature of the findings carried out on the honey samples of this study are organoleptic and chemical.

The order to determine water content, it is used the refractometer. On the lower prism of the refractometer it is placed a drop of sample honey and the chamber is sealed immediately after that. At that moment, the refraction index can be read on the scale.

Depending on this index, based on Chataway's tables which accompany the refractometer, it is established the percentage of dry substance and the concentration of in water.

The measurement of the purity is performed by weighting the filter before and after filtering 5 g of honey in water solution. The filter retains the foreign bodies which must be eliminated during purification process. That is mainly wax scrap, propolis fragments, dust and animal or plants remains.

Determining the acidity helps appreciating the honey freshness level and can be accomplished by titration. Honey acidity is expressed in levels of acidity, namely the centimeters of NaOH 0,1N needed to neutralize the acidity in 10 g of honey.

## RESULTS AND DISCUSSIONS

The honey has a series of specific sensorial features: aspect without froth or visible foreign bodies, a colour from fade colourless to light yellow, golden yellow, orange-yellow, dark-yellow, dark brown, smell and taste specific to honey, with more or less prominent flavor, sweet taste, homogenous fluid viscous and crystalized consistency (Şindilar, E., 2000).

The determination of the concentration in water of different types of honey was performed considering that the honey was preserved in rooms with high humidity level. For that, it was used a sample made of 1 kg of each type of honey which was transfused to colourless glass containers, without making wax cover. In order to maintain the constant humidity, the glass recipients had been placed on a workbench next to a quantity of 3 kg of coarse salt. This method was used as it is well known that salt maintains a high wettability in a working chamber.

The honey samples have been kept at different temperatures (4°C, 25°C – room temperature and natural light) analyzing it on prior established intervals (when packaging, after 6 months, after 12 months).

The organoleptic assessment was done for all the mentioned batches, having as reference the standard for each honey type (acacia honey, sunflower honey and rape honey). The data analyzed from the organoleptic point of view, under specified conditions of temperature, humidity and light, are mentioned in Table 1 and Table 2.

By keeping the analyzed types of honey at room temperature, in open packages (having contact with the air), in natural light and high humidity, the honey suffers a gradual and visible degradation. In case of the sun-flower honey, the degradation is total. By keeping the analyzed types of honey at 4°C, in sealed glass recipients, the honey degradation is visibly stopped. Under these conditions, in the case of the rape honey, it was noticed that it starts crystalizing after 5 months since starting the storing. It is then followed by the sunflower honey, where the crystallization starts after 7 months. The samples of acacia honey did not crystalized after 6 months of preservation at 4°C.

The colour was assessed by direct visual examination, in day-light, on a white background, of a quantity of honey introduced in a colourless glass test tube of 16 mm. The smell and taste were assessed by smelling and tasting the sample. It was noted the flavor savour (acacia, sun-flower and rape) and its intensity (strong, well distinguishable,

moderate, discrete). Furthermore, it was appreciated the intensity of the sweet taste (strong, well distinguishable, and moderate) and any secondary flavors (sour, bitter, astringent, fad, etc).

The consistency is appreciated based on how the honey flows on a glass rod, mentioning its state: watery, thin fluid, fluid-viscous, sticky.

The three types of analyzed honey were subjected to the purity test right after being brought to the lab. As a result, it was noticed that the analyzed honey had an average purity level, fine impurities being visible on the filter (wax, insects or other impurities) which may later on lead to the degradation of honey.

During preservation period, the water content constantly increases with approximately 28% to the sunflower honey, which starts the fermentation process (together with the yeast activity and high temperature) – Figure 1.

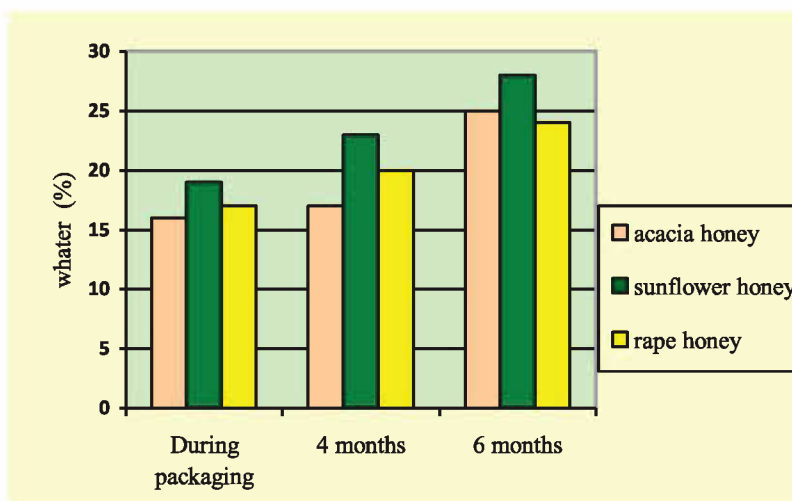


Figure 1 Determining the water content in the analyzed types of honey

At the analyzed samples, it was noted that, because of a higher water content right from the very beginning, the sunflower honey, fermented after 12 months of preservation under improper conditions. Fermentation was visible, as well as froth and an acid smell, not specific to the analyzed type of honey, which resulted from the carbon dioxide release.

Table 1

Organoleptic analysis of certain types of honey improperly preserved

Types of honey	Colour		Smell, taste		Consistency				
	during packaging	6 months	12 months	during packaging	6 months	12 months			
acacia honey	near colorless	yellow light	intense yellow	during packaging sweet taste, characteristic acacia honey	sweet taste	uneven, sweet acidic touch	during packaging uniform fluid-thin	uniform viscous	intense viscous (stiky)
sunflower honey	golden yellow	yellow-orange	dark yellow to brown	sweet taste specific sunflower honey	uneven, sweet acidic touch	heavy pungent fermentation	uniform fluid	uneven viscous	conglomerate foaming
rape honey	yellow	intense yellow	dark yellow	sweet taste intense specific	sweet taste specific	sweet taste	uniform fluid	viscous	viscous, uneven

Table 2

Organoleptic analysis of certain types of honey properly preserved

Types of honey	Colour		Smell, taste		Consistency				
	during packaging	6 months	12 months	during packaging	6 months	12 months			
acacia honey	near colorless	near colorless	yellow limon	during packaging sweet taste characteristic acacia honey	sweet taste	sweet taste	during packaging uniform fluid no signs crystallization	uniform viscous	uniform viscous
sunflower honey	golden yellow	golden yellow	yellow-orange	sweet taste, specific	sweet taste, specific	sweet taste, specific	uniform fluid	viscous	occurrence of crystallization
rape honey	yellow light	yellow light	dark yellow	sweet taste	sweet taste specific	sweet taste, specific	uniform fluid or viscous crystallized	occurrence of crystallization	coarse crystallization

The continuous change in colour is also due to the alteration of acidity (Table 3).

One can see that at 4°C, there are no major changes of acidity (there were no changes noticed in the acacia honey in terms of this parameter).

Table 3

Analysis acidity of honey in different storage conditions

Types of honey	Acidity ( ml NaOH sol.1N/100g)				
	During packaging	6 months		12 months	
		4°C	25°C	4°C	25°C
acacia honey	4	4	5	4	6
sunflower honey	5	5	6	6	10
rape honey	4	4	5	5	6

Still, at high temperature, the acidity of the analyzed honey also increases. In the case of the sunflower honey the acidity can reach up to 10 ml NaOH solution 1N/100g.

### CONCLUSIONS

At normal temperature (25°C), slight organoleptic transformations are noted starting from a preservation period of 6 months and continuing until month 12.

After 6 months of preservation, the sunflower changes its consistence becoming viscous and presenting some white spots. After 2 months, it becomes an intensely fermented yellowish conglomerated mass.

Keeping the analyzed honey types at 4°C in sealed glass recipients visibly stops the degradation of honey. Under these conditions, the rape honey crystalized the fastest (3 months since preservation, being followed by the sunflower honey which presented signs of crystallization after 6 months), while the acacia honey did not crystalized at all during the preservation period of 12 months.

By determining the water content, it was highlighted the higher content of this parameter in the sunflower honey and the hygroscopic character of the analyzed types of honey under preservation conditions of high humidity.

The determination of acidity under different preservation conditions of the honey samples showed once again the rapid degradation of sunflower honey, if preserved improperly. It was noticed that, even though honey cannot lose its properties in low temperature conditions (the colour remains specific to each type of analyzed honey, and so is the taste and the smell – until the 6<sup>th</sup> month of preservation), the consistency suffers major changes.

Thus, acacia and sunflower honey becomes more viscous while big visible crystals appear in the rape honey, so that after 12 months of preservation in a cool room the honey is totally crystalized

The quality biotechnical characteristics are compromise when the honey is preserved in inadequate conditions after 4 months or decrease under conditions of preservation in cool temperature after 12 month.



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THE STUDY OF PHENOPHASES IN SOME VARIETIES OF APPLES  
GROWN IN THE SOUTHERN AREA OF THE COUNTRY

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**Keywords:** *growth, fructification, variety, apple.*

**ABSTRACT**

*Expanding and changing old varieties with new varieties is necessary, so the apple culture presents a great interest for our country.*

*Flowering begins with variety Remus, then bloom Prima and Parmen auriu and last flowering varieties were Jonathan and Red Delicious.*

*Fruits maturation takes place in July at Remus and variety Parmen auriu in September and October.*

*Achieved significant production in the south of Romania, varieties Remus, Prima and Parmen auriu.*

**INTRODUCTION**

Today tree plantations are established mainly in specific areas, and new orchards are made only species listed in assortment.

The establishment of new orchards of apple should be taken into account that the apple is a species demanding soil and air humidity (65% - 70%) and annual rainfall of over 650 mm.

Have highlighted very valuable varieties whose fruits are required both in internal and external trade.

S. Maric et al. (2013) studied the properties of seven genotypes of apple native who grew up in a region of Serbia, so the two of them, namely Budimka and Kozara presents high quality fruit and good resistance to scab.

Lukic M. et al. (2013) presents an overview through the past, present and future of the apple breeding program, the Research Institute of Fruit.

Johnson D. et al. (2007) in the program at East Malling have selected different rootstocks. The authors obtained a rootstock that is AR. 680-2 same with M 9, rootstocks which induces an increase in fruit.

The selections AR 801-11 AR 835-11 trees produce the same effect as the rootstock M 26, and good productivity.

Jadczyk E. et al. (2007) emphasize the behavior of the variety Golden Delicious grafted on four rootstocks namely: M 9 EMLA, M 27, P 22 and PB - 4.

Balan V. et al. (2006) noted, that a plant leaf surface is connected to shoot growth.

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## MATERIALS AND METHODS

Experience includes five apple varieties studied namely Remus; Prima; Parmen auriu; Red Delicious; Jonathan.

Varieties were grafted on rootstock M 9, and was chosen as the control variety Red delicious variety.

Crown shape was determined Drapeau Marchand

The experiment was located in the Botanical Garden of Craiova.

The distances used in the experiment were: 3.5 m between rows and 2.0 m at a time

Between the years 2011 - 2013 were made the following observations and measurements:

- Diameter and height of trees;
- The total number of annual branches and length of these branches;
- Phenological observations;
- The number of inflorescences per branch control;
- The number of open flowers on branch control;
- The total number of fruits on branch control;
- The number of fruit remaining on branch control after the fall of physiological, establishing the percentage of the total remaining fruit related.

## RESULTS AND DISCUSSIONS

Concerning the decoration of trees with branches, we can rely on the annual that tree of the Prima variety shows average number of 49 branches annual garnish on the branch control.

The trees of the varieties Remus and Jonathan showed middle garnish namely forming 42 branches and 38 annual control.

In general there was a lower garnish variety Parmen auriu with 35 annual branches, especially witness Red Delicious with just 22 annual branches garnish.

If we look at the distribution of annual increases in length categories namely: 0-10 cm, 10-30 cm, 30-60 cm and 60 cm, we find the following.

It was observed that the percentage Remus variety of branches up to 10 cm was 83.3%, and the remaining of 11.9% were measured 10-30 cm and 0.04% branches of 30-60 cm.

Variety Jonathan recorded 73.6% long branches up to 10 cm, and 19.4% of the branches are growing up to 30 cm.

The varieties Parmen auriu and Red Delicious, showed branches an increase of up to 10 cm at a rate of 57.1 to 72.7%, the remaining 27.3 to 28.5% had increased to 30 cm. Variety Parmen auriu presented and 30-60 cm long branches or a percentage of 0.14%.

Analyzing total length of the annual apple varieties studied, we obtained the following results. The largest annual increases length control arm achieved trees in the Prima variety (750.0 cm) beyond the control of 375.0%, (Figure 1).

Annual increases amount reaches values between 500.0 to 620.0 cm at varieties Remus, Jonathan and Parmen auriu.

The variety Red Delicious witness had an overall increase of 200.0 cm branch, proves the weakest vigour of growth.

Regarding the number of inflorescences occurred in snake control, we found that more than 170 flowering we got variety Remus, then 140 and 130 varieties Prima and Parmen auriu and varieties Red Delicious and Jonathan had a number of 102 and 110 inflorescences.

Also, the average number of flowers was recorded at variety Remus of 900, then 739 and 700 to varieties Prima and Parmen auriu.

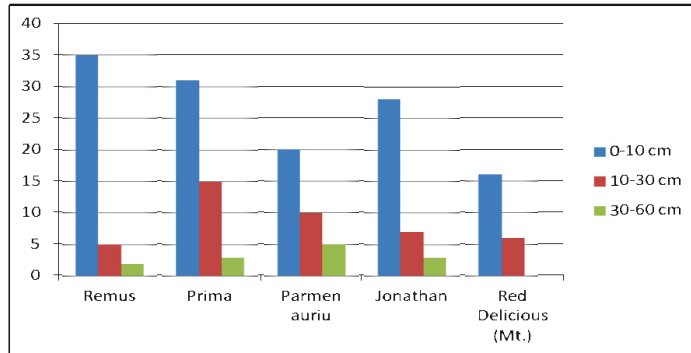


Figure 1. Distribution of annual increases in category length (Years 2011-2013)

The lowest number of flowers per branch was observed in cultivar Red Delicious.

After the flower petals fall, I counted the flowers about the control branch. Most fruit varieties were Remus and Prima with 95 and 75.

A slightly smaller number of fruits joined Jonathan and Red Delicious varieties, that a number of 58 and 45 fruit related.

It is found that the highest percentage of fruits of all varieties of flowers open stands with earlier ripening.

The average number of fruits in the inflorescence varies between 0.46 and 0.52 to 0.53.

After the fall physiological June, were the fruits of the control arm, leaving the tree a total of 60 fruit per cultivar Remus, ie 6.6% of the flowers open.

To variety Prima and Parmen auriu and remained 50 and 48 fruits that is a percentage of 6.7 to 6.8% of the flowers open.

Varieties Red Delicious and Jonathan recorded the lowest number of fruits, namely 42 and 35 fruit that is from 7.8 to 8.2% of the flowers open.

Duration intense growth of shoots, length of phenophase downturn and shoot growth cessation time varies from species to species, from variety to variety, from season to season.

From the records, we obtained a daily increase of 1.30 cm shoots to varieties Parmen auriu and Red Delicious and a minimal increase of 0.85 cm variety Jonathan. Duration of shoot growth was greater variety Red Delicious of 65 days, of 60 days and 63 to varieties Prima and Parmen auriu, and Jonathan varieties and Remus growth period 56 - 57 days (Table 1).

At the end of growth, the longest shoots reached a varieties Red Delicious and Parmen auriu of 53 and 55 cm.

A growing small final shoot observed to variety Jonathan of 36 cm and average shoot growth had Remus and Prima varieties. In conclusion, there is a correlation between the number of shoots and their length in the growth of the tree.

The winter varieties had a greater length of shoots but the number of shoots on snake control, was the lowest.

Table 1

## Observations on the dynamics of growth of shoots \*

Nr. crt.	Variety	The final length of the shoots of control (cm)	Growth period (days)	Daily growth of shoots (cm) (cm)	
				Average	Maximum
1.	Remus	43	57	0,65	1,10
2.	Prima	49	63	0,70	1,10
3.	Parmen auriu	53	60	0,75	1,30
4.	Jonathan	36	56	0,72	0,85
5.	Red Delicious (Mt.)	55	65	0,55	1,30

\*2011-2013

These changes increase the fruit varies from one variety to another. Growth curve of the fruit shows some upward maximum growth and then a slowdown in diameter.

In terms of fruit size represented by their diameter, the first place is occupied by the varieties Red Delicious and Remus with an average increase of 0.080 to 0.095 cm and Parmen auriu, Prima with an increase of 0.75 cm. Fruits with a smaller diameter were noted for the variety Jonathan.

### CONCLUSIONS

A great length of annual growth tree on branch control recorded of the Prima variety.

Varieties Parmen auriu and Jonathan had large percentages of actual fruit branches and Red Delicious variety a small percentage of actual fruit branches.

The winter varieties had a greater length of shoots, but the number of shoots on branch control, was the lowest.

In the southern conditions to ensure continuous reserves fruit can be retained assortment varieties: Remus, Prima and Parmen auriu.

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**COMPARATIVE STUDY REGARDING THE AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF SOME HYBRID ELITES FOR TABLE GRAPES OBTAINED FROM SELF-POLLINATION VICTORIA X VICTORIA**

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*Keywords:* agrobiological and technological descriptors, hybrid elites, parental forms

**ABSTRACT**

*This paper aims to highlight the superior agro biological and technological potential of the ten hybrid elites, obtained by self-pollination of the Victoria variety (Victoria x Victoria), visually attractive, expressed by very large berry, beautifully coloured, with special taste. Determining of the agrobiological and technological value of the elites for table grapes, obtained by the above-mentioned procedure, was done through a comparative study of these with the variety used as genitor, contributing to the knowledge, in addition to the qualitative traits of the parental form too hybrid crossing potential of transmitted characters.*

**INTRODUCTION**

It is known that Victoria is the most appreciated variety for fresh consumption in the country and not only, thanks its pleasant aspect, expressed by large berry, beautifully colored, with special taste, good resistance to low the temperatures in spring; therefore, these characteristics were oriented and have been obtained new genotypes for table grapes (Oprea St. et al. 1989, Damian et al. 1997, Gribaudo & Grando 2005).

The varieties for table grapes are primarily appreciated for the size of the bunches and especially of the berry, reason for that aims at obtaining of some genotypes with the weight of 100 berry that to exceed 800 g, specific to the self-pollination variety (Victoria).

Varieties belonging to *Vitis vinifera* shows the largest differences in terms the flavor of the berries, their content in sugar, the acidity, the color of the must, among themselves, as well depending on pedoclimatic conditions in which they are cultivated (Neagu M. I. 1975, Cichi et al. 2010).

The large number of grapes on a vine plant has a great practical significance, as it constitutes alongside weight average one of the the components of the yield. Without a representative number of grapes on the vine plant, at the varieties for table grapes it can not speak by the obtaining of the quality production (Onea Cristina, 2014).

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Alongside with the large number of bunches per vine plant, average weight of a bunch may be considered the second element that determines production of grapes per the vine plant, which in turn determines the production of grapes that can be obtained per a hectare.

## MATERIAL AND METHODS

Among the elites resulted by self-pollination of the Victoria variety, 10 genotypes were selected, which have been highlighted in the comparative field by at least two characters, the same or superior in comparison with the paternal variety (A1, A5, A6, A9, A16, A17, A19, A25, A27, A31).

*Viability of the buds* was determined by two methods: the counting, in the vegetation period, all of the eyes on the vine plant and by cutting of the buds. Was made the average of the data obtained in three years (2011-2013). It was determined *the percentage of fertile shoots* from the total number of shoots per vine plant, observations was carried out at all shoots on the 10 vine plant.

Still in the category of the descriptors agrobiologic, there were calculated and the *Coefficients of fertility* (descriptors introduced by I.C.V.V. Valley Călugărească):

- *absolute* (C.F.A.) - representing the ratio between the number of bunches on the vine plant and the number of fertile shoots;
- *relative* (C.F.R.) - representing the ratio between the number of bunches on the vine plant and the total number of shoots.

The *Indices of productivity* were also calculated. These are of two kinds: absolute and relative. The Indices of productivity (descriptors introduced by I.C.V.V. Valley Călugărească) are represented by:

- *absolute productivity index* (I.P.A.), which expresses the product between coefficient of absolute fertility (C.F.A.) and the average weight of a bunch;
- *relative productivity index* (I.P.R.), which expresses the product between coefficient of relative fertility (C.F.R.) and the average weight of a bunch;

Regarding the determinations carried out concerning the technological descriptors these consisted in the repeated weighings of the grapes on 10 vine plant for a period of three years (2011-2013). *Weight of grape bunches*, according to the codes OIV 502, IBPGR 6.2.25.; ICVV 15.1., was determined at full ripening, by weighing of the grapes bunch coming from the 10 shoots belonging to 10 different vine plant, from the same genotype. *The weight of one berry* (codes OIV 503; IBPGR 6.2.26) was determined by weighing of 100 berries, 10 berries from the middle area of 10 grapes, from 10 different vine plants.

Still in the category of the descriptors technological has been determined *the sugars content of the must*, by refractometer, by realizing a sample mean from to all the grapes from 10 shoots from 10 vine plants. *The content of the must in acidity* was determined by performing of a medium sample resulted from healthy and turgid berries from all grapes from 10 shoots. Was calculated the glucoacidimetric index, which represents the ratio between sugar (%)/total acidity.

## RESULTS AND DISCUSSIONS

Elites obtained by the self-pollination of the Victoria variety were started in vegetation by a high percentage of viable buds. In this hybrid crossing have been elites with 100% viability (A1, A6, A9). Overall, elites have proved resistant to the low temperatures during winter (-23,4°C), the most vulnerable being elites A31 and A19, with the percentage of dead eyes 25-24% similar to the Victoria variety (figure 1).



The percentage of fertile shoots on vine plant has varied to the each elite, from 27%-33% (elites A5, A16, A31), to 42%-56% (elites A1, A25, A9, A17, Victoria), until 64%-70% (elites A27, A19, A6). Over the average of the genotypes at this descriptor were framed 6 elites and the reference variety. Depending on the frost resistance, based on the percentage of viable buds (figure1) elites obtained by self-pollination of the Victoria variety are framing thus:

- with very good resistance: A1, A6, A9;
- with good resistance: A17, A27, A16, A25, A19, A5;
- with medium resistance: A 31.

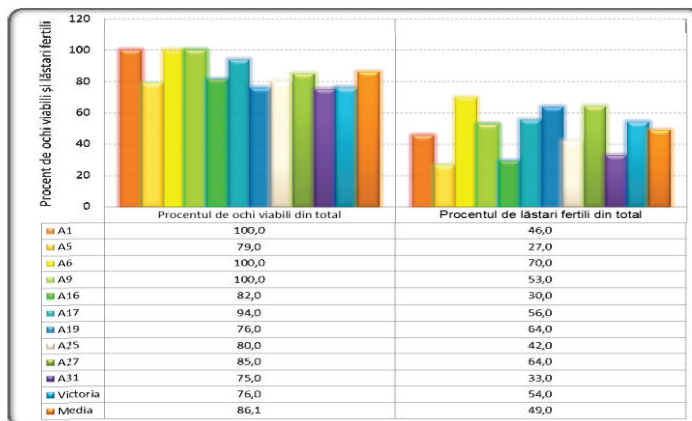


Figure 1. Viability and fertility of obtained elites by self - pollination Victoria variety

In relation to the fertility of the shoots, expressed by the percentage of fertile shoots (figure 1) can appreciate that elites obtained by the self-pollination of the Victoria variety are grouped as follows:

- with percentage very small of fertile shoots: A1, A5, A16, A25 and A31;
- with percentage small of fertile shoots: A17 and A 9;
- with percentage medium of fertile shoots: A6, A 19 and A 27.

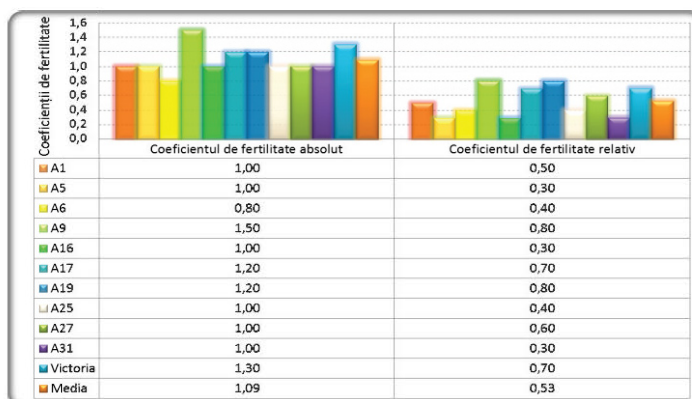


Figure 2. Coefficients of absolute and relative fertility of the elites in self-pollination Victoria crossing and parental variety

Coefficients of fertility calculated (figure 2) had values close for all forms studied. *Coefficient of absolute fertility* (C.F.A.) below the average has registered only elite A6 (0,8), and six elites which were close by the value 1 (A1, A5, A17, A25, A27, A31). Only elite A9 has exceeded the genitor variety to this characteristic.

*Coefficient of relative fertility* (C.F.R.) has varied, between the values 0,3 (A5, A16, A31) and 0,8 (A9, A19), being influenced by the burst of the vegetation, of the viability of the buds and the morphological characters.

At the elites resulted by the self-pollination of the Victoria variety, the values recorded at the descriptor *absolute productivity index* (I.P.A) (figure 3) the genotypes fall into the following categories:

- *medium* (values 100-300 ) in which are framing A31 and A25;
- *large* (values 300-500) in which are framing A6, A9, A16;
- *very large* (values 300-500) in which are framing A1, A5, A 17, A19 and A27.

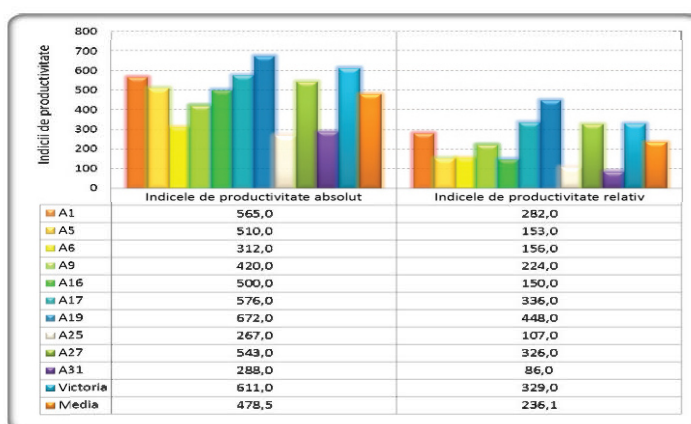


Figure 3. Indices of productivity: absolute and relative of the elites from hybrid crossing A

Calculating the indices of relative productivity (I.P.R.) led to the classification of genotypes of this hybrid crossing in the following classes:

1. - *small* < 100 : elite A31;
2. - *medium* 100-300: elites A1, A5, A6, A9, A16, A25;
3. - *large* 300-500: elites A17, A25, A27 and Victoria variety.

The weighings repeated of the bunches on 10 vines, over a period of three years (2011-2013), have led to the following considerations: Victoria variety, known for the size of the bunch was exceeded in the average weight by the six of the 10 hybrid elites (A1, A5, A16, A17, A19, A27) selected from the hybrid crossing A, with 10 g (A17), up to 95 g (A1).

Grapes smaller, under 300 g have recorded the four hybrid descendants: A25, A9, A25 and A31, elites which have been highlighted by other characters. Concerning to this trait, elites have been divided into two categories, each with 5 genotypes (figure 4).

1. With the grapes under 400 g weight;
2. With the grapes over 400 g weight.

A characteristic particularly appreciated to this combination is the weight of the berry, very varied to the 10 descendants (figure 4). Two elites had weight of 100 berry exceeding 1 kg, one elite (A27) more than 800 g, exceeding with about 200 g the Victoria variety and the average, and seven elites were framed in values lower than 600 g.

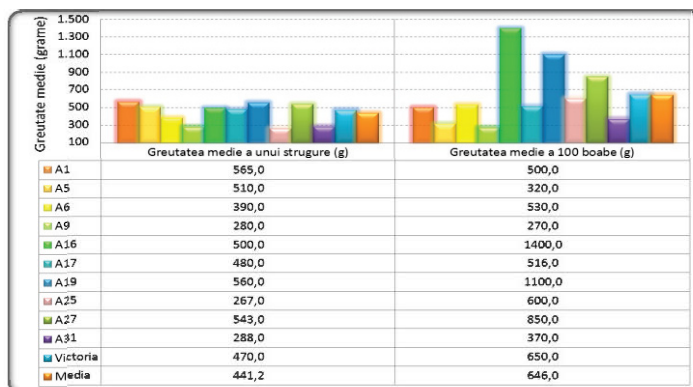


Figure 4. Influence of genotype on the average weight of the grapes and berries

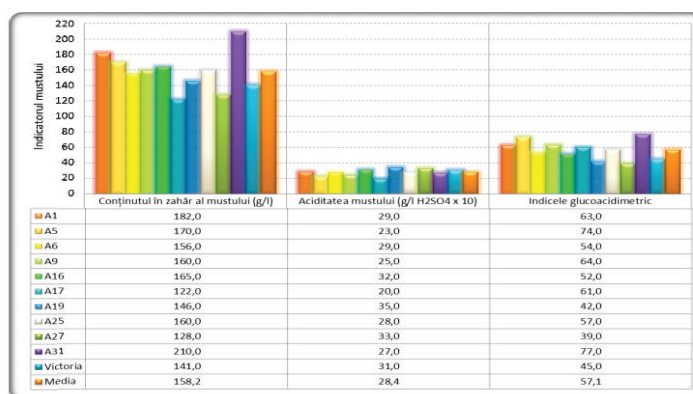


Figure 5. Influence of genotype on the composition of the must

The accumulation in sugars of the must at the hybrid descendants A it has been reported to the concentration of the Victoria variety, the principal witness and genitor. Thus, elites were divided into two categories:

1. With more concentrated sugars than the Victoria variety (141 g/l), includes elites: A1, A5, A9, A6, A16, A19, A25, A31;
2. With the value of the sugars lower than of the Victoria variety, includes elites: A17, A27.

Reported to the average of the genotypes presented, Victoria variety was situated under the their average, and six elites over the average value. Noteworthy elite A31, which accumulated 210 g/l sugars in must with the glucoacidimetric index corresponding of the value 77( figure 5). The acidity of the must had presented close values for all genotypes studied, significant differences being between elites A19 (3,5 g/l H<sub>2</sub>SO<sub>4</sub>) and A17 (2,0 g/l H<sub>2</sub>SO<sub>4</sub>). Glucoacidimetric index has presented the highest value (77) to hybrid elite A31, due to content very high in sugars. Elite A19 (44 glucoacidimetric index value) it was very close to the Victoria variety (45 glucoacidimetric index value) at this indicator.

## CONCLUSIONS

Conclusions regarding the study of the **agrobiological characteristics** of the elites resulted by self-pollination of the Victoria variety (*cod A*) highlights the following:

- *fertility of shoots*, expressed by the percentage of fertile shoots, are framing the elites obtained by self-pollination of the Victoria variety thus:

- with percentage very small of fertile shoots: A1, A5, A16, A25 and A31;
- with percentage small of fertile shoots: A17 and A ;
- with percentage medium of fertile shoots: A6, A19 and A 27;

- the recorded values to the descriptor *absolute productivity index* (I.P.A.) is framing the genotypes within the following categories:

- *medium* (values 100-300 ) in which are framing A31 and A25;
- *large* (values 300-500) in which are framing A6, A9, A16;
- *very large* (values 300-500) in which are framing A1, A5, A 17, A19 and A27.

Depending on frost resistance, on the basis of the percentage of viable buds, elites resulted by self-pollination of the variety Victoria are framing as follows:

- with very good resistance: A1, A6, A9;
- with good resistance: A17, A27, A16, A25, A19, A5;
- with medium resistance: A 31 similar to the genitor.

Conclusions regarding the study of the **technological characteristics** of the elites resulted by self-pollination of the Victoria variety (*cod A*) highlights the following:

-Victoria variety was exceeded in average weight of 100 berries by six from 10 selected hybrid elites (A1, A5, A16, A17, A19, A27);

- *the content in sugars* higher than to the variety Victoria (141 g/l), have elites: A1, A5, A9, A6, A16, A19, A25, A31;

- it was found that there is a very significant positive correlation and linear between the weight of the 100 berries (g) and the weight of the one bunch (g) to the all elites studied;

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**THE EFFECT TEMPORARY PROTECTION UPON THE  
QUANTITATIVE AND QUALITATIVE ELEMENTS AT THE CULTURE  
CAPSICUM ON SANDY SOILS**

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**Keywords:** *Capsicum, protection, production, quality*

**ABSTRACT**

*At capsicum were studied in different cultivation methods: 1 - unprotected cultures; 2 - soil mulching with polyethylene; 3 - to protect plants with polyethylene tunnel; 4 - protecting the plants with polyethylene tunnel and soil mulching with polyethylene; 5 - to protect plants with foil type Agril and 6 - protect plants with Agril and soil with polyethylene mulch. The protect method has influenced the pace of growth of plants, number of fruits/plant, total production and in the dynamics and some biochemical components in the fruit. The best production results were obtained by protecting plants with foil type Agril and soil with polyethylene mulch (34.4 t/ha and 8.5 fruits/plant).*

**INTRODUCTION**

The sandy soils in the southern Oltenia enjoys a particularly high potential of heat which can satisfy the most demanding vegetable species compared to the heat factor (Voinea, M. et al., 1977). It is known that, carrying out normal metabolic processes in plants is achieved only if ensure suitable environmental conditions requirements for each species. Special attention should be given to particularly the lethal minimum temperatures as well as the minimum and maximum biological ones.

The permissible extreme limits for plant growth of capsicum are between 15-35<sup>0</sup>C the optimal temperature being comprised between 22-25<sup>0</sup> C, and at temperatures below 15<sup>0</sup> C are affected the growth roots and the buds (Voican V., Lăcătuș V., 1998). Linking the fruit is strong influenced by environmental conditions: light, temperature, carbon dioxide, humidity, soil fertility conditions. More often than not under shelter, flower abortion occurs because of low the temperatures, below 10<sup>0</sup> C the night and under 15<sup>0</sup>C day. Increasing the temperature at 40-500 c even for a short period of time is cause the abortion of flowers, especially when humidity is very low (Lăcătuș V., 2012).

Analysis of minimum thermal values recorded in the southern Oltenia reveals a decrease in April-May, which could influence the of vegetable crops and the precocity (Ifrim Aurelia et al., 2003).

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Removing or reducing the undesirable effects arising in the early part of the period from vegetation can be achieved by applying of technologists suitable of cultivation including temporary protection in area has a significant role. Also, through the protection of culture are obtained earlier productions, in a period when demand is greater than the offer.

### MATERIALS AND METHODS

In specific conditions on sandy soils in the southern Oltenia were taken in study for culture of Capsicum, the following variants:

- V1 - unprotected cultures
- V2 - soil mulching with polyethylene
- V3 - to protect plants with polyethylene tunnel
- V4 - protecting the plants with polyethylene tunnel and soil mulching with polyethylene
- V5 - to protect plants with foil type Agril
- V6 - protect plants with Agril and soil with polyethylene mulch

The planting in the shelter was made on 12 April. Once with the planting has been installed, and the appropriate protection system. The system of protection was held until May 22. Determinations were made on the pace of growth of plants, number of pructe/plant, the quantity and quality of production. From the fruits of Capsicum at maturity for consumption were made the following determinations: the water and the dry substance total through method gravimetric, solubil dry substance by the method refractometric, total carbohydrates by method Soxhlet Fehling and vitamin C by the method iodometric. At each harvest was determine number of the fruit and determined the weight of the production on each variant side's in 4 repetitions.

The results were calculated and statistically interpreted.

### RESULTS AND DISCUSSIONS

Analyzing the limits of temperature recorded during the months of April-May we can draw the conclusion that there is a risk that the temperatures exceed the limits extreme and capsicum plants are affected (table 1).

Table 1

The limits of temperature recorded during period April- may

The year	Specification	The temperature °C	
		April	May
2011	The minimum	-0.1	2.5
	The maximum	25.8	27.3
	The average	11.9	16.1
2012	The minimum	-3.7	7.9
	The maximum	29.4	31.8
	The average	13.9	17.2
2013	The minimum	3.1	6.7
	The maximum	32.2	31.8
	The average	14.3	20.0

Three years of research were different in terms of climate. Average temperatures in April were between 11.9 – 14.3°C, and the average temperature of the month of May between 16.1-20.0°C. The year 2011 was the coldest during April-May, and 2013 was the

warmest. This has influenced differently the culture of Capsicum. In April the absolute temperatures recorded values between  $-3.7$  and  $3.1^{\circ}\text{C}$ , and in May the absolute temperatures recorded values between  $2.5$ - $7.9^{\circ}\text{C}$ . Steps should therefore be taken to protect culture in this period. Maximum temperatures are slightly higher than those optimal and for the research are necessary of methods and materials in order to protect of the plants and a microclimate close of to optimal.

At the time removal protection system was determined the height the plant of Capsicum. Microclimate conditions created by the different methods of protecting influenced differently the pace of growth of the plants (table 2).

The influence of the system of protections upon increases  
at plants of Capsicum

Table 2

The variant	The plant height		Difference cm	Significance
	cm	%		
Unprotected	23.8	100	Mt.	
Mulch	27.2	114	+3.4	
Polyethylene tunnel	29.7	125	+5.9	
Polyethylene tunnel+mulch	29.2	123	+5.4	
Agryl	29.6	124	+5.8	
Agryl+mulch	34.4	145	+10.6	**

LSD 5% = 7.06 cm; LSD 1% = 10.04 cm; LSD 0,1% = 14.55 cm

Unprotected plants, directly subject to the action of the climatic factors have recorded the lowest growth rate. At the removal of the protection system the plants have measured a height of 23.8 cm. Mulch the soil with polyethylene contributed to increased temperature in soil and accelerated the pace of growth. In these conditions the plants of Capsicum have measured 27.2 inches. Microclimate conditions created by protecting plants with polyethylene tunnel were favourable for the growth of the Capsicum plants. Also by protecting plants with Agryl has accelerated the pace of growth. The largest height had the plants protected with mulch + Agryl (34.4 cm). The face of unprotected plants, by protecting with Agryl+ mulch increased by 10.6 cm, distinct growth of significant statistically. The temperatures in the period between April and May and the method of protection influenced the precocity production of the Capsicum (table 3).

Production of Capsicum in dynamics depending  
on the method of protection

Table 3

The variant	The production (t/ha) obtained in the period:						
	Until 15.06	16- 30.06	1- 15.07	16- 31.07	1- 15.08	16- 31.08	After 1.09
Unprotected	0.2	5.3	5.0	11.2	3.6	3.7	5.2
Mulch	0.3	7.3	7.5	10.4	3.0	3.9	4.7
Polyethylene tunnel	-	3.3	5.2	12.4	4.0	5.6	4.6
Polyethylene tunnel+mulch	0.7	5.8	6.8	11.0	4.1	5.7	5.6
Agryl	0.8	7.7	8.3	11.2	3.9	4.8	5.5
Agryl+mulch	1.8	10.2	7.1	11.1	3.6	4.6	5.3

Until June 15, at the unprotected plants were harvested 0.2 t/ha, while at mulch were harvested 0.3 t/ha. By protecting with polyethylene of the plant has skyrocketed the temperature under tunnel, flowers have aborted and production been delayed. In these conditions of temperature not were obtained productions until June 15. The Agryl, through porous nature leave water and air to penetrate inside and creates a microclimate favourable to growth and fructification of the plants. In these conditions there have obtained the biggest early productions. The productions were of 0.8 t/ha to culture covered with Agryl. The precocity of the production grew converging of the plants with Agryl and soil with polyethylene (1.8 t/ha were obtained up to this date). Noting the importance of mulch in increasing precocity of the production. And in July they maintained high productions in the variants protected with Agryl and Agryl with mulch. In The following period differences between the production variants were small.

The number of fruit/plant and the total production were influenced by the method of protection (table 4). At the unprotected plants were harvested 6.6 fruit/plant and at plants in soil mulched with polyethylene were harvested 7.6 fruit/plant.

Table 4

The influence of the method of protecting upon  
the number of fruits/plant and upon the of total production  
of Capsicum (average 2011-2013)

The variant	The number of fruits/plant	The production (t/ha)	Difference t/ha	Significance
Unprotected	6.6	33.6	Mt.	
Mulch	7.5	37.1	+3.6	
Polyethylene tunnel	6.5	35.1	+1.5	
Polyethylene tunnel+mulch	7.6	39.7	+6.1	
Agryl	8.4	42.2	+8.6	*
Agryl+mulch	8.5	43.7	+10.1	**

LSD 5% = 6.70 t/ha; LSD 1% = 9.29 t/ha; LSD 0,1% = 12.82 t/ha

At the protected plants with polyethylene tunnel have been harvested the same number of fruit since the first flowers were lost due to high temperatures which were registered under the tunnel. Under the Agryl was obtained the largest number of fruits/plant.

The lowest production was of 33.6 t/ha and was obtained at unprotected plants. Through the soil with polyethylene mulch was obtained a production increase of 3.6 t/ha, concerned a production of 37.1t/ha. By protecting with tunnel of polyethylene was achieved a production of 35.1 t/ha, and at protect with mulch and polyethylene tunnel the production has been of 39.7 t/ha. The increasing number of fruits/plant at of protecting with the Agryl determined and getting the biggest productions in these conditions. Through using of the Agryl to protect plants has achieved a production of 42,2 t/ha, resulting in an increase of production of 8.6 t/ha compared to unprotected, significant statistically.

Through protection with polyethylene mulch and Agryl was achieved most production, 43.7 t/ha, resulting in an increase of production of 10.1 t/ha compared to unprotected, distinctly significant statistically.



The protect of the plants of Capsicum is justified by productions made. Soil mulch with polyethylene provides significant increases of the production regardless of the method of perotejare of the plants.

The results obtained on the accumulation of biochemical components in the main fruits of Capsicum are presented in table 5.

Table 5

The biochemical composition the of fruits of Capsicum depending the protection method

The variant	Total dry substance (%)	Water (%)	Solubil dry substance (%)	Carbohy-drates (%)	Vitamin C (mg/100 g f.s)
Unprotected	6.9	93.1	5.7	4.0	81.9
Mulch	7.9	92.1	6.3	4.4	81.4
Polyethylene tunnel	8.7	91.3	6.8	4.8	95.5
Polyethylene tunnel+mulch	8.7	91.3	6.6	4.5	101.7
Agryl	7.4	92.6	6.5	4.4	107.4
Agryl+mulch	10.1	89.9	6.9	5.4	113.5

Total dry substance content in the fruit was between 6.9% at unprotected crop and 10.1% in those from culture protected with Agryl and mulch. With the accumulation of total dry substance in fruit decreases the amount of water. The literature indicate for the fruits of Capsicum a total dry substance content of between 7-13%. In the study of the variants the fruits of Capsicum have accumulated a quantity of dry substance within the limits. The total dry substance content in protected variants was higher compared to the unprotected variant. Total dry substance accumulation and amount of water from the fruit is influenced by culture conditions. At high temperatures but with moisture in the soil total dry substance remains more great because it lowers the intensity of the process of respiration (Tony Kellock et al., 1995).

The dry substance presented values from 5.7% in the unprotected variant and 6.9% in variant protected with Agryl and mulch. In Capsicum fruits was determined a carbohydrate content of 4.0% in unprotected variant and between 4.4-5.4% in the protected variations. The results obtained show a higher content of quality indices in the protected variations. In these variants, the plants have started faster in vegetation due to the microclimate created by protecting it with a direct influence on the processes of assimilation.

Vitamin C is the main vitamin synthesized of the plants. All varieties of peppers contain large amounts of vitamin C. The literature highlights the environmental content of vitamin C of 130 mg/100 g fresh substance (Gherghi A.. et al., 1983). The content of vitamin C in fruits was 81.4-81.9 mg/100 g fresh substance of unprotected variants, 95.5 mg/100 g fresh substance in fruit from the plants protected with polyethylene tunnel, 101.7 mg/100 g fresh substance to protect the tunnel from polyethylene and mulch, 107.4 mg/100 g fresh substance to protect the Agryl and 113.5 mg/100 g fresh substance to those protected with Agryl with mulch.

## CONCLUSIONS

The protection system used influences differently the microclimate conditions contributing to the increasing number of fruits/plant and the production increase. Unprotected plants, directly subject to the action of the climatic factors have recorded the lowest growth rate and the largest height had the plants protected with mulch + Agril.

Through the adding of the mulch to protect the plants or polyethylene tunnel or agril increases precocity and total production.

The best results both in terms of quantity and precocity yield of Capsicum were obtained by protecting crops with Agril and mulch. Under these conditions until June 15, was obtained by a production of 1.8 t/ha and the total production was 43.7 t/ha.

At the culture of Capsicum best results with regard of the fruit quality were obtained in the variants protected with mulch and tunnel of polyethylene and mulch with Agril, in which fruits have accumulated 10.5-12.4% dry substance, 89.5 total-87.6% water, 6.7% dry substance in powder, 3.64-4.9% carbohydrates, 120.56-127.6 mg per 100 g s.p. vitamin C.

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## EVALUATION OF POLLEN GERMINATION CAPACITY OF SOME KIWI GENOTYPES (*ACTINIDIA SPP.*)

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**Keywords:** Pollen germination, *Actinidia spp.*, pollinators, kiwi.

### ABSTRACT

*The evaluation of pollen germination capacity is one essential criteria for kiwi pollinator's characterization. This study was carried out to evaluate pollen quality of nine genotypes of kiwi (*Actinidia spp.*). Germination rate and pollen tube growth in a culture medium containing 20% sucrose, 5 ppm boric acid ( $H_3BO_3$ ) and 1% agar were registered after 3, 6 and 9 hours (H) of incubation. Results showed that in all kiwi genotypes the germination rate and pollen tube growth varied according to the incubation period. The highest percentage of germination (72%) was recorded after 9 H of incubation for R9P19, R10P9 and Nz. Among the kiwi genotypes studied, five types (R1P4, R1P17, R9P19, R10P9 and Nz) appears to be suitable pollinators with respect to the criteria investigated.*

### INTRODUCTION

All *Actinidia* species are apparently dioecious, thus the consequences of dioecism are that the female and male plants are both required, female and male plants must flower at the same time, pollen must be transferred from male to female flowers by bees or mechanical pollination.

The *Actinidia* female flowers have several functional stigmata in the central region, surrounded by anthers that produce sterile pollen. The male flowers consist of a rudimentary and non-functional pistil and a large number of stamens with anthers that produce viable pollen grains. Pollination is very important for fruiting performed mainly by bees (Ferguson 2007) and necessary, as fruit size is affected by the number of seed set.

Pollen physiology especially germination and viability has received considerable attention for its application in reproductive physiology (Falasca et al. 2010), conservation of germplasm (González-Benito et al. 2004), pollination and fruiting (Nunes et al. 2001, Bettiol Neto et al. 2009) and plant breeding (Cruz et al. 2008, Chagas et al. 2010, Novo et al. 2010), with varied aims and objective. For example in breeding programs of pollinator (male) kiwi plants, for selecting the ones which would have high ability to produce pollen and flowering periods that coincide with the current fruit producing varieties (Novo et al. 2010).

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Pollen is a useful source of diverse alleles within a gene pool and may be an effective propagule for gene banks. The ease of pollen storage and shipment and the potential for its immediate use provide researchers with increased options when designing their breeding programs (Gayle 2011).

For the evaluation of pollen viability several methods can be mentioned: evaluation by using colorimetric tests like acetic carmine staining or TTC (triphenyl tetrazolium chloride) (Domingues et al. 1999, Badii et al. 2013), incubation in Baker solution (Oliveira et al. 2001) or the germination test in culture medium (Franzon and Raseira 2006, Pio et al. 2007).

Evaluating the viability of pollen through germination in culture medium *in vitro* is practical and accurate (Einhardt et al. 2006).

Tests for *in vitro* germination were performed for different fruit species, such as Annonaceae (Rosell et al. 1999, Bettiol Neto et al. 2009), citrus (Pio et al. 2007, Ramos et al. 2008), apple (Nunes et al. 2001, Dantas et al. 2005), passion fruit (Cruz et al. 2008) Myrtaceae (Franzon et al. 2005, Franzon and Raseira 2006), including kiwi (Abreu and Oliveira 2004).

In this study, we examined kiwi pollen grains for *in vitro* germinability of nine kiwi genotypes (*Actinidia* spp.) with the aim of identifying the most suitable pollinators for kiwi female orchard establishment. This information is expected to be useful for programs plant breeding, genetics, and gene bank conservation that need pollen viability tests.

## MATERIAL AND METHODS

This study was carried out to evaluate pollen quality of nine genotypes of kiwi (*Actinidia* spp.) (Table1). The pollen's germinability tests were made using fresh pollen from the kiwi collection cultivars of Pomology Department at the Faculty of Horticulture from București. During the flowering period (June-July) the anthers of some kiwi genotype were picked out in Petri dishes and transferred to the laboratory.

The pollen was released on paper, placed in Petri dishes and stored at 4°C in a refrigerator until use. Pollen germination and pollen tube growth were determined by sowing pollen grains on germinating media. The culture medium was heated to near-boiling (95°C), and then distributed on Petri dishes (10 ml dish). The pollen grains of each variety were evenly distributed on the surface of the culture medium using a brush. Pollen was incubated under dark conditions at 25°C in a semi-solid culture medium containing 20% sucrose, 5 ppm boric acid (H<sub>3</sub>BO<sub>3</sub>) and 1% agar for 3, 6 and 9 hours (H).

Table 1

Studied kiwi genotypes

Number	Specie	Genotype
1	<i>Actinidia deliciosa</i>	R1P4
2	<i>Actinidia deliciosa</i>	R1P7
3	<i>Actinidia deliciosa</i>	R1P8
4	<i>Actinidia deliciosa</i>	R1P17
5	<i>Actinidia deliciosa</i>	Nz
6	<i>Actinidia arguta</i>	R8P5
7	<i>Actinidia arguta</i>	R9P17
8	<i>Actinidia arguta</i>	R9P19
9	<i>Actinidia arguta</i>	R10P9

The pollen's germinability tests were made twice, successively every 5 days, using fresh pollen put directly out of the anther. In our study we also used stored pollen grains kept in refrigerator (pollen from New Zealand which in our study it is marked Nz).

Germination was recorded by counting three fields under an optical microscope with 100-fold amplification (Figure 1). A pollen grain was considered germinated when pollen tube length was at least equal to or greater than the grain diameter. Germination percentage (%) was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field of view.

### RESULTS AND DISCUSSIONS

The observations made under photonic microscope were that pollen germination rates and pollen tube growth for the different kiwi genotypes were not synchronous and heterogeneous. In the same medium, we observed different pollen tube lengths.

Under germination conditions described and starting from 3h, the grains of pollen start to emerge a pollen tube where its length varied according to the incubation period (Figure1).

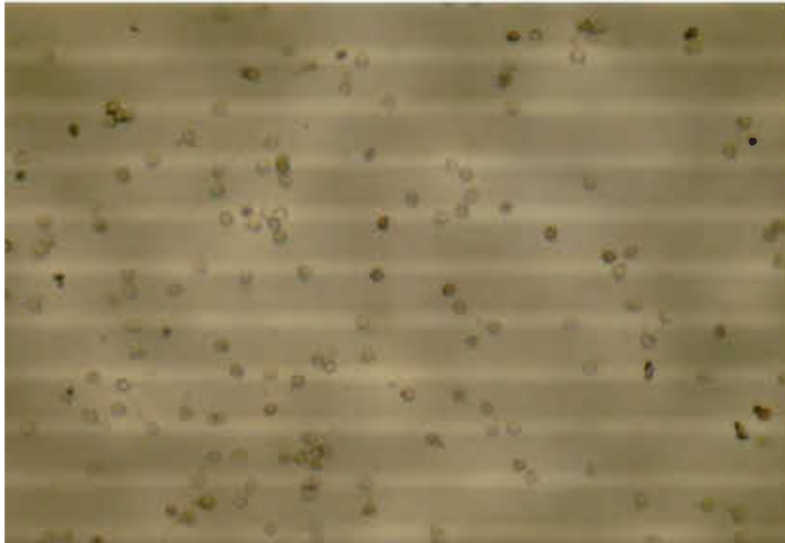


Figure 1. Kiwi pollen grain of the *Actinidia deliciosa* (Nz), in culture medium for *in vitro* germination test, after 3 hours of incubation

Results showed significant differences among pollen tube length kiwi genotypes for germination rate (Figure 2). In all kiwi types, germination rate varied according to the incubation periods (Table 2).

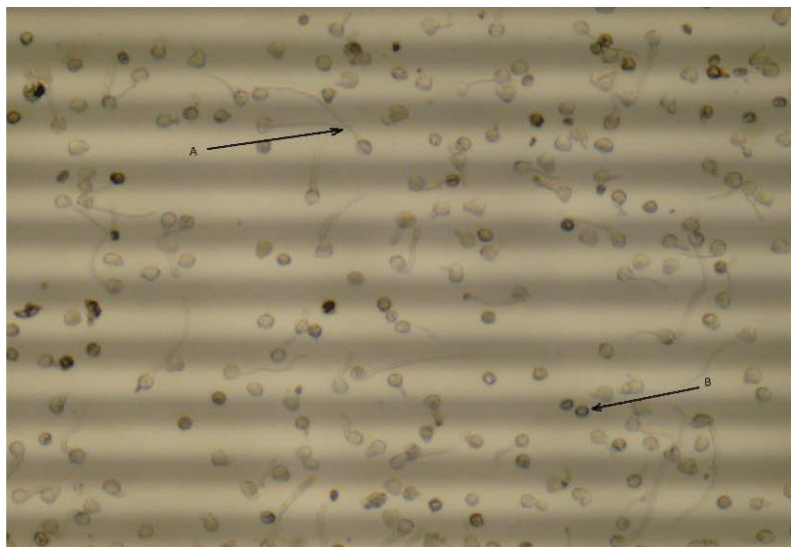


Figure 2. Kiwi pollen grain of the *Actinidia deliciosa* (Nz), in culture medium for *in vitro* germination test. Germinated (A) and non-germinated pollen grains (B), after 9 hours of incubation.

Germination rate after 3h ranged from 5 to 28%. The lowest value was found in R9P17, while the highest one was obtained in Nz. Germination rate after 6h varied between 15 (R9P17) and 64% (Nz). After 9h, the highest germination percentage (72%) was recorded in R9P19, R10P9 and Nz, whereas the lowest one (20%) was found in R9P17 (Table 2).

Table 2  
Germination rates after 3, 6, and 9 hours (H) of incubation for *Actinidia* spp.

Specie	Genotype	Germinations rates (%) after		
		3h	6h	9h
<i>Actinidia deliciosa</i>	R1P4	21	52	68
<i>Actinidia deliciosa</i>	R1P7	12	23	33
<i>Actinidia deliciosa</i>	R1P8	12	20	30
<i>Actinidia deliciosa</i>	R1P17	20	45	59
<i>Actinidia deliciosa</i>	Nz	28	64	72
<i>Actinidia arguta</i>	R8P5	17	34	44
<i>Actinidia arguta</i>	R9P17	5	15	20
<i>Actinidia arguta</i>	R9P19	25	60	72
<i>Actinidia arguta</i>	R10P9	22	59	72

A positive effect of the addition of boric acid and sucrose concentrations between 10 and 20 % was also mentioned for apple (Nunes et al. 2001) and for pear where studying

the germination of pear pollen grains, Chagas et al. (2010) also observed that the presence of boron in the culture medium was essential.

### CONCLUSIONS

The results in this study indicate the presence of boron in the culture medium stimulated pollen germination. Boron has multiple effects, as discussed by Herrera-Rodríguez et al. (2010), and its presence in the culture medium induces the formation of a sugar complex, and improving pollen germination, promoting their growth (Herrera-Rodríguez et al. 2010).

In this study the high pollen germination rate *in vitro* suggests that also stored pollen can result in effective viability. Though, more studies on effective fruiting using preserved pollen grains still need to be approached to kiwi. Results of the present study strongly supported this approach.

The five kiwi genotypes (R1P4, R1P17, R9P19, R10P9 and Nz) appear to have sufficient pollen germination and can be used on kiwi female pollination and breeding programs; however, this needs to be tested by *in vivo* pollinations for yield.

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## CHEMICAL COMPOSITION AND SENSORY CHARACTERISTICS OF RAW DISTILLED FROM PLUMS

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**Keywords:** *distilled, components, area, time,*

### ABSTRACT

*Summary of our research on exploitation through , chemical composition and sensory characteristics of raw distilled from plums such as Agen, Gras Romanesc, Stanley and Tuleu dulce, grown in Samburesti-Dobroteasa showed that fruits of these varieties is a staple good quality distilled. Raw distillates with the most complex composition are obtained from the fruit varieties Agen and the Gras Romanesc, because they have a high potential for accumulation of sugars. Made aging in oak barrels, these spirits enrich their composition, especially if we refer to esters and aldehydes, impressing by the flavor they have. Our observations also noticed that we have a raw material (marc) of good quality, but, if the alcoholic fermentation and distillation is faulty, distillates obtained ceases to be quality.*

### INTRODUCTION

Among the tree species, plum occupy large areas in Romania, with a long tradition in the cultivation of this species. Prunes have a high food value. In their chemical constitution, there are useful substances for human consumption (nutrition). They contain per 100 g fresh substance, water (73-86%), dry matter (26-33%), sugar (9-16%), organic acids (0, 39-2%), protein (0, 22 to 1.07%), pectin (0.35 to 0.95%), tanoide (0.6 - 0.25%), ascorbic acid (0.20 - 14%) and have energy value of between 44 - 89 per 100 g of fresh.

Plums contain a number of vitamins: provitamin A ( $\beta$ caroten) 0.05 -0.60 mg / 100 g, ascorbic acid (vitamin C) 1 to 23 mg / 100 g, Vitamin B1 20 - 200 y / 100 g, vitamin B2 20 - 90 y / 100 g, Biotin 0.6 y / 100 g, vitamin PP 0.2 to 0.6 y / 100 g Pantothenic acid 0.12 to 0.24 mg / 100 g. Plums contain 100 g ash: Cl (0.0015 mg), I (0.007 mg), P (22.8 mg), Na (2.2 mg), K (167 mg), Ca (13, 3 mg) , Mg (13 mg), Fe (0.14 mg), Mn (0, 14 mg).

As a result of this composition they are widely used for fresh consumption, semi and industrialized. All these have been highlighted by numerous studies: Popa A, 1985, 2002, Radulescu Paula 2010; Pomohaci N. and al., 2009, 1999; Wittkowski, R. and al., 2000; Tourliere, SD, 1978, Curbelo, Garcia, AS, 2006, Bonepas, C., Gauthier, P., 2008.

Yet more than 40 - 45% of production for obtaining distillates, which are known as brandy, plum brandy, horinca, etc. These distilled, by their chemical composition and olfactogustative characteristics are superior to those obtained from cereals. Measurements

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and observations made by us to the plum distillates from Samburesti – Dobroteasa, Olt County, attests that.

### **MATERIAL AND METHODS**

Marc distillates were obtained from plum varieties belonging: Agen, Gras Romanesc, Stanley and Tuleu dulce, grown in the area Samburesti - Dobroteasa, Olt County. The chemical composition of raw, or shortly distillates aged in oak barrels (220 l), was determined by laboratory analysis using the methods recommended by the OIV and those in standards in Romania. Organoleptic assessment was made using compensation system points from 1 to 20. At plum marc was a double distillation.

### **RESULTS AND DISCUSSIONS**

Results plum distillate is appreciated when raw (immediately after distillation) as one of the finest spirits. Substances contained in raw distillates of different origins plums. A part from fruits, mostly formed during alcoholic fermentation of the pulp of plum, while others are formed during distillation. Besides alcohol, water extract and raw ash plum distillate containing volatile substances such as acids (particularly acetic acid), aldehydes (especially acetic aldehyde), furfural, esters (mostly in ethyl acetate) and alcohols, butyl and amyl alcohols predominating. The quality of raw plum distillate is determined by the extent to which these substances enter the composition, as a result, they serve to characterize them. The proportion of these components depends on the plums quality - raw material, mode of fermentation and distillation mode, climatic offer made to areas where fruits grow. The alcoholic strength of raw distillate of plums, belonging to different varieties, depends on the potential for accumulation of sugars in fruits and how fermentation and distillation of marc is made; it oscillates between 47 vol% and 55 vol% alcohol. Smaller alcoholic concentrations are found in raw distillates made of marcs of Stanley variety.

Total acidity values are between 164 and 185 mg (acetic acid) to 100 mg anhydrous alcohol. Aldehydes are found in proportions of 40 to 48 mg per 100 ml anhydrous alcohol. Plum distillate esters were determined from values of 35 to 114 mg (ethyl acetate) in 100 ml anhydrous alcohol.

Furfural, although falling within aldehydes, during the analysis of distilled spirits is metered separately. It's thought, in moderate proportions, to contribute to a more complex, pleasant flavor. When there is excessive amounts of it, it has got a negative influence.

Raw distillation of plum, belonging to different varieties contain very little furfural (0.11 to 2.06 mg per 100 ml anhydrous alcohol). Somewhat higher amounts (2.06 ml / 100 ml anhydrous alcohol) were found in the distillate derived from Tuleu dulce variety plums. Higher alcohols are found in quantities ranging from 264 to 304 ml (isobutyl alcohol) in 100 ml anhydrous alcohol. The extract has values ranging between 340 and 604 mg / l.

Ashes, due to content in the extract, have got great variation limits, from 103 to 290 mg / l. Non-alcoholic coefficient, representing the sum of content in acids, aldehydes, esters, furfural and higher alcohols; the sum esters plus higher alcohols and the report higher alcohols / esters give us information about the qualitative value of raw materials, the manner in which alcoholic fermentation was conducted, when and how distillation took place, but help us, also, diagnose the qualitative value gained by aging raw plum distillate. Sensory qualities of raw distilled of superior quality from plums, should come to the organoleptic assessment as clear liquids, colorless or colored to dark yellow with characteristic taste and pleasant, alcoholic smell.

Sometimes we meet situations when, although raw plum distillates are clear and colorless, they have a bitter, metallic taste, very bad, and, if they are available in oak, they acquire a blue- black color as ink, repellent. This shows that distillates contain large proportions of iron and sometimes copper. Excessive content of heavy metals explains the emergence of bitter, unpleasant taste, and the blue - black color, by forming ferric combed in contact with oak wood. The two heavy metals (iron and copper) can normally be found in raw distillates as traces, but excessive enrichment of iron and copper of the distillate comes on one hand from the distillation installations, on the other, from the borhots in which sulfur is found in large quantities and during the process of distillation corrodes the installations.

Copper, in amounts of 2.5 to 3 mg / l, which pass sanitary laws, does not affect sensitive the taste into bad taste, the color or degree of clarity of plum distillate, on the contrary, is a catalyst for the oxidation - reduction processes after which distillates enrich sensitively their olfacto-gustative qualities.

The presence of copper is put, in this situation, in relation to the degree of purity of the copper processing or how it is made the distillation unit, knowing that metals are corroded more so, more impure are the conditions.

Unsatisfying olfacto-gustative qualities are registered also when the plum distillates come from borhots smelling of mold, hint of lag or beginning of deterioration. The taste and smell of smoked betrays inadequate leadership of the distillation process. Table 2 summarizes the composition and organoleptic characteristics of plum distillate after 6 months (180 days) of aging in oak barrels with a capacity of 220 liters.

Since about the process of aging we will be back with another work, in this we summarize a few general issues. It can easily detach that received good marks from the judges for flavors tasting were rich coating agreeable, coming from plums from which distillate was produced, and the wealth of aldehydes and esters gained during the operation of distillation and then during aging.

Distillates from the variety Agen plums were most appreciated. Immediately after were ranked distillates from Gras Romanesc plum varieties and Tuleu dulce. They impressed by the accuracy and smoothness of flavor, the lack of aggressiveness of alcohol, the ideal balance between alcohol, acidity and extract, but especially by their vocation to aging. It is a testimony to the fact that when the raw material is first quality, the fermentation process was well managed and the distillation and aging process is properly controlled by the technologist, the distillates obtained are of real quality.

## CONCLUSIONS

The chemical composition of raw plum distillate and their sensory attributes are complex and different, being dependent of the culture area of the trees, the plum variety, the time of harvest, the process of alcoholic fermentation and distillation.

Agen plum varieties, Gras Romanesc, Tuleu dulce and Stanley can be the raw material for spirits.

Distillates with a balanced and harmonious composition and that - best enjoyed organoleptic evaluation are those from fruit varieties Agen and Gras Romanesc, followed closely by those from Tuleu dulce fruit variety.

Plum crude distillates are suitable for aging in small oak capacity vessels (220 l), enriching - and enhancing composition and sensory characteristics.

Table 1  
Medium variations and limits of oscillation of the main components of raw distillates Plum (2010 - 2011)

Number of samples analyzed	Variety	In mg per 100 ml of anhydrous										mg / L	
		vol% alcoholic strength at 15 °C	Total acidity (acetic acid)	Esters (ethyl acetate)	Furfural	Higher alcohols (alcohol utility)	Soft coefficient	Sum + esters and superior alcohols	Higher alcohol esters	Aldehydes (acetaldehyde)	Extract	Ash	
6	Agen	52 – 55	17 – 56	35 – 36	0.11-0.21	66-190	133.11-302.21	71-297	1.88-5.27	15-20	12-210	9-45	
6	Gras Romanesc	51 – 53	16 – 47	38 – 41	0.12-0.28	64-150	138.12-265.28	102-191	1.68-3.65	20-27	14-106	8-40	
6	Stanley	46 – 48	18 – 49	37 – 39	0.14-0.34	61-104	146.14-226.34	98-143	1.64-2.66	30-34	12-104	7-56	
6	Tuleu dulce	50 – 54	15 – 50	39 – 46	0.13-0.31	63-130	136.13-252.31	102-176	1.61-2.82	19-26	13-104	7-47	

Table 2  
Composition and organoleptic characteristics of plum distillate after 6 months of aging in oak barrels with a capacity of 220 liters

Variety	° Alcoholic title vol % at 15 C	pH	In mg / L distilled			In mg at 100 ml anhydrous alcohol							Esters + superior alcohols	superior alcohols / esters	Organoleptic assessment notes 1-20
			Extract	Ash	Total	Total acidity (acetic acid)	Aldehyde (acet aldehyd)	Esters (etyl acetate)	Furzuroi	Higher alcohols (alc. izobuty)	Non-alcoholic coefficient				
Agen plum distilled	53	3.80	98	8	1.36	106.30	38.40	253	0.4	343	741.10	596	1.35	19.96	
Gras Romanesc plum distilled	52	3.60	96	10	1.84	122.10	39.40	296	0.5	359	817.00	655	1.21	19.62	
Stanley plum distilled	47	3.56	90	7.5	1.25	66.40	33.19	194	0.9	205	499.49	399	1.05	18.20	
Tuleu dulce plum distilled	52	3.75	95	8	1.31	89.20	37.52	232	0.7	287	646.42	512	1.23	19.52	

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## EVALUATION OF THE INFLUENCE OF CLIMATIC FACTORS ON FRUIT QUALITY OF PEACH IN THE CONDITIONS OF SANDY SOILS

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*Keywords: sandy soils, peach, quality, climate change*

### ABSTRACT

*Horticultural plants are very good conditions for growth and maturation on sandy soils. It can speak of a real basin horticultural in this area due to earliness that you print the climatic conditions. Fruit quality is influenced by climatic conditions manifested during experimentation. Peach tree is considered the most studied species, fruit growing on sandy soils, with an impressive number of varieties differing in maturation period, biochemical composition, form and color of the fruit. Peaches are sources of carbohydrates, water, C vitamin etc.*

*Quality indexes and climatic factors studied were significant correlations determined that highlight their influence on the achievement of production, qualitatively superior.*

*With average temperature increase in the region, varieties, tardive, Jerseyland, and Redhaven have begun to mature fruit, with almost 12 days earlier. The trend of reduction of the vegetation period takes place after a polynomial regression equation, with an significant correlation factor ( $r = .65 *$ ) to the variety Jerseyland, and after linear regression equation, with an very significant correlation factor ( $r = 0.81 **$ ) variety, Redhaven. The best quality results were obtained under the conditions of 2006, to amount of rainfall of 330mm and 3175 C0 the amount degrees of temperature during fruit growth and maturation.*

### INTRODUCTION

On sandy soils along time were cultivated many plant species, with an impressive number of varieties.

The main objective of the research has been establishment culture technology in order to achieve the economically efficient production. Alongside achieving the quantitative determination of the quality of agricultural products was another objective of the research. Horticultural plants are very good conditions for growth and maturation on sandy soils. It can speak of a real basin horticultural in this area due to earliness that you print the climatic conditions. Whether the technological factors can be largely controlled, climatic factors are highly variable and can influence positively or negatively the quality of production.

During the same species in different components biochemical contents differ depending on the variety and conditions agropedoclimatic (Mihaela Croitoru 2000, 2001, Durău Anica& Croitoru Mihaela 2007, Durău Anica et al. 2009, Todd W. 2009, Garcia-

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Montiel F. 2010, Fachinello J.C. et al. 2012, Croitoru Mihaela et al., 2014). Vitamin C content of fruit varies very widely depending the species, variety and the conditions agropedoclimatic (Gherghe A., et al., 1983). In recent years, speaks very much of change global climate, desertification and aridity in many regions (due to increased temperature and lack of rainfall) as is the area, sandy soils in southern Oltenia. Climate change affects both growing and developing plants and achieving production quantity and quality. The results on peach fruit quality sandy soils obtained emphasizes the role of climatic conditions in obtaining high-quality harvest.

### **MATERIAL AND METHODS**

Starting from these premises were studied various peach varieties in order to determine the quality of production under the impact of climatic factors.

Varieties studied: Cora, Delta, Romamer, Springold, Cardinal, Jerseyland, Redhaven, Collins, Florin.

Were made the following observations and measurements:

- total dry matter and water (%) - gravimetric method;
- soluble dry matter (%) - refractometry method;
- carbohydrate (%) - Soxleth Reagent method;
- titratable acidity (malic acid g/100g f.s.) - titrimetric method;
- C vitamin (mg/100 g f.s.)- iodometric method;
- recording climatic elements of CCDCPN Dăbuleni weather station.

### **RESULTS AND DISCUSSIONS**

Peach tree is considered the most studied species, fruit growing on sandy soils, with an impressive number of varieties differing in maturation period, biochemical composition, form and color of the fruit. Peaches are sources of carbohydrates, water, C vitamin.

Results obtained concerning to the quality of the fruit in 2011-2012 are shown in Table 1. Peach fruit has an average content of total dry matter of 12.44%, with values of 13.30% for the variety Delta and 14, 10% Cora variety. Carbohydrates are an essential part of the peach fruit, content ranging from 7.43% Jerseyland variety and 8.98% Romamer variety, with an average of 8.47% varieties and literature indicates an average of 10.50%. Peach fruit has a balanced acidity 0.54g malic acid / 100g fresh substances and have an average content of soluble dray matter of 10.80% being very juicy and sweet to maturity consumption.

C vitamin content was between 7.10mg for the Springold variety and 11.88mg Collins variety, with an average of 9.57 mg.

The varieties Cardinal and Redhaven Jerseyland remain a standard of quality and are considered as varieties witness in the assortment recommended for sandy soils. Analyzing the fruit period maturation over a period of 25 years it can be seen the influence of climate change on fruit maturation data. Cardinal variety is early variety that fruits and matures in late June, early July. All this time maturation period remains constant correlation factor is insignificant.

With average temperature increase in the area tardive varieties, and Redhaven, Jerseyland have begun to mature fruit about 12 days earlier. The trend of reduction of the vegetation period takes place after a polynomial regression equation, with an significant



Table 1

The influence of variety on the biochemical composition of fruits of peach  
(2011-2013)

Variety	Water (%)	Total dray matter (%)	Soluble dray matter (%)	Titratable acidity (g acid malic /100g f.s.*)	Carbo-hydrate (%)	C vitamin (mg/100g f.s.)
Cora	85.90	14.10	10.35	0.45	8.62	9.46
Delta	86.70	13.30	11.6	0.51	8.73	9.90
Romamer	88.80	11.20	9.8	0.68	8.98	8.16
Springold	89.03	10.97	10.4	0.43	8.77	7.10
Cardinal	87.05	12.95	11.8	0.58	8.75	10.56
Jerseyland	88.62	11.38	8.55	0.44	7.43	9.90
Redhaven	87.70	12.30	11.9	0.59	8.73	8.58
Collins	87.15	12.85	11.11	0.63	8.68	11.88
Florin	87.11	12.89	11.65	0.53	7.50	10.56
Average varieties	87.56	12.44	10.80	0.54	8.47	9.57
Values from literature	82-91	9-18	8.8-15.5	0.65	10.50	5-18

correlation factor ( $r = 0.65 *$ ) after a variety Jerseyland linear regression equation with a factor highly significant correlation ( $r = 0.81 **$ ) variety Redhaven, (Figure 1).

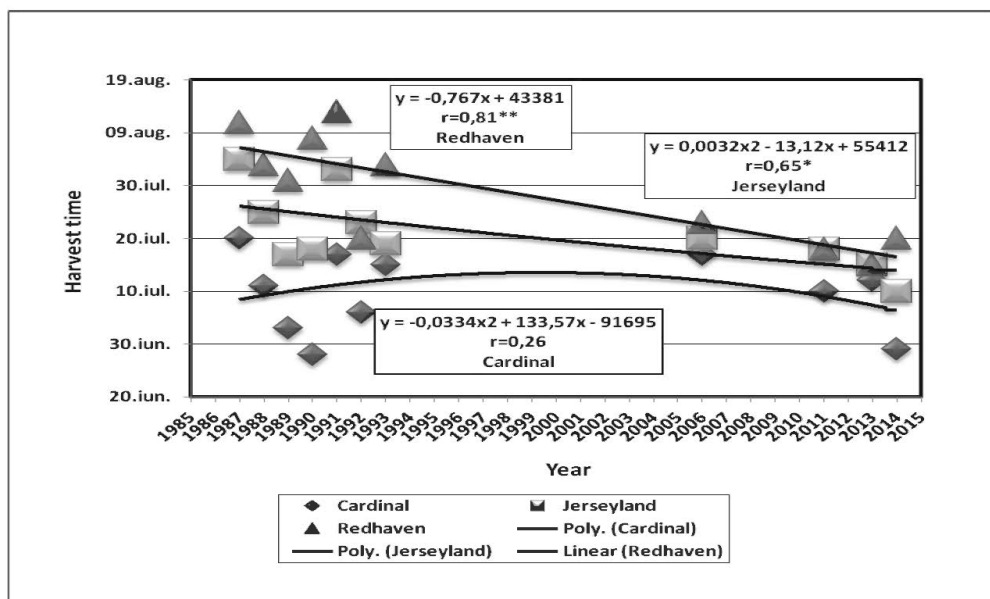


Figure 1. Change in date of harvest during 1985-2014, some peach varieties on sandy soils

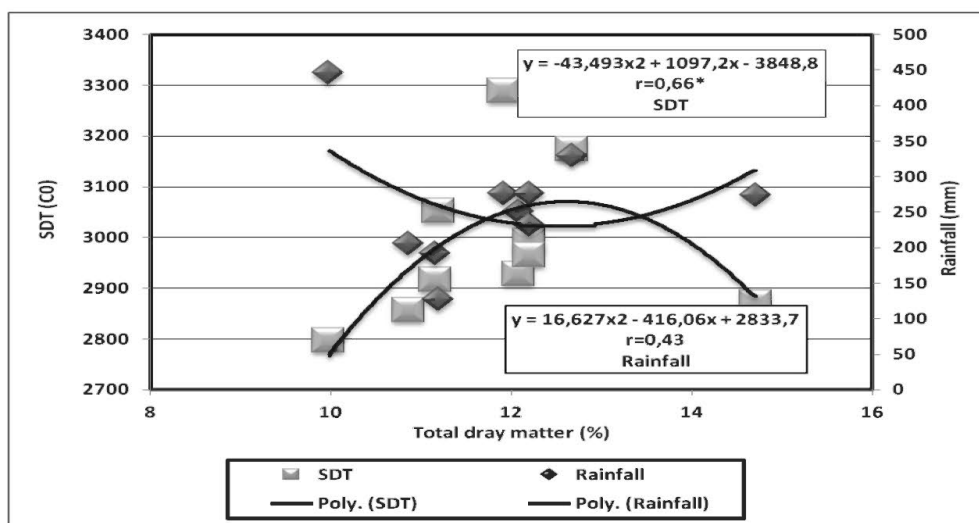


Figure 2. Influence of years of study on total dry matter accumulation in peach fruit

By shortening the period of vegetation, to these varieties is amended, the rate of accumulation of the various biochemical components of fruits. The total dry matter

accumulation Cardinal variety is given by a linear regression equation with a correlation factor significantly ( $r = 0.69 *$ ) and cultivars Redhaven Jerseyland and is observed a trend of declining dry matter content total after polynomial regression equations with correlation factors insignificant (Figure 2).

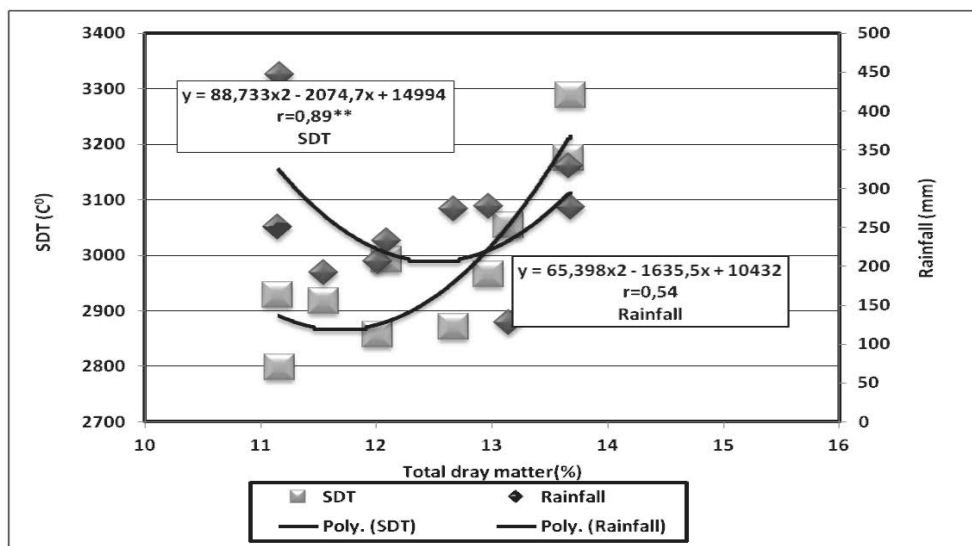


Figure 3. Influence of climatic conditions on total dry matter accumulation in fruit of peach (1989-2013)

Quantity of rainfall and the sum of degrees the temperature of the vegetation also influences dry matter accumulation in peach fruits. The best quality results were obtained under the conditions of 2006, when there were recorded a rainfall of 330 mm and 3175 °C sum of degrees of temperature (SDT), Figure 3.

### CONCLUSIONS

Fruit quality is influenced by climatic conditions manifested during experimentation.

Between the quality indicators studied and climatic factors were determined significant correlations, which highlights their determining influence in achieving higher production quality.

The best quality results were obtained under the conditions of 2006, when there were recorded a rainfall of 330 mm and 3175 °C sum of degrees of temperature (SDT).

In the action of climatic factors in the sandy soils by judicious choice of variety within the species and the application of advanced cultivation technologies, peach yields obtained are competitive in the market compared to the yields obtained in other parts of the country.

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## THE CHARACTERIZATION OF SOUTHERN OLTENIA SANDY SOILS IN THE CONTEXT OF CLIMATE CHANGE AND USE IN CULTURE FRUIT GROWING PLANTS

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**Keywords:** climatic factors, environmental conditions , effects of climate change on soil.

### ABSTRACT

*This paper aims to analyze the characteristics of sandy soils in southern Oltenia and their influence on fruit plants in the context of climate change.*

*The better awareness on sands and sandy soils has been a major concern, both worldwide and in our country. Growing on sandy land had as a main goal the need to increase agricultural production.*

*These sands and sandy soils are also known as sands and sandy soils in southern Oltenia.*

*On the sandy soils in southern Oltenia, stone fruit species give the best results. Peach has exhibited valence as the most valuable species. In this respect it should be given due attention to maintaining and even increasing crop areas by applying appropriate technologies and, of course, the continuous improvement of assortment to increase fruit quantity and quality of them.*

### INTRODUCTION

Psamo-pelitic soil come from the Protisoils class, (from the Greek psalmos sand), these soils are defined by an A horizon (Ao, Au, I) formed on parent material consisted of sandy deposits, refreshed by wind, with at least in the first 50 cm of coarse texture (clay <6%) or medium coarse (clay: 6 ÷ 12%).

In the Romanian System of Soil Taxonomy SRTS - 2003, Psamo-pelitic soil are classified as Protisoils, class name and definition remaining identical to the previous classification (SRCS 1980).

Psamo-pelitic soil includes the following subtypes: distric, eutric, calcaric, molic, umbric, gleic, sodic. Choice assortment of tree species with high suitability of psamo-pelitic ecopedological conditions is essential in obtaining high yields, safe and stable, to a better use of these soils.

In terms of climate, the sandy soil, left of the river Jiu, is framed, by Köppen, in the CFX climate province, with a strong continental characteristic with slight Mediterranean influence, manifested by a pronounced dry, from July to September and a normal precipitation quantity in May and June. The average annual temperature is 11.1 °C.

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By classifying counties in Romania by the size and the potential effects of climate change based on Downing and Patwardhan (2003), Szöcs Emse (2011) carried out the classification according to their vulnerability and scored Dolj, Olt, Buzau, Mehedinți, Teleorman, Giurgiu, Tulcea, Călărași, Ialomița, Brăila, Vrancea, in highly vulnerable areas.

In order to efficiently capitalize sandy soils, which occupy large areas in southern Romania, concerns on the field, including objectives, studying the behavior of certain species of fruit trees with a high potential for adaptability in terms of ecopedological poorly fertile sandy soils in order to obtain competitive quality fruit on the market (Hoza D. et al., 1998; Baciu A., 2005).

Existing climatic conditions and agrophytotechnical measures, specific for the agricultural system, practiced on sandy soils, led to permanent and long-term measures, to prevent and combat this natural process of degradation of sandy soils cultivated with fruit plants (Fidegelli C., Rigo G., 1995).

### MATERIAL AND METHODS

The preparation of the irrigation system Sadova - Corabia, about 36,000 ha of sandy soils were leveled or shaped. In these areas, the former dune fields were taken by flat fields, wavy fields or have been even leveled. Small bumps that remain after these works do not exceed 1.2 m and the transition from positive to negative forms is done on long distances with slopes.

Through the modeling - leveling work, the prerequisites for achieving uniformity natural fertility sandy soils were created.

Sandy soils have been and are a source of increasing the agricultural area of the country. Physical and chemical properties of sandy soils have suffered major changes after arranging them and the edaphic factor should be treated with more caution. Research conducted on sandy soils, in their natural state, revealed the origin of sandy material and size and mineralogical composition of its chemical and physical properties, chemical analysis of ground water (Ion Petre, 1988).

These works aimed to achieve the previously set parameters, with the aim of capitalizing sandy land. For this purpose, it could not be taken into account the preservation and conservation of soil fertility status. Thus, in many cases, existing humus soil horizon, initially above the soil surface, mixed with soil mass or was buried at depths between 0,3-1,5m. Soils not affected by work-leveling modeling, so unchanged anthropogenic, are found entirely local.

Pedological, a typical psamosol, characteristic also to sands in their natural state, is characterized by an Ao horizon, 25-30 cm thick, very light in color due to low organic matter content. It is followed by a transitional horizon A / C (20-25 cm) and then a C horizon, whose thickness can exceed 130 cm (Gheorghe D. et al., 2002; Gheorghe D., 2005 - Table 6).

The study was undertaken at SCCCNP Dăbuleni and in terms of climate, the southern extremity of the area with sandy soils, left of Jiu, is affected by drought and heat, the frequency of dry years, in terms of agriculture, exceeding 40%. (Gheorghe D., 2007).

In the sandy soils of southern Oltenia, the average P of yearly rainfall is 540 mm, temperature 11,2°C and potential evapo-transpiration ETP of 700-755 mm. These values lead to a moisture index De Martonne of  $I = 25-26$ , the P-ETP aridity indices of 160 ... -210 mm and 100P / ETP of 72-75%. Index value within the sandy soils of the Sadova - Corabia arrangement include the zone in the semiarid and excessively dry climate type. Environmental conditions offered by sandy soils in southern Oltenia grant earliness in the ripening of fruit, with 7-10 days ahead of other areas. The opportunity to exploit the weak fertile sandy soils, unsuitable for certain crops, the need for fruit supply for the area and early production are several factors that call for the development of fruit growing on these soils (A. Baciu et al., 1997).

The research was conducted by analyzing the influence of climatic factors on the soil in Dăbuleni area and prevention by protective measures for the proper development of fruit plants.

### RESULTS AND DISCUSSIONS

Unlocking the agro-ecological potential of sandy soils in southern Oltenia by growing fruit trees is currently done differently, depending on the nature of soils, their fertility and assortment of fruit trees species, highly adaptable to the ecopedological conditions, the mechanization degree, the degree of physico-chemical improvement and agroproductive attributes and last but not least, the greater financial effort that investments are made and productions obtained.

In terms of lithology, sandy soils in southern Oltenia are arranged in terraces of different widths, composed of loess and loess material deposited alternatively, with layers of sand and gravel. Under these deposits appears a Levantine older material, generally made of clay or marl clays rich in CaCO<sub>3</sub>. Size composition is different on dunes and interdunes. Maxim I. 1966 shows that sand dune material is cohesive and non-cohesive, having a physical clay content between 5.8 to 8.4% and over 90% coarse sand and fine sand. The interdune physical clay content reaches 12.1%, or 15.8%, and the percentage of coarse sand and fine sand drops to 80-75%.

Existing water resources are insufficient for optimal plant growth and fruit development, drought being predominant during the growing season. The results obtained under non-irrigation research shows that getting production from fruit plants grown on sandy soils is random, which is dependent on the amount of rainfall and its distribution in time, and production increases due to irrigation are substantial (Table 1).

On sandy soils, drought periods may occur at shorter intervals of time than other types of soil. The rather limited available moisture on these terrains determine the essential characteristic of applying splashing - small watering rules, applied at short intervals.

Table 1  
Climate characterization of sandy soils in southern Oltenia area during the growing season (1985-2002)

Month	Climatic indices					
	12 p / (t+10) (De Martonne)		100 p / ETP % (Donciu)		( p/t)10 (Seleaninov)	
	Value	Grade	Value	Grade	Value	Grade
IV	24,8	moderately dry	79,5	dry	1,5	sufficient
V	26,1	moderately dry	55,8	very dry	1,1	sufficient
VI	22,0	semiarid	42,6	excessive dry	0,9	insufficient
VII	20,2	semiarid	31,4	excessive dry	0,8	insufficient
VIII	11,1	arid	23,2	excessive dry	0,5	uncertain agriculture
IX	13,1	arid	50,6	very dry	0,7	uncertain agriculture
Average (1985-2002)	19,6	semiarid	47,2	excessive dry	0,9	insufficient

Research has revealed high levels of evapotranspiration due to high temperatures, dry winds and low atmospheric humidity during the active period of growing plants.

Another factor with climatic implications in the culture system practiced in a particular area is the wind, whose action on sandy soils gives rise to the phenomenon called wind erosion or deflation.

From about 46 560 ha of sandy soils showing textures in terms of exposure to deflation we have the following situation:

- 28440 ha with a 'very high' - 'high' exposure grade - sands and psamosoils;
- 7350 ha with a 'moderate' - 'low' exposure grade - cambic chernozems;
- 2230 ha with 'low' exposure grade - brown clay - iluvial;
- 8540 ha with an 'absent' exposure grade - chernozem, chernozem cambic and preluvisols

Sandy soils (the most exposed to deflation) predominate in the central-west and south-west, this being the territory SCDCPN Dăbuleni marked on the map, near the towns Dăbuleni and Calarasi.

The intensifying of the phenomenon of deflation after 1990 is the main cause of the emergence of new forms of ownership of agricultural land, leading to the abusive cutting of protective forest, deforestation of large tracts of forest, vineyards and orchards and farming of the land without proper technology measures to prevent and combat this phenomenon.

The damage brought to farming by this phenomenon, specific for sandy soils, can be summarized as follows: uprooting of plants, plant trauma by drifted sand covering fruit crops, plant photosynthetic capacity reduced due to the dust deposited on leaves, humus soil degradation, in nutrients, fine soil particles (dust and clay) and the fertile soil covering by sand.

Research done Croitoru M. (2002) emphasizes the close link that exists between wind speed and intensity of deflation. Sandy soils from the hydrological planning Sadova - Corabia are permanently subjected to wind erosion, mostly during the spring - summer months: March, April, May, June and July.

In general, it is considered that particles from a sandy soil, uncohesive and completely dry at the surface, are moved by wind speeds of 3.5-4.5 m / s at ground level, which corresponds to their weather vane station of 5-6 m / s.

Table 2

Intensity classes of deflation depending on wind speed

Crt. nr.	Wind speed		Intensity class	Effects on soil and plant
	(m/s)	Characteristic		
1	5-6	weak	insignificant	weak
2	7-8	moderate	significant - moderate	moderate
3	9-10	high	strong	important
4	11	very high	very strong	strong

Preventing and combating deflation and wind erosion constitutes a particularly important measure in strengthening and retaining sandy soils.



The location agroforestry belts perpendicular to the prevailing wind is considered as a first step with permanent and long-term characteristic to prevent and combat this natural process of degradation of sandy soils.

Research carried out for this purpose showed that wind-protected area behind the agroforestry curtain is about 20 times the height of the curtain.

As a first step, with a permanent and long term characteristic, to prevent and combat this natural process of sandy soil degradation, on areas occupied by fruit plant, rye strips are recommended, and along with the antideflational effect they constitute a source of organic matter by incorporating in the ground, in the rye phase bellows.

Tree species with good results on sandy soils in southern Oltenia and that have contributed greatly to reducing the phenomenon of deflation and sand fixation phones were peach, plum, apricot, cherry and apple.

### CONCLUSIONS

Soil is the main means of production in agriculture.

Sandy soils have been and are a source of increasing agricultural area of the country.

Better sands and sandy soils have been a major concern, for the world and our country.

On the sandy soils in southern Oltenia, stone species give the best results. Peach has exhibited valence as the most valuable species.

It is necessary to pay due attention to maintain and even increase crop areas by applying appropriate technologies and of course the continuous improvement of assortment to increase fruit quantity and quality.

In the wild stone species, peach, apricot and almond have exhibited valence as the most valuable species.

Given the fact that climatic elements created on sandy soils of Dăbuleni are similar and even higher than the main growing areas of peach, apricot and almond in the country, it can be appreciated that the area through which prints early fruit ripening is a favorable area of culture for peach, apricot and almond.

If we consider the requirements of apricot, peach and almond to the environment, taking into account climate data easily influenced or undirected, we can say that these tree species succeed very well in the sandy soils.

Through the earliness that climatic conditions imprint on species grown on sands, price recovery fruit is high in appearance, being considered early vegetables.

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## EFFICIENCY OF INVESTMENT SOLUTIONS FOR TABLE GRAPES PRODUCTION OF VERY EARLY AND EARLY RIPENING VARIETIES

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*Keywords: table grapes, production, investments, efficiency*

### ABSTRACT

*Development of table grapes production in Bulgaria - a sector with tradition and innovative potential had marked a negative tendency for two decades. The severe drop of cultivated vineyards - from 8999 ha in 2001 to 2248 ha in 2012 and the inadequate level of the average yields resulted in reduced level of satisfaction of the market demands with local production. Overcoming the negative trend requires increased investment activity which under the conditions of significant need of capital and high risk imposes the clarification of investment choices. The present study makes an assessment of the effectiveness of different investment solutions in table grapes production. The values of the indicators net present value, profitability index and payback period are compared on the basis of developed theoretical models of agricultural systems for table grapes production of very early and early ripening varieties.*

### INTRODUCTION

The dynamic development of table grapes growing in Bulgaria from the early 1920-ies to the late 1960-ies defined the position of our country as a leading producer and exporter of table grapes in Europe (Cholakov, 1989, Marinov et al., 1999, Dimitrova et al., 2007). In 1968 the area of table grapes reached 53 500 ha, representing 26.4% of the total area planted with vines. Average for the period 1961-65, the exports of table grapes amounted to 184 thousand tons as only for 1965 it was 260 thousand tons, which ranked Bulgaria first in exported quantity among the main countries-exporters worldwide.

The increased migration of labor force from rural to urban areas in the early 1970-ies led to negative trends in the sector development, which continued in the coming decades. The processes of abandonment of vineyards speeded up, resulting that the area of cultivated table grapes decreased to 17 900 ha in 1985 and 13 670 ha in 1996 (Marinov et al., 1999).

The complex impact of a number of factors, including unstable macroeconomic environment, the lack of a state policy with clearly defined priorities in the agricultural sector, the negative demographic trends and the low purchasing power of the population, deepened the problems of table grapes viticulture. The total harvested area of vineyards

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with table grapes varieties continued to decline, reaching 8999 ha in 2001 and only 2248 ha in 2012 (4% of the total harvested vineyards in the country). The production of table grapes dropped to 12 934 tons on the average for the period 2007-2012, covering only 71.6% of the consumed quantity in the country. The limited production volume, under the impact of the strong reduction of the vineyards and low level of productivity per unit of area (5011 kg ha<sup>-1</sup>), not corresponding to the productive potential of the cultivated table grape varieties reflected on the consumption of table grapes, which decreased from 34.1 kg per capita in 1965 to 2.3 kg in 2012. To overcome the negative trend of development and to increase the food supply of the population and developing the potential, which table grapes growing had for creating employment (Dimitrova et al., 2007, Borisov and Radev, 2011, Borisov and Radev, 2012, MAF, 2013) required improvement of the investment climate in the sector as well as upgrading the technological level of production. Renovation of varietal structure in accordance with the consumers' demands, ensuring the stability of the entrepreneurial income determined the framework of the investment choices.

The objective of this study was to assess the effectiveness of different investment alternatives in the production of table grapes from very early and early ripening varieties.

### MATERIALS AND METHODS

The efficiency of the investment solutions was evaluated based on developed models for farms producing table grapes of red and white seed varieties classified by the period of reaching ripeness for consumption of the fruit in the following way (Roychev, 2012):

- very early ripening table grape varieties (August, 03 – August, 15) – Super ran Bolgar, Armira, Misket Rusenski, Nadezhda, Cardinal;
- early ripening table grape varieties (August, 13 – August, 29) – Maritsa, Dunav, Brestovitsa, Velika, Victoria, Diana.

The production process was modeled with 10 ha area of cultivation and privately owned equipment. The selected technological variants specific for table grapes production in the northern and southern parts of the country included the following parameters: ground improved Guillot training system with planting distance 2.20 x 1.30 m (density 3,500 vines ha<sup>-1</sup>) and semi-high modified Mozer training system with stem height h 0.80 cm and planting distance 2.50 x 1.30 m (3080 vines ha<sup>-1</sup>). The calculations were performed by MS Excel, divided into three interrelated modules: creation and cultivation of the vine plantation until reaching fruit-bearing stage; growing fruit-bearing vineyard and a detailed budget of the cash flows.

The influence of the soil and climatic conditions of the region on the quantity and quality of yield was modeled by determining the percentage of the quantity of product quality by class [Extra Class, Class I, Class II and discard (Ordinance No. 16)] from the total value of yield according to the type of training. The relative weights were as follows: For the variant of ground training system - 35%, 45%, 15% and 5% while for the semi-high training variant - 50%, 40%, 5% and 5%.

The realization prices used for evaluation of the produce were based on the official weekly information of the State Commission on Commodity Exchanges and Wholesale Markets for 2010-2013 in the following order:

- for the very early ripening varieties 0.99 EUR kg<sup>-1</sup> for Extra Class, 0.83 EUR kg<sup>-1</sup> for Class I, 0.66 EUR kg<sup>-1</sup> for Class II and 0.26 EUR kg<sup>-1</sup> for non-standard production;
- for the early ripening varieties: Maritsa, Diana and Dunav – 0.93 EUR kg<sup>-1</sup> for Extra Class, 0.78 EUR kg<sup>-1</sup> for Class I, 0.62 EUR kg<sup>-1</sup> for Class II, for Victoria and Velika varieties –0.86 EUR kg<sup>-1</sup> for Extra Class, 0.72 EUR kg<sup>-1</sup> for Class I, 0.57 EUR kg<sup>-1</sup> for

Class II, for Brestovitsa variety –0.82 EUR kg<sup>-1</sup> for Extra Class, 0.68 EUR kg<sup>-1</sup> for Class I, 0.54 EUR kg<sup>-1</sup> for Class II and 0.26 EUR kg<sup>-1</sup> for the non-standard production from all varieties. The greater differentiation in the price level of this group of varieties was the result of the wholesale prices dynamics depending on the growth in the quantity of the supplied production on the market.

The comparative economic analysis of the efficiency of investment solutions was carried out using the method of net present value (NPV) and the indicators profitability index with discounting (PI) and payback period of investment (PBP).

The net present value of the evaluated variants was defined by the following formula:

$$NPV = \sum NCF_n \times DF_{tn} - I, \text{ where}$$

**NCF** – net cash flows for n<sup>th</sup> year;

**DF<sub>tn</sub>** = 1/(1+r)<sup>n</sup>, where,

**r** – discount rate;

**n** – number of time intervals, years in this case.

**I** – initial amount of the investment costs.

The discount factor was 6.57% established as an arithmetic average between the annual interest rate on long-term loans, announced by the Bulgarian National Bank and the annual average inflation rate set by the National Statistical Institute.

The returns per unit of invested capital during the period of the project was found by the profitability index by the formula:

$$PI = \sum NCF_{nd} / I_m, \text{ where:}$$

**NCF<sub>nd</sub>** – discounted net cash flows;

**I<sub>m</sub>** – the updated total value of the investment.

The payback period was derived by comparing the accumulated amount of updated annual net cash flows with the updated value of investments:

$$PBP = \sum NCF_{nd} = I_m.$$

## RESULTS AND DISCUSSIONS

The varietal composition of table grapes in Bulgaria, with leading share in its structure, of Bolgar variety (43%), had determined the mass supply of table grapes in September and October, realized under the intense pressure of imports from neighboring countries - Turkey and Greece and the competition of the offered seasonal fruit- substitutes. These factors, not only have an impact in the reduction of the productions prices level but they put the realization of the economic interests of the grape growers highly dependent on the climatic conditions of the year and the established market situation. The ways for risk reduction and realization of stable and attractive profitability by farmers included the need for technological modernization of production and the improvement of the structure of varieties, mainly in the direction of extending the period of supply of the produce beyond the season of the mass production. Due to the typical moderate-continental climate for the country, the opportunities for large scale cultivation of late-ripening table grape varieties (September, 16 - October, 28) were limited (Nikov et al., 1990). As a result of researches for many years in the field of vine selection, the country had a wide range of early ripening varieties of superior appearance and excellent organoleptic qualities. At this stage of the sector development more widely distributed and known to the consumer were the table varieties Super ran Bolgar, Cardinal and Brestovitsa, as the area of the first two varieties at the end of 1995 took respectively 5.6% and 2.3% of the total area of table grapes in the country (Abracheva et al., 1997).

The obtained results from the comparative analysis on the efficiency of investment alternatives with ground training system are presented in Table. 1. In the group of the very early ripening varieties Nadezhda variety stood out with the highest values of the indicators net present value and profitability index as well as the shortest payback period. As a result of the high level of the theoretical average yield calculated on the basis of the potential fertility of the variety and the high realization price, the obtained increase in the difference of the total amount of discounted net cash flows and the value of the initial investment compared to the other varieties in this group ranged from 30.2% to 229.2% and concerning the investment profitability - from 25.4% to 150.0%.

Table 1

Efficiency of investment solutions for table grapes production  
– ground training system

Variety	Indicators					
	Average yield, kg ha <sup>-1</sup>	Investment costs, EUR	Operational costs, EUR	NPV, EUR	PI, EUR	PBP, years
<b>I. Very early ripening</b>						
Super ran Bolgar	13986	177166.65	39710.32	527100.00	2.19	8.0
Armira	17483	177166.65	42132.94	755373.01	2.91	6.10
Misket Rusenski	15732	177166.65	40921.63	641236.50	2.56	7.3
Nadezhda	20976	177166.65	44555.56	983646.03	3.65	6.1
Cardinal	10488	177166.65	37287.70	298826.97	1.46	9.8
<b>II. Early ripening</b>						
Maritsa	17480	177166.65	42132.94	679486.26	2.68	7.3
Diana	17480	177166.65	42132.94	679486.26	2.68	7.3
Dunav	16082	177166.65	41163.89	594166.89	2.41	8.1
Victoria	17480	177166.65	42132.94	588976.52	2.39	7.8
Velika	20976	177166.65	44555.56	784426.27	3.01	6.10
Brestovitsa	15732	177166.65	40921.63	443865.74	1.93	8.6

*Source: own calculations*

The lower efficiency of the invested capital in the production of table grapes of Cardinal variety was mainly due to the determined in an expert way level of the average yield, which was by 33.4% to 100.0% less than the potential of the other varieties.

In the group of early ripening varieties the best indicators of the investment choice had Velika variety for which the sum of the net present value exceeded the level of the indicator calculated for the other variants from 15.4% to 76.7%, and the increase in the amount of revenue per unit of invested funds ranged from 1.29 to 2.38 EUR.

The indicators for assessing the effectiveness of investment choices had the lowest values for the traditionally grown in our country Brestovitsa variety under the simultaneous effect of the smaller quantity of the calculated theoretical average yield and the lower level of the possible realization price, determined by the fact that the grapes of this variety in most areas of the country matured in the last week of August, when the mass supply of table grapes had begun.

The estimated profitability and return on investment alternatives based on semi-high training system, presented in Table 2, put at the top positions again both varieties Nadezhda (from the group of very early ripening) and Velika (from the early ripening

varieties), as regardless the different price conditions, the parameters of the investment choices in both variants were significantly closer. The net present value of the investment in the production of table grapes of Velika variety exceeded that of Nadezhda variety with 285 250.10 EUR, due to the higher level of the theoretical average yield - with 3693 kg ha<sup>-1</sup>. The profitability of the investment was higher for Nadezhda variety (by 1.4%) as a result of the higher operating costs (5.9%) for Velika variety that followed the growth rate of productivity per unit area.

Table 2

Efficiency of investment solutions for table grapes production  
– semi-high training system

Variety	Indicators					
	Average yield, kg ha <sup>-1</sup>	Investment costs, EUR	Operational costs, EUR	NPV, EUR	PI, EUR	PBP, years
<b>I. Very early ripening</b>						
Super ran Bolgar	15385	178843.55	37193.42	661502.37	2.62	8.11
Armira	17539	178843.55	38816.20	806227.40	3.08	7.7
Misket Rusenski	16924	178843.55	38258.34	759929.95	2.93	7.6
Nadezhda	24000	178843.55	43157.05	1212688.69	4.37	6.8
Cardinal	12308	178843.55	35063.55	464647.22	1.99	8.6
<b>II. Early ripening</b>						
Maritsa	19077	178843.55	39749.25	851399.65	3.22	7.3
Diana	18462	178843.55	39323.28	779173.14	2.99	7.7
Dunav	18462	178843.55	39323.28	779173.14	2.99	7.7
Victoria	21539	178843.55	41453.15	854004.01	3.23	7.4
Velika	27693	178843.55	45712.87	1497938.79	4.31	6.8
Brestovitsa	17847	178843.55	38894.54	599112.85	2.42	8.10

Source: own calculations

The comparison of the values of the analyzed parameters - net present value, profitability index and payback period obtained for both studied technological variants determined the higher level of efficiency of investments in semi-high training systems. Although the evaluation of the indicator investment costs in this training system exceeded the level, found for the ground training systems due to the longer period of no fruit-bearing (4 years, compared to 3 years for the ground training system), the saving of resources during the utilization period of the plantation as a result of the elimination of labor-intensive activities for hilling up and down and the smaller density of vines per hectare in addition to the increased level of productivity per vine resulted in the higher profitability and shorter payback period in this type of training compared to the ground one.

The values of the profitability index in the very early ripening varieties grown on stem formation exceeded by 0.17 EUR to 0.72 EUR this indicator compared to the ground training system. In the group of the early ripening varieties that increase was within the range from 0.31 EUR to 1.30 EUR.

## CONCLUSIONS

The results of the comparative economic analysis on the effectiveness of different investment solutions in the production of table grapes from very early ripening and early ripening table grape varieties showed good profitability in all studied variants, which demonstrated the innovative potential of the sector.

The renovation of the varietal structure as a strategic measure for increasing the competitiveness of production required improving of the investment climate, stabilizing and expanding the production of vine propagation material from the newly-selected table grape varieties, organizational and economic restructuring and development of integration links in the supply chain.

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## THE EFFECT OF ANTIOXIDANT SUBSTANCES AND HUMIC ACIDS ON THE SEEDS GERMINATION OF SOME VEGETABLE SPECIES

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**Keywords:** seeds, antioxidants, humic acids

### ABSTRACT

*The Solanum lycopersicum L. seeds, Rio Grande variety, Cucumis sativus, Kybria F1 hybrid and Cucurbita pepo, Opal F1 hybrid were germinated in water and in solution of grape seeds extract and humic acids.*

*This study aimed to observe the action mode of these compounds (with polyphenols and with humic acids) on the process of germination, root system development and growth of seedlings.*

*In the observations there was found that the extract from the seeds of Vitis vinifera has greatly influenced the percentage of germinated seeds, and the seeds treated with humic acids recorded also higher values than the control plant. The root system of plants resulting from the variants with polyphenols and humic acids had a better development compared with the control plant. The variant with humic acids determined seedlings that had a balanced growth in terms of root system and the average weight of seedlings.*

### INTRODUCTION

The seeds germination is a process through which they initiate a new cycle of ontogenetic development when favourable environmental conditions are provided.

The seed germinated when a new plant emerged in terms of agronomic aspect, but also in terms of physiological aspect, it is considered that the germination process is completed when the radicle has penetrated the cracks of the soil tegument.

After Mazliak (1982) the seed removal from rest is achieved in favourable environmental conditions (temperature, humidity, light) that determine the activity of genes involved in the biosynthesis of DNA or RNA which encode the enzymes that catalyze the reactions for this process: the biosynthesis of the stimulating hormones and of enzymes.

The practical aspect of the germination process occurs after the seed was put in contact with the soil or water that forms the soil solution and it takes place the seed imbibition. During this process, the phenolic substances in the tegument diffuse into the soil solution, thus there is eliminated one of the factors that induce the seminal rest.

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After several days of imbibitions, the embryo radicle penetrates outwards by micropyle or pericarp ruptures marking the end of the germination and the early plant growth process.

Regarding the seed germination of different vegetable plant species, there have been conducted many studies that aimed at presenting different aspects related to this complete and complex process.

For the solanaceous vegetable species there may be mentioned the following aspects as a result of the research activity:

- the seeds of the grown varieties and hybrids of tomatoes do not have any vegetative rest unlike those of the wild forms that have a low rest, caused by the presence of abscisic acid conferring the adaptation to survival of wild species in unfavourable environmental conditions (Weyers 1985);
- the pepper seeds have a rest period of 20-25 days which can be eliminated by delaying with 10 days their extraction from fruits or it can be shortened by their exposure to 24°C temperature for 2-3 weeks (Randle and Hanma 1981);
- the seeds of pepper and eggplant have a similar chemical structure with a high fat content 20-28%, 15-18% proteins, 15-19% cellulose, about 3% of soluble carbohydrates with no starch, in contrast to that of tomatoes which have a high content of starch 14-15%; thus the germination process is more rapid at tomatoes;
- for the solanaceous vegetable plant seed germination, the temperature and the amount of degrees/day have a great importance.

## MATERIAL AND METHODS

This study was conducted in the Laboratory of Horticulture of the Faculty of Agriculture and Horticulture, Craiova, Romania, in 2012 using seeds of tomatoes, cucumbers and marrow that were introduced in the Jacobsen germinator in three variants. The variant was represented by the germination environment, water, polyphenols from the seeds of *Vitis vinifera* and humic acids. Each variant had four repetitions with 50 seeds for each repetition. The seeds were placed in Petri pots containing 5 ml of solution each in a concentration of 0.5%, and then they were placed on the germinator at a constant temperature of 25°C.

## RESULTS AND DISCUSSIONS

The *Vitis vinifera* seeds extract caused an insignificant percentage of germination between the species of the Cucurbitaceae family (cucumber and marrow) but a significant one, after 4 days of germination between the tomato and the other two species (51% for tomatoes and 20-21% for cucumbers and marrow).

It was noted that in the first 4 days after placing the seeds of tomato on the germinator, they germinated at a higher percentage than the other two species, after which in the next few days these percentages tend to become equal.

The humic acid absorbed by the tomato seeds determined a germination rate ranging between 45% after 4 days, 70% after 8 days and 85% after 14 days (table 1).

The lowest value is recorded by the control plant and the higher values are recorded by the variant with humic acids and with grape seed extract.

The percentage of germinated seeds at cucumber was 10% after 4 days, 70% after 8 days and 75% after 14 days at the variant with humic acids. The highest values were also recorded at the variant with extract of the *Vitis vinifera* seeds.

The marrow recorded values ranging between 10% and 78% at the variant 3 according to the number of days after which the observations were made. The best results were also recorded at the variant with *Vitis vinifera* seed extract.

Table 1

The percentage of seeds of tomato, cucumber and marrow germinated in water, extract from the *Vitis vinifera* seeds and humic acids

Variant/Variant characteristics	Germination percentage (%)			
	No. of days	Tomatoes	Cucumber	Marrows
V1- Water	4	20	15	16
	8	64	66	67
	14	85	85	84
V2- Extract from the <i>Vitis vinifera</i> seeds	4	51	20	21
	8	86	75	73
	14	70	94	96
V3- Humic acids solution	4	45	10	10
	8	70	70	71
	14	85	75	78

There were no differences between species. The significant difference was after 4 days between the tomatoes and the other two species of the Cucurbitaceae family for the variant 2 and 3.

The absorption of humic substances at the seeds has a positive role on the process of germination. As the humic substances enter the seed cells, the intensity of respiration increases and the cell division is accelerated. The same respiratory processes determine the formation of the root meristem.

Smidova (1962) observed that the extract of humic acid increased the rate of germination of wheat seeds. Dixit and Kishore (1967) found an increased rate of germination in barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and wheat (*Triticum* L. aestivum). Ishwaran and Chonker (1971) observed that the soybean seeds have germinated in greater percentage at the variant treated with humic acids. Piccolo A. et al. (1993) observed no increase in the percentage of germinated seeds of lettuce or tomato treated with humic acids in Petri pots obtained from oxidized lignite. There was no evidence that the humic substances increased the viability of the two species seeds.

In this study, the measurements of the growth elements of the tomato seedlings have been carried out after two weeks. The plant height, the length of the root system and the average weight of tomato plants was measured (table 2 and 3).

The studied vegetable species recorded higher values for the height of seedlings at the two experimental variants compared to the control variant (table 2).

The best results are observed at V3, being significant for cucumbers and marrows (5.48 cm for cucumber and 5.20 cm for marrow).

In terms of root system length the tomatoes recorded higher values than the other two species. Higher values are observed at the V3 (humic acids).

The roots had no big length compared to the control plant, but had a significant number of secondary roots (data not presented) fact that is observed in the fresh weight of the roots (table 3).

Table 2

The determinations on seedlings from the germinator at 15 days after wetting  
(absolute values)

Var.	Plants height (cm)			The root system lenght (cm)		
	Tomatoe	Cucumbers	Marrows	Tomatoe	Cucumbers	Marrows
V1	4.54	4.08	4.51	5.52	5.50	5.61
V2	4.84	4.20	4.82	5.90	5.64	5.78
V3	4.92	5.48	5.20	6.86	5.82	5.99

It ranged from 20 mg to 37 mg at tomatoes and from 260-300 mg to 335-400 mg at cucumbers and marrow.

The plants treated with humic acids have a stronger development than those treated with polyphenols because the humic acids molecule comprises a chinoid group of 4 simple bonds and 4 double bonds. In fact this part of the molecule is a cloud of valence electrons placed on the defined energy level. When receiving an amount of solar energy these electrons move to a higher level of energy. This repeated action helps to accumulate solar energy; the humic acids contain specific groups of peptides and carbohydrates.

The peptide group structure is very similar to the lipid structure which surrounds the walls of the cell. As a result, it can easily interact with the cell membrane, forming a protective net around it. During its growth and development, a cell is always exposed to stress (nutrient, hydric, heat stress) but it is protected by the humic acid.

Table 3

The fresh weight (mg) of roots of tomato, cucumber and marrow germinated in water,  
extract from *Vitis vinifera* seeds and humic acids

Variant/Variant characteristics	The fresh weight (mg) of roots:		
	Tomatoes	Cucumbers	Marrows
V1- Water	20	260	300
V2- Extract from the <i>Vitis vinifera</i> seeds	32	275	355
V3- Humic acids solution	37	335	400

The application of humic acid and polyphenols of *Vitis vinifera* seeds at the seeds germination increased the number of germinated seeds in comparison with the control plant (water) and determined a better development of the root system of the plants. These results are similar to those of David et al. (1994), Sanders et al. (1990) and Malik and Azam (1985) who found an increase in the weight of roots after the application of humic acid by various methods.

The growth of the roots number and length was observed at various cultures grown on sand or on nutrient solution and which were treated with humic acid and fulvic acids from coal. The observations were made on species such as: beans (*Phaseolus vulgaris* L.) Schnitzer and Piapst, 1967; cucumber (*Cucumis sativus* L.) Rauthan and Schnitzer, 1981;

pepper (*Capsicum annum L.*) Sanchez-Conde and Ortega, 1968; tomatoes (*Lycopersicon esculenta L.*) Sladky, 1959; Lineham, 1976; Adani et al. 1998; cucumbers (*Cucumis sativus L.*) and marrows (*Cucurbita pepo*) Hartwigsen and Evans, 2000.

The secondary roots length increased significantly according to the findings of Malik and Azam 1985 and Mylonas and McCants 1980 who reported an increase of 500% in the wheat root length and in the secondary root length of tobacco. Vaughan 1974 suggested that a higher growth of the secondary root results from the formation of HA complexes of iron in the plant tissues that prevent the cease of the root growth.

The number of secondary roots was not affected by the application of humic acids. This contrasts with the increasing number of secondary roots observed by O Donnell 1973 in the cuttings of geranium and by Mylonas and McCants 1980 in tobacco. This discrepancy may be due to the natural differences in the growth patterns of the tested species or due to the application method.

### CONCLUSIONS

The plants treated with polyphenols absorbed during the germination and growth period an amount of total polyphenols (10 mg %) which have a beneficial role on the grown plants (data not presented). The antioxidant role of polyphenols contributes to the removal of oxidative stress that occurs during the germination period and in the first days of plant growth.

The application of polyphenols and humic acids as a treatment in order to stimulate the seeds germination had an effect of increasing the height of the seedlings, the increase in length of the root system and its fresh weight in the three species, especially for the cucumbers and marrows.

Further studies will be conducted with observations in the cultures in order to determine if certain morphological, physiological or biochemical changes are noted for the three species.

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## RESEARCH ON THE INFLUENCE OF TREATMENT WITH SPRAYGARD OF QUALITY SEEDLINGS

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**Keywords:** *adjuvant, transplanting, survival, transpiration*

### ABSTRACT

*Achieving quality seedlings, as prerequisite for subsequent favorable production results is a major objective.*

*Spraygard adjuvant is formulated in a single coating based on a synthetic resin polymer di-1-p-menthene and alcohol ethoxylated with many uses in the agricultural and horticultural practice. By application to the plant and leaves it forms a coating of persisting from 2 days to 2 weeks. The presence of this film has the side effect of reducing transpiration and, consequently, a better water management in the plant.*

*The results presented in this paper showed that the survival rate of 100% for transplanting tomatoes and pepper seedlings, combined with favorable growth and further development may advise treatment with Spraygard 1% to transplanted seedlings.*

### INTRODUCTION

Obtaining the quality seedlings represents a major objective as a premise for further positive production results favorable to each vegetables producer. Therefore, it has been shown to be necessary the use of different bioactive treatments in order to improve the gripping rate at transplanters seedlings, an improvement in the seedlings' metabolism in incipient phases of growth and development, which may lead to a reduction in obtaining the seedlings, as well as diminishing the costs of production.

In the course of time, in our country, the bioactive treatments showed good results in accelerating or inhibiting the growth of seedlings. Thus, Chilom Pelagia (1976) has successfully used such substances in order to obtain greenhouse tomato seedlings and in 1994, Ruxandra Ciofu (by Ciofu Ruxandra, 1994) and her fellow workers have tested the stimulation of seedlings with substances of vegetable origin which were environmentally inoffensive (Moldstim, Pavstim, Ecostim), obtaining this way, very good results for cucumbers, tomatoes, peppers and eggplants.

Being known the fact that the horticol system is based on the highly intensive technologies of culture, not seldom, they threaten the integrity and food security, and the current concepts of integrated horticulture as well as the biological fertilizers and adjuvants require reconsideration of the organic horticultural practices which are used to reduce the environmental pollution, nowadays the use of bioactive substances "environmentally friendly" representing a priority for producers.

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## MATERIAL AND METHODS

During the 11<sup>th</sup> of March to the 25<sup>th</sup> of April 2013, within the sector of seedlings production at the Faculty of Horticulture - Bucharest there were set up important experiences whose main objective was the testing of the product Spraygard, manufactured by Nufarm - Australia in different concentrations on three vegetable species in order to recommend it to different seedling producers from Romania.

Spraygard adjuvant has a unique formula in a single coating based on the synthetic resin that is "environmentally friendly" and the polymer di-1-p-menthene and ethoxylated alcohol by applying it on the plant and on its leaves forms a pellicle that persists 2 days up to 2 weeks, having as a side effect the reduction of perspiration and, therefore, a better water management within the plant.

This fact causes the physiological chain reactions whose results are being expressed by increasing the plant resistance to stress factors such as the drought and the cold. The effect of reducing perspiration recommends to apply the product strictly in the leaves, when one plants the vegetables seedlings in a concentration of 2% in order to increase the fixing rate (\*\*\*) Manufacturer's data sheet).

The biological material used consisted in three vegetable species which are different as ecology and physiology such as cabbage, tomatoes and peppers.

Experience has involved the installation of three experimental devices, mono-factorial mounted following the linear alignment method of blocks with four repetitions, as follows:

Experience 1 considered the application when transplanting on cabbage seedlings (Gloria F1), a treatment with Spraygard in a concentration of 1%.

Experience 2 considered the application when transplanting on the seedlings of tomato (Missouri) a treatment with Spraygard in a concentration of 1%.

Experience 3 took into account the application at the time of transplanting on the seedlings of peppers (Galben superior), a treatment with Spraygard in a concentration of 1%.

Each experience resulted in the following: V0 - untreated seedlings; V1 - seedlings treated with 1% concentration.

The total number of plants in the experiment was 80, for each experience being 40 plants, each variant being awarded with a total of five plants per repetition. In order to produce the necessary seedlings for the foundation of the experiences there were made frequent crops in boxes, in a hot greenhouse, in the 9<sup>th</sup> of March. Optimal conditions and seeds with high germinal energy, lead to a faster emergence, massively, for all the three species, after 6 days in cabbage and tomato (the 15<sup>th</sup> of March) and after 8 days for peppers (the 17<sup>th</sup> of March).

The seedlings transplanting was made in Jiffy pots of 150 cmc., in a fertile substrate specialized on Kekkila type at 8-10 days after the mass emergence (the 25<sup>th</sup> of March). During the vegetation period there was applied a specific agro-technique for seedling production: daily ventilation, watering, weeds weeding. Maintenance works were applied uniformly to all the studied variants.

During the experiments there were made certain observations and measurements on plant growth and development at two different times: at one week after transplanting (the 2<sup>nd</sup> of March) and at the end of the experience, when the seedlings have reached the optimum for best planted (the 25<sup>th</sup> of April).

Observations and direct measurements: the rate of grip transplantation; the number of true leaves which were formed; length and the vegetative mass air; total mass plantings; root mass and volume. The data obtained were analyzed and calculated as an average of the measurements taken on three plants per repetition.





The results obtained at the end of the experiment (one month after transplanting and applying the treatment) showed that the application of Spraygard positively influences the growth and contributes to the balanced development of seedlings, regardless of species. Thus, it is noted that the growth increase has a percentage of 21.01% for peppers, 19.12% for cabbage and 17.04% for tomato (Table 2 and Figure 2).

Table 2

**The growth and development of seedlings of cabbage, tomatoes and peppers a month after transplanting**

Species	Variant	Plant height (cm)	Crown diameter (cm)	Number of formed leaves	Internodes length (cm)
Cabbage	V <sub>0</sub>	18.3	0.55	6.5	2.81
	V <sub>1</sub>	21.8	0.78	8.7	2.50
Tomato	V <sub>0</sub>	22.3	0.58	5.5	4.05
	V <sub>1</sub>	26.1	0.65	7	3.73
Pepper	V <sub>0</sub>	13.8	0.36	5.2	2.65
	V <sub>1</sub>	16.07	0.48	6.7	2.40

The diameter for bale was superior for the treated variants whatever the species are. Along with the number of leaves, this parameter can be considered an indicator of development as it implies the existence of accumulations as a permanent deposit. The achieved growth has a value of 41.8% for cabbage, 33.3% to 12.7% for V<sub>1</sub> peppers and tomatoes. The number of formed leaves followed the same trend, being higher in the variants treated with Spraygard compared with the not treated variants, with 2.2 cabbage leaves, 1.5 tomato leaves and 1.5 for V<sub>1</sub> peppers leaves.



Figure 2 Seedlings of pepper, cabbage and tomatoes at the end of the experiment

The accumulation performed by plants quantified through the mass aerial plant, root mass, root total weight and volume represent indirect indicators that allow the evaluation of physiological and metabolic status of the plant (Table 3).

Table 3

**Root mass and volume of seed sprouts, tomatoes and peppers the time of planting**

Species	Variant	Total Mass (g)	Mass aerial part (g)	Root Mass (g)	Rot volume (cm <sup>3</sup> )
Cabbage	V <sub>0</sub>	9.4	8.2	1.2	0.8
	V <sub>1</sub>	11.2	9.8	1.4	1.2
Tomato	V <sub>0</sub>	7.7	6.8	0.9	2.5
	V <sub>1</sub>	8.5	7.3	1.2	2.7
Pepper	V <sub>0</sub>	7.1	6.4	0.7	0.8
	V <sub>1</sub>	8.4	7.2	1.2	1.2

Globally, one may consider that these results are clearly indicating the superiority of the plants treated with Spraygard accumulation, regardless of the species. For the cabbage seedlings, these accumulations realized by the plants in the V<sub>1</sub> in comparison with those in the V<sub>0</sub> are of 1.8 g in the total mass, 1.6 g for the weight of the aerial part and the roots mass of 0.2 g. For the tomato seedlings, the accumulations are of 0.8 g for the total mass, 0.5 g for the mass of aerial part and 0.3 g for root mass. For pepper, the plants in V<sub>1</sub> have achieved mass accumulation of 1.3 g for the total mass, 0.8 g for the mass of aerial part and 0.5 g for the root mass. The root volume recorded an increase concerning the variants treated with Spraygard. These were as follows: 0.4 cm<sup>3</sup> for peppers and cabbage and 0.2 cm<sup>3</sup> for tomatoes. The total chlorophyll content of leaves recorded an increase in V<sub>1</sub> for all the tested species, being observed as well in the accumulations of dry substance (Table 4). These results support the mass accumulations realised by plants under Spraygard treatment, but not reaching a direct connection. This is due to the fact that there are other factors which influence the plant's growth and development from the experience.

Table 4

The physiological plant indicators at the end of the experiment

Species	Variant	Total chlorophyll (mg/100 g mv)	Dry substance %
Cabbage	V <sub>0</sub>	62.98	15.13
	V <sub>1</sub>	78.76	16.02
Tomato	V <sub>0</sub>	66.96	17.24
	V <sub>1</sub>	82.42	19.07
Pepper	V <sub>0</sub>	59.97	12.01
	V <sub>1</sub>	79.78	13.22

### CONCLUSIONS

The results obtained in the first moment of analysis revealed that each species had its own behavior regarding the application or not of Spraygard treatments.

The application of Spraygard treatments in the first time of analysis showed a balanced development at seedlings of cabbage and tomatoes while the results of pepper seedlings highlight the fact that they follow a process of inhibition.

The results obtained at the end of the experiment showed that the application of Spraygard had positively influenced the growth and contributed to the balanced development of seedlings, whatever the species is.

The accumulations realized by plants (aerial part mass, root mass, total mass) and root volume are indicators that allow indirect assessment of physiological and metabolic state of the plant, these results regarding the status of these indicators for seedlings of experiences which clearly indicate the superiority of metabolism for plants treated with Spraygard, whatever the species is.

The total chlorophyll content of leaves and dry substance accumulation record growths for variants treated with Spraygard, whatever the species is.

The results come to support mass accumulation of plants, subject of the treatment with Spraygard.

At the end of the experiment, the age of the seedlings was 40 days for cabbage and tomatoes and 38 days for peppers, these being easily reduced for cabbage (with 4-5 days), tomatoes (with 10 - 15 days), but considerably for peppers (with at least 20 days) in comparison with the data in the scientific literature.

The survival rate of 100% for both tomatoes and peppers as in conjunction with favorable growth and development of plants represent plausible arguments, recommending the use of Spraygard 1 % as a treatment in the seedlings transplant.

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## INFLUENCE OF FERTILIZATION ON GROWTH AND DEVELOPMENT OF TOMATO SEEDLINGS

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*Keywords:* leaf, moment, root, photosynthesis, transpiration

### ABSTRACT

*This paper presents results of research related to foliar fertilization, single or combined, of tomato seedlings with Cropmax 0.05% and Razormin 0.1%.*

*The results showed that Razormin 0.1% fertilization yielded of the seedlings of tomato a good quality with a growing and developing balanced and strong roots. These results are supported by physiological and biochemical processes in plants intensely expressed in this variant.*

### INTRODUCTION

During the last years, amid to the development of integrated horticulture concepts, it was required reconsideration of fertilizers used in horticultural practice.

This fact comes to bring our country vegetable production to European Community directives for reducing environmental pollution as a result of horticultural practices, knowing that horticultural system requires highly intensive culture technologies which, not infrequently, endanger their security and the environment integrity .

For the production of quality seedlings using a wide range of complex foliar fertilizer to ensure a balanced growth and development and therefore high quality as the prerequisite for the success of commercial crops.

With very good results in vegetable grown practices , can use different foliar fertilizers such as product fertilo- stimulator Cropmax, applying at least two treatments especially to solanaceous and cucurbits species (Maria Dinu et al..2008, 2002 Pelagia Chilom et all., 2000).

### MATERIAL AND METHODS

Experience established in 2014 under a private sector for the production of seedlings found in the village of Palanca village Florești - Stoenesti, Giurgiu County, 20 km south-west of Bucharest, had as main objective the testing of simple and combined fertilizers action, Razormin and Cropmax, on tomato seedlings production in purposes of drawing up recommendation for seedling producers in Romania on their use as supportive

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treatment in the growth rate of seedlings, improving metabolism and implications in reducing the cost of seedlings production.

Razormin is an environmentally friendly biostimulatory with rooting effect. The composition is complex and balanced and primarily induces root system development and later development of vegetative part through cell division. We find in his composition free amino acids and polysaccharides, such components favoring nutrient absorption and leading to the further good development of the plant.

Cropmax is a complex nutrient superconcentrates for foliar fertilization, 100% natural, that, according to EU organic product certificate issued by BCS - KO - Germany. It has a complex composition, containing macro and micro elements, amino acids, growth promoters, vitamins and enzymes.

Experience, monofactorial with 4 variants considered the application of simple and combined fertilizers Razormin 0.1% and Cropmax 0.05% in tomato seedlings production (Marissa F1) in two distinct moments: 3 and 5 weeks after emergence (7 March and 17 March). Experimental variants were: V1 - unfertilized seedlings; V2 - Cropmax 0.05% fertilized seedlings (March 7 and March 17); V3 - Razormin 0.1% fertilized seedlings (March 7 and March 17); V4 - Razormin 0.1% and Cropmax 0.05% fertilized seedlings (March 7) (March 17). The experiment was installed after the linear blocs method with 4 repetitions. The total number of plants in the experiment was 240, each variant had a total of 60 plants, 15 plants per repetition.

Sowing was done directly into pallets alveolar (socket  $\varnothing = 8.5$  cm) on February 7. Because heat and water were provided optimal emergence occurred after 10 days on 17februarie. During the growing season applied specific agrotechnics for seedling production: daily ventilation, watering, weeding weeds. Particular attention was paid to keep the temperature at a level of 16 – 17 °C to 28°C day and 14-17 °C at night. To prevent fall of the seedlings on March 20 was applied treatment with CE Bravo 0.2%.

Particular attention was given to quench seedlings since March 20, by lowering the temperature of the space to 12 - 14°C at night and about 18 to 22 °C day and reduced watering. This work is absolutely necessary to accustom seedlings with non fatal variable stress condition they will encounter in the place of cultivation and allow the formation of "stress memory" (Agnieszka Sekar et al., 2012).

During the course experiences were made observations and measurements on plant growth and development at two different times:

- before second fertilization (March 17, after a month of emergence);
- at the end of the experience, when the seedlings have reached the optimum for good planting (March 25).

Through direct observations and measurements were determined:

- Biometric parameters of seedlings: plant height; the number of true leaves; vegetative mass aerial; total mass of plant ; root mass and volume;
- Measurements of the intensity of the main physiological processes (photosynthesis, transpiration, stomatal conductance) at the end of the experiment. We used the system of photosynthesis LC pro +. The data were calculated and analyzed as averages of measurements taken over 8 readings per repetition. The measurements were performed on the middle third of the active leaves of the plant.
- Biochemical determinations in the active leaf taken from the middle third; dosing assimilating pigments, spectrophotometric method modified Schopfer (Schopfer, 1989).

## RESULTS AND DISCUSSIONS

The results of the analysis of the first time - March 17 - (one month after the emergence and and 10 days after the first fertilization) are shown in Table 1. Tomatoes, from this time showed important nuances as a result of the application of fertilizers in the sense that V1 plants recorded biometric parameters and indicators lower than those of fertilized variants.

Plant height showed an amplitude of 3.8 cm, ranged from 13.6 cm to V1 and 17.4 cm to V4. The number of leaves was a significant variation from 4.2 leaves (V1) to 5 leaves (V2). Frequency of leaves is relatively constant leaves (0.3 leaves / cm PA V1 and V2 and 0.29 leaves / cm PA V3), but with 0.27 leaves / cm PA to V4. Context analysis reveals V3 (fertilized with Razormin) as a variant in which seedlings had a balanced growth and development and place in inferior position to other variants, the V1 unfertilized.

The growth and development of the tomato seedlings  
at 10 days after the first fertilization

Table 1

Variants	Plant height -HPA - (cm)	No. of leaves	Frequency of leaves (no./ cm. HPA)
V <sub>1</sub>	13.6	4.2	0.30
V <sub>2</sub>	16.4	5.0	0.30
V <sub>3</sub>	16.6	4.8	0.29
V <sub>4</sub>	17.4	4.8	0.27

To determine the overall effect of the fertilization program applied to the seedlings were made observations and measurements at one week after of the second fertilization, respectively on March 25. The results obtained are shown in Tables 2 and 3, and Figures 1 and 2.

Growth of tomato seedlings after one week to the second fertilization

Table 2

Variants	No. of leaves	Plant height HPA (cm)	Root length HR (cm)	The total length of the plant- HT (cm)	Frequency of leaves (no./ cm. HPA)
V <sub>1</sub>	6.4	34.6	21.2	55.8	0.18
V <sub>2</sub>	6.0	38.6	15.8	54.4	0.15
V <sub>3</sub>	8.4	32.4	18.4	50.8	0.26
V <sub>4</sub>	6.0	37.6	15.6	53.2	0.16

Applied fertilization program determined differences in seedling growth. The number of leaves varied, detaching V3 with 8.4 leaves, the following being V1 with 6.4 leaves. The lowest number of leaves -6, were formed to the V2 and V4 seedlings. Plant height showed large differences, with an amplitude of 6.2 cm, determined by V2 (38.6 cm) and V3 (32.4 cm). Except V3, all other variants fertilized achieved higher growth then V1.

Root length is also very different from 15.6 cm - V1 to 21.2 - V4. On the whole, can be seen that fertilized plants variants realized root lengths smaller than the unfertilized variant.

The total length of the plant show differences, but we can not specify a clear influence Figure 1. The greatest total length of plants was determined at V1 - 55.8 cm, and the lowest V3 - 50.8 cm.

Frequency leaves place V3, with 0.26 leaves formed, on the first place. Low frequency conducted by V2 plants can leads to the conclusion that 0.5% Cropmax fertilization induce elongation of plants.

Regarding the growth results, we can say that the balanced growth realized a V3 plants which, due to a lower height, formed the largest number of leaves, the best frequency of leaves and the best growth of roots. In contrast, plants of V2 had the unbalanced growth though realized a superior heights compared to other variants, recorded values for the remaining indicators are among the lowest.

From Figures 1 and 2 it can be seen that the applied fertilization program tomato seedlings has significant influence ( $R^2 = 0.4859$ ) on the growth of roots but this influence is not transmitted further and the total length of the plant.

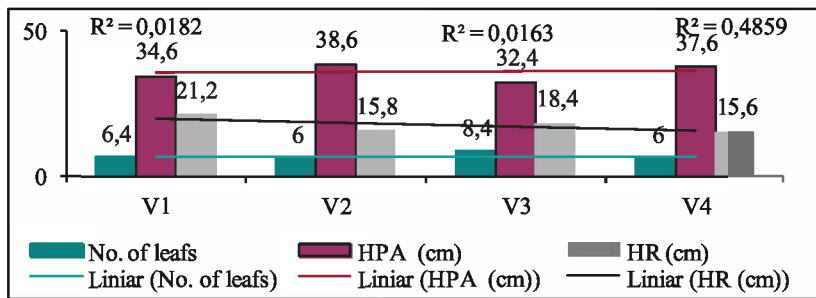


Figure 1 Influence of fertilization program on the growth of tomato seedlings

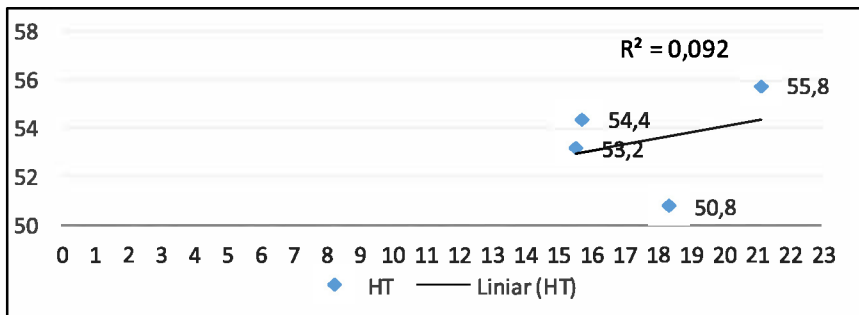


Figure 2 The relationship between root and total length of tomato seedlings

Developing of the seedlings at one week after second fertilization was measured by various indicators of mass, volume and diameter package. The results obtained are shown in Table 3. V3 is noted that all indicators study recorded the highest values. At the opposite pole is placed V4 that, except mass of aerial vegetativ part, all other indicators showed lower values.



Table 3

Development of tomato seedlings after one week to the second fertilization

Variants	mass of aerial part (g)	Mass of root Mrad (g)	Total mass of plant (g)	Volume of root (cm <sup>3</sup> )	Diameter of stem (mm)
V <sub>1</sub>	11.5	7.5	19.0	8.0	6.0
V <sub>2</sub>	12.0	7.0	19.0	8.0	6.0
V <sub>3</sub>	16.0	7.5	23.5	8.5	7.0
V <sub>4</sub>	12.5	6.0	18.5	5.5	6.0

Results regarding the physiology of the seedlings differentially fertilized are shown in Table 4. As can be seen, leaf temperature was relatively constant (25.8 to 26.2 °C) and light intensity was 890-900  $\mu\text{mol}/\text{m}^2/\text{s}$ .

Table 4

Physiology of tomato seedlings at one week after the second fertilization

Var.	Photosynthesis intensity - A [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]	Transpiration intensity - E [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]	Photosynthetic efficiency - A/E	Stomatal conductance - GS [ $\mu\text{mol}/\text{m}^2/\text{s}$ ]
V <sub>1</sub>	12.13	1.60	7.58	0.09
V <sub>2</sub>	13.14	2.72	4.83	0.14
V <sub>3</sub>	13.39	1.74	7.69	0.13
V <sub>4</sub>	12.29	2.67	4.60	0.095

Analysis of physiological indicators recorded of seedlings fertilized differentiated emphasizes again V3. The recorded values supported a balanced physiology (A = 13.39  $\text{mmol}/\text{m}^2/\text{s}$ ; E = 1.74  $\text{mmol}/\text{m}^2/\text{s}$ ; Gs = 0.11; A/E = 7.69). This is supported by the superior and balanced growth and development of seedlings of this variant. A low physiological activity was determined in V1. In this variant expressing intensity values of the physiological processes are minimal, the existence of plants and accumulations made based on a good photosynthetic efficiency (A/E = 7.58).

The biochemistry of plants (Table 5) complete picture of results achieved under physiological aspect.

Table 5

Biochemistry of tomato seedling leaves at one week after the second fertilization

Variants	Assimilating pigments (mg%)		
	Chlorophyll a	Chlorophyll b	Carotenes
V <sub>1</sub>	106.17	39.27	6.17
V <sub>2</sub>	171.66	68.81	6.51
V <sub>3</sub>	190.37	75.16	6.95
V <sub>4</sub>	180.07	68.21	6.09

The tomato leaf biochemistry and plant physiology maintains close morphometry. It highlights again V3 with highest content on assimilating pigments and carotenes (chlorophyll a - 190.37 mg%, chlorophyll b - 75.16 mg% carotenes - 6.95 mg%). In a less

favorable situation is V1 which recorded the lowest content of chlorophyll pigments chlorophyll a - 106 mg%; chlorophyll b 39.27 mg%).

### CONCLUSIONS

Tomato seedlings, from one week to the first fertilization showed important nuances as a result of fertilizers application, in the sense that the unfertilized plants recorded biometric parameters and indicators lower than those of fertilized variants. Context analysis reveals V3 (fertilized with Razormin) as a variant in which, seedlings had the balanced growth.

At the second moment of analysis, applied fertilization program caused major differences between the studied variants concerning the growth and development of plants.

In terms of growth, on the whole, the fertilized variants are superior to most of the studied parameters, except root lengths, consistently exceeds the unfertilized variant.

The most balance growth and development realized plants to the V3 - fertilized with Razormin 0.1% which, due to a lower height, formed the largest number of leaves, best leaf frequency, best root growth and all mass indicators and stem diameter had the highest values of the experimental device.

Physiology and biochemistry analysis of differentially fertilized seedlings, V3 emphasizes again that, for all the parameters studied showed the highest values. This intense physiological activity is supported by upper and balanced growth and development of seedlings of this variant.

Carefully analyzing the results and interpretations made, we estimate that 0.1% Razormin fertilization determined obtaining of the best seedlings by stimulating root system formation.

### ACKNOWLEDGMENT

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## RESEARCH ON THE BEHAVIOR OF LETTUCE CULTIVATION OF PERLITE SUBSTRATE

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**Keywords:** *variety, growing, grains*

### ABSTRACT

*Although worldwide hydroponics industry are strongly, in our country, with lower investment but retaining basic principles can grow hydroponic culture systems through minor adjustments to existing spaces. Multitude of technological options available clearly raises the issue, whichever is most appropriate for a given situation. Therefore, the main objective of the research is to test the behavior of several lettuce cultivars on two grains of perlite (4 and 5 mm), for hydroponic system, on bags placed horizontally and fertigated in open systems.*

*Throughout the experiment remained a tendency of inhibiting the growth of lettuce plants on perlite substrate with grain size of 5 mm. Therefore, regardless of the variety tested, suitable for the culture expansion is the perlite substrate with 4 mm grain.*

### INTRODUCTION

Despite the high costs, hydroponic cultures can also be used in Romanian horticulture because it covers the requirements of the market and it responds to the constraints of the urban life. The concept of hydroponic horticulture is known in our country at a theoretical level since 1956 (Maier I., 1969, Atanasiu N., 2002, Ruxandra Ciofu, 2003).

Even though they are not official, nowadays, in Romania, there are some insignificant growers of vegetables and flowers who applied different technologies of soilless cultures in their small farms of glasshouses and solariums. Perlite and coconut soilless cultures are most seen in Romania and the most cultivated vegetables are the cucumber, the tomato and the pepper.

The many technological options available, clearly raises the issue whichever is most appropriate for a given situation. Therefore, the main objective of the research is to test the behavior of several lettuce cultivars on two grains of perlite (4 and 5 mm), in hydroponic system, on the bags placed horizontally and fertigation in open systems.

### MATERIAL AND METHODS

The experimental device was placed inside a vegetation house to Horticulture Faculty - Veterinary Medicine Bucharest, autumn cycle, the establishment on 24 September and harvest on October 28.

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Experimental cultures were established in perlite bags placed in PVC trays placed suspended on metallic supports with a good slope to drain excess nutrient solution resulted in draining substrate. Collect of drained solution was made in buckets placed on the gutter end.

The experimental monofactorial device, with two experiences, was established on the linear blocks method and comprised 50 bags of perlite, 25 for each experience. For each one, was considered one granulation of the perlite, 4 respectively 5 mmm. The data collected and observations were made on a total of 20 plants / variant.

To experiment were chosen three varieties of lettuce grown in the currently nationally and internationally sortiment: White Boston, Lollo Rossa and Blonde du Cazard. The experimental variants resulted therefore are shown in Table 1.

Table 1

Experimental variants

Experience	Experimental variants	Specification	Perlit granulation (G) ø (mm)
I	V1	White Boston	4
	V2	Lollo Rossa	
	V3	Blonde du Cazard	
II	V1	White Boston	5
	V2	Lollo Rossa	
	V3	Blonde du Cazard	

To establish cultures were used seedlings of lettuce produced in cold greenhouse and picked in a fertile substrate, based on peat, in 80 cm<sup>3</sup> plastic pots.

Perlite bags had 100/25/20 cm and were delivered by a Romanian company, Procema S.A.

In order to ensure an appropriate nutrition level, four plants were planted by bag (Figure 1), so that the distance between plants was 25 cm. For planting, on the top of the bags were made with knife four cross-shaped slits, large enough to allow planting seedlings. With 48 hours before planting, bags were moistened to capacity because, at planting moment, the seedlings need to find a good active substrate humidity (65-75% effective humidity of the substrate).



Figure 1 Aspect to the crop establishment

Applied care works of the culture were the current to the protected culture, respectively, ventilation, monitoring plant health, plant fertigation. Fertigation system was the drip irrigation with pipe placed to the base of each plant (Figure 1). To prepare solution was chosen Universol Green 10/06/23 + 2.7MgO + TE, a fertilizer with high water solubility, high quality and versatility. In the first week after planting, the plants were

fertigated for three times a day, at 10; 13; 16, with a 0.8% solution of Universol Green, 200 ml / plant / day. Since the second week until the harvest, the fertigation was done on the same orar schedule, but Green Universol solution concentration was 1%, 300 ml / plant / day.

In the experimental period have made a series of observations and measurements that allow us to evaluate the behavior of the three varieties of lettuce grown on different substrate perlite grains: at planting - growth of the seedlings of lettuce and at five weeks after planting (36 days) when we began harvesting plants.

Through direct observations and measurements were determined:

- Plant height
- Number of formed leaves
- Mass and diameter of aerial vegetative part of plant (rosette);
- Mass and volume of plants roots
- Total mass of seedlings ;

Foliar analysis involved the determination of unchanged forms in CH<sub>3</sub>COOH extract 2% (1: 20): N-NO<sub>3</sub><sup>-</sup> - spectrophotometrically; P-PO<sub>4</sub><sup>3-</sup> - spectrophotometrically; K<sup>+</sup> - flam photometric and volumetric determination of vitamin C.

Results of the growth of seedlings at planting are shown in Table 2 and Figure 2.

In terms of height, it ranged between 13.9 cm at V1 and 12.3 cm to V2.

The number of leaves had the same type of variation, the largest being the White Boston and the lowest at Lollo Rossa. Good foliar plant frequency of V1 shows a balanced growth, growth that is supported by a volume of 5.4 cm<sup>3</sup> root.

Regarding the seedlings growth, may remark, that until at the earliest stages of vegetation exist a hierarchy of varieties grown. White Boston is proving to be the most vigorous followed by Blonde du Cazard. Variety has a least significant influence on the formation of aerial vegetative part of lettuce seedlings.

Table 2

Growth indicators of lettuce seedlings at planting (24.09.2013)

Variant	Height of the aerial part HPA (cm)	No. of leaves	Frequency of leaves (nr/ cm. HPA)	Root volume (cm <sup>3</sup> )
V1 - White Boston	13,9	5,8	0,4	5,4
V2 - Lollo Rossa	12,3	3,6	0,3	3,8
V3 - Blonde du Cazard	12,5	4,0	0,3	5,0

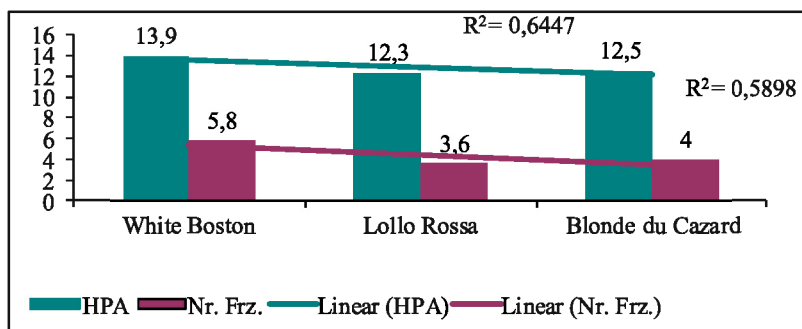


Figure 2 The influence of variety on the growth of lettuce seedlings

The results obtained at the next time of analysis – five weeks after planting were made last measurement of growth as most working variants were reached optimum harvesting phase, White Boston and Blonde du Cazard. The results are shown in Table 3.

To the grain of 4 mm, can be seen V1 which has the highest plants, while V3 (Blonde du Cazard) has the shorter plants (20.6 cm). The perlite grain size of 5 mm is kept the same trend of variation with the first version with highest plants at V1 (22.4 cm) and V3 in last place with 18.8 cm height of the aerial part.

The number of leaves, although it is important determined of the genetic factors, changes within a single kind of variety from a substrate grain to another. The highest number of leaves was recorded at the V1 on 4 mm perlite granulation (14.2 leaves) and the lowest in the V2, at the grain size of 5 mm (9.6 leaves). More generally, it can be seen that the trend is maintained for inhibiting plant growth in grain substrate of 5mm.

Tabelul 3

Growth indicators of of lettuce plants five weeks after planting

Variant	Height of the aerial part HPA (cm)		No. of leaves	
	Φ=4 mm G4	Φ=5 mm G5	Φ=4 mm G4	Φ=5 mm G5
V1 - White Boston	23,8	22,4	14,2	12,4
V2 - Lollo Rossa	22,4	21,4	10,2	9,6
V3 - Blonde du Cazard	20,6	18,8	11,2	9,8

Results on growth and quality of lettuce rosettes are shown in Table 4.

In terms of rosettes mass, the best results were recorded in V1, at the G4. Plants of this variant have made the biggest mass of rosettes (188.4 g) and largest diameter (31.8 cm). In terms of mass variation, the worst is Lollo Rosso with 162.6 g (G4) and 150,4 g at the G5. At this variety, the weaker results were compensated by the larger diameter of rosettes and, considering that this is one of the criteria for assess visual quality of lettuce rosettes (about 30 cm regardless of grain perlite), we conclude that lower mass doesn't become an obstacle for a better use of rosettes. Blonde du Cazard rosettes although it has greater mass, they are more compact and have a smaller diameter and because of this were most poorly placed. At the G5, V3 rosettes were obtained with the smallest diameter from experience (25.6 cm).

Regarding these quality indicators of rosettes can be seen that the grain size of 4mm yielded the most favorable results for all lettuce varieties studied.

Table 4

Indicators of of growth and quality of lettuce rosettes five weeks after planting

Varianta	Mass of rosette (g)		Diameter of rosette (cm)	
	Φ=4 mm G4	Φ=5 mm G5	Φ=4 mm G4	Φ=5 mm G5
V1 - White Boston	188,4	174,2	31,8	28,8
V2 - Lollo Rossa	162,6	150,4	30,2	29,6
V3 - Blonde du Cazard	176,8	166,4	27,6	25,6

Root mass and volume results are relevant because they can explain the differences between both versions of perlite grain size and are presented in Table 5 and Figure 3. The analysis results can be seen that these indicators preserved the trends sketched above. The most favorable results were obtained for all variants on G4 but especially the V1, where the root mass was 48.6 g, and 34.4 cm<sup>3</sup> volume root. Lollo Rossa, regarding the root mass, retains trend of aerial parts mass. The V2 on the G5 substrate recorded the smallest mass of roots, only 30.6 g, and the lowest root volume of 20.4 cm<sup>3</sup>.

From Figure 3 it can be seen that between the mass and volume of the lettuce plant roots are very strong bond,  $R^2 = 0.9312$ .

Table 5

Mass and volume of roots indicators of lettuce plants at five weeks after planting

Variant	Mass roots (g)		Volume roots (cm <sup>3</sup> )	
	Φ=4 mm G4	Φ=5 mm G5	Φ=4 mm G4	Φ=5 mm G5
V1 - White Boston	48,6	44,2	34,4	31,2
V2 - Lollo Rossa	34,8	30,6	23,6	20,4
V3 - Blonde du Cazard	36,8	32,4	26,6	25,4

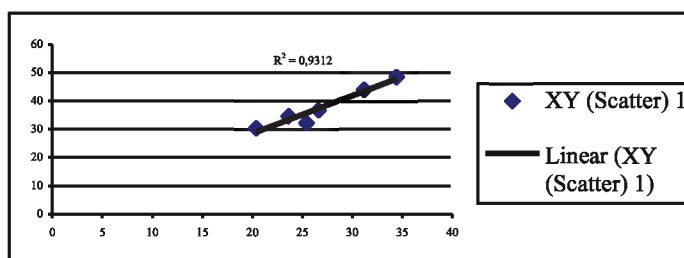


Figure 3 Relationship between mass and volume roots of lettuce plant

Foliar analysis (table 6) showed that, in terms of food security, studied variants lettuce plants obtained, have the N-NO<sub>3</sub> content well below the permissible limits. The nitrate content of the LMA does not exceed 2,000 ppm (in the field); 3000 ppm (in the greenhouse) Order No. 293/640/2001 regarding Safety and quality fresh fruits and vegetables for human consumption. Phosphorus and potassium content is favorable, especially for growing variants V1 and V2 on the G4, and the results are good for the V3 on G5. Vitamin C content was generally low in (mean values for lettuce 4 mg %). The highest content of vitamin C was recorded in V1, to the G4 (1.10 mg %) and lowest in Blonde du Cazard of 0.4 to 0.42 mg%.

Table 7

## Foliar analysis and nutritional quality

Varianta	N-NO <sub>3</sub> <sup>-</sup> ppm		P-PO <sub>4</sub> <sup>3-</sup> ppm		K <sup>+</sup> ppm		Vitamina C mg%	
	Φ=4 mm G4	Φ=5 mm G5	Φ=4 mm G4	Φ=5 mm G5	Φ=4 mm G4	Φ=5 mm G5	Φ=4 mm G4	Φ=5 mm G5
	V1 - White Boston	63,00	95,00	84,40	58,80	1224	1066	1,10
V2 - Lollo Rossa	75,50	104,50	59,60	69,20	1148	1220	0,88	0,66
V3 - Blonde du Cazard	74,50	69,50	68,07	78,40	914	1144	0,42	0,40

**CONCLUSIONS**

Regarding the seedlings growth can be seen that, even at the earliest stages of vegetation, that there is a hierarchy of the cultivar grown. White Boston is proving to be the most vigorous followed by Blonde du Cazard and, last, place Lollo Rossa, variety (genetic determination) having at least a significant influence on the formation of the aerial vegetative part.

The results of the plant growth and quality of the lettuce rosettes can be seen that on the grain size of 4 mm were produced the most favorable results for all studied varieties of lettuce.

In terms of mass the worst variety is Lollo Rosso on whichever substrate but, this is offset by the larger diameter of the rosettes and does not become an obstacle to better use of rosettes.

V3 (Blonde du Cazard) although it has greater mass rosettes, they are more compact and have a smaller diameter, that this variety was the most poorly placed, specially cultivated on perlite grain substrate of 5 mm.

Root mass and volume results are relevant because they can explain the differences between both versions perlite grain size. It is maintained the anterior trend, with the most favorable results have been obtained for all variants on the G4 but especially for the V1; between mass and volume lettuce plant roots are very strong correlation,  $R^2 = 0.9312$ .

Foliar analysis showed that, in terms of food safety, lettuce plants, have the N-NO<sub>3</sub> content well below the permissible limits, phosphorus and potassium content is favorable, especially for growing variants V1 and V2 on the G4 and the vitamin C content was generally low (in the V1 to the G4 1.10 mg % and 0.4 mg % Blonde du Cazard on G5).

Throughout the experimentation period has remained a tendency of inhibiting plant growth on the substrate perlite grain size of 5 mm. Given that this consideration applies to all varieties tested, we recommend to expand the lettuce crop, perlite substrate with 4 mm grain size.

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**THE EFFECT OF TEMPERATURE VARIATIONS ON UNCONVENTIONAL  
CULTURE OF TOMATO PRODUCTIONS**

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**Keywords:** *tomatoes, perlite, yield*

**ABSTRACT**

*The tomatoes hold a priority place in greenhouse crops. Tomato crops applied in unconventional system is constantly expanding both in the world and in Romania on the one hand because crop yields achieved are far superior on the ground and on the other can be grown the whole year. In addition extreme heat of summer severely affects greenhouse tomato production so are necessaries further studies to solve this problem. In this context, for maintenance of adequate temperature and the best conditions for growth and development of tomato in the greenhouse were monitored key determinants of crops in the greenhouse.*

*Thus in this study we followed the effect of temperature variations on tomato plant growth and development, early and total production of tomatoes, along with the monitoring of the vegetation, light, humidity, atmospheric, fertigation regime, concentration of CO<sub>2</sub>.*

**INTRODUCTION**

Growing tomatoes in greenhouse is widely practiced in both the world and in Romania. Increased production was based on improving production efficiency due to technical progress in this area and the use of modern technologies.

Concerns for finding economical solutions for producing tomatoes were mentioned by many researchers. The nutrient substrate used in unconventional crops is chosen depending on culture technology. A constant concern of researchers and vegetable growers worldwide is growing and providing organic substrate, reusable, suitability characteristics and maintain good moisture and adequate ventilation in the roots. Solutions most appropriate and economical while performing farmers are taking interest to obtain yields that are bigger and provide market as early productions and quality.

In the current conditions in Romania, the farmers use in the modern technology the artificial substrates (mineral wool).

If the problem of growing substrate was largely resolved the medium factors from greenhouse are very difficult to control without a computer controller that provide tomato

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plants an climate in ensuring adequate vegetative growth and also to optimum conditions for fructification. It is known that the optimal temperature conditions tomato growth and fruiting are between 20-24°C by day and 18°C at night (Ciofu Ruxandra and all, 2003).

Tomato plants have, within certain limits, the ability to integrate temperature. Plants exposed to a fluctuating temperature regime often suffer no overall loss of yield when compared with those grown in a constant regime having the same mean temperature (Hurd and Graves, 1984; Khayat et al., 1985; de Koning, 1988, 1990, cited by Adams S. R. and all., 2001)

We appreciate that the issue of monitoring of greenhouse environmental factors has been largely solved can thus provide both heat in winter and cooling in summer by using advanced technologies. However, in Romania and in other parts of the world, cooling costs are almost similar cultural space heating costs as outdoor temperatures during the summer months from June to August, often reach 38-40° C and greenhouse can record temperatures above 45°C. These elevated temperatures have a pernicious influence on production. In this study we followed the effect of temperature variation in emissions throughout the period of growth and development of tomato plants grown in the greenhouse on different nutrient substrate in order to warn tomato producers on the importance of ensuring optimal conditions of temperature.

### MATERIAL AND METHODS

The study was carried out in Hortinvest Recearces Centre in Faculty of Horticulture Bucharest during January-August 2013.

The biological material used was the hybrid tomato Gaheris.

Experience consisted of the following experiments presented in Table 1.

Table 1

Experimental variants

Variants	Substrate type	Quantity of perlite in mattresses	The length of the mattress
V1	Perlit 100%	30 l/ sac	1 m
V2	Peat 100 %	30 l/ sac	1 m
V3	Perlit 25% + Peat 75%	30 l/ sac	1 m
V4	Perlit 50% + Peat 50%	30 l/ sac	1 m
V5	Perlit 75% + Peat 25 %	30 l/ sac	1 m

Culture was established in unheated greenhouse. Of each variant we use six mattresses of 1 m long for where we had planted each three plants. Total number of mattresses use for experiment was 30.

The distance between the mattresses was 90 cm and 35 cm between plants so, the plant density resulted was 31,746 plants per ha. Therefore, for each plant we had assured 10 l perlite substrate with granulation of 4 mm diameter. Also, for peat and for mixed perlite and peat we had used 10 l substrate for each plant. Mattresses had contained 30 l of substrate.

Mattresses were made of biodegradable polyethylene, triple laminated, composed of two layers, colored black inside and white outside. Mattresses have a length of 1 m and a width of 20 cm. This mattress was furnished from SC PROCEMA PERLIT SRL, Romania.

The fertilizing recipe was modified with tomato phenophase. In the first phenophase, immediate period after planting, for each plant we gave amount 50 ml of solution per fertigation during 2 weeks. Daily we had administrated a number of six watering. I increased the amount

of solution per plant as the plants increased in height, so had administrated between 150 and 200 ml depending on temperature and light.

During the growing season were conducted observations and determinations so: recording the temperature; increase in height of tomato plant; number of leaves; number of leaves until the first inflorescence; number of leaves between inflorescences; average distance between inflorescences; dynamics of inflorescence; determine the number of inflorescences and flowers in blossom; percent of binding of fruit; early production; the quantity of fruit harvested per plant; the average fruit per harvest; the total production; distribution by quality; determination of storage capacity; determination of fruit firmness.

Fruit production was determined by weighing.

For each determination was made statistical analysis

### RESULTS AND DISCUSSIONS

The temperatures in the greenhouse, at 60 days after planting were kept constant at 22°C during the day and 18°C at night. Around 12<sup>oo</sup> the temperatures from greenhouse have not risen above the 22°C. We observed that there were differences in vegetative growth on the type of substrate used. Thus, we observed a positive significant increase in V2 when we used Peat substrate (table 2). Also, we can remark that the plants of V1 had grew the least and the percent to V1 (control) was 96,6% with negatively statistical significances (table 3).

Table 2					Table 3				
Synthesis of results regarding height of tomatoes plant after 60 days					Synthesis of results regarding height of tomatoes plant after 80 days				
VARIANTS	Height (cm)	Differences (cm)	Significance (%)		VARIANTS	Height (cm)	Differences (cm)	Signif. (%)	
V (0) average	60.93	0.00	100.00	Mt	V(0) average	121.26	0.00	100.00	Mt
V (1)	57.30	-3.63	94.05	N	V(1)	117.13	-4.13	96.60	O
V (2)	66.33	5.41	108.87	*	V(2)	126.77	5.51	104.54	**
V (3)	60.67	-0.26	99.57	N	V(3)	121.03	-0.23	99.81	N
V (4)	61.33	0.41	100.67	N	V(4)	121.27	0.01	100.01	N
V (5)	59.00	-1.93	96.84	N	V(5)	120.10	-1.16	99.04	N
<i>DL5%</i> =	4.300	<i>DL5% in %</i> =	7.0577		<i>DL5%</i> =	2.930	<i>DL5% in %</i> =	2.4163	
<i>DL1%</i> =	6.260	<i>DL1% in %</i> =	10.2746		<i>DL1%</i> =	4.270	<i>DL1% in %</i> =	3.5214	
<i>DL01%</i> =	9.380	<i>DL01% in %</i> =	15.3956		<i>DL01%</i> =	6.400	<i>DL01% in %</i> =	5.2779	
Table 4					Table 5				
Synthesis of results regarding height of tomatoes plant after 100 days					Synthesis of results regarding height of tomatoes plant after 120 days				
VARIANTS	Height (cm)	Differences (cm)	Significance (%)		VARIANTS	Height (cm)	Differences (cm)	Signif. (%)	
V (0) average	139.03	0.00	100.00	Mt	V(0) average	162.53	0.00	100.00	Mt
V (1)	131.43	-7.59	94.54	O	V(1)	162.33	-0.20	99.88	N
V (2)	143.17	4.14	102.98	N	V(2)	172.33	9.80	106.03	**
V (3)	144.77	5.74	104.13	*	V(3)	162.80	0.27	100.16	N
V (4)	137.23	-1.79	98.71	N	V(4)	158.00	-4.53	97.21	N
V (5)	138.53	-0.49	99.65	N	V(5)	157.20	-5.33	96.72	N
<i>DL5%</i> =	5.530	<i>DL5% in %</i> =	3.9777		<i>DL5%</i> =	6.700	<i>DL5% in %</i> =	4.1222	
<i>DL1%</i> =	8.050	<i>DL1% in %</i> =	5.7903		<i>DL1%</i> =	9.750	<i>DL1% in %</i> =	5.9988	
<i>DL01%</i> =	12.050	<i>DL01% in %</i> =	8.6674		<i>DL01%</i> =	14.610	<i>DL01% in %</i> =	8.9889	

At 150 days after planting the smallest height was recorded at V1 were used the perlite substrate and the biggest was at V2. In this period the temperature doesn't fluctuate.

Table 6

Synthesis of results regarding height of tomatoes plant after 150 days

VARIANTS	Height (cm)	Differences (cm)	Differences (%)	Significance
V(0) MEDIA	223.53	0.00	100.00	Mt
V(1)	191.00	-32.53	85.45	OO
V(2)	248.33	24.80	111.09	*
V(3)	236.67	13.13	105.88	N
V(4)	218.00	-5.53	97.52	N
V(5)	223.67	0.13	100.06	N

DL 5% =	18.210	DL5% in % =	8.1464
DL 1% =	26.510	DL1% in % =	11.8595
DL01% =	39.710	DL01% in % =	17.7647

In June and July the temperature in the middle day has grown above 37 -39° C and in night 22-23°C. We can remark that the flowering and fruiting was drastic affected. The percentage of binding was 100% at the first and second inflorescences but at the third, fourth and fifth inflorescence the percentage was very low (figure 1).

In May and first decade of June we had harvest the biggest product at the V1 so, we can appreciate that this variant give the earliest production (figure 2).

Also we can remark that in the period July-August, when temperatures exceed 37 degrees we had obtained lower percentage of binding was also and fruit size.

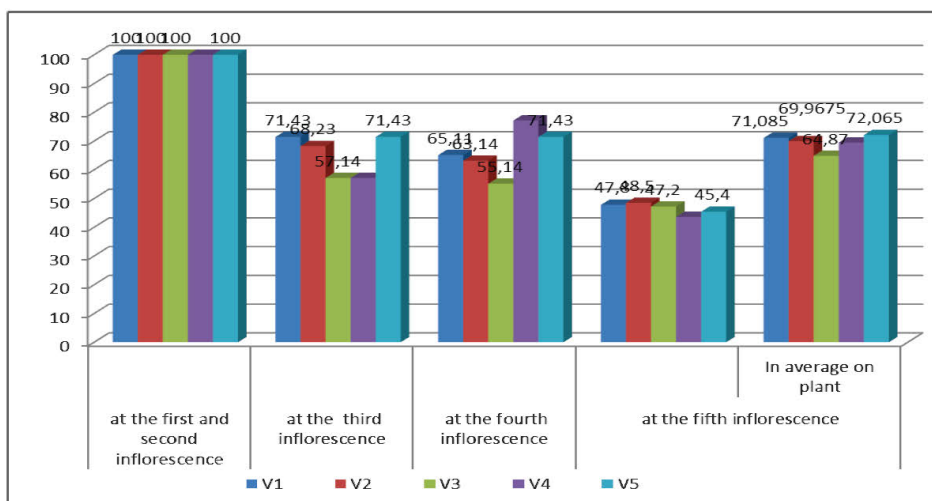


Figure 1. Percent of binding at the tomatoes grown in greenhouse

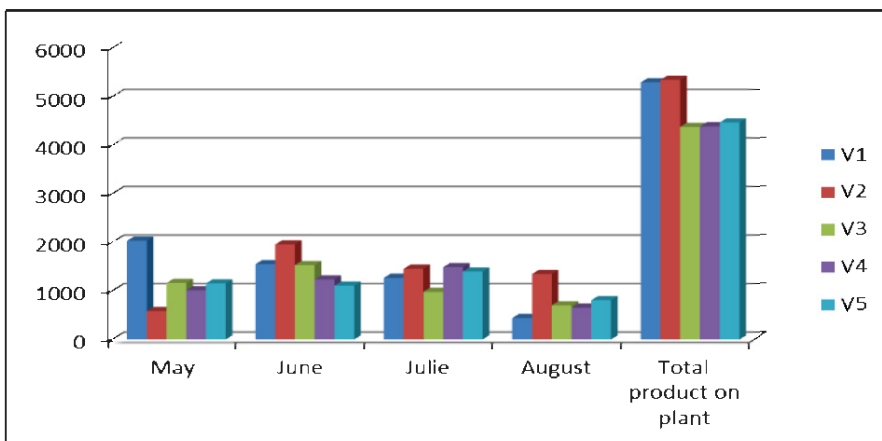


Figure 2. Total production of tomato obtained during June-August

Regarding the medium mass on fruit we can observe that in May at all variants was between 145.2 g (V1) and 148,8 (V2). In June and July the medium mass of fruit was lower (figure 3).

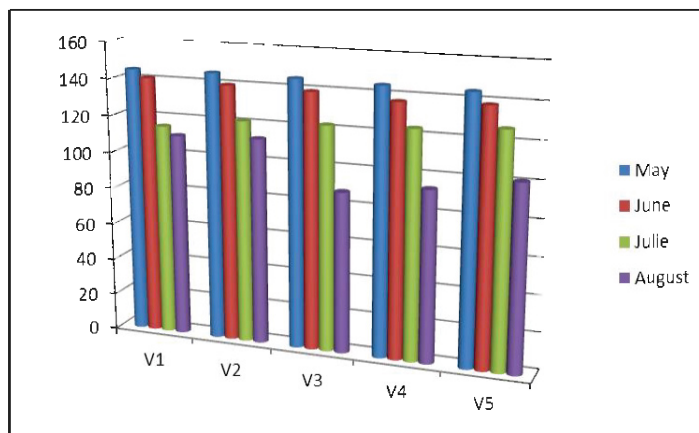


Figure 3. Average weight of tomato fruit

### CONCLUSIONS

The adequate temperature during vegetative growth favored the tomato plants grown on the mattresses with peat (57.30 cm) also and all variants when I used combination of peat with perlite after 60 days.

After 150 days the tomatoes height had 248.8 cm at V2 (on mattresses with peat) and 191 cm at V1 (on mattresses with perlite).

The distance between inflorescence was big at variant with peat comparative with perlite and combination of perlite with peat.

The binding percent was biggest in the first period of tomato plants vegetation when had assured favorable temperature for plants.

During July-August, in middle day, when in the greenhouse temperatures of over 35 degrees the fructification was affected.

Fruits were smaller in the period July-August, most fruits are under the marketing standard.

#### **ACKNOWLEDGMENT**

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INFLUENCE OF MALOLACTIC FERMENTATION ON SENSORIAL  
ATTRIBUTES OF RED WINES OBTAINED IN GALICEA MARE  
VITICULTURAL AREA

Dumitrescu Raluca<sup>1</sup>, Popa Daniela<sup>2</sup>, Baduca C.<sup>2</sup>

*Keywords: red wine, sensorial attributes, malolactic fermentation*

**ABSTRACT**

Research conducted for 4 years showed that the major interest is to have a fast start and completion of malolactic fermentation. Specific climatic conditions of the vineyard are generally favourable for spontaneous malolactic fermentation, because they allow a very good maturation of the grapes and proper sanitary condition. Instead, spontaneous malolactic fermentation does not always guarantee the best results in the sensory aspect. The use of selected lactic acid bacteria starter cultures resulted in shortening the malolactic fermentation process. Both types of malolactic fermentation has some advantages: spontaneous malolactic fermentation is economical but has uneven results regarding the sensory aspect: malolactic fermentation conducted with selected lactic acid bacteria minimizes the risk of delay, accidental stops or sensory deviations.

**INTRODUCTION**

The understanding of the complex transformation of grape must into wine requires the monitoring and controlling of the many steps of this process (Di Gennaro S.F. et al., 2013). Malolactic fermentation (MLF) is an integral step in red winemaking that results in a decrease in acidity and can also influence additional wine quality parameters (Burns T. and Osborne J.P., 2013). Lactic acid bacteria, such as *Oenococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus* spp., promote MLF, and although it is regarded as a secondary fermentation process, it plays a primary role in the production of most red wines, contributing to the complexity of the wine taste and flavour (Di Gennaro S.F. et al., 2013). MLF usually takes place after alcoholic fermentation (AF), so the role of lactic acid bacteria (LAB) such as *O. oeni* is affected by the composition of the wine, the direct result of the yeasts work (Reguant C. et al., 2005). Lactic acid bacteria remaining in wine after the end of MLF could cause microbiological problems and promote the production of undesirable volatile compounds, such as volatile acidity, mousy taint, bitterness, geranium notes, oily and slimy texture, overt buttery characters (Bartowsky E.J. and Pretorius I.S., 2008). Malolactic fermentation (MLF) can help to preserve the quality of wine by hampering the development of *Brettanomyces* yeast (Gerbaux V. et al., 2009). Variations in

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the effect of MLF on wine sensory and chemical properties has been found to arise from the choice of bacterial strain and wine matrix composition/style. (Costello J.P. et al., 2012). Sulphur dioxide is added to wine as an antioxidant and to prevent the growth of detrimental microorganisms. However, the SO<sub>2</sub> concentration should not be very high, because it can inhibit LAB during MLF (Carrete et al., 2002).

### **MATERIAL AND METHODS**

This paper presents the results of a study on malolactic fermentation of red wines produced in the viticultural area Galicea Mare in three varieties: Cabernet Sauvignon, Merlot and Black Fetească. The study was conducted during 2011 - 2014, both under the wine cellar and the laboratory conditions and targeted two driving modes of malolactic fermentation: selected lactic acid bacteria and indigenous lactic acid bacteria correlated with the quality of the grapes, as expressed by their degree of maturity and health. The study followed the influence of the two types of lactic bacteria on the conduct of malolactic fermentation and sensory characteristics of wine, given their use as quality red wines. As selected lactic bacteria it used two commercial preparations inoculated in wine, compliance management protocols recommended by the manufacturer.

### **RESULTS AND DISCUSSIONS**

Viticultural area Galicea Mare in Dolj county, is situated in one of the most southern and warm wine regions in Romania. Climate conditions specific for viticultural area Galicea Mare, characterized by rich resources of heat and light and by low precipitation regime in the summer are especially favorable for red wine varieties. In these circumstances, grapes accumulate a large proportion of sugar and anthocyanin, so wines produced in this area have a high alcoholic strength and are intensely colored. Specific climatic conditions of the vineyard have a strong influence on grape ripening and harvesting. Table 1 presents a situation harvesting grapes in vineyard area Galicea Mare in 2011 – 2014. Data in the table show important differences from one year to another in the time of harvest of red wine grapes. Thus, it is very clear that 2014 was very different from previous years as a result of adverse conditions, the most cool and rainy of all, leading to a difficult grape maturation, evidenced by lower contents sugar and lower values of pH, as well as a later period of harvest. Analysis of the data relating to the preceding years showing a very good ripening of the grapes, as evidenced by the large sugar content and relatively high values of pH, generally around 3.3 to 3.4, which is an acid overall quite low, which is very relevant for conducting malolactic fermentation. Another aspect, which shows strong evidence of Table 1, is related to the relatively early time of harvesting, the daily temperatures are favorable in order to allow the development of alcoholic fermentation, and thereafter, the malolactic fermentation before the late fall and thus, before the significant decrease of temperatures. This is of great importance in making decisions about the direction and control of malolactic fermentation.

Preliminary results for 2014 show that given the conditions of a very rainy and cool year for all the three years preceding the survey, harvesting grapes was clearly delayed by an average of three weeks to years 2012 and 2013 but the most important thing is that the grapes had at harvest a lower sugar content of 10 to 40 g/L, depending on the variety and time of harvest. If the Cabernet Sauvignon grape harvest began in 2014, on 4 October, 3 days later from 2011, ended a week later because of rain that led to discontinuation of two times with 3 or 4 days. A similar situation was at Merlot, which in 2014 began gathering at with indigenous bacteria. In the favorable years when the grapes are aged in very good



condition and very good health condition, the period of harvest is early autumn and the same time as in 2013 (October 1) but ended two days later than the previous year. What distinguish the most the two years was the sugar content, of 15 to 20 g/L lower and a lower pH in 2014, as a result of a weak ripening of the grapes. Instead, the Feteasca Neagra harvest was earlier than other years, not because of good maturation of the grapes but to reduce losses caused by adverse weather conditions during the year and during the wine harvest. In these circumstances, the previous year, harvest of Feteasca Neagra started two days earlier and 3 g/L less sugar but ended six days earlier and 34 g/L less sugar content and the pH was reduced to about 0.3. In these conditions, the malolactic fermentability of wines from 2014 was much weaker than in previous years.

The most important objective pursued was to identify optimal solutions for conducting malolactic fermentation in a short time after alcoholic fermentation, given the importance of this issue and organoleptic and technological implications of malolactic fermentation in duration. Table 2 presents data on the duration of malolactic fermentation in wines from three varieties, from 2011 to 2013 for both types of fermentation: selected lactic bacteria and lactic bacteria indigenous. The data in the table show that the wines of 2011, the duration of malolactic fermentation with selected lactic bacteria ranged from 34 days (in Feteasca Neagra) and 40 days (Cabernet Sauvignon), while the indigenous lactic bacteria during fermentation ranged between 42 days (Feteasca Neagra) and 48 days (Cabernet Sauvignon). In 2012, the duration of malolactic fermentation was shorter in all wines, with 4-5 days regardless of the type of lactic bacteria involved. Again, the lasting of malolactic fermentation with selected lactic bacteria was shorter than fermentation with indigenous lactic bacteria up to eight days for Cabernet Sauvignon wines and Feteasca Neagra, up to 7 days for Merlot. At red wines from 2013, during malolactic fermentation with lactic acid bacteria selected ranged from 32 days (in Feteasca Neagra) and 38 days (Cabernet Sauvignon), while the indigenous lactic acid bacteria during fermentation ranged from 40 days (in Feteasca Neagra) and 46 days (Cabernet Sauvignon). So, in 2013, the difference in duration was 6-8 days for fermentation with selected lactic acid bacteria. This is very important in terms of technology as quicker completion of malolactic fermentation allows timely implementation of other technological operations transfuse and physico-chemical stabilization and especially biology. A wine that has not completed malolactic fermentation is microbiologically unstable and exposed to many accidents as it remained unprotected from antiseptic point of view and malic acid is very unstable biological. The short duration of the fermentation with selected lactic bacteria is due to decrease of lag phase, on the one hand, and to a higher population density of the starter culture of bacteria used for inoculation of the wine compared with the population of indigenous lactic bacteria, on the other part. For 2014, the situation is much different from previous years, regardless of the type and species of lactic acid bacteria because of adverse weather conditions. Data on malolactic fermentation course in 2014 cannot be submitted because they are irrelevant, while the malolactic fermentation was not completed but preliminary results indicate durations of malolactic fermentation with 20 to 30 days higher than previous years or even higher at some versions of indigenous lactic bacteria, both because of lower pH values, and because higher doses of sulphitation 30-50 mg/L, imposed by weaker health of the grapes in 2014.

Efficiency of selected lactic bacteria is indisputable, especially in bad weather conditions, with cold and rainy autumn, the risks are increased when lactic fermentation sulphitation doses used in wine are low, the malolactic fermentation resulting in good condition with minor risks even with indigenous lactic bacteria.

Table 1

## Harvesting grapes in vineyard area Galicea Mare

Year	Cabernet Sauvignon			Merlot			Feteasca neagra		
	Sugar, g/L	pH	Harvest	Sugar, g/L	pH	Harvest	Sugar, g/L	pH	Harvest
2011	212 – 228	3.2 – 3.3	1.X – 15.X	208 – 220	3.22 – 3.28	8.X – 18.X	210 – 232	3.26 – 3.33	28.IX – 6.X
2012	230 – 248	3.34 – 3.40	16.IX – 23.IX	228 – 244	3.30 – 3.38	20.IX – 26.IX	233 – 250	3.38 – 3.44	10.IX – 15.IX
2013	227 – 242	3.32 – 3.32	26.IX – 12.X	221 – 240	3.31 – 3.36	1.X – 18.X	203 – 246	3.34 – 3.42	18.IX – 29.IX
2014	202 – 218	3.10 – 3.16	4.X – 22.X	205 – 220	3.12 – 3.22	1.X – 20.X	200 – 212	3.04 – 3.10	16.IX – 23.IX

Table 2

## Duration of malolactic fermentation in red wines de Galicea Mare

Year	Cabernet Sauvignon		Merlot		Feteasca neagra	
	Selected lactic acid bacteria	Indigenous lactic acid bacteria	Selected lactic acid bacteria	Indigenous lactic acid bacteria	Selected lactic acid bacteria	Indigenous lactic acid bacteria
2011	40	48	38	45	34	42
2012	36	44	33	40	30	38
2013	38	46	36	42	32	40

The second important objective of the study was to assess the influence of malolactic fermentation on sensory characteristics of red wines produced in the area of Great Galicea for 3 wine varieties. During the 3 years of the study it was noted that selected lactic bacteria have a favorable influence on sensory characteristics of red wines, regardless of variety or year. The malolactic fermentation by selected lactic bacteria leads to the improvement of taste and odor characteristics, in this very young stage of the evolution when the wines are lack in finesse. Also the malolactic fermentation accomplished by indigenous lactic bacteria derived from grapes, positively influences the sensory characteristics of red wines, reducing astringency, acidity, vegetable flavors, intensifying fruity flavour and aromatic complexity, but selected lactic bacteria lead to more intense flavors very agreeable, with notes of cranberry and currant (in Cabernet Sauvignon wines), black cherry and cherry (the Merlot wines) and cherries and plums (Feteasca Neagra wines). Figures 1 and 2 are presented sensory profiles of wines from Cabernet Sauvignon and Merlot, the harvest of 2012, before and after malolactic fermentation carried out in both versions, with selected lactic bacteria and indigenous lactic bacteria and in Figure 3 are presented the sensory profiles for two Feteasca Neagra wines, 2013 after both malolactic fermentation.

From these figures it is clear that both types of malolactic fermentation of wine significantly improves the sensory profile, but not to the same extent. Each sensory characteristic examined changes after malolactic fermentation, for the purpose of intensifying or diminishing the perception of that, each type of lactic bacteria influence over some features rather than others, as can be seen in the three figures.

—◆— Before MLF —●— LAB s —◆— LAB i

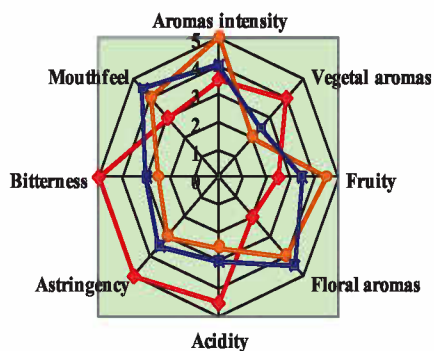


Figure 1 – Sensory profile of Cabernet Sauvignon wines

—◆— Before MLF —●— LAB s —◆— LAB i

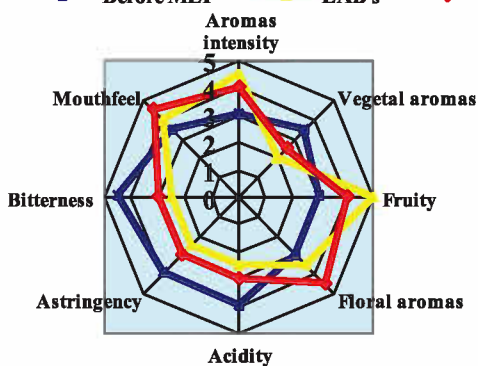


Figure 2 – Sensory profile of Merlot wines

—◆— LAB s —◆— LAB i

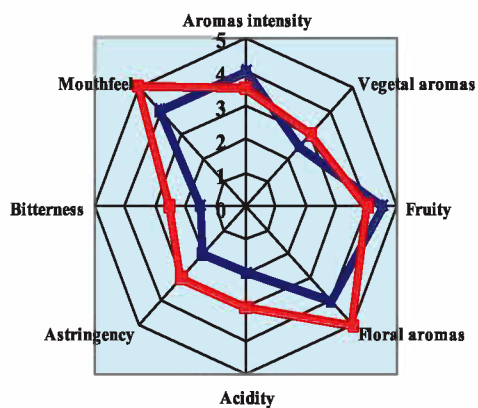


Figure 3 – Sensory profile of Fetească neagră wines after MLF

## CONCLUSIONS

The study conducted has revealed that malolactic fermentation is a major technological step for obtaining quality red wines in viticultural area Galicea Mare. Climatic conditions in the first three years of the study were extremely favorable and allowed a very good maturation of the grapes. Under these conditions, well ripened grapes healthy, harvested in early autumn with low sulphitation doses used the malolactic fermentation of wine with indigenous lactic bacteria run smoothly without crashes. Instead, in 2014, a very rainy and cold year, the grape maturation was poor, their health was significantly affected by blight attack and especially gray mold, so it took the use of higher doses of sulphitation. Under these conditions, native malolactic fermentation with lactic bacteria is seriously disturbed and the risk of accidents are major, so the use of starter cultures of selected lactic bacteria is a mandatory option. Even during good years for wine, selected lactic bacteria have proved useful, leading to shortening malolactic fermentation up to 6-8 days. From the sensory point of view, the use of selected lactic bacteria led to a decrease sensations of astringency, bitterness, acidity and vegetal aromas, grassy, in parallel with increased fruity, floral aromas and smoothness.

Compared with indigenous lactic bacteria, the selected ones led to a higher flavor intensity but aromatic complexity of wines was lower. In these circumstances, the decision to choose for a certain way of leading the malolactic fermentation depends on many factors, among which are primordial quality of the grapes at harvest and desired sensory profile of the future wine.

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## THE USE OF PHYSICAL FACTORS IN ORDER TO OBTAIN SAFE LIQUID FOOD

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**Keywords:** yeast, liquid food, corona discharge

### ABSTRACT

*The detecting of microorganisms in foods is very important because, sometimes, these could affect the safety and quality of products. In this paper we have study the behavior of yeasts population after unconventional treatment with physical factors.*

*The pure yeasts culture isolated from grape juice (from Stefanesti, Arges) was treated with negative corona discharge. In the corona discharge, the electrical field is strongly located around electrode (in our study, the negative electrode made from wolfram wire). We used different values of the exposition times and the intensity of discharge current for experiments. The total number of viable cells (CFU/ml) was determined after each treatment.*

*We observed that the injection of negative ions inside the culture medium of yeast produces the decrease of viable cell, proportional to the corona irradiation dose.*

### INTRODUCTION

The microorganisms from food could cause human health risks and may be a problem to quality of food products. This is the reason for necessity of good sterilization process. Because the traditional thermal process of liquid foods can cause physical and chemical undesired changes of products, new methods are developed: electronic pasteurization, high-pressure processing, irradiation and pulsed electric field technology (Deliu et al. 2013). With adequate control methods, the food industry will produce safe food products for consumers (Ravishankar&Maks 2007).

One of the classical methods is the thermal treatment. Both low temperature for long time and high temperature for short time can due to sensory and physical and chemical changes of liquid foods properties.

One of the unconventional methods is microwave heating, which offer several advantages: speed of heating, energy saving, higher quality of taste, texture and nutritional content of products (Deliu et al. 2013). However the microwave heating process depend of food properties (density, salt content, dielectric properties) (Albert et al. 2009).

Others unconventional methods have some inconveniences, but can have an advantage, too. This is the reason to prospect alternate methods, less expensive, easier, better than classical ones.

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The technologies of wine preparation assume some sterilization phases. The grape juice is usually got ready by sterile filtration (Delfini and Formica, 2001), this treatment conserves the composition of must. Other techniques include centrifugation, freezing or chemical sterilization with antimicrobial compounds.

The treatment with negative corona discharge can become a useful method to stabilize the final product in wine preparation.

The exposure in electrical field is an alternative method to sterilisation of fresh grape juice in wine making process (Aubrecht et al, 1999).

## MATERIAL AND METHODS

In this paper was used a yeast strain isolated from grape juice (Stefanesti – Arges vineyard).

The grape juice dilution was inoculated on solid medium (GYP: glucose - yeast extract – peptone – agar medium) by streak plate technique for obtaining isolated colonies. After 24 h incubation at 30°C, some strains with distinct cultural and colonial characteristics was isolated from mixed culture and preserved on the GYP solid medium slants.

A pure culture of yeast in liquid medium (GYP - glucose - yeast extract – peptone) was obtained in 24 h at 30°C. The yeast suspension was homogenized and treated with negative corona discharge with the aid of certain device.

A witness lot (without exposure) to compare the results was used (W). The experiments were repeated for three times.

The electrical field was obtained with negative electrode made from wolfram wire, with  $10^{-2}$  mm diameter, introduced in a quartz capilar; the active electrode, the part of wire, out of capilar is 1cm long. The distance between the electrodes could be modified (in our experiments, the distances was 3 cm). The voltage applied on electrodes was read on an electrostatic voltmeter and the current intensity on a digital microammpermeter. The set-up for experimental study of corona discharge on yeast culture is like in Figure 1

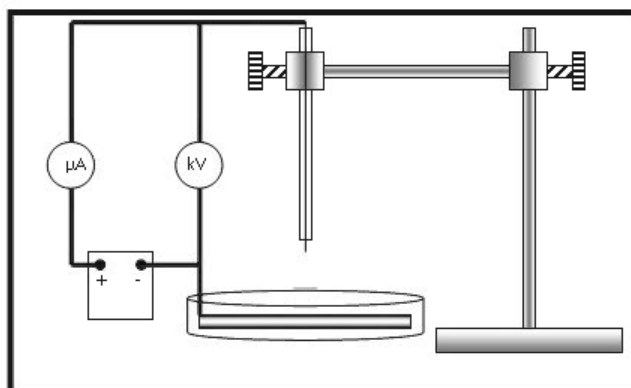


Fig.1 Experimental set-up

The electrical discharge is discontinuous, like current pulses with the frequency proportional direct with medium intensity of electrical current. The exposure dose represents the product between electric intensity ( $5\mu\text{A}$ ) and exposure time; in such experiments this is more important than each term (I or t).

Different values of the exposure time were used in order to establish distinct values of discharge current during experiments. Thus, four experimental variants were used, depend of exposure time: the yeast suspension was treated for 15, 30, 60 and 90 minutes, the current intensity was 5  $\mu$ A.

The experimental variants were:

- V1 – variant with time of exposure 15 minutes; dose:  $45 \cdot 10^{-4}$ C
- V2 – variant with time of exposure 30 minutes; dose  $90 \cdot 10^{-4}$ C
- V3 – variant with time of exposure 60 minutes; dose  $180 \cdot 10^{-4}$ C
- V4 – variant with time of exposure 90 minutes; dose  $270 \cdot 10^{-4}$ C.

After exposure the yeast suspensions, both exposure and witness ones, were used for estimate the viable cells number (colony forming units per millilitre – CFU/ml). The samples were diluted from  $10^{-1}$  to  $10^{-6}$  and 0.2 ml of each appropriate dilution was uniform inoculated on the surface of solid medium. Three repetitions from each dilution were used. Inoculated plates were incubated at 30°C for 24 h.

The number of colonies was established with Funke Gerber colony counter and CFU/ml were calculated for each variants according to all values, amount of inoculated dilution and dilution factor. The results are presented in Figure 2 in terms of percentage relative witness values.

### RESULTS AND DISCUSSIONS

The number of viable cells in suspensions varies inversely proportional with exposure time and electric dose. The number of colony forming units per millilitre decreases by 88.29% to 64.21% (Figure 2). The most significant decline was registered for 90 minutes of exposure to negative corona discharge (variant V4, Figure 3). The shortest time of exposure (variant V1) determined the smallest differences beside witness sample.

Other experiments with yeast treated with negative corona discharge also showed decrease number of viable cells, estimated with Thoma chamber (Oprescu et al. 2006).

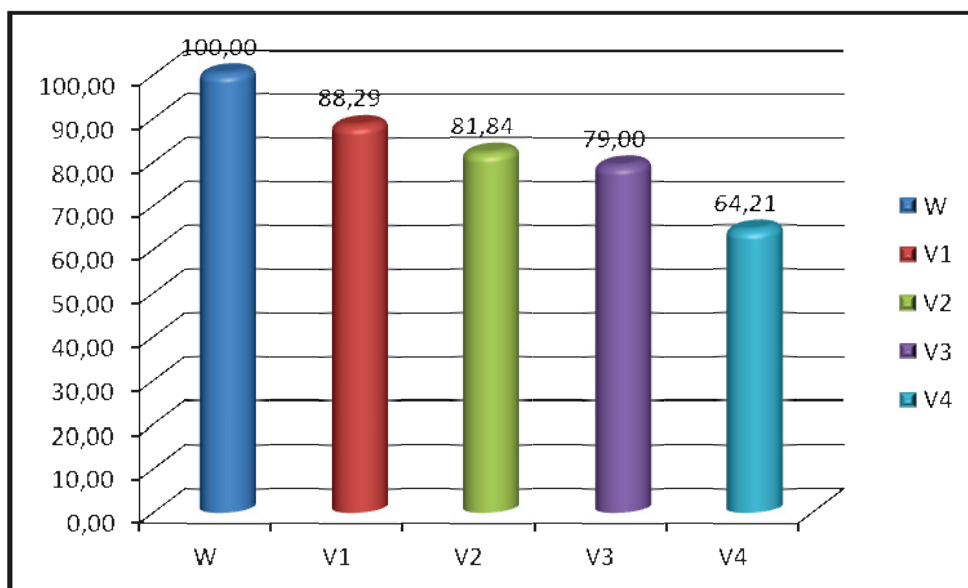


Figure 2. Yeast viable cells after corona discharge treatment

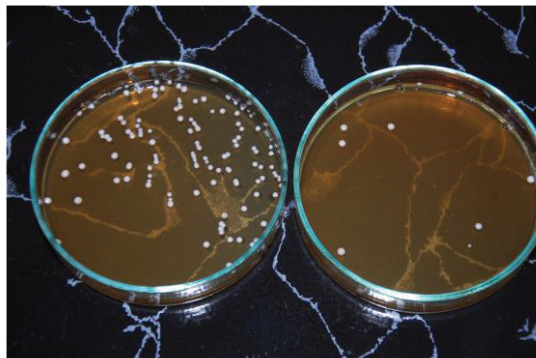


Figure 3. Yeast cultures after 90 minutes corona discharge treatment (dilution  $10^{-5}$  and  $10^{-6}$ )

The experiments realized with yeasts isolated from milk showed a significant decrease of CFU/ml (from 33% to 100%) under the microwave treatment (Deliu et al. 2013), but the heating of product must be noticed; the loss of viability can occur during the heating if the temperature exceed certain level. Also, the liquid medium composition is very important.

The experiments realized with the presented device used doses from  $45 \cdot 10^{-4} \text{C}$  to  $270 \cdot 10^{-4} \text{C}$  and determined less strict decrease of CFU/ml, but during the negative corona discharge the yeast cells were in liquid culture medium (GYP), not in their natural one, the grape juice.

Homogenous experiments with microorganisms from other liquid foods, in their natural medium or in liquid culture medium, supplemented with physical and chemical tests to demonstrate changes of liquid, can indicate the proper method for next level.

### CONCLUSIONS

The treatment of yeast cells in suspension with negative corona discharge determined the decrease of number of viable cells while exposure time increases.

The most significant decrease was registered for the longest exposure time.

Considering these results, we intend to continue research with negative and positive corona discharge exposure for liquid food (such wine, milk).

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**PRELIMINARY STUDY REGARDING THE INFLUENCE OF COLOUR  
LIGHT OVER THE CHARACTERISTICS OF TOMATOES, CUCUMBER  
AND CARROTS IN STORAGE SPACES**

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**Keywords:** *vegetables, chromatic characteristics, red, yellow, green colour.*

**ABSTRACT**

*This study have in view the influence of colour light over the organoleptic and physico-chemical properties of vegetables keep at difference light colour in storage spaces. We have in view 3 species: tomatoes, cucumber and carrots, and used in storage spaces red light for tomatoeses, green light for cucumbers and yellow light for carrots. For each light treatment, the first group of vegetables were irradiated with color light at an intensity of  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 7 days at 20°C (80–90% relative humidity). The second group of vegetables, which was non-illuminated with light, was stored at 20°C, 7 days in the dark. The third group of vegetables was analysed in fresh form after provided from the market.*

**INTRODUCTION**

Vegetables are food products with high water content, 75-96%, low content in dry substances and high level of enzymes, vitamins, mineral substances with important role in human nutrition. (I.F.Radu, 1985).

In storage process of vegetables it could be record significantly change qualitative and quantitative in chemical composition, especially decrease in vitamins and pro-vitamins, which are inherently unstable (Cavalcante, 1991; Goldman et al., 1983).

Preservation process should be acceptable if the quality and nutrition are maintained. Factors such as temperature, light, exposure to oxygen, food matrix, acidity, water content, and enzymes as well as other antioxidants and pro-oxidants can influence oxidation (Burton, 1989; Goldman et al., 1983).

Light is one of the most important factors in post-harvest evolution of fruits and vegetables. Light can prolong with 3 days self-life of vegetables like broccoli, cabbage and

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asparagus, or decrease the self-light of olives, citrus, forest fruits. Recently, UV irradiations have effect in delay of chlorophyll degradation and floret yellowing in green fruits and vegetables after harvest. (Costa et al., 2006; Aiamla-or et al. 2010). Moreover, the combination of UV-C and hot air was effective to increase the levels of phenolics and ascorbic acid, and enhance the activity of enzymes involved in removing reactive oxygen species (Lemoine et al., 2010).

A lot of studies have in view the influence of colour light over the composition of fruits and vegetables in storage spaces. Feng Xu et al., 2014, show the effect of blue light treatment on quality, antioxidant capacity and enzyme activity of strawberry fruit stored at 5°C. Blue light illumination increased the colour index, respiration rate and ethylene production in strawberries during storage. The treatment also enhanced the activities of antioxidant enzymes including superoxide dismutase, catalase and ascorbate peroxidase, and maintained lower levels of superoxide anion, hydrogen peroxide, and malondialdehyde. Red and blue light have the greatest impact on plant growth and biosynthesis of secondary metabolites because they are the major energy sources for photosynthetic CO<sub>2</sub> assimilation in plants (Lin et al., 2013).

The propose of this paper concern in a comparative study regard the influence of red light, normal light and dark over the tomatoes composition, influence of green light, normal light and dark over the cucumbers composition and influence of yellow light, normal light and dark over the carrots composition.

## MATERIAL AND METHODS

In experiments was used fresh tomatoes, cucumber and carrots provide from the market. A room with control temperature it was used to storage the vegetables in colour light and dark during one week. For colour light it was provide standard colour wavelength bulb with 650 nm for red light; 589 nm for yellow light and 510 nm for green light.

For each light treatment, the first group of vegetables were irradiated with colour light at an intensity of 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 7 days at 20°C (80–90% relative humidity). The second group of vegetables, which was non-illuminated with light, was stored at 20°C in the dark. The third group of vegetables was analysed in fresh form after provided from the market.

For each vegetable it was determinate dry substance, water content, acidity, pH, ash, ash alkalinity, sugar content.

In determination was follow the protocols:

- dry substance and water content: weight accurately 5 g of each well mixed samples in a previously dried and tarred moisture dish. Place the dish in an air oven maintained at 105±2°C and dry at least 2 hours. Cool in a dessicator and weigh. Repeat the process of heating, cooling, and weighing until the difference between two successive weighing is less than 1 mg. Record the lowest weight.

- acidity: a) colourless or slightly coloured solution – take 10 g well mixed juice, dilute to 250 ml with neutralised or distillate water. Titrate with 0.1 N NaOH using 0.3 ml phenolphthalein for each 100 ml of the solution to pink end point persisting for 30 seconds. b) highly coloured solutions – dilute known weight of sample with neutralized water and titrate to just before end point with 0.1 N alkali using 0.3 ml phenolphthalein for each 100 ml titrated. pH: was determinate by measuring the electrode potential between glass and references electrodes; pH meter was standardised using standard pH buffers.

- ash: weight 5 g sample in a platinum dish (standard 7-8 cm), dry on a water bath, ignite on a burner and ash in a muffle furnace at 525-550°C for 4-6 hrs till a white ash is

obtained. Cool the dish and weigh. Keep in muffle furnace again 1 hr, cool and weigh. Note the lowest weight and calculate total ash.

- alkalinity of the ash : in a Berzelius flask weigh 1 g of ash, 25 ml of 0.1 N HCl. Dissolve by warming on a water bath, cool and titrate the excess acid with 0.1N NaOH using methyl orange indicator. Let the alkali consumed be B ml. Carry out blank titration using 10 ml of 0.1 N HCl and let the blank value A ml.

- sugar content was determinate using Atago digital 10791 Refractometer.

The experiment was repeated twice and had a completely randomized design. Fisher's protected least significant difference (LSD) procedures were used to detect and separate the mean treatment differences at  $P=0.05$ . The program MiniTab ver 13 was used to conduct the analysis of variance.

### RESULTS AND DISCUSSIONS

All of the vegetables take in study have high water content. Light from storage spaces produce a decrease of water content at tomatoes, cucumbers and an increase at carrots (table 1). Dark produce an increase of water content at tomatoes and carrots and decrease cucumber. Water loss or gains in both of condition compare with fresh vegetables was minor, it is impossible to generate a conclusion which of the methods is better.

Table 1

Water content in analysed samples

Water content (%)					
Fresh tomatoes	94.86%	Fresh carrots	87.34%	Fresh cucumber	96.32%
Tomatoes storage in red color 7 days	93.12%	Carrots storage in yellow light 7 days	87.55%	Cucumber storage in green light 7 days	95.75%
Tomatoes storage in dark 7 days	95.41%	Carrots storage in dark 7 days	87.58%	Cucumber storage in dark 7 days	94.10%

Dry substances have the similar evolution as water content. Can be remark that red colour induce an increase of dry substance at tomatoes, yellow colour induce a decrease of dry substance at carrots and green colour increase the dry substances at cucumbers (table 2). Dark storage spaces produce dry substance lose at tomatoes and carrots and improve the content of dry substances at cucumbers. After 7 days to storage in spaces with colour light tomatoes and carrots increase the dry substance than storage in dark places, for cucumbers the results not recommend expenses with electricity, is better for keep in dark spaces.

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. The results present in table 3 shows that in both methods the content in minerals was nearly the same. It was recorded a small decrease at all the samples keep in colour light. Explication consist in organic process occurred in colour light. In a next study we propose to analyse the enzymatic activity and content in colour pigments.

Table 2

## Dry substance in analysed samples

Dry substance (%)					
Fresh tomatoes	5.14%	Fresh carrots	12.66%	Fresh cucumbers	3.68%
Tomatoes storage in red color 7 days	6.88%	Carrots storage in yellow light 7 days	12.45%	Cucumbers storage in green light 7 days	4.25%
Tomatoes storage in dark 7 days	4.59%	Carrots storage in dark 7 days	12.42%	Cucumbers storage in dark 7 days	5.9%

Table 3

## Ash content

Ash content (%)					
Fresh tomatoes	0.6932%	Fresh carrots	0.7366%	Fresh cucumbers	0.7260%
Tomatoes storage in red color 7 days	0.6724%	Carrots storage in yellow light 7 days	0.7304%	Cucumbers storage in green light 7 days	0.7258%
Tomatoes storage in dark 7 days	0.6846%	Carrots storage in dark 7 days	0.7345%	Cucumbers storage in dark 7 days	0.7236%

Table 4

## Ash alkalinity (ml HCl 0.1 N)

Ash alkalinity (ml HCl 0.1 N)					
Fresh tomatoes	11.43 ml	Fresh carrots	0.56 ml	Fresh cucumbers	6.67 ml
Tomatoes storage in red color 7 days	13.49 ml	Carrots storage in yellow light 7 days	2.017 ml	Cucumbers storage in green light 7 days	5.51 ml
Tomatoes storage in dark 7 days	11.58 ml	Carrots storage in dark 7 days	7.34 ml	Cucumbers storage in dark 7 days	5.95 ml

Carrots record a high increase of ash alkalinity in storage places. Carrots keep in yellow light record an increase with 260% and carrots keep in dark record an increase with 1210% than control sample. At tomatoes ash alkalinity record small increase and at cucumbers the values record decrease.

An important analyses witch permit to compare storage systems was sugarcontent. Sugar content decrease at all of samples compare with control sample. The postharvest determine consume of sugars from all of the vegetables by physiological process: respiration and transpiration. An important factor in sugar decrease is temperature. In both system temperature was the same 20°C. At tomatoes sugar decrease was low at red light

system than dark system, for cucumbers and carrots sugar loss was lower in dark system than green light respective yellow light system. Results was in concordance with (Feng Xu, 2014) content in sugar of strawberry irradiated with blue colour record a decrease during the storage process.

Table 5

Sugar content

Sugar content (Refractive Index, 20°C)					
Fresh tomatoes	1.4038 RI	Fresh carrots	1.6135 RI	Fresh cucumbers	1.4045 RI
Tomatoes storage in red color 7 days	1.4035 RI	Carrots storage in yellow light 7 days	1.6126 RI	Cucumbers storage in green light 7 days	1.4032 RI
Tomatoes storage in dark 7 days	1.4032 RI	Carrots storage in dark 7 days	1.6132 RI	Cucumbers storage in dark 7 days	1.4038 RI

Table 6

Total acidity

Total acidity (ml NaOH 0.1N.100 g <sup>-1</sup> )					
Fresh tomatoes	22.74 ml	Fresh carrots	55.48 ml	Fresh cucumbers	50.03 ml
Tomatoes storage in red color 7 days	30 ml	Carrots storage in yellow light 7 days	31.01 ml	Cucumbers storage in green light 7 days	27.28 ml
Tomatoes storage in dark 7 days	38.19 ml	Carrots storage in dark 7 days	39.83 ml	Cucumbers storage in dark 7 days	30.93 ml

Total acidity is an index which provide information about the alteration of vegetables during storage time. Tomatoes present an augmentation of total acidity in red colour light storage and in dark storage. However the red colour light stop the increase of acidity than the dark storage system. Cucumbers and carrots record a decrease of total acidity in both system compare with the control. Storage in colour light improve the quality of cucumbers and carrots, level of total acidity was reduced than the samples storage in dark spaces. In conclusion, storage in colour light of all vegetables take in study improve the quality of food product and expand the self-life. Results are similar with (Feng Xu, 2014) total acidity content diminished in strawberries storage in colour light.

**CONCLUSIONS**

For tomatoes the irradiation with red light in storage spaces increase the content in dry substances, ash alkalinity, total acidity and decrease water content, sugar content, ash. Red colour irradiation improve the quality of tomatoes and increase the storage period. Tomatoes keep in dark places had nearest characteristics with tomatoes red colour irradiated, but sugar content are lower and total acidity are higher than samples keep in red

light spaces. In conclusion experimental results recommend tomatoes storage in spaces illuminated with red colour.

For carrots, yellow light increase the content in water, ash alkalinity and decrease the content in dry substances, ash, sugar content and acidity. A special remark can be made over the content in ash alkalinity, yellow colour determine an increase with 200% than control. Next study will provide more information about chemical transformation produce during storage process. Except the content in ash alkalinity the results was nearest in both system dark and yellow light. Total acidity and sugar content recommend to keep the carrots in storage spaces illuminated with yellow light.

For cucumbers, green light increase the content in dry substances and decrease the content in water, ash, alkalinity ash, sugar and total acidity. Results was close in both system dark and green light. Total acidity present a high decrease in samples irradiated with green colour than the samples storage in dark. Sugar content decrease in samples irradiated more than in samples keep in dark. In conclusion spaces irradiated with green colour are better than dark spaces to conserve cucumbers for fresh consumption.

In conclusion, the preliminary study regarding the influence of colour light over the characteristics of tomatoes, cucumbers and carrots can proposed these method for storage places even a supplemental light source generate electricity cost, but improve the quality of the products during the storage period.

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## **STUDY ON RED WINES CONTAMINATION WITH DIFFERENT TYPES OF MOULDS**

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**Keywords:** *Phanerochaete, Penicillium chrysogenum, Penicillium expansum*

### **ABSTRACT**

*Red wine is the drink produced exclusively by alcoholic fermentation complete or partial, of fresh grapes crushed or uncrushed or the must of fresh grapes ("Law of Vine and Wine", 244/2002). Quality red wines are wines that are made from black grapes and have special sensory characteristics compared to other wines. These few considerations highlight the desirability and importance of the study of the production of quality red wines from superior quality parameters. The study was conducted in 2012-2013, with developing practical aspects at Technological Educational Institute of Larisa, Greece and aimed primarily the influence of processes on red wines. The paper presents this subject because even though we are in the century of advanced technology, there are wine contamination with microorganisms. One of the problems of current technology is the contamination of their wines through cork. The goal was to infect red wines with three different types of fungi and to track and analyze their progress (Rădulescu Axenia, 2012)*

### **INTRODUCTION**

Wine is a delicate product that can be deeply affected by the temperature at which it is stored and transported, by contact with oxygen that gets into wine during the production and bottling of bottles. They may also be contaminated with various organic compounds, which may accidentally get into the bottle through the cork stoppers. All these factors can negatively influence a tasting. The main culprit is a compound called 2,4,6-trichloroanisol or shortened: TCA. Wine lovers, most often refers simply to the smell of cork. (Șepilici Georgeta, 1994).

The purpose of this paper is to follow the red wine contamination with three different types of fungi, isolated from samples obtained from contaminated cork (corkwood) stoppers. The types are: *Phanerochaete chrysosporium* (a), *Penicillium chrysogenum* (b), *Penicillium expansum* (c). (Figure 1: a, b, c)

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## MATERIAL AND METHODS

Two types of wine were used for experiment development: (i) Semi-sweet Burgundy, (ii) Semi-dry Fetească Neagră.

Each type of wine was divided in four Erlenmayer glasses, obtaining eight samples. Two samples were preserved intact and the remaining samples were inoculated with the three types of mold. Then the glasses were agitated for three weeks at 25°C and 150 rotations per minute in incubated shaker.

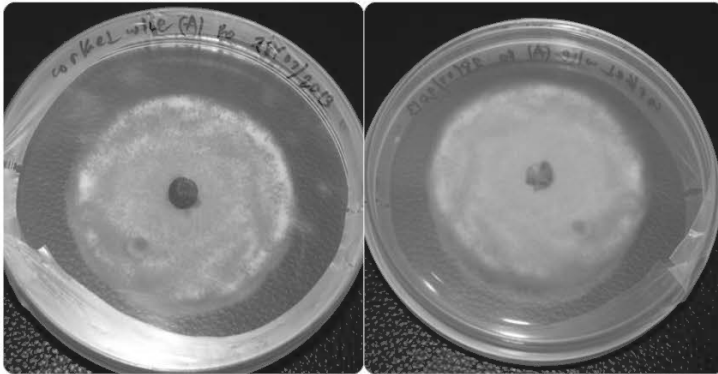


Figure 1.a) *Phanerochaete chrysosporium*

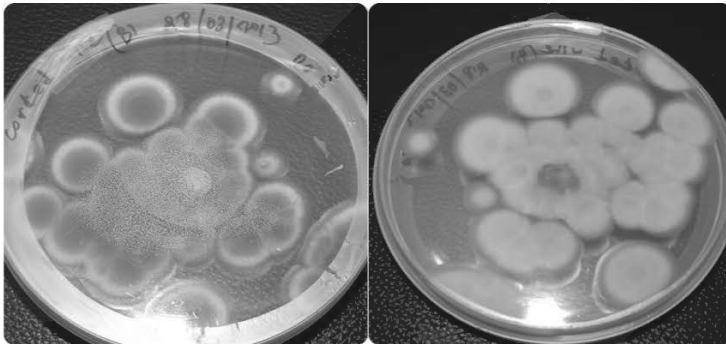


Figure 1.b) *Penicillium chrysogenum*



Figure 1.c) *Penicillium expansum*



The goal was to obtain from each type of wine a healthy sample, the second with *Phanerochaete chrysosporium*, the third with *Penicillium chrysogenum* and the fourth with *Penicillium expansum*.

Microorganisms were isolated from damaged cork samples, plated fragments into PDA plates (potatoes dextrose agar).

The next analysis were conduct for wine samples:

a) *Total SO<sub>2</sub> (mg/L)* : OIV-MA-AS323-04B

Sulfur dioxide is determined by direct titration with iodine. The combined sulfur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulfur dioxide, it gives the total sulfur dioxide. Total sulfur dioxide is defined as the total of all the various forms of sulfur dioxide present in the wine, either in the free state or combined with their constituents.

b) *Free SO<sub>2</sub> (mg/L)* Reference: OIV-MA-AS323-04B

Free sulfur dioxide is defined as the sulfur dioxide present in the must or wine in the following forms: H<sub>2</sub>SO<sub>3</sub>, HSO<sub>3</sub><sup>-</sup>, whose equilibrium as a function of pH and temperature is:



c) *Total acidity (gr H<sub>2</sub>SO<sub>4</sub>/L)* Reference: OIV-MA-AS313-01

The total acidity of the wine is the sum of its titratable acidities when it is titrated to pH 7 against a standard alkaline solution. Carbon dioxide is not included in the total acidity. The method consists in titration with bromothymol blue as indicator and comparison with an end-point color standard.

d) *Volatile acidity (gr CH<sub>3</sub>COOH/L)* Reference: OIV-MA-AS313-02

The volatile acidity is derived from the acids of the acetic series present in wine in the free state and combined as salts. Principle: Carbon dioxide is first removed from the wine. Volatile acids are separated from the wine by steam distillation and titrated using standard sodium hydroxide. The acidity of free and combined sulfur dioxide distilled under these conditions should be subtracted from the acidity of the distillate. The acidity of any ascorbic acid, which may have been added to the wine, must also be subtracted. (Ribereau Gayon, 2012).

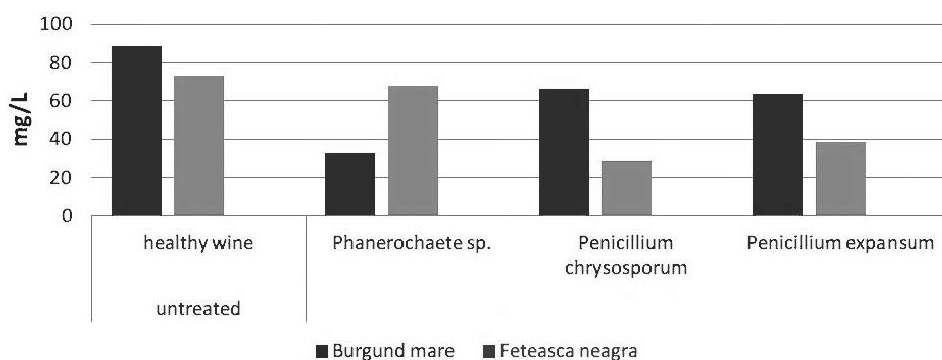
## RESULTS AND DISCUSSIONS

Four types of analysis were carried out on the eight samples of wine, these are: Total-SO<sub>2</sub>, Free-SO<sub>2</sub>, Total Acidity and Volatile Acidity.

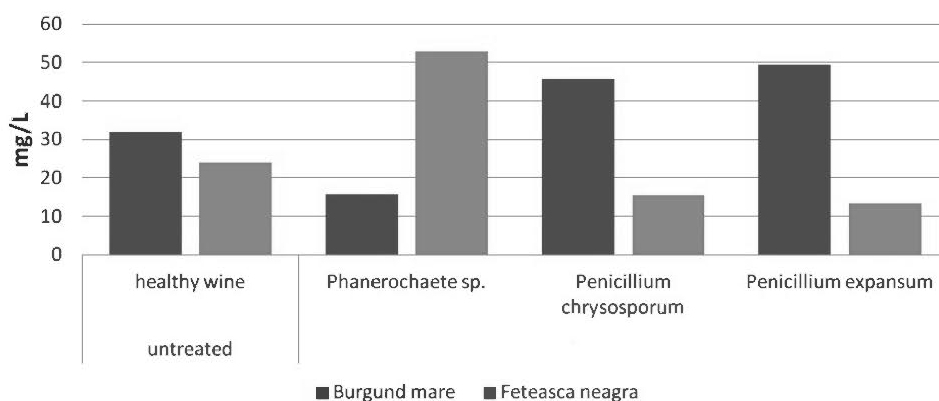
Figure 4 shows the variation of Total-SO<sub>2</sub> for each type of fungi. It can be seen that *Phanerochaete chrysosporium* creates a decrease in SO<sub>2</sub> in Burgundy wine, for almost 63% compared with the control wine. Also the two types of *Penicillium* produce a decrease in Feteasca Neagra wine for 60%. The value of Total-SO<sub>2</sub> should be framed between 150-300 mg/L. The maximum value for all wines is 88.8 so the wines were not damaged.

According to Figure 5 we see a decrease in Free SO<sub>2</sub> in samples contaminated with microorganisms, because of the antioxidant reaction and antiseptic characteristics of SO<sub>2</sub>. Also the *Phanerochaete chrysosporium* produce a double increase of the value in Feteasca Neagra wine (Panagou, 2007).

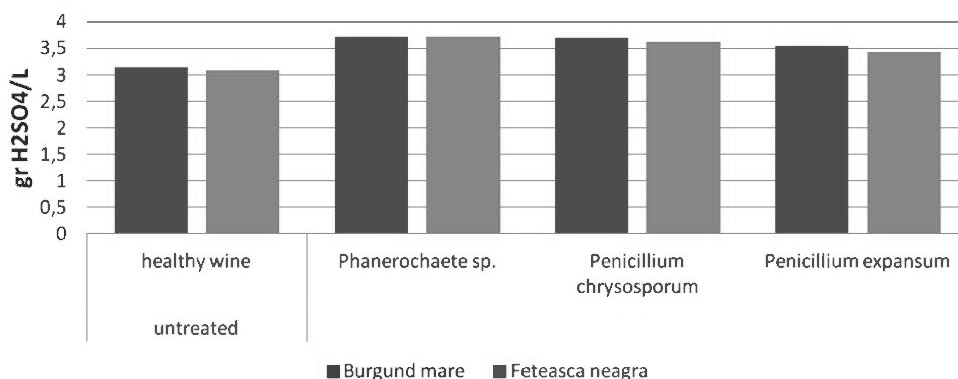
Figure 6 represents the variation of Total Acidity for the wines. It can be observe that molds produce acid and overall acidity is higher so the taste is acidulated. At the Burgund wine we can see an increase of 18% of Total Acidity due to *Phanerochaete chrysosporium* and at the Feteasca Neagra wine an increase of 20% of the total acidity.



**Figure 2. Total SO<sub>2</sub> content in control and contaminated samples**



**Figure 3. Free SO<sub>2</sub> content in control and contaminated samples**



**Figure 4. Total acidity content in control and contaminated samples**

Wines with higher Volatile Acidity of 1.5 g/L for the red ones are not good for consumption (Cotea D.V., 2010). According to Figure 7 the Volatile Acidity presents lower values than 1.5 g/L and this is good, because all three types of fungi consume volatile acids and CO<sub>2</sub> as a source of nutrition.

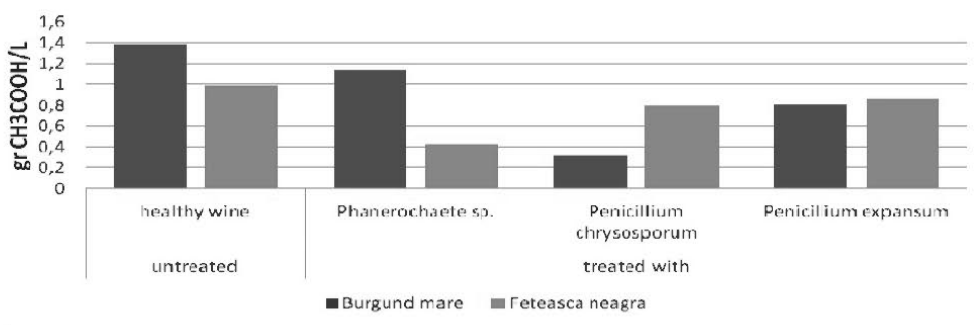


Figure 5. Volatile acidity content in control and contaminated samples

### CONCLUSIONS

In this paper we have seen that the wine retains some physicochemical properties even if it has been infected with microorganisms. However they have affected in some measure the acidity, the taste, the Free SO<sub>2</sub> and Total SO<sub>2</sub>, but it doesn't mean it cannot be consumed by costumers. It is apparent that *Phanerochaete chrysosporium* created a decrease in Total and Free-SO<sub>2</sub> in Burgundy wine and an increase of the values in Feteasca Neagra wine, it also created a slight increase of the Total Acidity and a decrease of Volatile Acidity in both types of wines. Results are in according with study realized by Vaghelas, 2012. *Penicillium chrysosporium* and *Penicillium expansum* behaved the same and produced nearly the same changes of the parameters. They produced a decrease of the Total-SO<sub>2</sub> values, an increase of Free-SO<sub>2</sub> in the Burgundy wine and a decrease in Feteasca Neagra wine, a slight increase of the Total Acidity, and the value for Volatile Acidity in Burgundy wine was with 77% lower than the control sample, because of *Penicillium chrysosporium*.

Results obtained in our study was in conformity with (Vaghelas, 2012; Lahali, 2005) Regarding the value of total acidity for all types of wines is maximum 4.07 g/L, so this means that he wine was not contaminated, the wine is not acidulated. The value of total SO<sub>2</sub> should be framed between 150-300 mg/l. According to the quantity of total SO<sub>2</sub> for all types of wines is maximum 89.6 mg/l, so the wines were not damaged. The amount of ash for healthy wine should be framed in concentration range 1.3 to 4 g/l. According to the quantity of ash for healthy wines raise the value to maximum 1 g/L, this means that the microorganisms metabolized the total solids.

The obtained values, either high or low, does not affect the health of consumers.

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**RESCUING AND PROMOTION OF SOME VALUABLE OLD VARIETIES  
OF VINES FROM DRĂGĂȘANI VINEYARD**

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*Keywords: ampelographic descriptors, old varieties, verification plots*

**ABSTRACT**

*The study was conducted between the years 2010-2013 in the plantation of Drăgășani vineyard and had pursued rescuing and promotion of valuable old varieties of vines local or cultivated over time in the vineyard. The varieties studied were 'Teișor', 'România' and 'Moroștină'. In conducting this study were used modern ampelographic descriptors. Those old varieties of vines were multiplied in the grafting and planted in the verification plots, intended for the new plantations in order to be used by the winegrowers around the world.*

**INTRODUCTION**

In Romania, the local varieties have begun to be very hard to find, because to rejuvenate the vine plantations with noble varieties appeared new market (Gorjan, 2013).

Currently, the Romanian space remaining nearly 30 local varieties, which by their uniqueness, are a precious of the national patrimony. When Romania entered the European community assumes clearly affirmation of the value romanian old varieties, so that our country to occupy the place it deserves in within the international of values exchanges. In this context, the studies of identification, conservation and capitalization in the first place the autochthonous vine varieties are absolutely necessary and highly topical (Pușcă, 2005).

The studied varieties are found in very limited in the Drăgășani vineyard but also in other of the Romanian vineyard, namely 'Teișor', 'România' and 'Moroștină'.

The study was conducted using modern descriptors for a more ample to know of these varieties is located in regress with the passage of time, order to highlight their the valuable characters.

**MATERIAL AND METHODS**

The study followed the technology of description *on farm*, in the regarding old vine varieties.

Study aimed identification of three of vines varieties which are endangered in the plantation of the Drăgășani vineyard for rescuing and promoting them.

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Has been studied phenology of the variety and the agroproductive characteristics analyzed were: fertile shoots, vigor hub, production grapes/ hub, the average weight of grapes, quality grapes (Varga et al., 2005).

The technological elements were: weight of 100 berries, sugar content in the grape, acidity ( $H_2SO_4$ ) (Gorjan, 2012).

For the thorough description of their were used descriptors OIV; UPOV; Bioversity-IBPGR from the list of descriptors *varieties* and *Vitis* species, 2009.

## RESULTS AND DISCUSSIONS

In the period 2010 - 2013 were identified these old varieties of vines in Drăgășani vineyard in many lands under vines in the vineyard, in precise points at different farmers.

The variety 'Românie' is a romanian variety, cultivated in the land of our country for a long time, unfortunately, he began to be endangered both in Drăgășani vineyard and other vineyards in the country. It is said that it was discovered in 1946 year at Drăgășani on the property general Teodorinii, after his return after the Soviet front. In the Drăgășani vineyard we may meet you Research and Development Station for Viticulture and Vinification into a collection ampelographic and a few private vineyard plantations.

In the Drăgășani vineyard, this variety has been identified in the land viticultural Sutești-Pietroasa (point Bobocea), Drăgășani-Bârsanu (point Iulia) and Călina (point Mateescu). Have been identified and localized twelve of vines hubs of this variety, in plantations very old approximately 50-90 years, in admixture biological with other varieties old vines, planting distance between rows is 1,8 m and 1,0 m between the hubs.

Location of this variety in the Drăgășani-Romania vineyard: the land viticultural Sutești-Pietroasa (44°40'46 N, 24°12'02 E, 197 m alt.); the land viticultural Drăgășani-Bârsanu (44°41'03 N, 24°15'07 E, 185 m alt.); the land viticultural Călina (44°43'06 N, 24°13'37 E, 296 m alt.).

The variety 'Teișor' is a variety spread on our land long before the phylloxera disaster, being localized in vineyards of Oltenia and Moldova. At present this variety is less common, being endangered being found in the ampelographic collections of the Research Station and very rarely in the private plantations.

In the Drăgășani vineyard we meet in ampelographic collection of Researchers and Development Station for Viticulture and Vinification Drăgășani and in a small number in the private vineyard plantations. This variety has been identified and located in private plantations in the vineyard, the land viticultural Sutești-Pietroasa (point Bobocea). We have identified a small number of hubs in the number four, into the old plantation of vines approximately 90 years, in admixture biological with other varieties, planting distance between rows is 1.6 m, and 1.0 m between the hubs.

Location in the Drăgășani-Romania vineyard: the land viticultural Sutești-Pietroasa (44°40'50 N 24°12'11 E 222 m alt.).

Moroștina' for is a variety endangered of the Romania vineyards. Its origin would be from the Oriental countries, is known as 'Leșească'. It is a prefiloxeric variety, very old, cultivated over time in Romania. In the Drăgășani vineyard as well as the other is found really hard, so it is a variety that must be promoted and preserved *on farm, in situ, ex situ*.

In the Drăgășani vineyard, the land viticultural Sutești-Pietroasa (point Bobocea) has been identified and located in a very old plantation of over 90 years, in admixture biological with other varieties, planting distance between rows is 1.6 m, and 1.0 m between the hubs. We identified three hubs from this old variety and were taken in the study.

Location in the Drăgășani-Romania vineyard: the land viticultural Sutești-Pietroasa (44°40'47 N, 24°12'02 E, 196 m alt.).

The phenological data studied in this period are presented in Table 1.

Table 1

The data average phenological 2010-2013

Variety	Bud burst	Full bloom	Beginning of berry ripening	Full maturity of the berry	Beginning of wood maturity
'România'	13.04	29.05 – 09.06	10.08	18.09	12.08
'Teșor'	10.04	30.05 – 07.06	06.08	12.09	09.08
'Morostină'	12.04	28.05 – 06.06	06.08	19.09	09.08

The main characteristics agroproductive and technological demonstrates that these varieties are very valuable, their direction of production is destined for obtaining quality white wines (Table 2).

Table 2

The main characteristics agroproductive and technological  
2010-2013

Specification	U.M.	Variety 'România'	Variety 'Teșor'	Variety 'Morostină'
Fertile shoots	%	91	80	77
Vigor hub	-	High	medium	medium
Production grapes/ hub	Kg	4,500	3,200	4,500
The average weight of grapes	G	210	170	245
Quality of grapes	%	90	90	90
Weight of 100 berries	G	220	174	272
Sugar content in must	g/l	210	210	210
Acidity (H <sub>2</sub> SO <sub>4</sub> )	g/l	7,8	4,3	5,2

The variety 'România' presents specific descriptors to obtain white wines and rosé. The main morphological descriptors studying are: the opening of the young shoot tip is fully open, attitude (before tying) of the shoot is semi-erect. The adult leaf is medium, pentagonal with five lobes. The cross section of the woody shoot is circular. The woody shoot has a color brownish-yellow. The flower is fully developed stamens and fully developed gynoecium. The bunch has shape cilindrico-conical. The berry shape is globose. The phenological characteristics are medium, the autumn coloration of leaves being yellow reddish. Related to agrobiological characteristics, they present a vigor medium of shoots growth. The physiological characteristics shows us that this variety is resistant both to drought as well as *Plasmopara*, *Oidium*, *Botyitis*. Sugar content in must is medium, with a low/medium acidity (Figure 1 and 2).

The variety 'Teșor' presents distinct characters to obtain quality white wines. Has the following characters: the opening of the young shoot tip is half open, attitude (before tying) of the shoot is semi-erect. The adult leaf is slow, entire, circular shape. The cross section of the woody shoot is circular. The woody shoot has a color brown. The flower is fully developed stamens and fully developed gynoecium. The bunch has shape cylindrical. The berry is globose. The phenological characteristics are medium, the autumn coloration of leaves being yellow. The vigor of shoot growth and growth of lateral shoots is medium.

Has a good resistance to drought and disease. Sugar content in must is high and the total acidity of must is low (Figure 3 and 4).

'Moroștină' is a variety for the production of quality white wines. Has the following morphological characters: the opening of the young shoot tip is half open, attitude (before tying) of the shoot is erect. The adult leaf is large, cordate/ pentagonal shape, entire or five lobe. The woody shoot has a brownish-yellow color, having a circular cross section. The flower is reflexed stamens and fully developed gynoecium. The bunch has shape cilindrico-conical. The berry shape is globose (Figure 5 and 6). The phenological characteristics are medium, the autumn coloration of leaves being yellow. The agrobiological characteristics exhibited a strong of shoot growth and growth of lateral shoots. The physiological characteristics highlights the fact that is resistant to drought, *Plasmopara* and *Botrytis*. The technological characteristics are generally medium (Table 3). (Gorjan, 2013).

### CONCLUSIONS

The old varieties of wines taken in the study are very valuable in terms of agroproductive and technological, direction of their production is to obtain quality white wines.

Being varieties of a certain value, they were preserved *on farm*, *in situ* and *ex situ* in ampelographic collections, aiming at in the future recovery the traditional assortment of Drăgășani vineyard as well as in other vineyards of Romania.

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\*\*\* Code des caractères descriptifs de variétés et espèces de *Vitis*, 2009, 2 – eme édition de la liste des descripteurs OIV Paris, Franța.



Table 3

## The main characters of the varieties studied

Variety	'România'		'Teișor'		'Moroștină'		Codes OIV, UPOV, Bioversity- IBPGR
	Expression character	Notes	Expression character	Notes	Expression character	Notes	
Characteristics	1	2	3	4	5	6	7
0							
Morphological descriptors:							
Young shoot:							
Young shoot: opening of the shoot tip	fully open	5	half open	3	half open	3	001; 2; 6.1.1
Shoot:							
Shoot: attitude (before tying)	semi-erect	3	semi-erect	3	erect	1	006; 9; 6.1.5
Shoot: number of consecutive tendrils	2 or less	1	2 or less	1	2 or less	1	016, -; 6.1.14
Mature leaf:							
Mature leaf: size of blade	medium	5	small	3	large	7	065; 17; 6.1.21
Mature leaf: shape of blade	pentagonal	3	circular	4	cordate/ pentagonal	1/3	067; 18; 6.1.22
Mature leaf: number of lobes	5	3	one (entire leaf)-3	1/2	one (entire leaf)-5	1/3	068; 20; 6.1.23
Woody shoot:							
Woody shoot: cross section	circular	1	circular	1	circular	1	101, -, -
Woody shoot: main color	brownish- yellow	2/1	brownish	2	brownish-yellow	2/1	103, 44, 6.1.42
Flower:							
Flower: sexual organs	fully developed stamens and fully developed gynoecium	3	fully developed stamens and fully developed gynoecium	3	reflexed stamens and fully developed gynoecium	4	151; 16; 6.2.1
Bunch:							
Bunch: shape	cylindrical -conical	1/2	cylindrical	1	cylindrical - conical	1/2	208; -, -
Berry:							
Berry: shape	globose	2	globose	2	globose	2	223; 36; 6.2.6
Phenological characteristics:							
Time of bud burst	medium	5	medium	5	medium	5	301; 1; 7.1.1
Time of full bloom	medium	5	medium	5	medium	5	302; -, 6.2.21
Time of beginning of berry ripening (veraison)	medium	5	medium	5	medium	5	303; 31; 7.1.4
Time of full physiological maturity of the berry	medium	5	medium	5	medium	5	304, -, 7.1.10

	0	1	2	3	4	5	6	7
Time of beginning of wood maturity		medium	5	medium	5	medium	5	305; -; -
Autumn coloration of leaves		yellow reddish	1/2	yellow	1	yellow	1	306; -; -
Agrobiological characteristics:								
Vigor of shoot growth		medium	5	medium	5	strong	7	351; -; -
Growth of lateral shoots		medium	5	medium	5	strong	7	352; -; 7.1.11
Physiological characteristics:								
Leaf: degree of resistance to <i>Plasmopara</i>		high	7	high	7	high	7	452; -; 9.2.3
Cluster: degree of resistance to <i>Plasmopara</i>		high	7	high	7	high	7	453; -; 9.2.4
Leaf: degree of resistance to <i>Oidium</i>		high	7	high	7	high	7	455; -; 9.2.5
Cluster: degree of resistance to <i>Oidium</i>		high	7	high	7	medium	5	456; -; 9.2.6
Leaf: degree of resistance to <i>Botrytis</i>		high	7	high	7	high	7	458; -; 9.2.1
Cluster: degree of resistance to <i>Botrytis</i>		high	7	high	7	high	7	459; -; 9.2.2
Technological characteristics:								
Sugar content of must		medium	5	high	7	medium	5	505; -; 7.1.17
Total acidity of must		low/medium	3/5	low	3	medium	5	506; -; 7.1.18



Figure 1 'Romănie' Bunch



Leaf



Figure 3 'Teișor' Bunch



Figure 4 'Teișor' Leaf



Figure 5 'Morosștină' Bunch



Figure 6 'Morosștină' Leaf

## COMPARATIVE STUDY OF AFFINITY AND HARDENING METHODS OF GRAFTED CUTTINGS

Anatoli Iliev.<sup>1</sup>, Violeta Dimitrova<sup>1</sup>, Peykov V.<sup>1</sup>

**Keywords:** *vine propagation material, technology, affinity, hardening*

### ABSTRACT

*The studies were carried out at the Institute of Viticulture and Enology – Pleven, in the period 2011-2013. The main biological indicators of red vine varieties Rubin and Storgozia (selected in IVE-Pleven) grafted on the world-famous grape rootstocks 110 Richter and 44-53 Malegue were traced for testing the affinity. Better results were obtained for Rubin variety grafted on the rootstock 110 R, while for Storgozia variety – on 44-53 M. The study of the grafted and waxed cuttings hardening before rooting was carried out with Misket Kaylashki and Storgozia varieties grafted on rootstock SO4. Two variants of grafted cuttings hardening were tested - in water and in sawdust. The indicators germination and yield of vines were traced.*

### INTRODUCTION

With the implementation of the operational techniques of grafting on American cultural vine varieties, it was set the issue of studying the affinity of *Vitis vinifera* varieties to the vine rootstocks depending on the genotype of both components and the cultivation conditions. That problem had occurred already at the beginning of grafting however it is still relevant nowadays due to the active selection works in the countries with traditional viticulture and the need of determining the appropriate rootstock combination for the respective soil and weather conditions (Volpe et al., 1990; Trifonova and Hristov, 2007; Regner, 2011). According to Pavlyuchenko et al., 2007 it had not yet been found available and highly reliable method for predicting the affinity, thus determining the appropriate rootstock combinations was carried out by the method of direct experiment. This study presents the results of testing the affinity of selected at IVE-Pleven varieties to used rootstocks in viticultural practice.

Subject of permanent study is also the technology for vine propagation material production (Radulov 1979, Panya 2007, Dimitrova et al. 2009, Smith et al. 2012). In its essence affinity and technology are interrelated and are determining each other - affinity is studied through the different steps of the technology and at the same affinity with its specifics is manifested through the technology. As the problem of the grafted cuttings

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hardening before rooting being an essential part of the technology needs to be clarified here the initial results of this important step shall be presented.

Objective of the study - comparative study of the affinity of wine grape varieties Rubin and Storgozia and methods of the grafted cuttings hardening.

### MATERIAL AND METHODS

The studies were carried out at the Production-Experimental Base of IVE – Pleven. The affinity was studied in the period 2011-2013 with varieties, selected at IVE – Pleven: Rubin (Nebiolo x Syrah) – red wine variety and Storgozia (Buket x Save Vilar 12375) – red wine variety with enhanced resistance to powdery mildew and low winter temperatures. The vine rootstocks included in the experiment were Berlandieri x Rupestris 110 Rihter (110 R) and Riparia x (Cordifolia x Rupestris) 44-53 Malegou (44-53 M).

The study on hardening of the grafted cuttings was carried out with Misket Kaylashki variety grafted to Berlandieri x Riparia SO4 (Misket Kaylashki/SO4) in two variants – in water (V1) and in sawdust (V2). The hardening in water was conducted in containers with water level 3-5 cm. In variant V2, after the second waxing, the cuttings were returned in the boxes and placed in a medium of highly wetted sawdust. Only the cuttings, waxed twice, were subjected to hardening.

The grafted cuttings were rooted with open waxed part in two lining beds (Radulov, 1979).

### RESULTS AND DISCUSSIONS

#### *Comparative Study on the Affinity of Rubin and Storgozia Varieties*

The callus formation of the grafted components is one of the main indicators when studying the affinity. The studies on the nature and degree of callus formation when grafting Rubin and Storgozia varieties on 110 R and 44-53 M rootstocks after the completion of the stratification showed that the rate of cuttings with full round callus at the site of grafting was higher in the variants with 44-53 M rootstock. The grafted cuttings of Rubin/44-53 M variety exceeded those with 110 R for the whole period of the study by 35.4% on the average, with variation per years from 17.5 to 58.7% (Figure 1a). For Storgozia variety that difference was significantly less – 6.6% on the average, with variation per years from 5 to 15% (Figure 1b).

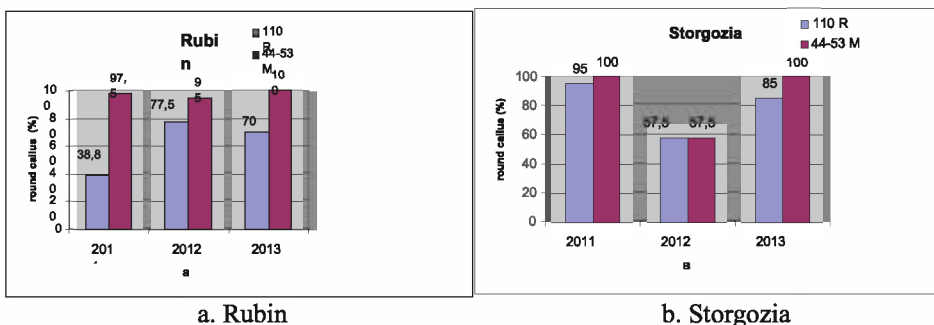


Figure 1. Formation of round callus on the site of grafting

Following up the callus formation at the foot of the grafted cuttings from the different variants it was found that the formed round callus with 44-53 M rootstock

exceeded that of 110 R rootstock by 15.4% on the average for Rubin variety and 14.2% for Stargozia variety (Figure 2 a and b).

The best germinating rate in dynamics totally per years was reported in the variant Stargozia x 110 R – 81.2% (Figure 3). In the other 3 variants there was no difference in the rate of germinating shoots - from 72.0 to 72.7% on the average for the period of the study. The reported higher values for 2011 were notable but they were probably due to the complex of favorable environmental factors after rooting, as well as before that during the processes of stratification and hardening.

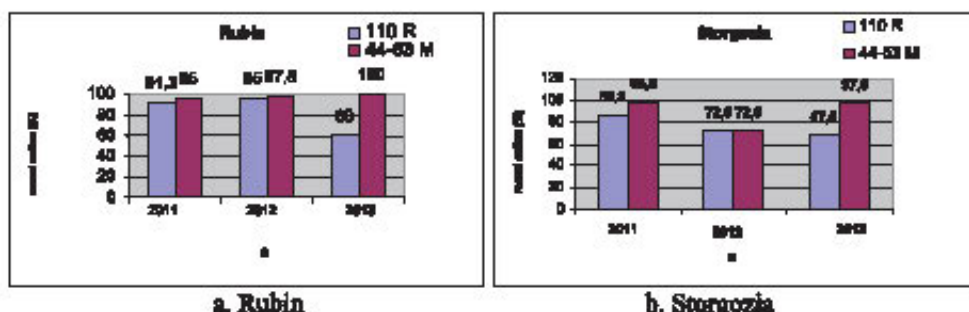


Figure 2. Callus formation at the foot of the rootstock cutting

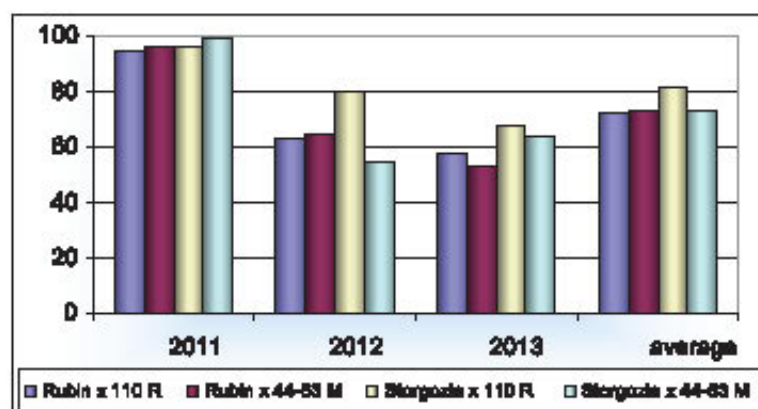


Figure 3. Changes in the germinating rates of the grafted cuttings

In the production of grafted rooted vines the most important economic indicator was the percentage of obtained standard vines.

Experimental data showed that the highest yield of standard vines was obtained in the combination Stargozia x 44-53 M – 50.4%. For the rest 3 variants the rate was a little bit lower however in very close ranges from 43.3 to 47.3% (Figure 4).

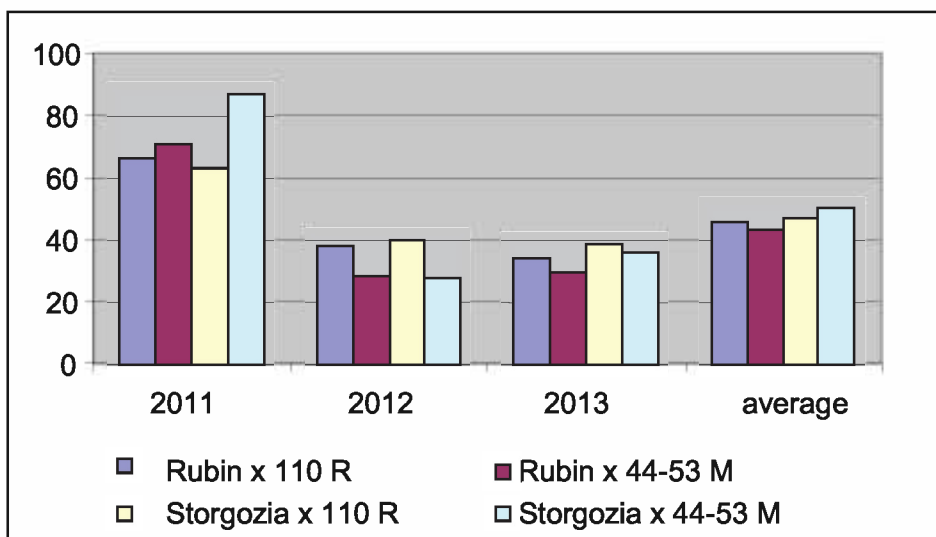


Figure 4. Yield of first-class grafted rooted vines %

When grafting Rubin and Storgozia varieties on 110 R and 44-53 M rootstocks, the rate of cuttings with full round callus on the site of grafting was higher in the variants with 44-53 M rootstock, while the germinating rate of the shoots was the most pronounced in Storgozia x 110 R – 81.2%. On the average for the three years of the study the highest yield of first-class vines was obtained when grafting Storgozia variety to 44-53 M rootstock – 50.4 %.

#### *Testing of Grafted Cuttings Hardening Methods*

The results of hardening in water were known (Radulov, 1979), but the variant of hardening in damp sawdust was of interest too. Technologically, this variant was more easily feasible, associated with less labour, and suggested reducing the cost of materials.

Data on shoot germination of the grafted cuttings in the nursery in 2011 are presented in Figure 5. The difference in the first recording on May, 28 between the variants of hardening of the grafted and waxed cuttings in water and in sawdust was 5.8% in favor of the variant of hardening in water. Subsequently, the difference dropped rapidly down for a period of 10 days (the second recording) to 2.7% and during the last recording on July, 11 there was gradually equalization of the variants. Data for 2012 were similar with the results for 2011. Probably when soaked in water the physiological moisture of the tissues of the grafted cuttings was better restored compared to the variant of hardening in damp sawdust that explained the better start of shoots germination.

As a summarizing indicator in the production of vine propagation material, the percentage of first-class grafted rooted vines was essential to determine the effectiveness of the technological improvements. Figure 6 presents the data on the yield of the produced vines. Despite the better start of shoots germination in the variant of soaking the grafted cuttings in water (V1), the difference in yield was insignificant - 1.2%. When hardening the grafted cuttings in damp sawdust it was obtained 45.5% grafted rooted vines, while in the variant V2 – 46.7%. The study was in its early stages and the results were mostly preliminary.

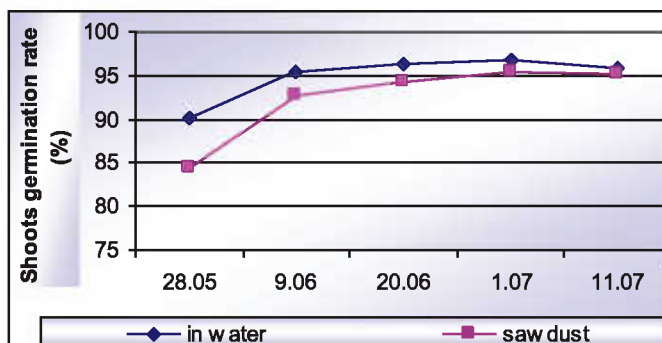


Figure 5. Shoots germination rate in dynamics of Misket Kaylashki/SO4 variety upon hardening of the grafted and waxed cuttings in water and in sawdust (2011)

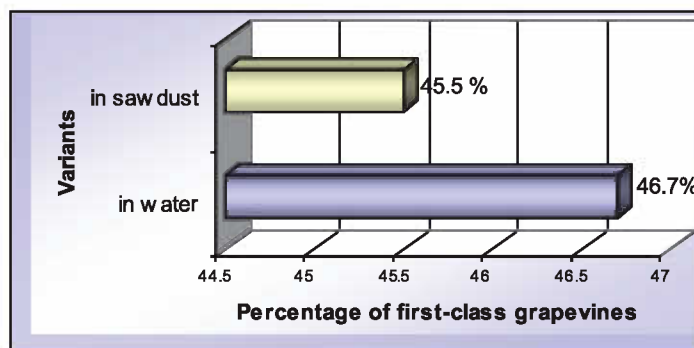


Figure 6. Yield of Misket Kaylashki/SO4 variety vines upon hardening in water and in sawdust

With the open way of cultivating the waxed cuttings, they were subjected to much higher stress with respect to temperature effects compared to the classical way of cultivating. Further studies should be undertaken to follow-up the changes in the water content of the component tissues to be grafted before and during hardening and later when rooted as well as to test the response of a larger number of varieties.

### CONCLUSIONS

When grafting Rubin and Storgozia varieties on 110 R and 44-53 M rootstocks, the rate of cuttings with full round callus on the site of grafting was higher in the variants with 44-53 M rootstock, while the germinating rate of the shoots was the most pronounced in Storgozia x 110 R – 81.2%. On the average for the three years of the study the highest yield of first-class vines was obtained when grafting Storgozia variety to 44-53 M rootstock – 50.4 %.

In a comparative testing of hardening of the grafted cuttings in water and in damp sawdust of Misket Kaylashki/SO4 variety it was obtained 45.5% grafted rooted vines in hardening in damp sawdust and 46.7% in soaking in water.

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**RESEARCH ON THE INFLUENCE OF THE QUANTITY OF  
FERTILIZERS AND IRRIGATION USED ON THE TOMATO  
PRODUCTION IN A GREENHOUSE ENVIRONMENT AND  
ACCORDING TO THE CROP'S SUBSTRATE**

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*Keywords: variety, irrigation standard, crop substrate*

**ABSTRACT**

*The study was conducted within the research center Hortinvest Bucharest of the USAMV University in the first cycle of the tomato crop. I have used the hybrid biological material Cindel. I have also used three types of crop substrate, namely mineral wool, coir and perlite with grain size of 4 mm. The crop was set up on February 12, 2014.*

*The main goal of this research was to identify the best irrigation norms on culture in order to achieve a sustainable crop production with a precocious development.*

*After the research I noticed that I have obtained a more precocious development on the variety grown on the perlite substrate. On the other hand, the variety grown on mineral wool and coir substrate had a stronger vegetative growth, but the fructification was delayed for 17-18 days.*

*The research subject is also supported by the research program POSDRU /159/1.5/S/ 132765.*

**INTRODUCTION**

Regarding the improvement of culture system and technologies culture of greenhouse vegetable at world level, it can be found the extension of soilless culture using different substrates. Soilless culture have a series of advantages as: rigorously and scheduled control of mineral nutrition and water supply, a better control of pest and diseases, thermo energy saving, due to the elimination of soil steam disinfection, elimination of soil tillage etc.(*Choux Cl., Cl.Foury, 1994, Lemaire F., 1991*).

Foliar fertilization is applied during the vegetation period and its role is to ensure for the plant a good supply of nutritive elements and to prevent nutritional disorders.

Foliar fertilization represents a fast and efficient way to correct certain plant nutrition deficiencies, especially in case of some elements for which ground application has no immediate effect or the deficiencies appear in advanced stages of vegetation when ground application is difficult.

It is also a way to stimulate plants, contributing to the quantity and quality growth of the production, increases plants' resistance to different diseases, reestablishes

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immediately the nutritional disorder and reduces the quantity of fertilizer that is applied on the ground. (Hoza Gheorghita et al, 2008).

Some of the reasons why hydroponics is being adapted around the world for food production are the following: no soil is needed for hydroponics; the water stays in the system and can be reused - thus, a lower water requirement; it is possible to control the nutrition levels in their entirety - thus, lower nutrition requirements; no nutrition pollution is released into the environment because of the controlled system; stable and high yields; pests and diseases are easier to get rid of than in soil because of the container's mobility; ease of harvesting; PH balance of nutrient solution can be easily set.

The consumption of nutrient solution in the case of hydroponic growing is an important technological and economic matter. It should be emphasized from the beginning the fact that all variants of crops within the hydroponic growing are characterized by low water consumption, which is very important for the expansion of horticultural production in where water resources are insufficient for the classical horticulture. In the long term, in the context of global warming, the matter of economic use of natural resources is becoming more important, especially in the case of water used for human consumption and irrigation.

The consumption of water (nutrient solution) for unconventional crops depends on the species, phenophase plant height and the surface of the appliance foliar, temperature, light, atmospheric humidity and soil. The nutrient solution flows from the place where it's prepared, from the bottom of the plants, throughout the drip irrigation system. The fitted nozzles of the drip irrigation systems have flow rates of 2/4l/hour at a water pressure of 1 bar. The according flow rates are ensured by activating the irrigation system for a preset period of time.

The daily water consumption of tomato plants, in the crop installations with a solution circulated open system) is between 1.5 l and 6 l for vigorous plants, topped inflorescences 6-12, in the summer months (June-July). This consumption is not evenly distributed for 24 h. The plants begin to consume the nutrient solution right after the intensity of light begins to exceed (for instance, at 7-7/30 in the summer mornings). The peak consumption takes places at about 13:00 in the afternoon and the nutrient consumption starts decreasing until the sun sets, when it reaches insignificant values.

In the case of tomatoes crops grown in greenhouses or with the help of solar energy, based on organic substrate with higher water retention (peat), the first watering must be applied with 45-60 minutes before the sunrise, until the substrate is saturated and drains outside about 15 to 20% of the administered amount.

The second operation must be programmed at about 60 min. before the peak consumption is achieved and the last watering (about 10-15% of the daily amount) must take place at night.

For the substrate crops with low water retention (perlite, sand) the day time amount of fertigation is split into a large number of waterings, in small or large amounts. In this case, increasing the standard day time irrigation can be achieved by increasing the number of waterings and using a very precise amount. (Nicolae Eugen Atanasiu, 2009)

## MATERIAL AND METHODS

The study was conducted within the Bucharest research center Hortinvest, of the USAMV, in the first cycle of the tomato crop. The crop was set up on February 12, 2014. The biological material used was Cindel hybrid, a hybrid with an indeterminate growth, characterized by earliness of crop, vigorous port and red, uniform harvest fruits with an average weight between 120 and 130 g.

Tomato culture was founded in an unconventional way on February 12, 2014, using as crop substrate mattresses filled with perlite and peat moss in various experimental variants shown in Table 1.

The main goal of this research was to identify the best irrigation norms on culture in order to achieve a sustainable crop production with a precocious development.

Table 1

Experimental variants

Variants	Type of substrate	Amount of nutrient solution required at each irrigation	Time period between waterings
V1	mineral wool	50 ml	60 minutes
V2	mineral wool	100 ml	60 minutes
V3	coir	50 ml	60 minutes
V4	coir	100 ml	60 minutes
V5	perlite with grain size of 4 mm	50 ml	60 minutes
V6	perlite with grain size of 4 mm	100 ml	60 minutes

Coir is the leftover material after the fibres have been removed from the outermost shell of the coconut. Coir is a 100% natural grow and flowering medium. Coconut Coir is colonized with trichoderma Fungi, which protects roots and stimulates root growth. It has perfect air-to-water ratio, plant roots thrive in this environment, coir has a high cation exchange, meaning it can store unused minerals to be released to the plant as and when it requires it. Coir is available in many forms, most common is coco peat, which has the appearance and texture of soil but contains no mineral content

Perlite is a volcanic rock that has been superheated into very lightweight expanded glass pebbles. It is used loose or in plastic sleeves immersed in the water. It is also used in potting soil mixes to decrease soil density. Perlite has similar properties and uses to vermiculite but, in general, holds more air and less water. It is a fusion of granite, obsidian, pumice and basalt.

Mineral wool products can hold large quantities of water and air that aid root growth and nutrient uptake in hydroponics; their fibrous nature also provides a good mechanical structure to hold the plant stable. (*Tom Alexander, Don Parker – 1994*)

The main observations and measurements were: monitoring of environmental factors, temperature, light, moisture content throughout the whole experimental period; dynamics of plant growth; early and total production.

## RESULTS AND DISCUSSIONS

On the basis of the data obtained in this study we can observe that the height of the tomato plants was lower in the case of the variant grown on perlite substrate.

The percentage of the average experience in the case of irrigation with 50 ml/ watering was 94.52% at 50 days after planting, 91.13% at 70 days after planting, 93.52% at 90 days after planting and 91.82% at 120 days after planting. The biggest growth was observed in V1 (Table 2).

Table 2

Dynamics of crop growth and the growth rate of the tomato plants  
(for plants irrigated with 50 ml nutrient solution)

Variants	After 50 days (cm)	Growth rate over the average (%)	After 70 days (cm)	Growth rate over the average (%)	After 90 days (cm)	Growth rate over the average (%)	After 120 days (cm)	Growth rate over the average (%)
V1	65.67	104.33	86.33	101.52	153.60	102.85	188.67	96.58
V3	63.67	101.15	91.3	107.36	154.77	103.63	218	111.60
V5	59.5	94.52	77.5	91.13	139.67	93.52	179.37	91.82
Average	62.95	100	85.04	100	149.35	100	195.35	100

Table 3

Dynamics of tomato plants growth in the irrigation process with 100 ml nutrient solution  
and the rate over the average crop

Variants	After 50 days (cm)	Growth rate over the average (%)	After 70 days (cm)	Growth rate over the average (%)	After 90 days (cm)	Growth rate over the average (%)	After 120 days (cm)	Growth rate over the average (%)
V1	67.0	104.89	88.3	101.91	161.67	103.07	200.33	94.75
V3	64.33	100.71	92.4	106.60	161.23	102.79	236.67	111.93
V5	63.3	97.57	79.3	91.49	147.67	94.14	197.33	93.32
Average	64.88	100	86.68	100	156.86	100	211.44	100

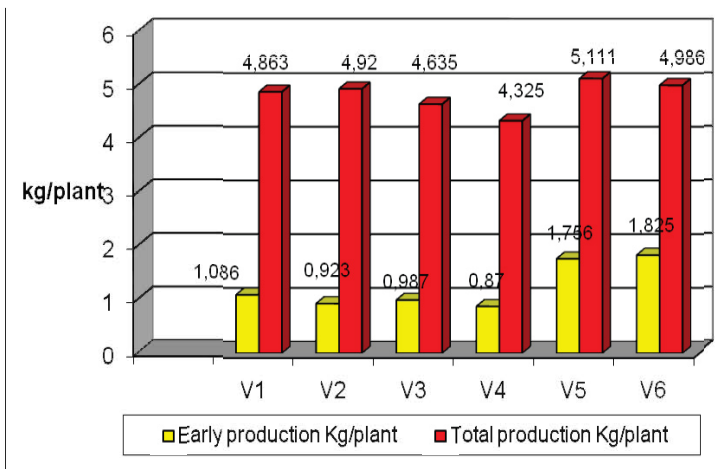


Figure 1. Early and total production of the tomato plants

In the irrigation process with 100 l/watering, it was easily observed that there were no differences in height over the variations irrigated with 50 ml/watering.

It is also noticeable that in the case of this variant, the 4 mm perlite substrate had the lowest increase (table 3).

Analyzing the resulted crops we can notice in figure 1 that the largest early crops were obtained in variants 5 and 6, the case of the perlite of 4 mm grain substrate that was irrigated with 50 ml, respectively 100 ml/watering. The largest crop was achieved in the case of Variant 5 (5,111 kg/plant). We can notice that there weren't many differences between the variants irrigated with 100ml/watering and the ones irrigated with 50 ml/watering, regardless of the nutrient substrate used.

### CONCLUSIONS

In variants grown on mineral wool substrate, the plants showed increases of 188.67 cm in the case of V1, that was irrigated with 50 ml/plant and 200.33 cm in the case of the variants irrigated with 100 ml/plant.

The difference in growth between the variant irrigated with 100 ml solution/plant and the one irrigated with 50 ml/plant was of only 11.66 cm.

In the variant cultivated on coir substrate there was a small difference of 0.33 cm in growth between the variant irrigated with 50 ml solution/plant and the one irrigated with 100 ml/plant.

The smallest increase in plant height was observed in the case of the variants grown on the perlite substrate irrigated with 50 ml solution/plant: 179.35 cm and in the case of the variants irrigated with 100 ml/plant : 197.33 cm.

In the variants grown on perlite substrate the growth difference between the one irrigated with 50 ml/plant and other with 100 ml/plant was of only 17.96 cm.

The early production was larger in the case of the variant grown on perlite substrate and irrigated with 100 ml/plant.

The largest total production was obtained in the case of V5, irrigated with 50 ml/plant.

### **ACKNOWLEDGMENT**

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## A MODERN SYSTEM FOR URBAN HORTICULTURE - CONTAINERIZED PLANTS

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*Keywords:* horticulture, ornamentals, container, trees

### ABSTRACT

*The container plant growing is a trend and a necessity for Romania and other countries experiencing multiple aspects of modernizing society. Advantages offered by containerized plants refer to the possibility of growing very diverse crops in confined spaces and at acceptable costs, with the possibility of recycling the containers. Containers used in this study are made of plastic (29 types), ceramics (10 types) and wood (3 types) and have different sizes and colors. Depending on the volume and size of the containers were grouped as: large (69.1 to 395.4 liters), medium (14.3 to 43.3 liters) and small containers (under 6.4 liters). The amount of substrate distributed into containers varied between 8.5 and 303.2 kg for each one. In these containers were cultivated 17 species and cultivars of ornamental plants and fruit trees that have developed normally for 8 to 17 years. Plants were influenced in their growing by the size of containers, the degree of compaction of the substrate and the temperature achieved inside of the container.*

### INTRODUCTION

The container-grown plants are present in the human history from ancient times. Archaeological and written evidence proving the use of containers in the royal gardens of Egypt's Queen Hatshepsut (1500 BC), during Pharaoh Thutmose III time (1450 BC), in the Hanging Gardens of Babylon under King Nebuchadnezzar II (604-563 BC), etc. (Janick, 2003).

The original container gardens were "hanging gardens". The containers did not actually "hang" but were plants that when planted would hang over the edge of the terrace or platform in which they were planted. An inexact translation of the word "hanging" „comes from the Greek word "kremastos" or the Latin word "pensilis," which mean not just "hanging", but "overhanging" as in the case of a terrace or balcony" (Prior, 2012).

This term, meaning the container for plants and has appeared in Romania between 1980 - 1990 only. In villages and towns, plants in containers (boxes, pots, etc.) are used for a long time to flower plants, ornamentals, etc.

Plant containers are also known as very diversified containers used widely in the confined spaces for the growth of plants of various different purposes (Myers, 2013; Paulsen 2010; Steward, 2010).

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Among the advantages offered by growing plants in container can be mentioned the following ones (Lieberman, 2010, Mills, 2013):

- occupy small spaces and plants can be recycled for a long time;
- plants can be arranged according to various schemes and can be transported to different places;
- ornamental and medicinal plants, vegetables, fruits and vines of different habitus can be grown;
- containers are cost effective as uses and costs;
- growing plants in containers use higher technical level and ensure normal growth, development and health status of plants;
- plant growing into containers ensures maximum security for long term conservation of *ex situ* genetic resources (NCGR Corvallis - Oregon; University of Craiova - SCDP Vâlcea; RIFG Pitesti - Mărăcineni, etc).

Human society in which we live determines many traumatic problems due to loss of plants people love the most and even substantial losses of species (Pearson, 2013; Jackson 2014).

Logical answer to such problems is to establish safe, easy and economical possibilities to reduce losses and effects by container growing plants.

In this paper, we propose to establish solutions for growing different plants in containers under the conditions from Northern Oltenia, at the University of Craiova – SCDP Vâlcea and S.C. Dumbrăvoș S.R.L. Rm.Vâlcea.

## MATERIAL AND METHODS

The study was conducted during 1996 - 2014 period and included the use of containers of different sizes and composition where different multi-annual plants were grown (ornamental plants and fruit trees). The containers were made of plastic, ceramic and wood. A total of 500 containers were used (of different sizes), numerous species and cultivars were tested. In the present paper, we tested a number of 17 species (with 9 cultivars).

The plants were grown in containers disposed as:

- containers above ground;
- buried containers (for plants that can be used for conservation as genetic resources).
- The containers contained a mixed substrate, which consisted in:
  - neutralized peat (1/3 of the total amount of substrate);
  - river sand (1/3 of the total amount of substrate);
  - forest soil (1/6 of the total amount of substrate);
  - manure (1/6 of the total amount of substrate).

The substrate mixture had a pH of between 6.9 and 7.1. Plants were fertilized annually with NPK fertilizers and watering was done from April to September, every 2 to 3 days, with 4 to 6 liters of water per container.

Observations and measurements were carried out for containers, substrate and plants. The size index was determined for the containers as  $Si = (D+d+h)/3$ , where  $D$ ,  $d$  and  $h$  are the container dimensions. The container volumes and capacity in liquids were also determined.

Nutritional substrate amount was determined based on the size of the container ( $1\text{m}^3 = 860\text{ kg}$ ), the degree of compaction and temperature in the container. In case of plants the age, surface area, trunk cross-sectional area, plant height, crown diameter, crown shape were recorded. Each species was represented by 5 - 10 containerized plants as repetitions.



## RESULTS AND DISCUSSIONS

The study was carried out using plastic (29), ceramic (10) and wooden containers (3). Container shapes was different (reverse conical, parallelogram, ovoid, parallelepiped, etc (Table 1). Depending on the size index ( $I_m$ ) and volumes the containers can be classified as:

- large containers ( $I_m > 45$  cm, volumes from 69,075 to 395,375cm<sup>3</sup> and liquid capacity from 69.1 to 395.4 liters);
- medium containers ( $I_m$  between 25 to 45 cm, volumes from 14,306 to 43,200 cm<sup>3</sup> volume and liquid capacity from 14.3 to 43.2 liters).
- small containers ( $I_m < 25$  cm, volume  $< 6,358$  cm<sup>3</sup> and liquid capacity  $< 6.4$  liters).

The plastic containers meet all three size categories, but the industry works very different types of container sizes.

Ceramic containers are usually of medium and small sizes, rarely larger (amphorae), because they are difficult to produce, are heavy and break easily.

The containers made of wood are manufactured in the form of crates or boxes and need metal or plastic inner lining not to rot in contact with water. These containers come in different sizes. In some cases they can reach over 500 kg substrate per container.

In most countries the container capacity is given in liters or gallons depending of the liquid volume they can hold.

The substrate is particularly important for plant growth, The substrate typically does not occupy the entire volume of the container (2 to 8 cm from the height of the container is empty). Depending on the container size the amount of substrate ranges from 50.3 to 303.2 kg in case of large containers, from 9.3 to 38.9 kg for medium sized ones and less than 8.5 kg for small containers.

The color of containers is different and has a definite role to ornamental plants, raising or reducing their visual effect.

In the trial different ornamental plants and fruit trees grown in containers were used (Table 2). The plants are aged between 8 (*Castanea sativa* cv. Marsol) to 17 years old (*Ginkgo biloba* and *Malus niedzwetzkyana*).

The plastic containers were utilized for these plants and due to their large sizes the amount of substrate varied between 69.1 kg (*Abies concolor* and *Prunus cerasifera* cv. Red Castle) and 303.2 kg (*Magnolia grandiflora*). Plants grew and developed according to the species and cultivar characteristics and the size of the containers (Table 2).

The highest value for trunk-cross sectional area (TCSA) was recorded in case of *Quercus palustris* (158.3 cm<sup>2</sup>) and the lowest for *Prunus cerasifera* cv. Red Castle (9.6 cm<sup>2</sup>). High values of TCSA were also registered in case of *Prunus armeniaca* cv. Comandor (132.6 cm<sup>2</sup>), *Prunus persica* cv. Superbă de toamnă (100.2 cm<sup>2</sup>), *Magnolia grandiflora* (81.7 cm<sup>2</sup>) etc. *Viburnum opulus* and *Laurus nobilis* plants developed multi stems and therefore have bush shapes.

Plant height ranged from 1.4 m (*Laurus nobilis*) to 6.0 m (*Quercus palustris*). High plants were also recorded for *Magnolia grandiflora* (4.5 m) and *Photinia beauverdiana* (3.2 m) but these have stability problems when strong winds occur.

Most plants were trained with cylindrical (*Quercus palustris*, *Magnolia grandiflora*, *Photinia beauverdiana*) and broad-conical shaped crowns (*Ginkgo biloba*, *Juglans regia*, etc.). Only several species had globular or reverse pyramid crowns.

Normal growth of the containerized plants is dependent on the degree of soil or substrate compaction from the container and the temperatures recorded in the containers (Table 3).

Table 1

## Types of containers with different ornamentals and fruit trees

Container type	Container shape	Container size group	Size index ( $S_i = \frac{D+d+H}{3}$ ) (cm)	Container volume (cm <sup>3</sup> )	Liquid capacity (l)	Substrate weight (kg)*		Container color
						full complete	with slope	
Plastic	- reverse conical - parallelogram (reverse)	large	>45.0	69,075 - 395,375	69.1 - 395.4	59.4 - 340.0	50.3 - 303.2	black, grey, green, brown, blue
		medium	25.0 - 45.0	14,306 - 43,200	14.3 - 43.2	12.3 - 31.2	9.3 - 26.0	
		small	< 25.0	< 6,358	< 6.4	2.5 - 5.5	2.3 - 4.8	
Ceramic	- reverse conical - ovoid - parallelogram (reverse) - amphora	large	70.0	150,720	150.7	129.6	108.9	dominant brown
		medium	26.7 - 40.0	14,719 - 49,676	14.7 - 49.6	126.0 - 42.6	11.0 - 38.9	
		small	< 25.3	< 12,309	< 12.3	< 10.6	< 8.5	
Wood	- parallelepiped	medium	36.7 - 46.7	48,000 - 100,000	-	41.3 - 86.0	38.7 - 71.0	various colors

\* 860 kg/m<sup>3</sup> (0.86 g/cm<sup>3</sup>)

Table 2.

Growth characteristics of ornamentals and fruit trees grown in containers  
(8-16 years old)

No.	Species and cultivar	Age (years)	Trunk cross-sectional area (cm <sup>2</sup> )	Plant height (m)	Crown diameter (cm)	Plant Shape
1	<i>Quercus palustris</i>	12	158.3	6.0	95	Cylindrical
2	<i>Abies concolor</i>	11	54.1	2.5	130	Conical
3	<i>Magnolia grandiflora</i>	15	81.7	4.5	220	Cylindrical
4	<i>Photinia beauverdana</i>	12	31.2	3.2	150	Cylindrical
5	<i>Juniperus chinensis</i> 'Pyramidalis'	15	28.3	2.8	56	Conical
6	<i>Ginkgo biloba</i>	17	37.4	2.1	140	broad-conical
7	<i>Malus niedzwetzkyana</i>	17	26.4	1.9	110	broad-conical
8	<i>Castanea sativa</i> cv. Marsol	8	18.8	1.6	86	broad-conical
9	<i>Juglans regia</i> cv. Jupânești	15	28.3	1.8	108	broad-conical
10	<i>Prunus cerasifera</i> cv. Red Castle	10	9.6	1.5	80	globular
11	<i>Prunus domestica</i> cv. Tuleu timpuriu	16	50.2	2.1	190	reverse pyramid
12	<i>Prunus domestica</i> cv. Helena	16	84.9	2.1	200	reverse pyramid
13	<i>Prunus persica</i> cv. Springold	15	86.5	2.3	260	broad-conical
14	<i>Prunus persica</i> cv. Superbă de toamnă	16	100.2	2.1	220	broad-conical
15	<i>Prunus armeniaca</i> cv. Comandor	16	132.6	2.1	245	broad-conical
16	<i>Viburnum opulus</i> cv. Bulgăre de zăpadă	14	3 stems	1.5	120	globular
17	<i>Laurus nobilis</i>	14	3 stems	1.4	100	globular

Table 3

The degree of soil compaction and differences of temperature recorded for plastic containers with different plants

No.	Species and cultivars	Plant age (years)	Substrate weight (kg)	The degree of compaction of the substrate in the container (kg/force)			Temperature (°C)			Difference between temperature in the field - soil and container edge	
				Center of container	Container edge	Difference (±)	Center of container	Container edge	Difference (±)	Field (soil) (°C)	Difference from the edge of the container (°C)
1	<i>Juniperus chinensis</i> 'Pyramidalis'	15	91.8	11.8	8.9	-2.9	19	23	+4	20.2	+2.8
2	<i>Ginkgo biloba</i>	17	91.8	9.5	6.8	-2.7	21	23	+2	20.2	+2.8
3	<i>Juglans regia</i>	15	96.9	10.5	8.5	-2.0	23	25	+2	20.2	+4.8
4	<i>Magnolia grandiflora</i>	15	303.2	9.5	7.2	-2.3	19	21	+2	20.2	+0.8
5	<i>Malus niedzwetzkyana</i>	17	91.8	10.6	8.4	-2.2	23	24	+1	20.2	+3.8
6	<i>Prunus cerasifera</i> cv. Red Castle	10	69.1	8.8	6.8	-2.0	22	25	+3	20.2	+4.8
7	<i>Abies concolor</i>	11	69.1	12.5	10.9	-1.6	19	23	+4	20.2	+2.8
8	<i>Prunus domestica</i> cv. Flora*	16	91.6	12.6	10.3	-2.3	21	21	-	20.2	+0.8
9	Level of soil compaction in the field ( $\bar{x}$ )	-	-	7.74	-	-	-	-	-	-	-

\*Container berried in the soil

The degree of compaction of substrate in the center of the container varied between 8.8 and 12.6 kg force and the edge of the container from 6.8 to 10.8 kg force, regardless of species. Settling differences are smaller at the edge of the container with 1.6 to 2.9 kg force and this phenomenon has major importance in relation with temperatures inside containers.

At the edge of the container the substrate is loose because the roots had high density here, and then started to rot. This means that every 2 to 3 years is necessary to apply root pruning at the inner edge of the container followed by changing or improving substrate composition.

In the case of large plastic containers it was found that the substrate temperature is different in the center and the edge of the container (Table 3). The temperature in the center of the container is always lower with 1 to 4°C than at the edge of the substrate. At the same time, the temperature of the surrounding soil is lower or similar to that of the center of the container, while the temperature is higher at the edge of the container with 0.8 to 4.8°C than in soil. This issue affects the behavior of the plant during the summer time when air temperatures exceed 30°C, but especially in the winter when temperatures drop below -20°C and cause damages to the roots.

## CONCLUSIONS

In Romania, the containerized plants soared in the last decade particularly by using most of them as ornamentals in confined spaces or for propagation of some of them for their edible products. The containers used are of different sizes and chemical compositions.

In this trial were used:

- plastic containers (29 types);
- ceramic containers (10 types);
- wooden containers (3 types).

After the volume and size of studied containers, they were classified into three classes:

- large containers (69.1 to 395.4 liters);
- medium containers (14.3 to 43.2 liters);
- small containers (less than 6.4 liters).

The container size influenced the amount of nutrient substrate, from 50.3 to 303.2 kg in case of large containers; from 9.3 to 38.9 kg for medium containers and less than 8.5 kg for small containers.

The species and cultivars of plants grown in large containers (aged 8 to 17 years) behaved satisfactorily (SST = 9.6 to 158.3 cm<sup>2</sup>; plant height from 1.4 to 6.0 m; crown diameters of 80 to 260 cm).

Plants were influenced in their growth by container size, degree of compaction of the substrate nutrient and temperature differences between the center and edge of containers.

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## **THE STUDY OF THE MAIN PHENOLIC PARAMETERS OF RED WINES DURING THEIR EVOLUTION**

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**Keywords:** *red wines, grapes, anthocyanin complex, colour, maturation, aging*

### **ABSTRACT**

*In red wines, the most important changes in the phases of maturation and aging are produced on phenolic compounds. This paper study the main phenolic parameters in red wines during their evolution Negru de Drăgășani and Fetească neagră wines from Drăgășani vineyard was study. During the maturation and aging of red wines there are some important changes taking place in the phenolic composition.*

### **INTRODUCTION**

The red wines with a normal composition improve their quality attributes as they progress through stages of maturation and aging. Winemakers from a commercial winery observed sensory differences in Cabernet Sauvignon wines made from three pruning treatments in a single vineyard, particularly in mouth feel characteristics (H.E. Holt et al., 2008).

At this stage of maturity, the transformations that took place under the moderate influence of oxygen, and at the state of aging, there are important transformations, with effects of compositional and organoleptic nature, occurred in conditions of a deeper and deeper reducing process.

In red wines, the most important changes in the phases of maturation and aging are produced on phenolic compounds. During the maturation of wines, under the influence of alcohol, acids and other characteristic substances, there are extracted, from the timber of the vessels especially tannins and some aromatic constituents.

Through bridges of C-C type occur also the associations between anthocyanins and non-hydrolyzed tannins (catechins), but also combinations of anthocyanin and products resulting from condensing leucoanthocyanins between them, or between them and their catechins (Cotea D.V.si col.-1988 citat de Muntean Camelia 2012).

The colour of combinations between anthocyanins and tannins is less influenced by PH, SO<sub>2</sub> oxidation. This aspect is explained by the protection of anthocyanins by tannins, as antioxidants. (Glories Y. – 1998, Ribereau-Gayon P. et al. – 1998, Vivas N., et.al. 2000).

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## MATERIAL AND METHODS

The researches were conducted on wines made from Romanian grapes Negru de Dragasani and Fetească Neagră from Drăgășani vineyard, matured in oak barrels for 12 months and bottled in bottles.

By applying modern methods of analysis and control (spectrophotometry) at 3,6,12,15 and 18 months, there have been made analyzes on the wines regarding to: total contents of anthocyanin; contents of free and combined anthocyanin; proportions of yellow, red and blue pigments on which there have been calculated chromatic attributes (intensity colour, colour tone, proportions of flavilium cations); contents of total polyphenols and tannins.

## RESULTS AND DISCUSSIONS

The data on the development of phenolic components of Negru de Drăgășani wine during 12 months of evolution and wood maturation and during 6 months of aging in the bottle, are quantified in Table 1.

Negru de Drăgășani wine shows, since the beginning, a phenolic and "robust" composition, with real possibilities to evolve very positively during vessel maturation and aging in bottle.

Throughout the period of maturation and aging of wine, anthocyanins content decreases continuously, so that in 12 months maturing period, these colour components are reduced by 130 mg./l, and during aging, by another 64 mg/l, which represents a total loss of 24.18%.

Free anthocyanins, in percentage values, are decreasing from 70% - in the case of the wine aged 3 months to 65% - at the time of bottling, reaching 58% - after 18 months of maturation and aging. Throughout the period of maturation and aging (together) combined anthocyanins increased from 241.6 mg/l to 256.3 mg/l. It results that the rate of combination of anthocyanins in polymer formation is more intense during the stage of maturation and slower during the phase of aging. It results also that oxygen is the main factor involved in the polymerization of anthocyanins.

Both during maturation and aging, there are occurring important combinations between anthocyanins and tannins, rates of these reactions being higher during maturation.

The contents of yellow-orange, red and blue pigments are decreasing progressively over the two stages of evolution, which is found in the values of optical densities at wavelengths of 420, 520, 620 nm

The evolutions of the three components of chromatic spectrum are better expressed by the percentages, comparing to the dye intensity values. From this point of view, the situation is different in the case of the absolute participation of the pigments.

Thus, in relation with the general evolution of the chromatic complex, the yellow component is growing, the red component decreases continuously and the blue component knows oscillations, with a general trend of growth.

These changes of the anthocyanin complex components accurately reflect in the values of chromatic characteristics of wine during maturation and ageing times. In this way, the staining intensity ( $I_c$ ) decreases continuously from 1628 (at 3 months) to 1217 (at 18 months).

This development of the color intensity is driven by the diminution of the color intensity of red component, which cannot be compensated by the increasing of the proportion of yellow-orange and blue pigments.



Table 1

The evolution of polyphenolic characteristics of Negru de Drăgășani wine during stages of maturation and aging

Anthocyanin sizes		Age of wines – months –				
		3	6	12	15	18
		Maturation			Aging	
Anthocyanin complex	Total anthocyanins mg/l	804	767	674	627	610
	Free anthocyanins mg/l	562,4	521,2	438,8	376,0	353,7
	Free anthocyanins %	70	68	65	60	58
	Combined anthocyanins mg/l	241,6	245,8	236,2	251,0	256,3
	Combined anthocyanins %	30	32	35	40	42
Colour	DO 420 nm	0,486	0,461	0,439	0,431	0,402
	DO 520 nm	0,920	0,820	0,771	0,705	0,659
	DO 620 nm	0,222	0,201	0,194	0,159	0,156
	Yellow pigments %	29,8	31,0	31,2	33,2	33,0
	Red pigments %	56,5	55,3	54,9	54,4	54,1
	Blue Pigments %	13,7	13,7	13,9	12,4	12,9
	Ic	1,628	1,482	1,404	1,295	1,217
	Tc	0,527	0,560	0,568	0,610	0,609
dA%	61,47	59,61	58,88	58,14	57,65	

Color tone value is increasing from 0.527 (3 months) to 0.609 (at 18 months), a situation realized by increasing the proportions of yellow pigment and a drop of red pigment. In this way, as compared visually, improved color become more and more as time passes. Flavilium cations decrease from 61.47% (3 months) to 57.65% (18 months).

The values of the Flavilium cations proportions at different stages of aging, although declining, reflect beside an intense color a very beautiful and attractive ruby red color with an obvious shine.

Polyphenols and tannins (Table 2) are increasing during aging in wooden bowl and down the stage of aging. Increases of these phenolic compounds during maturation stage are based on drawings of some phenolic constituents from the staves of vessels.

The states of the tannins and their implications for the compositional and sensory values are found in the values of specific indices. In this sense, very condensed and condensed tannins (HCl index %), evolve in a steady increasing, with more sustained rates during maturation stage.

Astringent tannins, being able to combine with gelatin (gelatin index) are going down, quite uniform, during both stages.

This development means a continuous decrease of the sensation of astringency, which in organoleptic aspect represents a real advantage.

The tannins combined with some salts and polysaccharides (EtOH index% or ethanol) are increasing in the same direction with the passage of time of maturation and aging.

The consequence of this development index of ethanol is continuously enhancing the character of softness, fullness and expressiveness of the Negru de Drăgășani wine.

The evolution of the phenolic components of the Fetească neagră wine, during maturation and aging stages is presented in Table 3.

Table 2

The evolution of the polyphenols complex from Negru de Drăgășani wines

Polyphenolic sizes		Age of wines – months -				
		3	6	12	15	18
		Maturation			Aging	
Total Polyphenols and total tannins	Total polyphenol g/l	3,27	3,41	3,45	3,39	3,32
	Tannin g/l	2,82	2,93	3,10	3,04	2,99
Indici de tanin	HCl Index %	30,3	31,5	32,2	32,3	32,0
	Gelatine Index %	51,9	50,7	49,3	48,1	48,0
	EtOH Index %	17,2	18,4	19,5	19,5	19,8

Table 3

Evolution of the chromatic and polyphenic characteristics of Fetească Neagră wine during stages of maturation and aging

Antocyanin sizes		Age of wines – months –				
		3	6	12	15	18
		Maturation			Aging	
Antocyanin complex	Total antocyanins mg/l	677	605	573	554	528
	Free antocyanins mg/l	494	411	349	316	285
	Free antocyanins %	72	66	59	55	52
	Combined antocyanins mg/l	183	194	224	238	243
	Combined antocyanins %	28	34	41	45	48
Colour	DO 420 nm	0,444	0,419	0,389	0,376	0,355
	DO 520 nm	0,807	0,741	0,677	0,602	0,548
	DO 620 nm	0,141	0,138	0,135	0,124	0,117
	Yellow pigments %	31,9	32,3	32,4	34,1	34,8
	Red pigments %	58,0	57,1	56,4	54,6	53,7
	Blue Pigments %	10,1	10,6	11,2	11,3	11,5
	Ic	1,392	1,298	1,201	1,102	1,020
	Tc	0,550	0,565	0,574	0,624	0,647
dA%	63,7	62,5	61,3	58,46	56,9	

On the whole it appears also in this case similar developments as meanings as in the previous cases, but starting to maturing and aging from lower contents in: anthocyanins (677 mg/l) staining intensity (1.392) polyphenols (2.82 g/l), tannins (2.63 g/l).

After 18 months of primary winemaking, the content of colored anthocyanin content is at the level of 528 mg/l, providing a normal color for high class red wine.

Inside the complex anthocyanins after that period mentioned above, the proportion of free anthocyanins is 52% and that of the combined anthocyanin is 48%.

The values of the tonality and Flavilium cations (0.647 and, respectively 56.9%) being in context with other color sizes, are signifying at Fetească Neagră wine a vivid color, bright, "warm" and very attractive.

Tannin indicators, at their turn, are showing taste characters, to the highest demands.

Table 4

The evolution of polyphenols complex from Fetească Neagră wines

Polyphenolic sizes		Vârsta vinurilor – luni –				
		3	6	12	15	18
		Maturare			Învechire	
Total polyphenols and tannins	Total polyphenols g/l	2,82	3,11	3,26	3,20	3,19
	Tannin g/l	2,63	2,87	2,95	2,95	2,93
Tannin index	HCl Index %	11,2	12,4	16,4	18,4	18,3
	Gelatine index %	41,1	40,6	38,3	38,0	37,1
	EtOH Index %	17,7	18,4	18,6	19,1	19,4

Total polyphenols and tannins (Table no. 4) in contents somewhat lower than Negru de Drăgășani wine, keeping the proportions, are having evolutions similar to those from the previous analysis.

The values of the tannin indexes are showing that for the same ages of wines, Fetească Neagră has better organoleptic characteristics than the wine made from the variety of Negru de Drăgășani, meaning that is less astringent (gelatin index%), more "soft" and more "round" (EtOH index%).

## CONCLUSIONS

During the maturation and aging of red wines there are some important changes taking place in the phenolic composition. In colorants, free anthocyanins are decreasing, and the combined ones are increasing.

There are decreasing the proportions of yellow and red pigments, but the relationship between them changes from one stage to another, in the advantage of the yellow coloring, the consequence being the decreased intensity and the increased tonality. The proportions of the flavilium cations are decreasing continuously, due to the decline of the red pigments contents.

During maturation are increasing the contents of the total polyphenols and tannins, these constituents are decreasing insignificantly during aging.

During maturation and aging is increasing the proportions of condensed tannins (index HCl), are decreasing the contents of astringent tannins (gelatin index), and also are increasing the tannins combined with the polysaccharides, which has effects on the improvement of taste qualities.

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## PROPAGATION AND THE EFFECT OF FOLIAR TREATMENTS WITH BIOFERTILIZERS ON GROWTH OF POMEGRANATE PLANTS

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*Keywords: Punica granatum L., propagation, cuttings, foliar treatments*

### ABSTRACT

*The paper presents the results of the research on the propagation by seed and vegetative propagation by cuttings of Punica granatum L. species, in order to obtain plants for growing in pots and for using in interior decoration, due to the fact that they are plants highly appreciated for the decorative flowers and fruits. There was observed also the influence of foliar treatments with some biofertilizers on the growth of pomegranate plants obtained from seeds. The percentage of germination was higher at the variant of using the seeds with pulp, while the lowest germination was recorded for the seeds without pulp, kept in warm water for 12 hours. Regarding the percentage of rooting and quality of rooted cuttings, the best results were obtained in the perlite substrate.*

*The highest values in terms of plant height, number of shoots per plant and leaves length were recorded by the plants that were treated with Cropmax (0.1%).*

### INTRODUCTION

*Punica granatum L. (Punicaceae family) is a species native from the south-west of Asia and from the Mediterranean region; it is a shrub or small tree of 2-5 m high. It has lanceolate or oblong-linear, deciduous, glossy leaves, arranged opposite or alternate, 3-8 cm long and green colour. The red-orange flowers, simple or double, are solitary or 2-3 united at the top of the ramifications. The spherical fruits of 5-12 cm in diameter and of reddish-yellow colour, are edible.*

It is known as a decorative plant by flowers and fruits, grown in pots, used in the decoration of apartments, and during summer the plants can be placed in terraces, balconies or in the garden in spaces with sunny exposure. In addition to the use as an ornamental plant, it has an edible and medicinal importance (Jurenka, 2008). Due to its high content in polyphenols and anthocyanins and to the high antioxidant capacity, the pomegranate fruits are important for the human health (Gil et al., 2000; Drogoudi et al., 2005).

The propagation of pomegranate is made through seeds, but it is not indicated due to the high variability of the plants and fruits characters. In order to obtain a high percentage of germination, an uniform and rapid germination, there are necessary treatments such as: stratification of seeds at low temperatures (5°C), immersion the seeds in concentrated H<sub>2</sub>SO<sub>4</sub>, followed by cold stratification, etc. (Rawat et al., 2010; Olmez et al., 2007).

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Numerous studies have been conducted on the vegetative propagation by cuttings, aiming at the observation of the different rooting stimulators influence, of the harvest period, of the substrate, of the type of cuttings, on the rooting capacity of different varieties of pomegranate (Sandhu et al. 1991; LaFaver & Ching, 1998; Popovic et al. 1999; Tibet & Onur, 1999; Melgarejo et al., 2000; Sharma et al., 2009; Owis, 2010; Alikhani et al., 2011).

In order to obtain a rapid and mass production of planting material, there have been conducted many studies and researches on micro-propagation of the pomegranate and there have been carried out protocols for the regeneration of plantlets in vitro, either by organogenesis or by somatic embryogenesis (Shailaja & Madhuri, 1997; Sharon & Sinha, 2000; Deepika & Kanwar, 2010; Patil et al., 2011; Chauhan & Kanwar, 2012).

This paper presents the results of the generative and vegetative by cuttings propagation and the effect of foliar treatments with biofertilizer products, in different concentrations, on the growth of *Punica granatum* L. plants.

### MATERIAL AND METHODS

For the generative propagation, the biological material consisted of seeds obtained from the fruits (pomegranate) purchased commercially. The seeds were separated from the fruit, washed in water, dried in the shade and stored in polyethylene bags until the start of experiments. In order to stimulate the germination, a part of the seeds were treated with Atonik, a growth stimulator in the form of concentrated aqueous solution, and the other part of seeds were held in warm water for 12 hours. The seeds were sown in alveolar trays, in March 2011, in the greenhouse, in a standard, light, permeable substrate, with 100 for each experimental variant.

The experimental variants were: V<sub>1</sub> (control - untreated seeds without pulp); V<sub>2</sub> (seeds without pulp that were soaked for 8 hours in Atonik 0.5%); V<sub>3</sub> (seeds without pulp kept in warm water for 12 hours); V<sub>4</sub> (seeds with pulp). After sowing, the watering was done by spraying. The observations on the germination process have been carried out daily for 40 days after sowing and aimed at the identification of the germination moment, duration and dynamics of the process and at the determination of the percentage of germination for each experimental variant.

For the vegetative propagation, the biological material for the placement of experiments was collected from healthy mother plants, cultivated in pots, in greenhouse conditions. There have been made shoot cuttings at the beginning of June, which have been shaped and then treated with a bio-stimulator for rooting (Radistim). For rooting of the cuttings there were used two substrates: perlite and a mixture of peat and perlite (1:1). The greenhouse temperature was 25°C and the relative humidity was 85%.

The observations and measurements aimed at the influence of the substrate on the rooting percentage of cuttings; biometric measurements of root system and aerial part of the rooted cuttings, in the moment of planting in pots.

At the beginning of June, the plants obtained from the seeds were moved to pots with a diameter of 10 cm. Biometric measurements were carried out in the moment of planting in pots, regarding the number and length of roots and height of the seedlings.

Two weeks after planting in pots there were conducted foliar treatments with biofertilizer products - Bionat Plus, Cropmax (natural, ecological products) and Kristalon - applied every two weeks from May to September. The experimental variants were: control (untreated plants); V<sub>1</sub> (Cropmax 0.1%); V<sub>2</sub> (Bionat Plus 0.2%) and V<sub>3</sub> (Kristalon 0.1%). The treatments were applied in the morning.

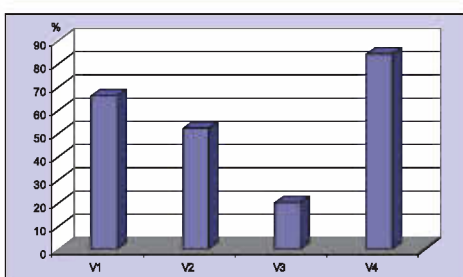
In the period July-November 2011, observations and biometric measurements have been made on the morphological characters (the average height of the plants, the

average number of shoots per plant, the average length of the shoots, number of leaves, the average length of leaves), in order to assess the influence of the biofertilizer products on the growth process of the *Punica granatum* L. plants.

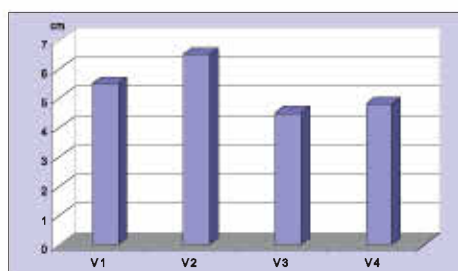
## RESULTS AND DISCUSSIONS

Regarding the propagation by seeds, there was found that the germination of pomegranate seeds started after 13 days from sowing at V<sub>2</sub> (Atonik 0.5%) and after 15 days at V<sub>1</sub> (control), while at V<sub>3</sub> (seeds held in warm water) and V<sub>4</sub> (seeds with pulp), the seeds germinated after 18 days from sowing. The highest percentage of germination has been recorded at V<sub>4</sub> (84%) and V<sub>1</sub> (66%), compared to the variant of which the seeds were treated with Atonik 0.5%, which recorded a germination percentage of 52%. The warm water treatment applied to seeds did not stimulate the germination, there was recorded the lowest percentage of germination - 20% at V<sub>3</sub> (graph 1).

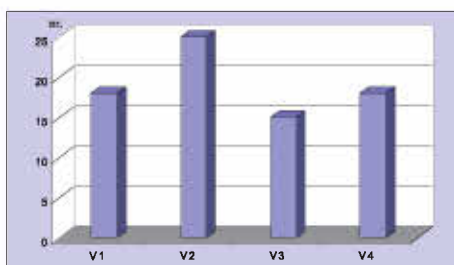
In the moment of planting in pots, biometric measurements were carried out on the root system (the average number and length of roots) and on the aerial parts of the seedlings (the average stem height). The average plant height ranged from 4.5 cm at V<sub>3</sub> - warm water and 6.5 cm at V<sub>2</sub> - Atonik 0.5% (graph 2). The highest average number of roots (25.0) was found at V<sub>2</sub>, and the lowest average number of roots (15.0) at V<sub>3</sub>. The average number of roots was 18 at the other variants. The average length of the root system ranged from 17.5 cm at V<sub>1</sub> (control) and 23.5 cm at V<sub>2</sub> (graphs 3 and 4).



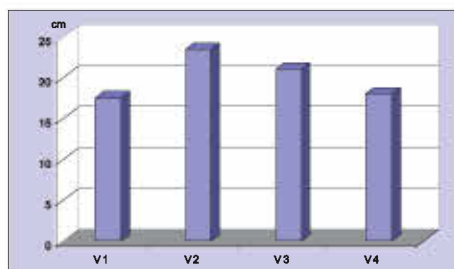
Graph 1. The percentage of germination



Graph 2. The average height of plants



Graph 3. The average number of roots



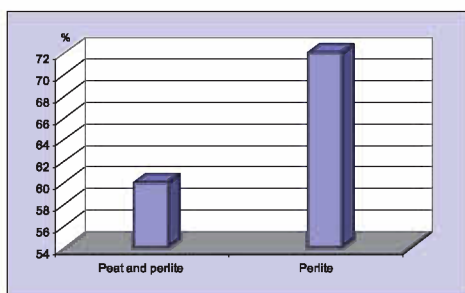
Graph 4. The average length of roots

Regarding the vegetative propagation there was found that the percentage of rooting, appreciated after 97 days from the harvesting of cuttings and planting in the rooting substrate, was 72% in perlite and 60% in the substrate formed of a mix of peat and perlite (1:1) (graph 5).

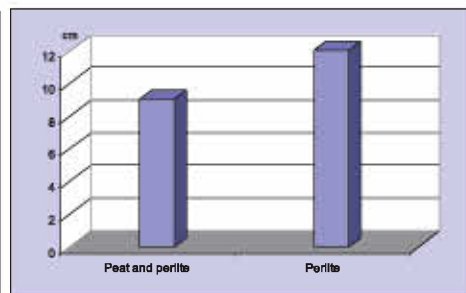
It was found that the perlite substrate had a positive influence on both the percentage of rooting of the cuttings and on the root system and the height of the rooted

cuttings. Regarding the height of cuttings there was recorded an average value of 9.0 cm in the substrate of peat and perlite, and 11.7 cm in the perlite substrate (graph 6).

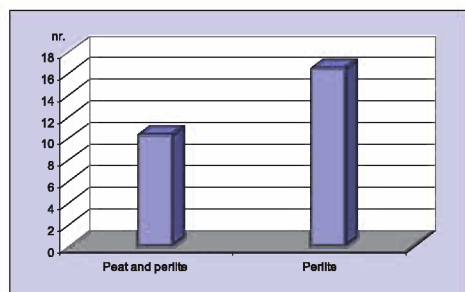
The highest number of roots was found in perlite (16.1), compared with the peat and perlite substrate (9.8). The average length of roots at the rooted cuttings in peat and perlite was of 4.0 cm, and at those rooted in perlite of 8.3 cm (graphs 7 and 8).



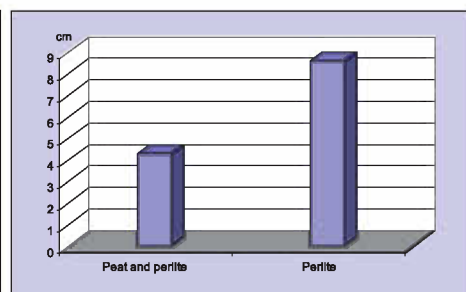
Graph 5. Percentage of rooting



Graph 6. The average height of rooted cuttings



Graph 7. The average number of roots



Graph 8. The average length of roots

The results on the dynamics of plant growth obtained from seeds show that the highest average plant height was recorded at V<sub>1</sub> (Cropmax 0.1%), followed by V<sub>2</sub> (Bionat Plus 0.2%) and V<sub>3</sub> (Kristalon 0.1%), and the lowest value was recorded at the untreated plants (control). Regarding the data recorded in the July-September period, on the vegetative growths of plants, there is found that the highest increase in height at the plants fertilized with Kristalon 0.1% and Cropmax 0.1% (10.7 cm) compared to the untreated plants, which recorded a growth increase of 8.9 cm.

In the September-November period, the growth rate was much reduced compared to the previous period, when the plants treated with Bionat Plus 0.2% and Cropmax 0.1% recorded the highest increase (4.5-4.9 cm).

At the end of the evaluation period of the influence of foliar biofertilizers on the average height of plants, there is found an average height of 33.9 cm at the plants treated with Cropmax 0.1%, the lowest height (29.9 cm), being recorded at the control plants (graph 9).

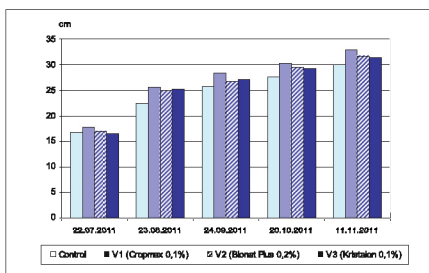
There was also observed the influence of the foliar biofertilizers on the degree of plant branching, shoots length, number of leaves per plant and on the leaves length.

In terms of the average number of shoots, the plants fertilized with Cropmax 0.1% recorded 2.5 shoots/plant, followed by those treated with Kristalon 0.1% - 2 shoots/plant, the lowest number of shoots being recorded at the control plants - 1.3 shoots/plant (graph

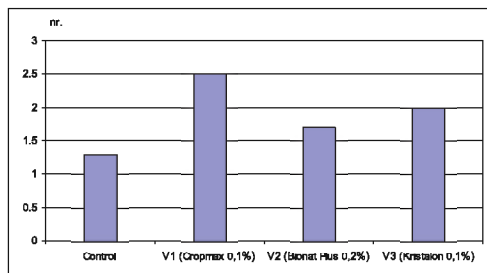


10). The average length of the shoots was lower at all the variants that were treated compared to the control plants (14.5 cm), ranging from 4.5 cm at V<sub>3</sub> - Kristalon 0.1% and 11.9 cm at V<sub>1</sub> - Cropmax 0.1% (graph 11).

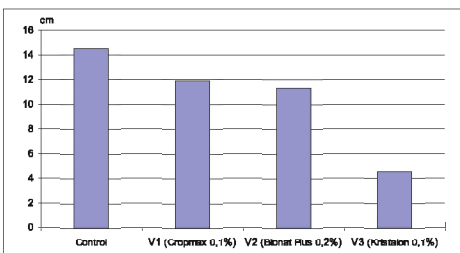
The data from graph 12, concerning on the average number of leaves per plant show the largest number of leaves (58.0), at the plants treated with Bionat Plus 0.2%, and the lowest number of leaves (37.4) recorded at the control plants. The average length of the leaves had similar values at the analyzed variants, ranging from 3.9 cm at the treated plants with Bionat Plus 0.2% and 4.4 cm at the plants treated with Cropmax 0.1%.



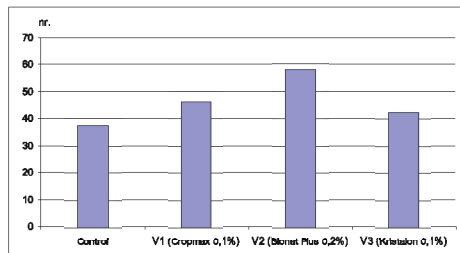
Graph 9. The average height of plants



Graph 10. The average number of shoots



Graph 11. The average length of shoots



Graph 12. The average number of leaves/plant

## CONCLUSIONS

Regarding the generative propagation there was found that at the variant where there were used seeds with pulp, the highest germination percentage (84%) was recorded. The warm water treatment applied to seeds did not stimulate the germination, resulting the lowest germination percentage (20%).

The rooting percentage of the cuttings was higher in perlite (72%), a substrate that had a positive influence on the root system and on the height of the rooted cuttings.

The analyzed morphological characters (plant height, number of shoots per plant and leaves length), recorded the highest values at the plants that were treated with Cropmax (0.1%).

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## USE OF INDUSTRIAL TOMATO WASTES AS FOOD INGREDIENTS IN THE MANUFACTURE OF SOME EMULSION-TYPE SAUSAGES

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**Keywords:** *tomato waste, sausages, drying, lycopene*

### ABSTRACT

*The conventional method for tomato processing generates high quantities of wastes consisting of skins and seeds which can be recovered and used as ingredients in the food industry. These wastes contain high amount of lycopene, other carotenoids and fibers. This paper describe experiments performed on the dehydration of tomato waste at different temperatures and on the incorporation of the powder obtained after dehydration and grinding of tomato waste into sausages at 5% and 10% addition levels in order to obtain a new product enriched in lycopene and other bioactive compounds.*

### INTRODUCTION

Tomatoes and tomato products are rich in lycopene and other carotenoids such as  $\beta$ -carotene, phytoene, phytofluene and lutein. Tomato skins also contain large amounts of carotenoids, especially lycopene. There is ample evidence supporting the health benefits of carotenoids. Numerous epidemiological studies have suggested that the intake of foods containing lycopene in the diet is inversely correlated with the incidence of cardiovascular diseases and various cancers (Shi et al., 2000).

A major challenge for the food industry is the optimization of processes in terms of recovery and recycling of waste after processing. The conventional methods of tomatoes processing generate large amounts of thermally treated waste. Skins and seeds are the main waste and they contain large amounts of biologically active substances. The importance of these compounds is increasing due to their extensive use in foods, cosmetics and pharmaceuticals, as a result of European directives that support the use of natural compounds in detriment of those synthetic (Vági et al., 2007). Recycling or reusing these wastes accumulated during processing and available in large quantities can reduce costs. Getting carotenoids such as lycopene from tomato skin can be a good solution for reuse of this waste from the tomato processing industry. Lycopene can be extracted from tomato waste using organic solvent (Breithaupt, 2004; Calvo et al., 2007; Ishida et al., 2005 or supercritical fluid Gómez-Prieto et al., 2003; Rozzi et al., 2002; Topal et al., 2006). The concentration of lycopene depends on the source and the conditions of extraction. Extracts obtained with supercritical CO<sub>2</sub> contain no residual solvent, but this technology is expensive.

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Improving tomato waste value is possible by developing processes to convert this waste into new food ingredients. A relatively simple recovery technology would be to produce a waste powder which may be directly incorporated in foods.

The addition of tomatoes, tomato products or lycopene to the meat could lead to products with health benefits. Few studies have been reported on the use of tomato products and lycopene in meat products. Candogan 2002 reported on the use of tomato paste in beef meatball while Deda et al. 2007 in frankfurters.

Yilmaz et al. 2002 studied the influence of the tomato juice addition on the composition and quality of low fat sausages. Østerlie and Lerfall 2005 studied the effect of lycopene addition on the evolution of minced meat quality during storage while (Sánchez-Escalante et al. 2003) analyzed the influence of oleoresins and frozen tomato pulp addition on the color and the odor of beef patties.

The aim of this study was to determine the effects of the addition of dried tomato waste (husks and seeds) on the texture and sensory properties of smoked sausages. A study has been also carried out on the influence of the drying temperature on the quality of the dehydrated tomato waste.

## MATERIALS AND METHOD

### *Raw material and preparation of dehydrated tomato wastes*

Tomato wastes used in this study (skins and seeds) were obtained from Leader International S.A., a fruits and vegetables processing plant from Caracal, Olt county. Tomato wastes were dried in an oven at various temperatures (60, 70, 80, 90 and 100 °C) until their moisture content reached 12% wet basis (wb).

The drying air speed was kept constant. Initial moisture content of the tomato waste (%) was determined gravimetrically by drying the sample in an oven at 105° C. The titratable acidity (expressed as % citric acid) was determined by titration of the aqueous extract of tomato waste with 0.1 N sodium hydroxide using phenolphthalein as indicator.

Dried tomato waste were cooled, crushed, packed in polyethylene bags lined with aluminum foil and stored under ambient conditions for further analysis and use.

### *Rehydration characteristics of dried tomato waste*

Rehydration experiments for dried samples at 60, 70, 80, 90 and 100 °C were carried out in distilled water at temperature of 25 and 100°C. Approximately 5 g of dried sample was added to 50 ml distilled water in a 100 ml beaker. The actual rehydration duration was 24 hours for the rehydration at 25°C and 5 min for the rehydration at 100°C. Weights of dried and rehydrated samples were measured using an electronic digital balance (Kern, model ABJ 220, Kern & Sohn GmbH, Stuttgart, Germany).

The mass of dehydrated ( $m_D$ ) and rehydrated samples ( $m_R$ ) were used to determine the following rehydration characteristics:

$$\text{Rehydration ratio, } RR = \frac{m_R}{m_D}$$

$$\text{Rehydration coefficient, } C_{\text{reh}} = \frac{m_R \times (100 - A)}{m_D \times (100 - B)}$$

where:

A = moisture content of samples before dehydration, % (wb);

B = moisture content of dehydrated samples, % (wb).

#### ***Determination of $\beta$ -carotene and lycopene content in dried tomato waste***

Determination was based on a spectrophotometric analysis following the method developed by Nagata and Yamashita (1992) for the simultaneous determination of chlorophyll and carotenoids in tomato fruits. Sixteen milliliters of acetone–hexane (4:6) solvents were added to 1.0 g of minced tomato wastes and mixed in a test-tube. Automatically, two phases separated, and an aliquot was taken from the upper solution for measurement of optical density at 663, 645, 505, and 453 nm in a spectrophotometer (Varian Cary 50 UV-Vis, Varian Co., USA). Lycopene and  $\beta$ -carotene contents were calculated according to the equations: Lycopene (mg/100 ml of extract) =  $-0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$ ;  $\beta$ -Carotene (mg 100/ml of extract) =  $0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$ . Lycopene and  $\beta$ -carotene were finally expressed as mg/kg fresh weight (fw).

#### ***Supplementation of the sausage with dried tomato waste***

The experiment was carried out at S.C. Casa Corina S.R.L. and consisted in the production of three types of sausages, based on the recipe and the technology of the summer sausage. Two types of sausage were made with addition of dried tomato waste, with addition levels of 5% and 10% compared with the witness without addition. Dried tomato wastes were incorporated into the mixture and samples were processed just like the witness. Figure 1 presents the mixture of the sausage with addition of dried tomato waste and the bars of sausage before the heat treatment.



Figura 1. a) mixture of sausage with addition of dried tomato waste; b) bars of sausage before the heat treatment.

#### ***Sausage quality assessment***

pH and moisture content were determined at the three sausage samples. The pH was measured with a Hanna HI 255 pH meter (USA) in the homogenate prepared from one gram of sample and 9 mL of distilled water while moisture content was determined by drying a homogenized test portion to constant mass at 103°C (ISO 1442, 1997).

### **RESULTS AND DISCUSSION**

#### ***Quality assessment of the dried tomato wastes***

A first study was conducted in order to evaluate the effect of the drying temperature on the quality of the dehydrated tomato waste.

Tomato wastes used in this study had an initial moisture content of 70% and a titratable acidity of 0.21% expressed as citric acid. Rehydration behavior was assessed in

terms of the ability of the product to regain water lost through dehydration. Rehydration ratios and rehydration coefficients of the dried samples obtained at different temperatures are presented in Table 1. It should be noted that a higher rehydration ratio indicates a superior product. Rehydration ratio ranged between 4.35 and 5.40 for the samples dried at different temperatures. Tomato waste dried at lower temperature showed a greater rehydration ratio compared to the samples dried at higher temperatures. This can be explained by the fact that at lower temperatures the cellular destruction and the water displacement occur with lower intensity, and thus the material has a greater ability to reabsorb water. Similar to the rehydration ratio, a higher coefficient of rehydration indicates a superior product and, as the temperature decreased, the coefficients of rehydration increased.

Table 1  
Rehydration ratios and rehydration coefficients of tomato wastes dehydrated at different temperatures

Temperature (°C)	60	70	80	90	100
Rehydration ratio at 25°C	5,40	5,19	4,77	4,42	4,35
Rehydration coefficient at 25°C	184,09	176,93	162,61	150,68	148,30
Rehydration ratio at 100°C	5,86	5,22	5,10	4,78	4,49
Rehydration coefficient at 25°C	199,77	177,95	173,86	162,95	153,07



Figura 2. Appearance of tomato waste after drying (a) and after grinding of the dried sample (b)

Table 2  
Lycopene and  $\beta$ -carotene content of dried tomato waste at different temperatures

Temperature (°C)	60	70	80	90	100
Lycopene (mg/100 g)	51,06	50,44	48,87	46,33	42,19
$\beta$ -Carotene (mg/100 g)	9,56	8,87	8,02	7,28	6,72

Dehydrated tomato wastes contained 42.19-51.06 mg /100 g lycopene while  $\beta$ -carotene content was between 6.72 and 9.56 mg /100 g (Table 2). There were significant differences in color between the samples dried at different temperatures. Drying tomato wastes at higher temperatures led to brown products, with darker color, due to the non-enzymatic darkening phenomena (caramelization, Maillard reactions) which occur at higher temperatures.



### ***Effect of dried tomato waste addition on the sausage quality***

Dehydrated tomato waste had a pH of 4.32 while the pH of the witness was 5.95. The incorporation of dried tomato waste caused a slight decrease in pH of the sausage, decrease which was dependent on the addition level. The sample with 5% addition level had a pH of 5.68 whereas the sample made with 10% tomato waste addition had a pH of 5.35. As expected, the addition of dehydrated tomato wastes determined the decrease of moisture content of the sausage. The witness had a moisture content of 48.7%, the sample with 5% addition level showed a moisture content of 46.76%, while the sample with 10% addition level registered a moisture content of 44.83%. This decrease of moisture content can be observed, the bars with dried tomato waste addition being firmer relative to the witness. From the sensorial point of view, the addition stands out in the sliced product as fragments of rehydrated skins (basically dried tomato waste absorbed moisture from the mixture of the sausage), but also the addition influences the taste of the product, giving it a sweeter taste and a certain acidity. It can be said that the product is acceptable, although we can make the following observations:

- dried tomato wastes require a more advanced grinding in order to obtain a fine powder, such as by rehydration in the mixture of sausage, to avoid the reconstitution of large pieces of skins which may confer some unpleasant fibrousness;
- the mixture of the sausage requires a proper blending for the uniform distribution of the fine powder, so that the visual effect be similar to an addition of paprika.



Figura 2. The appearance of the sausage samples (the sample with 10% addition of dried tomato waste and the witness)

### **CONCLUSIONS**

It was developed a new type of sausage enriched in lycopene and other bioactive compounds by the addition of tomato skins and seeds resulted as waste from the tomato processing industry. The product contains levels of lycopene which may increase the intake of this carotenoid in the diet. The direct use of the waste from processing of tomatoes, thus avoiding the extraction of lycopene, has clear benefits. Also tomato skins contain fibers and their addition to meat products can lead to healthier products. Research can be continued to study the effects of adding dried tomato waste to inhibit lipid oxidation during maturation and storage of meat products and even the possibility of partial replacement of nitrite by the addition of dehydrated tomato skins in dry fermented sausages and other meat products. Dehydration of tomato wastes (skins and seeds) at lower temperatures (60°C) leads to a better product, with intense orange color which reminds of paprika, without shades of brown and with higher rehydration capacity.

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**MONITORING OF NITRITES AND NITRATES CONTENT IN SEVERAL  
VEGETABLE SPECIES IN THE CARACAL AREA, DOLJ COUNTY.  
CASE STUDY ON EXPOSURE TO NITRATES  
FROM FOODS AND WATER**

Violeta Nour\*

*Keywords: nitrates, nitrites, vegetables, water, risk assessment*

**ABSTRACT**

*Population exposure to chemical hazards comes from both environmental factors but also from foods. Effects of certain residues and contaminants are even more complex and harder to estimate as these factors act simultaneously and cumulatively. This study evaluates the risk of exposure to nitrates for people living in the Caracal area. Nitrates come both from foods of animal origin and from vegetables as well as from drinking water derived from local unsupervised sources, heavily polluted with nitrogen compounds. In this paper it was calculated the estimated daily dose (EDD) for two scenarios, separately for adults and children. The risk for nitrates intake, quantified in the form of Hazard Ratio, was below one for adults, while for children HR was above but close to one. The risk can be reduced by drinking water from controlled sources and by consuming vegetables from the group "with lower risk" in terms of nitrate levels.*

**INTRODUCTION**

Exposure to nitrates and nitrites is very different, varying with age, weight, eating habits, season (winter salad is rich in nitrates), climatic conditions (forcing crops by applying fertilizer), variety, method of cultivation (greenhouse/open space). Effects of certain residues and contaminants are even more complex and harder to estimate as these factors act simultaneously and cumulatively.

Vegetable products may contain large amounts of nitrates as a result of crop fertilization in order to obtain high yields. Among these, lettuce, spinach, potatoes contain the highest amounts of nitrate. These levels are highly variable depending on variety, season, fertilization, climatic conditions, etc.

It has been estimated that vegetables are a major source of human exposure to nitrates contributing by approx. 40 to 90% of the average daily intake (Ximenes et al., 2000; Eichholzer & Gutzwiller, 2003). The concentrations of nitrates in vegetables can vary between 1 and 10000 mg/kg based on the biological properties of the species, variety, light intensity, soil composition, temperature, density of growth, humidity, plant maturity,

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growth period, time of harvest, size of vegetables, shelf life, edible portion of the plant and nitrogen sources (Fytianos & Zarogiannis, 1999).

There may be significant differences even between different samples of the same variety of vegetable. De Martin and Restani (2003) showed that green leafy vegetables accumulate the highest amounts of nitrate (the concentrations may reach 6000 mg/kg). Nitrite content of most vegetables is relatively low and typically in the range of 0-2 mg/kg.

Nitrite levels of fruits are low compared to vegetables, usually remaining below 10 mg/kg. Nitrite contents of fruits were found just below 1 mg/kg (Nabrzyski & Gajewska 1994; Susin et al., 2006).

It was estimated that the contribution of milk and dairy products at the overall ingestion of nitrates/nitrites is very low (Nikolas et al., 1997; Luf, 2002). Most milk samples contained nitrate in quantities not exceeding 2 mg/kg while nitrites were present only in trace amounts or they were not detected at all.

Nitrate concentrations in bread and cereals are in the range 4-20 mg/kg, nitrite concentrations remaining below 1 mg/kg (Nabrzyski et al., 1990; Ysart et al., 1999). At present, maximum limits for nitrates in foods have been established in the European Union only for spinach, lettuce and baby products. Limits established depend on the season of harvest and conditions (place) of culture, higher concentrations being permitted at the plants grown during winter and in greenhouse conditions.

Drinking water is considered as the second source of nitrates in the diet after vegetables (Belitz & Grosch, 1999; Caballero Mesa & Rubio Armendariz, 2003; WHO, 2004). According to the results of various studies, 20% of the total intake of nitrates is derived from the consumption of drinking water (White, 1983). In order to ensure the safety of drinking water, maximum permitted levels were established for nitrates and nitrites (50 mg/l and 0.5 mg/l respectively).

## **MATERIALS AND METHOD**

### ***Monitoring the nitrates and nitrites content***

To estimate the intake of nitrates and nitrites from vegetables, a total of 60 samples of vegetables were analyzed, especially those that have been incriminated in the scientific literature as the most loaded with nitrates (lettuce, spinach, beetroot, white radish, carrots, cabbage, tomatoes).

The plant material was purchased from private producers, having different origins and cultivation methods. Measurements were performed in accordance with STAS 11581-83. The method consists in the determination of nitrites by measuring the intensity of the color formed in the reaction of diazotization between sulfanilic acid and nitrites from the aqueous extract of the sample and coupling with alpha-naphthylamine.

Total nitrites content is determined using a standard curve, while nitrates content is calculated from the difference between total nitrites content and initial nitrites content.

Readings were made with a Metertek SP-830 spectrophotometer at a wavelength of 520 nm, relative to a blank in which the sample solution was replaced with water.

### ***Risk assessment of exposure to nitrates***

For risk assessment, expressed by Hazard Ratio (HR), first is evaluated the Estimated Daily Dose (EDD) by adding together the components from water, vegetables and meat products. These components were compared to see which one is the main cause of the risk.

EDD for nitrates can be evaluated by summing the effects produced by ingestion of water contaminated with nitrates, of vegetables and of meat products (Coquet, 2007). Two scenarios can be made, one for adults and one for children.

For adults, a person of mass 70 kg is taking into account, which consumes 2 liters of water/day (nitrates = 90 mg NO<sub>3</sub>/kg), 75 g carrots/day (nitrates = 38 mg NO<sub>3</sub>/kg), 50 g spinach/day (nitrates = 72 mg NO<sub>3</sub>/kg), 50 g salad/day (nitrates = 100 mg NO<sub>3</sub>/kg), 50 g beetroot/day (nitrates = 152 mg NO<sub>3</sub>/kg), 150 g tomatoes/day (nitrates = 10 mg NO<sub>3</sub>/kg) and 50 g meat products/day (nitrates = 35 mg NO<sub>3</sub>/kg) (Roman, 2005). It was considered an exposure rate of 50% of the total time, assuming that the pollution occurred relatively recently and the person was born in another part of the country, unpolluted.

For children, a child of mass 35 kg and 12 years old is taking into account, which consumes 1.5 liters of water (nitrates = 90 mg NO<sub>3</sub>/kg), 75 g carrots/day (nitrates = 38 mg NO<sub>3</sub>/kg), 50 g spinach/day (nitrates = 72 mg NO<sub>3</sub>/kg), 50 g salad/day (nitrates = 100 mg NO<sub>3</sub>/kg), 50 g beetroot/day (nitrates = 152 mg NO<sub>3</sub>/kg), 150 g tomatoes/day (nitrates = 10 mg NO<sub>3</sub>/kg) and 50 g meat products/day (nitrates = 35 mg NO<sub>3</sub>/kg) (Roman, 2005). It was considered an exposure rate of 80% of the total time, as the time of life the child lived in this polluted environment.

EDD was estimated using the following formula (Coquet, 2007):

$$EDD = \frac{\sum \text{Concentration} \times \text{Amount eaten} \times \text{Exposure rate}}{\text{Body weight}} \quad [1]$$

Risk characterization is done by the Hazard Ratio (HR):

$$HR = \text{Estimated Daily Dose} / \text{Acceptable Daily Intake} \quad [2]$$

In order to estimate the exposure through the ingestion of water, 150 samples of water from local sources in the Caracal area were analyzed.

## RESULTS AND DISCUSSION

### *Monitoring the nitrates and nitrites content*

The results obtained for the vegetables analyzed were found to be lower than the maximum allowed. The nitrate content in the salad analyzed is about 20 times lower than the maximum level established by UE Regulation 1881/2006 for summer salad obtained in greenhouse. The same for spinach, values are much lower than the values specified in that order, i.e. 2500 mg NO<sub>3</sub>/kg.

Table 2  
The concentration of nitrates and nitrites in the studied species (mg/kg)

No.	Product	Nitrites	Nitrates	Nitrite ions	Nitrate ions
1	Green salad	0.27±0.15	152.59±131.65	0.18±0.10	93.54≥80.7
2	Spinach	0.46±0.67	118.06±108.93	0.31±0.44	72.37≥66.77
3	Carrot	0.39±0.43	62.43±83.59	0.26±0.29	38.27≥51.24
4	Red beet	16.09±20.74	247.75±172.60	10.73±13.83	151.87≥105.85
5	White radish	0.37±0.34	185.81±159.77	0.25±0.22	113.90≥97.94
6	Cabbage	0.22±0.26	45.36±21.80	0.15±0.17	27.81≥13.36
7	Tomato	0.36≥0.16	16.55≥5.77	0.24≥0.11	10.14≥3.53

The nitrite content varied between 0.151 mg NO<sub>2</sub>/kg (cabbage) and 0.310 mg NO<sub>2</sub>/kg (spinach), except red beet which registered 10.73 mg NO<sub>2</sub>/kg.

A relatively high content of nitrates was determined in red beet, white cabbage, salad and spinach (table 2). Relatively low values were found in cabbage and tomatoes and

intermediate values in carrots. Salad presented an average content of 93.53 mg NO<sub>3</sub>/kg, spinach 72.37 mg NO<sub>3</sub>/kg, and white radish 113.90 mg NO<sub>3</sub>/kg. Red beet presented an unusually high content of nitrate ions (151.87 mg/kg), while carrots contained 38.27 mg NO<sub>3</sub>/kg and tomatoes only 10.14 mg NO<sub>3</sub>/kg.

**Risk assessment of exposure to nitrates**

Using formula [1] EDD was estimated for adults and in a similar manner for children.

Considering an exposure rate of 100% we obtained:

$$EDD = \frac{\sum \text{Concentration} \times \text{Amount eaten} \times \text{Exposure rate}}{\text{Body weight}}$$

$$EDD_{\text{adult}} = \frac{90\text{mg}/1 \times 21}{70} + \frac{38\text{mg}/\text{kg} \times 0.075\text{kg} + 72\text{mg}/\text{kg} \times 0.05\text{kg} + 100\text{mg}/\text{kg} \times 0.05\text{kg}}{70} + \frac{152\text{mg}/\text{kg} \times 0.05\text{kg} + 10\text{mg}/\text{kg} \times 0.15\text{kg}}{70} + \frac{35\text{mg}/\text{kg} \times 0.05\text{kg}}{70}$$

$$EDD_{\text{adult}} = 2.57 + 0.293 + 0.025 = 2.888$$

If, however, the exposure rate is 50%, then EDD<sub>adult</sub>=1.444.

In a similar manner, for children and for an exposure rate of 100% we obtained:

$$EDD_{\text{child}} = \frac{90\text{mg}/1 \times 1.51}{35} + \frac{38\text{mg}/\text{kg} \times 0.075\text{kg} + 72\text{mg}/\text{kg} \times 0.05\text{kg} + 100\text{mg}/\text{kg} \times 0.05\text{kg}}{35} + \frac{152\text{mg}/\text{kg} \times 0.05\text{kg} + 10\text{mg}/\text{kg} \times 0.15\text{kg}}{35} + \frac{35\text{mg}/\text{kg} \times 0.05\text{kg}}{35} =$$

$$EDD_{\text{child}} = 3.855 + 0.586 + 0.05 = 4.491$$

For an exposure rate of 80 % we obtained:

$$EDD_{\text{child}} = 4.491 \times 0.8 = 3.59$$

Plotting each component of the EDD, it can be seen that although drinking water has the lowest concentration limits (maximum allowed by the law 458/2006 is 50 mg nitrate/l), it still contributes most to EDD due to the large amount consumed (Figure 4).

The characterization of the risk is done through the value of Hazard Ratio (HR):

$$HR_{\text{adults}} = \text{Estimated Daily Dose}/\text{Acceptable Daily Intake} = 2.888/3.7 = 0.78$$

$$HR_{\text{children}} = \text{Estimated Daily Dose}/\text{Acceptable Daily Intake} = 4.491/3.7 = 1.213 > 1$$

Table 3

EDD and HR estimated for adults and children for nitrates

	Adults	Children
EDD	2.888	4.491
HR	0.78	1.213

Following the scenarios conducted, a set of conclusions can be drawn:

- the risk of nitrates intake in adults is low;
- the risk of nitrate intake in children is higher than one, so the risk at children is present and is even greater as the child is smaller (body weight is decisive in this respect).

Due to the high proportion of the rural population which drinks water from uncontrolled sources, the risk of exposure to nitrate is a collective one. In all cases water has a major contribution to HR and therefore it is the component that must be removed fastest.

The variability of this risk is still great due to the eating habits of the population, the permanent variability of groundwater quality, the different age of the subjects and the variability of the conversion rate of nitrate to nitrite.

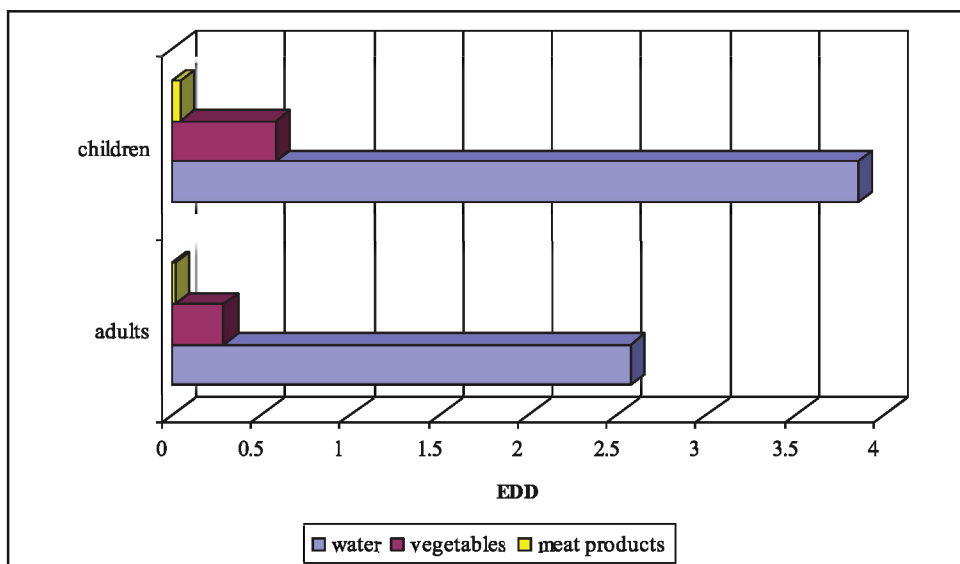


Figure 4. Graphical representation of the components contributing to Estimated Daily Dose (EDD).

### CONCLUSIONS

From the evaluation of the quality of foods containing nitrates (water, vegetables, meat products) it results that all three components contribute to some extent to the presence of the risk of exposure to nitrates to the population in a certain geographic area.

A significant proportion of the rural population uses water from uncontrolled sources. It is urgently needed to develop water networks at the level of small communities. As a transitional measure, each resident must control the household drinking water and must avoid using it in case it does not meet the standards.

Some of the dietary vegetables (lettuce, spinach, red beet) can make a high contribution to the risk of exposure to nitrates. Other vegetables like carrots, tomatoes, cucumbers, present a lower risk in this regard. Commercial meat products also contribute to the overall risk. Although the limits imposed by law for water are much lower than for meat products or vegetables, water has a high contribution to the risk of exposure to nitrates due to the high amounts consumed.

Even if water, vegetables and meat products taken separately have no effect on human health, the association of these three components can lead in some cases to a high risk, especially on children.

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## EFFECT OF PLANT GROWTH REGULATOR ON PREHARVEST FRUIT DROP ON THE GOLDEN REINDERS APPLE VARIETY

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*Keywords: Apple, fruit drop, growth regulator, NAA, preharvest spray.*

### ABSTRACT

*The experimental plot is placed in the orchard "Dacfruct" Ltd. founded in 2006. The study subject of the experience was Golden Reinders apple variety grafted on M 9. The trees were trained as slender spindles. The distance of plantation is 3.5 x 1.2 m.*

*The research was conducted during the period of 2013 year. The aim of this study was to evaluate the effectiveness of grower regulator agent in reducing fruit drop et in Golden Reinders apple trees. The tested agents were Obsthormon 24a (NAA), which was sprayed in one time in preharvest period. During the research, it was studied the quantity of dropped fruits under the trees and percentage of total fruit yield.*

*It was established that, in the 2013 year, one treatment with Obsthormon 24a in dose of 500 ml/ha have a significant effect on fruit drop and fruit quality.*

### INTRODUCTION

'Golden delicious' and its clones was one of the most popular and commercially important apple cultivars in Moldova where it makes up about 20% of the national crop. Preharvest fruit drop can reduce production of apple cultivars in commercial orchards by as much as 30% (Marini et al., 1993; Yuan & Carbaugh, 2007. Losses resulting from preharvest fruit drop can be mitigated by applying either naphthaleneacetic acid (NAA) to delay drop.

Naphthaleneacetic acid (NAA) is an auxin-type growth regulator that primarily is used to reduce preharvest drop. NAA does not strengthen up the fruit attachment, but only prevents further loosening from the fruit stem. When it is used to reduce drop, it does not delay ripening Kvikliene et al., 2010; Schupp & Greene, 2004; Yuan & Li, 2008.

The aim of this study was to assess the effects of Obsthormon 24a (NAA) used during the preharvest period, on fruit drop and fruit quality.

### MATERIAL AND METHODS

The researches were made during the period of 2013 year in the apple orchard founded in autumn 2006 at the company "Dacfruct" Ltd. with "knip boom" type apple trees crown formation. The study object of experience was Golden Reinders apple tree variety

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grafted on M9 rootstock. The crown was conducted on ameliorated thin spindle system. Distance of plantation is 3.5 x 1.2 m.

The chemical growth regulator used was Obsthormon 24a, containing 84 g/l active ingredient NAA, the preparation by the „L. Gobbi Ltd.” producer from Italy. To optimize the fruit dropping of the apple trees were experimented the following variants: V<sub>1</sub> - Control variant- without chemical treatments; V<sub>2</sub> - Obsthormon 24a - 400 ml/ha; V<sub>3</sub> - Obsthormon 24a - 500 ml/ha.

On experimental section in accordance with the experiences scheme in the second and in the third variants were made a single treatment (09/15/13) with a dose of 400 ml/ha and 500 ml/ha. Fruits drop was expressed as quantity dropped under the trees and percentage of total fruit yield.

## RESULTS AND DISCUSSIONS

In the fall before collecting fruits, apple orchards register a premature dropping. Premature fruit dropping is registered when apple fruit are not harvested in the optimally set terms. To exclude this phenomenon, or reducing the amount of the fallen fruit, it is recommended to treat the trees with the fruit-based growth regulator NAA 10-20 days before harvest.

Investigations conducted demonstrated that during treatment (09/04/2013) the amount of fruit in the trees crown on apple variety Golden Reinders was 103-105 pcs (tab. 1). This amount of fruit in the trees crown is considered optimal to achieve consistent and qualitative annually productions. If in the control variant, the total amount of fruit from the tree crown was 104 units, then in the version treated with the growth regulator Obsthormon 24a ranged between 103-105 pcs/tree. A slight increase in the amount of fruit per tree was recorded from variant 3, where was planned the treatment with growth regulator Obsthormon 24a in dose of 500 ml/ha - 105 pcs/tree, compared to variant where was treated with Obsthormon 24a in dose of 400 ml/ha - 103 pcs/tree.

Table 1  
The influence of growth regulator Obsthormon 24a on the quantity of fruits before and after treatment in the trees of the variety Golden Reinders, average fruit weight and diameter

Nr.	Variants	Quantity of fruits, pieces			The average weight, g	The average diameter, mm
		total per tree at treatment	on trees at harvest	dropped under the trees		
1.	Control, without treatment	104	86	18	167.0	72.0
2.	Obsthormon 24a, 400 ml/ha	103	98	5	168.0	72.3
3.	Obsthormon 24a, 500 ml/ha	105	102	3	167.8	72.2

This slight difference between variations in study 2 pcs /tree allowed us to mount the experiment for testing the growth regulator Obsthormon 24a to prevent premature fruit dropping in the fall before harvest. On the day of collection (09/15/2013) has been noticed that the amount of fruits in the trees crown in the variants in study was 86 to 102 pcs. A smaller amount of fruits in the crown of trees was recorded for control variant - without treatment, where the studied index is 86 pcs /tree.



In the variants treated with the growth regulator Obsthormon 24a, the amount of fruit in the crown of trees was 98-102 pcs. In the variant where is made the treatment with Obsthormon 24a in dose of 500 ml/ha were recorded maximal values 102 pcs/tree and in the variant treated with Obsthormon 24a in dose of 400 ml/ha - 98 pcs/tree. The difference between the amount of fruit in variant Obsthormon 24a in dose of 400 ml/ha and control variant-without treatment was 12 pcs/tree, and between the variant Obsthormon 24a in dose of 500 ml/ha and variant control -16 pcs/tree.

The most important index of apple plantation treatment with growth regulator to prevent fruit dropping is the amount of fruit fallen to the ground from the crown. The conducted investigations show that in variant control is recorded the largest amount of fruit fallen - 18 pcs/tree. The treatments with growth regulator Obsthormon 24a decreased essential premature fruit dropping until harvest. When the treatment with Obsthormon 24a was made in dose of 400 ml/ha amount of fallen fruit was 5 pcs and when the doses was 500 ml/ha was only 3 fruits.

Therefore, treating trees with growth regulator Obsthormon 24a improved physiological processes that occurred in plants, didn't allowed to form suber layer between stalk and fruit bearing formations and prevented their premature dropping.

The use of growth regulator Obsthormon 24a had the least influence on the weight and the average diameter of the fruit because on the ground fall the largest fruits which usually have an early maturity. The average fruit weight in variant control was 167.0 g and diameter was 72.0 mm. In the variant treated with Obsthormon 24a in dose of 400 ml/ha indexes studied were increased respectively by 6.0% and 4.2% and where the treatment with Obsthormon 24a was in dose of 500 ml/ha with 4.8% and 2 8%.

Studying the influence of treatment dose on the indicators investigated, recorded, an insignificant difference between variant Obsthormon 24a in dose of 400 ml/ha and the dose of 500 ml/ha. If in the variant Obsthormon 24a in dose 400 ml/ha amount of fruit prematurely fallen from trees crown was 5 pcs/tree, then in the variant Obsthormon 24a in dose of 500 ml/ha was 3 pcs/tree. The average diameter of the fruit weight was the same.

In apple orchards, the fruit production is one of the major index to the effectiveness of technological elements.

The conducted investigations (tabel 2) demonstrate that total fruit production in the variants studied was from 17.30 to 17.53 kg/tree. The results show that the treatment made with Obsthormon 24a growth regulator did not influence the total production registered per tree. If the productivity per tree in control variant was 17.36 kg, and the variants treated with Obsthormon 24a - 17.30 - 17.56 kg/tree, then it is confirmed by statistical analysis as between variants in the study there wasn't a significant difference.

Further studying the difference between production harvested from the tree and fallen on the ground, we record that a positive co-report records the variants treated with growth regulator Obsthormon 24a.

If in the control variant where no treatment was carried the production of fruits harvested from tree was 14.26 kg and the one collected from the ground was 3.10 kg, then the use of Obsthormon 24a had a positive influence on production remaining in trees crown.

The growth regulator Obsthormon 24a has naphthaleneacetic acid as an active ingredient which increases the amount of hormones formed in the plant. As a result of physiological changes that occur in the plant is delayed suber layer formation between fruit formation and fruit peduncle, which prevents their premature dropping.

When the treatment with growth regulator Obsthormon 24a was applied the fruit production being harvested from crown increased from 16.45 to 16.99 kg/tree and of the soil collected decreased recording values of 0.54 - 0.85 kg/tree. This difference between the

control, untreated and treated variants with Obsthormon growth regulator 24a is also confirmed by statistical data.

Studying the influence of growth regulator Obsthormon 24a depending on the used dose, we record a slight increase in the amount of fruit collected from the tree such as, in variant Obsthormon 24a in dose of 500 ml/ha - 16.99 kg compared with in the variant Obsthormon 24a in dose of 400 ml/ha - 16.45 kg, but statistical processing data do not show a significant difference between variants 2 and 3.

Table 2

The influence of growth regulators Obsthormon 24a on fruit production obtained from a tree of Golden Reinders apple variety on harvest

Nr.	Variants	Production, kg/tree			Share production fallen, %
		On the tree	Dropped from the tree	total	
1.	Control, without treatment	14.26	3.10	17.36	17.85
2.	Obsthormon 24a, 400 ml/ha	16.45	0.85	17.30	4.91
3.	Obsthormon 24a, 500 ml/ha	16.99	0.54	17.53	3.08
LSD 5%		0.60	0.17	0.67	-

The conducted investigations show that the largest share of fallen fruit was recorded in the control, untreated - 17.85%. On the next place with an essential difference is the variant Obsthormon 24a in dose of 400 ml/ha - 4.91% and then the variant Obsthormon 24a in dose of 500 ml/ ha - 3.08%.

Studying the fruit production per unit area, we record the index in study is closely correlated with the amount of trees per hectare and the productivity of a tree.

The data of table 3 shoes that growth regulator Obsthormon 24a did not influence the total production of fruits and was almost the same as in the control variant without treatment. In control variant fruit production was 39.68 t/ha, in the variant treated with Obsthormon 24a in dose 400 ml/ha - 39.54 t/ha and with Obsthormon 24a in dose of 500 ml/ha - 40.07 t/ha. It is obvious that from the amount of total production, some fell on the ground until the harvest, and the other was collected in the crown of trees. The ratio of the production amount collected from the ground and the one from crown of the tree is different and has been influenced by growth regulator Obsthormon 24a whose active ingredient is naphthaleneacetic acid.

In the control variant, the production collected from trees crown was 32.30 t/ha and harvested from the ground for industrialization 7.38 t/ha, or 22.8% of the share of quality.

The treatment with growth regulator Obsthormon 24a positively influenced on maintaining maximum production of apples in trees crown. When treating with Obsthormon 24a in dose of 400 ml/ha the production collected from crown was 37.52 t/ha and from the soil was 2.02 t/ha, which represented 5.3% of the share of quality. In the variant Obsthormon 24ain dose of 500 ml/ha the share of low quality production collected from the soil was 3.3% compared with that collected from the crown.

The effectiveness of treating with growth regulator Obsthormon 24a is demonstrated by the difference in production collected from the treated variants and the

version control without treatment. When treating with the growth regulator Obsthormon 24a in dose of 400 ml/ha, the difference between the production collected from the crown and the control variant was 5.22 t/ha and in the variant Obsthormon 24a in dose of 500 ml/ha increased, registering 6.49 t/ha.

Table 3

The influence of Obsthormon 24a growth regulators on fruit production obtained from a unit area of the variety Golden Reinders at harvest

Nr.	Variants	Production, t/ha			The difference between the production left and control
		total	dropped under the tree	in the tree	
1.	Control, without treatment	39.68	7.38	32.30	-
2.	Obsthormon 24a, 400 ml/ha	39.54	2.02	37.52	+5.22
3.	Obsthormon 24a, 500 ml/ha	40.07	1.28	38.79	+6.49
	LSD 5%	1.68	0.24	1.20	-

The obtained results show that between the variants where we use growth regulators to prevent fruit drop as Obsthormon 24a, more convincing results are obtained if the dose is 500 ml/ha, and there was a more favorable co-ratio between the amount of fruit collected from the crown and the sol.

In practice, it's a known fact that the new agro links developed should contribute not only to increase yield, but also to the quality (firmness, soluble dry substance, etc.), or at least not diminish it. For treating the winter varieties of apples with the growth regulator based on NAA, the recommended period is 10 -20 days before harvest. Basically, during this period the flesh firmness is 8.8 to 8.9 kg/cm<sup>2</sup>.

The conducted investigations (tab. 4) demonstrate that apple fruit flesh firmness on Golden Reinders variety 10 days before harvest was 8.5 to 8.6 kg/cm<sup>2</sup>. Basically, difference in the firmness of the flesh in that period wasn't recorded. In version control pulp firmness was 8.6 kg/cm<sup>2</sup>, and in the variants where was expected treatment with growth regulator Obsthormon 24a was 8.5 to 8.6 kg/cm<sup>2</sup>.

At the time of harvest (10 days after treatment) the Golden Reinders variety flesh firmness decreased to 7.1 to 7.7 kg/cm<sup>2</sup>. The smallest firmness has been in variant control - 7.1 kg/cm<sup>2</sup>.

When treating with growth regulator Obsthormon 24a, the pulp firmness was 7.5-7.7 kg/cm<sup>2</sup>. This demonstrates that preparations based on naphthaleneacetic acid inhibits physiological processes of formation of ethylene and increases fruit firmness. These fruits can be stored in refrigerated rooms for a long period of time.

Dropped fruits on the ground have a much lower firmness compared to the fruits from the trees crown. The firmness of the fruit in the studied variants ranged from 6.0-6.4 kg/cm<sup>2</sup>. So, the difference in firmness of fruit fallen on the ground and the ones collected from the trees crown was 1.1-1.3 kg/cm<sup>2</sup>. The lowest value was recorded in the control variant - 1.1 kg/cm<sup>2</sup>. Variants treated with growth regulator Obsthormon 24a showed a greater determination being 1.3 kg/cm<sup>2</sup>.

The data obtained demonstrates that the amount of soluble dry substances on fruits of the trees at Golden Reinders variety was 12.6 to 13.1%.

The highest value of the weight soluble dry substance was obtained in the control, no treatment variant- 13.1%. When treating with Obsthormon 24a is noticed a decrease of 0.4-0.5% of the index in the study compared to the control variant, representing 12.6 to 12.7%. This demonstrates that Obsthormon 24a increased fruit firmness and decreased the amount of soluble solids.

Table 4

The influence of growth regulators Obsthormon 24 on firmness and soluble solids content of fruits of Golden Reinders apple variety

Nr.	Variants	Firmness, kg / cm <sup>2</sup>			Soluble solids (% harvest)	
		at treatment	at harvest	dropped fruits	fruits of the tree	dropped fruits
1.	Control, without treatment	8.6	7.1	6.0	13.1	14.4
2.	Obsthormon 24a, 400 ml/ha	8.5	7.5	6.2	12.6	14.2
3.	Obsthormon 24a, 500 ml/ha	8.6	7.7	6.4	12.7	14.1

Dropped fruits on the ground have a smaller firm and a high content of soluble solids, representing the variations in the study from 14.1 to 14.4%. The amount of soluble solids demonstrates that fruit fell to the ground with a degree of maturation before, and can only be used for industrialization.

### CONCLUSIONS

The treatments made with growth regulator based of NAA 10 days before harvesting decreased essentially the amount of dropped fruits.

The fruit firmness and soluble solids content recorded higher values in variants treated with Obsthormon 24a then in the control variant.

The smallest amount of dropped fruits at harvest was recorded in the variant Obsthormon 24a in dose of 500 ml/ha.

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**EVALUATION OF AMPELOMETRIC PARAMETERS THAT DETERMINES THE FORM AND DEPTH LATERAL SINUSES AT MATURE LEAF OF THE SOME HYBRID ELITES OBTAINED FROM HYBRID CROSSING MUSCAT IANTARNÎI X CANNER**

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*Keywords: ampelometric descriptors, hybrid crossing, leaf of grapevine*

**ABSTRACT**

*In order to describe ampelographic the new genotypes for table grapes, obtained by way of intraspecific hybridization, were used the following descriptors ampelometric: length of veins (N1, N2, N3, N4), angles between main veins ( $\alpha$ ,  $\beta$ ,  $\gamma$ ), distances from the base of upper lateral sinuses to petiole point (d1 and d2), the ratio between length of the veins N2, N3, N4 and length of the main vein, and the ratios d1/N2, d2/N3 respectively.*

*The evaluation of ampelometric parameters at the mature leaves, coming from the hybrid descendants of the hybrid crossing Muscat Iantarnii x Canner (HI), has contributed to a better understanding the way in which these characters are transmitted in lineage, the mature leaf representing the principal vegetative organ for describing and recognizing the varieties of grape-vines.*

**INTRODUCTION**

Over time there have been many concerns for understanding, describing and differentiating varieties of grapevines. Until the beginning nineteenth century varieties presentation is limited exclusively to botanical description of vegetative organs, particularly the bunch, each author using their own methods and terminology, which often were generating the confusions (Olteanu I. et al. 2012, Damian Doina et al. 2011).

The relationship between the shape of leaf and angles which are made by the main veins between them is revealed by Herman Goethe in 1867 (quoted by Galet P., 1990) reason why suggests that the description and characterization of grapevine varieties to be based on the architecture of the leaf, considered to be the main ampelographic organ.

In 1936, M. Lazarevski (quoted by Mîndrilă, 2009), shall draw up on the same principle a determiner for 257 fruitful vine varieties, regarding essential characters that have ampelometric value.

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For the description of varieties Rodrigues M. A. (quoted by Vintilescu Monica, 2012) proposes, in 1955, filometric method making the measurements on the graph paper of the representative 9 points of the leaf.

Based on the ampelographic measurements made Galet get specific ratios on which encodes, reaching to an ampelographic index, specific to each variety (Oşlobeanu M. et al., 1980 quoted by Olteanu I. et al. 2002).

In our country the ampelometric method was used for the first time in 1937 by Billeau A., who drew up ampelometric diagram of the *Vitis vinifera* leaf for 33 varieties the most widespread in the culture at that time specifying the that this method can complete the botanical description of varieties using the numerical values, specific to each variety (Stroe Marinela, 2012).

### MATERIAL AND METHODS

The method consists in choosing of 10 adult leaves (it is considered the inserted leaf on the main shoot between nodes 9-12), measurement of the length of main vein (N1, N2, N3, N4), the angles which are made by the main veins between them ( $\alpha$  – between N1 and N2,  $\beta$  –between N2 and N3,  $\gamma$  –between N3 and N4), is measured distance between the petiole point and the base of the lateral sinuses ( $d_1$  and  $d_2$ ). Based on the resulted measurements the ratios values are established ( $N_2/N_1$ ,  $N_3/N_1$ ,  $N_4/N_1$ ), is determined the sum of the angles between the veins in view of their interpretation ( $\sum \alpha + \beta$  and  $\sum \alpha + \beta + \gamma$ ), it is determined the ratios between distances sinuses and the length of the main vein  $d_1/N_2$  and  $d_2/N_3$ . The ratios between the length of the veins  $N_2/N_1=A$ ,  $N_3/N_1=B$ ,  $N_4/N_1=C$  have been grouped into ten classes of variation, from 0.01 to 1.00 and on the basis of those values the shape of leaf can be: *Circular*: A = 0.91-1.00; B = 0.71-0.80; C = 0.41-0.50; *Kidney-Shaped*: A = 0.91-1.00; B = 0.51-0.90; C = 0.31-0.60; *Cordate*: A = 0.51-0.71; B = 0.31-0.50; C = 0.11- 0.30; *Wedge-shaped*: A = 0.71-0.90; B = 0.51-0.70; C = 0.21-0.50; *Tronconic*: A = 0.81 -1.00; B = 0.51 - 0.60; C = 0.31- 0.50. Based on the ratios values A, B, C and the value of the angles  $\sum \alpha + \beta$  and  $\sum \alpha + \beta + \gamma$  it can be determined the fundamental shape of the blade on based of the diagram conceived by Galet: circular, kidney-shaped, cordate, wedge-shaped, pentagonal, and the intermediate types: circular-kidney-shaped, wedge-shaped-cordate, wedge-shaped -pentagonal, circular-wedge-shaped. With regard the angles between the veins after the calculating the amounts  $\gamma + \beta$  then  $\gamma + \beta + \gamma$  are established classes of variation which are encoded by notes from 0 to 9. Depth of the lateral sinuses is determined by calculating the values of ratios between  $d_1/N_2$  and  $d_2/N_3$  for which they were established 10 classes of variation encoded with figures from 0 to 9.

The data recorded have been interpreted statistically using statistical test of significance Duncan for a probability of error P less than or equal to 0.05.

### RESULTS AND DISCUSSIONS

By applying of the statistical calculation provided by Duncan test for the length of main vein N1 it is found that two elites (HI17, HI19) values were significantly different comparative with the paternal genitor variety Canner. Values close to the Canner variety with the vein N1 exceeding 10 cm meet at the genotypes HI11 and Muscat Iantarnii (figure 1).

Vein length N2 is significantly different in the ratio of the genitors and hybrid descendants, exception done only elite HI11, with intermediate values. And in the case of the vein N3 statistical differences only meet between leaf of the varieties Canner, Muscat Iantarnii and HI9, HI13, HI19 genotypes. The measurements value of the vein N4 establishes that Muscat Iantarnii variety is insured statistically compared to the values of other genotypes, except elite HI17, with the values between a and b.

Regarding ratios N2 / N1 (\*21-Table 1) values range between 0.78 (elite HI 9) and 0.87 (elite HI19) compared to the recorded values by the parental varieties 0.89 (Muscat Iantarnii) and 0.82 (Canner); for N3/N1 (\*31-Table 1) have been recorded values between 0.54 (HI9) and 0.60 (elite HI19) compared to 0.63 (Muscat Iantarnii) and 0.56 (Canner) and for the N4/N1 (\*41-Table 1) the values range between 0.32 (HI11 and HI13 elites) and 0.36 (elite HI19) compared to genitors values 0.40 and 0.31. Codes assigned to these values obtained range between 135 and 246. Genitors have received codes 136 (maternal variety) and 146 (paternal variety), table 1.

Differences results by measuring the angle  $\alpha$  (between N1 and N2) and angle  $\beta$  (between N2 and N3), were statistically insured, between the values of the Muscat Iantarnii variety and elites HI17 and HI9 being significant differences. Compared to the variety Canner, the opening of the angles is not significant compared to descendants. Codes assigned according to the values obtained ranged between 34-47 compared to genitors that have the codes 56 and 35 (Table 1).

In the case of the  $\alpha + \beta + \gamma$  angles, HI11 elite differ significantly by HI9 elite (lowest value of the sum of the angles between the veins) but and comparative with other genotypes, insignificant being only the difference compared with Muscat Iantarnii (figure 2).

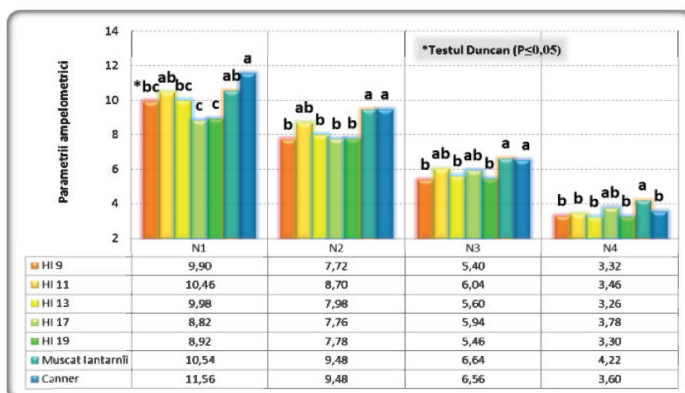


Figure 1. Influence of the HI genotypes on some ampelometric parameters (main veins)

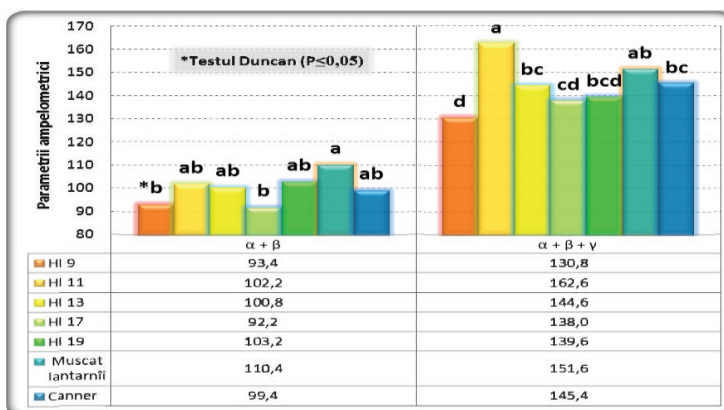


Figure 2. Influence of the HI genotypes on some ampelometric parameters (sum of the angles)

Table 1

Ampelometric parameters that determines the shape of mature leaf to hybrid elites in *Muscat Iantarnii* x *Canner* hybrid crossing compared to genitors

No. ct.	Elite/ genitor	Ampelometric Parameters										Codes			Shape Of Leaf
		N1	N2	N3	N4	$\alpha + \beta$	$\alpha + \beta + \gamma$	*21	*31	*41	$\sum \alpha + \beta$	$\sum \alpha + \beta + \gamma$	Report ABC		
1.	HI 9	9.9	7.7	5.4	3.3	93	131	0.78	0.54	0.33	3	4	246	Wedge-shaped	
2.	HI 11	10.7	8.7	6.0	3.5	102	163	0.81	0.56	0.32	4	7	146	Wedge-shaped - pentagonal	
3.	HI 13	10.0	8.0	5.6	3.3	100	145	0.79	0.56	0.32	3	5	246	Wedge-shaped	
4.	HI 17	8.8	7.8	5.9	3.8	92	138	0.87	0.66	0.42	3	4	135	Wedge-shaped	
5.	HI 19	8.9	7.8	5.5	3.3	103	140	0.87	0.60	0.36	4	4	146	Wedge-shaped - pentagonal	
6.	Muscat Iantarnii	10.5	9.5	6.6	4.2	111	152	0.89	0.63	0.40	5	6	136	Circular	
7.	Canner	11.6	9.5	6.6	3.6	99	145	0.82	0.56	0.31	3	5	146	Wedge-shaped	

\*N1, N2, N3, N4 –are expressed in centimeters.



In relation to *the depth of upper lateral sinuses* (determined based on the distance from the base of upper lateral sinuses to the petiole point denoted - d1) is observed that the differences between Muscat Iantarnii variety and other genotypes are statistically assured, the differences being significant, at the both extreme being the maternal variety gernitor Canner (d) with the lowest value (2.76 cm) and the paternal genitor Muscat Iantarnii variety with d1 very high (5.26 cm). Three elites (HI9, HI11, HI 17) significant differences have been registered comparative with both genitors (Figure 3).

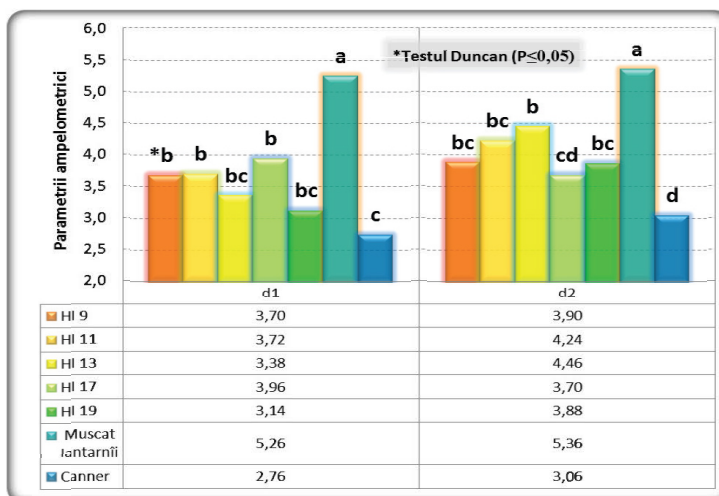


Figure 3. Influence of the HI genotypes on some ampelometric parameters (distances d1 and d2)

Table 2

Ampelometric parameters that define the depth of lateral sinuses of mature leaf to hybrid elites in *Muscat Iantarnii x Canner* hybrid crossing compared to genitors

No. crt.	Elite/ genitor	Ampelometric Parameters				Codes		Depth of sinuses	
		d1	d2	d1/N2	d2/N3	d1/N2	d2/N3	Upper	Lower
1.	HI 9	3.7	3.9	0.48	0.72	5	2	accentuated	poorly sketched
2.	HI 11	3.7	4.2	0.42	0.70	5	3	accentuated	poorly sketched
3.	HI 13	3.4	4.5	0.42	0.79	5	2	accentuated	poorly sketched
4.	HI 17	4.0	3.7	0.51	0.63	4	3	accentuated	poorly sketched
5.	HI 19	3.1	3.9	0.40	0.71	6	2	deep	poorly sketched
6.	Muscat Iantarnii	5.3	5.4	0.55	0.81	4	1	accentuated	superficial
7.	Canner	2.8	3.1	0.29	0.46	7	5	deep	accentuated

The values of distances  $d_2$  (the distance from the base of the lower lateral sinuses at the petiole point - descriptor for the determination of the depth of the lower lateral sinuses) indicates a particularly significant difference between the varieties used in hybridization and HI13 elite, which in turn is different than HI17 elite. The ratio  $d_1 / N_2$  has ranged between 0.40 (HI19) and 0.51 (HI17) compared to the genitors which have 0.55 (maternal variety) and 0.29 (paternal variety). Concerning  $d_2 / N_3$ , the values varies from 0.63 (HI17) and 0.79 (HI13) compared to 0.81 (Muscat Iantarnîi) and Canner (0.46). Attributed codes are: **52** (elite HI9), **53** (elite HI11), **52** (elite HI13), **43** (elite HI17), **62** (elite HI19) framing the leaves from accentuated/poorly sketched to deep/poorly sketched.

The parental varieties have received the codes **41** (accentuated/superficial) and **75** (deep/accentuated) which indicate that Muscat Iantarnîi has imposed in transmitting this character (Table 2).

### CONCLUSIONS

Assessment of the ampelometric parameters to the mature leaves derived from hybrid descendants of the hybrid crossing Muscat Iantarnîi x Canner (HI) has contributed to a better understanding of the way in which are transmitted these characters in lineage;

- *mature leaves* are *small*, only HI11 elite had the mature leaf of medium size, like the maternal genitor;

- the paternal genitor variety (Canner) has transmitted in F1 generation to 4 descendants (HI9, HI13, HI17, HI19) the *shape of blade* wedge-shaped, one elite (HI 11) has presented the leaf with pentagonal shape, different from the both parents;

- all the genotypes presented have the leaves with five lobes.

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**ASSESSMENT OF THE ANTIBACTERIAL ACTION OF DRY RED AND WHITE WINES PRODUCED IN THE MINIȘ-MĂDERAT VINEYARD**

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**Keywords:** red wine, white wine, antibacterial effect, bacterial cultures.

**ABSTRACT**

The antimicrobial effects of wine are long known, and its antibacterial activity largely demonstrates the efficiency of its digestive properties. Previous research attests the promising results of the antibacterial effect of wine, but are still quite contradictory.

In this paper, the antimicrobial activity of wines was determined by the agar diffusion method, being assessed on two bacterial species: *Bacillus subtilis* and *Escherichia coli*. From the results it appears that the antibacterial effect of the Burgund wine is stronger than the antibacterial effect of the Riesling Italian white wine, refuting previous studies showing that white wine solutions have a stronger antibacterial effect than red wine solutions. From the correlation of the antibacterial activity with the chemical analysis applied to wines tested we concluded that the antimicrobial effect is due to anthocyanins present in the Burgund red wine, respectively to catechin tannins present in Riesling Italian white wine.

**INTRODUCTION**

Numerous studies on the effects of moderate wine consumption on health have revealed a multitude of beneficial properties of proanthocyanidins present in wine with applications in nutrition and medicine.

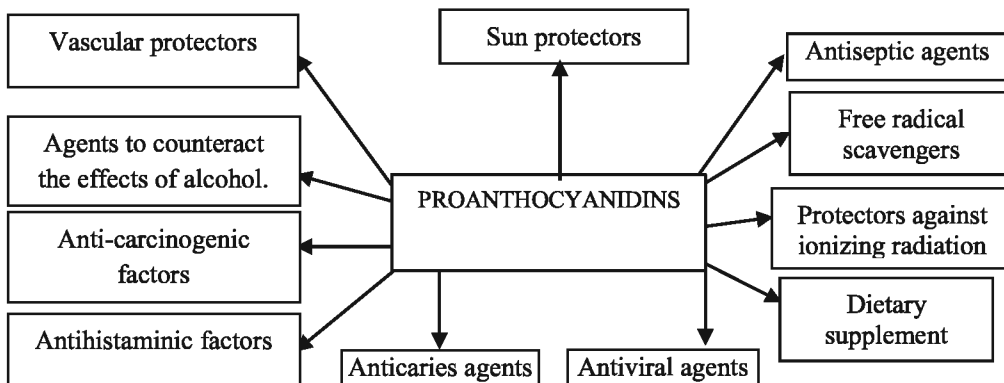


Figure 1. The positive effects of proanthocyanidins in wine (Masquelier J. and Delaunay D., 1965)

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The antimicrobial effects of wine are long known, and its antibacterial activity largely demonstrates the efficiency of its digestive properties. The following is a brief history of the main arguments for the antimicrobial effect of wine.

During the cholera epidemic that hit Paris in 1886, Rabusteau found that people who regularly drank wine during meals were more protected from the scourge compared to those who didn't. During the cholera epidemic in Paris in 1892, Viennese physician Alois Pick conducted a series of experiments that have become famous, by which he proved – using himself as a test person – the effect of wine on cholera vibrios (*Vibrio cholerae*). He found that cholera vibrio infected water can be consumed without any danger if mixed with equal parts wine, 15 minutes before consumption. Both white and red wines gave the same results; important was only to observe the vibrio contact time with the wine (Teodorescu I.C. et al., 1966).

In 1907, Sabrazes and Mercandier seeded different types of Bordeaux wines with Eberth bacilli (*Salmonella typhi*), noting that they are destroyed after a variable time – 20 minutes to two hours – depending on the number of germs inoculated. In the case of wine dilution with water, a longer time is required for the destruction of the bacilli.

Until the nineteenth century, Bordeaux red wine was used in hospitals and in private practice for upset stomachs and diarrhea. Wine also destroys the dysentery bacillus (*Shigella dysenteriae*) and the typhus microbe (*Rickettsia prowazekii*) (Dejeu L., 2000).

In 1945, Schreb studied the action of red wine on the typhous bacillus (*Salmonella typhi*), the paratyphoid bacillus (*Salmonella paratyphi*) and the colibacillus (*Escherichia coli*). In his experiments, he varied the time of contact of the germ and wine and the proportion of the mixture, coming to the conclusion that the distillation residue, in particular, tannins and acids contained in wine, are those which have the most important role in the bactericidal action of wine (Masquelier J., 1982).

In 1956, H. Gachot, after resuming Schreb's experiments, managed to identify what substances in wines have a destructive action on the typhous bacillus (*Salmonella typhi*), noting that it is not the alcohol that has a bactericidal effect, but the organic acids, particularly, the malic acid. Moreover, he pointed out that wines that did not undergo malolactic fermentation (with poorer malic acid contents) have a poorer bactericidal effect than malolactic fermented wines (rich in malic acid).

Masquelier J. (1982, 1985, 1987) conducted more thorough research over two decades, managing to partially clear the problem by the following statements:

- organics acids have a minor bactericidal influence;
- sulfur dioxide, according to the doses used, does not affect germs; even in the absence of sulfur dioxide, wine has a bactericidal activity;
- the main role in the bactericidal action of wine is held by dyes (anthocyanins and flavonoids). Thus the wine's bactericidal effect increases if yellow pigments (flavonoids) or red pigments (anthocyanins) are added to wine;
- the reduction (loss) of bactericidal properties of red and white wines is due to their discoloring using vegetable charcoal;
- grape must does not have a bactericidal action. (Masquelier J., apud. Mihalca Al. et al., 2010)

In 1995, a U.S. study was conducted that revealed the digestive properties of red and white wines with, comparing the antimicrobial effects of wine with those of bismuth salicylate. Red and white wines were compared with a bismuth salicylate solution, with an alcoholic solution with sterilized water, the study taking place on some bacteria responsible for the onset of the diarrheal disease (*Escherichia coli*, *Salmonella enteritidis*, *Shigella*

*sanei*). Thus it was proved that red and white wines have an antibacterial effect superior to all other solutions tested. The 10% ethanol solution had no antibacterial effect, and is compared to a sterile water solution. Contrary to expectations, white wine solutions reduced the number of bacterial colonies faster than red wine solutions (Weisse M. E. et al., 1995).

A Danish study showed the protective effect of moderate wine consumption on *Helicobacter pylori* infections, a major cause for type B gastritis and closely associated with duodenal and gastric ulcers. Furthermore, the study shows that infections with *Helicobacter pylori* have a lower incidence among consumers of wine compared to beer drinkers (Pillot J., 2002). Recent studies on the antimicrobial activity of musts, wines and phenolic extracts from grapes, must and wine were conducted by several authors with remarkable results. (Jayaprakasha G. K. et al., 2003; Papadopoulou C. et al., 2005; Baydar N. G. et al., 2006; Xia E. Q. et al., 2010).

## MATERIALS AND METHODS

The antimicrobial activity, as determined by the agar diffusion method with disks impregnated by filter paper (diffusion method), was tested on two species of bacteria : *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922). The cultivation medium (AMB - agar meat broth) was acquired from Merck (Germany). The bacterial strains were purchased from the Cantacuzino Institute, Bucharest (Romania), and were maintained in the microbiology laboratory of the faculty, being regularly inoculated on fresh AMB media by transplanting.

The antibacterial activity was estimated by the diffusion method (Bauer A.W. et al., 1966). To this end, slices of qualitative filter paper with a diameter of 10 mm are prepared, which are sterilized in a Petri dish, in an oven. Afterwards, these are soaked with test solutions (Burgund red wine, Riesling italian white wine and 10% alcohol solution). Filter paper discs were impregnated with a volume of 50 µl of the test solution.

Antibacterial activity is tested for the following solutions:

- dry Burgund red wine with an alcoholic strength of 10%, obtained at the Miniş-Măderat vineyard, 2012 harvest;
- dry Riesling italian white wine with an alcoholic strength of 10%, obtained at the Miniş-Măderat vineyard, 2012 harvest;
- reference alcoholic solution with a concentration of 10% alcohol.

Culture medium: AMB (agar meat broth)

20 ml of nutrient agar, liquefied and cooled to 45° C is poured in sterile Petri dishes. Prior to solidification, it is seeded with 1 ml of the liquid microbial culture. The dish is rotated so as to obtain an even layer of culture. The dishes are dried in a thermostat at 37°C for one hour. Then, a sterile clamp is applied to the surface of the medium, consisting of 5 filter paper discs impregnated with the test solutions. Thermostating takes 24 hours at 37°C to finish. The reading is done by measuring the diameter of the inhibition area. The higher it is, the more the bacterial strain is sensitive to the test solution. (Bularda D. M., 2000).

For microbiologically tested wines, the following physicochemical parameters were determined: alcohol content, total acidity, malic acid content, free and total SO<sub>2</sub> content, anthocyanins and tannins content.

## RESULTS AND DISCUSSIONS

Products tested (Burgund red wine and Riesling italian white wine) have shown bactericidal activity on the *Bacillus subtilis* bacterial species; in addition, Burgund red wine showed bactericidal activity also on the *Escherichia coli* bacterial species, as shown in Table 1 and in Figures 2a, 2b and 3a:

Table 1

Inhibition areas

Inhibition areas (mm)		
Reference Microorganisms	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
10% alcohol solution	ND	ND
Dry Burgund red wine	19	11
Dry Riesling italian white wine	16	ND

ND – not detected

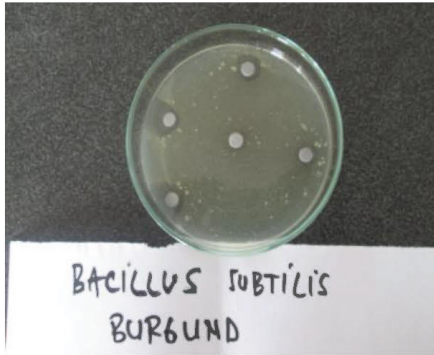


Figure 2a. Burgund red wine tested on a *Bacillus subtilis* culture

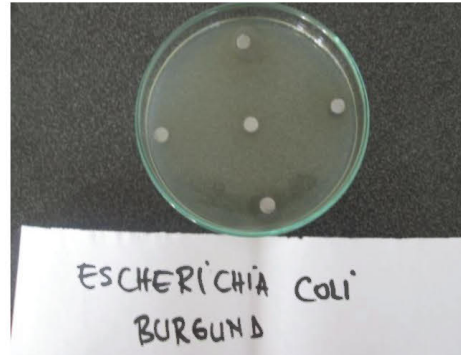


Figure 2b. Burgund red wine tested on a *Escherichia coli* culture

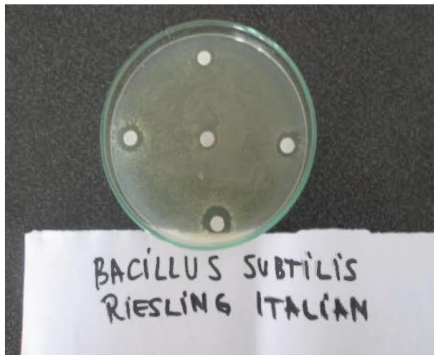


Figure 3a. Riesling Italian white wine tested on a *Bacillus subtilis* culture

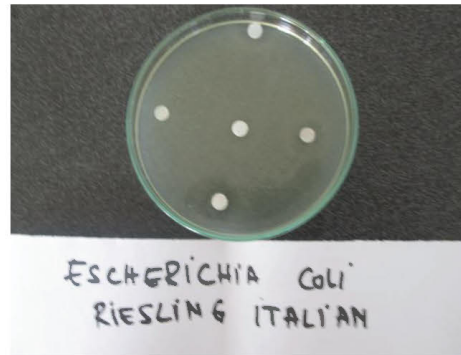


Figure 3b. Riesling Italian white wine tested on a *Escherichia coli* culture

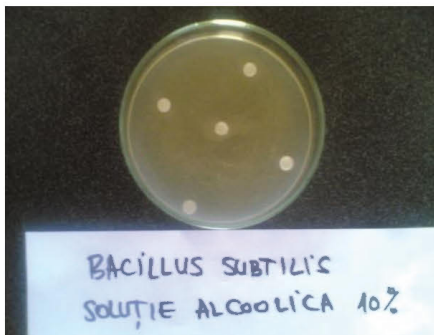


Figure 4a. 10% alcohol solution tested on a *Bacillus subtilis* culture

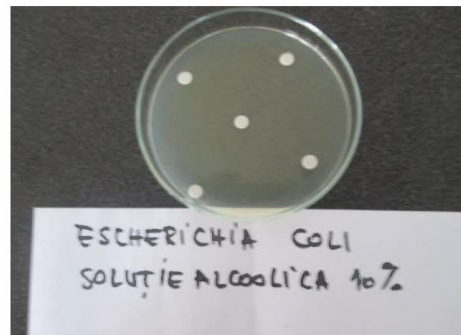


Figure 4b. 10% alcohol solution tested on a *Escherichia coli* culture

On the (Gram- positive) *Bacillus subtilis* bacterial culture both the Burgund red wine and the Riesling italian white wine developed very well defined inhibition areas, as can be seen in Figures 2a and 3a. On the same *Bacillus subtilis* culture, the 10% alcoholic solution does not develop inhibition areas, which means that it has no antibacterial effect. On the (Gram-negative) *Escherichia coli* bacterial culture only Burgund red wine developed inhibition areas, but less pronounced compared to the inhibition areas developed on the *Bacillus subtilis* culture. Instead, Riesling italian white wine and the 10% alcoholic solution develop inhibition areas, having an antibacterial effect on the *Escherichia coli* culture. Under these conditions, the *Escherichia coli* culture is sensitive only to the treatment with Burgund red wine.

To elucidate the different behavior of Riesling italian and Burgund wines to the bacterial species *Bacillus subtilis* and *Escherichia coli*, we performed chemical analyses for these wines.

Table 2

Chemical analysis of wines produces in the Miniş-Mäderat vineyard, 2012 harvest

Type of wine	Alcohol (% vol.)	Total acidity g/l H <sub>2</sub> SO <sub>4</sub>	Malic acid (g/l)	SO <sub>2</sub> (mg/l)		Anthocyanins (mg/l)	Catechin tannins (mg/l)
				free	total		
1. Riesling italian	10	5.8	1.2	8	40	0	415
2. Burgund	10	4.2	traces	7	28	545	2350

Table 2 shows that the antibacterial effect of red wine (Burgund) and white wine (Riesling italian) is not due to alcohol. The data presented in the table show that Burgund wine, although not containing malic acid (because it is consumed by malolactic fermentation) and having quite modest sulfur dioxide contents, has a pronounced antibacterial activity. On the other hand, Riesling italian wine, although it has a relatively high content of malic acid and a higher concentration of sulfur dioxide, has a modest antibacterial activity. From these considerations it follows that malic acid and sulfur dioxide concentration are not able to potentiate the bactericidal effect of wine. This enables us to state that the antimicrobial effect is due to the anthocyanins present in significant concentrations in Burgund red wine, and the catechin tannins present in the Riesling italian white wine, respectively.

### CONCLUSIONS

This study proves that red and white wines have an antimicrobial activity, especially on Gram-positive bacteria of the species *Bacillus subtilis*.

Based on the results, we are making the following conclusions:

- the antibacterial effect of wines is not due to the alcohol content (wines were compared with an alcoholic solution of the same concentration);
- the antibacterial effect of wines is not due to the malic acid content, as argued by previous research; for the Burgund wine, which does not contain malic acid, the inhibition area is better defined and measures 19 mm, compared to the 16 mm of the Riesling italian white wine;
- sulfur dioxide, according to the doses used, does not affect the antibacterial effect of wine; even if Riesling italian white wine has a higher content of sulfur dioxide

- compared to the Burgund wine, the described antimicrobial effect in the inhibition area is weaker;
- the antibacterial effect of Burgund red wine is stronger than the antibacterial effect of Riesling italian white wine, refuting previous studies which show that white wine solutions have a stronger antibacterial effect than red wine solutions;
- Burgund red wine inhibits the growth of coliform bacteria (*Escherichia coli*), this explaining its digestive properties.

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**RESEARCH REGARDING THE INFLUENCE OF THE NUTRIENT MIXTURE ON THE MORPHOLOGICAL CHARACTERISTICS OF *WEIGELIA ROSEA* LINDL. SEEDLINGS OBTAINED FROM MOTHER PLANTS**

Poșta Daniela Sabina\*<sup>1</sup>, Dorin Camen\*<sup>1</sup>, Nicolae I.<sup>2</sup>

**Keywords:** *heel cutting, nutrient mixture, vegetative reproduction*

**ABSTRACT**

*Weigelia rosea* originates in Northern China, Korea and Manchuria and can grow up to 5 m long. It is one of the most decorative and used species of its cast. It is highly decorative if planted in groups. The experience was aimed to identify the best trial variant of the nutrient mixture (manure, peat and sand) on this species. The measurements were made on seedlings (stem length, collar diameter, number of leaves, foliar surface). The correlation index used to calculate the foliar surface was 0.654.

**INTRODUCTION**

The genus is named after the German scientist Christian Ehrenfried Weigel and contains about 12 species spread in North America and East Asia. The species are highly appreciated in green areas due to the long-lasting blooming period and to colourful flower abundance. They have isolated 15 flavonoids from the leaves and flowers in 4 of the genus species (Chin-Sung Chang 1997). *Weigelia rosea* belongs to the Family *Caprifoliaceae*: it is a shrub which can reach, in Romania, 3 m in height. It is one of the most decorative and frequently cultivated species of the genus. The most spectacular effects are when planting in groups. It takes well mowing, it vegetates on all soil types, in full light, and it is resistant to frost (Iliescu Ana Felicia, 1998). Together with vegetative multiplication (Dejeu Mariana, D. Zacharias, Lenuța Kish, 2003), green or dry cuttings, they also use *in vitro* multiplication (Cantor Maria, Lenuța Kish, 2006).

**MATERIAL AND METHODS**

The cuttings were harvested and prepared on October 5, 2011, in the park of the Teaching Basis of Timișoara, Romania. They measured 10-12 cm; we used heel cuttings. The rooting substrate used in cutting rooting was river sand. The cuttings were introduced in the rooting substrate keeping a 5-cm distance between the rows and 2 cm between them.

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In the greenhouse, we ensured a temperature of 20-22°C and moisture of 85%. To root cuttings, we used the biostimulator Radistim 2 (Pošta Daniela Sabina, 2012). The treatment with indolylacetic acid increased the number of rooted cuttings and root length (Grace, 2011). The seedlings were transplanted on January 15, 2012, using the following nutrient mixtures (Buta Erzsebet & Cantor Maria, 2011):

V<sub>1</sub> – 50% manure; 40% peat; 10% sand; V<sub>2</sub> – 40% manure; 40% peat; 20% sand;  
 V<sub>3</sub> – 40% manure; 30% peat; 30% sand; V<sub>4</sub> – 30% manure; 30% peat; 40% sand.

On May 15, 2012, to emphasise the influence of the nutrient mixture on seedling growth, we made biometric measurements (Pošta Daniela Sabina, 2012) on the following parameters: stem height, collar diameter, number of leaves, foliar surface. The correction coefficient used in the calculus of foliar surface was 0.654.

## RESULTS AND DISCUSSIONS

The nutrient mixture used in the production of biological material in ornamental trees and shrubs species should ensure the fertility level necessary to plant growth and development and, in this type of vegetative multiplication, be adequate thus contributing to optimum moisture regime (i.e., soil air and gas). To do so, we fragmented in a different way the three components of the nutrient mixture and produced 4 trial variants used in the two trial cycles. Assessing the influence of the composition of the nutrient mixture on the physiological and biochemical processes in the plant, on the growth and development of the ontogenetic cycle of the species were quantified by biometric measurements of some morphological features such as stem height, collar diameter in seedlings, number of leaves, leaf length and width, and foliar surface. Trial results in the 4 trial variants of nutrient mixture used in cutting multiplication are shown in Table 1.

Table 1  
 Mean values of morphological features in *Weigelia rosea* Lindl. On May 15  
 (150 days after cutting)

Nutrient mixture trial variant		Stem height (cm)	Collar diameter (mm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Foliar surface (cm <sup>2</sup> )
V <sub>1</sub> 50% manure 40% peat 10% sand	x ±	15.19 ±	1.60 ± 0.07	6.36 ±	6.01 ±	2.74 ±	70.62 ±
	sx	0.12		0.22	0.06	0.14	4.22
	s <sup>2</sup>	0.38	0.13	1.25	0.11	0.51	0.44
	s%	4.09	22.76	17.62	5.70	26.14	29.91
V <sub>2</sub> 40% manure 40% peat 20% sand	x ±	17.54 ±	1.77 ± 0.01	9.85 ±	6.47 ±	3.35 ±	142.94 ±
	sx	0.21		0.36	0.05	0.05	2.53
	s <sup>2</sup>	1.16	0.004	3.26	0.08	0.06	0.60
	s%	6.14	3.57	18.34	4.51	7.61	8.85
V <sub>3</sub> 40% manure 30% peat 30% sand	x ±	19.82 ±	1.97 ± 0.01	14.44 ±	7.07 ±	3.76 ±	262.99 ±
	sx	0.14		0.32	0.05	0.06	2.28
	s <sup>2</sup>	0.49	0.005	2.60	0.08	0.09	0.30
	s%	3.55	3.69	11.16	4.21	8.14	4.33
V <sub>4</sub> 30% manure 30% peat 40% sand	x ±	20.55 ±	2.06 ± 0.01	18.13 ±	7.44 ±	4.17 ±	380.48 ±
	sx	0.07		0.29	0.05	0.05	1.22
	s <sup>2</sup>	0.14	0.007	2.19	0.07	0.06	0.37
	s%	1.82	4.14	8.16	3.72	6.17	1.61

Analysing the trial results presented in Table 1 regarding the influence of the nutrient mixture composition on some morphological features in *Weigelia rosea* Lindl.

Seedlings, we can see in trial variant V<sub>4</sub> (30% manure, 30% peat and 40% sand) superior values of these morphological features compared to the other trial variants.

Tables 2-7 show syntheses of trial results and the significance of differences in morphological features in *Weigelia rosea* Lindl. cuttings 150 days after cutting.

Table 2

Synthesis of trial results and significance of differences in transplanted seedling height in *Weigelia rosea* Lindl. (150 days after cutting)

Nutrient mixture trial variant	Mean seedling height (cm)	Relative seedling height (%)	Difference (cm)	Significance
50% manure : 40% peat : 10% sand	15.19	82.83	-3.14	ooo
40% manure : 40% peat : 20% sand	17.61	96.05	-0.72	o
40% manure : 30% peat : 30% sand	19.84	108.17	1.49	***
30% manure : 30% peat : 40% sand	20.71	112.94	2.37	***
Control variant	18.34	100.00	0.00	-

DL<sub>5%</sub> = 0.63 cm;

DL<sub>1%</sub> = 0.91 cm;

DL<sub>0.1%</sub> = 1.34 cm.

As for the trial results presented in Table 2, there are very distinctly significant positive differences in seedling size in the nutrient trial variants V<sub>3</sub> (40% manure : 30% peat : 30% sand) and V<sub>4</sub> (30% manure : 30% peat : 40% sand) compared to the control variant. Trial data regarding collar diameter in *Weigelia rosea* Lindl. seedlings are shown in Table 3.

Table 3

Synthesis of trial results and significance of differences in transplanted seedling collar diameter in *Weigelia rosea* Lindl. (150 days after cutting)

Nutrient mixture trial variant	Mean collar diameter (mm)	Relative collar diameter (%)	Difference (cm)	Significance
50% manure : 40% peat : 10% sand	1.55	83.80	-0.30	ooo
40% manure : 40% peat : 20% sand	1.78	96.32	-0.06	-
40% manure : 30% peat : 30% sand	1.97	106.90	0.12	**
30% manure : 30% peat : 40% sand	2.09	113.00	0.24	***
Control variant	1.84	100.00	0.00	-

DL<sub>5%</sub> = 0.11 mm;

DL<sub>1%</sub> = 0.15 mm;

DL<sub>0.1%</sub> = 0.22 mm.

Analysing trial results presented in Table 3 regarding the influence of the nutrient mixture composition on collar diameter in the seedlings we can see in the trial variant V<sub>4</sub> (30% manure, 30% peat and 40% sand) a very distinctly significant positive difference (0.24 cm) compared to the control variant and a very distinctly significant negative difference in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand).

Table 4 presents a synthesis of trial results and significance of differences in transplanted seedling number of leaves in *Weigelia rosea* Lindl.

Table 4.

Synthesis of experimental results and significance of differences in transplanted seedling number of leaves in *Weigelia rosea* Lindl. (150 days after cutting)

Trial variant	Mean number of leaves	Relative number of leaves (%)	Difference	Significance
50% manure : 40% peat : 10% sand	6.52	52.48	-5.90	ooo
40% manure : 40% peat : 20% sand	10.10	81.24	-2.33	ooo
40% manure : 30% peat : 30% sand	14.75	118.65	2.31	***
30% manure : 30% peat : 40% sand	18.35	147.61	5.91	***
Control variant	12.43	100.00	0.00	-

DL<sub>5%</sub> = 0.94;

DL<sub>1%</sub> = 1.36;

DL<sub>0.1%</sub> = 2.00.

Using a nutrient mixture rich in organic matter and balanced from the point of view of friability results in the growth and development of a larger number of leaves per plant with very distinctly significant positive differences compared to the control variant in the trial variants V<sub>3</sub> (40% manure : 30% peat : 30% sand) (2.31) and V<sub>4</sub> (30% manure : 30% peat : 40% sand) (5.91). Mean values of leaf length in the 4 trial variants of nutrient mixtures used in the production of biological material in *Weigelia rosea* Lindl. are shown in Table 5.

Table 5.

Synthesis of experimental results and significance of differences in transplanted seedling leaf length in *Weigelia rosea* Lindl. (150 days after cutting)

Trial variant	Mean leaf length (cm)	Relative leaf length (%)	Difference (cm)	Significance
50% manure : 40% peat : 10% sand	6.05	88.88	-0.75	ooo
40% manure : 40% peat : 20% sand	6.49	95.34	-0.31	o
40% manure : 30% peat : 30% sand	7.15	104.92	0.33	*
30% manure : 30% peat : 40% sand	7.55	110.86	0.74	***
Control variant	6.81	100.00	0.00	-

DL<sub>5%</sub> = 0.24 cm;

DL<sub>1%</sub> = 0.35 cm;

DL<sub>0.1%</sub> = 0.51 cm.

Analysing the trial results presented in Table 5, we can see that in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand) there is a very distinctly significant positive difference compared to the control variant from the point of view of leaf length.

Trial results regarding mean values of leaf width in the seedlings and their significance compared to the control variant are shown in Table 6.

Table 6.

Synthesis of experimental results and significance of differences in transplanted seedling leaf width in *Weigelia rosea* Lindl. (150 days after cutting)

Trial variant	Mean leaf width (cm)	Relative leaf width (%)	Difference (cm)	Significance
50% manure : 40% peat : 10% sand	2.69	76.43	-0.82	ooo
40% manure : 40% peat : 20% sand	3.37	95.83	-0.14	-
40% manure : 30% peat : 30% sand	3.81	108.50	0.29	-
30% manure : 30% peat : 40% sand	4.19	119.30	0.67	*
Control variant	3.51	100.00	0.00	-

DL<sub>5%</sub> = 0.57 cm;

DL<sub>1%</sub> = 0.82 cm;

DL<sub>0.1%</sub> = 1.21 cm.

As for the results presented in Table 6, we can see that in the trial variant of nutrient mixture V<sub>4</sub> (30% manure : 30% peat : 40% sand) there is a significant positive difference compare to the control variant from the perspective of seedling leaf width in *Weigelia rosea* Lindl. Foliar surface is one of the morphological features of importance in plant growth and development because it contributes substantially in the synthesis of organic substances. Trial data regarding this morphological feature and the differences between trial variants are presented in Table 7.

Table 7.

Synthesis of experimental results and significance of differences in transplanted seedling foliar surface in *Weigelia rosea* Lindl. (150 days after cutting)

Trial variant	Leaf foliar surface (cm <sup>2</sup> )	Relative leaf foliar surface (%)	Difference (cm <sup>2</sup> )	Significance
50% manure : 40% peat : 10% sand	71.15	33.14	-143.49	ooo
40% manure : 40% peat : 20% sand	143.72	66.96	-70.91	ooo
40% manure : 30% peat : 30% sand	263.06	122.55	48.41	***
30% manure : 30% peat : 40% sand	380.62	177.33	165.98	***
Control variant	214.64	100.00	0.00	-

DL<sub>5%</sub> = 22.58 cm<sup>2</sup>;

DL<sub>1%</sub> = 32.47 cm<sup>2</sup>;

DL<sub>0.1%</sub> = 47.76 cm<sup>2</sup>.

Analysing trial data presented in Table 7, we can see very distinctly significant positive differences compared to the control variant in the trial variant V<sub>3</sub> (40% manure : 30% peat : 30% sand) (48.41 cm<sup>2</sup>) and particularly in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand) (165.98 cm).

## CONCLUSIONS

As far as seedling size is concerned 150 days after cutting, there are very distinctly significant positive differences in the nutrient mixture trial variants V<sub>3</sub> (40% manure : 30% peat : 30% sand) and V<sub>4</sub> (30% manure : 30% peat : 40% sand) compared to the control variant. As for the influence of the nutrient mixture composition on collar diameter in the seedlings, we can see in the trial variant V<sub>4</sub> (30% manure: 30% peat : 40% sand) a very distinctly significant positive difference and a very distinctly significant negative difference in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand) compared to the control variant.

From the perspective of seedling leaf width in *Weigelia rosea* Lindl., we can see that, in the nutrient mixture trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand) there is a distinctly significant positive difference compared to the control variant.

Analysing trial results regarding leaf area, we can see very distinctly significant positive differences in the trial variant V<sub>3</sub> (48.41 cm<sup>2</sup>) and particularly in the trial variant V<sub>4</sub> (165.98 cm<sup>2</sup>) compared to the control variant.

The analysis of trial results regarding the influence of the nutrient mixture composition on some morphological features of the seedlings of *Weigelia rosea* Lindl. from cutting to transplanting points to values superior to the control variant in the trial variants V<sub>4</sub> (30% manure : 30% peat : 40% sand).

Trial results from transplanting to removal to containers show that in the trial variant V<sub>4</sub> (30% manure: 30% peat : 40% sand) fertilised with Razormin there are values superior to the other trial variants in the features analysed.

In conclusion, we can see that the trial variant V<sub>4</sub> (30% manure: 30% peat : 40% sand) produced the best morphological features compared to the other trial variants, particularly V<sub>1</sub> (50% manure : 40% peat : 10% sand).

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**RESEARCH REGARDING THE INFLUENCE OF SOME FOLIAR AND PHASE FERTILISERS ON SOME MORPHOLOGICAL FEATURES OF *WEIGELIA ROSEA* LINDL. SEEDLINGS OBTAINED VEGETATIVELY**

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*Keywords: heel cutting, foliar and phase fertilisers, vegetative multiplication*

**ABSTRACT**

*Weigelia rosea* is a species original from North China, Korea and Manchuria where it reaches 5 m in height. The trial aimed at identifying the best foliar and phase fertiliser (Nutri leaf 20:20:20, Atonik, Radifarm, Razormin) for this species. Biometric measurements were made on seedlings (stem height, collar diameter, number of leaves, foliar surface). The correction coefficient used in the calculus of foliar surface was 0.654.

**INTRODUCTION**

*Weigelia rosea* belongs to the Family *Caprifoliaceae*; it is a shrub which, in Romania, can reach 3 m in height. This shrub has young stems with two rows of hairs. The buds are small and opposed. The leaves are elliptic, ovate or obovate, acute, short-petioled, 5-10 cm long, minutely serrate, slightly pubescent on the upper face, pubescent on the under face along the nerves. The flowers are grouped into axial cymes with 5 petals and persisting. The corolla is urceolate, about 3 cm long, pink, with 5 rounded lobes, divergent. The fruit is a capsule with 2 valves (Stanescu et al, 1997). There are four varieties: "Tango" with red flowers, "Variegata" with whitish leaves and purple flowers; "Alexandra" with reddish leaves and red flowers; "Red prince" with red flowers (Iliescu Ana Felicia, 1998). In Romania, it is appreciated as ornamental species due to its beautifully coloured flowers and fruits that winter on the branches. It is used in parks and gardens, and it is planted isolated, in groups, or as fences. *In vitro* multiplication of the species *Weigelia florida* resulted in an increase of both quality and quantity of the seedlings (Angela C. Ghrist et al, 1991).

**MATERIAL AND METHODS**

The biological material used in the trials was represented by seedlings of the species *Weigelia rosea* using different foliar and phase fertilisers. The cuttings were harvested and prepared on October 5, 2011, from the park of the Teaching Basis of Timișoara. They measured 10-12 cm; we used the heel cutting type. The rooting substrate used in the rooting of the cuttings was river sand. The seedlings were transplanted from the

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rooting substrate on January 15, 2012, using a nutrient mixture of manure, peat and sand in different shares (Pošta Daniela Sabina et al, 2014). By fractioning differently the three components of the nutrient mixture, we obtained 4 variants that we used in the two trial cycles: we monitored the influence of the nutrient mixture composition and of foliar and phase fertilisers on the physiological and biochemical processes in the plant. One hundred and fifty days after cutting, on May 2012, in order to emphasise the influence of the nutrient mixture composition on seedling growth, we made biometric measurements of the studied features: stem height, collar diameter, number of leaves, foliar surface. The correction coefficient used in the calculus of foliar surface was 0.654. After biometric measurements were over, on the same day (May 15, 2012), the seedlings were transplanted again and fertilised foliarly and phasally every 10 days with Nutri leaf 20:20:20 – 0.1%, Atonik – 0.25 ml/1 l water, Radifarm – 0.2-0.3 l/100 l solution, applying 2-4 l solution/plant, Razormin – 0.05% until October 10, 2012, when the seedlings were transplanted into containers (Roşca I., 2010 & Pošta Daniela Sabina).

## RESULTS AND DISCUSSIONS

Tables 1 to 7 present a synthesis of trial results and the significance of differences in morphological features in the seedlings of *Weigelia rosea* Lindl. The mean values of morphological features (stem height, collar diameter, number of leaves, foliar surface) are shown in Table 1.

Table 1.  
Mean values of morphological features in *Weigelia rosea* Lindl. 180 days after first transplanting

Trial variant		Stem height (cm)	Collar diameter (mm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Foliar surface (cm <sup>2</sup> )
V <sub>1</sub> 50% manure 40% peat 10% sand Fertilised with Nutri leaf	$\bar{x} \pm$	17.36 ±	1.71 ± 0.07	10.48 ±	6.73 ±	3.60 ±	168.75 ±
	sx	0.10		0.26	0.04	0.10	5.50
	s <sup>2</sup>	0.25	0.12	1.69	0.04	0.25	0.58
	s%	2.92	20.57	12.39	3.15	14.09	16.32
V <sub>2</sub> 40% manure 40% peat 20% sand Fertilised with Atonik	$\bar{x} \pm$	19.73 ±	1.85 ± 0.01	12.78 ±	6.99 ±	4.19 ±	247.84 ±
	sx	0.18		0.25	0.06	0.03	2.00
	s <sup>2</sup>	0.82	0.004	1.59	0.10	0.03	0.11
	s%	4.60	3.59	9.87	4.74	4.21	4.03
V <sub>3</sub> 40% manure 30% peat 30% sand Fertilised with Radifarm	$\bar{x} \pm$	22.37 ±	2.09 ± 0.01	17.78 ±	7.53 ±	4.55 ±	409.37 ±
	sx	0.10		0.41	0.05	0.02	4.94
	s <sup>2</sup>	0.29	0.004	4.21	0.07	0.01	0.61
	s%	2.42	3.25	11.53	3.67	2.83	6.03
V <sub>4</sub> 30% manure 30% peat 40% sand Fertilised with Razormin	$\bar{x} \pm$	23.46 ±	2.17 ±	22.34 ±	8.36 ±	5.24 ±	647.60 ±
	sx	0.11	0.008	0.29	0.11	0.07	9.48
	s <sup>2</sup>	0.33	0.001	2.15	0.35	0.13	2.27
	s%	2.44	1.89	6.56	7.16	7.03	7.32



As far as stem height is concerned, we can see that the highest value was in the variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) (23.46 cm), while the lowest was in the variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) (17.36 cm). As for the collar diameter, the values ranged between 1.71 mm in the variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) and 2.17 mm in the variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin). As for foliar surface, the variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) recorded again the highest value (647.60 cm<sup>2</sup>) compared to V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) (168.75 cm<sup>2</sup>). Analysing the trial results presented in Table 1 (regarding the influence of the nutrient mixture composition on some morphological features of the seedlings of *Weigelia rosea* Lindl. 180 days after first transplanting), we can see that, in the variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin), the values are superior to the values of the other trial variants. As for the trial results presented in Table 2, we can see there a very significantly positive differences in seedling height in the trial variants V<sub>3</sub> (40% manure : 30% peat : 30% sand + Radifarm) and V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) compared to the control variant (trial mean).

Table 2.  
Synthesis of trial results and significance of differences in transplanted seedling height in *Weigelia rosea* Lindl.

Trial variant	Mean seedling height (cm)	Relative seedling height (%)	Difference (cm)	Significance
50% manure : 40% peat : 10% sand Nutri leaf	17.38	83.66	-3.39	○○○
40% manure : 40% peat : 20% sand Atonik	19.76	95.10	-1.01	○○
40% manure : 30% peat : 30% sand Radifarm	22.37	107.64	1.58	***
30% manure : 30% peat : 40% sand Razormin	23.60	113.59	2.82	***
Trial mean (Control)	20.78	100.00	0.00	-

DL<sub>5%</sub> = 0.55 cm;                      DL<sub>1%</sub> = 0.79 cm;                      DL<sub>0.1%</sub> = 1.16 cm.

Table 3 presents trial data regarding collar diameter in the seedlings. Analysing the results, we can say that there are very distinctly significant positive differences in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) and very distinctly significant negative differences in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) compared to the control variant.

Table 3.  
Synthesis of trial results and significance of differences in transplanted seedling collar diameter in *Weigelia rosea* Lindl.

Trial variant	Mean collar diameter (mm)	Relative collar diameter (%)	Difference (cm)	Significance
50% manure:40%peat:10% sand Nutri leaf	1.71	87.04	-0.25	○○○
40% manure :40% peat : 20% sand Atonik	1.85	94.64	-0.10	-
40% manure:30%peat:30% sand Radifarm	2.10	106.93	0.13	**
30% manure:30%peat:40% sand Razormin	2.18	111.38	0.22	***
Trial mean (Control)	1.96	100.00	0.00	-

DL<sub>5%</sub> = 0.09 mm;                      DL<sub>1%</sub> = 0.14 mm;                      DL<sub>0.1%</sub> = 0.21 mm.

From the point of view of the number of leaves in the transplanted seedlings, trial results show very distinctly significant negative differences in the trial variants V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) and V<sub>2</sub> (40% manure : 40% peat : 20% sand + Atonik), and very distinctly significant positive differences in the trial variants V<sub>3</sub> (40% manure : 30% peat : 30% sand + Radifarm) and V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) compared to the control (Table 4).

Table 4.

Synthesis of trial results and significance of differences in transplanted seedling leaf number in *Weigelia rosea* Lindl.

Trial variant	Mean number of leaves	Relative number of leaves (%)	Difference	Significance
50% manure : 40% peat : 10% sand Nutri leaf	10.55	65.64	-5.52	ooo
40% manure : 40% peat : 20% sand Atonik	12.90	80.26	-3.17	ooo
40% manure : 30% peat : 30% sand Radifarm	18.18	113.13	2.11	***
30% manure : 30% peat : 40% sand Razormin	22.65	140.96	6.58	***
Trial mean (Control)	16.07	100.00	0.00	-

DL<sub>5%</sub> = 0.78;

DL<sub>1%</sub> = 1.13;

DL<sub>0.1%</sub> = 1.66.

As for trial results in transplanted seedling leaf length, there are distinctly significant positive differences in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) (8.38 cm) and significantly negative differences in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) (6.73 cm) compared to the control (Table 5).

Table 5.

Synthesis of trial results and significance of differences in transplanted seedling leaf length in *Weigelia rosea* Lindl.

Trial variant	Mean leaf length (cm)	Relative leaf length (%)	Difference (cm)	Significance
50% manure:40%peat:10% sand Nutri leaf	6.73	90.68	-0.69	o
40% manure: 40% peat : 20% sand Atonik	7.01	94.45	-0.41	-
40% manure:30%peat:30% sand Radifarm	7.57	102.03	0.15	-
30% manure:30%peat:40% sand Razormin	8.38	112.83	0.95	**
Trial mean (Control)	7.42	100.00	0.00	-

DL<sub>5%</sub> = 0.56 cm;

DL<sub>1%</sub> = 0.81 cm;

DL<sub>0.1%</sub> = 1.19 cm.

From the point of view of trial results in seedling leaf width (Table 6), there are distinctly significant positive differences in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) and distinctly significant negative differences in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) compared to the control. Mean leaf width oscillated between 3.64 cm in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) and 5.24 cm in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin).

Table 6.  
Synthesis of trial results and significance of differences in transplanted seedling leaf width in *Weigelia rosea* Lindl.

Trial variant	Mean leaf width (cm)	Relative leaf width (%)	Difference (cm)	Significance
50% manure : 40% peat : 10% sand Nutri leaf	3.64	82.52	-0.77	oo
40% manure : 40% peat : 20% sand Atonik	4.20	95.14	-0.21	-
40% manure : 30% peat : 30% sand Radifarm	4.57	103.58	0.15	-
30% manure : 30% peat : 40% sand Razormin	5.24	118.75	0.82	**
Trial mean (Control)	4.41	100.00	0.00	-

DL<sub>5%</sub> = 0.42 cm; DL<sub>1%</sub> = 0.61 cm; DL<sub>0.1%</sub> = 0.90 cm.

Table 7 shows data regarding the mean foliar surface of the transplanted seedlings. Analysing the results, we can see that the largest foliar surface was in the trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) (650.70 cm<sup>2</sup>), while the smallest one was in the trial variant V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) (170.42 cm<sup>2</sup>). The trial variant V<sub>4</sub> (30% manure : 30% peat : 40% sand + Razormin) shows very distinctly significant positive differences, while the trial variants V<sub>1</sub> (50% manure : 40% peat : 10% sand + Nutri leaf) and V<sub>2</sub> (40% manure : 40% peat : 20% sand + Atonik) show very distinctly significant negative differences.

Table 7.  
Synthesis of trial results and significance of differences in transplanted seedling foliar surface in *Weigelia rosea* Lindl.

Trial variant	Mean leaf foliar surface (cm <sup>2</sup> )	Relative leaf foliar surface (%)	Difference (cm <sup>2</sup> )	Significance
50% manure:40%peat:10% sand Nutri leaf	170.42	46.05	-199.60	ooo
40% manure : 40% peat : 20% sand Atonik	248.29	67.10	-121.70	ooo
40% manure : 30% peat : 30% sand Radifarm	410.57	110.97	40.57	*
30% manure: 30% peat : 40% sand Razormin	650.70	175.87	280.71	***
Trial mean (Control)	369.99	100.00	0.00	-

DL<sub>5%</sub> = 36.63 cm<sup>2</sup>; DL<sub>1%</sub> = 52.67 cm<sup>2</sup>; DL<sub>0.1%</sub> = 77.47 cm<sup>2</sup>.

### CONCLUSIONS

Regarding the trial results from first transplanting to transplanting into containers in seedling height, there are very significant positive differences in the trial variants V<sub>3</sub> and V<sub>4</sub> compared to the control. The highest value was in the trial variant V<sub>4</sub> (23.46 cm), while the lowest value was in the trial variant V<sub>1</sub> (17.36 cm).

Analysing the trial results regarding seedling collar diameter, there are very distinctly significant positive differences in the trial variant V<sub>4</sub> and very distinctly significant negative differences in the trial variant V<sub>1</sub> compared to the control.

As for trial results in leaf width, there are distinctly significant positive differences in the trial variant V<sub>4</sub> and distinctly significant negative differences in the trial variant V<sub>1</sub> compared to the control variant. Mean leaf width recorded values between 3.64 cm in the trial variant V<sub>1</sub> and 5.24 cm in the trial variant V<sub>4</sub>.

Regarding the number of leaves per transplanted seedling, trial results show very distinctly significant negative differences in the trial variants V<sub>1</sub> and V<sub>2</sub>, and very distinctly significant positive differences in the variants V<sub>3</sub> and V<sub>4</sub> compared to the control. As far as the foliar surface is concerned, the trial variant V<sub>4</sub> recorded the highest value (647.60 cm<sup>2</sup>) compared to the trial variant V<sub>1</sub> (168.75 cm<sup>2</sup>).

Analysing trial results from first transplanting to the transplanting into containers, we can see there are superior values of the features analysed in the trial variant V<sub>4</sub> compared to the other trial variants.

The conclusion is that the trial variant V<sub>4</sub> resulted in superior values of all morphological features studied compared to the other trial variants, particularly the trial variant V<sub>1</sub>.

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## STUDY ON THE TECHNOLOGICAL IMPROVEMENT OF VINE GROWING IN NURSERY

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**Keywords:** *vine, technology, nursery, weeds, herbicides*

### ABSTRACT

*The results from the researches carried out in the vine nursery at the Institute of Viticulture and Enology, Pleven, Bulgaria are presented. Different depths of rooting of the cuttings and their impact on the main biometric indicators were tested. Best results were obtained with exposed waxed part of the cuttings, 7-8 cm and 15-16 cm long. The efficiency and selectivity of herbicides: Wing P and Gardoprim Plus Gold 500 SC at doses of 0.4 and 0.6 l/da were studied. It was found that they controlled efficiently the annual weeds, did not cause phytotoxic reaction and did not have any inhibitory effect on bud germination. Increased yield of rooted vines from the treated variants was accounted.*

### INTRODUCTION

By rooting of the grafted cuttings with an exposed waxed part, the studies in our country were focused on determining the optimum factors influencing the regenerative processes in the vine nursery - types of waxes, irrigation regime, organic and mineral fertilizers, herbicides (Dimitrova et al., 2007, 2013; Encheva and Dimitrova, 2007; Prodanova-Marinova, N., 2012; Tsvetanov and Kumanov, 2011).

The application of waxes allowed the waxed part to remain exposed and the grafted cuttings to be planted at a shallow depth in the soil. With this change in the technology, the issue of the appropriate depth of planting and then rooting and development of transplanted cuttings had to be clarified.

The aggressive competition of weeds in the vine nursery necessitated well-timed fight for their destruction. The effect of herbicides Wing P and Gardoprim Plus Gold 500 SC on vines has been little studied. Researches had shown that Gardoprim Plus Gold 500 SC was selective for the vine and could successfully replace simazine (Sarpe, N. et al., 2007). The active substances, pendimethalin, s-metolachlor and terbuthylazine separately or in combinations, in the form of other commercial products, were recommended for weeds control in vineyards (Bordelon, B., 2011; Sarpe, N, 2011). Over the past decade the possibility for their application in vine nurseries had been investigated.

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Objective of the study – testing different planting depth and rooting of the grafted cuttings and study the efficiency and selectivity of the herbicides *Wing P* and *Gardoprим Plus Gold 500 SC*.

## MATERIAL AND METHODS

### Testing Different Planting Depth and Rooting of the Grafted Cuttings

The study was carried out with Misket Kaylashki variety (white, wine variety, selection of IVE-Pleven) grafted to Berlandieri x Riparia SO4 rootstock (Misket Kaylashki/SO4) in the period 2007-2009. The grafted cuttings were planted in the nursery of the Production-Experimental Base on soil type – leached chernozem, in the following variants: C (control) – in ridges (in accordance with the conventional technology by covering the grafts with soil 3-4 cm above the cut); V1 – in ridges (by covering the cuttings with soil to the level of the grafts cut); V2 – in semi-formed ridges (the grafted cuttings had exposed top part 7-8 cm); V3 - 15-16 cm).

Study the Efficiency and Selectivity of the Herbicides *Wing P* and *Gardoprим Plus Gold 500 SC*

The efficiency and selectivity of *Wing P* (250 g/l pendimethalin + 212.5 g/l dimethenamid P) and *Gardoprим Plus Gold 500 SC* (312.5 g/l, s-metolachlor + 177.5 g/l terbuthylazine) were studied. The variants were as follows: V1 – *Wing P* (0.4 l/da); V2 – *Wing P* (0.6 l/da); V3 - *Gardoprим Plus Gold 500 SC* (0.4 l/da); V4 - *Gardoprим Plus Gold 500 SC* (0.6 l/da); C – weeded untreated control. The herbicides were introduced immediately after planting of the cuttings and just before the first sprinkling of the nursery. The control was maintained by thrice manual weeding out.

The experiment was carried out with Misket Kaylashki/SO4 cuttings on soil type leached chernozem. The weeds density (pc./ m<sup>2</sup>) was followed in dynamics (on 30-ieth, 60-ieth and 90-ieth day post treatment). The germination ratio and the yield of grafted rooted vines ratio compared to the planted cuttings were counted.

Data on germination ratio and yield of rooted vines were processed by analysis of variance (Dimova, D., E. Marinkov, 1999).

## RESULTS AND DISCUSSIONS

### Testing Different Planting Depth and Rooting of the Grafted Cuttings

The data on shoots germination ratio of the variants with different depth of planting of the grafted cuttings in the nursery are presented in Figure 1 for the year 2009, however they were representative for 2007 and 2008 too. It was observed a highly expressed contrast between the control and the three variants from the beginning of the shoots germination until the last date of counting. The developed shoots ratio during the first counting on June, 01 was the lowest in the control - 29.4%. In the other variants this indicator was within the range of 79.5% and 86.5%, i.e. about 2.5 times higher than the number of germinated shoots compared to the control. During the subsequent counting (June, 10 and July, 09), the difference had dropped down insignificantly between the control and the experimental variants.

The observed contrast between the control and the different depths of rooting was a result of the slow response of the grafted cuttings in closed ridges due to temperature differences in the zone of the graft and the depth available to the basal part of the cuttings.

Data on the rate of first-class vines were clearly differentiated and followed the logic of the results on shoots germination in the nursery. The lowest ratio of grafted rooted vines was recorded in the control - 30.1% (Figure 2). In V1, vine yield exceeded the control by 9%.

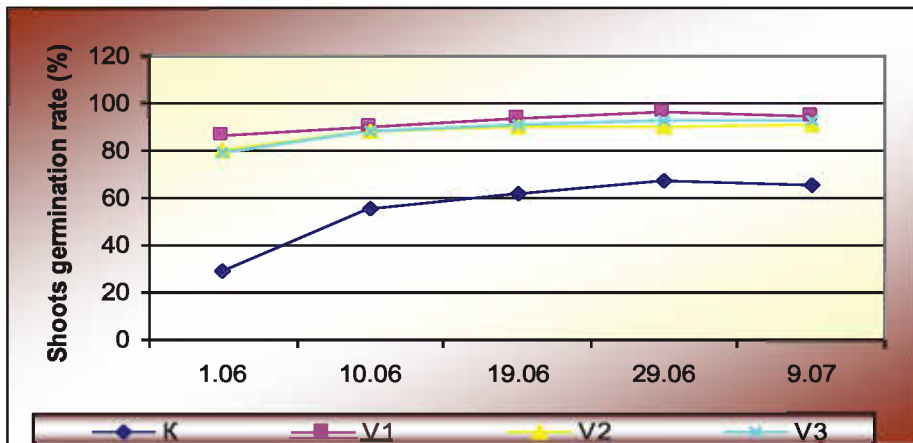


Figure 1. Dynamics of shoots germination of Misket Kaylashki variety of cuttings planted at different depths

In the variants of rooting the cuttings with exposed waxed part 7-8 cm and 15-16 cm, the ratio of first-class vines was approximately twice higher. Obviously, the obtained data were in favor of the smaller depth of planting and rooting of the grafted cuttings. Shoots germinated much faster compared to those rooted in accordance with the conventional technology. The smaller depth where the heels of the grafted cuttings were located determined the more favorable conditions for root formation – soil temperature, moisture and aeration. The physiological processes of the shoots and the roots growth were activated based on the correlations of the roots and the parts above the ground.

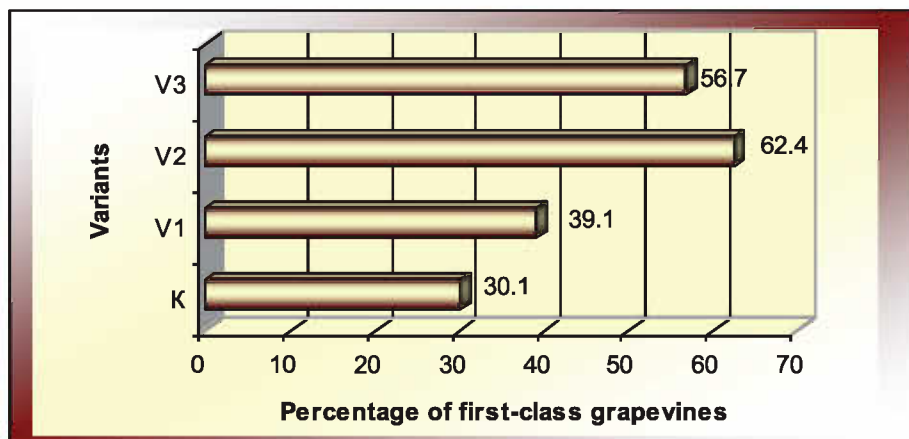


Figure 2. Yield of first-class vines (%) at different depth of rooting of Misket Kaylashki/SO4 variety grafted cuttings

*Study the Efficiency and Selectivity of the Herbicides Wing P and Gardoprим Plus Gold 500 SC*

The tested herbicides effectively controlled the annual weed species with the exception of *Xanthium strumarium* (L.). The high soil moisture in combination with

leached chernozem created conditions for shorter effect of Wing P. Sixty days after the treatment, the weeds density in V1 increased by 0.23 pc./m<sup>2</sup>, while in V2 – by 0.3 pc./m<sup>2</sup> (Figure 3). On the ninetieth day it was already insignificantly exceeding the counted density of the control (2 pc./m<sup>2</sup>). Gardoprim Plus Gold exhibited high herbicidal effects and long-term action. Ninety days after the treatment the density of the weeds had still remained unchanged and was significantly smaller compared to the control.

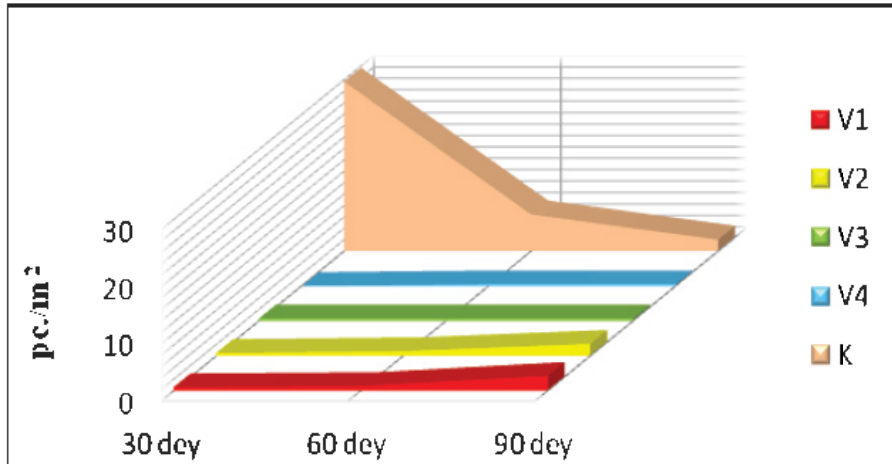


Figure 3. Dynamics of the total weeds density on the average for the period 2011 – 2013

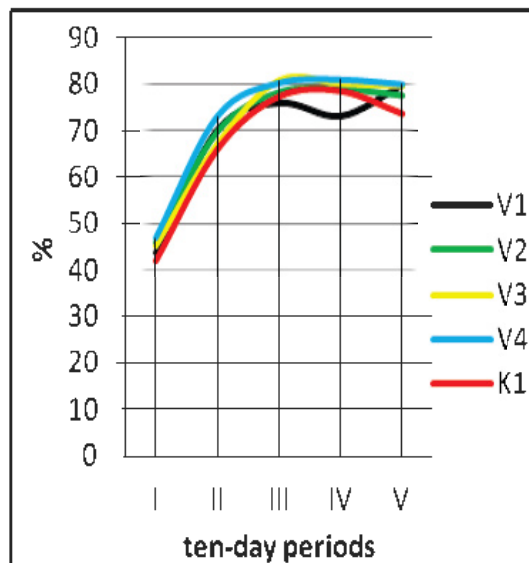


Figure 4. Germination rate dynamics of the grafted cuttings on the average for the period 2011 – 2013



Throughout the whole period of the study Wing P and Gardoprim Plus Gold had shown high efficiency against the annual weeds and selectivity towards the grafted vine cuttings. The direct falling of the solutions on developing buds did not lead to negative consequences for the young shoots and did not cause phytotoxic response. Shoots germination in all variants of treatment and the control occurred at approximately equal rates (Figure. 4). In the first decade after treatment the germination rate in the treated variants varied between 43.87 (V1) and 46.94 (V4) and did not differ significantly from the control (GD 5% = 7.283; GD 1% = 10.596; GD 0.1% = 15.921).

Later the germination rate increased in all variants and the control reaching its final values fifty days after planting the cuttings in the nursery. At the end of the fifth ten-day period the differences in the values of this indicator in the treated variants were insignificant. The germination rate in all variants exceeded the control, however provability was not established (GD 5% = 10.679; GD 1% = 15.537; GD 0.1% = 23.344).

The higher rate of germinated cuttings in the treated variants determined the higher yield of grafted rooted vines. The results showed that it surpassed the manually weeded control from 4.81% (V1) to 6.52% (V3). The highest rates of this indicator were reported in the variants with Gardoprim Plus Gold. The differences between them and the control were mathematically proven (GD 5% = 5.679; GD 1% = 8.263; GD 0.1% = 12.416).

## CONCLUSIONS

In testing the different depth of planting and rooting of Misket Kaylashki/SO4 variety grafted cuttings on soil type leached chernozem, the yield of first-class grafted vines in the variants with exposed waxed part of the rooted cuttings 7-8 cm (62.4%) and 15-16 cm (56.7%) was twice higher compared to the conventional way of cultivation in ridges (30.1%).

The herbicides Wing P and Gardoprim Plus Gold 500 SC at doses of 0.4 and 0.6 l/da control efficiently the annual weeds during the first ninety days after planting the cuttings. The direct falling of the herbicide solutions on developing buds did not cause phytotoxic response and did not inhibit the grafted cuttings germination. Wing P and Gardoprim Plus Gold 500 SC in the applied doses did not affect adversely the yield of rooted vines but created conditions for its increase compared to the technology of cuttings cultivation with manual weeding.

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**RESEARCH REGARDING THE EFFECT OF GROWTH  
REGULATORS OVER THE IN VITRO MORPHOGENETIC  
REACTION AT *ORIGANUM MAJORANA***

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*Key words: Origanum majorana, "in vitro", explants, microshoots, growth regulators*

**ABSTRACT**

*The goal of this work was to develop a protocol for obtaining a high regeneration rate by testing the response of different explants of Origanum majorana species on different culture media. Results showed that explants of apices stems represent optimal source of inoculums. Research in the evaluation of morphogenetic capacity of explants on different nutrient regeneration formulas have shown that the best results in terms of percentage of explants started the trend was obtained on the version supplemented with 1 mg/l BAP. The greatest number of shoots (16,2 microshoots/explant) was observed in the media variant that contained 2 mg/l BAP in combination with 0,5 mg/l NAA. Shoots were multiplied by subculture on the same medium. The shoots were rooted also on the multiplication medium. The rooted plantlets were acclimatized and successfully fortified in pots registering a survival rate of 87%.*

**INTRODUCTION**

Marjoram (*Origanum majorana*) is a perennial medicinal and aromatic plants belonging to *Lamiaceae* family. *Origanum* genus comprises approximately 38 species, most of which are originate in Mediterranean region. The marjoram essential oils have antibacterials (Vokou et al.,1993) and antifungals (Paster et al., 1993) actions. Many of them are commercially exploited, their demand is increasing (Schiuma, 1993).

Conventional techniques for vegetative propagation at *Origanum majorana* based on cuttings are difficult because low rates of rooting. *In vitro* cells and tissues cultures assure a unique opportunity to manipulate morphogenesis in a perfectly controlled medium, thus offering a powerful complementary instrument that can help in overcoming such problems.

There is a few information about *in vitro* culture of *Origanum* species in the current literature (Kumari and Saradhi, 1992; Crutis et al., 1996; Iyer and Pai, 2000; Tisserat and Silman, 2000; Minnas, 2001).

Therefore, in this paper, the researches were directed towards establishing an *in vitro* propagation system with increased efficiency for the production of plants intended extraction of secondary metabolites used in the pharmaceutical industry.

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## MATERIALS AND METHODS

The biological material derived from *Origanum majorana* species has been multiplied *in vitro* to yield neoplantlets regenerated by tissue culture.

Explants used to initiate tissue culture was represented by nodes and apexes stems. The defoliated shoots were first washed in tap water and were sterilized in 6% HgCl<sub>2</sub> for 10 minutes, after which 3 rinses were performed in sterile distilled water.

After sterilization of plant material, the explants were sampled and inoculated on MS culture medium supplemented with various concentrations of BAP (0.5, 1.0, 1.5, 2.0 mg/l). As a carbon source was used dextrose (40 g/l) and as a source of iron was used NaFeEDTA (32 g/l). For solidification of the culture medium was used agar (7 g/l).

The culture media were sterilized by autoclaving at 120°C temperature for 20 minutes. Before autoclaving, the pH registered in a culture medium was adjusted to 5.6-5.8.

The reactivity of explants was evaluated after a month of culture.

In order to culture proliferation, the explants by a node taken from microshoots obtained in the initiation cultures phase were inoculated on MS culture medium supplemented with various concentrations of BAP (0.1, 0.5, 1, 2 mg/l) in combination with NAA (0, 0.1, 0.5 mg/l). The cultures were transferred each 4 weeks on fresh media.

Rooting phase was not necessary, microshoots developing roots on multiplication medium.

For growth, multiplication and rooting of explants have ensured in the growing room controlled conditions of temperature (22-24°C), photoperiod (16 hours) and light intensity (2 500 lx).

The *in vitro* rooted plants were transferred *ex vitro* in order to be acclimatization at their natural environment.

The results obtained regards *in vitro* reactivity of marjoram explants were expressed in percent and was statistic interpreted using the Duncan test. Multiplication rate was expressed as number of differentiated shoots on the explant. The acclimatization percentage of *in vitro* regenerated plant was calculated as the ratio between the number of acclimatized plants viable and total number of *in vitro* regenerated plants.

## RESULTS AND DISCUSSIONS

To establish a reproducible protocol for *in vitro* multiplication was necessary the selection of explant and of optimal culture medium to induce a higher number of shoots. In comparative analyzes aimed morphogenetic competence of explants from different origins (nodes and apexes stems), in interaction with different culture media at which BAP concentration varied, the results obtained showed that both types of explants analyzed have the ability to regenerate shoots under *in vitro* conditions, but the highest morphogenetic potential was observed in the case of apexes stems. They had the highest regeneration ability on MS basal medium supplemented with 1 mg/l BAP.

On the variant of the medium supplemented with 0.5 mg/l BAP, regeneration percentage registered lower values compared to the variant discussed above. Also, increasing BAP concentration had a negative effect on the development of explants, the lowest values of the percentage of growth yield in the presence of 1.5 mg/l BAP.

As regards the use of 0 and 2 mg/l BAP concentrations were observed inhibitory effects expressed as a null percentage of explants grown (fig. 1).

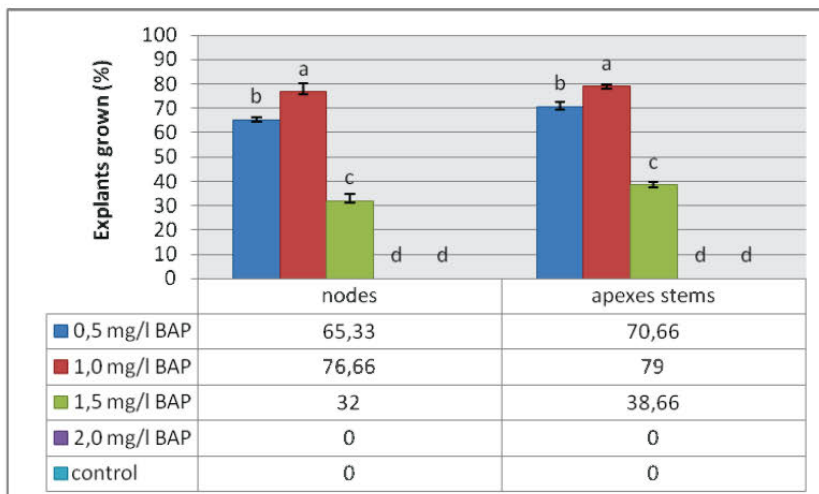


Fig. 1. Marjoram explants reactivity depending on type of explant used and composition of culture medium

Values are means  $\pm$  s. d. based on three repetitions; bars indicate standard deviations; small letters represent interpreting the significance of differences by using Duncan test,  $p < 0.05$

The results registered in shoots regeneration experiments at *Origanum majorana* species reflected the important role that it has hormonal balance in overall of the factors that determine the expression of regenerative potential in *in vitro* culture of a node explants sampled from microshoots obtained in the initiation phase of cultures, a conclusive evidence in this regard being different effects of the same basal medium supplemented with various combinations and concentrations of growth hormones.

Variants of the medium that allowed stimulation of regenerative processes are characterized by the presence of BAP in combination with NAA. The most adequate hormonal balance for this purpose has proved to be 2 mg/l BAP / 0.5 mg/l NAA (fig. 2).

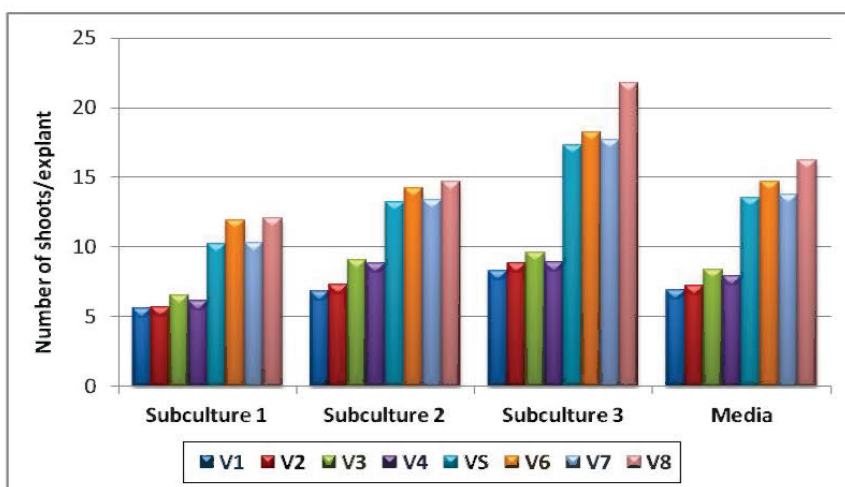


Fig. 2. The influence of combination and concentration of growth hormone on *in vitro* multiplication rate of *Origanum majorana* species

After approximately 30 days, the shoots obtained were transferred to fresh medium that supported the regenerative processes by determining a good proliferation of shoots.

The observations made during three successive subcultivation showed that there was recorded an increase of the multiplication rate up to 21.8 shoots/explant in the case of third subculture.

From the qualitative point of view, the biological material resulting from the regeneration of explants had a normal morphology, without vitrification aspects, necrosis or callus differentiation (fig. 3).



Fig. 3. Biological material multiplied *in vitro*

The rooting phase was not necessary, the association between BAP and NAA determining also the appearance of the roots inside the nutrient medium, but also the development of aerial roots (fig. 4). Achievement of this process allows elimination of *in vitro* rooting phase from standard propagation technology by tissue culture, determining cost reductions, the phase being recognized as very expensive. Also, simultaneously with *in vitro* multiplication occurs rooting, in which case, the period of planting material production through tissue culture shall be reduced by less than 30 days.



Fig. 4. The emergence of roots inside the nutrient medium (right) and the development of aerial roots (left) at microshoots regenerated on culture medium supplemented with BAP and NAA

*In vitro* regenerated plants had a vigorous root system, allowing their passage in the next phase, of acclimatization, in order to accommodate at *ex vitro* conditions.

The best results with regard to acclimatization of *Origanum majorana* plants has been obtained when have been used pills of peat type Jiffy (V.2) (87% acclimated plants). When using the mixture of peat, perlite and manure in proportion of 2: 1: 1 (V.1), the percentage of acclimatized plants was lower (56%), this substrate however ensures a good fortification of acclimated plants (fig. 5 and 6).

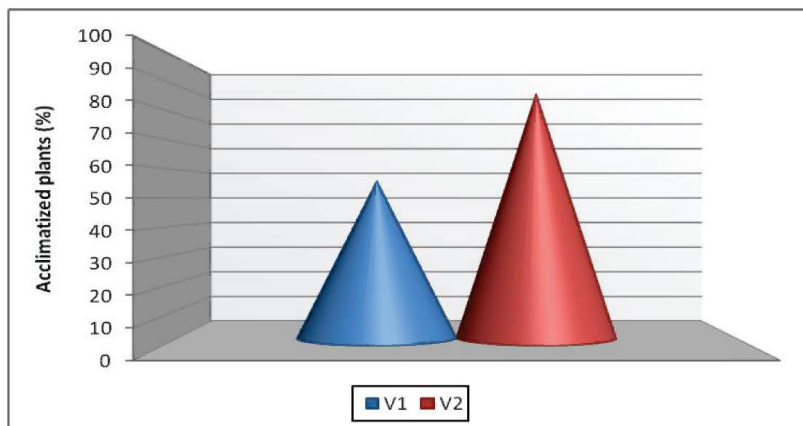


Fig. 5. The influence of substrate on acclimatization at *ex vitro* conditions of *Origanum majorana* regenerants

After the acclimatization and fortification at pots, the plants were transplanted to greenhouse for further their growth and development (fig. 6). Mention the fact that plants obtained by *in vitro* propagation have preserved morphological characteristics of donor plant.



Fig. 6. *Origanum majorana* plants fortified in pots and on soil

We conclude that results obtained throughout *in vitro* regeneration phases, confirming preliminary studies and certify that for *Origanum majorana* species, cloning by tissue culture is viable. Micropropagation technique represents an effective alternative to

propagating for species under study, in order to create a permanent source to extract of some secondary metabolites for pharmaceutical industry.

### CONCLUSIONS

In comparative analyzes aimed morphogenetic competence of explants from different origins (nodes, apexes stems), in interaction with different types of culture media is recommended for *in vitro* multiplication explants represented by apexes stems.

Among abiotic factors, nutritive medium composition influenced differently induction of morphogenesis. The most efficient proved to be MS basal nutritive medium supplemented with 1 mg/l BAP.

At *Origanum majorana* species value of *in vitro* regeneration rate was influenced by hormonal balance, the highest number of shoots/explant were obtained on variant of medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.

The rooting phase was not necessary, microshoots developing roots on multiplication medium.

The yield on acclimatization of species under study was influenced by nutritive support used. Optimal conditions for acclimatization have been found those based on the use of peat pills as substrate nutrient which ensured a percentage of 87% acclimatized plants. The mixture of peat, manure and perlite in a proportion of 2: 1: 1 is recommended for fortification of vitroplants in pots.

The results obtained throughout *in vitro* regeneration phases confirm preliminary studies and certify that for *Origanum majorana* species, micropropagation technique constitutes an advantageous alternative that enables rapid obtaining of high quality planting material.

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**THE SUBSTRATES INFLUENCE ON INTENSITY AND QUALITY  
MINERAL NUTRITION OF GRAPEVINES CUTTINGS, INITIAL  
MATERIAL G1**

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*Keywords: organic, mineral, nutrition, quality, intensity*

**ABSTRACT**

*The content of rooting substrates is very important for the production of grapevines cuttings. These must provide the nutrients necessary for optimum growth and development of shoots and roots. In this paper were used five rooting substrates: 100% mineral, 75-25% mineralo-organic, homogeneous (50 % organic + 50 % mineral), 75-25% organo-mineral and 100% organic. On each substrate were planted cuttings from the following clones: Muscat d'Adda 22 St, Feteasca regala 72 St, Feteasca alba 97 St., Muscat Ottonel 16 St, Feteasca neagra 6 St. The plants development was different on each rooting substrate depending on the organic matter and mineral content, intensity and quality of mineral nutrition, and also being differentiated on the variety (genotype) and their preferability for certain soil types suitable to natural conditions of adaptation and acclimatization.*

**INTRODUCTION**

In vegetation pots or in culture substrates, the assimilable minerals necessary of grapevine plants or rooted cuttings it cover from existing reserves in culture substrates by adding organic fertilizers (manure, peat, compost) at the formation of mixtures and from fertilizers produced in industries (chemicals or minerals) which completes periodic the needs of plants.

The assurance of cultivated plants necessities from these substrates known a dynamics that depends on one side of the amount of substrate who is at the disposal of the plant (the size of the vegetation pots) and ensures, in the first years of vegetation, all necessary nutrients but also a substrate depletion, and on the other side by the manage mode of growing and development processes and the control of environmental factors in protected spaces, so by the intensity of these processes and factors.

The high complexity of the influences which have the factors and vegetation conditions in their interactions on mineral nutrition and on the plants needs for fertilizer leads to obtained the experimental data that are used to establish the doses of fertilizers.

A number of principles (Borlan Z. et al., 1982) should be considered in establishing the fertilization recipes in protected spaces and not only:

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- the volume of the vegetal phytomass is the result of the action of all factors and vegetation conditions;
- optimization and harmonization of factors and vegetation conditions make it possible to obtaining a high productivity;
- the productivity level obtained reflect the degree of positive interaction of factors and vegetation conditions;
- the positive interaction degree of the biological, physical and chemical factors of vegetation increases with the level of vegetal phytomass, their role in the interactions being greater than their present quantitative separated;
- the positive interaction of factors and vegetation conditions lead to specific consumption of fertilizers that decrease gradually with the vegetation phytomass level due to complete metabolization in plants of the nutritive elements and their incorporation into useful organic substances of constitution and reserve;
- optimization by an superior agrotechnics of a biological and physical factors determine a better mobilization of nutritive substances from the substrate and superior capitalization of them in plants;
- the doses and the effect of fertilizers gradually decreases after a curve who tends toward a invariably minimum level, during the improvement of the soil or substrates insurance state with nutritive substances in effective or potential assimilable forms for plants.

Also, should be taken into account, unlike farms, in protected spaces and especially in vegetation plots the active roots of plants (radicular hairs) explores directly the the surface of soil particles who contain in absorbed stage the nutritive substances and that they come into contact with the solution that contein nutrient ions in indirectly assimilated forms. Nutrients mobilization by the activity of microorganisms and chemical processes, as well as the ions diffusion and migration processes in soil is influenced by environmental factors with the predictable dynamics (controlled) during the vegetation period.

## MATERIAL AND METHODS

Rooting substrate as technical factor, along with other technical factors, biological and ecological, has the most important role in the nutrition process of grapevine plants planted in pots.

In order to establish the mixes recipes for the substrates adequate for rooting of cuttings of one eye from biological material of superior quality (G0, G1 and G2), in protected spaces, and to determine the nutritional regime, the intensity and quality of mineral nutrition during vegetation, was created an experimental device that took into account the following two factors:

**A Factor - the nutrient mixture, with graduations**

- A1- mineral mixture, 100% (0-3 mm washed sand)
- A2- mineralo-organic mixture, 75% mineral, 25 % organic
- A3- homogeneous mixture, 50 % organic + 50 % mineral
- A4- organo-mineral mixture, 75 % organic + 25 % mineral
- A5- organic mixture, 100%, 50 % manure + 75 % peat

**B Factor - varieties, with graduations**

- B1- Muscat d'Adda 22 St.
- B2- Feteasca regala 72 St.
- B3- Feteasca alba 97 St.
- B4- Muscat Ottonel 16 St.
- B5- Feteasca neagra 6 St.

The experience is bifactorial of type 5x5. By combining the factors are resulted 25 variants, each experimental variant being randomized into repetition arranged together, continued from 2013 (Radulescu I. et al, 2013).

During the experiment took place the steps of the cuttings stratification, acclimatization and transfer on the nutritional substrates in order to strengthen.

The studies conducted have focused on one part determination of rooting substrates content in organic matter and nutrients and their influence on vegetative growth, through biometric measurements, respectively the amount of annually wood removed from cutting and the appreciation of the maturation degree of cuttings young shoots by soluble carbohydrate through the content of soluble carbohydrate starch, total water, crude protein and total nitrogen and the appreciation of the cuttings phytoproductivity by the determination of the vegetative mass of the shoots in full vegetation, and on the other hand targeted the intensity and quality of mineral nutrition in different growth phenophase.

The mineral nutrition intensity and quality of the viticultural initial material G1 planted in pots in greenhouse, was made by quantitative foliar diagnosis at the blooming and veraison, with leaf sampling located next to bunches, determining the content of total N, P and K by the laboratory analysis and the global nutrition and food balance through mathematical calculations.

Based on determination of nitrogen, phosphorus and potassium total forms have established the global nutrition as the sum of the three basic elements, expressed in % at the dry matter.

## RESULTS AND DISCUSSIONS

Mineral nutrition intensity and quality at the blooming and veraison (NPK - %, global nutrition - %, alimentary equilibrium – NPK).

The intensity and quality of the grapevine mineral nutrition, determined by the quantitative foliar diagnosis at the blooming and veraison is correlated with other factors of vegetation (soil-substrate, climate conditions, maintenance work) and considering the fact that the plants are in a continues biological process, the results refer only the time when the probes were prelevated.

Analyzing the average values during the vegetation (Table 1), 2.85 % N, 0.29 % P and 1.33 % K compared with the critical level set by Lagatu 1934 and after Maumere-Dulac, 1948, of 2.5% N, 0.22% P and 1.25% K, the aprovisionation supply of plants was superior in all the nutrients without record values that indicate the abundance or excess; if we appreciate after Levy (cited by Davidescu D. and Davidescu Velicica, 1981), which

Table 1  
Mineral nutrition intensity and quality at blooming and veraison at the  
G1- Initial materials

Varieties/Clones	Blooming-veraison			Global aliment.	Nutritional equilibrium		
	N%	P%	K%		Blooming-veraison		
				N%	P%	K%	
Feteasca alba 97 St	2.97	0.21	1.34	4.5	65	5	30
Feteasca regala 72 St	3.17	0.28	1.64	5.1	63	6	31
White varieties average	2.97	0.26	1.52	4.8	66	8	26
Tamaioasa romaneasca	2.62	0.27	0.93	3.8	69	8	23
Aromatic varieties average	2.72	0.27	1.52	4.1	67	8	25
Feteasca neagra 6 St	2.85	0.39	1.50	4.7	59	10	31
Black varieties average	2.92	0.34	1.31	4.5	65	8	27
General average	2.85	0.29	1.33	4.5	65	8	27

established in France a reference average of 2.5% N, 0.5% P and 2.5% K and an nutritive echilibrium of 45.5% N, 9% P<sub>2</sub>O<sub>5</sub>, 45.5 % K<sub>2</sub>O, the obtained results indicate a

nutritive equilibrium favorable for nitrogen and in detriment for phosphorus and potassium which does not affect the quality of propagation material found in greenhouses, where the vegetation period is extended and the balance can be change and improve.

The medium content obtained at blooming- veraison: 2.85 % N; 0.29 % P; 1.33 % K. The average content after Velicica and David Davidescu 1999: 1.5-2.6 % N, 0.7 % P<sub>2</sub>O<sub>5</sub> (0.31 % P) and 0.9 % K<sub>2</sub>O (0.55 % K), (fcP =0.436; fcK = 0.41). Interpreting after these authors are distinguish the higher content of leaf in nitrogen and potassium and relatively close in phosphorus, which corresponds, at some point, with symptoms appeared on the leaves, shiny appearance and corrugating, abundance specific symptom (or potassium excess), a phenomenon that not led to leaves damage.

A comparative analysis of varieties groups, underline a preference of all white wines varieties, in special the superior white wine, for nitrogen nutriment and potassium and lesser for phosphorus, while the red wine varieties have a higher preferences for phosphorus nutriment potassium and lesser, but still high, for the potassium. Flavored varieties have a lower preferences for nitrogen and phosphorus nutriment and higher for potassium. This element is used from grapevine in the growing process of the leaves and shoots. Total nitrogen content of the foliar material was influenced, primarily, by the culture substrate and in the second place by the genotype (Table 2). In the case of all studied clones was observed an increase of a total nitrogen content from foliar material as the nutrient is fertile in organic matter.

Table 2

Total nitrogen content (N%) from foliar material on different substrates

Varieties	Substrate					Average
	A1	A2	A3	A4	A5	
Muscat d'Adda 22 St	2.12	2.43	2.49	3.08	3.98	2.82
Feteasca Regala 72 St	2.41	3.00	3.38	3.83	4.60	3.44
Feteasca alba 97 St	2.15	2.57	3.34	3.41	4.02	3.10
Muscat Ottonel 16 St	1.78	2.00	3.06	3.39	3.35	2.72
Feteasca neagra 6 St	2.00	2.08	3.01	3.76	4.25	3.02
Average	2.09	2.42	3.06	3.49	4.04	3.02
FCDF	1.17	1.04	0.67	0.31	-0.29	0.58

$$FCDF; N = 1,4-0,0256 \times N_f^3$$

From the data presented in the Table 2, concerning the foliar material content in N %, was observed that the harvested leaves were recorded a medium content of 3.02 % for N, with variations between 2.09 and 4.04 %, in general with higher compared to medium content of 1.5 to 2.6 % N, optimum established for grapevine cultivated in normal culture conditions. It should be noted that the variants which substrate mainly mineral it fits in these limits while those with substrates predominantly organic record high contents of N% with the growth of the amount of organic matter, which shows the grapevine preferences for stony soils and also of the cuttings for rooted on such substrates.

Another important constituent of plant tissue is phosphorus (P). It enters into the nucleic acids composition and intervenes in cell proliferation. The phosphorus plays an important role in photosynthesis. Like in the case of nitrogen, phosphorus assimilation in leaf was correlated with organic matter content from culture substrate. It has been noted that the studied genotypes reacted positively (accumulation of 0.39 % P) for variant A5 (100 % organic) compared with the A1 substrate (100 % mineral) to which phosphorus content was 0.13 %.

As a result of studies (Table 3), it can be appreciated that the substrates with 50 % and even 75 % organic matter favours the phosphorus assimilation in the leaf (blooming-veraison), the recorded values fits into a good supply of this element, 0.29 % P in A3, and 0.32 % P in A4 compared to an optimum of 0.31 % P in P<sub>2</sub>O<sub>5</sub>. The extreme substrates, mineral and organic 100 % present the disadvantage that one favors a weak assimilation and the other a strong assimilation, situation which may lead to a deficiency or excess and also to poor assimilation or blocking other nutritive elements from soil. If the organic substrates can be removed also from other considerations, the mineral substrates can be accepted from physical considerations and chemically improved through rational and proper fertilization.

Table 3

The phosphorus content (P%) at vegetal material on different culture substrates

Varieties	Substrate					Average
	A1	A2	A3	A4	A5	
Muscat d' Adda 22 St	0.14	0.16	0.23	0.28	0.34	0.23
Feteasca Regala 72 St	0.15	0.25	0.28	0.41	0.39	0.30
Feteasca alba 97 St	0.16	0.20	0.23	0.25	0.40	0.25
Muscat Ottonel 16 St	0.09	0.10	0.39	0.39	0.49	0.29
Feteasca neagra 6 St	0.10	0.15	0.31	0.28	0.33	0.23
Average	0.13	0.17	0.29	0.32	0.39	0.26
FCDF	1.39	1.38	1.31	1.28	1.18	1.33

$$FCDF; P = 1,4 - 3,75 \times P_f^3$$

Between the clones used in the experiment (Table 3) were recorded differences, in the sense that Feteasca Regala 72 St and Muscat Ottonel 16 St assimilated, to an optimal value, the phosphorus from soil, Feteasca alba 97 St assimilated phosphorus at the inferior limit of optimum, while Feteasca neagra 6 St and Muscat d' Adda 22 St registered suboptimal values.

The potassium (K), another vital element in the development of the grapevine, being directly involved in the metabolic processes of the plant. It acts as a cation (K<sup>+</sup>) with other cations, such as calcium and magnesium. The potassium ion, K<sup>+</sup>, is present in the composition of each phase of the soil (liquid or solid), it being involved in the development of many cellular processes: carbohydrates and aminoacids synthesis, cells multiplication, maintaining the osmotic pressure, reserve substances biosynthesis, reduction of perspiration, etc. The foliar material content variation in potassium depending on the genotype and the used substrate is shown in table 4.

Table 4

The K% content from plant material on different cultured substrates

Varieties	Substrate					Average
	A1	A2	A3	A4	A5	
Muscat d' Adda 22 St	0.93	0.87	0.89	0.89	0.79	0.87
Feteasca Regala 72 St	0.69	0.77	0.66	0.75	0.64	0.70
Feteasca alba 97 St	0.76	0.81	0.71	0.74	0.94	0.79
Muscat Ottonel 16 St	0.58	0.54	0.66	0.82	0.74	0.67
Feteasca neagra 6 St	0.36	0.46	0.56	0.51	0.58	0.49
Average	0.66	0.69	0.70	0.74	0.74	0.70
FCDF	1.17	1.16	1.16	1.14	1.14	1.15

$$FCDC, K = 1,4 - 0,3 \times K_f - 0,1 \times K_f^3$$

The content of K % (of K<sub>2</sub>O) from leaves at the time of diagnoses (average blooming-veraison) was 0.7 % and varied little, from 0.66-0.69 % on the mineral and mainly mineral substrates, at 0.74 % on those organic and preponderant organic, the containing almost double compared to the optimal one of 0.37 % in the field culture, that showing an excess absorption of potassium especially on the organic substrates, which evidently is due to the nitrogen-potassium tandem and to the synergism between the two elements and reinforces the conviction that the use of the mixtures predominantly organic at the grapevine cuttings rooting in pots vegetation vases in protected spaces are not quite matched.

Regarding the clones used in this experiment, except Muscat Adda 22 St clone, at which the amount of potassium from leaf isn't influenced by the nature of the rooting substrate, at the percentage in K increases with the increasing of organic component from the substrate. Value, superior accumulation in potassium were recorded at Muscat Adda 22 St clone (0.87 %), while at the clone Feteasca neagra the potassium from foliar material was minimal (0.49 %). After the K % contents from leaves it can be established a hierarchy of varieties susceptibility to the potassium abundance and excess, namely: the least susceptible are those for red wine are (which records and most frequently the symptoms of potassium excess, so are the most sensitive) followed by those aromatic, white wine and table varieties. Among the white wines, endurance at potassium abundance or excess increases with varieties quality, from the table white wine varieties at the wines varieties of superior quality.

Based on experimental results obtained for foliar diagnosis, were calculated for each nutrient analyzed N, P, K the correction factor of foliar diagnosis- FCDF used to assess the fertilizer DOExp.

FCDF = 1.4 - hx Ef - ix EF3, where Ef is the element content by leaves. FCDF varies from 1, values considered normal, to 1.3-1.4, values considered under optimal and which corresponding increases the fertilizer DOExp.

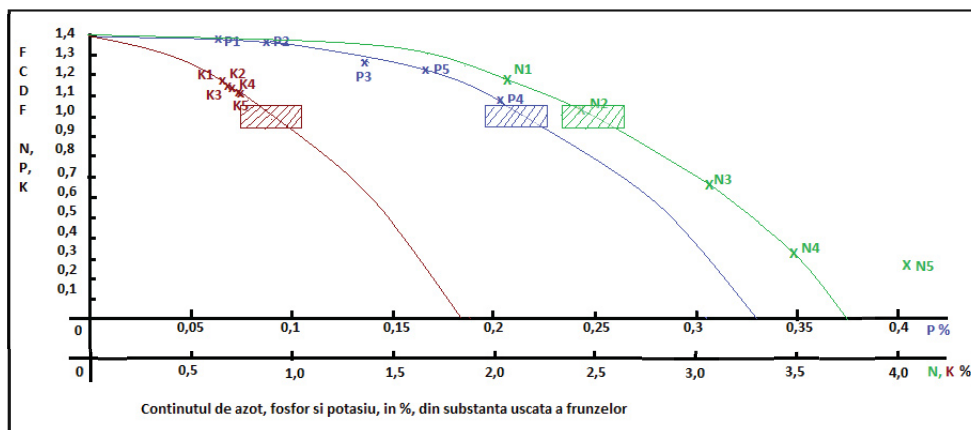


Figure 1. FCDF of the experimental optimal dose of N, P<sub>2</sub>O<sub>5</sub> si K<sub>2</sub>O at grapevine that produce strings / graft eye depending to the contents of N, P and K from leaves dry substances (average blooming-veraison).

FCDF for N, based on the medium content for N % from vegetal material, the coefficient which decreases with increasing of organic matter from culture substrate, has been on average the value of 0.58, with variations depending on substrate of culture, from 1.17 to 1.04 on mineral substrates, at 0.31 - (-0.29) on the organics.

FCDF for P, based on the medium content for P % from vegetal material, the coefficient which decreases with increasing of organic matter content from culture substrate, and which records in media values of 1.33, with variations depending on the culture substrate from 1.39 to 1.31 on mineral substrates at 1.31 to 1.18 on the organics, which shows a largest necessity of phosphorus fertilizer on the mineral substrates.

FCDF for K, based on the medium content for K % from vegetal material, recorded in media the value of 1.15 with variations depending on the culture substrate from 1.17 - 1.16 on mineral substrates to 1.14 on the organics, the values very close to the optimal, which shows a lower necessity for potassium fertilizer of the mineral substrates.

### CONCLUSIONS

Plant status supply was proved experimentally through the media content of 2.85 % N, 0.29 % P and 1.33 % K and was superior in all the nutritive elements without record values that indicate excess, but the results indicate nutrient balance for nitrogen and phosphorus and in the detriment of phosphorus differentiated by the varieties preferences.

The total nitrogen content from foliar material is determined by the culture substrate nature, the organic component favoring the synthesis and the accumulation of the nitrogen compounds; a substrate with 75 % and even 50 % organic matter favors the assimilation of phosphorus in leaf (during from blooming to veraison), registred values falling into the limits of a good supply with this element; with the exception of Muscat Adda 22 St clone, wherein the amount of potassium from leaves isn't influenced by the nature of the rooting substrate, on the other studied clones the percentage content in K decreases while the organic component from the substrate is reduced.

In the mixture from the vegetation pots is a great nonuniformity of a nutritive elements. The organic matter added to the mixture was in large quantity and is still untransformed in nutrients and the necessities of the nitrogen fertilizer application is not appropriate.

The substrate content in mobile phosphorus (PAL) show a low supply state to medium in phosphorus and an increased necessity for application of the fertilizers based on phosphorus.

The content of the substrate in mobile potassium (KAL) show a very low supply in potassium and an urgent necessity for application of fertilizers based on potassium.

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## GROWTH AND FRUCTIFICATION POTENTIAL OF SOME APPLE VARIETIES WITH GENETIC RESISTANCE TO DISEASES, GRAFTED ON MM 106 ROOTSTOCK

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*Keywords: apple, productivity, environmental protection.*

### ABSTRACT

*The research conducted at the Voinești Fruit Growing Research and Development Center, during 2011-2013, highlighted the growth and fructification potential for apple varieties with genetic resistance to diseases: Redix, Iris, Irisem, Real, Remar, Inedit, Voinicel and Florina, grafted on MM 106 rootstock, cultivated in intensive system (1250 trees/ha). The growth vigor during the 9th year of age of the trees indicated significant differences among the studied apple varieties related to growth in thickness of the trunk, size of the trees and crown volume. The productive potential of the studied varieties had values between 25 and 40 tones/ha for trees of 7-9 years old. The varieties that produced more than 30 tones/ha (Redix, Iris, Remar, Inedit, Real) can be considered as apple varieties with high productive potential. The level of costs for the phyto-sanitary treatments applied to the studied apple varieties is lower by more than 55%, compared to the sensitive assortment.*

### INTRODUCTION

The apple assortment has changed significantly during the last decades, as varieties that meet the continuous growing demand began to be promoted. A special case is represented by the promotion of apple varieties that are genetically resistant to diseases (Branîște, Andrieș 1990, Hoza and all. 2009) which represent for the new plantations links of the modern economic technology (Petre et al. 2005, 2010), with immediate effect through total or partial elimination of the fungicides (Petre, Andrieș, Petre, 2005).

The gradual change in the assortment through promoting apple varieties with genetic resistance to diseases depends on various characteristics that the varieties may display (Bramiște, Andrieș 1990, Petre 2000). The superiority of the new varieties must mainly address the requirements of the producer, sensitive to economic efficiency, growth vigor, high productive potential, various consumption periods etc, as well as the taste of the consumer.

The important progress obtained during the study of apple varieties with genetic resistance to diseases, conducted at the Voinești Research and Development center, allowed the study of the behavior within an orchard of the trees from 8 apple varieties resistant to diseases, in order to promote them within the assortment, compared to 2 control varieties, sensitive to diseases: Jonathan și Golden delicious.

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## MATERIAL AND METHODS

The research was conducted within apple orchards belonging to the Voinesti Center, experimental base no.1, during 2011 – 2013, highlighted the growth and fructification potential of some apple varieties with genetic resistance to diseases, as the main factor in obtaining productions adapted to the requirements of the European quality standards. Eight apple varieties resistant to diseases were studied: Redix, Iris, Remar, Inedit, Voinicel, Real, Irisem, created by the R&D center, and Florina variety, compared to the sensitive varieties Jonathan and Golden delicious. All studied varieties were grafted on the MM 106 rootstock. The trees were planted during 2005, at a distance of 4 x 2 m (1250 trees/ha), the crown was shaped as spindle-bush freely flattened along the row.

Observations and measurements were made related to the growth in thickness of the trunk, crown size, production and average fruit weight.

The soil from the orchard was brown, weakly eutric cambisol, with loamy texture with a weak acid pH (5.7 – 5.9); the humus content was medium at the surface (2.0 – 2.9%), medium supplied with nitrogen and weakly supplied with phosphorus and potassium.

The climatic conditions were favorable for the growth and fructification of the trees, characterized by an annual average temperature with 1.0°C higher than the normal local temperature, (8.8°C), with total annual rainfall of 755 mm.

The soil from the orchard was unreaclaimed on the interval and maintained worked on the row of trees. In order to combat pests, 6 - 8 annual treatments were applied, using insect powder only. The other works were executed accordingly to the technology specific to intensive apple orchards.

## RESULTS AND DISCUSSIONS

Tree vigor for the studied apple varieties resistant to diseases was quantitatively expressed through the volume of the annual vegetative growth as trunk size, height and crown size, these being determined by the vigor of the variety, degree of soil fertility, planting distance, the rootstock being the unchanged factor.

The growth vigor during the 9<sup>th</sup> year of age of the trees, when the growth potential was well defined, indicated the fact that there were significant differences among the studied apple varieties related to growth in thickness of the trunk, height and crown diameter (Table 1).

Related to trunk growth during the 9<sup>th</sup> year, evaluated as the area of the trunk section, measured at the height of 30 cm from the ground, rather great differences were recorded among the varieties. The variation limits were between 66.44 cm<sup>2</sup> for Voinicel variety and 165.05 cm<sup>2</sup> for Florina, which was the most vigorous among the varieties genetically resistant to diseases, followed by Redix.

Crown volume depended on the size of the trees, being greatly influenced by the tree vigor.

The tree height during the 9<sup>th</sup> year since plating had values between 310 cm for the varieties Iris and Voinicel and 380 cm for Florina, compared to the varieties Jonathan and Golden delicious, taken as control varieties, for which tree height was of 340 cm, respectively 360 cm, when the height was limited by fructification pruning executed during the rest period of the trees.

The thickness of the fruit wall had appropriate values for all studied varieties, between 160 and 230 cm.

Table 1

Growth potential for some apple varieties, grafted on MM 106 rootstock,  
during the 9<sup>th</sup> year since plating

No	Variety	Area of trunk section (cm <sup>2</sup> )	Tree size (cm)		Crown volume (cm)		
			Height	Weight	Per ha	Dif.± compared to Mt.1	Dif.± compared to Mt.2
1	Jonathan (Mt.1)	130.63	340	230	16.675	-	- 375
2	Golden delicious (Mt.2)	140.95	360	220	17.050	+375	-
3	Florina	165.05	380	130	18.975	+2300 <sup>xx</sup>	+1925 <sup>x</sup>
4	Inedit	83.28	320	175	11.812	-4863 <sup>000</sup>	-5238 <sup>000</sup>
5	Iris	100.24	310	180	11.700	-4975 <sup>000</sup>	-5350 <sup>000</sup>
6	Irisem	89.87	320	190	12.825	-3850 <sup>000</sup>	-4225 <sup>000</sup>
7	Real	105.63	340	215	15.587	-1088	-1463
8	Redix	149.49	370	220	17.600	+925	+550
9	Remar	122.66	350	215	16.662	-13	-388
10	Voinicel	66.44	310	160	10.400	-6275 <sup>000</sup>	-6650 <sup>000</sup>

DL 5% = 1680cm; DL 1% = 2310cm; DL 0,1% = 3150cm.

Based on the data related to tree vigor, crown volume per unit of area was calculated.

Crown volume per unit of area recorded a rather high variability among varieties, from 10.400 cm/ha for Voinicel to 18.975 cm/ha for Florina, being considered the most vigorous among the apple varieties with genetic resistance to diseases.

Lower values for the crown volume per unit of area were recorded also for the varieties Inedit, Iris and Irisem. For the other varieties, crown volume per unit of area had values close to the ones recorded for Jonathan and Golden delicious, the control varieties (16.675 – 17.050 cm/ha).

The statistical calculus recorded significant positive distinct differences compared to Jonathan, as the control variety 1, and significant positive differences compared to Golden delicious, as the control variety 2, only for the variety Florina, considered the most vigorous among the apple varieties with genetic resistance to diseases. The other varieties had lower values for the crown volume, under the control level.

The productive potential of the varieties and the level of the production obtained per variety during 2011 – 2013, are presented in table 2.

The recording of the annual production showed differences between the production capacities of the studied apple varieties. Analyzing the average production of the years 7-9 since planting, it could be observed that out of the 8 studied apple varieties cultivated in intensive system, the varieties Iris and Remar largely fructified every year, with productions over 40 t/ha. Smaller harvests were recorded for the varieties Voinicel, Irisem and Florina (28.1 – 29.3 t/ha). They were considered to have high potential also the varieties that produced over 33 t/ha, meaning Inedit, Real and Redix. The varieties Jonathan and Golden delicious, the controls, recorded a three year average production of 26.1 t/ha, respectively 30.2 t/ha. The statistically computed data confirmed very significant positive

differences compared to Jonathan for the majority of the studied varieties. Compared to Golden delicious, the resistant varieties Inedit, Iris, Remar recorded very significant positive differences, while Real and Redix recorded significant distinct positive differences.

Table 2

Fruit production obtained for the apple varieties with genetic resistance to diseases during the years 7 – 9 since planting (1.250 trees/ha)

No	Variety	Production obtained (t/ha), in the year			Average of the years 7- 8 (t/ha)	Difference ±	
		7 2011	8 2012	9 2013		Compared to Mt.1	Compar ed to Mt.2
1	Jonathan (Mt.1)	30.3	25.6	28.3	26.1	-	-4.1 <sup>000</sup>
2	Golden delicious (Mt.2)	43.1	19.5	28.0	30.2	+4.1 <sup>xx</sup>	-
3	Florina	30.4	28.0	25.8	28.1	+2.0	-2.1
4	Inedit	45.5	33.3	29.5	36.1	+10.1 <sup>xxx</sup>	+5.9 <sup>xxx</sup>
5	Iris	47.8	45.3	37.6	43.6	+17.5 <sup>xxx</sup>	+13.4
6	Irisem	30.3	28.0	29.5	29.3	+3.2 <sup>xx</sup>	-0.9
7	Real	40.5	35.4	26.0	34.0	+7.9 <sup>xxx</sup>	+3.8 <sup>xx</sup>
8	Redix	38.4	35.9	25.8	33.4	+7.3 <sup>xxx</sup>	+3.2 <sup>xx</sup>
9	Remar	45.5	29.1	35.5	40.0	+13.9 <sup>xxx</sup>	+9.8 <sup>xxx</sup>
10	Voinicel	28.9	32.4	23.6	28.3	+2.2	-1.9

DL 5% = 2.26t; DL 1% = 3.10t; DL 0.1% = 4.22t.

The productions recorded for Florina and Voinicel were not statistically ensured, varieties for which the degree of fruit bud differentiation was less obvious.

It must be mentioned the fact that the varieties Voinicel and Irisem had a much lower crown volume than the other varieties. The planting of these varieties at smaller distances along the rows would ensure a higher planting density and, as a consequence, a larger production, which would allow them to be included in the list of high productive potential apple varieties.

The fruit growing assortments undergo a permanent change, the place of the varieties with weaker commercial characteristics being taken by the new homologated varieties that meet the continuous growing demand of the consumers.

The creation of a large number of apple varieties with genetic resistance to diseases, both within Romanian fruit growing centers and foreign varieties, the concept related to promoting them into culture, acquired new meanings that contribute to a faster capitalization for the producers and to the creation of apple lots which are less polluted, for the benefit of the consumer.

The studies and researches conducted at the Voinesti center present to the apple growers a group of varieties with different maturation periods, which cover a great part of the apple consumption with the production obtained from the apple varieties genetically resistant to diseases.

Nowadays, the basic assortment includes the resistant varieties: Romus 1, Romus 3, Romus 4, Prima, Pionier, Voinea, Ciprian, Florina, which are reproduced into the plant nurseries across the country. Some of the mentioned varieties, even though they comply for

the moment with the resistance, productivity and fruit quality requirements, could be replaced as new varieties appear, more valuable from the point of view of the production and of fruit quality.

The studied apple varieties, created at the Voinesti Center, can meet a great part of the consumption period, along with certain existing varieties with genetic resistant to diseases, already known and appreciated on the market.

Table 3 presents the apple varieties with genetic resistance to diseases that were studied during 2011–2013 and the manner in which these varieties fit among the genetically resistant apple varieties from the current assortment.

Depending on the maturation and fruit consumption periods, the studied varieties that were noted for genetic resistance to diseases, productivity and fruit quality are fitted differently into the varietal conveyor for the Dambovita fruit growing region.

Table 3

The consumption period of the studied varieties, fitted into the varietal conveyor of the resistant apple varieties, cultivated in the influence region of the Voinesti Fruit Growing Center

Variety	Month																											
	VII			VIII			IX			X			XI			XII			I			II			III			
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Romus 1		•	•																									
Romus 2		•	•																									
Romus 3				•	•																							
Irisem							•	•																				
Real							•	•																				
Prima							•	•	•																			
Remar										•	•	•	•	•														
Iris										•	•	•	•	•														
Voinea										•	•	•	•															
Voinicel										•	•	•	•	•	•	•												
Pionier										•	•	•	•	•	•	•												
Inedit										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Generos										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Redix										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Florina										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	

Thus, after the varieties Romus 1, Romus 3 and Romus 4, Irisem and Real, can be introduced, with fruit with pleasant aspect, maturation during the last decade of August – first decade of September.

The Remar variety fits between Voinea and Pionier, with the chance of replacing Voinea with Remar, as this variety presents higher quality related to taste and color of the

fruit. Moreover, for autumn, the varieties Iris and Voinicel can be noted, which can be reproduced together with Pionier, variety that can be replaced.

The variety Redix completes a consumption period that lasts until December – January. The variety Inedit exceeds the consumption period of the variety Redix, being close to the one of Florina.

Shifting to apple varieties with genetic resistance to diseases will be required gradually, as a consequence of the economic efficiency, but also as because these represent the main factor in obtaining ecological productions.

### CONCLUSIONS

Growth vigor of the trees during the 9<sup>th</sup> year since planting, represented by the area of the trunk section, recorded extreme values between 66.44cm<sup>2</sup> for Voinicel and 165.05 cm<sup>2</sup> for Florina.

Crown volume per unit of area varied between 10,400 m<sup>3</sup>/ha for Voinicel, as the least vigorous, and 18,975 cm<sup>3</sup>/ha, for Florina, as the most vigorous among the genetically resistant apple varieties.

The highest productive potential was recorded during the years 7 – 9 since planting for the varieties Iris and Remar, with production values of more than 40 t/ha, but also for the varieties Inedit, Real and Redix with 33 t/ha.

The apple varieties homologated at the Voinesti Research and Development center cover most of the consumption season together with other resistant varieties that are already known and appreciated on the market; they fit differently into the conveyor recommended for the Dambovită fruit growing region.

By promoting genetically resistant apple varieties, one can obtain economic results beneficial for the producers, environmental production and apple fruit with less pesticide residue, which are more and more demanded by the consumers.

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## PARAMETERS OF FRUIT QUALITY FOR SOME GENETICALLY RESISTANT TO DISEASES APPLE VARIETIES

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*Keywords:* apple, fruit quality, biochemical components

### ABSTRACT

*The research conducted at the Voinești Fruit Growing Research and Development Center, during 2011-2013, had the purpose to establish apple fruit quality, in order to promote within the culture the most valuable varieties, which could correspond to the quality standards of a commercial variety, genetically resistant to diseases, with pleasant taste and aspect, large fruit and high fructification potential. The apple varieties genetically resistant to diseases: Remar, Real, Irisem, Florina, had large fruit with average weight of 170-185 g, followed by Redix and Voinicel with fruit of 160-165g. The content of biochemical components was within the corresponding limits: dry substance of 12-15%, total carbohydrates 11.5-14.0%, acidity 0.20-0.36% apple acid and vitamin C with values of 7-9.3 mg/100g fresh fruit. The quality of the apple fruit for the studied varieties can also be noted through low level of pesticides, compared to any variety of sensitive to diseases apples.*

### INTRODUCTION

During the last decades, the apple assortment has changed significantly, as varieties that met the continuous demands of the market and consumers began to be promoted (Ionescu 2011, Hoza 2008, Petre & Petre Valeria 2006).

In order to be promoted within the culture, the apple varieties must comply with a series of conditions, first of all of productivity and especially of fruit quality, mainly for the fresh consumption. Due to this, the pleasant aspect of the fruit must be associated with a pleasant taste, which is due to the main biochemical components; the apples, which are vegetal food, contain almost all the necessary substances for the human body, being included in all food diets (Chira & Chira Lenuța 2008).

Recently, there has been the tendency to introduce within the assortment genetically resistant varieties, which enhance the simplification of the culture technology; this tendency has the purpose of reducing the production costs. For the majority of apple varieties that are genetically resistant to diseases, fruit quality for these varieties is comparable to the quality of standard varieties. For the regions with apple culture tradition in Romania, genetically resistant varieties have been created, that are adapted to local soil and climatic conditions and that are appropriate for research and large scale culture, in

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order to complete the assortment depending on their maturation period. For this purpose, the experiment presented as follows was conducted.

The research conducted at the and Development Centre, during 2011 – 2013, highlights the qualitative features of the apple fruit from physical and biochemical point of view, which define the food quality conditions of certain apple varieties with genetic resistance to local or foreign diseases.

### MATERIAL AND METHODS

The research was conducted during 2011 – 2013, at the Voinești Fruit Growing Research and Development Centre, seven apple varieties with genetic resistance to diseases being studied, creations of the Voinești center: Redix, Iris, Remar, Inedit, Voinicel, Real, Irisem and Florina as foreign variety, compared to the varieties Jonathan as Golden delicious, considered as control varieties. All the varieties were grafted on the MM 106 rootstock, cultivated in intensive system (1.250 trees/ha).

The soil from the orchard was unreaclaimed on the interval and maintained worked on the row of trees. It is brown, weakly eutric-cambisols, with loamy texture with weak acid pH (5.7 – 5.9). The humus content was medium at the surface (2.0 – 2.9%), medium supplied with nitrogen and weakly supplied with phosphorus and potassium.

The climatic conditions were favorable for the growth and fructification of the trees, characterized by an average annual temperature higher with 1.0°C, than the normal local temperature (8.8°C), with total annual rainfall of 755 mm.

In order to combat pests, 6 - 8 annual treatments were applied, using insect powder only. The other works were executed accordingly to the technology specific to intensive apple orchards.

The quality of the fruit was highlighted by the biomass of the fruit, firmness and content in carbohydrates, sugars, acidity and vitamin C.

### RESULTS AND DISCUSSIONS

Apple quality is expressed both through biometric indicators and especially through a series of biochemical components according to which it can be directed and capitalized. If the fruit are for fresh consumption, apart from the shape, size and color, the apples must have a taste accepted by the consumer. According to the region, it can be balanced, sweeter or sour; the consumers usually keep to a tendency that is difficult to change. As a result, the fruit offer must be close to what it is consumed, in order to have capitalization potential.

If the fruit are for industrial processing, the quality is evaluated from the point of view of the biochemical composition, according to the content in soluble dry substance and/or content in vitamin C, components that influence the quality of the finished product.

**The size of the fruit**, associated with an intense and uniform color (red – Jonathan type, yellow – Golden delicious type) and a symmetric shape, gives the fruit the necessary aspect and attractiveness. The study conducted on the apple varieties created at the Research and Development Center highlighted a genetic variability related to the size of the fruit. It was observed a variability of the fruit size between varieties with different ripening periods.

It is appreciated that an average fruit size of 170-180g is consistent with a modern apple variety. The studied apple varieties had a fruit size appropriate for promotion within the culture.

In order to identify the varieties with large fruit, weighing was performed during every year. From the data recorded and presented in table 1, it can be observed that the largest fruit were obtained for the varieties Remar, Real, Irisem, Florina (171-185 g), followed by Redix, Voinicel (155-160 g). The smallest fruit were obtained for the varieties Iris and Inedit (148-151 g, table 1).



Compared to the varieties Jonathan and Golden delicious taken as control variants, fruit biomass for the majority of the apple varieties with genetic diseases resistance recorded high values (160-175g). For Jonathan and Golden delicious varieties, fruit biomass was 155, respectively 165g.

Table 1

Fruit biomass for some apple varieties, at harvest  
(2011-2013)

No.	Variety	Fruit biomass (g)			
		2011	2012	2013	Media
1	Jonathan (Mt1)	167	156	142	155
2	Golden delicious (Mt2)	165	170	160	165
3	Florina	175	170	168	171
4	Inedit	154	149	150	151
5	Iris	148	150	145	148
6	Irisem	185	183	184	184
7	Real	181	175	172	176
8	Redix	158	160	162	160
9	Remar	183	189	183	185
10	Voinicel	159	154	152	155

The size of the fruit during the three years of study was greatly influenced by the size of the harvest and climatic conditions of each year, mainly by the amount of rainfall during the fruit growth period.

For the majority of apple varieties, larger fruit were obtained during the first year of study, while smaller fruit were obtained during the third year.

Pulp firmness is considered appropriate when it is compact, crispy or fine, with uniformly colored (table 2).

Table 2

Fruit penetration resistance for some apple varieties, at harvest  
(2011-2013)

No.	Variety	Fruit firmness at harvest (kgf/cm <sup>2</sup> )			Average
		2011	2012	2013	
1	Jonathan (Mt1)	6.30	6.81	7.10	6.74
2	Golden delicious (Mt2)	7.28	7.63	8.15	7.69
3	Florina	8.13	7.75	8.10	7.99
4	Inedit	7.35	7.63	8.10	7.69
5	Iris	8.10	8.05	7.83	7.99
6	Irisem	6.75	6.80	6.40	6.65
7	Real	6.58	6.96	7.00	6.85
8	Redix	7.63	8.21	7.80	7.88
9	Remar	8.00	7.93	7.91	7.95
10	Voinicel	6.20	6.63	6.72	6.52

It is considered to be inappropriate a low firmness (flourish) and is generally associated with overripe fruits. Moreover, a rough texture, coarse, with areas of hardness, is negatively evaluated.

From the data recorded in table 2, it resulted that there were varieties that produced firm fruit, such as: Remar, Redix, Florina, Inedit, Iris, with medium penetration resistance values between 7.69 and 7.99 kgf/cm<sup>2</sup> and varieties such as: Voinicel, Irisem, Real, whose medium penetration resistance was much lower, 6.52-6.85 kgf/cm<sup>2</sup>. For Jonathan and Golden delicious, the control varieties, pulp firmness had average values of 6.74 – 7.69 kgf/cm<sup>2</sup>.

From the point of view of pulp firmness, it was observed that neither variety had a constant behavior during the research period, the results being influenced both the variety and by the climatic conditions during one year.

#### **Biochemical fruit content.**

The studied apple varieties will be promoted into culture if they comply with the productivity and fruit quality conditions, mainly for fresh consumption. Due to this, the pleasant aspect of the fruit must be associated with a pleasant taste, which is greatly due to the main biochemical components.

A fruit with high sugar content becomes tasteless, as a fruit with high acidity becomes astringent. In order to be consumed and appreciated, the fruit must have a balance between the main biochemical components.

From the data presented in table 3, it could be noticed that, for the studied apple varieties, the dry substance content was 12.3% for Florina, the highest content being recorded for Iris with 14.6%. For the majority of varieties, the dry substance content had values between 12.5 and 13.1%.

Table 3

Biochemical characteristics of some apple varieties with genetic resistance to diseases  
(2011-2013)

No.	Variety	Dry substance (%)	Total carbohydrates (%)	Acidity apple acid (%)	Vitamin C mg /100 g
1	Florina	12.3	12.90	0.36	7.97
2	Inedit	13.1	12.70	0.25	5.58
3	Iris	14.6	13.10	0.22	9.26
4	Irisem	12.8	14.00	0.24	7.84
5	Real	12.5	13.25	0.30	7.30
6	Redix	13.0	12.60	0.28	7.79
7	Remar	12.8	11.50	0.18	6.80
8	Voinicel	12.8	11.64	0.32	7.42

The total carbohydrates content varied from 11.50% for Remar and 14.0% for Irisem. The majority of apple varieties contained more than 11.50% total carbohydrates.

The acidity had values between 0.18% apple acid for Remar and 0.36% apple acid for Florina. For the other apple varieties, the content was between 0.22-0.32% apple acid.

Generally, the consumer prefers the fruit obtained from varieties that present a balance between the sugar content and acidity, ultimately having a balanced taste.

The vitamin C content varied from 5.58mg/100g fresh fruit, for *Inedit* and 9.26 mg/100g fresh fruit, for *Iris*. From the point of view of vitamin C content, the 8 studied varieties can be grouped as follows:

- 5.58-6.80mg/100g fresh fruit – 2 varieties (*Inedit* and *Remar*);
- 7.30-7.79mg/100g fresh fruit – 3 varieties (*Real*, *Voinicel*, *Redix*);
- 7.84-9.26mg/100g fresh fruit – 3 varieties (*Irisem*, *Florina*, *Iris*).

For the fresh consumption, the following genetically resistant varieties are considered: *Real* and *Irisem* as summer varieties; *Remar* as autumn variety and *Florina* as winter variety, with fruits of more than 170g biomass and appropriate firmness. The fruit from the *Inedit* variety must also be considered for fresh consumption, which has fruit with winter maturation period; even though the size is lower, the fruit can be capitalized especially for the students as they are less polluted due to the low number of phyto-sanitary treatments.

Depending on the biochemical components, the fruit from *Iris* and *Voinicel* can also be considered, with lower fruit, but balanced biochemical content, appropriate for the production of fresh juices, a constantly increasing demand.

The apple varieties genetically resistant to diseases are appreciated by the consumers for the fresh consumption and production of juices, as a result of their high fruit quality, high productive potential, with important economic advantages and low level of environmental pollution.

## CONCLUSIONS

The size of the fruit was influenced by the size of the harvest and the climatic conditions for each year. The largest fruit were obtained for the varieties *Remar*, *Real*, *Irisem*, *Florina* (171-185 g), followed by *Redix* and *Voinicel* varieties (155-160 g), while the smallest ones for *Iris* and *Inedit* (148-151 g);

Fruit firmness was higher for the varieties *Remar*, *Redix*, *Florina*, *Iris*, with a medium penetration resistance between 7.69 and 7.99 kgf/cm<sup>2</sup>, whilst for the varieties *Voinicel*, *Irisem* and *Real*, the medium penetration resistance was much lower, 6.52-6.85 kgf/cm<sup>2</sup>.

The biochemical content of the fruit, during 2011-2013, showed that:

- The highest content in dry substance was recorded for the variety *Iris*, with 14.6%, while for the majority of studied apple varieties the values were between 12.5 and 13.1%;

- The total carbohydrates content varied from 11.5% for *Remar* and 14% for *Irisem*, with values greater than 11.5% for the majority of varieties;

- The fruit acidity had values between 0.18% apple acid for the variety *Remar* and 0,36% apple acid for *Florina*, the other varieties recording a balanced acidity, with values of 0.22-0.32% apple acid;

- The vitamin C content varied between 5.58 mg/100g fresh fruit for the variety *Inedit* and 9.26 58 mg/100g fresh fruit for *Iris*.

The apple varieties recently homologated, together with *Florina*, grafted on MM106 rootstock, present a series of superior features, expressed mainly through genetic resistance to diseases, but also through high fruit quality, high productive potential, with important economic advantages.

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**RESEARCH ON THE INFLUENCE OF THE MAINTENANCE SYSTEM  
OF SOIL ON MOISTURE DYNAMICS IN THE VINEYARDS FROM  
SOUTHERN OLTENIA**

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*Keywords: sandy soils, mulch, moisture dynamics*

**ABSTRACT**

*The moisture content of the soil were superior in the variant with partial mulch between rows and herbicide on row of vines, during the whole period of vegetation. It follows that, the mulch of husks protects soil efficiently, thus preventing its loss through evaporation at the road surface, thus helping to conserve water on the soil profile during the growing season. Higher values, the moisture content of the soil, were maintained and in the variant where used the mulch vegetative on all surface, until September, when the vegetative mulch entered to putrefaction. In August-October period variant with minimal soil works registered higher values comparative variants with vegetative mulch and field black.*

**INTRODUCTION**

The soil moisture and of substrate of culture is represent the amount of water in the soil-substrate, making it one of the most important parameters for the successful cultivation of plants. Optimal soil moisture varies according to species and vegetative stage of vegetation because the consumption is different (Alexandrescu I. 1966, Popa V. Gh. 1977; Popa V. Gh. 1975, 1977; Dejeu L., 1986; Popa V. Gh., 1991). The loss of water in soil by evaporation at its surface can be enhanced through soil energy works carried out at the wrong time.

The main source of water in the soil is precipitation (rain and snow). Viticulture in our country is possible at an amount of rainfall between 500-700 mm, of which 250 to 300 mm during the period of vegetation distributed in the form of useful rainfall (Țârdea C., Dejeu L., 1995). In the case of water demand deficit is through irrigation ( Ploae P., 1994).

**MATERIAL AND METHODS**

The researches were carried out in a wine-growing plantation, established in 2009, with the Roşioară variety. Planting distances 2.2/1.2 m.

The experimental variants were:

V1 - field black (autumn ploughing, spring ploughing, 5 mechanical weeding between rows, 5 manual weeding on row.

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V2 - total mulch with plant debris (spread straw or other plant debris on all surface and with thickness of 10 cm).

V3-partial mulch between rows with husks of grapes with a thickness of 10 cm, two herbicide in vegetative.

V4-minimum tillage (autumn ploughing, deep spring mechanical weeding, 2-3 total herbicide).

Noted that it was not supplemented water through irrigation.

## RESULTS AND DISCUSSIONS

In 2012 year the values of soil moisture, at the beginning of the growing season, oscillated very little from one variant to another being, however, in favor variants in which the soil was protected with mulch (Figure 1). Rainfall registered in the months of May and June, although appreciable values were recorded, failed to maintain the ground water

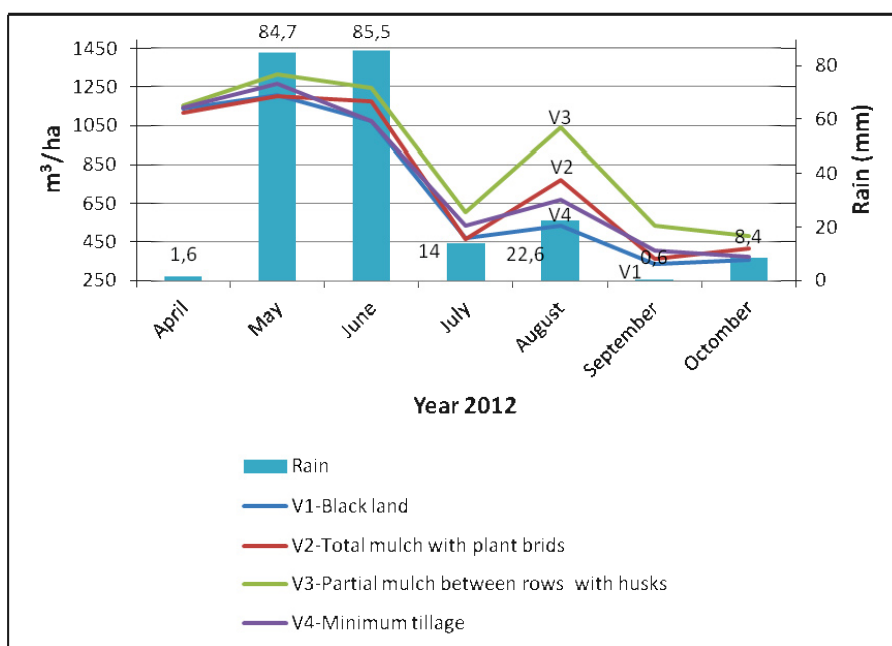


Figure 1. Soil moisture dynamics depending on the maintenance system of vineyards on sandy soils in 2012

reserve at a high level, during the summer, during July-October, by the gradually. In the version with partial mulch on the range with mulch of husks of grapes and herbicide the moisture content values were superior throughout the period of vegetation.

Higher values, the moisture content of the soil, were maintained and in the variant used to mulch vegetative until September, when this entered to putrefaction. At the end of the growing season (October), a result of very small quantities of rainfall during the months of July to October, soil moisture has not been restored. In 2013 year, the rainfalls registered in the winter have reworked soil water reserve such that, at the beginning of the growing season (April-June) the moisture content of the soil record slightly higher values in the classic variant field black, due to the fact that the earth was mellow and well managed to accumulate and store water much better (Figure 2). As the temperature values have grown

so plant water consumption and evapotranspiration process intensified (July-September), and the water content of the version with field black was greatly reduced.

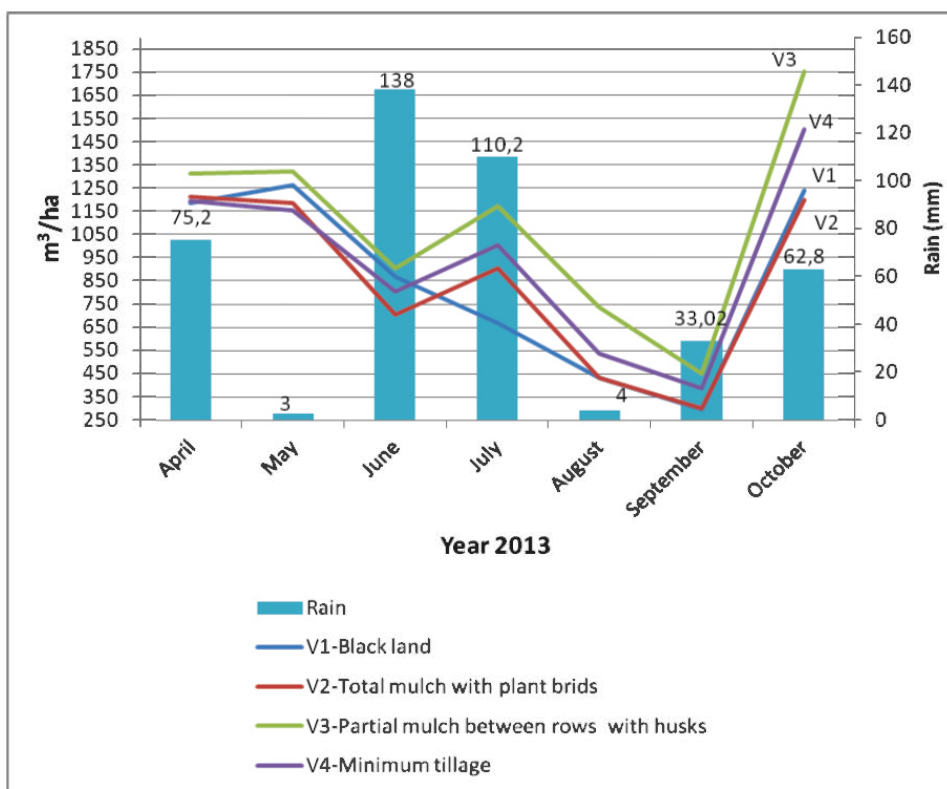


Figure 2. Soil moisture dynamics depending on the maintenance system of vineyards on sandy soils in 2013

It has also reduced the water content and the variant in which the vegetative mulch used since it went into decay, and mulch's role has diminished. Starting in July, the water content of the soil, in the variant in which the used husks of grapes mulch was superior to the other variants.

It follows that, by the husks of grapes mulch protects soil also efficiently, contributing to water conservation on soil profile, preventing its loss through evaporation at the soil surface. By eliminating mechanical works of the soil decreases fuel consumption. At the end of the growing season (October) the water content of the soil was approximately the same as at the beginning of the growing season of about 100 mm of rainfalls during the months of September and October.

### CONCLUSIONS

In 2012 year the moisture content of the soil were superior in the variant with partial mulch with husks of grapes and herbicide on row, during the whole period of vegetation. Higher values, the moisture content of the soil, were maintained and in the variant used to mulch plant vegetative, until September, when the plants entered to

putrefaction. In august-October period with minimal soil works recorded higher values of variants with vegetative mulch and field of black.

In 2013 year the rainfall registered in the winter have reworked soil water reserve such that, at the beginning of the growing season (April-June) the moisture content of the soil were superior in the version with partial mulch with husks of grapes on the range and herbicides on row, during the whole period of vegetation. Higher values, the moisture content of the soil, were maintained and in the variant used to mulch vegetative, until September, when entered the plant in putrefaction.

As the temperature values have grown so plant water consumption and evapotranspiration process intensified (July-September), and the water content of the variant with field black was greatly reduced. It has also reduced the water content and the variant in which the mulch in vegetable scraps used since it went into decay.

Starting in July, the water content of the soil, in the variant in which the used husks of grapes mulch was superior to the other variants. It follows that, by husks of grapes mulch protect efficiently, thus preventing its loss through evaporation at the soil surface, thus helping to conserve water on the soil profile during the growing season.

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## THE INFLUENCE OF PRESSURING CYCLIC PROCESS ON TENDERIZING BEEF PASTRAMI

Roșca A.<sup>1\*</sup>, Roșca Daniela<sup>2</sup>

*Keywords: pressuring cyclic process, beef raw meat tenderizing, Warner-Bratzler method*

### ABSTRACT

*The paper presents the mechanical characteristics of raw meat's mechanical tenderizing process in order to produce Romanian traditional cured-smoked product 'Beef Pastrami' type. These characteristics are based on the diagrams obtained by using Werner-Bratzler testing method of the final product, before and after tenderizing process by using pressuring cyclic process. The tenderizing process performed to decrease the duration of curing / marinating period consists in 0...10 bar successive pressuring and de - pressuring cyclic processing. The paper presents characteristic shear force amount obtained by using Werner - Bratzler testing method for the tenderized and cured-smoked final product, in comparison with the initial no tenderized meat sample.*

### INTRODUCTION

*Pastrama* is a popular delicatessen meat traditionally in Romania made from lamb, sheep, mutton, goat and also from pork and beef. *Pastramă* was originally created as a way to preserve meat before modern refrigeration. For pastrami, the raw meat is brined, partly dried, seasoned with various herbs and spices, then smoked and steamed. At the beginning, *pastramă* is a speciality from Wallachia made from young ram's meat. The word *pastramă* is etymologically rooted in the Romanian *a păstra* which means "to keep" or "to preserve". But the word is maybe more ancient and come from the latin *pastor* who means *shepherd*; so *Pastramă* is *shepherd's meat* of lamb or mutton. The *Pastramă* was introduced by Romans to the city of Caesarea Mazaca in Anatolia, known as *pastron*. This recipe may be the origin of *pastirma*.

In 455 AD the Gepids under king Ardarich conquered Pannonia, settled for two centuries in Transylvania. The Gepids was destroyed by the attack of Lombards in 567 AD. At this time, the lombards discovered *pastramă*, known now as *bresaola*.

The *Pastramă* was introduced to the United States in a wave of Romanian Jewish immigration from Romania in the second half of the 19th century. Early references in English used the spelling "*pastrama*", closer to the Romanian original. The modified "*pastrami*" spelling likely was introduced to sound related to the Italian salami.

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Traditionally, *pastrami* is made from beef brisket (which comes from the lower chest of the steer), or from beef navel (a small piece cut from the muscle known as the plate). Traditional *Pastrami* is a cured meat, meaning that it has been quickly injected with brine usually containing preservation additives (in industrial process) or otherwise infused for long time with brine (in homemade or small enterprise process) (<http://en.wikipedia.org/wiki/Pastrami>).

Both beef brisket and beef navel are tenderless parts of the animal's carcass.

Therefore in the industrial process the meat is tenderized for 4-8 hours in massaging vacuum equipment (maximum relative vacuum up to - 0,7 bar) (Rosca & Rosca 2012, Rosca 2014).

For the same reason, in homemade or small enterprise process, the meat is tenderized for 1-3 weeks in high concentration brine containing additional flavors added.

Then, traditional *pastrami* is cold smoked, and finally dried in ventilated cold air (<http://en.wikipedia.org/wiki/Tenderizing>).

Tenderizing is a process to break down collagens in meat to make it more palatable for consumption. There are several ways to tenderize meat: mechanical tenderization, such as pounding or piercing; the tenderization that occurs through cooking, such as braising; tenderizers in the form of naturally occurring enzymes, which can be added to food before cooking (examples of enzymes used for tenderizing: papain from papaya; marinating the meat with vinegar, wine, lemon juice, buttermilk or yogurt; brining the meat in a salt solution (brine); dry aging of meat at 0 to 2°C) (Tyszkiewicz & Klossowska 1996, McGee 2004).

In order to reduce the tendering process as much is possible, this paper presents a novel tenderizing method based on pressuring cyclic processing.

## MATERIAL AND METHODS

**Processing method and equipment.** In order to produce *Beef Pastrami*, commercial beef brisket (14 pieces) was used (according Animal Slaughter Certificate: cow, 7 years, individual small farm).

Two of these pieces were used to produce *Beef Pastrami* respecting traditional home-made or small enterprise process: the meat was 4 times pierced (Figure 1), then infused for 12 days in 12% concentration brine, then cold smoked in several steps during 24 hours, and finally, dried in free ventilated air for 24 hours.

The piercing step was realized by using the *Multi-needle piercing device* (Figure 2), that in principle consists in 120 needles ( $\varnothing 5$ , 20° conical sharp) disposed in the same shape and reciprocity distance as into the industrial brine injection equipment (Rosca & Rosca 2014).



Figure 1. Sheep brisket piercing



Figure 2. Multi-needle piercing device

In actual massaging vacuum processing equipment the vacuum level do not exceed - 0,7 bar. American and west-European meat tenderizing recent research papers recommend increasing the vacuum level up to - 0,95 bar, or high pressure process up to 5000 bar (Institute of Food Technologists 1981, Rosca & Rosca 2012, Rosca 2014, Xargayó et al. 2011).

The novel tenderizing method proposed in this paper consists in several cyclic pressuring and de-pressuring steps of the raw meat and the brine, too, into a pressure vessel. During the pressuring process, the pressure level is 3 times higher than during brine injection in industrial equipment, and 2-3 times than the dynamic pressing during the massaging industrial process.

In order to put in evidence the influence of pressuring cyclic process (PCP) on meat tenderization, *Experimental Equipment for PCP* (EE-PCP) was used. *Experimental Equipment for PCP* and *Multi-needle piercing device* were designed and made by Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Agriculture and Horticulture - *UTEFIL*, in collaboration with Environmental Protection in Industry within Faculty of Electrical Engineering, within the University of Craiova (Rosca & Rosca 2012, 2013, 2014).

In principle, EE-PCP (Figure 3) is composed in a pressuring (and vacuuming, too, if it is necessary) process hydraulic cylinder (PHC) consisting in a cylindrical vessel (inner diameter  $\varnothing$  80; length 180 mm) made in stainless steel W1.4571 and a piston made in food grade Teflon. The pressuring processing hydraulic cylinder is provided with a manometer gauge (0...12 bar; 1,6 precision class), and a manovacuumeter gauge (-1...1,5 bar; 2,5 precision class) when vacuuming process is actuated. In order to evacuate the liquid / gas excess before and after PCP, the piston is provided with G1/4" tap connected to Rilsan  $\varnothing$ 8 tube (Rosca & Rosca 2014).

In order to actuate the pressuring process into EE-PCP, universal testing machine *Lloyds Instruments Plus 5kN* was used (Figure 3).

Pressuring cyclic method consists in the following processing steps:

- The raw beef brisket 4 times pierced (as was presented above) is introduced into the PHC of the EE-PCP that contain 12% salted concentration brine (proportion 2:1 for raw beef brisket, and brine, respectively).
- Each pressuring cycle lasts 16 min consists in 8 consecutive steps, each lasting 2 min: slow pressuring (during 1 min) up to 10 bar, followed by maintaining for 1 min at this pressure level, followed by fast de-pressuring up to the ambient atmosphere (Figure 4).
- For this paper were used 2, 3, 4, 5 and 6, respectively, pressuring cycles that last 32 min (PCP 32), 48 min (PCP 48), 64 min (PCP 64), 80 min (PCP 80), 96 min (PCP 96) and 112 min (PCP 112), respectively.
- Before starting the last 16 min pressuring cyclic process, the used brine was replaced by a fresh cold one.



Figure 3. *Experimental Equipment for PCP* mounted on *Lloyds Instruments Plus 5kN*

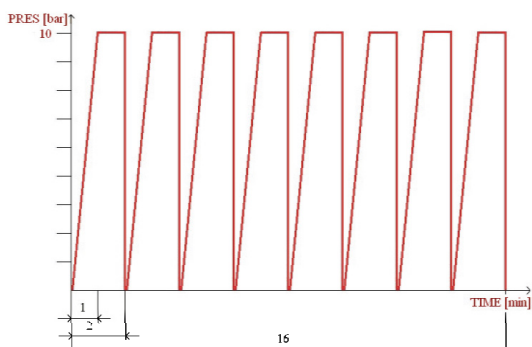


Figure 4. Pressuring cycle process diagram

For each pressuring cycle process lasting 32 min, 48 min, 64 min, 80 min, 96 min and 112 min, respectively, two raw beef brisket were used. All the twelve raw beef brisket tenderized by using pressuring cyclic process were smoked and dried in the same time (and technological conditions) with the pieces used to obtain *Beef Pastrami* by using traditional method.

The most relevant and utilized destructive texture and tenderness tests are puncture/ penetration test, compression test, and Warner-Bratzler shear test. The shear force behavior gives information about tenderness, as well as the bite characteristic products. The jig consists of a rigid frame supporting a shear bar. The jig acts in direct compression for slicing / shearing tests on products (Institute of Food Technologists, 1981, Tyszkiewicz & Klossowska 1996, [www.lloyd-instruments.co.uk](http://www.lloyd-instruments.co.uk), Rosca & Rosca 2013, 2014).

To perform interdisciplinary researches concerning general texture and tenderness analysis, universal testing machines *Lloyd Instruments LRXPlus 5*, cell force - extension 0,5 class accuracy (*UTEFIL* within Faculty of Agriculture and Horticulture in Craiova), is used since several years ago to perform comparative texture measurements (Rosca & Rosca 2013).

Due to collaboration between *UTEFIL* and Environmental Engineering Laboratory within Faculty of Electrical Engineering, an *experimental Warner - Bratzler equipment* was made: special rigid frame (made in food-grade Teflon) supporting a shear bar that permits interchangeable Warner - Bratzler shear blades (V and square plate cut blade made in stainless steel DIN W1.4571) to slide fit into the frame ((Rosca & Rosca 2013, Rosca & Simion 2014).

During this experiment, 100mm/min cutting speed was used.

*Beef Pastrami* obtained by using raw beef brisket tenderized after PCP 96, during Warner - Bratzler testing shear force, is presented in Figure 5.



Figure 5. *Beef Pastrami* made by PCP 96 during Warner - Bratzler testing shear force

## RESULTS AND DISCUSSIONS

In order to determine the influence of tenderizing process on the final product tenderness, *Beef Pastrami* pieces made by using traditional homemade method, and tenderized by using PCP, respectively, were tested according Warner - Bratzler shear force method. During the Warner - Bratzler shear force tests, each of all 14 pieces of *Beef Pastrami* were sliced in 6 parts.

Representative Warner - Bratzler shear force diagram is presented in Figure 6.

In Table 1 are presented the maximum shear force amount and the shear force average for each of the seven of *Beef Pastrami* types. In Table 1 there are presented too, the decrease of percentage average shear force (in comparison with traditional homemade *Beef Pastrami*'s tenderness) by using each processing method, that demonstrate the tenderness increase of the final product, that was initial tenderized by using pressuring cyclic process.

Table 1 presents a synthesis of the influence of pressuring cyclic process on *Beef Pastrami* final tenderness':

- in comparison with traditional homemade *Beef Pastrami*'s tenderness, PCP 32 method determines a small (6,11%) tenderness' increasing of the final product;
- in comparison with traditional homemade *Beef Pastrami*, an important fast increasing (from 12,34% to 26,57%) of the final product tenderness' is observed when PCP 48, PCP 64 and VCP 80 were used;
- instead, slow increase (from 26,57%...to 33,23%) of the final product *Beef Pastrami* tenderness' is observed when PCP 80, PCP 96 and PCP 112, in comparison with traditional home-made *Beef Pastrami*'s tenderness.

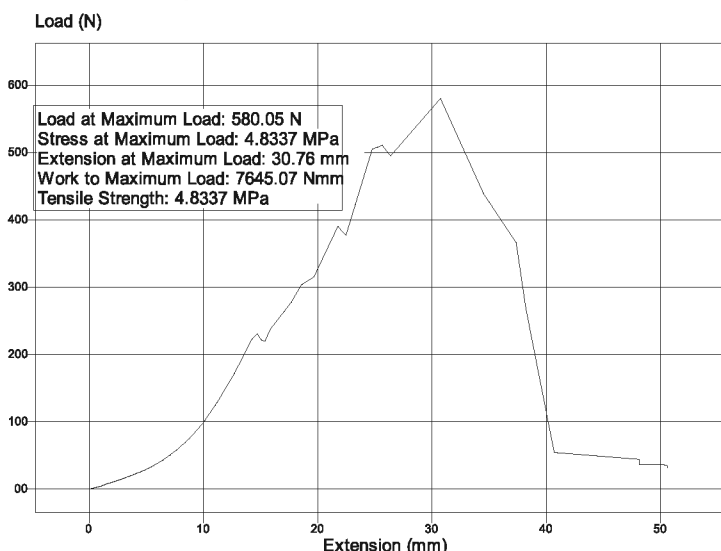


Figure 6. Warner - Bratzler shear force test for *Beef Pastrami* made by using pressuring cyclic PCP140 method

Table 1

Warner - Bratzler shear force for *Beef Pastrami* types

Sample code	Maximum shear force min...max amount, N	Shear force average, N	Decrease of shear force average, %
TRAD	783,49...882,11	832,78	-
PCP 32	743,68...828,34	780,46	6,11
PCP 48	693,82...765,27	729,66	12,34
PCP 64	631,19...715,02	662,45	19,78
PCP 80	565,24...643,18	607,53	26,57
PCP 96	543,18...618,32	578,12	30,09
PCP 112	519,17...587,43	554,57	33,23

## CONCLUSIONS

Cyclic pressuring process represents a novel method to obtain increasing of *Beef Pastrami* tenderness'. Due to pressure level, and fast pressuring and de - pressuring steps, too, cyclic pressuring process effect's is similar with vacuum cyclic processing (Rosca &. Rosca 2014) and represents a much more intensively tenderizing method than massaging vacuum equipment in industrial processing. Cyclic pressuring process determines much smaller salt marinating / period, only 96 - 112 min, in comparison with 4 - 8 hours in massaging vacuum

equipment in industrial processing, or 1 - 2 weeks salting / marinating in homemade or small enterprise processing.

As one of the most recommended analyze method, the Warner - Bratzler shear force test offers the certitude of influence of cyclic pressuring process on meat final products tenderness'.

This paper opens further experimental researches concerning the influence of similar cyclic pressuring process (higher pressure level, shorter or longer pressuring and de - pressuring) to produce *Beef Pastrami*, by using other much more tenderless parts of animal's carcass.

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\*\*\*[www.lloyd-instruments.co.uk](http://www.lloyd-instruments.co.uk)

\*\*\*<http://en.wikipedia.org/wiki/Pastrami>

\*\*\* <http://en.wikipedia.org/wiki/Tenderizing>



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## THE INFLUENCE OF PRESSURING CYCLIC PROCESS ON TENDERIZING SHEEP PASTRAMI

Roșca A.<sup>1\*</sup>, Roșca Daniela<sup>2</sup>

*Keywords: pressuring cyclic process, sheep raw meat tenderizing, Warner-Bratzler method*

### ABSTRACT

*The paper presents the mechanical characteristics of raw meat's mechanical tenderizing process in order to produce Romanian traditional cured-smoked product "Sheep Pastrami" type. These characteristics are based on the diagrams obtained by using Warner-Bratzler testing method of final product, before and after tenderizing process by using pressuring cyclic process. The tenderizing process performed to decrease the duration of cured marinating period consists in 0...10 bar successive pressuring and de - pressuring cyclic processing. The paper presents characteristic shear force amount obtained by using Warner-Bratzler testing method for the tenderized and cured-smoked final product, in comparison with the initial no tenderized meat sample.*

### INTRODUCTION

*Pastrami* (Turkish: *pastırma*, Romanian: *pastramă*, Yiddish: *pastróme*) is a popular delicatessen meat usually made from beef, and sometimes from pork, sheep, mutton or turkey. The raw meat is brined, partially dried, seasoned with various herbs and spices, then smoked and/or steamed. The method for making *pastrami* was originally used to preserve beef from spoiling in a time before modern refrigeration (in fact, the word "*pastrami*" comes from the Romanian *păstra*, to preserve). The first versions of this preparation date back to the Ottoman Empire, where Turkish people salted and dried beef and sheep. Beef and sheep plates are the traditional primal cut for making *pastrami*, but these days it is more common to see made from beef's and sheep's brisket and breast (<http://en.wikipedia.org/wiki/Pastrami>).

The Romanian specialty was introduced to the United States in a wave of Romanian Jewish immigration from Bessarabia and Romania in the second half of the 19th century, via the Yiddish (pronounced *pastróme*). Early references in English used the spelling "*pastrama*", closer to the Romanian original. Among Jewish Romanians, goose breasts were commonly made into *pastrami* because they were inexpensive. Beef navels and briskets, and sheep brisket and breast were cheaper than goose meat in America, so the Romanian Jews in America adapted their recipe and began to make the cheaper beef and sheep *pastrami*, as they usually done in Romania (<http://en.wikipedia.org/wiki/Pastrami>).

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Traditional *Pastrami* is a cured meat, meaning that it has been quickly injected with brine usually containing preservation additives (in industrial process) or otherwise infused for long time with brine (in homemade or small enterprise process). Both sheep brisket and sheep breast are tenderless parts of the animal's carcass (<http://en.wikipedia.org/wiki/Tenderizing>).

Therefore in the industrial process the meat is tenderized for 4-6 hours in massaging intermittent vacuum equipment (maximum relative vacuum -0,65 bar).

For the same reason, in homemade or small enterprise process, the meat is tenderized for 1-2 weeks in high concentration brine containing additional flavors added (marinated).

Then, traditional *pastrami* is cold smoked, and finally dried in ventilated cold air (<http://en.wikipedia.org/wiki/Tenderizing>).

Tenderizing is a process to break down collagens in meat to make it more palatable for consumption. There are several ways to tenderize meat: mechanical tenderization, such as pounding or piercing; the tenderization that occurs through cooking, such as braising; tenderizers in the form of naturally occurring enzymes, which can be added to food before cooking (examples of enzymes used for tenderizing: papain from papaya; marinating the meat with vinegar, wine, lemon juice, buttermilk or yogurt; brining the meat in a salt solution (brine); dry aging of meat at 0 to 2°C) (Tyszkiewicz &. Klossowska 1996, McGee 2004).

In order to reduce the tendering process as much is possible, this paper presents a novel tenderizing method based on pressuring cyclic processing.

## MATERIAL AND METHODS

### Processing method and equipment

In order to produce *Sheep Pastrami*, 14 pieces of sheep brisket and breast were used (Animal Slaughter Certificate: 2 sheep, 11 months, 28 kg in carcass, individual small farm).

Two of these pieces were used to produce *Sheep Pastrami* respecting traditional home-made or small enterprise process: the meat was pierced for 2 times (Figure 1), then infused for 8 days in 12% concentration brine, then cold smoked in several steps during 1 day, and finally, dried in free ventilated air for 24 hours.

The piercing step was realized by using the *Multi-needle piercing device* (Figure 2), that in principle consists in 120 needles ( $\varnothing 5$ , 20° conical sharp) disposed in the same shape and reciprocity distance as into the industrial brine injection equipment (Rosca &. Rosca 2014).



Figure 1. Sheep meat piercing

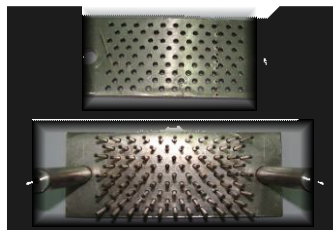


Figure 2. Multi-needle piercing device

In actual massaging vacuum processing equipment the vacuum level do not exceed - 0,7 bar. Recent American and west-European meat tenderizing research papers recommend to increase the vacuum level up to - 0,95 bar, or high pressure process up to 5000 bar (Institute of Food Technologists 1981, Rosca &. Rosca 2012, Rosca 2014, Xargayó et al. 2011).



The novel tenderizing method proposed in this paper consists in several cyclic pressuring and de-pressuring steps of the raw meat and the brine, too, into a pressure vessel. During the pressuring process, the pressure level is 3 times higher than during brine injection in industrial equipment, and 2-3 times than the dynamic pressing during the massaging industrial process.

In order to put in evidence the influence of pressuring cyclic process (PCP) on meat tenderization, *Experimental Equipment for PCP* (EE-PCP) was used. *Experimental Equipment for PCP* and *Multi-needle piercing device* were designed and made by Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Agriculture and Horticulture - *UTEFIL*, in collaboration with Environmental Protection in Industry within Faculty of Electrical Engineering, within the University of Craiova (Rosca & Rosca 2012, 2013, 2014).

In principle, EE-PCP (Figure 3) is composed in a pressuring (and vacuuming, too, if it is necessary) process hydraulic cylinder (PHC) consisting in a cylindrical vessel (inner diameter  $\varnothing$  80; length 180 mm) made in stainless steel W1.4571 and a piston made in food grade Teflon. The pressuring processing hydraulic cylinder is provided with a manometer gauge (0...12 bar; 1,6 precision class), and a manovacuumeter gauge (-1...1,5 bar; 2,5 precision class) when vacuuming process is actuated. In order to evacuate the liquid / gas excess before and after PCP, the piston is provided with G1/4" tap connected to Rilsan  $\varnothing$ 8 tube (Rosca & Rosca 2014).

In order to actuate the pressuring process into EE-PCP, an universal testing machine *Lloyds Instruments Plus 5kN* was used (Figure 3).



Figure 3. *Experimental Equipment for PCP* mounted on *Lloyds Instruments Plus 5kN*

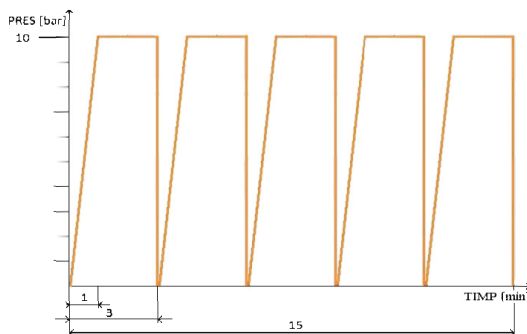


Figure 4. Pressuring cycle process diagram

Pressuring cyclic method consists in the following processing steps:

- The raw sheep brisket 2 times pierced (as was presented above) is introduced into the PHC of the EE-PCP that contain 12% salted concentration brine (proportion 2:1 for raw sheep brisket, and brine, respectively).

- Each pressuring cycle lasts 15 min consists in 5 consecutive steps, each lasting 3 min: slow pressuring (during 1 min) up to 10 bar, followed by maintaining for 2 min at this pressure level, followed by fast de-pressuring up to the ambient atmosphere (Figure 4).

- For this paper were used 3, 4, 5, 6, 7 and 8, respectively, pressuring cycles that last 45 min (PCP 45), 60 min (PCP 60), 75 min (PCP 75), 90 min (PCP 90), 105 min (PCP 105) and 120 min (PCP 120), respectively.

- Before starting the last 15 min pressuring cyclic process, the used brine was replaced by a fresh cold one.

For each pressuring cycle process lasting 45 min, 60 min, 75 min, 90 min, 105 min and 120 min, respectively, two raw sheep brisket were used. All the twelve raw sheep

brisket tenderized by using pressuring cyclic process were smoked and dried in the same time (and technological conditions) with the pieces used to obtain *Sheep Pastrami* by using traditional method.

#### **Tenderness evaluation by using Warner-Bratzler method**

The most relevant and utilized destructive texture and tenderness tests are puncture/ penetration test, compression test, and Warner-Bratzler shear test. The shear force behavior gives information about tenderness, as well as the bite characteristic products. The jig consists of a rigid frame supporting a shear bar. The jig acts in direct compression for slicing / shearing tests on products (Institute of Food Technologists, 1981, Tyszkiewicz & Klossowska 1996, Rosca & Rosca 2013, 2014, [www.lloyd-instruments.co.uk](http://www.lloyd-instruments.co.uk)).

To perform interdisciplinary researches concerning general texture and tenderness analysis, universal testing machines *Lloyd Instruments LRXPlus 5*, cell force - extension 0,5 class accuracy (*UTEFIL* within Faculty of Agriculture and Horticulture in Craiova), is used since several years ago to perform comparative texture measurements (Rosca & Rosca 2013).

Due to collaboration between *UTEFIL* and Environmental Engineering Laboratory within Faculty of Electrical Engineering, an *experimental Warner - Bratzler equipment* was made: special rigid frame (made in food-grade Teflon) supporting a shear bar that permits interchangeable Warner - Bratzler shear blades (V and square plate cut blade made in stainless steel DIN W1.4571) to slide fit into the frame ((Rosca & Rosca 2013, Rosca & Simion 2014).

During this experiment, 100mm/min cutting speed was used.

*Sheep Pastrami* obtained by using raw sheep brisket and breast pieces tenderized during pressuring cyclic process lasting 75 min (PCP 75), 90 min (PCP 90), 105 min (PCP 105) and 120 min (PCP 120), during Warner - Bratzler testing shear force, are presented in Figure 5.

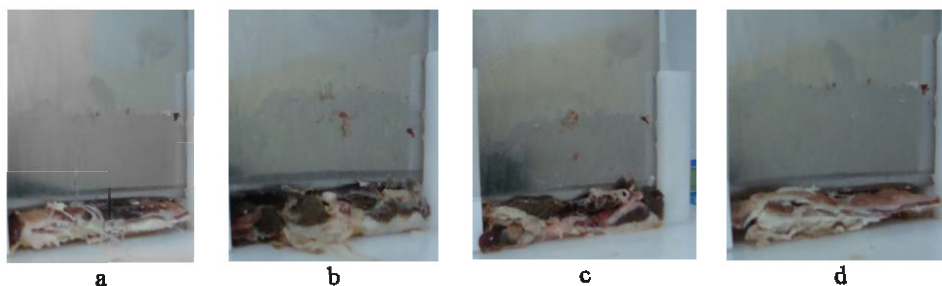


Figure 5. *Sheep Pastrami* obtained by using raw sheep brisket and breast pieces after tenderizing PCP 75 (a), PCP 90 (b), PCP 105 (c) and PCP 120 (d), during Warner - Bratzler testing shear force

### **RESULTS AND DISCUSSIONS**

In order to determine the influence of tenderizing process on the final product tenderness, *Sheep Pastrami* pieces made by using traditional homemade method, and tenderized by using PCP, respectively, were tested according Warner - Bratzler shear force method. During the Warner - Bratzler shear force tests, each of all 14 pieces of *Sheep Pastrami* were sliced in 6 parts.

Representative Warner - Bratzler shear force diagram is presented in Figure 6.

In Table 1 are presented the maximum shear force amount and the shear force average for each of the four of *Sheep Pastrami* types. In Table 1 is presented too, the decrease of percentage average shear force (in comparison with traditional homemade

*Sheep Pastrami*'s tenderness) by using each processing method, that demonstrate the tenderness increase of the final product, that was initial tenderized by using pressuring cyclic process.

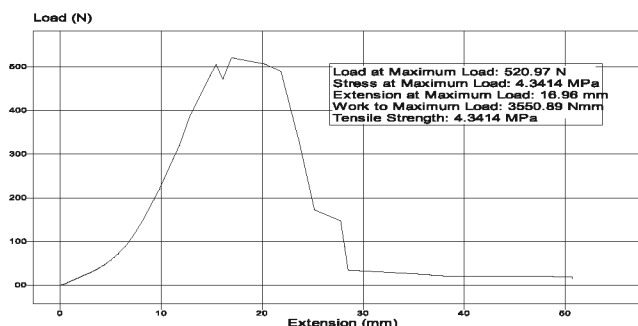


Figure 6. Warner - Bratzler shear force test for *Sheep Pastrami* made by using pressuring cyclic PCP 75 method

Table 1

Warner - Bratzler shear force for *Sheep Pastrami* types

Sample code	Maximum shear force min...max amount, N	Shear force average, N	Decrease of shear force average, %
TRAD	606,37...713,58	657,83	-
PCP 45	584,12...673,57	624,27	5,15
PCP 60	539,78...641,33	589,39	10,43
PCP 75	504,35...586,13	535,44	18,64
PCP 90	439,77...518,45	479,51	27,12
PCP 105	429,48...503,11	457,80	30,44
PCP 120	411,05...482,58	445,29	32,37

Table 1 presents a synthesis of the influence of cyclic vacuum process on *Sheep Pastrami* final tenderness': -In comparison with traditional homemade *Sheep Pastrami*'s tenderness, PCP 45 method determines a small (5,15%) tenderness' increasing of the final product;

-In comparison with traditional homemade *Sheep Pastrami*, an important fast increasing (from 5,15% to 27,12%) of the final product tenderness' is observed when PCP 45, PCP 60, PCP 75 and PCP 90 were used;

-Instead, slow increase (from 27,12% ... to 32,37%) of the final product *Sheep Pastrami* tenderness' is observed when PCP 90, PCP 105 and PCP 120, in comparison with traditional home-made *Sheep Pastrami*'s tenderness.

## CONCLUSIONS

Cyclic pressuring process represents a novel method to obtain increasing of *Sheep Pastrami* tenderness.

Due to pressure level, and fast pressuring and de - pressuring steps, too, cyclic pressuring process effect's is similar with vacuum cyclic processing (Rosca &. Rosca 2014) and represents a much more intensively tenderizing method than massaging vacuum equipment in industrial processing.

Cyclic pressuring process determines much smaller salt marinating / tenderizing period, only 90 - 120 min, in comparison with 4 - 6 hours in massaging vacuum equipment in industrial processing, or 1 - 2 weeks salting / marinating in homemade or small enterprise processing.

As one of the most recommended analyze method, the Warner - Bratzler shear force test offers the certitude of influence of cyclic pressuring process on meat final products tenderness'.

This paper opens further experimental researches concerning the influence of similar cyclic pressuring process (higher pressure level, shorter or longer pressuring and de - pressuring) to produce *Sheep Pastrami*, by using other much more tenderless parts of animal's carcass.

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## THE INFLUENCE OF CITRIC ACID ACROSS THE OXIDATIVE STATUS OF YOGHURT FROM COW MILK

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**Keywords:** *Yoghurt, NAD, FMN, citric acid.*

### ABSTRACT

*This paper presents the partial results from the big study related by oxidative status of cow yoghurt that can registered the majors changed when it is added the natural citric acid and synthesis citric acid.*

*The influence of antioxidants and oxidants present or added in to caw yoghurt on any oxidoreductases cofactors - like as NAD and FMN - are significant and the study on these oxidoreductase are normal and necessary. The work paper is part of a complex study able for improve the red ox process that occur in the processing of food (cow milk). The work paper is a side of complex study regarding the effects of natural and synthetic food additives on the lot of foods.*

### INTRODUCTION

Nicotinamide adenine dinucleotide, abbreviated NAD<sup>+</sup>, is a coenzyme found in all living cells. The compound is a dinucleotide, since it consists of two nucleotides joined through their phosphate groups: with one nucleotide containing an adenosine ring, and the other containing nicotinamide (Belitz E., M.Grosch, 1999).

In metabolism, NAD<sup>+</sup> is involved in redox reactions, carrying electrons from one reaction to another (Leonte M., T. Florea, 1998). The coenzyme is therefore found in two forms in cells: NAD<sup>+</sup> is an oxidizing agent – it accepts electrons from other molecules and becomes reduced, this reaction forms NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD<sup>+</sup> (Dawson MC 1987).

The midpoint potential of the NAD<sup>+</sup>/NADH redox pair is -0.32 volts, which makes NADH a strong reducing agent. The reaction is easily reversible, when NADH reduces another molecule and is re-oxidized to NAD<sup>+</sup>. This means the coenzyme can continuously cycle between the NAD<sup>+</sup> and NADH forms without being consumed.

After use the natural or synthesis additive (the natural and synthetic additive from citric acid), the composition of yoghurt and him oxidative status will be changed and this change can be proved using the UV-Vis spectroscopy (like as cheapest analysis method). The new effects create by the citric acid can be a good indicator of oxidative status and this indicator can be used in to new obtained products, in the frame of new technological

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process. Through a good observation of the variation of anaerobical and aerobical conditions it is easy to establish the best variant of preservation for the new yoghurt. The optimal variation of enzymatic cofactor can determine the optimal rate of acidification for this cow yoghurt. This rate of acidification can be used to determine the best way of preservation for this functional food (Savescu P. 2006)

### MATERIAL AND METHODS

For to quantify the changed oxidative status on the cow yoghurt and the content of  $\text{NAD}^+$ ,  $\text{NADH}+\text{H}^+$  and  $\text{FMN}^+$  and  $\text{FMNH}+\text{H}^+$  on added task with natural and synthetic's citric acid it is constituted nine experimental variants:

- V1 -DANONE Yoghurt with 0.1% fat,
- V2 -DANONE Yoghurt with 0.1% fat added with Natural Citric Acid,
- V3 - DANONE Yoghurt with 0.1% fat added with Synthetic Citric Acid
- V4 - Napolact Yoghurt with 3.5% fat,
- V5 - Napolact Yoghurt with 3.5% fat added with Natural Citric Acid,
- V6 - Napolact Yoghurt with 3.5% fat added with Synthetic Citric Acid,
- V7 - Covalact Yoghurt with 5% fat,
- V8 - Covalact Yoghurt with 5% fat added with Natural Citric Acid,
- V9 - Covalact Yoghurt with 5% fat added with Synthetic Citric Acid,

The samples were cleaned (for the interference substances) and were spectrophotometer in the nearly UV ranges. The variations of molecular absorption spectra were recording in report by the wave-length. Then, these molecular absorption spectra were analysed, help by the statistical soft „SPSS for Windows 11.0”, the deviation from the base variant, the analysis of the mean square for the obtain data and establish mathematic what is the best preserved variant for the natural yoghurt. Before the spectrometry task the samples were prepared in the same conditions of temperature, pressure and for spectrometry task it used an digital spectrophotometer UNICAM 2 UV-Vis, with 1cm cuvette broad and the automatically change of deuterium lamp with tungsten lamp at 325nm (this mechanism was set up before analysis).

Both  $\text{NAD}^+$  and  $\text{NADH}$  absorb strongly in the ultraviolet due to the adenine base. The peak absorption of  $\text{NAD}^+$  is at a wavelength of 259 nanometers (nm), with an extinction coefficient of  $16,900 \text{ M}^{-1}\text{cm}^{-1}$ .  $\text{NADH}$  also absorbs at higher wavelengths, with a second peak in UV absorption at 339 nm with an extinction coefficient of  $6,220 \text{ M}^{-1}\text{cm}^{-1}$ . This difference in the ultraviolet absorption spectra between the oxidized and reduced forms of the coenzymes at higher wavelengths makes it simple to measure the conversion of one to another in enzyme assays – by measuring the amount of UV absorption at 340 nm using a spectrophotometer. (Dawson MC).

For to establish the peak absorption of  $\text{FMN}^+$  and  $\text{FMNH}+\text{H}^+$  for these yoghurts were used the Pure Analysis Biochemical Reagent from specialized Chinese Firm “Flavin Coenzymes”. The used method for establish these peaks was “The Unique Addition Method”

For decreasing the limits of errors, the obtained results were replayed in to auto-tracking and save in to files .qnt format and convert with the soft Visio ver.2.

### RESULTS AND DISCUSSIONS

Result as the analysis were obtained the concentration for  $\text{NAD}^+$  and  $\text{NADH}+\text{H}^+$  and the concentration for  $\text{FMN}^+$  and  $\text{FMNH}+\text{H}^+$  from yoghurts - experimental variants like as the figures 1 and 2. When the fat content of the yogurt is higher, the conductivity is

lower. All the variants of fortified by synthetic citric acid (V3, V6, V9), there have been significant decreases in conductivity values (Savescu, P., 2006).

-In to range of Visible spectra (400-700nm) the best equilibra variants were the variant of yoghurt with 5% fat content (Savescu P., 2006).

-One of the most balanced experimental variants in terms of inducing agents of coenzymes oxidoreductazelor cofactorii involved in anaerobic and aerobic environment was the variant that use the citric acid (natural, from lemon) V8-the most powerful influences of this acid in report of concentration between oxidised forms and concentrations of reduced forms for these oxidoreductases

The greatest  $NAD^+$  and  $NADH+H^+$  content and activity were registered at the experimental variants V5 that use the cow yoghurt 3.5% fat with natural citric acid. (figure 1). For the values of the content and activity of  $FMN^+$  and  $FMNH+H^+$  it is clear: the variant V8 (that use the cow yoghurt with 5% fat and natural citric acid) is on top position (figure 2).

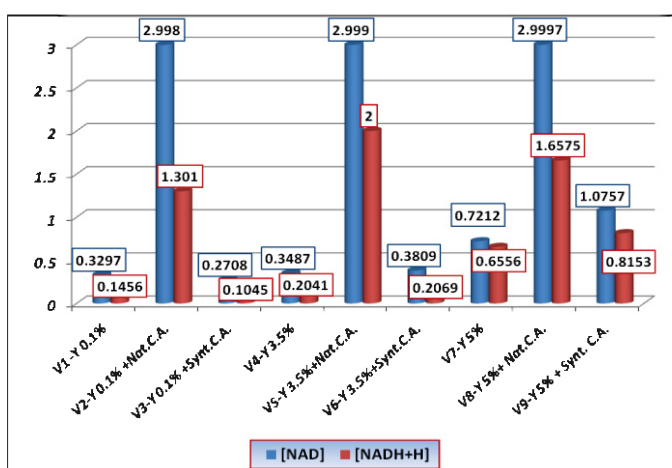


Figure 1 – The  $NAD^+$  and  $NADH+H^+$  content in the experimental variants

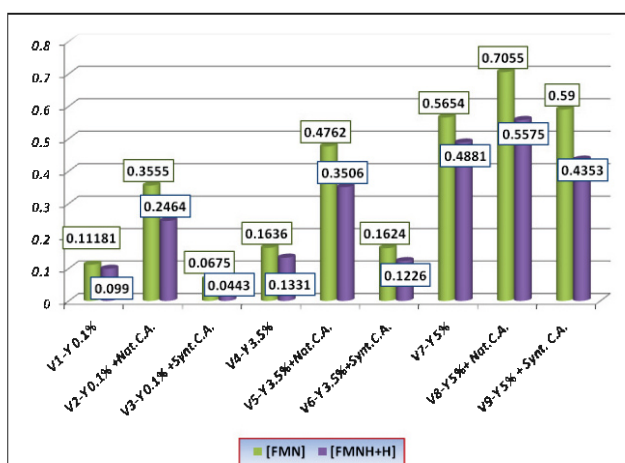


Figure 2 – The  $FMN^+$  and  $FMNH+H^+$  content in the experimental variants

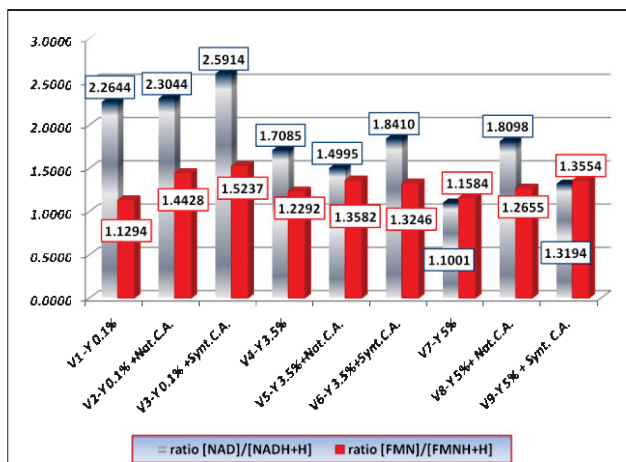


Figure 3 – The  $[NAD^+]/[NADH+H^+]$  and  $[FMN^+]/[FMNH+H^+]$  in the experimental variants

The most stable Variant into redox processes was the V7 (natural yoghurt with 5% fat) and the big activity linked of the redox processes was registered on V3 Variant (variant that use Yoghurt with 0.1%fat and Syntehetic citric acid)(figure 3).

### CONCLUSIONS

The method of analysis that use the UV VIS spectrometry can be a good and cheaper method of analyse than HPLC methods for determinate the concentration and effect of additives, the UV-VIS optical methods can be used for to determinate the best additive for the natural yoghurt and can be complete with FTIR spectrometry (for analysis the any isomers derivate from compounds of base).

In to range of Visible spectra (400-700nm) the best equilibra variants were the variant of yoghurt with 5% fat content.

One of the most balanced experimental variants in terms of inducing agents of coenzymes oxidoreductazelor cofactorii involved in anaerobic and aerobic environment was the variant that use the citric acid (natural, from lemon), V8.

The most stable Variant prepared for the redox processes was the V7 (natural yoghurt with 5% fat) and the big activity linked of the redox processes was registered on V3 Variant (variant that use Yoghurt with 0.1%fat and Synthetic citric acid).

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**COMPARATIVE TECHNOLOGICAL CHARACTERISTICS OF MERLOT  
CLONE 10/27 UNDER THE SOIL AND CLIMATIC CONDITIONS OF  
PLEVEN**

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*Keywords: vine, Merlot variety, clone, mechanical analysis, technological characteristics*

**ABSTRACT**

*Comparative study of the technological characteristics of Merlot clone 10/27 and the variety population was carried out. It was found that between the population and clone 10/27 of Merlot variety there were proven mathematical differences in the most important ampelographic indicators for the mechanical and chemical analysis of cluster and berry. The studied clone was superior compared to the control in average mass of cluster and yield per vine. In all studied vintages of clone 10/27 grapes accumulated on the average 0.6-1.0% more sugars, which accounted for about 4-6% more substrate. That determined the higher concentration of alcohol in the future wines more metabolites and extracting. Wines from Merlot 10/27 were the most preferred from all vintages, as they were suitable for the production of high quality Merlot red wines. The average tasting assessments of that sample were on the average with 1-2 scores higher than those of variety Merlot.*

**INTRODUCTION**

The changes of the mechanical and chemical analysis indicators of cluster and berry for each vine variety/clone are directly dependent on soil and climatic conditions in the micro-region of cultivation and the characteristics of the terroir (Reynier 2001). According to Redl (1991) fertility, yield, size and mass of the cluster, sugar content and acids in grapes are affected more strongly by the interaction terroir/year rather than terroir/clone.

In clonal selection of wine grape varieties besides the specific agro-biological and technological characteristics, the composition of the produced wines and their organoleptic profile is also of great importance.

The composition and quality of the raw material is crucial for obtaining quality wines (Calò et al., 1988; Gomez et al., 1994; Radulov et al., 2004.). Grapes indicators having the greatest impact on the future wine profile are accumulated fermentable sugars, titratable acidity, grape juice pH, the total phenolic compounds content, the concentration of aromatic components or their precursors (Beltran, Casellas 2005). Merlot is one of the most widely distributed and cultivated variety for red wines production (Clarke, Rande

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2001; Robinson 2006). Clonal selection of this second most important in France and in the world red wine variety began in 1955 in Bordeaux, where in 1958 the first collection of 120 variations, selected from various plantations with Merlot variety was established (Walter, Martelli 1997).

As a result of ongoing for decades large-scale selection and targeted clonal and sanitary selection of Merlot variety a positive effect had been achieved on fertility, yield and grapes quality. In France more than 20 clones of Merlot variety were selected while in Italy - more than 15 clones, most of which were focused on yield and grapes quality (Moretti 1998; Grenan et al., 2000; ENTAV 2007).

The study of agro-biological and technological characteristics and oenological potential of clones of Merlot variety had been the subject of numerous researches in different countries, regions and micro-regions (Matevska et al., 1974; Ferretti 1993; Guseynov et al., 2011; Sparacio et al., 2004; Bertrand, Kotseridis 2004; Fabbro et al., 2008; Necula et al., 2009).

**The objective** of this study was a comparative biometric and statistical analysis of the indicators of the mechanical and chemical analysis of the cluster, berry and wine of Merlot clone 10/27, grown in the region of Pleven, Central Northern Bulgaria.

## MATERIAL AND METHODS

Merlot 10/27 was obtained by individual selection of positive traits in 1972 in mother vineyard of Merlot variety in the region of Pleven. Its technological characteristics were studied in 2006-2008, as population of the variety was used for control.

The clone and the population were raised in clonal section of the Experimental Base at the Institute of Viticulture and Enology – Pleven. The vines were grafted to rootstock Berlandieri x Riparia CO4 and grown on ground training system - improved Guillot, at planting distance 2,20/1,30 m.

For finding regularity in the mechanical properties, sugar accumulation and qualitative indicators of the studied clone and the control, the vines were loaded with equal number of winter eyes.

Grapes mechanical analysis of the studied variants was performed during its technological ripeness in accordance with the methods described in Bulgarian Ampelography, volume 1 (Katerov et al., 1990).

For determining the structure and composition of the cluster and the berry the following indicators were accounted for a period of three years: average mass of a cluster (g); average weight of 100 berries (g); bunches and berries in a cluster (%); skins, seeds and mesocarp in a berry (%). Sugars (%) and titratable acidity (g/dm<sup>3</sup>) content was analyzed to characterize the grapes quality.

The obtained biometrical data for all studied indicators of the mechanical analysis were mathematically processed by analysis of variance (Dimova, Marinkov, 1999). The comparative evaluation of clone 10/27 and the population of Merlot variety was done based on the average values of the obtained results for the indicators and the presence of any significant differences of their proof.

Annually, 30 kg of grapes from the clone and the population of the variety were vinified in the Experimental Wine Cellar of IVE Pleven after the classical technology for producing red wines (Yankov et al., 1992; Amerine et al., 1972). The alcoholic fermentation of the experimental variants was carried out with dry pure culture wine yeasts *Saccharomyces cerevisiae* in an amount of 10 g/hl, temperature 28°C and daily control. After completion of the process the experimental wines were decanted and sulphited to 20 mg/dm<sup>3</sup> free SO<sub>2</sub>. The chemical composition of grapes and the wines was analyzed by conventional winemaking

methods (Ivanov et al., 1979). The differences in the organoleptic qualities of wines were determined by a 100-point scale by 9-member tasting committee (Tsvetanov 2001).

## RESULTS AND DISCUSSIONS

The indicators of the mechanical analysis are very important elements of the total evaluation of each vine clone, as they present in great detail its economic qualities. In the studied variants of Merlot variety, the mass of the cluster changed over the years of the research (2006-2008) in varying degrees. For Merlot – control, the variation was within the range from 185.5 g to 222.0 g, on the average – 204.2 g, while for clone 10/27–230.0 g / 253.9 g, on the average 239.6 g. Both for the clone and the control the year with less favorable conditions for increasing the mass of the cluster was 2006, and the best–2008. The mass of 100 berries was lower for the population – 153.33 g, in comparison with the clone 10/27, where it was 158.30 g.

Table 1

Comparative mechanical analysis of cluster and berry of clone 10/27 and population of the varieties Merlot for 2006-2008 periode

Clone	Year	Average weight per cluster, g	Average yield per vine, kg	Average weight per 100 berries, g
Population	2006	185.5	4.860	160.00
	2007	205.0	4.510	163.33
	2008	222.0	5.550	136.67
	average	204.2	4.973	153.33
Clone 10/27	2006	230.0	5.470	171.56
	2007	235.0	4.700	165.00
	2008	253.9	6.757	138.33
	average	239.6*	5.642*	158.30 <sup>n.s</sup>

The variation of this indicator over the years of the study was in a narrow range, except for 2008 when lower values were measured. The average yield per vine reflected the best all other indicators revealing the real productive potential of each variety (clone). On the average the yield per vine was higher from Merlot clone 10/27 – 5.642 kg, compared to the population of the variety – 4.973 kg. The same tendency for the yield was observed during each year of the study. The variation in the data by year and variants was significant, as again in 2008 the highest values for this indicator were obtained (Table 1).

The mechanical analysis of the clusters and berries revealed that the quantity of bunches 4.38 % (clone 10/27) – 4.76 % (population) and the berries 95.62 % (clone 10/27) – 95.24 % (population) were within the known parameters of wine grape varieties, as there were minor differences between the clone and control (Table 2).

The rates of the skins in the population and clone 10/27 had very similar values - 12.17 % and 11.78 %, respectively. For the seeds the relative values were 4.66 % (control) and 4.65 % (10/27). The data for the mesocarp reflected the numerical values of the previous two indicators. The small differences in the percentage of berries in the cluster, and mesocarp in the berries, determined the insignificant differences in the theoretical yield of must for clone 10/27 (79.91 %) and the population of Merlot variety (79.21 %).

The comparative statistical analysis of the biometric data from the mechanical analysis of the clusters and berries of the studied clone 10/27 and Merlot variety showed that only the average mass of a cluster and the summarizing indicator yield per vine was mathematically proven higher for clone 10/27 compared to the control. For the remaining indicators the differences between the two groups of plants were minimal, however always in favour of the newly selected clone 10/27 (Table 1, 2).

Table 2

Comparative mechanical analysis of cluster and berry of clone 10/27 and population of the varieties Merlot for 2006-2008 periode

Clone	Year	Mechanical analysis of:					Theoretical randeman
		Cluster		Berry			
		rachis	berries	skins	seeds	flesh	
		%	%	%	%	%	%
Population	2006	4.92	95.08	10.50	4.06	85.44	81.24
	2007	5.09	94.91	12.12	4.65	83.23	79.00
	2008	4.26	95.74	13.89	5.26	80.85	77.41
	average	4.76	95.24	12.17	4.66	83.17	79.21
Clone 10/27	2006	4.28	95.72	10.19	4.07	85.74	82.07
	2007	4.71	95.29	11.93	4.84	83.23	79.31
	2008	4.15	95.85	13.21	5.06	81.73	78.34
	average	4.38 <sup>n.s</sup>	95.62 <sup>n.s</sup>	11.78 <sup>n.s</sup>	4.65 <sup>n.s</sup>	83.57 <sup>n.s</sup>	79.91 <sup>n.s</sup>

From the data on the chemical composition of grape must it was seen that Merlot clone 10/27 had good sugars accumulation capability in the region of cultivation (22.13 % on the average), depending on weather conditions during the ripening period of the grapes, their amount varied from 21.30 % (2007) to 23.10 % (2006) (Table 3). In each vintage the quantity of fructose was prevailing, which was a good indicator of grapes ripeness. The amount of titratable acids in the must in both variants was very close, within the typical ratios for the variety. For Merlot clone 10/27, they were on the average 5.82 g/dm<sup>3</sup>, and for the control – 5.86 g/dm<sup>3</sup>.

Table 3

Comparative chemical analysis of grape must of clone 10/27 and population of the varieties Merlot for 2006-2008 periode

Variety	Year	Date of harvest	Sugars	Glucose	Fructose	Titratable acids	pH
			%	g/dm <sup>3</sup>	g/dm <sup>3</sup>	g/dm <sup>3</sup>	
Population	2006	26.09.	22.30	86.58	136.42	5.46	3.19
	2007	17.09.	20.60	96.70	104.30	6.38	3.56
	2008	20.09.	21.30	92.93	125.07	5.80	3.45
	average	21.09.	21.40	92.07	121.93	5.86	3.40
Merlot clone 10/27	2006	26.09.	23.10	72.18	155.82	5.53	3.32
	2007	17.09.	21.30	98.31	112.36	6.28	3.54
	2008	20.09.	22.00	86.63	126.37	5.65	3.37
	average	21.09 <sup>n.s</sup>	22.13*	85.71*	131.52*	5.82 <sup>n.s</sup>	3.41 <sup>n.s</sup>

The variation in the values of titratable acids over the years of the study was in a narrow range and always in direct inverse dependence on grape sugar content. The pH of the must during the individual years and average for the period of the study was within the normal limits for red wines. The obtained results showed that the composition of Merlot clone 10/27 must was suitable for making quality dry red wines. The comparative chemical analysis of grape must of the studied clone and Merlot variety population revealed the presence of mathematically proven differences in the individual indicators. Sugars content in the grapes of Merlot clone 10/27 was definitely higher. Statistically significant differences were also found between the clone and the population of the variety in the content of the monosaccharides glucose and fructose in the grapes.

Data on the chemical composition and organoleptic profile of wines made from the studied clone 10/27 and the control are presented in Table 4.

Table 4

Chemical composition of wines of clone 10/27 and population of the varieties Merlot for 2006-2008 periode

Indexes		Population of variety Merlot				Merlot clone 10/27			
		2006	2007	2008	average	2006	2007	2008	average
Alcohol	vol. %	13.38	12.36	12.78	12.84	13.86	12.78	13.20	13.28
Sugar-free extract	g/dm <sup>3</sup>	22.24	20.41	21.78	21.48	23.00	21.00	21.83	21.94
Titratable acids	g/dm <sup>3</sup>	6.40	5.67	5.25	5.77	6.00	5.50	5.43	5.64
Volatile acid	g/dm <sup>3</sup>	0.36	0.59	0.54	0.50	0.36	0.59	0.60	0.51
Anthocyan	mg/dm <sup>3</sup>	376.0	315.9	341.0	344.3	486.0	329.8	386.0	400.6
Total phenolic substances	g/dm <sup>3</sup>	3.60	2.71	2.43	2.41	3.50	2.80	2.64	2.98
pH		3.35	3.45	3.40	3.40	3.40	3.42	3.41	3.47
Taste evaluation		79.38	76.27	78.16	77.93	80.83	78.54	79.23	79.53

During the study period the alcoholic fermentation was fully completed in all experimental variants. Sugars in wine were about and slightly over 2 g/dm<sup>3</sup>, and the alcohol content corresponded to the initial sugars while the transformation ratio of the sugars into alcohol ranged from 0.59 to 0.60. The alcohol concentration in the variant Merlot 10/27 in all vintages was about 0.5 – 0.6% vol. greater compared to the control sample, which favoured its soft and mellow taste.

Titratable acids of the experimental wines were similar, as the control had on the average 0.1-0.2 g/dm<sup>3</sup> more acids than the studied clone. The difference was due to a greater degree of tartaric acid and, to a lesser extent, of malic acid. The wines from Merlot clone 10/27 made from the three vintages had higher sugar-free extract, respectively 23.00 g/dm<sup>3</sup> / 21.00 g/dm<sup>3</sup> / 21.83 g/dm<sup>3</sup>, at 22.64 g/dm<sup>3</sup> / 20.41 g/dm<sup>3</sup> / 21.78 g/dm<sup>3</sup> for the control. The differences contributed to greater harmony, fullness and mellowness of the wines from the studied clone 10/27. The values of the indicator amount of anthocyanins determining colour characteristics of the studied wines in all years and average for the

period were higher in Merlot clone 10/27, which had direct effect on the organoleptic profile. In 2007, the samples had the lowest colour intensity due to the cool, rainy autumn resulting in not enough ripe grapes and low tinctorial content. TFC concentrations were within the typical range for Merlot red wines. The volatile acidity and pH values varied slightly and were within the normal limits.

The organoleptic analysis of wines vintage 2006 found that wines from that year had the best quality. Tasting scores were 79.38 for the population and 80.83 for clone 10/27. These values corresponded to quality wines, displaying varietal specificity and positive in different directions. Wine from clone 10/27 was characterized by intense dark red color, with very well defined specific for the variety fruity fragrance, fresh, thick, soft, harmonious, suitable for aging.

In 2007, wines were relatively slightly coloured. Aromas were of medium intensity, fruity, somewhat slightly depressed. Wine taste was medium full, with outlined titratable acids and austere tannins in the aftertaste. Wine from Merlot variety had lower score – 76.27. It had fainter aroma, hints of “green” and raw vegetation. Wine taste was lighter; acids were outlined more than acceptable. Wine from clone 10/27 was darker red in colour, soft and had better expressed fruit flavor specific for the variety, and as a result it was assessed higher – 78.54.

Wines vintage 2008 possessed very good organoleptic characteristics. They were clear and shiny, pure ruby red in colour. The aroma was intense, very clean and fine, as the well outlined fruity nuances were dominating while the exquisite herbal notes were well integrated into the flavour harmony. The wines of the samples was well structured, harmonious, soft and well-balanced, the flavour was strong and relatively stable, with well-integrated acids and the aftertaste was relatively mild. When ranking it was preferred wine of Merlot clone 10/27 (score 79.23) compared to the control variant (score 78.16).

## CONCLUSIONS

Between the population and clone 10/27 of Merlot variety there were mathematically proven differences in the most important ampelographic indicators of the mechanical and chemical analysis of the cluster and berry. The studied clone was superior compared to the control in the average mass of the cluster, vine yield and sugars content in grapes.

In all studied vintages the grapes of Merlot clone 10/27 accumulated on the average 0.6-1.0% more sugars, representing about 4-6 % more substrate. This determined higher alcohol concentration in the future wines, more metabolites and extraction.

The differences in the chemical properties of the experimental wines led to differences in their organoleptic profile. In all vintages the wines from Merlot clone 10/27 were the most preferred, showing that the clone was suitable for the production of high quality red wines from Merlot variety. Average taste evaluations of this sample were on the average 1-2 scores higher than those of the population of Merlot.

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## THE INFLUENCE OF MECHANICAL TENDERIZING ON ROMANIAN TRADITIONAL PRODUCT *CEAFA PERPELITA*

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*Keywords: mechanical tenderization, traditional product Ceafa Perpelita, Warner-Bratzler method*

### ABSTRACT

*The paper presents the mechanical characteristics of raw meat's mechanical tenderizing process in order to produce Romanian traditional cured-smoked product Ceafa Perpelita type. These characteristics are based on experimental diagrams obtained by using Warner-Bratzler testing method of final product, after tenderizing process. The tenderizing process performed to decrease the necessary period for brining consists in passing several times the raw meat amongst rollers with cutting prongs, and cyclic impulsive pressing of the meat, respectively. The paper presents characteristic shear force amount obtained by using Warner-Bratzler testing method for the tenderized and cured-smoked final product, in comparison with the initial no tenderized meat sample.*

### INTRODUCTION

One of the major changes the meat industry has had to face in the past ten years has been the rapid development of sliced-packaged products. In the case of whole muscle cooked products, the food industry has given rise to a series of problems, which although already existed in the past have now greatly increased. Areas with weak binding, air holes, muscles that break or crumble will result in final product quality unsuitable for consumer desire (Xargayó et al. 2011).

Cohesion of the muscles takes place thanks to the myofibrillar proteins which have been extracted during the manufacturing process and which are found on the surface of the muscle. These proteins form the exudates and, due to their gelling capacity, act as glue between the muscles. It has been widely demonstrated in the pertinent literature that the greater number of proteins extracted, the greater the stability between muscles and therefore the better the sliceability and mastication. Extraction of myofibrillar proteins is achieved through two actions (Maddock 2008, Tyszkiewicz & Klossowska 1996, Xargayó et al. 2011):

- Chemical action: in brine composition, the presence of salt and phosphates increases the pH and the ionic strength of the medium, giving rise to the opening of the protein chains and facilitating their extraction.

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- Mechanical action: application of the mechanical process causes relaxation of the muscle structure and breaking up of the cells, making the membranes more permeable and increasing mobilization of the proteins up toward the surface of the muscle. The degree to which the muscle structure is opened will determine the final quantity of proteins present in the exudates. This opening of the structure is done by means of tenderization, pre-massage and massage.

S.C. AVI-GIIS SRL Stuparei is one of the most important small enterprise producers of meat products in Valcea County. Initially specialized in commercial cured-raw meats and cured-cooked products, in the last years SC AVI-GIIS SRL focused its effort to produce Romanians traditional produces cured-cooked *Ceafa Perpelita* type (pork collar butt). In principle, the processing technology of this cured-cooked product type consists in: wet curing phase of entire pieces of muscle meat in 15-20% curing salt concentration, *during 2-3 weeks*; drying / ripening phase in cold ventilation *for 6-8 hours*; cold smoke phase (around 20°C) *for 4-5 days*, followed by a short sequence of hot smoke phase (around 80°C), *for 4-6 hours* (Simion 2014).

According to Romanian legislation, sodium nitrite (NaNO<sub>2</sub>), sodium or potassium nitrate (NaNO<sub>3</sub>/KNO<sub>3</sub>), or any alternative curing substances are not permitted in traditional cured-cooked types products usual processing, and no brine injection is allowed.

In order *to reduce the wet curing phase as much is possible*, SC AVI-GIIS SRL decided to realize and to test two types of tenderizing machines: *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine* (Simion 2014).

## MATERIAL AND METHODS

### Processing method and equipment

In order to produce *Ceafa Perpelita*, 16 pieces of pork collar butt raw meat were used (Animal Slaughter Certificate: 8 pigs, 14 months, 70...80 kg in carcass, large farm).

To determine the influence of tenderizing process in reducing the too long wet curing phase of Romanians traditional produces cured-cooked type such as *Ceafa Perpelita*, the row meat was tenderized by using *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine*, too (Simion 2014).

To produce *Ceafa Perpelita* by using no-tenderized pork collar butt, raw meat was processed in the following traditional phases: wet curing phase of entire pieces of muscle meat in 15% curing salt concentration, *during 2 weeks*; drying / ripening phase in cold ventilation *for 6 hours*; cold smoke phase (around 20°C) *for 2 days*, followed by *4 hours* short sequence of hot smoke phase (around 80°C) (Simion 2014).

*Four roller tenderizer machine* is intended to increase the effective surface area for the extraction of muscle proteins during subsequent massage processes. In its operation, the machine perform superficial or deep cuts in the piece of meat that passes through a series of tenderizer roller, such as rotating rollers located at a small distance, subjecting it to compression and stretching, improves muscle protein extraction, resulting in improved adhesion between the muscles and the pieces of fat or rind incorporated into the meat mass. In principle, the machine consists of a pair of parallel tenderizing rollers (Figure 1), located at a short distance and rotated in opposite directions by a electro-mechanical transmission with the rollers fitted with a number of cutting prongs emerging from their surfaces, defining an elongated aperture through which the pressed meat passes, driven by the rollers and gravity. The distance between the prongs' a bottom of twin roller is 45mm, and 25mm between the prongs' edges of twin roller, respectively (Rosca et al. 2014, Simion 2014).

*Cyclic impulsive pressing machine* is a semi-continuous meat press machine for pressing meat products to tenderize the meat before marinating. In principle, *Cyclic impulsive pressing machine* (Figure 2) consists in a mechanical - pneumatical equipment, and a control

programmable automat. The mechanical - pneumatical equipment consists in a mobile upper plate, a fixed lower plate, pneumatical cylinder, electro-pneumatical actuating panel, and other accessories.

The control programmable automat permits parameters' configuration of the cyclic impulsive tenderize pressuring diagram (speed, pressure cycle time, release cycle time) when a certain pressure amount is supplied by the air compressor (Simion, 2014).

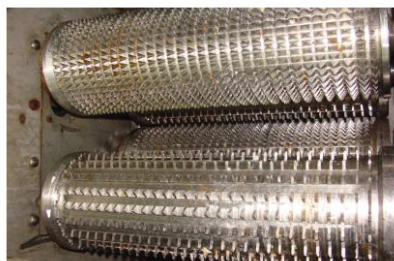


Figure 1. Four roller tenderizer machine (with cutting prongs)



Figure 2. Cyclic impulsive pressing machine (Teflon pads with pyramidal prongs)

In order to determine the influence of tenderizing process on *Ceafa Perpelita* tenderness' pork collar butt raw meat used to produce traditional cured-cooked product, five types of 6 samples were used:

-*Ceafa Perpelita* made with no tenderized pork collar butt (CEP-NO TEND);

-*Ceafa Perpelita* made after pork collar butt was tenderized by passing 5, and 10 successive, respectively times amongst the cutting prongs of the *Four roller tenderizer machine* (CEP-FRT 5x; CEP-FRT 10x);

-*Ceafa Perpelita* made after pork collar butt was tenderized by using *Cyclic impulsive pressing machine* in 30 pressing cycles, each consisting in 0,5s pressing periods, and 0,5s pauses periods, respectively (CEP-CIP 30-0,5-0,5);

-*Ceafa Perpelita* made after pork collar butt was tenderized by using *Cyclic impulsive pressing machine* in 30 pressing cycles, each consisting in 10s pressing periods, and 0,5s pauses periods, respectively (CEP-CIP 30-10-0,5).

After tenderizing process by using *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine*, respectively, the pork collar butt row meat was processed in the following phases: wet curing phase of entire pieces of muscle meat in 15% curing salt concentration, during *only 3 days*; drying / ripening phase in cold ventilation for *6 hours*; cold smoke phase (around 20°C) for *2 days*, followed by *4 hours* short sequence of hot smoke phase (around 80°C).

No-tenderized pork collar butt row meat and tenderized pork collar butt raw meat too, were used to produce Romanian traditional produce cured-cooked *Ceafa Perpelita* type, and then tested by using Warner - Bratzler shear force method.

#### **Tenderness evaluation by using Warner-Bratzler method**

The most relevant and utilized destructive texture and tenderness meat's tests are compression test, and Warner-Bratzler shear test. The shear force behavior gives information about tenderness, as well as the bite characteristic products ([www.lloyd-instruments.co.uk](http://www.lloyd-instruments.co.uk)).

During Warner-Bratzler test the jig and the shear blade acts in direct compression for slicing/shearing tests on products (Institute of Food Technologists 1991, Xianzhong 2011, Wheeler 2001).

To perform interdisciplinary researches concerning general texture and tenderness analysis, universal testing machines *Lloyd Instruments LRXPlus 5 (UTEFIL* within Faculty of Agri-culture and Horticulture in Craiova), is used since several years ago to perform comparative texture measurements (Rosca & Rosca 2013).

Due to collaboration between *UTEFIL* and Environmental Engineering Laboratory within Faculty of Electrical Engineering, an *experimental Warner - Bratzler equipment* was made: special rigid frame (made in food-grade Teflon) supporting a shear bar that permits interchangeable Warner - Bratzler shear blades (V and square plate cut blade made in stainless steel DIN W1.4571) to slide fit into the frame ((Rosca & Rosca 2013).

During this experiment, 100mm/min cutting speed was used.

*Ceafa Perpelita* obtained by using pork collar butt row meat pieces tenderized by using *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine* during Warner - Bratzler testing shear force, are presented in Figure 3.

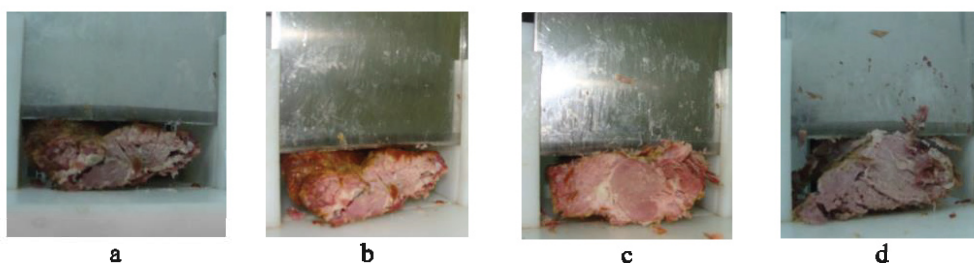


Figure 3. *Ceafa Perpelita* obtained by using pork collar butt row meat pieces after tenderizing, during Warner - Bratzler testing shear force:

CEP-FRT 5x (a), CEP-FRT 10x (b), CEP-CIP 30-0,5-0,5 (c), CEP-CIP 30-10-0,5 (d)

## RESULTS AND DISCUSSIONS

In order to determine the influence of tenderizing process on the final product tenderness, *Ceafa Perpelita* pieces made by using traditional homemade method (no tenderized), and tenderized by using CEP-FRT 5x, CEP-FRT 10x, CEP-CIP 30-0,5-0,5, CEP-CIP 30-10-0,5, respectively, were tested according Warner - Bratzler shear force method. During the Warner - Bratzler shear force tests, each of all 16 pieces of *Ceafa Perpelita* were sliced in 6 parts.

Representative Warner - Bratzler shear force diagram is presented in Figure 4.

In Table 1 are presented the maximum shear force amount and the shear force average for each of the five of *Ceafa Perpelita* types. In Table 1 is presented too, the decrease of percentage average shear force (in comparison with no tenderized traditional homemade *Ceafa Perpelita*'s tenderness) by using each processing method, that demonstrate the tenderness increase of the final product, that was initial tenderized by using the four tenderizing methods. Table 1 presents a synthesis of the influence of cyclic vacuum process on final *Ceafa Perpelita* tenderness':

- in comparison with traditional no - tenderized homemade *Ceafa Perpelita*'s tenderness, each one of CEP-FRT 5x, CEP-FRT 10x, CEP-CIP 30-0,5-0,5 and CEP-CIP 30-10-0,5 methods, determines a large tenderness' increasing of the final product;

- in comparison with *Cyclic impulsive pressing machine*, the tenderizing method *Four roller tenderizer machine* is more efficient, both in final product tenderness, and time processing and consumption energy, too.

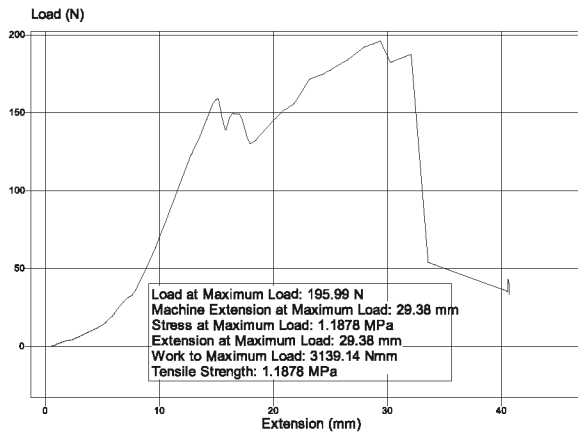


Figure 4. Warner - Bratzler shear force test for *Ceafa Perpelita* made by using CEP-CIP 30-10-0,5method

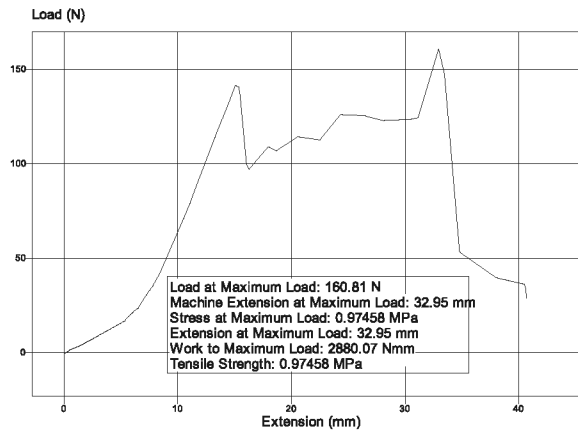


Figure 5. Warner - Bratzler shear force test for made *Ceafa Perpelita* by using CEP-FRT 10x method

Table 1

Warner - Bratzler shear force for *Ceafa Perpelita*

Sample code	Maximum shear force min...max amount, N	Shear force average, N	Decrease of shear force average, %
CEP- TRAD NO TEND	252,52...294,76	268,35	-
CEP-CIP 30-0,5-0,5	184,87...220,18	201,46	24,67
CEP-CIP 30-10-0,5	152,58...195,99	173,84	35,07
CEP-FRT 5x	136,25...177,55	158,67	40,47
CEP-FRT 10x	131,46...160,81	145,41	45,89

## CONCLUSIONS

Tenderizing by using *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing methods represent much more intensively tenderizing method than massaging vacuum equipment in industrial processing. Both methods determine much smaller salt marinating / brining period, in comparison with 4 - 6 hours in massaging vacuum equipment in industrial processing, or 1 - 2 weeks marinating / brining in homemade or small enterprise processing.

As one of the most recommended analyze method, the Warner - Bratzler shear force test offers the certitude of influence of *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing methods on meat final products tenderness'.

This paper opens further experimental researches concerning the influence of similar *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing (higher pressure level, shorter or longer impulsive pressuring steps) to produce *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing, by using other much more tenderless parts of animal's carcass.

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\*\*\* [www.lloyd-instruments.co.uk](http://www.lloyd-instruments.co.uk)

## THE INFLUENCE OF MECHANICAL TENDERIZING ON ROMANIAN TRADITIONAL PRODUCT *PULPA PERPELITA*

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*Keywords: mechanical tenderization, traditional product Pulpa Perpelita, Warner-Bratzler method*

### ABSTRACT

*The paper presents the mechanical characteristics of raw meat's mechanical tenderizing process in order to produce Romanian traditional cured-smoked product Pulpa Perpelita. These characteristics are based on experimental force – extension diagrams obtained by using Warner-Bratzler testing method of final product, after tenderizing process. The tenderizing process performed to decrease the period of cured marinating stage consists in passing several times the raw meat amongst rollers with cutting prongs, and cyclic impulsive pressing of the raw meat, respectively. The paper presents characteristic shear force amount obtained by using Warner-Bratzler testing method for the tenderized and cured-smoked final product, in comparison with the initial no tenderized meat sample.*

### INTRODUCTION

Tenderizing is a process to break down collagens in meat to make it more palatable for consumption. There are several ways to tenderize meat: mechanical tenderization, such as pounding or piercing; the tenderization that occurs through cooking, such as braising; tenderizers in the form of naturally occurring enzymes, which can be added to food before cooking (examples of enzymes used for tenderizing: papain from papaya, bromelain from pineapple and actinid in from kiwifruit; marinating the meat with vinegar, wine, lemon juice, buttermilk or yogurt; brining the meat in a salt solution (brine); dry aging of meat at 0 to 2°C (McGee 2004; <http://en.wikipedia.org/wiki/Tenderizing>).

Tenderization is the mechanical action of producing multiple cuts in the meat muscle in order to increase the surface area and thereby facilitate extraction and solubilization during the massaging phase. Softening of the muscle is also obtained, making the meat more adaptable to the cooking moulds. Tenderization, pre-massage and massage are closely inter-related, and not all products require the same mechanical action. Thus the mechanical action must be intensified and adapted in order to compensate for some of the negative consequences that may result in the product's quality. This will depend on the rest of the process and, above all, on the presentation and final quality of the product itself. In low-injection products where meat content represents more than 80% of the final

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composition, meat quality is a determining factor in mastication, while in more highly injected products, this is not as important as the process and technology used (Tyszkiewicz & Klossowska 1996, Xargayó et al. 2011).

By means of certain additives, aside from the above-mentioned salt and phosphates, such as carrageenan and vegetable gums, muscle texture can be slightly hardened and/or “plastified”, however this alone will not be sufficient to compensate for the meat’s lack of firmness. It has been observed that the *mechanical action of tenderization does have a positive effect on this type of meat, because the texture is less fragile due to an increased surface of contact between muscles*. Mechanical action: application of the mechanical process causes relaxation of the muscle structure and breaking up of the cells, making the membranes more permeable and increasing mobilization of the proteins up toward the surface of the muscle. The degree to which the muscle structure is opened will determine the final quantity of proteins present in the exudates. These openings of the structure are done by means of tenderization, pre-massage and massage (Maddock 2008, Tyszkiewicz & Klossowska 1996, Xargayó et al. 2011).

SC AVI-GIIS SRL Stuparei is a small enterprise for meat products in Valcea County, which was initially specialized in commercial cured-raw meats and cured - cooked products. In the last years SC AVI-GIIS SRL focused its effort to produce Romanians traditional produces cured-cooked *Pulpa Perpelita* type (pork leg set). In principle, the processing technology of this traditional cured-cooked product type consists in: wet curing phase of entire pieces of muscle meat in 15-20% curing salt concentration, *during 2-3 weeks*; drying / ripening phase in cold ventilation *for 6-8 hours*; cold smoke phase (around 20°C) *for 4-5 days*, followed by a short sequence of hot smoke phase (around 80°C), *for 4-6 hours* (Simion 2014).

According to Romanian legislation, sodium nitrite (NaNO<sub>2</sub>), sodium or potassium nitrate (NaNO<sub>3</sub>/KNO<sub>3</sub>), or any alternative curing substances are not permitted in traditional cured-cooked types products usual processing, and no brine injection is allowed.

In order to *reduce the wet curing phase as much is possible*, SC AVI-GIIS SRL decided to realize and to test two types of tenderizing machines: *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine* (Simion 2014).

## MATERIAL AND METHODS

### Processing method and equipment

In order to produce *Pulpa Perpelita*, 16 pieces of pork leg set raw meat were used (Animal Slaughter Certificate: 8 pigs, 14 months, 70...80 kg in carcass, large farm).

To determine the influence of tenderizing process in reducing the too long wet curing phase of Romanians traditional produces cured-cooked type such as *Pulpa Perpelita*, the row meat was tenderized by using *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine*, too (Simion 2014).

To produce *Ceafa Perpelita* by using no-tenderized pork leg set, raw meat was processed in the following traditional phases: wet curing phase of entire pieces of muscle meat in 15% curing salt concentration, *during 2 weeks*; drying / ripening phase in cold ventilation *for 6 hours*; cold smoke phase (around 20°C) *for 2 days*, followed by *4 hours* short sequence of hot smoke phase (around 80°C) (Simion 2014).

*Four roller tenderizer machine* perform superficial or deep cuts in the piece of meat that passes through a series of tenderizer roller, such as rotating rollers located at a small distance, subjecting it to compression and stretching, improves muscle protein extraction, resulting in improved adhesion between the muscles and the pieces of fat or rind incorporated into the meat mass. In principle, the machine consists of a pair of parallel



tenderizing rollers (Figure 1), located at a short distance and rotated in opposite directions by a electro-mechanical transmission with the rollers fitted with a number of cutting prongs emerging from their surfaces, defining an elongated aperture through which the pressed meat passes, driven by the rollers and gravity. The distance between the prongs' a bottom of twin roller is 45mm, and 25mm between the prongs' edges of twin roller, respectively (Rosca et al. 2014, Simion 2014).

*Cyclic impulsive pressing machine* is a semi-continuous meat press machine for pressing meat products to tenderize the meat before marinating. In principle, *Cyclic impulsive pressing machine* (Figure 2) consists in a mechanical - pneumatical equipment, and a control programmable automat. The mechanical - pneumatical equipment consists in a mobile upper plate, a fixed lower plate, pneumatical cylinder, electro-pneumatical actuating panel, and other accessories.

The control programmable automat permits parameters' configuration of the cyclic impulsive tenderize pressuring diagram (speed, pressure cycle time, release cycle time) when a certain pressure amount is supplied by the air compressor (Simion, 2014).

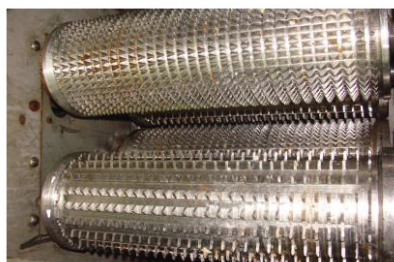


Figure 1. Four roller tenderizer machine



Figure 2. Cyclic impulsive pressing machine

In order to determine the influence of tenderizing process on *Pulpa Perpelita* tenderness' pork leg set raw meat used to produce traditional cured-cooked product, five types of 6 samples were used:

- Pulpa Perpelita* made with no tenderized pork leg set (PP-NO TEND);
- Pulpa Perpelita* made after pork leg set was tenderized by using *Cyclic impulsive pressing machine* in 30 pressing cycles, each consisting in 0,5s pressing periods, and 0,5s pauses periods, respectively (PP-CIP 30-0,5-0,5);
- Pulpa Perpelita* made after pork leg set was tenderized by using *Cyclic impulsive pressing machine* in 30 pressing cycles, each consisting in 10s pressing periods, and 0,5s pauses periods, respectively (PP-CIP 30-10-0,5);
- Pulpa Perpelita* made after pork leg set was tenderized by passing 5, and 10 successive, respectively times amongst the cutting prongs of the *Four roller tenderizer machine* (PP-FRT 5x; PP-FRT 10x);

After tenderizing process by using *Four roller tenderizer machine*, and *Cyclic impulsive pressing machine*, respectively, the pork leg set raw meat was processed in the following phases: wet curing phase of entire pieces of muscle meat in 15% curing salt concentration, during *only 3 days*; drying / ripening phase in cold ventilation for *6 hours*; cold smoke phase (around 20°C) for *2 days*, followed by *4 hours* short sequence of hot smoke phase (around 80°C).

No-tenderized pork leg set raw meat and tenderized pork leg set raw meat too, were used to produce Romanian traditional produce cured-cooked *Pulpa Perpelita* type, and then tested by using Warner - Bratzler shear force method.

### Tenderness evaluation by using Warner-Bratzler method

The most relevant and utilized destructive texture and tenderness meat's tests are compression test, and Warner-Bratzler shear test. The shear force behavior gives information about tenderness, as well as the bite characteristic products. During Warner-Bratzler test the jig and the shear blade acts in direct compression for slicing / shearing tests on products (Institute of Food Technologists 1991, Xianzhong 2011, Wheeler 2001, www.lloyd-instruments.co.uk).

To perform interdisciplinary researches concerning general texture and tenderness analysis, universal testing machines *Lloyd Instruments LRXPlus 5 (UTEFIL* within Faculty of Agriculture and Horticulture in Craiova), is used since several years ago to perform comparative texture measurements. Due to collaboration between *UTEFIL* and Environmental Engineering Laboratory within Faculty of Electrical Engineering, an *experimental Warner - Bratzler equipment* was made: special rigid frame supporting a shear bar that permits interchangeable Warner - Bratzler shear blades (V and square plate cut blade made in stainless steel DIN W1.4571) to slide fit into the frame ((Rosca & Rosca 2013). During this experiment, 100mm/min cutting speed was used.

*Pulpa Perpelita* obtained by using pork leg set raw meat pieces tenderized by using *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine*, respectively, during Warner - Bratzler testing shear force, are presented in Figure 3.

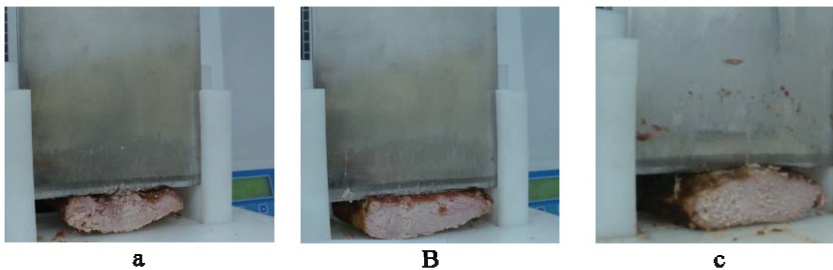


Figure 3. *Pulpa Perpelita* obtained by using pork leg set raw meat pieces after tenderizing, during Warner - Bratzler testing shear force: PP-CIP 30-0,5-0,5 (a), PP-CIP 30-10-0,5 (b) PP-FRT 10x (c)

### RESULTS AND DISCUSSIONS

In order to determine the influence of tenderizing process on the final product tenderness, *Pulpa Perpelita* pieces made by using traditional homemade method (no tenderized), and tenderized by using PP-CIP 30-0,5-0,5, PP-CIP 30-10-0,5, PP-FRT 5x and PP-FRT 10x, respectively, were tested according Warner - Bratzler shear force method. During the Warner - Bratzler shear force tests, each of all 16 pieces of *Pulpa Perpelita* were sliced in 6 parts.

Warner - Bratzler shear force Representative diagram is presented in Figure 4.

In Table 1 are presented the maximum shear force amount and the shear force average for each of the five types of *Pulpa Perpelita*. Table 1 presents too, the decrease of shear force per-centage average (in comparison with no tenderized traditional homemade *Pulpa Perpelita*'s tenderness) by using each processing method, that demonstrate the tenderness increase of the final product, that was initial tenderized by using the four tenderizing methods.

Table 1 presents a synthesis of the influence of mechanical tenderizing process on final *Pulpa Perpelita* tenderness':

-in comparison with traditional no - tenderized homemade *Pulpa Perpelita*'s tenderness, each one of proposed methods PP-CIP 30-0,5-0,5, PP-CIP 30-10-0,5, PP-FRT 5x and PP-FRT 10x, determines high tenderness' increasing of the final product;

-in comparison with *Cyclic impulsive pressing machine*, the tenderizing method *Four roller tenderizer machine* is much more efficient, both in final product tenderness, and time processing and energy consumption, too.

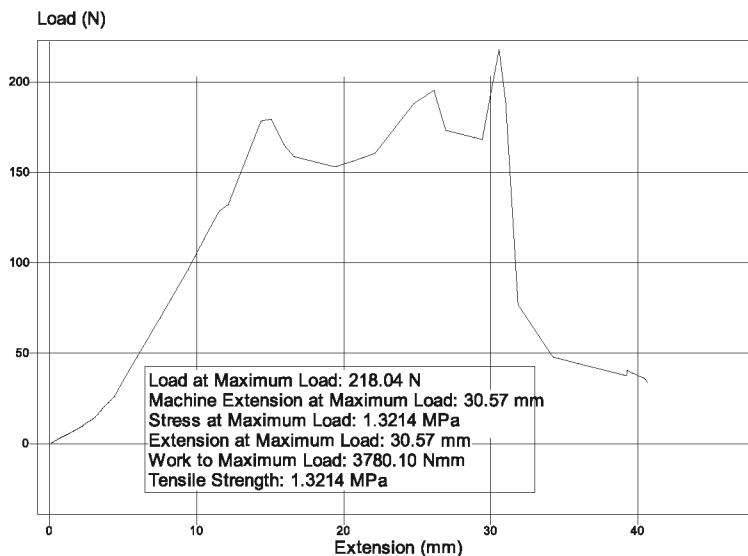


Figure 4. Warner - Bratzler shear force test for *Pulpa Perpelita* made by using PP-CIP 30-0,5-0,5method

Table 1

Warner - Bratzler shear force for *Pulpa Perpelita*

Sample code	Maximum shear force min...max amount, N	Shear force average, N	Decrease of shear force average, %
PP- TRAD NO TEND	348,83...384,52	363,55	-
PP-CIP 30-0,5-0,5	218,04...240,11	222,47	38,84
PP-CIP 30-10-0,5	165,86...194,49	179,78	50,68
PP-FRT 5x	147,33...189,76	169,84	53,44
PP-FRT 10x	132,76...164,57	151,36	58,41

## CONCLUSIONS

Tenderizing by using *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing methods represent much more intensively tenderizing method than massaging vacuum equipment in industrial processing. Both methods determine much smaller salt marinating / brining period, in comparison with 4 - 6 hours in massaging vacuum equipment in industrial processing, or 1 - 2 weeks marinating / brining in homemade or small enterprise processing.

As one of the most recommended analyze method, the Warner - Bratzler shear force test offers the certitude of influence of *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing methods on meat final products tenderness'.

This paper opens further experimental researches concerning the influence of similar *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing

(higher pressure level, shorter or longer impulsive pressuring steps) to produce *Cyclic impulsive pressing machine*, and *Four roller tenderizer machine* processing, by using other much more tenderless parts of animal's carcass.

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\*\*\* [www.lloyd-instruments.co.uk](http://www.lloyd-instruments.co.uk)

## **INFLUENCE OF MUST PROCESSING ON QUALITY NATURAL DISTILLATES**

Stoica Felicia, Muntean Camelia, Băducă C., Popescu Mitroi I.

*Key words: must clarity, wine, volatile compounds, distilled beverages*

### **ABSTRACT**

*Obtaining quality distillates is another variant of capitalization in wine production. The influence of must processing and different methods of clarification on the distilled quality were study.*

*Best distilled are obtained from wines with an alcoholic strength ranging from 8-9 vol% acidity than 6 g / l of volatile acidity as low un-reduced extract between 16 to 17 g / l. These conditions emphasize the positive content of volatile compounds and low proportions of methyl alcohol.*

### **INTRODUCTION**

Wine distillates are products obtained, as their name suggests, by distilling wine (Popa A., 1985).

Achieving quality distillates is conditioned by the use of wine-raw material namely obtained and a rational processing and distillation of raw material.

Regarding this issue, there have been many studies on the influence of technological factors on the composition and quality of distillates: quality wine, limpiditatea his time distillation, distillation system, storage condition (Nykänen L 1986).

The main way for the capitalization of distillate obtained from wine is that of their aging for the preparation vinars - romanian distilled (Stoica Felicia et al., 2009).

Quality distillate aged as finished product depends essentially on three factors: the quality of the raw material, the process of distillation of wine distillate the way aging (Lafon J., 1975, Ribereau Gayon P., 1971).

### **MATERIAL AND METHODS**

The main aspects of wine production - raw materials, their processing for distillation and obtained crude distillates were processing must (assembly) in the context of winemaking technology and wine clarity at the time of distillation

The wines used as work material were obtained by specific technology production this way: quality grapes harvested at technological maturity (minimum 156 g / l sugar and less than 5 g / l total acidity expressed in H<sub>2</sub> SO<sub>4</sub>), rapid processing of grapes (transport, crushing and crushing, separation must) with minimum protection of SO<sub>2</sub> (maximum 3 g SO<sub>2</sub> / 100 kg grapes), assembly must obtained without pressing the grapes with the fraction obtained from the first pressing, summary clarification of must by settling and decanting after 5-6 hours, fermentation at temperatures around 20 ° C and separation of the coarse deposit achieve 6-7 vol.% alcohol (70-80% fermented sugar), management of alcoholic

fermentation to dryness; maintain full and distillation of wine on it in the first 30-60 days after the end of alcoholic fermentation, while still present state of pronounced opalescence.

Distillation of wine made with Charante installation, removing overhead (2%) and tails. Alcoholic middle fraction ranged from 62-67 vol. %.

Determinations were performed on crude distillates contained in alcohol, methanol, acidity, aldehydes and main esters. Formal methods recommended by OIV - Paris and adopted ICVV were used.

## RESULTS AND DISCUSSIONS

The quality distillates, significant influence with the grape variety from which the wine - raw material obtained, holding a technology and processing operations must and wine. The data in Table 1 point to the differential assembly operation grape must fractions from the separation of the composition of its distillates. Fractions of pressure determined for the majority of the components increases and decreases in content to must obtained without pressing the grapes, fraction which is at the basis of the best distilled. The register increases quantitative at elements which at content increased, diminishing the quality distillates (aldehydes, furfural, methyl alcohol).

Table 1  
Characteristics of crude distillates, depending on the origin of wine-raw material

Determination	Provenience grape must		
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
Alcohol, vol. %	66,4	66,5	66,4
Total acidity, mg/L (acetic acid)	501	404	365
Volatile acidity, mg/L (acetic acid)	280	191	166
Free aldehydes, mg/L	75	108	109
Total aldehydes, mg/L	79	103	118
Esters, ml/L	790	445	415
Furfural, mg/L	3,0	3,1	3,8
Methyl alcohol, ml/L	0,51	0,81	0,77
Ethyl acetate, mg/L	280	193	184
Higher alcohols, mg/L	2298	1958	1412
n-propanol, mg/L	57,1	35,2	12,1
isobutanol, mg/L	273	246	164
1 methyl-2-butanol, mg/L	199	156	99
3 methyl-1butanol, mg/L	1771	1523	1151

M<sub>1</sub> - must obtained without pressing the grapes

M<sub>2</sub> - must obtained without pressing the grapes+ fraction obtained from the first pressing

M<sub>3</sub> - must obtained without pressing the grapes+ fraction obtained from the first and second pressing

Most components that prints distillate quality attributes (esters, ethyl acetate, n-propanol, isobutanol, etc.) recorded decreases.

Due to the wine-making technology, there is a high percentage grape must obtained without pressing the grapes made on the basis of the first fraction of the continuous press obviously reduced in this case.

This explains the relatively good quality of the distillate obtained by assembling the must obtained without pressing the grapes with the first part of the press.

Given the high productivity of must obtained without pressing the grapes, it is preferred that the wine-raw material for quality distillate is only achieved in this fraction.

The composition of the distillate is obviously influenced by the extent to which the assembly must is clarified before fermentation (Table 2).

Table 2

Content of crude distillates in volatile compounds obtained by different processing must

Compound, mg/L	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
Isobutyl alcohol	1570	765	793
Butyl alcohol,	109	84	95
Isoamyl alcohol	2008	1182	1244
Hexyl alcohol	10,4	4,4	4,6
Caprone ethyl	5,1	3,2	3,4
Ethyl caprylate	5,8	3,9	4,7
Caprin ethyl	6,8	1,3	1,7
ethyl succinate	3,3	1,4	1,6
Acetate $\beta$ -phenylethanol	0,6	0,3	0,3
$\beta$ -phenylethanol	8,3	5,8	4,3

M<sub>1</sub> - must obtained without pressing the grapes

M<sub>2</sub> - must obtained without pressing the grapes+ fraction obtained from the first pressing

M<sub>3</sub> - must obtained without pressing the grapes+ fraction obtained from the first and second pressing

Volatile compounds are found at very high alcohol content higher unfavorable quality (isoamyl alcohol and isobutanol even) if unclarified musts.

Clarification gravity, carried out with or without the addition of bentonite, lead to content in volatile handles close.

It can be concluded that the clarification by settling after about 6 hours is sufficient to achieve the quality of the distillates.

The processing of grape from which the wine-raw material influences a large extent and quality distillate composition (Table 3)

When the grape must has not been clarified, higher alcohol content is higher but methanol is significant proportions.

Clarification must - gravity or bentonite - leads generally - with good results, expressed in lower contents in methanol and higher alcohols.

Table 3

## Characteristics of crude distillate obtain by must different processing

Sample	Alcohol vol %	Acidity, mg/l	Methyl alcohol, ml/l	Esters, mg/l	Higher alcohols, mg/l
Must unclarified	60.21	301	357	878	3002
Gravity clarification for 6 hours	59.65	298	309	668	2747
Clarification with bentonite 1 g / l after 6 hours separated	60.71	312	238	757	2396
Fermented with bentonite 1 g / l	61.06	268	409	908	2913

A favorable situation is recorded and when grape must ferments together with bentonite. The advantage is given by the lowest the acid content and higher in esters. However, increases were registered in acidity, which is a negative point.

### CONCLUSIONS

Rapid processing of grapes to avoid high doses of SO<sub>2</sub>, is a basic condition for producing wines - good quality raw material.

Must obtain without pressing the grapes will assemble with the must at the press. This mixture will be used for producing wine - raw material - for distillates.

Grape must will be assembled and allow to stand for 6-8 hours in order to coarse clarification after running Settling further ensuring the protection of antiseptics and antioxidants, with the addition of SO<sub>2</sub> dose to 80-90 mg / l.

Immediately after exhaustion of the sugar content of the fermentation barrels must be filled completely. The wine must be keeping at the temperature being 10-13 ° C, to avoid the malolactic fermentation, which would result in the reduction of total acidity.

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**THE INFLUENCE OF REFRIGERATED STORAGE ON QUALITY INDICATORS OF VALUABLE VARIETIES OF GRAPES FOR THE TABLE**

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*Keywords: flavonoids, grape table, phenolic compounds*

**ABSTRACT**

*Post harvest, table grapes undergo a series of physical and chemical changes mainly influenced by preservation method and duration. During cooling and storage of grapes, physiological processes continue to unfold, but with reduced intensity compared to previous periods of growth and maturation of the fruit. Rate of biochemical reactions taking place in the fruit is determined by the intensity of breathing and sweating as well, as of both processes are affected by environmental conditions (temperature and relative air humidity). In this paper is monitored the influence of refrigerated storage (4°C temperature and relative humidity -75%) on the quality of grapes belonging to varieties of grapes for the table with middle-late maturity: Muscat de Hamburg, Muscat d'Adda, Italia, Bicane, Xenia and Tamina. The results showed that the grapes which are stored for a month under refrigerated conditions do not substantially alter the parameters of quality.*

**INTRODUCTION**

In the past years it has been found increasing consumer interest in rich antioxidants diets, being known their beneficial role for the human body. Grapes contain a variety of specific chemical compounds with a real antioxidative capacity, which belong to different chemical classes, including: phenols, terpenes, antibiotics, oils, resins, glycosides, sterols, alkaloids, saponins, many of which proved to be of great value for the food, pharmaceutical, cosmetic and agrochemical industry (Zhang et al., 2005).

The phenols are a large and complex group of secondary metabolites known for their contribution to pigmentation of various plant parts and fruits, especially helping to increase their food value, but also the role they play in plant resistance to biotic and abiotic stresses. The grapes destined for current consumption and especially the red ones contain appreciable quantities of phenolic compounds with pharmaceutical activity, antiallergenic, anticancer -in the skin and seeds of berries, while contributing in a fundamental way to the definition of specific sensory properties (Hooper et al., 2008). Epidemiological studies have shown that the anthocyanins, being in the form of red pigments, are found in the bean's skin (in a few cases are also in their pulp), in the form of red colored heterosides (at low

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values of pH) or blue (at high values of pH) and helps, with vitamin C, vitamin E and carotenoids, in protecting the tissue of "oxidative stress" by their action on free radicals often associated with cancer, cardiovascular diseases and inflammation ( Di Lorenzo et al., 2013, Milella et al., 2013). The accumulation of anthocyanins in grapes is recorded when veraison starts, following an upward curve until the maturation, under the influence of climatic factors. Also, it was observed that the anthocyanins biosynthesis can continue after harvest and storage of grapes at low temperature and, practically, postharvest treatments affect the synthesis or degradation of anthocyanins. Table grapes are perishable fruits at room temperature, perish process rapidly evolving, especially considering that they were harvested after registering optimal timing of harvest. Long-term storage is an issue, being recorded weight loss, discoloration, rachis and peduncle dehydration and the skin turgor loss, affecting the fruit quality. The purpose of this study is to evaluate the influence of storage conditions on the quality of vine varieties of table grapes, with special reference to the phenolic profile (polyphenols, flavonoids and anthocyanins total).

## MATERIALS AND METHODS

Plant material. were analyzed grapes belonging to six varieties of table grape-vines genetically related: Biance variety is the maternal genitor for Italia, Xenia and Tamina varieties, Muscat of Hamburg variety is the paternal genitor for the mentioned varieties and Muscat d'Adda was obtained by self-pollination Muscat of Hamburg. Varieties of grape-vines studied are found in the experimental field of the Ampelographic Collection of University of Agronomic Sciences and Veterinary Medicine, Bucharest and they have been conducted on the semi-stem; the type of pruning in the prior year was Guyot on semi-stem, with a load of 42 buds/vine. Varieties were watched during the course of phenological spectrum to ensure the health of crops and at harvest, on an average sample of 10 grape/10 shoots were performed chemical analysis: sugars (g/kg grapes), total acidity (g/l tartaric acid), gluco-acidometric index, polyphenols, flavonoids, anthocyanins fully present in both skin and seeds.

Grape sampling was performed at technological maturity (September 12) which corresponds to the conditions of the vineyard in 2013, the moment when maximum potential phenol was reached. Some of the samples were immediately analyzed, and the other part was stored for one month at refrigerated temperature (T-4<sup>0</sup>C, U%-75%), the tests being carried out after 34 days of harvesting (October 16). After harvesting, the grapes samples were processed immediately, separating skin, pulp and pips from 10 grapes berry/replicate and the phenolic compounds were obtained with an extractant ethanol: water: hydrochloric acid (70: 29: 1, v/v/v), 20 ml per sample. The Folin-Ciocalteu method was used for the determination of the total phenolic (Singleton and Rossi, 1965). The concentration of total phenolic is expressed as the mg gallic acid equivalent per kg grapes. All samples were prepared in triplicate. Total flavonoids content was evaluated according to a colorimetric assay with aluminum chloride.

For the determination of the total flavonoids, a colorimetric method using AlCl<sub>3</sub> was applied for the analysis of the fruit pulp and skin extracts (Zhishen et al., 1999). Catechin was used as the standard for the construction of a calibration curve and the concentrations are expressed as mg catechin /kg grapes. The determination of the total anthocyanins was realized by the method proposed by Di Stefano and Cravero, 1991. The samples were diluted with a solution consisting of 70/29/1 (v/v/v) ethanol/water/HCl (concentrated) and the absorbance was measured at 540 nm. Due to the lack of a malvidin-3-glucoside standard, the total anthocyanins contents are expressed as malvidin-3-glucoside equivalents and calculated using the following equation purposed by Di Stefano and Cravero, 1991. Total acidity and sugar content were made according to the official methods of O.I.V. (1990).

## RESULTS AND DISCUSSIONS

In this study, there was a differential behavior of the six varieties studied in terms of quality and quantity of grape harvest, although they were assigned the same vineyard agrotechnics. For relevant data, the results are based on an average weight of 100 berries, a parameter on which the variety show, in general, a significant influence.

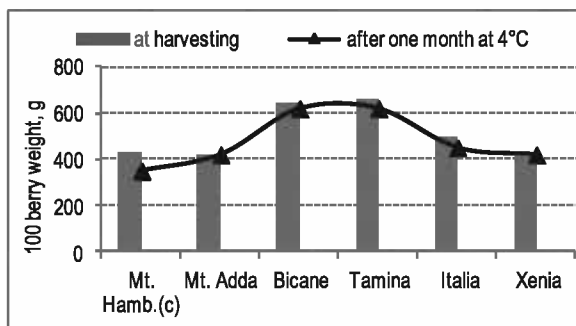


Figure 1 - Evolution of the weight of 100 berries in the experiment

For the refrigerated grapes, there was a decrease in this parameter, but without significant losses. Moreover, varieties Xenia and Muscat d'Adda have preserved skin turgor perfectly, average weight of 100 berries remaining virtually constant (Figure 1).

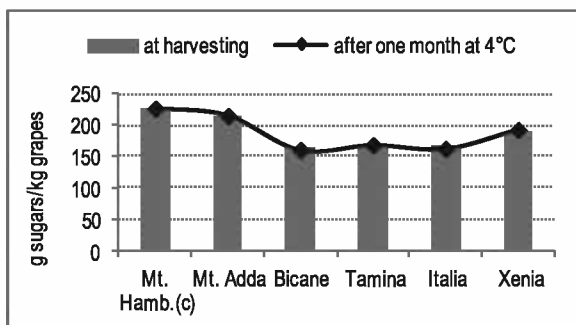


Figure 2 - Evaluation of the sugars content of the grapes varieties tested

Regarding the amount of sugar accumulated, it was noted that only variety Muscat d'Adda with 214 g/kg grapes and variety Xenia 188 g/kg grapes approaches the performance achieved by grape variety witness - Muscat of Hamburg (228 g/kg grapes). After cooling, this parameter knows quantitative changes less, both the reference variety and the other varieties, but unimportant (Figure 2). However, easing of carbohydrates, there was an increase in the total acidity of grape must after storage at 4°C and 75% relative humidity (Figure 3). The exception is Tamina variety which both quality parameters (sugars and titratable acidity) suffer a slight decrease. In general, table grape varieties can be harvested before full maturity, practically, at ripeness depending on gluco-acidometric index. Normally, this index is between 2.5 to 4.5, but in 2012-2013 wine year varieties showed values between 2.88 - 6.88, the highest values were signaled to the varieties Muscat of Hamburg and Muscat d'Adda (6.83 respectively, 6.88). After the storage (Figure 4), the parameter changes, in particular, for the above-mentioned two varieties (from 6.83 to 5.3, respectively 6.88 to 5.9).

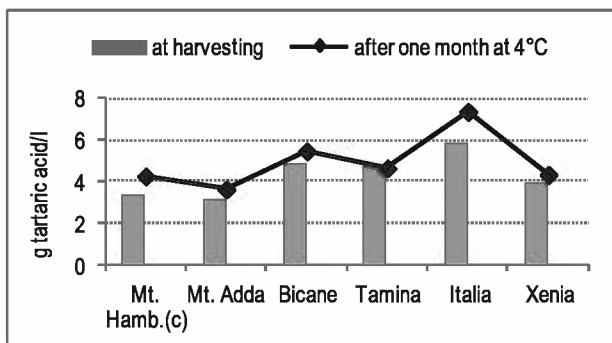


Figure 3 - Evaluation of the titratable acidity content of the grapes varieties tested

Currently, the focus is increasingly on the nutraceutical value of table grapes, due in particular to phenolic compounds accumulated in both the skin and seeds of the grapes. Analysis of the recorded data (Figure 5) showed a high phenolic potential at harvest for varieties of red grapes, polyphenols content being framed between 2.074 g gallic acid/kg of fresh weight (Muscat d'Adda variety) and 0.283 g gallic acid/kg fw (Bicane variety).

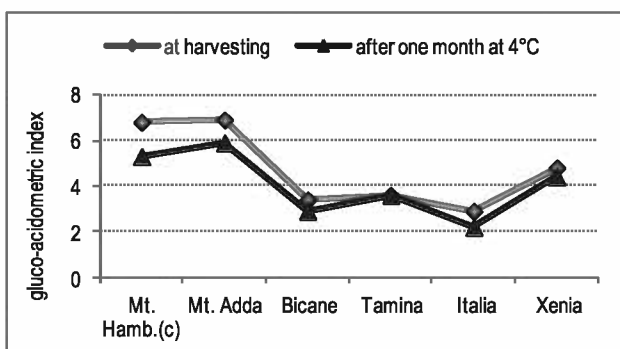


Figure 4 – Gluco-acidometric index variation of varieties tested

It's been noted that the variety Xenia, although white grape variety, has accumulated a remarkable amount of polyphenols, top of recorded both maternal variety, as well as compared with reference variety (paternal variety). Refrigeration grapes for a month led to degradation (oxidation) skin polyphenols of all varieties tested, the stronger has been at the Muscat d'Adda variety (Figure 5).

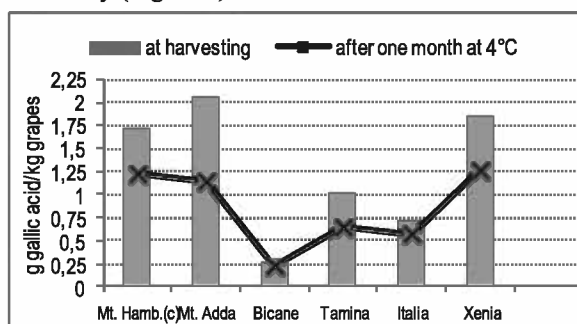


Figure 5 - Refrigeration influence on the content of total polyphenols from grapes

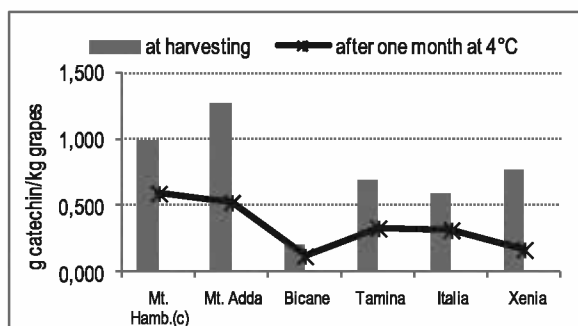


Figure 6 - Refrigeration influence on the content of total flavonoids in grapes

Although the total content of flavonoids in skin showed elevated at harvest (Figure 6), their content was halved after 30 days of storage, both red grapes, as well as those with yellow-green skin.

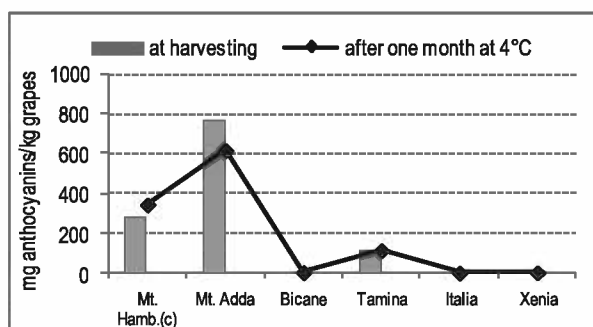


Figure 7 - Refrigeration influence on total anthocyanins content of grapes

At harvest, skin anthocyanins showed a similar trend to that of flavonoids, accumulating in significant amounts in the three varieties with red colored skin (Muscat d'Adda was observed with 764 mg malvidin-3-O glu/kg fw) – (Figure 7).

After a month of freezing, due to physical and chemical changes that occur in fruits after harvest, anthocyanin content in red varieties showed slightly higher values compared to the time of harvest; Muscat d'Adda is the exception that refrigeration for a month produces losses of anthocyanins content, polyphenols and flavonoids.

### CONCLUSIONS

After a storage period of over 30 days at 4°C and relative humidity of 75% phenolic potential was maintained at levels high enough to Muscat d'Adda (2.074 g gallic acid/kg of fw at harvest and 1.138 g gallic acid/kg fw after chilling), Xenia (1,864g gallic acid/ kg fw at harvest, 1.258g gallic acid/kg fw after a month) and the reference variety (1.727 g gallic acid/kg fw at harvest, 1.219 g gallic acid/kg fw after refrigeration).

Anthocyanin biosynthesis during chilling of grapes continued recording higher values compared to the time of harvest, except Muscat d'Adda (764 mg/kg fw, respectively 616.2 mg/kg fw).

The values obtained (polyphenols, flavonoids, anthocyanins) are maintained even after storage, the results also offering clues about nutritional and therapeutic value of these varieties.

We recommend taking Xenia crop varieties and Tamina, for their organoleptic and nutraceutical outstanding qualities, which equal and sometimes exceed that of the variety of parents Bicane and Muscat of Hamburg.

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## STUDY ON THE USE OF CERAMICS IN THE ARCHITECTURE OF PISTON PUMPS USED IN FOOD INDUSTRY

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*Keywords: ceramic, pump, piston flow rate*

### ABSTRACT

*The materials have played an important role in the development of the human kind during history. Some of them, even gave the name of the eras during history, such as The Stone Age, The Bronze Age and The Iron Age. After the industrial revolution iron cast and steel are the main materials used in construction machinery. In recent years, ceramic materials that should be used has always drawn the attention of specialists in engine construction.*

### INTRODUCTION

The first technical ceramic materials were used in 1938 by Herman Wünster for some parts of the hunting Messerschmitt 109 aircraft.

Ceramic materials were later used in the construction of vehicle bodies (Aerospatile, 29Lotus Elite), the fuselage of supersonic (Jaguar) or of the shield of the space shuttle (Colombia) (Burnete et al., 2001).

The first engines with ceramic elements were developed within the project "TACOM", launched and funded by the U.S. Army Tank and Automotive Command in 1975. The purpose of this research program was to produce adiabatic engines for trucks starting motors with a power of about unit 150 kW up to engines, for armoured vehicle over 1000kW. They were carried out under AMMRC (Army Materials and Mechanics Centre).

### MATERIAL AND METHODS

The pump drive speed is a key feature influencing directly the pump: flow, pressure, power and efficiency.

Unlike the centrifugal pumps, the piston pumps have the following advantages: they do not require starting implore; they have higher hydraulic efficiency.

In contrast to centrifugal pumps the piston pumps, have the following disadvantages: they have higher dimensions and mass; they require investment costs and higher operating at the same rate; they have as component parts as reciprocating piston

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valves etc. ; they have uneven flow; the impossibility of direct coupling with the drive motor; the anointing is more complicated (Bálc et al. 2013).

The pump with a single effect is the pump at which the piston has a single active facet which is in contact with the liquid. The opposite side is in contact with the air.

The action of pumping is produced only when the piston is driven in a single direction, the movement in the opposite direction of the piston is open.

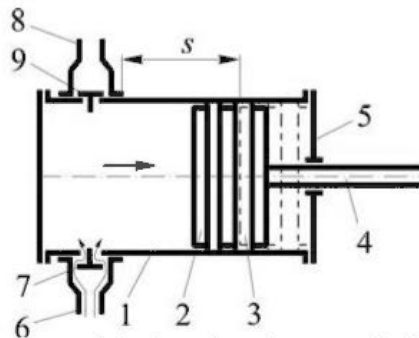


Figure 1. Piston pump of single-acting piston: 1-cylinder; 2-piston; 3-piston rings; 4-piston rod; 5-carter; 6-aspiration connection; 7-aspiration valve; 8-pressure side connection; 9-discharge valve.

During the movement of the piston to the right, the volume between the cylinder and the piston, located in front of the piston is increased thus the negative pressure is achieved.

Due to the depression created, the valve 9 is closed and the valve 7 is open allowing the liquid admission into the cylinder. The liquid will occupy the volume dislocated by the piston 2 by its displacement (Tisan, 2007).

At the movement of the piston to the left, the volume of liquid in the cylinder 1, before the piston is subjected to pressure. The pressure created determines the closure of the suction valve 7 and the opening of the discharge valve 9, allowing the liquid discharge through the nozzle 8. A further movement of the piston to the right will determine again the suction of the fluid in the pump cylinder and the movement of his left discharge.

The piston pump may have different mounting position, depending on the nature of the fluid as its temperature, viscosity, vacuum pressure, or discharge, etc.

The pump cylinder can be made of iron or steel. It can be placed horizontally or vertically. The pump cylinder is usually fixed by pressing it to the pump body. The pump body is made of cast iron, and higher pressures determine their composition to be made of cast steel or forged steel. The caps at the ends of the pump body 9 are fixed to it by means of screws arranged equidistantly. (Tisan, 2007).

## RESULTS AND DISCUSSIONS

This flow rate is theoretically corresponding to an ideal functioning.

$$Q = 60 S A n \left[ \frac{m^3}{h} \right] \quad (1)$$

$$Q = 60 \frac{\pi D^2}{4} S n \left[ \frac{m^3}{h} \right] \quad (2)$$



Where S is stroke A-cylinder surface [ $m^2$ ]; n-crankshaft speed [rpm]; D-cylinder diameter [m]. Real flow rate is:

$$Q_r = \eta Q \left[ \frac{m^3}{h} \right] \quad (3)$$

Where  $\eta$  is volumetric efficiency. Figure 2 show real flow rate, for D = 73 and S = 77 real flow rate is in figure 2.

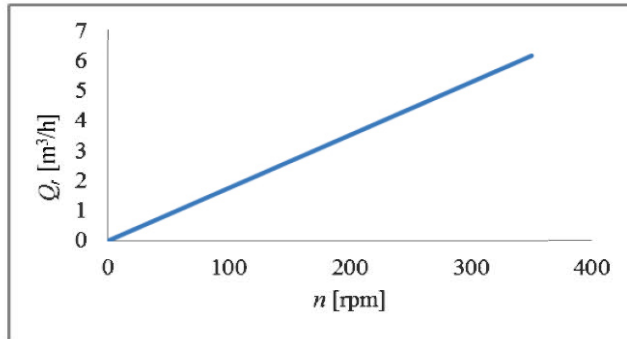


Figure 2. Real flow rate to crankshaft speed

Table 1

Technical characteristics of alumina (Şugar, 2007)

Nr. Crt.	Size	Value	U.M.
1	Aparent porosity	0,4	%
2	Aparent density	$3,5 \cdot 10^3$	$kg/m^3$
3	Resistance to breakege	$410 \cdot 10^6$	$N/m^2$
4	Elasticity mood	$220 \cdot 10^9$	$N/m^2$
5	Liniar expanding coeficient	$7 \cdot 9 \cdot 10^{-6}$	$K^{-1}$
6	Specific heat	790-1050	$J/kg \cdot K$
7	Thermic conductivity	10	$W/m \cdot K$
8	Melting temperature	2327	K
9	Resistance to termic shock	140	$^{\circ}C$
10	Entropia	50,92	$J/mol \cdot K$



Figure 3. Piston with ceramic crown

Our study focused on isolating the piston head with  $Al_2O_3$ . Table 1 show technical characteristics of alumina. Figure 3 show piston with ceramic crown.

### CONCLUSIONS

By using ceramic materials the mass of moving parts is decreasing, thus the inertia decreases as well in the dead spot.

Speed drive, which determines the speed of the piston and consequently the liquid, must take account of its characteristics: viscosity, density, temperature, pressure.

Usually this type of speed pump system is located around 300 rpm.

Volumetric efficiency takes into account primarily the inertia of the closing - opening the valve, then the leakage around the piston rings because they wear a shirt or cylinder, or even escape.

Piston pump flow is directly proportional to crankshaft speed

By using ceramic materials in construction increases reliability and reduces piston its mass, as a beginning to increase efficiency.

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## STUDY OF THE EVOLUTION OF RED WINES CHARACTERISTICS DURING AGING PROCESSES

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**Keywords:** red wines, aging, antocyanins, chromatography, color

### ABSTRACT

*This paper presents the phases of evolution of red wine and, in the same time, how much the physic and chemical characteristics of the wine, together with the chromatic ones, are influenced during the aging process. The totality of the anthocyanin content, the total and volatile acidity, as well as the coloring intensity from the beginning up until the end of the aging process were all analyzed and changes were recorded. In the begging of the aging process, red wines are more acid and the intensity of their color is lower. The younger the wines are, the higher the anthocyanin levels are, followed by a sudden drop of them in the wines that are 6 months older. The shade of the color varies from red-bluish or purple in young wines and tile red in older wines, presenting a higher absorbance at a value of 520 nm, rather than the one measured at the value of 420 nm. The absorbance values dropped at  $\lambda=520$ , accompanied by an increase of the absorbance at  $\lambda=420$  and 620 nm.*

### INTRODUCTION

Wine is one of the oldest beverages known by man. From all the fermented alcoholic beverages, wine is the only one that earned its reputation due to its attractive taste, the benefic effects over the human organism, getting a religious aura, ceremonious even and acknowledging its cultural valences (Mihai Macici, 2008).

The criteria that decides if a wine can be aged or not is various. The pH is one of these. Wines that have low pH values behave better during the aging process. Tannins and sugars also help the aging process. The proportion between sugars, acidity, phenols and water is decisive in the way in which the wine will behave in the aging process. Wine do not have only taste, but also fame. A famous wine will acquire more value throughout aging. Old wine is better. But for an old wine to be good, there is a set of conditions that need to be met. Not any wine can be aged. There are wines that need to be drank in maximum 2 years after bottling. After this time has passed the wine will start losing some of its properties of start developing defects (Rybureau Gayon, 2012).

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Quality is the basic asset of wines. Through wine quality it can be understood the whole of its agreeable assets, the measure in which the wine can be up to the levels of sensorial demands of the competent consumer (Mihai Macici, 2008).

In red wines the robustness and roundness are most appreciated and the lack of asperities, due to a high content of tannins and acidity. A condition in the quality of great red wines consists in aging in a container about 1-2 years. Aging wines in glass bottles contributes to the completeness of their quality.

A wine quality indicator represents the extract formed from what it remains after the volatile substances of the wine evaporates (water, alcohol, phenol esters, volatile acids etc). The extract offers the wine amplitude, corpulence, personality. Another important quality is represented by the color of the wine. Originating in the grapes, monomeric anthocyanins in young red wines contribute the majority of color and the supposed beneficial health effects related to their consumption, and as such they are recognized as one of the most important groups of phenolic metabolites in red wines (Cotea V. D., 2010).

### MATERIAL AND METHODS

We studied four types of wine made from red grapes varieties Burgund, Merlot, Cabernet Sauvignon and Feteasca Neagră, kept in glass bottles for 6 months and we monitored the evolution of their characteristics in different stages of aging at moment 0, 2 and 6 month after wine making process.

It was used in research investigation the following protocols:

- a) *Total acidity* ( $H_2SO_4$  gr.l<sup>-1</sup>) OIV-MA-AS313-01: The method consists in titration with NaOH in presence of red phenol as indicator, until the color turns pink
- b) *Volatile acidity* ( $CH_3COOH$  gr.l<sup>-1</sup>) OIV-MA-AS313-02 The method consists in training the volatile acid by titration with NaOH of the wine acidified with sulfuric acid in the presence of metal orange indicator up to pH=2,7
- c) *Total* ( $SO_2$  mg.l<sup>-1</sup>) OIV-MA-AS323-04B: Sulfur dioxide is determined by direct titration with iodine. The combined sulfur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulfur dioxide, it gives the total sulfur dioxide. Total sulfur dioxide is defined as the total of all the various forms of sulfur dioxide present in the wine, either in the free state or combined with their constituents.
- d) *Free  $SO_2$*  (mg.l<sup>-1</sup>) OIV-MA-AS323-04B Free sulfur dioxide is defined as the sulfur dioxide present in the must or wine in the following forms:  $H_2SO_3$ ,  $HSO_3^-$ , whose equilibrium as a function of pH and temperature.
- e) *The total dry extract from red wine*: The total dry extract means the total non-volatile content of dissolved or colloidal dispersed in wine, obtained by evaporation of the water at boiling point (M. Gheorghită, 1982).
- f) *Sugar content by Schoorl method*: Method consists in treating dealcoholized wine (by evaporation on a water steam bath up to a half of the initial volume) with a solution of basic lead acetate in order to precipitate protein substances, pectin, gums and mucilage and filtering the precipitate.
- g) *The determination of chromatic parameters by Glories method*: The method involves measuring the absorbance at wavelengths 520, 420 respectively 620 nm for determinate the composition on red, yellow and blue pigments of red wines.
- h) *The spectrophotometric determination of red anthocyanin substances* Anthocyanins, also known as anthocyanins, are water soluble flavonoid pigments that, depending on pH, and in some cases complexing agents, can contribute diverse colors such as red, purple and blue (Fei He et al, 2012). The method consist of direct measurement (at spectrophotometer) of

optical density of the solutions in 1 cm dish at a wavelength of 520 nm. The reference fluid will always be distilled water.

## RESULTS AND DISCUSSIONS

Figure 1 presents the variation of the total acidity of the four types of wine during the aging process. We can observe a more intense decrease in the first two months of aging, followed by a slight decrease in the 6<sup>th</sup> month of aging process. This decrease is due to the insolubility of the tartaric acid under the shape of potassium tartrate and neutral calcium tartrate. Another cause may be the transformation of the malic acid into lactic acid, by fermentation.

In figure 2 we can observe a continuous growth of the volatile acidity due to forming of the acetic acid through ethanol oxidation on a chemical and enzymatic way under the action of pathogen microorganisms. The volatile acidity also grows due to the presence of ethyl ethers of acetic acid, formed by metabolic processes of acetic bacteria. Lactic bacteria decompose residual sugars, tartaric acid and glycerol.

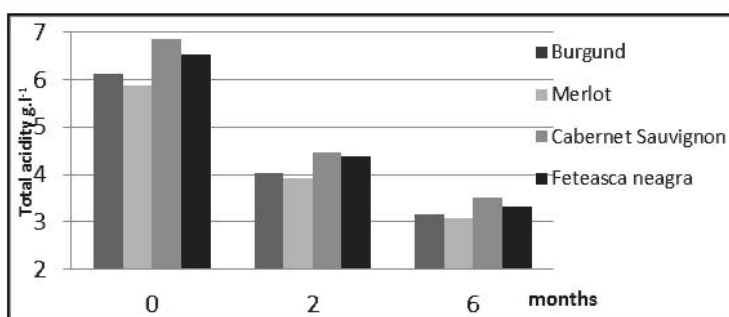


Figure 1 Evolution of total acidity of the four types of wines in various stages of aging

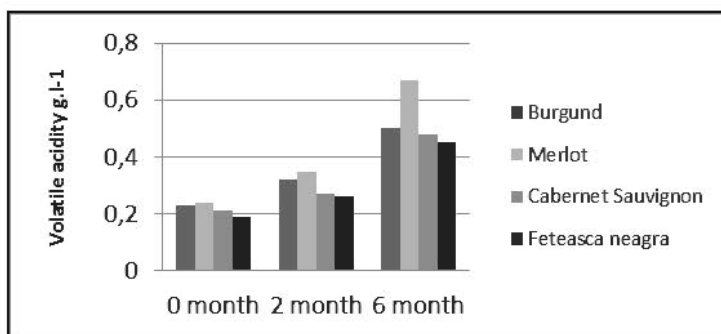


Figure 2 Evolution of total acidity of the four types of wines in various stages of aging

In table 1 is presented the variation of total dry extract of wines through aging processes. Throughout aging, the extract content shrinks somewhat, but the shrinking is not significant, this due to tartaric salts precipitation, condense and residence of phenolic compounds and partial coagulation of protean substances.

Figure 3 presents the variation of color intensity in wines. It may be observed that, through aging, absorbance values at  $\lambda=520$  nm drop, followed by the increase of absorbance at  $\lambda=420$  nm and 620 nm. This phenomenon occurs because of monomeric anthocyanin transformation into polymeric anthocyanin.

In the same time can be observed that, through aging the chromatic structure also modifies, due to color stabilization phenomenon. During wine aging process, the percent of yellow pigments grow and the percent of red pigments decreases, but both pigment categories are more balanced in wines aged to 2 and 6 month. Blue pigment class participate in coloring red wines in a smaller deal (between intervals 2.66 – 3.25 for Burgund, 1.9 – 2.72 for Merlot, 2.7 – 3.18 for Cabernet Sauvignon, 2.68 – 3.23 for Fetească Neagră. Red pigments are predominant both in moment 0 and as well in 2 and 6 month aging. The biggest percent of red pigments was recorded in Merlot red wine (67.59%). Yellow pigment class participates with less than 45% in red wine coloring.

Table 1

Total dry extract content in wines during aging process

Type of wine	Extract (g)	The total dry extract(g.l <sup>-1</sup> )
Burgund 0 month	1.407	28.14
Burgund 2 month	1.056	21.12
Burgund 6 month	0.965	19.21
Merlot 0 month	1.315	26.3
Merlot 2 month	0.9801	19.6
Merlot 6 month	0.949	18.98
Cabernet 0 month	1.6595	33.19
Cabernet 2 month	1.1525	23.05
Cabernet 6 month	1.011	20.22
Feteasca 0 month	1.541	30.82
Feteasca 2 month	1.085	21.7
Feteasca 6 month	0.982	19.64

Table 2

Results of chromatic parameters of red wines

Type of wine	I	T	Chromatic structure		
			% Yellow pigments	% Red pigments	% Blue pigments
Burgund 0 month	0.27	0.60	36.59	61.15	2.66
Burgund 6 month	0.26	0.69	39.65	56.73	2.88
Burgund 12 month	0.24	0.85	40.21	53.29	3.25
Merlot 0 month	0.22	0.43	29.27	67.59	1.90
Merlot 6 month	0.20	0.59	38.60	61.55	2.3
Merlot 12 month	0.18	0.84	45.39	54.05	2.72
Cabernet 0 month	0.30	0.62	36.93	59.13	2.7
Cabernet 2 month	0.28	0.73	40.43	55.29	2.96
Cabernet 6 month	0.27	0.84	43.44	51.93	3.18
Feteasca 0 month	0.25	0.65	38.28	59.08	2.68
Feteasca 2 month	0.24	0.75	40.88	54.79	2.88
Feteasca 6 month	0.22	0.87	46.18	53.23	3.23

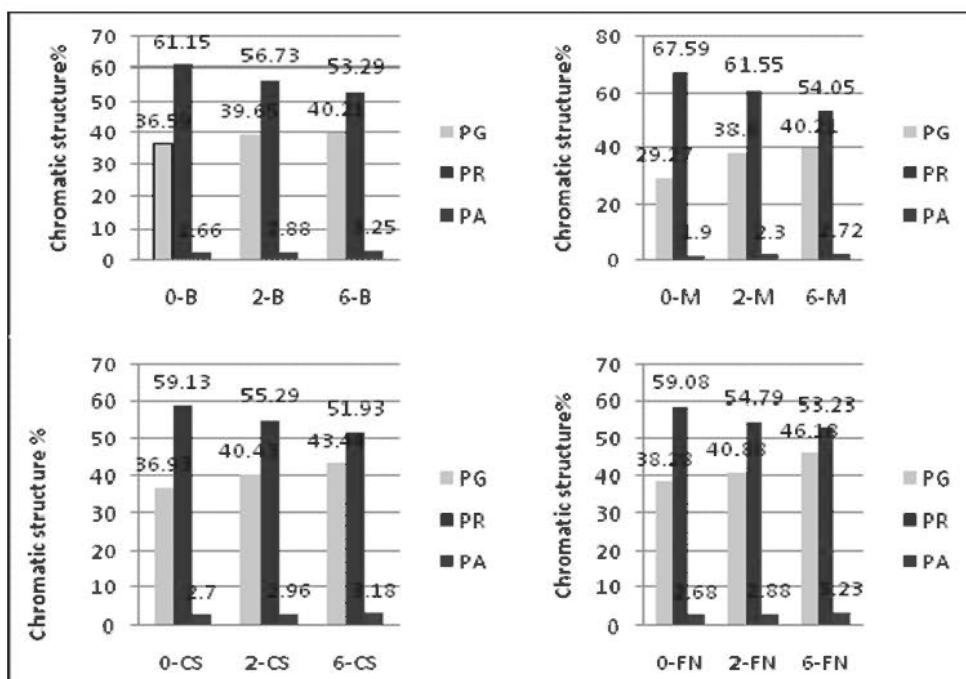


Figure 3. Evolution of chromatic structure of the four types of wines in various stages of aging (pg – yellow pigments, pr – red pigments, pa – blue pigments, M-Merlot, B-Burgund, CS-Cabernet Sauvignon, FN-Fetească Neagră)

In table 3 may be observed of anthocyanin content in red wines during the aging process. The anthocyanin content decreases slowly. Small contents of anthocyanin in aged red wines show that these compounds participate only in a small manner in coloring the wines. Their disappearance contributes to reduction in the wine's color, thus it is a disadvantage.

Table 3

Wine color structure evolution during aging process

Type of wine	Density at pH=0.6	Density at pH=3.5	$\Delta\epsilon$	Anthocyanin content (mg.l-1)
0-B	1.3087	0.8815	0.4272	165.06
2-B	1.1492	0.7501	0.3991	154.25
6-B	0.9788	0.6344	0.3444	133.28
0-M	1.1662	0.6991	0.4671	180.40
2-M	1.0034	0.5813	0.4221	163.09
6-M	0.8146	0.4513	0.3633	140.49
0-CS	1.5077	0.9661	0.5416	209.04
2-CS	1.3884	0.8891	0.4993	192.78
6-CS	1.2252	0.7835	0.4417	169.85
0-FN	1.257	0.8551	0.4019	155.32
2-FN	1.1347	0.7661	0.3686	142.52
6-FN	0.9314	0.6062	0.3252	125.95

## CONCLUSIONS

During wine aging over the length of 6 months there were a lot of processes that took place and modified the composition characteristic and organoleptic assets of the wine.

There were recorded changes of some measurements and physic-chemical indicators, like: total acidity, volatile acidity and extract content. Also modification took place in the level of chromatic structure of the red wines subjected to analysis.

It was determined that the wine keeping time is closely related with its composition and preserving conditions.

Through aging, the total acidity of wines gradually decreases. In the first two months of aging it can be observed a more significant decrease of acidity followed by a lighter decrease in 6 month aging. This decrease is due to the insolubility of the tartaric acid under the shape of potassium tartrate and neutral calcium tartrate under the influence of alcohol which is formed during fermentation processes. The decrease of acidity also takes place because of malic acid transformation into lactic acid, throughout malolactic fermentation and esterification processes.

The highest values of total acidity were recorded at 0 moment, especially for Cabernet Sauvignon red wine ( $6.85 \text{ g.l}^{-1}$ ), and the lowest values were observed at Merlot red wine, 6 months of aging ( $3.07 \text{ g.l}^{-1}$ ).

In the begging, the volatile acidity of red wines remains approximatively constant, followed by, after 6 months of aging, a sudden increase of it, due to acetic acid forming throughout ethanol oxidation, on chemical and enzymatic way, under the action of pathogen microorganisms. Volatile acidity grows also due to the presence of ethyl ethers of the acetic acid, formed in metabolic processes by acetic bacteria. Lactic bacteria decompose residual sugars, tartaric acid and glycerol.

The lowest level of volatile acidity is found in Fetească Neagră red wine at the beginning of aging process ( $0.19 \text{ g.l}^{-1}$ ), the highest values being recorded after 6 months of wine aging, especially in Merlot red wine ( $0.67 \text{ g.l}^{-1}$ ).

Through aging, the extract content shrinks somewhat, but the shrinking is not significant, this due to tartaric salts precipitation, condense and residence of phenolic compounds and partial coagulation of protean substances. The highest content of extract can be observed in Cabernet Sauvignon red wine ( $33.13 \text{ g.l}^{-1}$ ) and the lowest content was found in Merlot red wine ( $18.98 \text{ g.l}^{-1}$ ).

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## TECHNO-ECONOMIC EFFICIENCY OF SOME APRICOTS AND APPLES CULTIVARS IN THE MOARA DOMNEASCA FARM CONDITIONS

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*Keywords: production cost, economic efficiency, profit, rate of profitability*

### ABSTRACT

*Determination of economic efficiency of apricot studied cultivars 'Dacia', 'Tudor', 'Comandor' and apples ones: 'Romus 3', 'Generos' and 'Jonathan' starts from the following considered three experimental factors: cultivar (a), irrigation system (b) and fertilization (c). From the analysis of orchard operating costs where the cultivars above were tested, it result differences in production costs (lei/ha). Average sales prices were different between the cultivars studied, so the summer apple cultivar 'Romus 3' had an average price of 2,000 lei/t to 1,500 lei as 'Jonathan' and 'Geneos' cultivars had generated. The apricot 'Dacia' cultivar's sales price was of 2000 lei/t comparing to 'Tudor' and 'Comandor' who encountered only 1,500 lei/t., too.*

### INTRODUCTION

Making a modern fruit grow cannot be conceived without ensuring adequate hydric needs for fruit species alongside fertilization. Even in areas where 600-700 mm annual rainfall is recorded, there is a need to cover water deficiency during July-September, or sometimes in March and April, before and during flowering and fall in October, during the intense growth of roots. Irrigation with a drip system uses less water than sprinkler irrigation (Proebsting, 1994). The method of localized irrigation yielded a apricot fruit production increase of 11.06 kg in comparison with the method of micro irrigation with sprinkler which ensured a growth of 5.18 kg, both methods using 1m<sup>3</sup> of water (Iancu M., Leinar Septar, 2009).

Foliar fertilization is necessary to create a favorable nutrient medium in order to obtain high yields of profitable indicators of quality. Foliar fertilization with 0.1% Cropmax provided a significant increase in production while for 'Idared' variety a 6.2 t/ha (Gradinariu G., 2004).

Recent research on apricot and apple, in different experimental combinations of water regime and foliar fertilization levels showed significant increases in fruit production (t/ha) with the experimental factors studies underlying this thesis (Jeni Veronica Tiu et al., 2014).

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Positive results from economically point of view have been demonstrated in an experimental polifactorial manner (variety, rootstock, density, training system, hydric regime) for apple showed profit of 8357 lei/ha density variations 1666 trees/ha or 13370 lei/ha for versions 2500 trees/ha, higher than that achieved by the comparative technology (1250 trees/ha) with 5032 lei/ha in the first version of density and 10145 lei/ha in the second one (Căpraru F., 2010).

Similar results emerge from a study on the application of drip irrigation and microspraying located at various watering rate, the most effective being dripping version using of "Tack" dropper followed by variants with "Tipaz" dropper next by microspraying type with flow of 12 l/h and microspraying flow of 27 l/h (Septar Leinar, 2008).

### MATERIAL AND METHODS

The experimental plot was located in the Didactical Farm Station Belciugatele, didactical farm Moara Domneasca and included the study of two species: apricot grafted on Mirobolan and apple grafted on M9. The locality place is included in the Romanian Plain relief subdivision Vlăsiei, in the transition from steppe to forest area. The general relief is flat, with small bumps, and numerous depressions called dales, shapes and sizes. Groundwater is at depths of 6 to 10 m. Accumulated rainfall was 318.21 l/m<sup>2</sup> in 2011 and 401 l/m<sup>2</sup> in 2012 under the multiannual average 610.91 l/m<sup>2</sup> (1960-2010). The soil that was founded typical reddish preluvosoil where the experiments began (according SRTS-2012 Chrome Luvisol according to WRB-ST-1998).

The plantation was founded in 2004, planting distances 5 x 4m to apricot and 4 x 3,5m to apple. The experience was one trifactorial: factor A = variety (apricot: a1 – 'Dacia', a2 – 'Comandor', a3 – 'Tudor'; apple: a1-'Romus 3', a2-'Generos', a3-'Jonathan'), factor B = hydric regime (b1-unirrigated Control (Ct), b2- drip irrigation 4l/h) and factor C = foliar fertilizer (c1-unfertilized Control (Ct), c2-fertilized Cropmax 0.1%), placed after the linear method with 5 trees/variant.

Economic quantification of experimental results has been performed from prices and tariffs for the year 2012 in order to be conclusive. The production of fruit t/ha was calculated as the average of the years 2011-2012, starting from the average production (kg/tree) experimental variants carried out in repetition. In the study of economic calculation were stated the technological records for each culture and differentiated for each experimental variant (a - variety, b - hydric regime and c - level of fertilization) and were determined following economic factors: cost, income, profit and the rate of profitability. Specific culture technology for the two species (apricot and apple) was uniformly applied regarding: maintenance of trees (thinning and fructification pruning, gathering resulted branches), maintenance of the grass intervals, tillage and the phytosanitary treatments. Production costs (lei/ha) were calculated from the total expenditure/ha divided by the amount of fruit achieved. Compared to the cost price realized of total expenditure/ha, production unit selling price was different depending on the age of maturing varieties resulting income, profit (the difference between income and expenditure) and the percentage rate of profitability.

### RESULTS AND DISCUSSIONS

Higher fruit yields (t/ha) were recorded in the experimental variant b2c2 (irrigated + fertilized) with significant differences statistically assured in between 'Dacia' and 'Comandor' varieties comparing to variety 'Tudor' and variant b2c1 (irrigated and non fertilized) in the same order.

The experimental variant b1c1 Mt (non irrigated and non fertilized), although production was lower, 'Comandor' variety responded better to the experimental analyzed factors (Figure 2), ensuring significant differences from other varieties. Although fruit production gradually increased with the administration of the experimental factors, differences in fruit production (t/ha) between experimental variants b1c1 Mt (non irrigated + non fertilized) and b2c2 (irrigated + fertilized) are between 3.89 t/ha variety 'Comandor', 3.9 t/ha variety 'Tudor' and 4.75 t/ha variety 'Dacia'.



Figure 1. Determination on fruit production in experimental plot, 2012

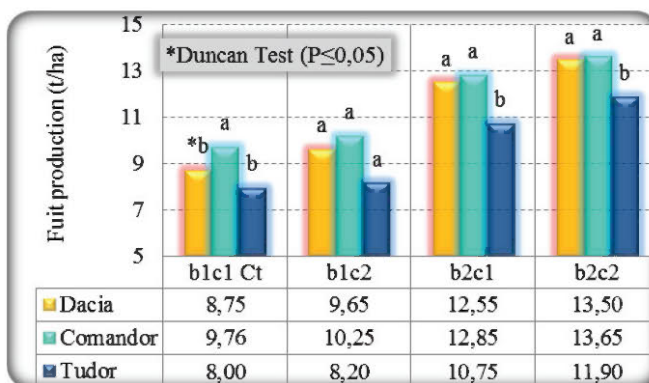


Figure 2. Influence of the apricot variety on fruit production by water and fertilizer regime

From the analysis of total expenses, results that irrigation raise the costs incurred on the part of the cost of irrigation, but also its maintenance and pumping. Total expenditure (lei/ha) are influenced by the work of collecting and sorting of fruit production. As much as production is higher, the specific costs are higher harvesting operation and therefore total expenditure. Significant differences emerged irrigated variants (b2c1) and irrigated + fertilized (b2c2) between 'Dacia' and 'Comandor' varieties comparative with 'Tudor' (Figure 3).

Cost price (lei/ha) resulted in total expenditure (lei/ha) / fruit production (t/ha) was different in the three species by applying experimental variants. Thus, differences in

production costs in the frame of applied variants are found in variety 'Comandor' 35 lei / ha, followed by the 'Dacia' with 124 lei/ha and 164 lei/ha for 'Tudor' (Table 1).

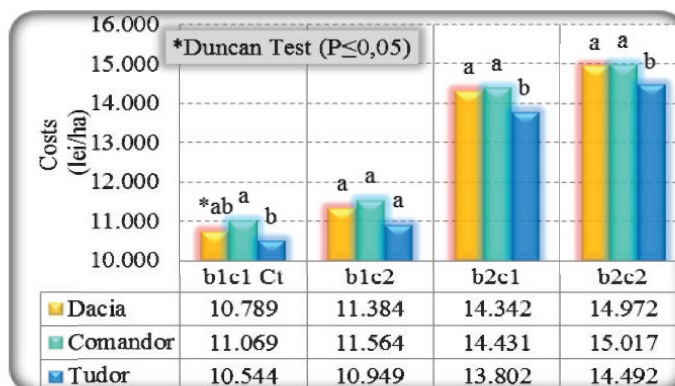


Figure 3. Influence of the apricot variety on costs by water and fertilizer regime

Table 1  
Cost price (lei/ha) for experimental variants at apricot

Dacia (a1)				Comandor (a2)				Tudor (a3)			
b1c1 Ct	b1c2	b2c1	b2c2	b1c1 Ct	b1c2	b2c1	b2c2	b1c1 Ct	b1c2	b2c1	b2c2
1233	1179	1142	1109	1135	1128	1123	1100	1381	1335	1283	1217

When calculating incomes, it should be noted that the average selling price of apricots was different depending on earliness, knowing that fruit earlier or late have the best price for sale. The average selling price for 'Dacia' variety was of 2.0 lei/kg, and for 'Comandor' and 'Tudor' was of 1.5 lei/kg. The highest incomes has made 'Dacia' variety depending on experimental factors gradual application, providing significant differences from the other two varieties separately (Figure 4). The biggest increase in income was 9500 lei/ha between control (b1c1Ct) and irrigated + fertilized variant (b2c2) variety 'Dacia', and 5850 lei/ha varieties 'Comandor' and 'Tudor' in the same variants (Figure 4).

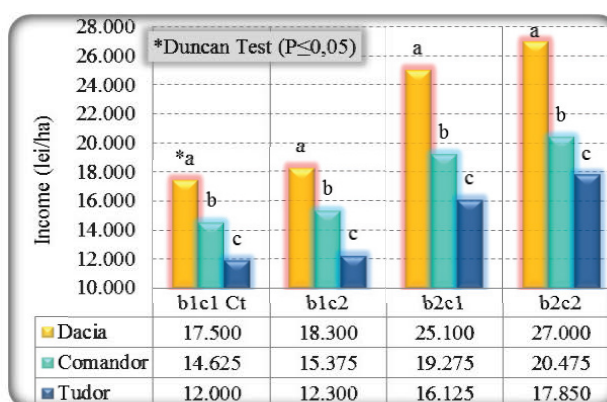


Figure 4. Influence of the apricot variety on income by water and fertilizer regime

The analysis of profit recorded emphasize that 'Dacia' was the most reliable variety in all experimental variants studied, the increases being progressive and distinctly significant (Figure 5), reaching almost double the sum to the control (b1c1Ct) and irrigated and fertilized variant (b2c2).

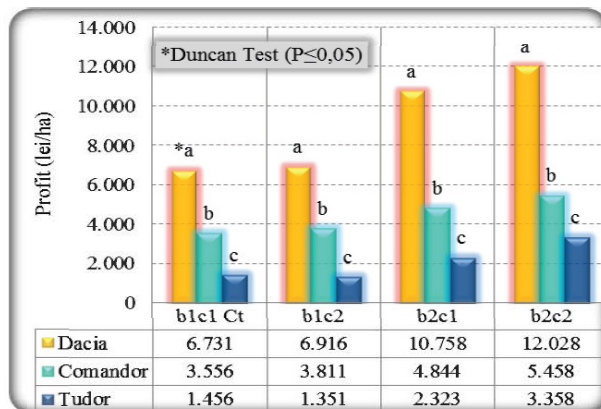


Figure 5. Influence of the apricot variety on profit by water and fertilizer regime

The return rate is a synthetic indicator, which in economic terms helps to delimited data results for variations. It expresses the true efficient use of resources. Rate of return distinct significant had 'Dacia' variety comparative to the other two varieties (Figure 6). But b1c2 variant (non irrigated + fertilized) return rate was less than or nearly equal to all varieties than to the control (b1c1Ct).

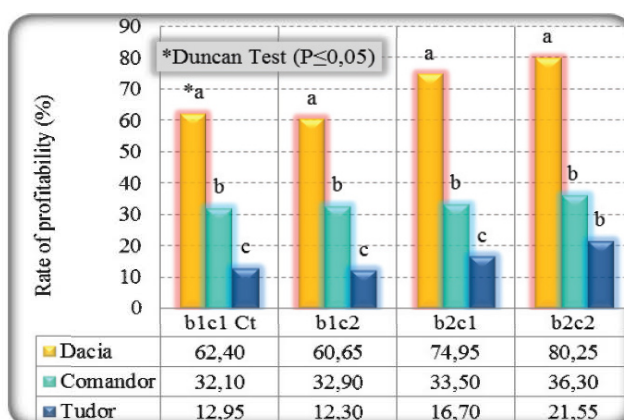


Figure 6. Influence of the apricot variety on rate profitability by water and fertilizer regime

Fruit production (t/ha) obtained at apple rich as applying fertilization and water regime, the most productive variety is 'Generos', which recorded in plus 8 t/ha and the variety 'Romus 3' which doubled his production of 9.0 t/ha from the control variant (b1c1Ct) to 18.3 t/ha in irrigated and fertilized variant (b2c2) (Figure 7). Significant differences appear in version control (b1c1Ct) and irrigated and fertilized variant (b2c2) (Figure 7).



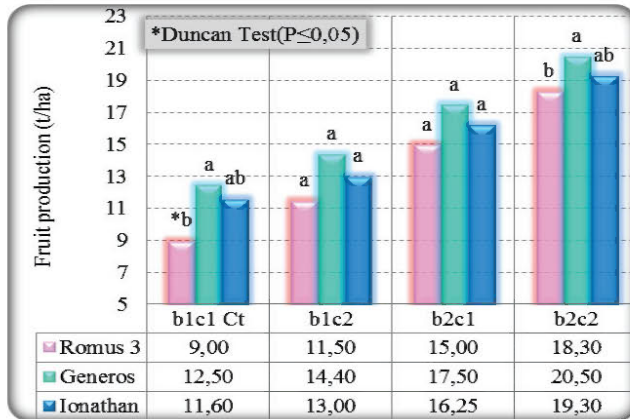


Figure 7. Influence of apple variety on fruit production by water and fertilizer regime

Among the three varieties studied, the variety 'Romus 3' has the smallest total expenditure on all experimental variants, followed by 'Jonathan' and little difference by 'Generos', significant differences in the control variant (b1c1Ct) and irrigated + fertilized (b2c2) (Figure 8).

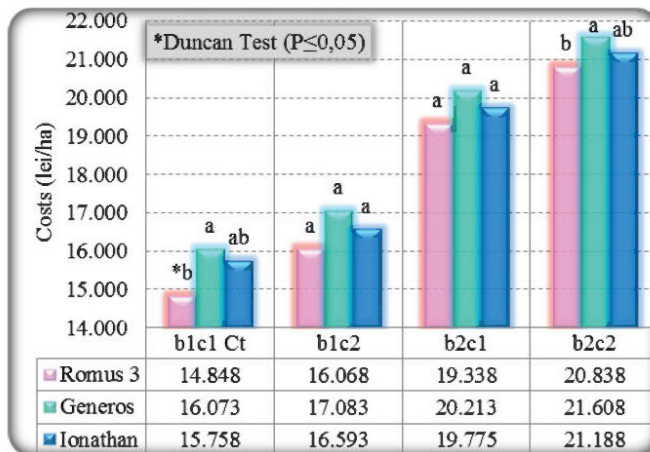


Figure 8. Influence of apple variety on costs by water and fertilizer regime

The highest price of the cost (lei/ha) calculated from experimental variants emerged in b1c1Ct version that dropped with fertilization application (b1c2), irrigation (b2c1) and irrigation + fertilization (b2c2). The largest differences between experimental factors visible variety 'Romus 3' with 511 lei/ha, 'Jonathan' with 261 lei/ha and 'Generos' with 231 lei/ha (Table 2).

Large income (lei/ha) assured at statistically level of 0.05% achieved 'Romus 3' variety in the version irrigated + fertilized (36600 lei/ha) more than the other two varieties (Figure 8) the differences of 5850 lei/ha compared to 'Generos' and 7650 lei/ha to 'Jonathan' (Figure 9), were due mainly to the sale price of the fruit of 2.0 lei/kg comparative to 1.5 lei/kg for 'Generos' and 'Jonathan' varieties justified by the fruit earliness.

Table 2

Cost price (lei/ha) for experimental variants at apple

Romus 3 (a1)				Generos (a2)				Ionathan (a3)			
b1c1 Ct	b1c2	b2c1	b2c2	b1c1 Ct	b1c2	b2c1	b2c2	b1c1 Ct	b1c2	b2c1	b2c2
1649	1397	1289	1138	1285	1186	1155	1054	1358	1276	1216	1097

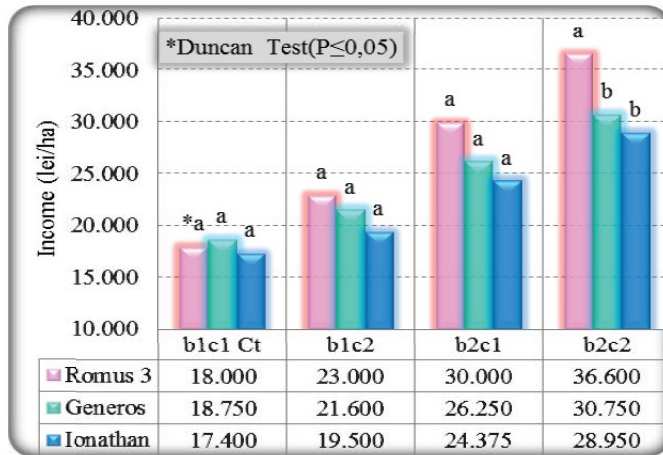


Figure 9. Influence of apple variety on income by water and fertilizer regime

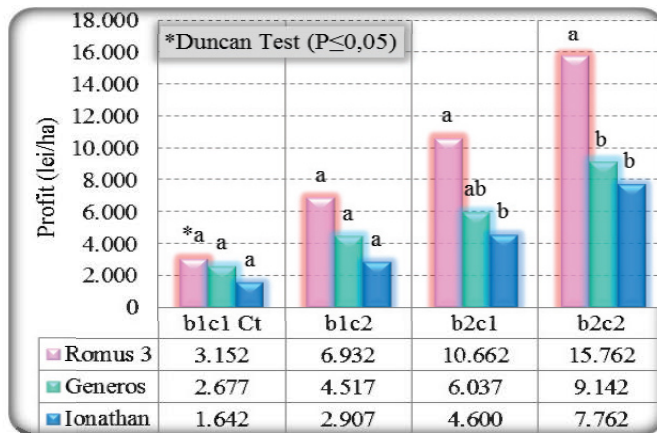


Figure 10. Influence of apple variety on profit by water and fertilizer regime

The most substantial profit (lei/ha) had registered variety 'Romus 3' - 15762 lei/ha with 12610 lei/ha more than the control variant (b1c1Ct), positive significance than the other varieties. The significant difference presents the same variety also in b2c1 version (irrigation+nonfertilized) (Figure 10).

Significant rate of return is obtained only in irrigated and non fertilized variant (b2c1) with the percentage of 20.9% and irrigated + fertilized variant (b2c2) with 75.6% for 'Romus 3' variety. Other varieties are profitable in smaller percentages, ranging from 16.6% (b1c1) to 42.5% (b2c2) for the 'Generos' variety and from 10.35% (b1c1) to 36.6% (b2c2) for 'Jonathan' (Figure 11).

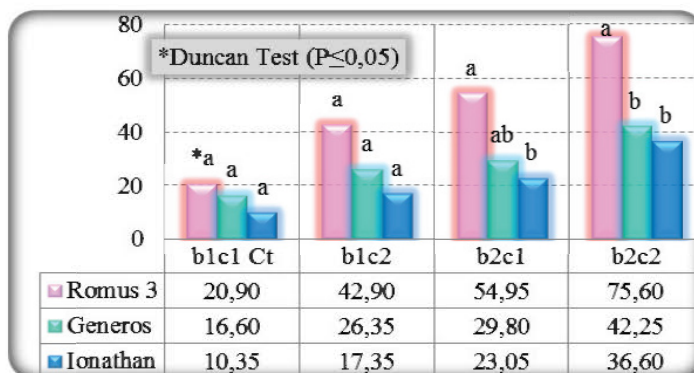


Figure 11. Influence of apple variety on rate of return by water and fertilizer regime

Table 3  
Values of simple correlation coefficients between specific elements of economic analysis at the apricot

Item	Fruit production (t/ha)	Income (lei/ha)	Costs (lei/ha)	Profit (lei/ha)	Rate of profitability (%)
Fruit production (t/ha)	1	0,819(**)	0,947(**)	0,585(**)	0,416(*)
Income (lei/ha)	0,819(**)	1	0,750(**)	0,934(**)	0,832(**)
Costs (lei/ha)	0,947(**)	0,750(**)	1	0,464(*)	0,268
Profit (lei/ha)	0,585(**)	0,934(**)	0,464(*)	1	0,969(**)
Rate of profitability (%)	0,416(*)	0,832(**)	0,268	0,969(**)	1

\*\* Correlation is significant at the 0.01 level

In the two species studied in this paper (apricot and apple) it were performed a series of correlations between items on the economic efficiency with respect to: fruit production (t/ha), income (lei/ha), expenses (lei/ha), profit (lei/ha) and the rate of profitability (%). The apricot is found significant correlations at a level of 0.01% between almost all the economic analysis, as follows: fruit production is correlated with income (lei/ha) with correlation coefficient of 0.819(\*\*), total costs (lei/ha) 0.947(\*\*) and profit (lei/ha) 0.585(\*\*). Weaker correlation exists between fruit production (t/ha) and the rate of profitability by the coefficient 0.416 (\*). Income (lei/ ha) significantly correlates with all elements analyzed: total costs 0.750(\*\*), with profit 0.934(\*\*) and the rate of profitability 0.832(\*\*) (Table 3). Strong significaton is between profit and rate of profitability by the existence of a high coefficient of correlation of 0.969 (\*\*). Weak correlation is with gross total costs (0.464\*) but also with the rate of profitability (0.268) (Table 3).



Table 4

Values of simple correlation coefficients between specific elements of economic analysis at the apple

Item	Fruit production (t/ha)	Income (lei/ha)	Costs (lei/ha)	Profit (lei/ha)	Rate of profitability (%)
Fruit production (t/ha)	1	0,828(**)	0,970(**)	0,651(**)	0,549(**)
Income (lei/ha)	0,828(**)	1	0,875(**)	0,959(**)	0,910(**)
Costs (lei/ha)	0,970(**)	0,875(**)	1	0,703(**)	0,601(**)
Profit (lei/ha)	0,651(**)	0,959(**)	0,703(**)	1	0,987(**)
Rate of profitability (%)	0,549(**)	0,910(**)	0,601(**)	0,987(**)	1

\*\* Correlation is significant at the 0.01 level

From table 4 results that for apple, all the economical analysis significantly correlate with each other. Lower values of simple correlation coefficients (r), although there are falling significantly stronger as the apricot between fruit production and the rate of profitability (0.549 \*\*) and between total costs and the rate of profitability (0.601 \*\*) (table 4).

### CONCLUSIONS

Fruit production gradually increased with the administration of the experimental factors, resulting in significant differences statistically assured. The apricot differences in fruit production (t/ha) between experimental variants b1c1Ct (irrigated + fertilized) and b2c2 (irrigated + fertilized) are between 3.89 t/ha at 'Comandor', 3.9 t/ha at 'Tudor' and 4.75 t/ha at 'Dacia'. Differences between the same experimental variants appear also in apple, the most productive variety is 'Generos', which recorded plus 8 t/ha and the variety 'Romus 3' which doubled his production from 9.0 t/ha to 18.3 t/ha.

Total costs (lei/ha) are directly proportional to fruit production (t/ha).

The higher cost prices (lei/ha) have emerged in b1c1Ct variant that dropped with fertilization application (b1c2), irrigation (b2c1) and irrigation + fertilization (b2c2).

Income (lei/ha) were influenced by the sale price of the fruit, the best price recorded the early varieties, resulting in significant differences in both apricot and apple.

Profit (lei/ha) and the rate of profitability (%) are directly proportional to income (lei/ha)

Variety Dacia is also reliable in standard culture conditions (b1c1Ct) with a rate of return of 62.4%, reaching in terms of irrigation + fertilization (b2c2) at a rate of 80.25%, providing significant differences from the other two varieties. Substantial profit (lei/ha) had variety 'Romus 3' 15762 lei/ha to 12610 lei/ha more than the control variant (b1c1Ct) positive significance than the other varieties, the rate of return of 20.9% in standard conditions (b1c1Ct) and 75.6% in b2c2 version.

There is a strong correlation between fruit production (t/ha) and the following economic indicators: income (lei/ha), total costs (lei/ha) and profit (lei/ha).

Weaker correlation was remarked between fruit production (t/ha) and the rate of return (%).

Correlates closely the income (lei/ha) with total costs (lei/ha), profit (lei/ha) and the rate of profitability (%)

A poor correlation noticed between total costs (lei/ha) and profit (lei/ha) and between total costs (lei/ha) and the rate of profitability (%).

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THE INFLUENCE OF CANOPY MANAGEMENT AND PRUNING LEVEL  
ON CHEMICAL AND SENSORY PROPERTIES OF MERLOT WINES  
FROM SÂMBUREȘTI VINEYARD

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*Keywords: pruning, quality, chemical composition, red wine*

**ABSTRACT**

*This paper presents the results regarding the fact that fruit load, and thus yield, significantly influences the quality Merlot wines, both in terms of compositional and sensory properties. Thus, a large load of buds/m<sup>2</sup>, which translates into high yield (10-12 t/ha), at least two weeks late ripening grapes process, leading to the accumulation of smaller proportions of sugar in the grapes and therefore less drinking wine. The small loads of buds/m<sup>2</sup> lead to low yields (6-7 tons/ha), early maturation of the grapes, the accumulation of large amounts of sugar and alcohol content in wine that can exceed 15% vol. Instead, the accumulation of phenolic compounds in grapes is weaker, the wines are less colorful, so it is necessary to extend the maceration time.*

**INTRODUCTION**

*Vitis vinifera* berry development is characterized by an initial phase where the fruit is small, hard and acidic, followed by a lag phase known as *veraison*. In the final phase, berries become larger, softer and sweeter and accumulate an array of organoleptic compounds (Sweetman et al. 2012). Grape and wine quality is influenced principally by environmental conditions (Pereira et al. 2007). It is acknowledged that a range of environmental constraints may restrict vigor and yield and thereby enhance the winemaking potential of the grape (Van Leeuwen et al. 2007). Unlike most plant crops, especially annuals, wine grape cultivars are often grown best under sub-optimal conditions (Van Leeuwen & Seguin 2006). Seasonal variations in air temperatures had limited effect on pro-anthocyanidins biosynthesized in grape berries (Cohen et al. 2012). The type of soil will impact on the extent of soil water redistribution, which in turn will buffer dehydration in the dry rootzone (Chaves M. et. al., 2010). Sugar accumulation in fruits is highly important for fruit quality because, in addition to imparting sweetness, sugar is the ultimate precursor for most quality-relevant components, such as acids, pigments, tannins, and aroma volatiles (Hall et al. 2011).

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## MATERIAL AND METHODS

The paper is based on the study of the influence of some technical viticultural elements on the quality of Merlot red wines from Sâmburești vineyard. The studied viticultural techniques were the following: canopy management (with two variants spur-pruned cordon and Guyot) and pruning level (with three loads of buds - 9, 12 and 15 buds/m<sup>2</sup>). These canopy management have been applied to cutting Merlot clone 181 grafted on two rootstocks SO4 and 1103 P. The study was conducted in 2013, on a 7-year-old plantation (4–th year of production) from Sâmburești vineyard, located on a plateau with slight exhibition southwest, with reddish-brown forest soil, rich in iron. In the plantation, the vines are distances of 2.2 x 1 m, which results in a density of 4.545 plants of vines/ha. For all 12 experimental variants was applied the same viticultural technique, the grapes were harvested on the same day and vinified at the University of Craiova, Faculty of Agriculture and Horticulture, using micro-vinification. To determine the production output, each vine was weighed (with a number of 20 hubs for each variant). Before the process of winemaking, for each variant was determined the sugar content and total acidity (g/l tartaric acid). The wine quality was appreciated by chemically analyzing the main parameters at the Central Laboratory for Quality Control and Wine Hygiene Valea Călugărească, Craiova Branch. Also sensory characteristics of the wines were determined by two rounds of wine tastings, made at 6 and 12 months by a panel of authorized tasters from the Faculty of Agriculture and Horticulture, Craiova.

## RESULTS AND DISCUSSIONS

Under normal conditions, the vine has an inverse relationship between the quantity and quality of production. The results of the study conducted in 2013 at Sâmburești vineyard on Merlot clone 181 shows that one of the largest technological factor – pruning level, respectively the fruit load – is influencing the performance. The data in Table 1 are relevant in this respect, and show that in both management systems, the load with more eyes (buds) left to cut is leading to an increase in the production of grapes, regardless of how reporting - on the 20 hubs, per hub or per ha - but there is not the same ratio of the production increase as well as the increase of the load.

Table 1

The influence of the management system and the cutting on the production of grapes

Clone/ rootstock	Canopy	Variants	Load/m <sup>2</sup>	Load /plant	kg/20 plants	kg/plant	kg/ha
181/SO <sub>4</sub>	Guyot	V1	9	20	22	1.1	5000
		V2	12	27	36	1.8	8180
		V3	15	34	45	2.25	10226
	Spur- pruned cordon	V1	9	20	20	1	4545
		V2	12	27	34	1.7	7727
		V3	15	34	42	2.1	9545
181/1103 P	Guyot	V1	9	20	28	1.4	6363
		V2	12	27	41	2.05	9317
		V3	15	34	54	2.7	12271
	Spur- pruned cordon	V1	9	20	26	1.3	5908
		V2	12	27	40	2	9090
		V3	15	34	51	2.55	11590

Thus, the lowest load, 9/m<sup>2</sup>, equivalent to 20 buds/hub, is leading to grape production of 1 - 1.4 kg/hub, meaning 4500-6200 kg/ha, that is, obviously, far below the potential of the variety and of the clone, so, economically speaking, this technique is not justified. Increasing fruit load to 12 buds/m<sup>2</sup>, equivalent to 27 buds/vine is leading to an increased production levels of 1.7 to 2.05 kg/vine, respectively from 7700 to 9300 kg/ha, very close to the optimal production potential of the variety and of clone. The highest load, 15 buds/m<sup>2</sup>, which corresponds to 34 buds/hub is leading to high yields of 2.1 to 2.7 kg/hub or 9500-12200 kg/ha, very good from economically point of view, but not recommended if we aim to obtain high quality wines. The data in Table 1 shows that for both combinations clone/rootstock, the canopy management Guyot leads to slightly grape production higher against spur-pruned cordon as canopy management system, regardless of the adopted fruit load. Also, for both types of control systems and for all 3 loads/plant, the production is higher, with 1000 to 2000 kg/ha for the combination of the clone 181 with the rootstock 1103 P, even if the type of the soil on which are located the two combinations is the same. Analysis of data concerning the chemical composition of the wines show an inverse relationship between the level of the production, determined, primarily by the load and the quality parameters of the wine. There are differences in values of the parameters concerning the composition of wines both between the taken combinations clone/rootstock and between management systems and loads of fruit, but the differences between the values of the composition parameter are smaller than the differences in the amount of grape production for the studied variants. The data for the main parameters on the composition, alcoholic concentration, indicate that for both combinations clone/rootstock and management in both systems, the higher alcoholic strength is at the variant with the lowest load of fruits, and it decreases with the increase of the production of grapes. From Table 2 it is noted that the combination of rootstock SO4 with clone 181, with Guyot canopy management system, at a load of 20 buds/vine, it can be obtained half production of grapes comparing with the load of 34 buds/vine; instead, the alcoholic concentration difference is only 0,5%. Also for the second management system, even if production at maximum fruit load is twice the minimum load, alcoholic concentration difference is only 0.9% vol. Situation is similar for the combination of rootstock 1103 P.

Table 2

Chemical composition of wines

Clone/ rootstock	Canopy	Loop/ m <sup>2</sup>	Alcohol, % vol.	Total acidity, g/L tartaric acid	Volatile acidity, g/L acetic acid	Glycerol, g/L	Total dry extract, g/L	Unreduced dry extract, g/L
181/SO <sub>4</sub>	Guyot	9	14.35	5.56	0.52	12.20	30.90	28.58
		12	14.26	5.22	0.45	12.02	30.65	27.71
		15	13.86	5.04	0.55	11.75	29.60	27.04
	Spur- pruned cordon	9	14.28	5.67	0.56	12.18	31.50	29.18
		12	14.23	5.32	0.53	12.06	29.70	27.18
		15	13.41	5.68	0.44	11.48	28.70	26.78
181/1103 P	Guyot	9	13.94	5.80	0.50	11.80	29.80	26.86
		12	13.15	6.21	0.49	11.24	29.00	26.31
		15	13.05	6.42	0.35	11.04	28.30	25.61
	Spur- pruned cordon	9	14.43	5.70	0.45	12.24	30.60	28.04
		12	14.17	6.03	0.40	12.15	30.40	27.14
		15	13.90	5.69	0.50	11.80	30.20	27.39

Regarding rootstocks and canopy management, at Guyot canopy management the alcohol concentration is higher in the wines made from the combination of rootstock SO4 with clone 181, comparing with wines made from the combination of rootstock P 1103, while the production of grapes is lower. In contrast, for spur-pruned cordon management system the alcohol concentration is higher for all the 3 loads of fruit, for the wines made from the combination of rootstock 1003 P with clone 181, even if the grape productions are higher. Regarding other parameters of the composition stands the very high content of wines in glycerol, above 11 g/L and, in some wines, just over 12 g/L. These values are in direct relation with high alcohol concentrations and are quite important because they are influencing the taste of wine, having an important role in reducing the sensation of astringency and perception of volume sensation. Lowest glycerol content is 11.04 g/L, in the variant which presented the lowest alcohol concentration (13.15% vol.), belonging to the combination 181/11003 P, Guyot management system, 34 eyes/hub and the largest glycerol content was 12.24 g/L, at the variant which presented the highest alcohol strength, belonging to the same combination, hut for spur-pruned cordon canopy management, 20 eyes/hub.

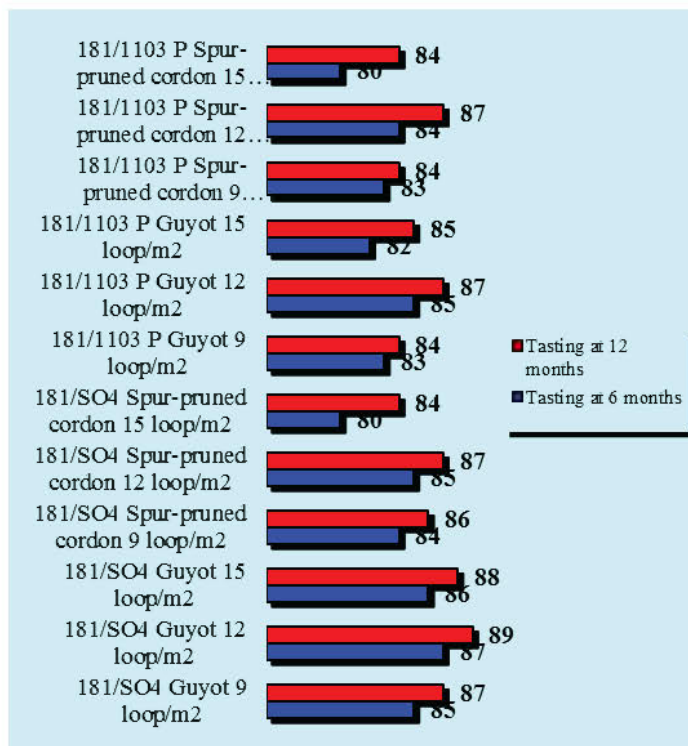


Figure 1. Wine tasting results

Total acidity of the wine, expressed as tartaric acid, has very low levels, in only 3 of the 12 experimental variants with more than 6 g/l, two of them (181/1103 P, Guyot, 12:15 eyes/m<sup>2</sup>) with lowest alcohol concentrations. Moreover, all three variants show grape yields over 9000 kg/ha. The volatile acidity of wines, expressed as acetic acid has good

values, ranging between 0.35 and 0.56 g/L, so less than half the limit imposed by the Romanian viticultural legislation for red wines. The main parameters of the composition of red wines include total dry extract content and dry extract unreduced. The data in Table 2 shows that for both combinations clone/rootstock and both management systems, content of total dry extract has the highest value in the variant with fruit load and hence the lower production and decreases with the increase of production. The highest content of total dry extract is 31.50 g/L, the version 181/SO4, spur-pruned cordon, 20 buds/plant, the only variant with more than 31 g/L, and the lowest is 28.3 g/L, at the variant 181/11003 P, Guyot management system, 34 buds/vine, which has the lowest alcohol concentration. The contents of the dry unreduced extract follows a parallel trend with the total dry extract, with lower values of 1-3 g/L. The highest content is 29.60 g/L, the only value above 29 g/L, at the same variant as the total dry extract and the lowest is 25.61 g/L, the only value below 25 g/L at the same variant as the total dry extract.

To determine the sensorial characteristics of the wine, they were subjected to tasting twice at the age of 6 months and 1 year. The results of these tastings show that for all 12 variants, the wines were rated at 1 year, achieving higher scores than the taste of the age of 6 months. From Figure 1 it appears that wines made from the combination 181/SO4 obtained higher scores in both tastings, regardless of system management and fruit load. Another important finding is that for both rootstock and both management systems, best rated wines in the tasting evaluation were obtained from version fruit load of 12 buds/m<sup>2</sup>, which resulted in average yields of grapes, regardless of the values of the composition parameters. So, no small grape production, nor very large, are not recommended because it leads to getting the best wines in terms of sensorial.

## CONCLUSIONS

The results of this study confirm our previous findings (Ursu et al. 2013, Băducă et al. 2012, 2013), on the relationship between the quantity and quality of grape production for the varieties and clones for quality red wines recently introduced at Sâmburești vineyard.

Thus, it was clearly evident that to achieve high-quality red wines, according to the right vocation of vineyard Sâmburești, it is recommended to obtain appropriate grape production to the optimum quality potential of the grapes. Application of severe cuts, leaving small loops, lead to lower grape yields, under the potential of the variety and the quality of wines, although from compositional point of view, shows that the best parameters to taste does not live up to expectations. Applying a pruning system which leaves a big load of buds leads to very high yields and low quality, both in terms of composition and sensory.

Therefore, it is strongly recommended to adopt pruning systems with average fruit load, in relation to the potential of the combination variety and clone/rootstock, soil and climate conditions specific to the vineyard.

Regarding the management system, it was found that the results concerning the quality of wine were different, depending on the rootstock. Thus, for the combination of rootstock SO4 with clone 181 were obtained qualitatively better results at Guyot canopy management, while in the combination clone 181 with 1103 P rootstock the best results were obtained at the spur-pruned cordon variants.

## ACKNOWLEDGMENT

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## INFLUENCE OF LOAD WITH YIELD NORMING ON THE QUANTITY AND QUALITY OF FRUIT CULTIVAR IDARED

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*Keywords: Apple, periodicity, chemical thinning, Quality*

### ABSTRACT

*Investigations were carried out in the years 2012-2014 in apple orchard in SA Zubresti planted in spring 2003 planting scheme 4x2 m. Trees are led by thin spindle-shaped crown. Apple fruit thinning was studied by chemical thinning, manual and mixed the soil Idared, grafted on rootstock M26. Norming application load the rod bears a major and stable on high quantity of high quality fruit, reducing the frequency of occurrence of fruiting process in apple trees.*

### INTRODUCTION

Apple fruit thinning is one of the most important techniques to improve fruit quality and preventing the formation of fruiting periodicity. A prerequisite for a harvest of high quality is an adequate number of flowers and fruit trees so that their chemical thinning is a common measure in commercial apple orchards (Wertheim 2000, Greene 2002).

Hand thinning of fruit is often impossible because of labor costs and labor limited. Therefore, the chemical thinning must be performed to ensure high fruit production. (Olien & Bukovac 1982).

Fruit thinning can increase their size and improve their color and quality. Hand thinning on strengthening stone is good practice for temperate fruits, but is costly and time consuming (Childers 1995).

### MATERIALS AND METHODS

Investigations were carried out in the years 2012- 2014 apple orchard company "Zubresti" SA, planted with trees grafted aged 2 years Zubresti around the village, district Strășeni. The plantations were conducted in spring 2003 Golden Delicious, Florina, Idared, grafted on rootstock M 26 distance 4x2 m planting trees.

We studied four fruit thinning variants:

Variant 1 - Witness untreated

Variant 2 - Management of chemicals when central fruit diameter of 10-12 mm are inflorescence Bioprzerzedzacz 060 SL formulation at a concentration of 0.075%.

Variant 3 - Management of chemicals when fruit diameter central inflorescence

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are 10-12 mm SL 060 Bioprzerzedzacz preparation concentration of 0.075% + hand thinning fruit.

Variant 4 - Manual fruit thinning is done after the fall of physiological, when fruits reach 16-18 mm in diameter.

Distribution of fruit harvest was determined at the end of the growing season to a height of 0-1, 1-2, and 2-3 m above the ground and in the togetherness of typical tree crowns.

The number of inflorescences was calculated by counting flower buttons 3 trees typical variant. The yield was determined for each tree individually, weighing 24 fruit production and making arithmetic. The average weight of a fruit was determined by weighing 100 to 100 fruits and dividing commercial quality of fruits was determined according to the European Standard for apples no.85 / 2004.

### RESULTS AND DISCUSSIONS

In 2012 the number of fruit varied fruit normalization method applied to 200 pcs/tree variant V2 186 pcs/tree V4 variant (Table 1).

At the site of the fruit 0-1 m highest percentage of fruit in this area is 52% V4 variant and variant V2 50%.

The percentage of fruit in different parts of the crown reveals a difference between variant control V1 37% and fruit thinning variants in the 2-3 m from 29% in V4 and variant variant V3.

In the year 2013 the number of fruit thinning variants significantly increased compared to 2012 and amounted to 200 pcs/tree variant with manual fruit thinning V4 216 pcs/tree variant with mixed fruit thinning V3.

Table 1

Fruit distribution crown Idared apple cultivar by thinning method  
reproductive organs, %  
(M26 Rootstock distance 4x2m planting, tree age 11 years, SA "Zubresti")

Variant	Total fruit from a tree, pieces	The distance from the soil, m		
		0-1	1-2	2-3
year 2012				
V <sub>1</sub>	190	44	37	19
V <sub>2</sub>	200	50	30	20
V <sub>3</sub>	190	38	36	26
V <sub>4</sub>	186	52	29	19
year 2013				
V <sub>1</sub>	158	45	40	15
V <sub>2</sub>	208	44	39	17
V <sub>3</sub>	216	44	38	18
V <sub>4</sub>	200	53	30	17
year 2014				
V <sub>1</sub>	33	44	40	16
V <sub>2</sub>	200	45	43	12
V <sub>3</sub>	196	43	42	15
V <sub>4</sub>	180	44	40	12

In the 0-1 m from the ground largest fruit weight was recorded variant V4 53% and the other 3 variants including variant control V1 share was around 44%. The guarded 1-2 m from ground level the highest percentage of fruit was recorded in variant control V1 40% and the lowest percentage of fruit in this area was identified in 30% V4 variant.

At the site of the fruit 2-3 m highest percentage was thinning trees of mixed fruit variant V3 by 18% (Table 1).

Location Idared variety of fruits in different parts of the crown is a small percentage difference between variants of fruit thinning, but big difference is in variant control where the percentage of fruit in the 2 to 3 m is 20% compared to variants with normalization of fruit load where the percentage of fruit is from 12% to V2 and V4 solution, and the solution mixed fruit thinning has been 15%.

Periodic thinning of fruits is of great importance both to make production quality and for reducing the periodicity of fruiting (Childers et al. 1995, Greene D. W. 2002, Olien & Bukovac 1982, Stopar M. 2004, Wertheim S. J. 2000).

Table 2

Fruit quality Idared apple cultivar by thinning method reproductive organs,%.  
(M26 Rootstock distance 4x2m planting, tree age 11 years, SA "Zubresti")

Variant	Fruit size categories		
	Extra	Categorie I	Categorie II
year 2012			
V <sub>1</sub>	11	19	65
V <sub>2</sub>	82	16	0
V <sub>3</sub>	86	10	2
V <sub>4</sub>	84	11	2
year 2013			
V <sub>1</sub>	25	30	45
V <sub>2</sub>	87	13	0
V <sub>3</sub>	95	5	0
V <sub>4</sub>	95	3	2
year 2014			
V <sub>1</sub>	28	33	39
V <sub>2</sub>	98	2	0
V <sub>3</sub>	97	3	0
V <sub>4</sub>	95	4	1

Idared variety in 2012 in variants thinning yielded fruit quality over 86 percent extra category compared to only 11% in the control variant. In 2013 30% of the fruit in variant control are category I compared with 13% in the variant with chemical treatment. Fruit variety Idared 2014 proved to be of higher quality variant where chemical thinning applied when fruits have reached 10-12 mm in diameter, extra category increased 98% (Table 2).

Idared variety as the biology is a variety with large fruit with variable shape from globular-flattened until the conical-globular (Cimpoieş, 2001).

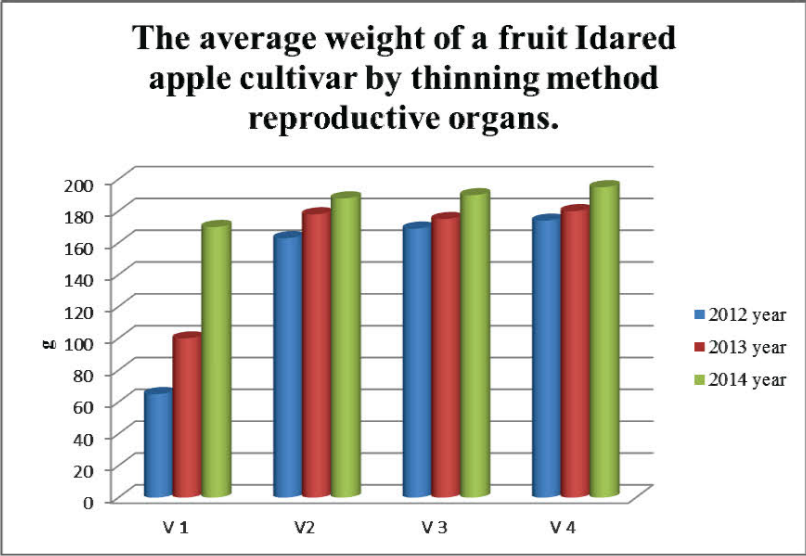


Figure 1. The average weight of a fruit Idared apple cultivar by thinning method reproductive organs (M26 Rootstock distance 4x2m planting, tree age 11 years, SA "Zubresti")

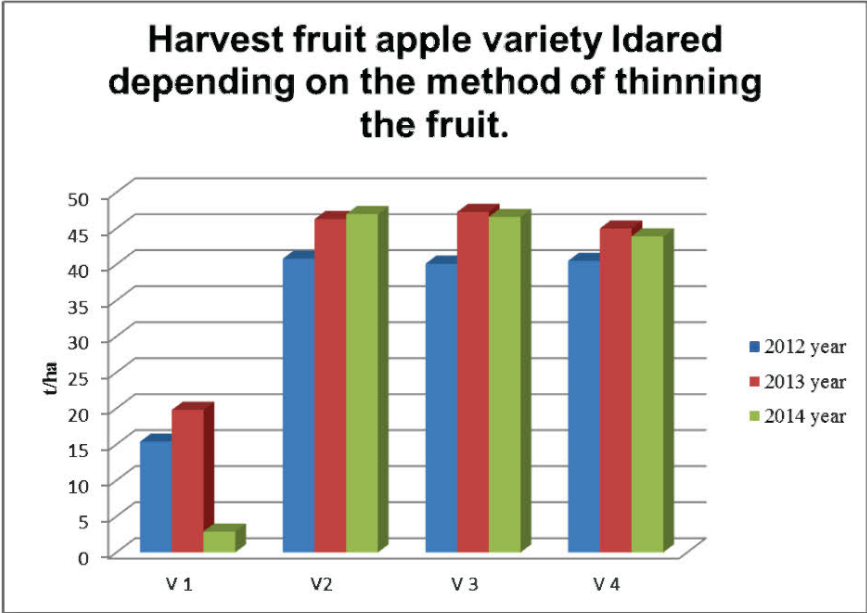


Figure 2. Harvest fruit apple variety Idared depending on the method of thinning the fruit (M26 Rootstock distance 4x2m planting, tree age 11 years, SA "Zubresti")

In the 2012, average fruit weight was determined by the number of fruits per tree and thinning method applied. Thus the lowest average fruit weight was recorded in variant control V1 65 g. Variants Thinning the fruit weight was 163 g fruit chemical thinning variant V2 174 g variant with manual fruit thinning V4 (Figure 1).

In the 2013 fruit weight in all variants increased. The biggest increase was recorded in variant V1 fruit where the fruit reached an average weight of 100 g at a fruit and variants with thinning increased fruit weight was not so increased, and amounted to 175 g V3 variant with mixed fruit thinning and most V4 variant with manual thinning fruit 180g (Figure 1).

In the 2014 the average weight of fruit increased as in 2013 and as of variant control V1 the highest average weight of 170 g fruit, but fruit number was very small only 33 pcs. The standardization of cargo variants fruit average weight increased by an average of 10 to 15 g more than the previous year.

Harvest the years 2012-2014 research in variant control V1R declined due to the number of fruit and fruiting periodicity emerging phenomenon where this year's harvest was 2.9 t / ha. The normalization variants in the same load with fruit fruit crop research period followed a path of ascent stable with growth in advanced chemical fruit thinning variant V2R central inflorescence when fruit is 10-12 mm in diameter, and averaged 44.7 t / ha (Figure 2).

## CONCLUSIONS

Location fruit trees crown is determined by the biology of the variety and fruit thinning method applied, so the research years from 2012 to 2014 at the variant V2 Idared variety of chemical fruit thinning fruits are placed evenly throughout the crown research period.

The highest quality fruit percent research period to optimal to load the rod standardization variants with a tendency to increase the amount of fruits extra quality variants V3 and V4.

The average weight of fruit cultivar Idared showed that applying different normalization methods to load the rod increases fruit weight, average weight studied thus period fruit thinning fruit variants accounted for 176 g variant V2 183 g in V4.

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**BIOMETRIC AND BIOCHEMICAL DETERMINATIONS IN SOME VARIETIES OF CHERRY ON SMALL ROOTSTOCK REGARDING THEIR BEHAVIOR IN A CULTURE IN THE VALCEA AREA**

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*Keywords: Cherry, biometrical determinations, biochemical determinations*

**ABSTRACT**

*In Romania, the cherry represents a traditional orchards culture with high ecological plasticity, being planted on alignments, interspersed with other species or isolated up to an altitude of 900 m Cherry leverages well sites and soils varied land that creates optimal conditions for expression of agrobiologic its potential. To contribute to zoning within the conditions of Vâlcea, some of the many varieties of cherry culture were performed in the Copăceni, Vâlcea County composed of four varieties on small rootstocks (Kordia, Simone, Regina and Summit).*

*In order to create the database on the potential of these varieties of cherry and their behavior in terms of growth and fructification and in terms of adaptation to the Vâlcea county were determined some biometric and biochemical biochemical parameters during the vegetation*

**INTRODUCTION**

In our country, the objectives in the fruit crops improvement programs are moving towards the growth of the agro-productive varieties performances and the improving of the crops quality, as well as the reducing of the resources allocated to the plant protection and diminishing of the fruit content of synthetic chemical compounds and, ultimately, the environment and consumers protection.

Starting with 1951, in Romania at the Bistrita Resort, began the improvement program regarding the cherry growth and its expansion after 1967 at the Research Institute for Fruit Growing in Pitesti-Maracineni and at the Fruit Growing Resort Iasi.

In this period, the cherry culture comes down only to varieties such as Bigarreau Donissen, Hedelfinger or some local ones such as Vartoase, Draganele de Pitesti, Pietroase Leordeni (Budan et al 2000, Braniste et al 2007).

Starting with 1970, there is an intense research for creating new varieties with different ripening periods, with a high fruit quality and a high productivity. By the year 1990, were approved the varieties Jubileu 30, Rubin, Timpuri de Bistrit, Rosi de Bistrita,

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cerna, Ponoare, Izverna. After this year, the research was aimed at creating new varieties with an earliness of fruiting, high fruit quality in terms of size, firmness, color and taste and with ripening periods at the ends of the cherries season. It was completed the approval of the varieties Amar de Galata, Amar de Maxut, Ana, Bucium, Cetatuaia, Catalina, Golia, Stefan, Oana, Tereza, Sublim etc.

An important role in improving the cherry culture in our country had the realization of the program implemented with the Michigan University (USA) after which were approved the varieties Rival, Satmarean, Timpurii de Cluj, Tarina, Pitic, Ilva, Dropia, Amanda, Nana (Budan et al 2000; Braniste et al, 2007).

The continuously increased consumption of fruits in our country, and especially cherries, has led to a research on the introduction in the culture of new cherry varieties. Thus, in this paper we want to highlight some results obtained in the early stage of the research regarding the behavior in culture of new cherry varieties on small rootstocks on an area of 1 Ha, in the area Valcea, locality Copaceni, located at the latitude 45, longitude 23.983 45°0'0" North, 23°58'59"''.

The varieties used for the study are obtained on the rootstock Gisela 5, quality A<sup>+</sup>:

- *Kordia*, a medium tree, with a broad, weeping crown, resistant to frost and drought, with a high productivity. The fruits are large, heart-shaped, oblong, bright red; the pulp is rocky, juicy, sweet and non-adherent to the seed. The ripening period: June 25 - July 5.

- *Simone*, medium tree, height and crown 4x4m. The fruit is large, juicy, tasty, dark red color. The ripening period: month of June.

- *Regina*, medium tree, with large crown, resistant to frost and drought, with a high productivity. The fruits are large, spherical with a bulging backside, blood-red color; the pulp is rocky, juicy, sweet and non-adherent to the seed. The ripening period: May 20 - 30.

- *Summit*, medium tree. Pyramidal crown with erect branches. Large fruit (6-8 g). Spherical heart-shaped slightly flattened. The bright red peel is attractive, after ripening becomes burgundy. The pulp is firm, semi-crispy, not prone to cracking and non-adherent to the seed. Sweet, aromatic, juicy taste.

## MATERIALS AND METHODS

For the biometric and biochemical determinations was used experimental material obtained from the varieties *Kordia*, *Simone*, *Regina* and *Summit*.

### **Biometric Determinations On The Cherry Plants**

The experimental biological material was subjected to measurements regarding:

- diameter under the grafting;
- diameter over the grafting;
- increase in height;
- diameter of the base crown;
- phenological determinations.

The results of the determinations are shown in the Table 1 and 2.

### **Biochemical Determinations On The Cherry Plants**

The biochemical determinations on the cherry plants consisted in determining the peroxidase enzymatic activity in the plants on the vegetative latency period (November - February) in order to obtain information related to how the low temperatures affect the plant metabolism during the determinations, between November and February.

*Determination of the peroxidase activity* EC 1.11.1.7: 5-10 g of plant tissue is weighed and is powdered fine with 50 mL of 2% NaCl solution. Quantitatively the pestle contents passes into a 100 mL volumetric flask and is brought to the mark with 2% NaCl



solution. It is allowed to stand for 30 minutes, during which is stirred a few times, and then filtered. Of the filtrate is taken 10 mL and is introduced into a conical flask, is added 10 mL of distilled water, 1 mL of hydrogen peroxide solution, 1 ml of guaiacol solution and is left for 30 minutes at the room temperature. Then it is measured the colored solution extinction at 420 nm in report with the distilled water. The peroxydase activity is expressed in extinction for 1 g of product (Dumitru et al, 2010, Ianculov et al, 2003).

## RESULTS AND DISCUSSIONS

From the biometric determinations carried out on the cherry plants, the following values were obtained, Table 1 and 2:

Table 1

Biometric determinations cherry

No. pos.	Variety	Diameter under the grafting, mm	Diameter over the grafting, mm	Diameter base crown, cm	Height, cm
1	Kordia	49,25	56,99	150	328
2		52.11	62.17	191	325
3		52.88	59.52	161	330
4		47.81	59.63	191	329
5		51.5	59.15	207	328
-	<b><math>\Sigma n</math></b>	<b>253.55</b>	<b>297.46</b>	<b>900</b>	<b>1640</b>
-	<b><math>x = (\Sigma n)/n</math></b>	<b>50.71</b>	<b>59.52</b>	<b>180</b>	<b>328</b>
1	Summit	45.6	56.52	187	310
2		52.62	55.05	89	304
3		39.15	40.74	203	310
4		47.9	47.25	217	299
5		49.08	52.28	207	302
-	<b><math>\Sigma n</math></b>	<b>234.35</b>	<b>251.84</b>	<b>903</b>	<b>1525</b>
-	<b><math>x = (\Sigma n)/n</math></b>	<b>46.87</b>	<b>50.36</b>	<b>180.6</b>	<b>305</b>
1	Regina	48.5	55.88	212	307
2		54.21	69.48	206	300
3		54.87	62.55	203	298
4		63.93	64.65	210	301
5		52.74	62.38	197	304
-	<b><math>\Sigma n</math></b>	<b>274.25</b>	<b>314.94</b>	<b>1028</b>	<b>1510</b>
-	<b><math>x = (\Sigma n)/n</math></b>	<b>54.81</b>	<b>62.98</b>	<b>205.6</b>	<b>302</b>
1	Simone	55.16	55.22	231	348
2		50.01	56.17	171	354
3		55.42	66.57	242	348
4		53.6	63.7	221	351
5		49.89	62.15	231	349
-	<b><math>\Sigma n</math></b>	<b>264.08</b>	<b>303.76</b>	<b>1096</b>	<b>1750</b>
-	<b><math>x = (\Sigma n)/n</math></b>	<b>52.81</b>	<b>60.75</b>	<b>219.2</b>	<b>350</b>

From the results obtained we observe the following:

The Regina cherry variety shows an average trunk diameter above the grafting of 62.98 mm, with 3.6% bigger than Simon, with 5.5% than Kordia and with 21% Summit.

The cherry crown presents an average size of 219 cm for Simon, 205.6 cm for Regina and 180 for Summit and Kordia.

The average height of the cherries is 350 cm for Simon, 328 cm for Kordia, 305 cm for Summit and 302 cm for Regina.

Phonologically the cherry varieties started in vegetation in March when the fruit buds began to swell and the bud opening happened in the middle of the month.

Were identified the branches with bouquets and the number of May bouquets and the start of blooming Table 2.

Table 2

Phenology determinations in cherry

No. pos.	Variety	Start bud opening	Start bloomed	No. branches bouquet	No. may Bouquets
1	Kordia	10.03	12.04-27.04	6	171
2				9	211
3				5	140
4				4	220
5				5	115
-	$\Sigma n$	-		<b>29</b>	<b>857</b>
-	$x = (\Sigma n)/n$			<b>5.8</b>	<b>171.4</b>
1	Summit	10.03	12.04-28.04	10	194
2				3	94
3				20	164
4				2	206
5				5	222
-	$\Sigma n$			<b>40</b>	<b>880</b>
-	$x = (\Sigma n)/n$			<b>8</b>	<b>176</b>
1	Regina	14.03	19.04-2.05	6	146
2				13	284
3				6	299
4				13	371
5				10	295
-	$\Sigma n$			<b>48</b>	<b>1395</b>
-	$x = (\Sigma n)/n$			<b>9.6</b>	<b>279</b>
1	Simone	15.03	19.04-4.05	7	265
2				3	125
3				5	277
4				5	223
5				2	53
-	$\Sigma n$			<b>22</b>	<b>943</b>
-	$x = (\Sigma n)/n$			<b>4.4</b>	<b>188.6</b>

The start of the bud opening for cherries started the earliest on 10.3 for the variety Kordia and Summit, and on 14 - 15.03 for Regina and Simone. The start of blooming was done on 12.04 until 27.04 for Kordia; 12.04 until 28.04 for Summit; 19.04 until 2.05 for Regina and 19.04 until 4.05 for Simone. The average number of branches bouquet was higher for Regina of 9.6 followed by Summit (8), Summit (8), Kordia (5.8) and Simone 4.4. The average number of May bouquets was of 279 for Regina, 188.6 for Simone, 176 for Summit and 171.4 for Kordia.

Regarding the enzymatic activity of the cherry plants, it was ranged between 4.11 and 9.53 absorbance units per gram of plant material.

The variation in the peroxydase concentration is a response of the plants to the stress provoked by the low temperature during winter, in the area it was registered a minimum temperature in November of 4.35°C, December of -4.57°C, January of -2.1°C and February of -1.98°C.

The decrease in temperature below 0°C leads in most cases to extracellular freezing. The plasma membrane forms a barrier against the growing ice crystals, causing the movement of water outside the cell, due to the lower chemical potential of the ice compared with that of the water. The stress generated by this cellular dehydration induced by freezing is extremely severe and the plant cells will lose most of the osmotically active water. It is believed that another important cause of the damage caused by freezing is the formation of oxygen free radicals (ROS) that the plants by their specific enzymes, known as antioxidant enzymes, contribute to their annihilation. The antioxidant enzymes in the plants are the catalase (CAT), super-oxy-dismutase (SOD), peroxydase (POX), peroxydase ascorbate (APX), glutathione S transferase (GCT).

In terms of plant resistance to low temperatures determined by measuring the peroxydase in the cherry samples registered during November-December 2013 and January-March 2014, is remarked Simone with a peroxydase activity of 9.53 extinction units per gram of plant product, Kordia with a value of 7.24, Regina with 5.32 and Summit with 4.11 extinction units per gram of plant product. The more intensive peroxydase activity for the variety Simone gives it a greater resistance to the stress caused by low temperatures.

The determination of the peroxydase enzymatic activity is considered a measure of the existing stress level, or a measure of evaluating the plant ability to respond adequately to this internal biochemical factor induced by the physiological condition of the plant.

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#### **CONCLUSIONS**

After the determinations carried out on the 4 varieties of cherry regarding their behavior in culture in the Valcea area, we remark the following:

- The variety Regina and Simone show an increase in the average diameter of the trunk over the graft, superior to the varieties Kordia and Summit;
- The crown diameter in the varieties Simone and Regina is superior to the varieties Summit and Kordia;
- The varieties Simone and Kordia have a plant height average superior to the varieties Summit and Kordia;
- The start of the bud opening for cherries started first for the varieties Kordia and Summit and then for Regina and Simone;
- The blooming started first for the varieties Kordia and Simone and then Summit that began blooming after 7 days;
- The average number of branches bouquet was higher for Regina of 9.6 followed by Summit (8), Kordia (5.8) and Simone 4.4.
- The average number of May bouquets was of 279 for Regina, of 188.6 for Simone, 176 for Summit and of 171.4 for Kordia;

- In terms of plant resistance to low temperatures determined by measuring the peroxydase activity, the Simone variety shows the best resistance to frost, followed by Kordia, Regina and Summit.

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## DEVELOPMENT A DIETARY SUPPLEMENT BASED ON MODIFICATION OF GREEK SALAD SYNTHESIS

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**Keywords:** *tomato, onion, oregano, dietary supplement*

### ABSTRACT

*The present work concern on design and development a dietary supplement based on local food. Greek salad is widely known salad around the world. The containing vegetables in Greek salad are, the tomato, the onion and the oregano. This salad consists a local food which are rich sources for a number of nutrients. Thus in our work is proposed a product made from tomato, carrot, onion and oregano and thanks to their valuable nutrients it is very beneficial for health and wellness. The proposed 'salad' has as ingredients lycopene from tomato, a powerful antioxidant with protective effects against cardiovascular risk, beta-carotene from carrots and phenolic compounds from onions, substances with strong antioxidant activity which prevent the action of free radicals; and a number of essential oils from oregano, which are involved in prevention of growth of various microorganisms. The proposed salad product, which could be manufactured by the extract of tomato, carrot, onion and oregano, will be available in powder form.*

### INTRODUCTION

Apart from the major food principles like protein, carbohydrates, and fats, large number of food items we consume consists of invaluable components in them known as phytonutrients or plant derived chemical substances. Although their caloric value is insignificant, inclusion in our diet in adequate levels is imperative since the potential benefits in terms of direct contribution to health promotion and disease prevention are enormous. Changes in lifestyle and dietary patterns have resulted in modern world illnesses like coronary artery disease, diabetes, stroke, cancers etc, in higher frequency than ever before. Several research and experimental epidemiological studies have clearly suggested that the trends in disease pattern linked to the diet we consume. Consequently, new interest has risen in the medicinal properties of food items like herbs, spices, vegetables, and fruits along with their peels.

Tomato (*Solanum lycopersicum*) comes from the Aztec word tomato, which literally means "swollen fruit". Although the exact date of domestic cultivation and

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consumption of tomatoes is not known, the first tomatoes might have had yellow color and size similar to the shape of cherry tomatoes. Early tomatoes were grown by the Aztecs of Central Mexico and were spread worldwide with the Spanish colonization of America. The first mention in European literature was in 1544 by an Italian herbalist Pietro Andrea Mattioli, who named tomato as pomo d'oro or "golden apple"(www.wikipedia.com.).

**Carrot:** Carrot (*Daucus carota*) belongs to the family of Umbelliferae. It is a fleshy, long conical orange, yellow or white depending on variety root and is believed to come from Afghanistan. In ancient Greece it was called Staflinos and was known for its medicinal properties. In early years, carrots were cultivated for their aromatic leaves and seeds and not their roots. In Europe, its cultivation began in the 13th century and carrots were purple due to some pigments. The cultivation of the orange carrots started in the Netherlands during the 17th century (wikipedia.com).

Onion (*Allium cepa*) is likely to come from Southeast Asia. Both the leaves and the bulb of the onion are edible and are characterized by their pungent taste and aroma which is limited by cooking. Onions are part of the human diet for millennia. In ancient Greece, athletes ate large quantities of onions because they believed that they help in blood balance. The Romans were rubbed with onions to strengthen their muscles. During the Middle Ages onions were so important foods that people were paying the rent with onions and gave them as gifts (wikipedia.com).

**Oregano:** Oregano (*Origanum vulgare*) is a plant native to the Mediterranean and Central Asia. In Greece, oregano is found in mountainous and rocky areas. It is used mainly as a spice and rarely as a tea for the treatment of coughs. From ancient times oregano was well known for its flavoring properties. In ancient Greece, oregano was termed as "the joy of the mountains" and considered a symbol of joy and happiness. Hippocrates used it for its antiseptic properties. During the Middle Ages, oregano was being chewed to relieve toothache, rheumatism and cough (wikipedia.com).

## MATERIAL AND METHODS

**Salad synthesis.** The Greek salad contains basic tomato, onion and oregano. In our case of the proposed product was added carrot. A tomato of 100gr *fresh weight*, 1 cup of carrots, ½ cup of onions and 30g of oregano broken leaves were used as raw material. Spray dryer could be used for drying of the above mentioned raw material. This method is very quickly compared to other methods of drying. Thus the raw material could be also turned into a dried powder in a single step. The ingredients of the proposed product were determined using a software program as is the Nutritionist Pro™ Diet Analysis. This software provides thorough nutrient analysis of diets, recipes, and menus with up-to-date food and nutrient data.

## RESULTS AND DISCUSSIONS

**Ingredients.** The proposed product consists of tomato, carrot, onion and oregano, vegetables and oregano, foods which are rich sources for a number of nutrients. The tomato is the richest source of lycopene (3mg/100g tomatoes) (Edwards, 2003) which is a red pigment responsible for its color and belongs to the group of carotenoids. Lycopene consists 80-90% of tomato carotenoids. According to pharmacokinetic studies, lycopene is better absorbed from processed tomato sources or tomato products than from raw tomatoes (Shi et al, 2000). Seventy percent of oregano oil also consists of a variety of phenolic compounds (Daferera, 2000; Dorman, 2000), namely *carvacrol*, *thymol*, and *eugenol* as well as their precursors, which are believed to be responsible for oregano's antibacterial,

antifungal, and antioxidant properties. (Dorman, et.al., 2000). Table 1 presents vegetable nutrient content (Nutritionist Pro™ Diet Analysis (AXXYA Systems)).

Table 1

Vegetable nutrient content (1 tomato, 1 cup of carrots, ½ cup of onions, 30g oregano)  
[Nutritionist Pro™ Diet Analysis (AXXYA Systems)]

Factor	Value	Factor	Value
Energy	110,35kcal	Dietary fiber	6,0g
Protein	3,09g	Sodium	92,73mg
Total carbohydrates	25,54g	Potassium	822,14mg
sugars	12,24g	Lycopene	3166,01µg
Total fats	0,74g	b-carotene	10722,45 µg
Saturated	0,15g	a-carotene	4366,16 µg

**Safety.** The designed and suggested product is a safe dietary supplement. However, it should always be used according to its directives after consultation of physician and/ or clinical dietitian. The suggested supplement should be avoided by people with known allergy/hypersensitivity to tomato and/ or lycopene or other components, the carrot and/ or constituents or other members of the family *Apiaceae*, onion and/ or components or other family members *Lilaceae* and the oregano. The suggested supplement should be used with caution by women in pregnancy or lactation, because of its content in vitamin A. Due to tomato content, this salad product should be used with caution by people with stomach ulcers and other disorders of the gastrointestinal tract (Jatoi, 2007; Clark, 2006), by people taking anticoagulants (Hsiao 2005) from those with hypotension and/ or individuals receiving antihypertensive treatment (Engelhard 2006; Vaishampayan 2007). Additionally, attention should be given and when it is consumed by women who receiving estrogens or hormone therapy due to possible negative interactions with lycopene; and men at increased risk of prostate cancer, because lycopene may cause a reduction in the levels of diagnostic markers (Vaishampayan 2007). Although the following are related with the consumption of large quantities of carrots (> 100gr daily), given the content of suggested salad in carrots, attention should be given when it is used by diabetic patients suffering from hypoglycemia (Gustafsson, 1994) and by people suffering from intestinal obstruction (Cummings 1978; Wisker et al., 1994). Attention should also be given when it is consumed by people with known allergy to allergens associated with birch pollen allergens, as carrot contains allergens similar to those from birch pollen (Ebner 1996). Also caution is advised (Hubbard et al., 2006) to patients with diabetes or hypoglycemia (Sharma 1977; 20.Mayer 2001) and hypotension in patients (Kalus 2000). In addition, due to the onion content of the suggested product, attention should be given when used or receiving anticoagulants (Hubbard et al., 2006) by diabetic patients (Sharma 1977; Mayer 2001) and by people suffering from hypotension (Kalus 2000). by people with blood disorders.

## CONCLUSIONS

**Usages-Indications.** This suggested product could be a great product for those people who do not particularly like vegetables, however, know the importance of them in diet; for children who do not eat vegetables but they need them for their growth; for athletes who want only the best; for busy people who do not always have time to buy and prepare vegetables either for their salads or for their meals; for travelers who want to have vegetables in their diet; and for the elderly and patients who cannot chew vegetables. The designed salad could be a unique formula for mothers and/ or wives who want to offer the best meals to their families and for chefs and restaurants who want to offer rich flavors with

nutritional value to their customers. Finally, the above mentioned Salad product is indispensable to all who have a poor diet, since it is rich in antioxidants, the importance of whose health has been recorded in a variety of scientific studies.

*Effectiveness.* Although some scientific findings seem quite promising for the effectiveness of the components of Salad Supplement, reliable evidence is very limited and extensive clinical studies are needed for thorough investigation of their possible actions.

*Antioxidant effects.* Lycopene is considered to be a powerful antioxidant (Stahl et al., 1998; Di Mascio et al., 1989). In a study of 57 diabetic patients, the effects of eating tomato juice (500ml/day) compared with the supplementation of vitamin E (800U/day) and vitamin C (500mg/day) on the oxidation of LDL-cholesterol were evaluated. Consumption of tomato juice was associated with a significant increase in plasma lycopene levels and reduced oxidation of LDL-cholesterol similar to that caused by the administration of the nutritional supplement (Upritchard, 2000). Porrini & Riso (Porrini et al., 2000) in a study of 9 adult women showed that daily consumption of 25g of tomatoes (7mg lycopene) for 7 days resulted in an increase in lycopene lymphocyte concentration and increased lymphocyte resistance to oxidative stress.

*Cardioprotective effects.* According to epidemiological studies, lycopene has been inversely associated with cardiovascular disease risk (Kristenson 1997; Kohlmeier 1997), which is believed to be due its antioxidant action (Stahl 1998; Di Mascio 1989). Steinberg & Chait 1998 evaluated the effects of consumption of tomato juice enriched with vitamins E, C and beta-carotene on the oxidation of blood lipids in a sample of smokers. According to the findings of the study, enriched with vitamins tomato juice was associated with reduced lipid hyperoxidation and LDL-cholesterol oxidation. In an dietary intervention of 19 healthy adults, the effect of lycopene from different dietary sources (tomato juice, tomato sauce, tomato oleoresin) on the oxidation of LDL-cholesterol was evaluated. Consumption of products rich in lycopene was associated with significant increase in lycopene blood levels and reduced lipid hyperoxidation and LDL-cholesterol oxidation, suggesting a possible role of lycopene in the reduction of cardiovascular risk (Agarwal et al., 1998). Engelhard et al. 2006 in a doubleblind clinical trial evaluated the effects of the consumption of an extract from tomato for eight weeks on blood pressure in 31 individuals with hypertension grade 1, who had no other disease and were not receiving antihypertensive treatment. Researchers reported a significant reduction in systolic and diastolic blood pressure after the consumption of the tomato extract, suggesting a possible beneficial effect of tomatoes on another cardiovascular risk factor.

*Antioxidant properties of carrot.* In clinical trial of 15 male smokers, the effects of eating foods rich in vitamin C and beta-carotene on the levels of vitamins in blood and oxidation of LDL-cholesterol were evaluated. During the first three weeks the participants consumed a free of vitamins drink and for the next three one drink containing orange juice (145mg vitamin C) and carrot juice (16mg beta-carotene). Juice consumption resulted in increased levels of vitamins in plasma. Although a decrease in one oxidation products was observed, juice consumption was not associated with the degree of LDL-cholesterol oxidation (Abbey 1995).

*Carot and Gastrointestinal effects.* Consumption of carrot-rice based rehydration solution was associated with statistically significant reduction in the duration of diarrhea episodes and fecal bulking compared with conventional glucose rehydration solutions (Pietschnig 1992).

*Insulin/ glucose effects.* Consumption of cooked carrots (200mg daily) was associated with statistically significant decrease in glucose levels and Insulin/ C-peptide responses of 10 healthy adult men (Gustafsson 1994).



*Onion- Antihypertensive effects.* Onion consumption has been associated with decrease in blood pressure. (Mayer 2001) in a study of 24 hypertensive subjects, evaluated the effects of a nutritional supplement, which contained onions against a placebo on blood pressure. The administration of the supplement was related with statistically significant decrease in systolic pressure. These effects were noticed both immediately and long-term after administration of the nutritional supplement for one week.

*Onion- Antidiabetic effects.* Onion has been proposed to have antidiabetic effects (Srinivasan et al., 2005). The effect of diet containing onions or green beans on blood glucose levels was evaluated in 20 diabetic patients. The patients ate for one week a certain diet (68% carbohydrate, 20% fat, 12% protein) and at sub-groups of 10 subjects, either 20g of fresh onions or fresh green beans 3 times daily for one week followed by one week eating the diet alone. Onion consumption was associated with statistically significant decrease in glucose levels compared with green bean consumption (Mayer 2001).

*Onion, Antihypercholesterolemic effects.* According to a clinical study, consumption of onions or onion oil products was associated with decline in cholesterol, fibrinogen and coagulation time (Bordia 1975).

*Oregano, effects on intestinal microflora.* The oral administration of an oregano oil supplement (200mg for 3 times daily) for 6 weeks in 13 subjects resulted in the elimination of parasites (*Blastocystis hominis*, *Entamoeba hartmanni*, *Endolimax nana*) to 77% of participants (Force et al., 2000).

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**THE AQUATIC AVIFAUNA OF URZICUȚA SETTLEMENT  
(DOLJ COUNTY)**

Bălescu Carmen Daniela\*<sup>1</sup>

**Keywords:** *aquatic birds, phenology, diversity, estimated number*

**ABSTRACT**

*The paper presents the results of the monitoring of aquatic birds in Urzicuța settlement (Dolj County) during July – August 2012, March 2013 – September 2014. It includes the taxonomic list, the phenological status, their seasonal dynamics, and their estimated number, closely connected to the environmental factors. The investigated aquatic ecosystems have a great avifaunal importance due to the diversity of the distinguished species: 43 species distributed in 8 orders and 14 families. Of these, 18 species are listed in Annex 1- Birds Directive 2009/147/EC, being considered important indicators in taking the most effective measures to conserve the habitats, to ensure their survival and reproduction in their area of distribution.*

**INTRODUCTION**

Urzicuța locality is situated in the central-southern area of Dolj County, in the central part of Desnățui Plain (subunit of Băileștilor Plain), on the interfluvium Desnățui-Baboia (Ghinea, 1998), at an altitude of 25-40 m (Câșlaru, 2011). It is comprised of two villages Urzicuța and Urzica Mare. Of the total area of 6072 ha, the overall area of the settlement, the terrestrial biotopes (arable land, thickets, grasslands, pastures, vineyards etc.) have an area of about 5981 ha and the aquatic ones (river, creek, lakes etc.) 91 ha.

Desnățui River runs through the village in the east part and Baboia creek in the south part. On the territory of Urzicuța settlement there is a series of ponds/lakes stretching about 3,5 km. They form Urzicuța Lake, known by the local people as "Village Pond". Urzicuța village center is divided by this lake into 2 parts. On certain areas the lake is surrounded by suitable aquatic vegetation (reed, rush, willow clumps etc). It is a eutrophic lake, rich in phyto and zooplankton, whose level decreases during dry summers. It is fueled by the groundwater and rainfall. In the south of the village there is Ionele Lake (Fig. 1), with an area of 3.2 ha. During 1971- 1989 it was known as "Băile Ionele" due to the mineral quality of the water and mud with therapeutic effects in the treatment of rheumatic diseases. This resort was abandoned after the 1990s. Today the lake is maintained by rain. In dry summers the water surface disappears, leaving behind some holes from which

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therapeutic mud is extracted. In the year 2000 Ionele Lake was designated as protected area of national interest (code 2396, Law 5/2000) due to the physicochemical and therapeutic quality of the water.



Figure 1. Urzicuța Ionele (Orig.)

This paper continues the study started last year (Bălescu & Ridiche, 2013) regarding the avifauna of Urzicuța locality. The monitoring of aquatic birds had the following objectives: enlarging the list of aquatic birds started last year; determining the phenological status, the seasonal dynamics of aquatic birds; the numerical evaluation of the populations of each species (comparison between the two years of study: 2013 and 2014); data regarding the breeding of aquatic species.

#### **MATERIAL AND METHODS**

The aquatic birds study was carried out based on the observations made at Urzicuța Lake and Ionele Lake as a result of the seasonal field trips in the periods: July and August 2012, March 2013 – September 2014.

For observing and identifying the birds, the binoculars (Norconia 10x50) and the guides (Peterson et al., 1989; Bruun et al., 1999) were used. The birds were photographed with a Canon Sx40HD camera and filmed with a Panasonic SDR-H20 video camera. Systematic list of species of birds was done after the model elaborated by Szabó-Szeley & Baczó (2006). All information taken from the field were supplemented with documentation activities performed in the laboratory and processed from a systematical, biological and ecological point of view.

#### **RESULTS AND DISCUSSIONS**

Although in the studied area the terrestrial habitats predominate over the aquatic and semi-aquatic ones (rivers, lakes, canals, wetlands, reed beds, wet meadows, etc.), the latter attract a large and specific number of birds, offering them a feeding, transit or nesting place.

43 species of birds distributed in 8 orders and 14 families were distinguished (Table 1). Subsequent observations have shown in addition to 2013, 8 aquatic species: *Phalacrocorax pygmaeus*, *Phalacrocorax carbo*, *Ardeola ralloides*, *Ardea alba*, *Ardea purpurea*, *Aythya ferina*, *Circus aeruginosus*, *Tringa glareola*.

The ecological factors have played an important role in the quantitative changes in bird populations and in the seasonal dynamics of the aquatic bird species. The anthropic impact on birds is low, although a large area of Urzicuța Lake is situated in the center of the village. Many species have been seen during the daytime performing their current activities without being disturbed by human presence.

The summer of 2013 was characterized by a dry year. Because Ionele Lake and large surfaces of Urzicuța Lake dried up, in these ecosystems were distinguished numerous examples of limicolous species and herons. The summer of 2014 was characterized by a rainy year. The rainfall in June and July exceeded normal limits. Therefore, the level of Urzicuța Lake was high, and Ionele Lake didn't dry up. Thus, good swimming and diving species prevailed (ducks, little grebes, moorhens, coots) and also good flying species (gulls, whiskered terns etc).

Each ecological season is characterized by numerous behavioral aspects of birds' life. The smallest number of species was recorded in the hiemal season (November 1 – March 1) when 9 species were observed with a small number of specimens: 3 species of Anatidae: *Anas platyrhynchos* (7-15 specimens), *Anas crecca* (5-7 specimens), *Cygnus olor* (4 adult and juvenile), 2 species of Ardeidae: *Ardea cinerea* (2-5 specimens), *Ardea alba* (1-3 specimens), 2 species of Rallidae: *Gallinula chloropus* (+10 specimens), *Fulica atra* (6-25 specimens), and one species of Phalacrocoracidae: *Phalacrocorax carbo* (1-6 specimens) and Laridae: *Chroicocephalus ridibundus* (4-6 specimens).

Table 1

List of systematic, distribution, phenology and effectives the aquatic birds of the Urzicuța locality (Dolj County) in July and August 2012, March 2013 – September 2014

No crt	Order, Family, Species	Distribution		Phenological status Urzicuța	Estimated number (specimens)	
		Urzicuța Lake	Ionele Lake		2013	2014 min-max
	Ord. PODICIPEDIFORMES Fam. Podicipedidae					
1	<i>Tachybaptus ruficollis</i>	*		SV, B	6 – 7	3 20
2.	<i>Podiceps cristatus</i>	*	*	P	4 – 5	3
	Ord. PELECANIFORMES Fam. Phalacrocoracidae					
3.	<i>Phalacrocorax carbo</i>	*	*	WV, P	–	1 6
4.	<i>Phalacrocorax pygmaeus</i>	*		SV	–	3 +35
	Ord. CICONIIFORMES Fam. Ardeidae					
5.	<i>Ixobrychus minutus</i>	*		SV, B	+5	3 +10
6.	<i>Nycticorax nycticorax</i>	*		SV, B	+100	6 +50
7.	<i>Ardeola ralloides</i>	*		SV	–	2 +5
8.	<i>Egretta garzetta</i>	*		SV, PB	+150	10 +70
9.	<i>Ardea alba</i>	*		SV, WV	–	1 +40
10.	<i>Ardea cinerea</i>	*	*	SV, WV	2 – 30	2 + 30
11.	<i>Ardea purpurea</i>	*		SV	–	6 15/20
	Fam. Ciconiidae					
12.	<i>Ciconia nigra</i>		*	P	25 – 35	2

13.	<i>Ciconia ciconia</i>	*	*	SV, B	dozens	6	20
	Fam. Threskiornithidae						
14.	<i>Plegadis falcinellus</i>	*		P	+12	3	5
	Ord. ANSERIFORMES Fam. Anatidae						
15.	<i>Cygnus olor</i>	*		PM, B	2 pairs	3	14
16.	<i>Anas crecca</i>	*	*	WV, P	12 – 30	5	25/ 30
17.	<i>Anas platyrhynchos</i>	*	*	PM, B	unevaluated	7	+200
18.	<i>Anas acuta</i>	*	*	P	12	7	25
19.	<i>Anas querquedula</i>	*	*	P, SV, B	6 – 16	6	40
20.	<i>Anas clypeata</i>	*	*	P	10 – 25	5	20/ 30
21.	<i>Aythya ferina</i>	*		P	–	4	+16
22.	<i>Aythya nyroca</i>	*	*	SV, B	+2 nesting pairs	+ 4 pairs 6	+40
	Ord. ACCIPITRIFORMES Fam. Accipitridae						
23.	<i>Circus aeruginosus</i>	*		SV, PB	–	1	4
	Ord. GRUIFORMES Fam. Rallidae						
24.	<i>Porzana porzana</i>	*		SV, PB	+2	1	3
25.	<i>Gallinula chloropus</i>	*		PM, B	unevaluated	10	300
26.	<i>Fulica atra</i>	*		PM, B	unevaluated	6	+400
	Ord. CHARADRIIFORMES Fam. Recurvirostridae						
27.	<i>Himantopus himantopus</i>	*	*	SV, B	50 – 80	3	30
	Fam. Charadriidae						
28.	<i>Charadrius dubius</i>	*	*	P	+10	2	5
29.	<i>Vanellus vanellus</i>	*	*	SV, B	130 – 150	5	+75
	Fam. Scolopacidae						
30.	<i>Philomachus pugnax</i>	*	*	P	+10	4	90/100
31.	<i>Limosa limosa</i>	*		P	+15	6	20
32.	<i>Tringa totanus</i>	*	*	P	+5	2	7
33.	<i>Tringa stagnatilis</i>	*		P	+10	4	8
34.	<i>Tringa glareola</i>		*	P	–		+4
35.	<i>Tringa ochropus</i>	*	*	SV, P, PB	5 – 20	3	10/15
36.	<i>Actitis hypoleucos</i>	*		SV, P, PB	+15	6	+10
	Fam. Laridae						
37.	<i>Chroicocephalus ridibundus</i>	*	*	MP, B	+350	4	+250
38.	<i>Larus cachinnans</i>	*	*	P	unevaluated	10	+75
	Fam. Sternidae						
39.	<i>Sterna hirundo</i>	*		P	unevaluated		+3
40.	<i>Chlidonias hybridus</i>	*	*	SV, B	+250 spec.	50	300
	Ord. PASSERIFORMES Fam. Sylviidae						
41.	<i>Locustella luscinioides</i>	*		SV, B	unevaluated	dozens	
42.	<i>Acrocephalus scirpaceus</i>	*		SV, B	unevaluated	dozens	
43.	<i>Acrocephalus arundinaceus</i>	*		SV, B	unevaluated	dozens	

#### Legend

Phenological status: SV-summer visitor; WV – winter visitor, P – passage species, S – sedentary species, PM – partial migratory species; B- breeding; PB - possible breeding.

Estimated number: min=minimum, max=maximum.

The prevernal season (March 1 – May 1) coincides with the spring migration, which can begin in mid-February until the first half of May. It is the season in which some species that stayed during the winter leave the area to nesting elsewhere (Great Egret - in February, Great Cormorant - in April, Common Teal - in May etc). Also, migratory birds



return from the wintering grounds in stages (February- May). Some remain to breeding; some are in transition towards nesting places.

This is the season when most migratory and sedentary birds start reproducing (summer visitors). Thus, we have noticed various reproductive manifestations such as: attracting partners for mating with a variety of songs, running across the water, swooping flights, nuptial dances etc.; we have noticed birds with different plants and sticks in their beak, sign of building nests in places as hidden as possible etc.

During the vernal season (May 1 – June 15) and aestival season (June 15 – July 15) the aquatic birds are busy with breeding activities. In some species May is the beginning of the prenuptial period (Little Bittern, Whiskered Tern), others hatch (*Fulica atra*, *Gallinula chloropus*) and others take care of their nestlings (*Gygis olor*) etc. Adults, nestlings and/ or juveniles are seen on the lake in search of food.

The months of June and July 2014 were characterized by the alternation of high temperatures with heavy rains, so that the water level of the lakes remained high. The rich trophic resource was sufficient both for the wild aquatic birds and for the domestic ones (ducks and geese). We haven't noticed major conflicts over food, the birds tolerating each other.

The beginning of the serotinal season (July 15 – September 15) coincides with the large increase in the number of the specimens. Other species that are not nesting in the area are seen for food and rest (Pygmy Cormorant, Great Egret, Purple Heron, various Charadriidae etc.) increasing the numbers of existing species. In July, we noticed that some nesting birds such as: Common Moorhen, Coot, Mallard etc. had nestlings at different stages of development. In late July and August we noticed significant agglomerations of Ardeidae, Anatidae, Rallidae, Laridae and Sternidae on different areas of the Urzicuța lake (Fig. 2, Fig. 3). We didn't notice compact groups of *Ardea purpurea*, *Ardeola ralloides*, *Ixobrychus minutus*. These species were at the edge of the reeds (between 1 and 4 individuals). Most limicolous species were stationed at the periphery of the Urzicuța Lake (in the south and in the north, towards the train station - where the water level was low), in the wet areas, along the spill channels, along Baboia creek and at Ionele Lake. While some limicolous birds recorded fewer specimens than the last year, at some of them we noticed a very large number of individuals (Table 1).

Thus, at Ionele Lake we noticed 90-100 specimens of *Philomachus pugnax* with different plumage color variations. It was the period of their plumage change. Their number exceeded by far the one recorded last year (+ 10 specimens). We have no reliable data on the breeding of this species in the investigated area. The species with the most significant number in 2014 (observed on August 20), which were also the dominant ones, are: *Fulica atra* +400 individuals, *Gallinula chloropus* + 250 individuals, *Chlidonias hybridus* 250-300 individuals, *Anas platyrhynchos* +200 individuals, *Chroicocephalus ridibundus* 200-250 individuals etc. This estimation comprises besides adults also immature specimens. Generally speaking, bird agglomerations represent a preparation of species for the autumn migration. It is the time when birds accumulate enough food to cope with migration to the wintering grounds.

During the autumnal season (September 15 – October 31) the summer visitors birds begin to leave the field. The autumn of 2013 was characterized by alternations of temperature, the temperature being sometimes very low, other times very high compared to normal values. The last week of September and the first week of October 2013 were characterized by a sudden drop in temperature, with rain and wind, the temperature reaching values that were not specific to those months. This affected the birds. Thus, most species of summer visitors have left earliest, in September.

Of the total number of identified species, 22 are nesting species and possible nesting. From the phenological point of view, 17 species are summer visitors. Many species of birds nesting in aquatic vegetation, in the reed: *Ixobrychus minutus* (common and constant in the habitat), *Fulica atra* (lays eggs 2-3 times a year), *Gallinula chloropus*, *Locustella luscinioides*, *Acrocephalus sp.* etc.



Figure 2. Urzicuța Lake. Agglomeration of aquatic birds (Orig.)

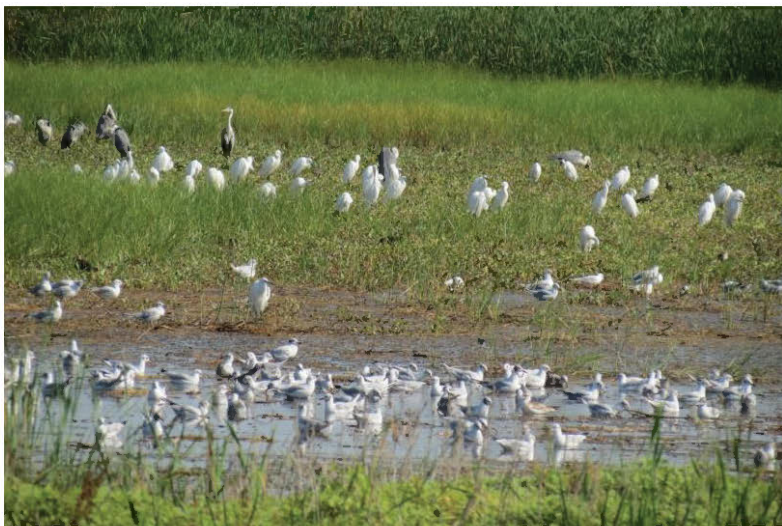


Figure 3. Urzicuța Lake. Agglomeration of aquatic birds (Orig.)

In 2014 two pairs of *Cygnus olor* were nested: one in the reeds in the north part of the lake (towards the train station), that pull out five nestlings, and the second pair in the reeds in the center of the lake, hatching eight nestlings. Subsequently two died. For *Tachybaptus*

*ruficollis* we observed four pairs of adults accompanied by three and four nestlings in three different areas of Urzicuța Lake. In late August, adults and juveniles were observed in association with coots, ducks etc. Due to their discrete life, it is possible to have more than four nesting pairs. *Chlidonias hybridus*, a common and constant species in the studied area, has nesting in a significant number of colonies on the surface of the aquatic vegetation. The nests built out of vegetation clumps were fixed by natant plants. In these floating nests, our attention was captured by two and three nestlings/ or juveniles that, while waiting for their parents to bring food, made loud sounds. *Vanellus vanellus* is breeding on grassy areas near the lakes and on farmland. In 2014, the first appearance in the village of the White Stork was reported on 27<sup>th</sup> of March. If in 2013 there were four pairs of breeding storks, hatching an overall of 12 nestlings, in 2014 there were three pairs of breeding storks, being seen 10 nestlings (Table 2). The fluctuations in number of the breeding pairs of storks and of the nestlings are the results of trophic supply, affected nests, weather conditions, etc. The dozens of specimens noticed in late August are due to the association with storks that came from neighboring villages.

Table 2

The situation of White Storks nesting pairs in Urzicuța locality

Village	Urzicuța				Urzica Mare		
	2013		2014		2013	2014	
Nests	1	1 1 1	3 occupied nests	1 1 1 1	2 occupied	1 nest	1 nest
Nestlings number	4	3 - 2	9 nestlings	3 - - 3	6 nestlings	3 nestlings	4 nestlings

We have no accurate data on species such as *Egretta garzetta*, *Tringa ochropus*, *Actitis hypoleucos* etc. We have not noticed any nestlings or nests. The presence of dozens of specimens, both adults and juveniles, in both years, indicated that they are potentially breeding species.

The research of every species can bring new information related to the feeding behavior, reproduction, defense etc., which is why the study should be continued, especially since Urzicuța locality is situated in a plain area, and it has a very surprising fauna.

### CONCLUSIONS

The aquatic ecosystems of Urzicuța locality (Dolj County) provide trophic resources, refuge and nesting place for a large number of aquatic birds. Out of the 43 aquatic species distinguished, 22 are breeding species and possibly breeding species.

The number of species and specimens vary annually and seasonally. The periods corresponding to migration (spring - autumn) are the richest in terms of avifauna. The climate and trophic resources remain the main factors which determine the presence or absence of birds in the village in certain periods, and the numerical variations within the same species.

On different areas of Urzicuța Lake, were observed in the serotinal season significant temporary agglomerations of some aquatic species.

As a result of the conducted study, 18 observed species can be found in Annex 1 of the Birds Directive 2009/147/EC, being used as indicators in the designation the Special Protection Areas (SPA) and also for conserving biodiversity. These are: *Phalacrocorax pygmaeus*, *Ixobrychus minutus*, *Nycticorax nycticorax*, *Ardeola ralloides*, *Egretta garzetta*,

*Ardea alba*, *Ardea purpurea*, *Ciconia nigra*, *Ciconia ciconia*, *Plegadis falcinellus*, *Aythya nyroca*, *Circus aeruginosus*, *Porzana porzana*, *Himantopus himantopus*, *Philomachus pugnax*, *Tringa glareola*, *Sterna hirundo*, *Chlidonias hybridus*.

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**NEW DATA ABOUT THE BIRD FAUNA OF URZICUȚA LOCALITY  
(DOLJ COUNTY)**

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*Keywords: birds, phenology, diversity, status of conservation*

**ABSTRACT**

*In this paper there are presented new information on the avifauna of Urzicuța locality-situated in the plain of Dolj county, information obtained after the study conducted in the period July-August 2012, March–August 2013 and continued until September 2014. Thus, the list of the highlighted birds reached 93 species distributed in 15 orders and 35 families. Due to existing protectionist laws, most birds in the investigated area have a secure status at European level (are safe).*

**INTRODUCTION**

Urzicuța locality is situated in the southern half of Dolj County (in the south-west of Romania), in the Băileștilor Plain at 44.2 degrees north latitude and 23.55 degrees east latitude (Câșlaru, 2011). It is formed of two villages: Urzicuța and Urzicuța Mare and has a total surface of 6072 ha.

The hidrography of the commune is represented by the Desnățui River in the eastern part, by the Baboia creek in the south part, by the chain of ponds/lakes Urzicuța and Ionele Lake (in the southern part).

The commune has no forests. The forest located in the eastern part, near Urzicuța Mare is included administratively in the neighbouring village, Giurguța. Therefore many forest birds are found in Urzicuța Mare. The existing wood vegetation is found along the roads and streets, in built-up areas, in some areas along the Urzicuța Lake etc. The spontaneous herbaceous vegetation (natural) and the controled one (cultivated with wheat, maize, barley, oats, sunflower, potatoes, sugar beet, etc.) prevail. The entire agricultural area has 5610 ha. The meadows (164 ha), with a steppe aspect, consist of xerophile grass. The vegetation of the Urzicuța Lake is diversified (*Phragmites communis*, *Typha latifolia*, *Carex sp.*, *Salix sp.* etc., various natant and submerged plants).

The studied area drew the attention by the variety of terrestrial, aquatic and strong anthropic ecosystems, which are in a close interdependence and between which there is a continuous transfer of substance and energy. The commune has the aspect of an anthropic

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steppe with isolated trees. Also, the Ionele Lake located within the commune, was declared a protected area of national interest (Code 2396, Law 5/2000) due to the physicochemical properties of water and due to the mud with curative effects. It has a balnear potential. In terms of ornithology, the study area has a variety of birds, some with a conservation interest at European level, which represent landmarks in the designation of Special Protection Areas (SPA), according to the Birds Directive 2009/147/EC. All these features determined us to continue the study of the fauna of Urzicuța locality.

The avifaunistic information in this paper complements the monitoring phase started last year (Bălescu & Ridiche, 2013). The subsequent research aimed: to clarify the phenological categories of species in the studied area (breeding/non-breeding, migratory/sedentary), to present information about the biology of terrestrial species; relative number of individuals belonging to certain terrestrial bird species; to present the conservation status of the species observed in the field. In the study, we will not insist on the aquatic birds, as they are the subject of another paper (Bălescu, 2014 – to be published).

### MATERIAL AND METHODS

The classic methodology was taken into account when monitoring the bird species and population. The field trips previously started (July and August 2012, March – August 2013) continued until September 2014 and took place during all seasons of the year. The direct observations (from fixed points or from movement), the species determinations, the photography and filming of various shots of bird life were conducted using the following equipment: binoculars (Norconia 10x50), guides for determining species (Peterson et al., 1989; Bruun et al., 1999), Canon Sx40HS digital camera and Panasonic SDR-H20 video camera. The bird list was supplemented with information from the locals obtained from field surveys. Many species were identified by songs. The species were classified using the Nomenclature of Birds in Romania (Szabó-Szeley & Baczó, 2006).

### RESULTS AND DISCUSSIONS

Within the terrestrial, aquatic, semi-aquatic and anthropic habitats of Urzicuța locality 93 bird species were identified, which are distributed in 15 orders and 35 families (Table 1). The previous obtained list was supplemented by another 13 species (*Phalacrocorax pygmaeus*, *Phalacrocorax carbo*, *Ardeola ralloides*, *Ardea alba*, *Ardea purpurea*, *Aythya ferina*, *Circus aeruginosus*, *Falco subbuteo*, *Tringa glareola*, *Riparia riparia*, *Phoenicurus ochruros*, *Muscicapa striata*, *Erithacus rubecula*). The bird list is always subject to changes due to the environmental and anthropogenic factors.

The most numerous observed species belong to the Passeriformes order: 35 species distributed in 14 families. The dominance of this order of species is due to the fact that the Passeriformes have the highest percentage in Romania. A large group of birds is the one of aquatic and semi-aquatic birds that is well represented in the area. Of the 43 aquatic species, the most numerous species belong to the Charadriiformes order (13 species), Ciconiiformes order (10 species) and Anseriformes with 8 species.

For some species, the observations are insufficient to draw definite conclusions on the phenological category and on the breeding/non-breeding status. The seasons dynamics, the environmental conditions can always influence their status. For example: if in 2013 the Hoopoe was a passage bird, in 2014 breeding in Urzicuța Mare village, in the trees neighbouring the agricultural land. It was seen at the edge of maize plantations in search of food.

In general the migratory species prevail: 40 summer visitors (in this category there were included the species which do not nesting in the area, but which are observed in the

summer in search of food and rest); 4 winter visitors; 21 are passage birds (seen in migration during spring and/or autumn), 2 species with irregular or accidental visits (they are seen seasonally, do not breeding in the studied area). The sedentary birds include 26 species (Table 1). Few species are strictly sedentary (*Streptopelia decaocto*, *Columba livia domestica*, *Passer domesticus*, *P. montanus* etc.). Many species are partially migratory (a part of the population of species leaves the nesting place and the other part remains during winter). Other species are erratic (after nesting, they move to other areas in search of food, such as woodpeckers, etc.).

The avifaunistic observations showed that most species of birds have been recorded in the prevernal season (March, April), when the spring migration occurs. In the serotinal season (July 15 – September 15) there is noticed an increase in the number of individuals and a concentration of the aquatic birds population. The winter is the season with the lowest number of species. The vernal and aestival season is characterized by a high number of summer visitor species and sedentary species. This confirms that the studied area offers proper conditions for breeding, feeding and resting.

Of the total recorded species, 59 are nesting and possible nesting species. In terms of phenology, 33 species are summer visitors and 26 species are sedentary (partially migratory). Not all the species are nesting in the same year.

In the research there were noticed many behaviours among birds, especially those related to breeding, feeding and habitat occupation etc. Since it is not possible to address all the problems, a few things will be mentioned. Regarding *Motacilla flava* species, which dominates in comparison to *Motacilla alba*, is a nesting bird in the area, formed of three subspecies: *Motacilla flava feldegg*, *M. f. flavissima* and *M. f. thunbergi*. The most important colony of Common House -Martin (*Delichon urbica*) was observed at the roof of the Cultural Centre in the center of Urzicuța village, where 27 pairs of birds nested in 2014. They hatched twice a year (May and August). The Pheasant (*Phasianus colchicus*) not nesting in the studied area. It was rarely seen on the agricultural land of Urzicuța Mare. During summer, *Galerida cristata* was seen in small number of specimens, 2-3 at the edge of the agricultural land. A higher number of specimens (4-8) have been reported in the cold season, in the anthropogenic habitat, roadsides, and along the Urzicuța Lake.

A characteristic of the area is the nesting of three species of falcons: the Red-footed Falcon - *Falco vespertinus* (a vulnerable species at European level), the Common Kestrel - *Falco tinnunculus*, and the Eurasian Hobby – *Falco subbuteo* (observed in this year: 2014) in the nests of Rooks (*Corvus frugilegus*). The nests are placed (at a height of about 9-15 m) in the poplars by the road in the immediate vicinity of the Urzicuța Lake. In 2014 we counted 35 nests which could be used. The competition between the falcons and *Corvus frugilegus* to occupy the nests begins in March, when *Falco tinnunculus* appears (on March 24, 2014 it was already in the area). *Falco vespertinus* appeared in the third week of April. On July 26, 2014 there were noticed juveniles of *Falco vespertinus* in 4 nests, of *Falco tinnunculus* in 5 nests, and of *Falco subbuteo* in a nest. We noticed 2 and 3 juveniles in the nest. The breeding of three species in the studied area is the result of good living conditions: they find the ready-made nests by the Corvidae and food is readily available both from the aquatic ecosystems and from the terrestrial agricultural ones. The competition with the crows and with other species continues after the nesting too. We have seen the spectacular hunting of the prey: from various insects (Diptera), frogs (*Pelophylax* sp.), lizards (*Lacerta taurica*, *L. viridis*), to mammals (*Ratus* sp., *Microtus arvalis*) etc. The Red-footed Falcon was seen in the afternoon (between 12 and 14 o'clock) with the prey in its beak or talons, in the nests with nestlings and juveniles. About these species, as well as

on the measures of maintaining the nesting population of falcons we will write further studies with new details.

Currently the birds are protected by numerous conventions, laws and directives. In the studied area, each species, from the observed ones, is included at least on one of the following lists: SPEC List (Species of European Conservation Concern - species subject to conservation concerns in Europe); The Birds Directive 2009/147/EC on the conservation of wild birds; the Bern Convention on the conservation of wildlife and natural habitats in Europe, to which Romania adhered by the Law 13 of 11 March 1993; the Bonn Convention on the conservation of migratory species of wild animals, ratified by Romania with the Law 13 of 8 January 1998; the Law 407/2006 - hunting and protection of hunting fund completed by the Law 197/2007; the Law 49/2011 for the approval of the Government Ordinance No 57/2007 regarding the regime of the natural protected areas, the conservation of natural habitats of wild fauna and flora etc. (Table 1). According to SPEC categories, the majority of bird species observed in Urzicuta locality (54 species) have a favourable status of conservation, being secure (Non Spec E and Non Spec category) (Table 1).

The presence of a large and diverse number of birds in natural and anthropogenic habitats of Urzicuta locality is the consequence of their adaptation to human actions, i.e. to the noise of sowing and threshing machines. After the plowing of the agricultural land, Laridae, Alaudidae, Corvidae etc. were observed on the field in search of food. The residents are excited about the variety of birds on the Urzicuta lake, located in the centre of the village. That is why, there were no interventions in the natural aquatic ecosystem. Along time, the surface of marsh vegetation spread in some areas. From the received information and own observations we noticed that species whose hunting is allowed – according to the Law No. 407/2006 (Table 1) are not subject to hunting in the studied area. Among the anthropogenic factors that have a negative effect on birds we may mention the grazing (the cattle destroy the existing nests on the ground, disturb the nesting when they go in the water), the intensive agriculture, the lack of forest etc. The most important cause of changing the population dynamics remains the ecological factor.

It is necessary for the population to be aware of the importance of these species in the ecosystems in which they live, and it is also necessary the transformation of the passive attitudes of locals into an active one regarding the current issues of protecting the habitats and the animals in order to maintain their biodiversity.

## CONCLUSIONS

The systematic list of bird species observed in the July-August 2012, March 2013-September 2014 period in Urzicuta locality includes 93 species distributed in 15 orders and 35 families.

The diversity of bird species recorded in a plain area, with no forest, is the result of the adaptation of birds to terrestrial, aquatic and anthropic habitats (being in a close interdependence), which provides favourable conditions for shelter, feeding and breeding.

In the studied area the migratory species prevail over the sedentary ones. For the 43 identified species, the aquatic species are representative. The number of species and specimens varies seasonally depending on the climate changes or trophic source. The effect of anthropic activities on birds is low.

Of the 59 nesting and possible nesting species reported to date, 12 species are included under the Birds Directive (Annex I): *Ixobrychus minutus*, *Nycticorax nycticorax*, *Egretta garzetta*, *Ciconia ciconia*, *Aythya nyroca*, *Falco vespertinus*, *Circus aeruginosus*, *Porzana porzana*, *Himantopus himantopus*, *Chlidonias hybridus*, *Dendrocopos syriacus*, *Lanius collurio*.



The conservation status of the bird species thus identified is regulated by several national and international law and convention, and the Birds Directive.

The concentration of a large number of valuable bird species in Urzicuța locality lead to the development of conservation measures by decision makers in accordance with the reality on the field in order to maintain and to protect the diversity of species.

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Table 1

Synoptic picture of the bird species found in the Urzicuța locality during July-August 2012, March 2013–September 2014  
(List systematic, biological and ecological characteristics, protection status)

No crt	Order, Family, Species	Ecologic type (habitat)	Phenology type Urzicuța	Observation	SPEC status (2004)	Protection statut				
						Birds Directive	Bern Conv.	Bonn Conv.	Law 407// 2006	Law 49/ 2011
	1.Ord. PODICIPEDIFORMES									
	1. Fam. Podicipedidae									
1	<i>Tachybaptus ruficollis</i>	aquatic	SV, B	frequent	NS		A II		A2	A4B
2.	<i>Podiceps cristatus</i>	aquatic	P	rare	NS		A III		A2	
	2. Ord. PELECANIFORMES									
	2. Fam. Phalacrocoracidae									
3.	<i>Phalacrocorax carbo</i>	aquatic	WV, P	rare; stationed in winter	NS		A III		A1	
4.	<i>Phalacrocorax pygmaeus</i>	aquatic	SV	for feeding and resting	1	Annex I	A II		A2	A3
	3. Ord. CICONIIFORMES									
	3. Fam. Ardeidae									
5.	<i>Ixobrychus minutus</i>	aquatic	SV, B	constant	3	A I	AII		A2	A3
6.	<i>Nycticorax nycticorax</i>	aquatic	SV, B	constant	3	A I	AII		A2	A3
7.	<i>Ardeola ralloides</i>	aquatic	SV	come for feeding and resting	3	A I	AII		A2	A3
8.	<i>Egretta garzetta</i>	aquatic	SV, PB	constant	NS	A I	AII		A2	A3
9.	<i>Ardea alba</i>	aquatic	SV, WV	come for feeding and resting; the wintering	NS	A I	AII		A2	A3
10.	<i>Ardea cinerea</i>	aquatic	SV, WV	come for feeding and resting; stationed during winter	NS		AIII		A2	
11.	<i>Ardea purpurea</i>	aquatic	SV	come for feeding and resting	3	A I	AII		A2	A3
	4. Fam. Ciconiidae									
12.	<i>Ciconia nigra</i>	aquatic	P	observed in August; in passage; rare	2	A I	AII		A2	A3
13.	<i>Ciconia ciconia</i>	aquatic	SV, B	constant; anthropogenic species	2	A I	AII		A2	A3
14.	<i>Plegadis falcinellus</i>	aquatic	P	rare	3	A I	AII		A2	A3
	5. Fam. Threskiornithidae									
	4. Ord. ANSERIFORMES									
	6. Fam. Anatidae									
15.	<i>Cygnus olor</i>	aquatic	PM, B	frequent	NSE		AIII		AII	A2
16.	<i>Anas crecca</i>	aquatic	WV, P	frequent; observed in winter and passage	NS		AIII		AII	A1
17.	<i>Anas platyrhynchos</i>	aquatic	PM, B	common	NS		AIII		AII	A1

18.	<i>Anas acuta</i>	aquatic	P	in passage of spring	3		AIII	AII	A1	
19.	<i>Anas querquedula</i>	aquatic	P, SV, B	constant; of nesting in 2014	3		AIII	AII	A1	
20.	<i>Anas clypeata</i>		P	more numerous in passage	3		AIII	AII	A1	
21.	<i>Aythya ferina</i>	aquatic	P	in passage of autumn	2		AIII	AII	A1	
22.	<i>Aythya nyroca</i>	aquatic	SV, B	constant	1	A I	AIII	AII	A2	A3
	5. Ord. ACCIPITRIFORMES									
	7. Fam. Accipitridae									
23.	<i>Circus aeruginosus</i>	aquatic	SV, PB	constantly observed in 2014	NS	AI	AII	AII	A2	A3
24.	<i>Accipiter gentilis</i>	terrestrial	WV, P	rare	NS		AII	AII	A2	
25.	<i>Buteo buteo</i>	terrestrial	ApN	came for food; with irregularly appearances	NS		AII	AII	A2	
	6. Ord. FALCONIFORMES									
	8. Fam. Falconidae									
26.	<i>Falco tinnunculus</i>	terrestrial	SV, B	constant; five nesting pairs in 2014	3		AII	AII	A2	A4B
27.	<i>Falco vespertinus</i>	terrestrial	SV, B	constant; four nesting pairs	3	AI	AII	AII	A2	A3
28.	<i>Falco subbuteo</i>	terrestrial	SV, B	a pair in 2014	NS		AII	AII	A2	A4B
	7. Ord. GALLIFORMES									
	9. Fam. Phasianidae									
29.	<i>Perdix perdix</i>	terrestrial	PM, B	+6 ind.; Urzica Mare; rare	3		AIII		A1	
30.	<i>Coturnix coturnix</i>	terrestrial	SV, B	frequent; +4 ind.; Urzica Mare;	3		AIII	AII	A1	
31.	<i>Phasianus colchicus</i>	terrestrial	Ap.N	not nesting; rare appearances	NS		AIII		A1	
	8. Ord. GRUIFORMES									
	10. Fam. Rallidae									
32.	<i>Porzana porzana</i>	aquatic	SV, P, B	discreetly; rare	NSE	A I	AII	AII	A2	A3
33.	<i>Gallinula chloropus</i>	aquatic	PM, B	common	NS		AIII		A1	
34.	<i>Fulica atra</i>	aquatic	PM,B	common	NS		AIII		A1	
	9. Ord. CHARADRIIFORMES									
	11. Fam. Recurvirostridae									
35.	<i>Himantopus himantopus</i>	aquatic	SV,B	constantly observed	NS	A I	AII	AII	A2	A3
	12. Fam. Charadriidae									
36.	<i>Charadrius dubius</i>	aquatic	P	not is constant observed	NS		AII	AII	A2	
37.	<i>Vanellus vanellus</i>	aquatic	SV, B	constant	2		AIII	AII	A2	
	13. Fam. Scolopacidae									
38.	<i>Philomachus pugnax</i>	aquatic	P	frequent; in serotinal season	NSE	A I	AIII	AII	A2	
39.	<i>Limosa limosa</i>	aquatic	P	frequent; in serotinal season	2		AIII	AII	A1	
40.	<i>Tringa totanus</i>	aquatic	P	frequent; in serotinal season	2		AIII	AII	A2	
41.	<i>Tringa stagnatilis</i>	aquatic	P	frequent; in August	NS		AII	AII	A2	
42.	<i>Tringa glareola</i>	aquatic	P	observed in August	3	A I	AII	AII	A2	A3

43.	<i>Tringa ochropus</i>	aquatic	P, SV, PB	frequent	NS	AII	AII	A2	
44.	<i>Actitis hypoleucos</i>	aquatic	P, SV, PB	frequent	3	AII	AII	A2	A4B
	14. Fam. Laridae								
45.	<i>Chroicocephalus ridibundus</i>	aquatic	PM, B	constant	NSE	AIII		A2	
46.	<i>Larus cachinnans</i>	aquatic	P	autumn is numerous in specimens	NSE			A2	
	15. Fam. Sternidae								
47.	<i>Sterna hirundo</i>	aquatic	P	rare	NS	A I	AII	A2	A3
48.	<i>Chlidonias hybridus</i>	aquatic	SV, B	common; constant	3	A I	AII	A2	A3
	10. Ord. COLUMBIFORMES								
	16. Fam. Columbidae								
49.	<i>Columba livia domestica</i>	terrestrial	S, B	common	NS				
50.	<i>Streptopelia decaocto</i>	terrestrial	S, B	common	NS	AIII		A2	
	11. Ord. CUCULIFORMES								
	17. Fam. Cuculidae								
51.	<i>Cuculus canorus</i>	terrestrial	SV, B	constant	NS	AIII		A2	
	12. Ord. STRIGIFORMES								
	18. Fam. Strigidae								
52.	<i>Athene noctua</i>	terrestrial	S, B	rare; in localities	3	AII		A2	A4B
53.	<i>Asio otus</i>	terrestrial	S, B	rare; in localities	NS	AII		A2	
	13. Ord. CORACIIFORMES								
	19. Fam. Meropidae								
54.	<i>Merops apiaster</i>	terrestrial	P	Ionele lake area; serotinal season; 5 individuals in flight	3	AII	AII	A2	A4B
	20. Fam. Upupidae								
55.	<i>Upupa epops</i>	terrestrial	SV, P, B	frequent; Urzica Mare; +2 ind.	3	AII		A2	A4B
	14. Ord. PICIFORMES								
	21. Fam. Picidae								
56.	<i>Picus viridis</i>	terrestrial	S, Er, B	1-2 ind.	2	AII		A2	A4B
57.	<i>Dendrocopos major</i>	terrestrial	S, Er, B	adults and juveniles, 3-4 ind.	NS	AII		A2	
58.	<i>Dendrocopos syriacus</i>	terrestrial	S, Er, B	adults and juveniles, 4-5 ind.	NSE	A I		A2	A3
	15. Ord. PASSERIFORMES								
	22. Fam. Alaudidae								
59.	<i>Galerida cristata</i>	terrestrial	S, B	rare; 2-8 ind.	3	AIII		A2	
60.	<i>Alauda arvensis</i>	terrestrial	SV, B	tens of exemplary	3	AIII		A1	
	23. Fam. Hirundinidae								
61.	<i>Riparia riparia</i>	terrestrial	P	10-16 ind.; Ionele lake area; serotinal season	3	AII		A2	
	62.								
62.	<i>Hirundo rustica</i>	terrestrial	SV, B	common, anthropogenic species,	3	AII		A2	

63.	<i>Delichon urbica</i>	terrestrial	SV, B	tens of exemplary common; anthropogenic species; tens of exemplary	3	AII	A2	
	24. Fam. Motacillidae							
64.	<i>Anthus campestris</i>	terrestrial	SV	rare; +2 ind.; Urzica Mare	3	AI	A2	A3
65.	<i>Motacilla flava</i>	terrestrial	SV, B	constant; +20 ind.	NS	AII	A2	A4B
66.	<i>Motacilla alba</i>	terrestrial	SV, B	rare; not constantly observed; +5 ind.	NS	AII	A2	A4B
	25. Fam. Turdidae							
67.	<i>Erythacus rubecula</i>	terrestrial	WV	willows and poplars in the Urzicuța lake; 1-2 ind.	NSE	AII	A2	A4B
68.	<i>Luscinia megarhynchos</i>	terrestrial	P	1-3 ind.; sound identification	NSE	AII	A2	
69.	<i>Phoenicurus ochruros</i>	terrestrial	P	orchards, gardens; Urzica Mare; 1-3 ind.	NS	AII	A2	A4B
70.	<i>Saxicola rubetra</i>	terrestrial	SV	rare; edge agricultural crop; Urzica Mare; +2 ind.	NSE	AII	A2	
	26. Fam. Sylviidae							
71.	<i>Locustella luscinioides</i>	aquatic	SV,B	frequent; species of reed	NSE	AII	A2	A4B
72.	<i>Acrocephalus scirpaceus</i>	aquatic	SV, B	frequent; reed species	NSE	AII	A2	
73.	<i>Acrocephalus arundinaceus</i>	aquatic	SV, B	common; reed species; tens of specimens;	NS	AII	A2	
74.	<i>Sylvia communis</i>	terrestrial	SV, B	frequent; 6 – 10 ind.;	NSE	AII	A2	
75.	<i>Sylvia atricapilla</i>	terrestrial	P	frequent in the passage; +4 ind.	NSE	AII	A2	
76.	<i>Sylvia curruca</i>	terrestrial	P	frequent in the passage; +6 ind.	NS	AII	A2	
77.	<i>Phylloscopus collybita</i>	terrestrial	P	frequent observed in the passage +4 ind.	NS	AII	A2	A4B
	27. Fam. Muscicapidae							
78.	<i>Muscicapa striata</i>	terrestrial	OV, B	frequent; 2-5 ind.	3	AII	A2	A4B
	28. Fam. Paridae							
79.	<i>Parus major</i>	terrestrial	S, B	common; tens of exemplary; more numerous autumn and winter	NS	AII	A2	
	29. Fam. Oriolidae							
80.	<i>Oriolus oriolus</i>	terrestrial	SV, B	frequent; 4-5 breeding pairs	NS	AII	A2	A4B
	30. Fam. Laniidae							
81.	<i>Lanius collurio</i>	terrestrial	SV, B	frequent; adults and juvenile; 8-10 ind.	2	AI	A2	A3
	31. Fam. Corvidae							
82.	<i>Pica pica</i>	terrestrial	S, B	common; 3-16 ind.	NS		A1	

83.	<i>Corvus monedula</i>	terrestrial	S, B	common; anthropogenic species; tens and hundreds of exemplary	NSE			A1
84.	<i>Corvus frugilegus</i>	terrestrial	S, B	common; tens and hundreds of exemplary	NS			A1
85.	<i>Corvus cornix</i>	terrestrial	S, B	common; effectives increase autumn and winter	NS			A1
	32. Fam. Sturnidae							
86.	<i>Sturnus vulgaris</i>	terrestrial	PM, B	common; overnight in reed, in large numbers	3			A1
	33. Fam. Passeridae							
87.	<i>Passer domesticus</i>	terrestrial	S, B	common, anthropogenic species, tens and hundreds of exemplary	3			A2
88.	<i>Passer hispaniolensis</i>	terrestrial	SV, B	frequent; breeding at nests of storks	NS		AIII	A2
89.	<i>Passer montanus</i>	terrestrial	S, B	common, anthropogenic species, tens and hundreds of exemplary	3		AIII	A2
	34. Fam. Fringillidae							
90.	<i>Fringilla coelebs</i>	terrestrial	PM, E, B	+6 ind.; number increases in autumn and winter with the arrival of other specimens	NSE		AIII	A2
91.	<i>Carduelis chloris</i>	terrestrial	S, B	rare; 6-8 ind.	NSE		AII	A2
92.	<i>Carduelis carduelis</i>	terrestrial	S, Er, B	frequent; 5-12 ind.	NS		AII	A2
	35. Fam. Emberizidae							
93.	<i>Emberiza calandra</i>	terrestrial	PM, B	common in agroecosystems; with tens of exemplary	2		AIII	A2

**Legend:**

**Phenologic status:** SV – summer visitor; WV – winter visitor, P – passage species, S – sedentary species, PM – partially migratory species, Er – erratic, B – breeding species; PB – probable breeding species. **Own observation:** in=individuals/exemplary.

**Spec Category:** 2 species included in SPEC 1 category = species of global conservation concern (*Phalacrocorax pygmaeus*, *Aythya nyroca*); 9 species included in SPEC 2 category = concentrated in Europe and with an unfavourable conservation status; 28 species in SPEC 3 category = not concentrated in Europe but with an unfavourable conservation status; 16 species in NSE category=species favourable conservation status are concentrated in Europe; 38 species in NS=NonSPEC category= have a favourable conservation status are not concentrated in Europe.

**Protection Statute:** A=Annex. **Bird Directive:** Annex I – species subject of special conservation measures concerning their habitat in order to ensure their survival and reproduction in their area of distribution (22 species in the investigated area are included in this Annex); **Bern Convention.** Annex II – strictly protected species (56 species), Annex III –protected species (29 species); **Bonn Convention:** according to the Annex II – 35 species are protected; **Law 407/2006:** Annex I= 19 species in the investigated area are subject hunting at certain periods; according to the Annex II – 72 protected species (not hunting). **Law 49/2011:** Annex 3 = species whose conservation requires the designation of Special Protection Areas (21 species) : Annex 4 B= species of national interest - requires strict protection (20 species).

**HISTO-ANATOMICAL STUDY OF *SIDERITIS SCARDICA* GRISEB.  
(*LAMIACEAE*) SPECIES**

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**Keywords:** *Sideritis scardica* Griseb., histo-anatomy, structure, *Sideritis herba*

**ABSTRACT**

*This paper presents the histo-anatomical study on the vegetative organs of Sideritis scardica Griseb. (Lamiaceae) species, given the development of pharmacognostic expertise. It also highlights the importance of Sideritis herba as a widely used natural product in the ethnopharmacology and phytotherapy of Balkan Peninsula.*

**INTRODUCTION**

*Sideritis scardica* Griseb. ironwort or (shepherd) mountain tea, *Lamiaceae* family, is a perennial, endemic and cultivated species in the Balkan Peninsula (Bulgaria, Greece, Albania, Macedonia) (Tadić et al. 2012b; Todorova & Trendafilova 2014).

From the phytochemical point of view, the medicinal product *Sideritis herba* contains a large amount of active principles such as essential oil rich in  $\alpha$ - and  $\beta$ -pinene, oct-1-en-3-ol, phenylacetaldehyde,  $\beta$ -bisabolene, benzyl benzoate (Kostadinova et al. 2007; Tadić et al. 2012b; Trendafilova et al. 2013), flavonoids – rutin and hypolaetin derivatives (Janeska et al. 2007; Samanidou et al. 2012), polyphenolic acids (Janeska et al. 2007; Petreska et al. 2011; Petreska Stanoeva et al. 2012; Samanidou et al. 2012), iridoids, diterpenoids (González-Burgos et al. 2011), triterpenoids, sterols, catechic tannin (Gîrd et al. 2006; González-Burgos et al. 2011; Samanidou et al. 2012), purine alkaloids (caffeine), heteroglycans (mucilages), coumarins, lignans, simple carbohydrates, fatty acids, proteins, enzymes, organic acids, vitamins, mineral salts (Gîrd et al. 2006; González-Burgos et al. 2011; Samanidou et al. 2012; Todorova & Trendafilova 2014).

*Sideritis herba* is widely used in the ethnopharmacology of Balkan Peninsula for its useful pharmacological properties: anti-bronchitis, antitussive, antiasthmatic (Todorova & Trendafilova 2014), antimicrobial, cicatrizing (González-Burgos et al. 2011; Tadić et al. 2012b), antioxidant and protective against cellular oxidative stress (Danesi et al. 2013; Todorova & Trendafilova 2014), cytotoxic, anti-inflammatory, analgesic, gastroprotective,

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antiulcerative, anticonvulsant, spasmolytic (González-Burgos et al. 2011; Tadić et al. 2012a), antidepressive acting on monoaminergic neurotransmission (Knörle 2012).

In the specialty papers, there are scarce and incomplete data concerning *S. scardica* histo-anatomy (Gîrd et al. 2006). For this reason and taking into account the medicinal relevance of the natural product *Sideritis herba*, our study contributes to the progress of the pharmacognostic expertise.

### MATERIAL AND METHODS

The vegetal material was harvested in August 2014 from *S. scardica* plants in blossom cultivated in Trigrad village, Rhodope Mountains, Bulgaria.

Biological material (roots, stems and leaves) fixation and preservation were made in 70% alcohol. Using botanical razor, the cross-sections were obtained by manually sectioning and after washing with distilled water, were submitted to the clarification process using 10% sodium hypochlorite (Javel water). Then, the clarifying agent was removed by washing with distilled water and the cross-sections were stained with Congo red–aniline sulfate mixture. Reactive induces various stains, depending on the chemical composition of cell membranes: pale red/blue for cellulose, yellow/brown for suberin, brown for lignified membranes (Andrei & Paraschivoiu 2003).

Stained and mounted cross-sections were analyzed on a Krüss binocular photon microscope at  $\times 4$ ,  $\times 10$  and  $\times 40$  objectives, and then photographed on a Nikon Eclipse 55i microscope with a high definition Nikon DS-Fi1 video camera. Images were captured, stored and analyzed using Image ProPlus 7 AMS software package (Media Cybernetics, Inc., Buckinghamshire, UK).

According to some classical authors (Toma & Rugină 1998), the description of microscopic cross-sections was achieved.

### RESULTS AND DISCUSSIONS

#### Structure of root

The root has a round shape and secondary structure, passed very early mainly on account of cambium. Rhizodermis with absorbent hairs exfoliate as much of the external bark. Exodermis is made of 1–2 layers with thick suberin walls. Cortical parenchyma is relatively thin with tangential-elongated cells. Phellogen that formed 2–3 layers of cork near the phloem tissue appear starting from one layer of endodermis and even of pericycle. The central cylinder is made of an external ring of secondary phloem and a central body of secondary xylem (Figure 1).

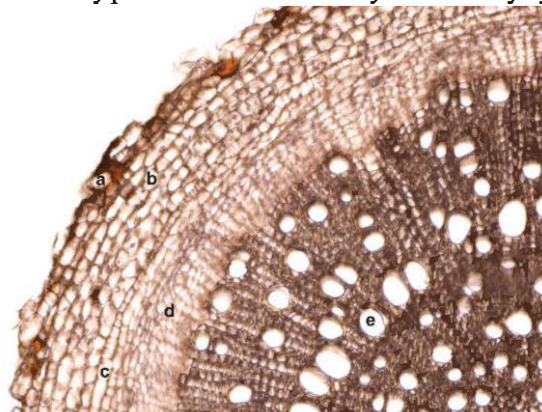


Figure 1. Cross-section through *S. scardica* root. Congo red–aniline sulfate staining,  $\times 100$ .  
(a) Exodermis; (b) Cortical parenchyma; (c) Endodermis; (d) Secondary phloem;  
(e) Secondary xylem.



The secondary phloem ring includes few conducting elements (sieve-tubes and annex cells) and numerous phloem fibers separated by phloem parenchyma cells and by parenchymatous-cellulosic medullary rays. The secondary xylem includes vessels with different diameter, irregularly dispersed in the fundamental libriform mass and separated by very large parenchymatous-lignified medullary rays. The centre of the root is compact, with several xylem vessels and many libriform fibers (Figure 2).

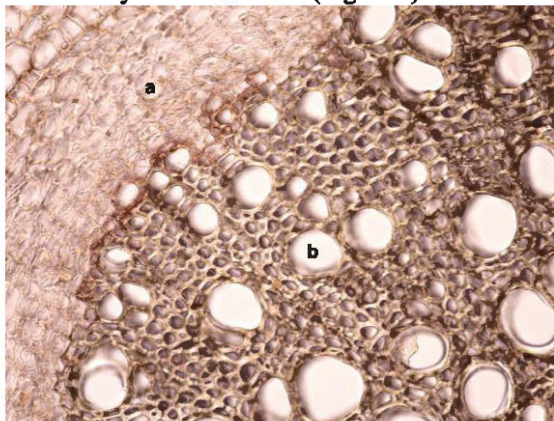


Figure 2. Cross-section through *S. scardica* root. Congo red–aniline sulfate staining,  $\times 200$ .  
(a) Secondary phloem; (b) Secondary xylem.

#### Structure of stem

The stem has round shape with four prominent ribs and secondary structure at the level of libero-ligneous conducting tissues (Figure 3).

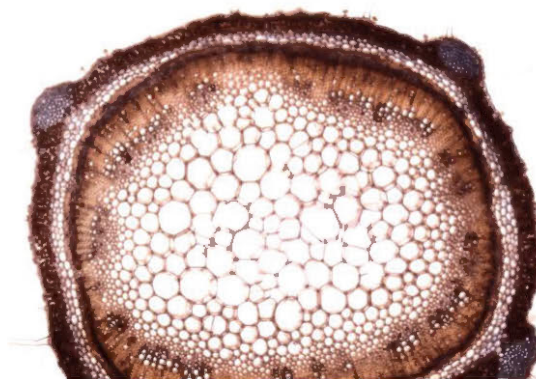


Figure 3. Cross-section through *S. scardica* stem, overview. Congo red–aniline sulfate staining,  $\times 40$ .

In cross-section, from the outside to the inside, the following histological sequence was highlighted:

The epidermis is made of one layer of flattened small cells, with thickened external and internal tangential walls and thin radial walls. Here and there, pluricellular tector hairs, stomates and glandular hairs are observed.

The angular collenchyma is well represented at the four ribs' level. Otherwise, chlorenchyma and cortical parenchyma to the inner part with periphloemic sclerenchyma caps were evidenced.

Organized in two concentric rings, the conducting tissues are predominantly from secondary origin.

The poorly developed phloem tissue is made of sieve tubes, few annex cells and phloem parenchyma. At this level, the medullary rays are pluricellular, uniseriate, cellulose.

The secondary xylem was developed starting from the libero-ligneous cambium. Into the libriform mass, the xylem tissue is well represented by secondary xylem vessels of big caliber arranged in radial rows. Few primary vessels are accompanied by xylem parenchyma. The medullary rays are pluricellular, uniseriate, lignified.

Into the central part of the stem, medullary parenchyma from meatus-type is well represented (Figures 4–6).



Figure 4. Cross-section through *S. scardica* stem. Congo red–aniline sulfate staining,  $\times 100$ .

(a) Epidermis with pluricellular tector hairs, stomates and glandular hairs; (b) Angular collenchyma; (c) Chlorenchyma (assimilatory parenchyma); (d) Inner (non-assimilatory) cortical parenchyma; (e) Periphloemic sclerenchyma caps; (f) Phloem; (g) Xylem; (h) Medullary parenchyma.

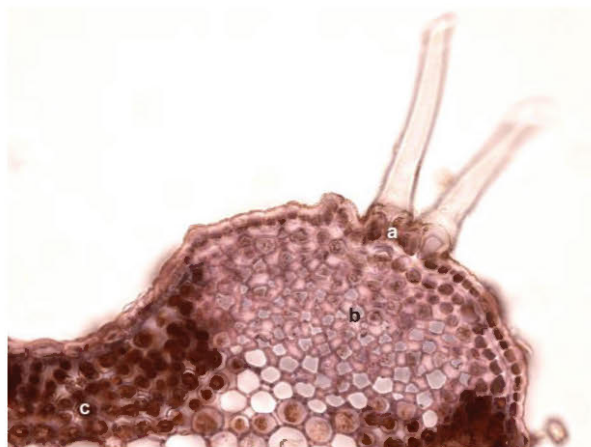


Figure 5. Cross-section through *S. scardica* stem. Congo red–aniline sulfate staining,  $\times 200$ .

(a) Epidermis with pluricellular tector hairs, stomates and glandular hairs; Angular collenchyma; (c) Chlorenchyma.

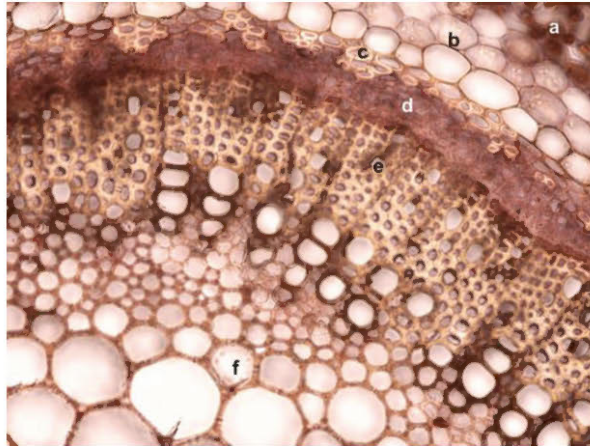


Figure 6. Cross-section through *S. scardica* stem. Congo red-aniline sulfate staining,  $\times 200$ .  
 (a) Chlorenchyma; (b) Inner cortical parenchyma; (c) Periphloemic sclerenchyma caps;  
 (d) Phloem; (e) Xylem; (f) Medullary parenchyma.

#### Structure of leaf's limb

The leaf's limb has bifacial dorsiventral structure from amphistomatic type. At the level of both upper and lower epidermis, anomocytic stomata are found (Figure 7).



Figure 6. Cross-section through *S. scardica* leaf's limb, overview. Congo red-aniline sulfate staining,  $\times 40$ .

The upper epidermis is made of one layer of heterodiametric big cells, with thickened external and internal tangential walls and thin radial walls. The external walls are bulged and covered by a thick cuticle. Pluricellular tector hairs, anomocytic stomata and glandular hairs are found from point to point.

The mesophyll is made of one layer of palisade tissue to the ventral side and 3–4 layers of lacunose parenchyma to the dorsal side. The palisade cells are tall, with big intercellular spaces. The lacunose parenchyma cells are small, with irregular shape and big aeriferous spaces. Collateral libero-ligneous fascicles are found into the leaf's mesophyll.

The lower epidermis is made of one layer of smaller cells compared to the upper epidermis, with thickened external and internal tangential walls, and thin radial walls. Long pluricellular tector hairs, anomocytic stomata and glandular hairs are found in patches.

The median rib is prominent to the dorsal side. One central big libero-ligneous conducting fascicle seated in a homogenous mass of fundamental parenchymatic tissue was found at the ribs' level. To the ventral side of the leaf's limb, the xylem tissue is made of xylem vessels disposed in radial rows and separated by cellulosic medullary rays. To the dorsal side, sieve tubes, annex cells and phloem parenchyma compose the phloem tissue (Figures 8 and 9).

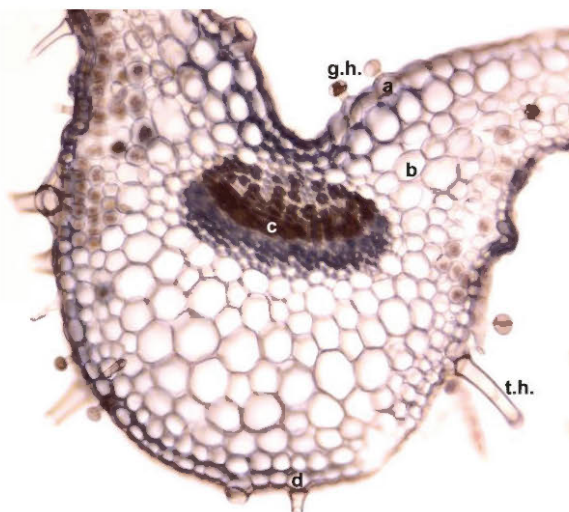


Figure 8. Cross-section through *S. scardica* leaf's median rib. Congo red–aniline sulfate staining,  $\times 100$ . (a) Upper epidermis; (b) Leaf's parenchyma; (c) Libero-ligneous fascicle; (d) Lower epidermis; (g,h) Glandular hair; (t.h.) Tector hair.

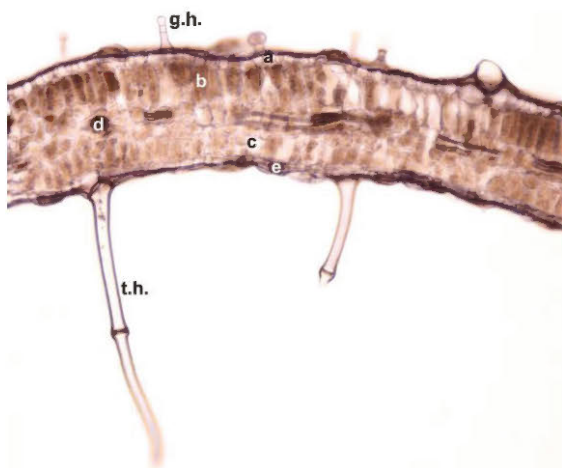


Figure 9. Cross-section through *S. scardica* leaf's limb. Congo red–aniline sulfate staining,  $\times 100$ . (a) Upper epidermis; (b) Palisade parenchyma; (c) Lacunose parenchyma; (d) Libero-ligneous fascicle; (e) Lower epidermis; (g,h) Glandular hair; (t.h.) Tector hair.

## CONCLUSIONS

The histo-anatomical study on the vegetative organs of *Sideritis scardica* species was achieved. The root has a round shape and secondary structure, due to the cambium activity. The stem exhibited round shape with four prominent ribs and secondary structure generated by libero-ligneous cambium. Prominent at the median rib's level, the leaf's limb has bifacial dorsiventral structure characterized by the presence of pluricellular tector hairs, anomocytic stomata and glandular hairs.

## ACKNOWLEDGMENTS

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## THE FIXATION OF THE INDIGOID CHROMOPHORE ON THE SUBSTRATUM OF A DIFFERENT TYLOSE TYPE

Bratu Magda Gabriela\*<sup>1</sup>

*Keywords: coloured thickening agent, tyloses, carmine-indigo, sulphanyl chloride.*

### ABSTRACT

*The study presents the synthesise of a coloured thickening agent obtained by covalent linking of tyloses (oxi-methylate celulosis which can contain other remnants of type  $\beta$  – hydroxieteric or type tip  $\alpha$  – carboxieteric) used as thickening agents with the indigoid chromophore using the carmine-indigo (the indigo acid 5,5' disulphonic)- acknowledged foodstuff colorant. The resulting additive, due to a high molecular mass, will have a reduced migration tendency and can safety be used to prepare foodstuffs with a much better texture and consistency. The colorant, fixed (immobilised) on tylosic support, would give on the consumer an enhanced desire and the psychological urge to try the product. The purpose of the study is to solve the two problems – the color and the texture of the food products by synthesing of the plyfunctional foodstuff colorants.*

### INTRODUCTION

In defining the structural-mechanical properties of a foodstuff product, the thickening agents have an important role because they improve the consistency of the products thanks to their capacity of thickening, jellification and stabilisation. By colouring these agents we solve two problems concerning the colour and the texture of the foodstuff product that contain them (Banu, 2000).

In the technology of using the coloured foodstuff additives the following aspects have to be known:

- their reaction, in the foodstuff products, with the physical-chemical factors of degradation during the processing and the depositing;
- the capacity of occlusion on some component support from the foodstuff products and of stability of the colouring as against the processes of migration;
- the modification of the balance hydrophilic-hydrophobic from their molecules depending on the nature of the substratum on which the occlusion is made;
- the increase of the stability of the additive coloured– support system by different auxiliary treatments (agitation, dilution, drying, sterilization, congelation, complexing with metallic ions);

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- the improvement of the qualities of conservation and stability on different intervals of temperature and humidity;
- the choice of the most favourable conditions of compatibilisation so that we eliminate the possibilities of migration of the colour on the supports that we use;
- the maintaining of the textural and colouristic properties induced in the foodstuff product during the processing and the depositing (Jieying & Shiping 1993, Wei et al. 1997).

### MATERIAL AND METHODS

The tests were realised on four varieties of tylose, from GmbH-England, a well-known firm recognised for the qualitative performances of the foodstuff additives it produces, such as: *TYLOPUR-MH-P2*- etherized normal compound, *TYLOPUR-MH-B-P2*- compound with a high degree of etherification, *TYLOPUR-C-P2*- etherized normal compound, *TYLOPUR-CB-P2*- compound with a high degree of etherification

Following, the four types of tylose will be represented schematically with the formulas 1-4, pointing out only the structural units of the remaining monomer. For the products presented the degree of poly-condensation is situated between 800-1000 (which is determined by the producer using the viscous-metric method).

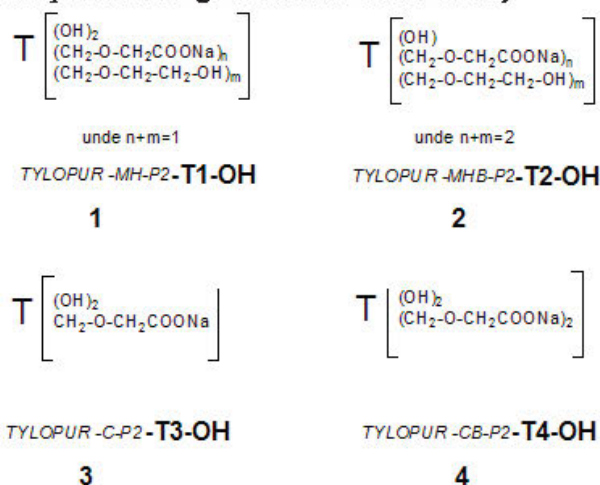


Figure 1. Types of tylose

As a reacting substance that includes the indigoid chromophore we have chosen the carmine-indigo (the indigo acid 5,5' disulphonic)- acknowledged foodstuff colorant. The alternative of functionalising/colouring of the tyloses tested in the laboratories is presented by the reaction presented in figure 2. The transformation of the disulphonic indigo acid 5,5' (carmine-indigo) (5) in the disulpho-chloride of the indigo acid 5,5' disulphuric (6) takes place by treating it with  $SOCl_2$  in molar proportion 1:1,05 in N-methyl pyrrolidone (the massic proportion of carmine-indigo: N-methyl pyrrolidone is of 1:15) at 70-90°C (exothermal reaction) in the presence of small quantities of N,N-di-methyl formamide as a catalyser. The reaction takes place in three or four hours after which the sulphinyl chloride in excess is distilled in void. We add tylose to the acid chloride solution (6) in N-methyl pyrrolidone. The mass of reaction is maintained at a temperature of 80-90°C and it is filtered in void. The precipitate obtained is washed repeatedly with cold ethanol and then dried. In all cases the filtrate is practically colourless ( $\lambda_{max}$  for indigo carmine = 600nm), which proves that the chromophore is fixed totally.



Data regarding the fixation of the indigoid chromophore on the substratum of a tylose type are presented in table no 1.

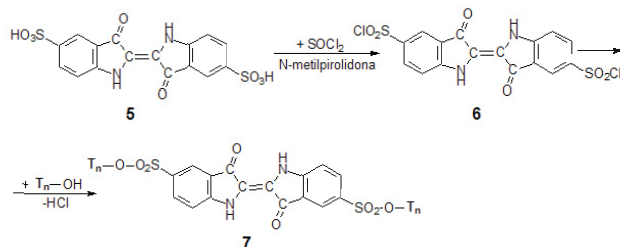


Figure 2. The functioning of the tylose with indigoid colorants

## RESULTS AND DISCUSSIONS

Analysing the dates in table no 1 it has been found that:

- the viscosity of the solutions of functionalised/coloured tylose is much smaller than that of the tyloses used as raw material. The values of the viscosity will be smaller if the proportion substratum: chromophor lowers. This is due to the powerful reticulation induced by the bifunctional reactant - the disulpho-chloride of the indigo acid 5,5' disulphuric.

- the proportion of colorant fixed on the substratum of tylose was established by determining the colorant that didn't react from the colourimetermination of the alcoholic tailings that resulted from the filtering of the coloured tylose ( $\epsilon=25000$ ); the conversions are big, the fixation of the indigoid chromophore on the substratum being quantitative.

## CONCLUSIONS

The functioning of the tylose of a foodstuff, cosmetic and pharmaceutical interest by treating it with disulpho-chlorure of the indigoid acid 5,5'disulphonic has proven to be a reaction that can be realised and that has great efficiency at a temperature of 70-80°C in a medium of N,N-dimethyl formamide (the conversion of the acid chloride is practically quantitative).

The sulpho-chlorination of the disulphonic indigoid acid 5, 5' takes place in vitro without previous intermediary isolation. 16 new coloured tiloses have been prepared with residual of an indigoid type (four different structural types). They haven't been mentioned in the professional literature, the presumptive coloured foodstuff additives being used as thickening agents. The coloured foodstuff additive introduced in the foodstuff compositions imprint to the products textural and colouristic qualities. Having a big molecular mass they eliminate the inconveniences connected to the tendency of migration of the colorant from a component to another in the foodstuff product.

The textural properties are also preserved in the conditions of the processing of the foodstuff products with different auxiliary treatments (agitation, dilution, drying, sterilisation, congelation, de-congelation). Thanks to the textural properties (the capacity of jellification, of thickening, of emulsifying, of foaming, of suspension, etc) induced in the foodstuff product, these colorant additives can be used for preparing the dietetic foodstuff product, with a low content of calories.

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Table 1

Experimental dates regarding the fixation of the indigoid chromophore on substrata of tyloze type

No.crt	Tyloze		g, N – methyl pyrrolidonne	Carmine-indigo		SOCl <sub>2</sub>		Viscosity in mPa·s, 2% in water Höppler, 20°		Code of product
	Tip	g		g	moli·10 <sup>5</sup>	g	moli·10 <sup>5</sup>	Reacting tyloze	Coloured tyloze	
1				0.1	26	0.04	27.3		600	B-MH-İ <sub>1</sub>
2		10	150	0.2	52	0.08	54.6	1000	500	B-MH-İ <sub>2</sub>
3				0.3	78	0.12	81.9		450	B-MH-İ <sub>3</sub>
4				1.5	390	0.6	409.5		400	B-MH-İ <sub>4</sub>
5				0.1	26	0.04	27.3		1900	B-MHB-İ <sub>1</sub>
6		10	150	0.2	52	0.08	54.6	3000	1400	B-MHB-İ <sub>2</sub>
7				0.3	78	0.12	81.9		1200	B-MHB-İ <sub>3</sub>
8				1.5	390	0.6	409.5		1100	B-MHB-İ <sub>4</sub>
9				0.1	26	0.04	27.3		6000	B-C-İ <sub>1</sub>
10		10	150	0.2	52	0.08	54.6	10000	5500	B-C-İ <sub>2</sub>
11				0.3	78	0.12	81.9		4800	B-C-İ <sub>3</sub>
12				1.5	390	0.6	409.5		4500	B-C-İ <sub>4</sub>
13				0.1	26	0.04	27.3		19000	B-CB-İ <sub>1</sub>
14		10	150	0.2	52	0.08	54.6	30000	18000	B-CB-İ <sub>2</sub>
15				0.3	78	0.12	81.9		16000	B-CB-İ <sub>3</sub>
16				1.5	390	0.6	409.5		15000	B-CB-İ <sub>4</sub>

## **ASSESSMENT OF HEAVY METALS IN THE GRAPE LEAVES**

Buzatu Gilda-Diana\*<sup>1</sup>

**Keywords:** *heavy metals, grape, leaves.*

### **ABSTRACT**

*Heavy metal pollution of agricultural soils is a major environmental problem that can affect plant productivity, food quality and human health. The aim of this study was to quantify the concentration of 19 heavy metals in the leaves of Pandur grape vine variety from CCDCPN Dăbuleni. As a conclusion, monitoring of heavy metal distribution data in soil and plant samples are very useful for becoming main references or guidelines in order to monitoring and avoid environmental pollution become worst in terms of quality of soil and also safety level for plants to be consumed.*

### **INTRODUCTION**

Heavy metals are naturally present in the environment, in high concentrations are toxic for plants, preventing their proper development. Heavy metals may enter the food chain as a result of their uptake by edible plants. So, determination of heavy metals in environmental samples is very important (Flora 2002).

Explaining the accumulation process of heavy metals in plant has led to wide research. Since 90's heavy metal pollution turns out to be a problem in some major viticulture regions. Comparative studies were done in the region of Brestnik-Plovdiv, in Jordan, in France, in Greece and Italy. Toxic effects of heavy metals have been well studied (Nakov et al. 2002, Flora 2002 Yang et al. 2002 Nordberg 2003 Massadeh et al. 2006).

Heavy metals affect many physiological and biochemical processes in plants such as photosynthesis, nitrogen assimilation, acceptance and metabolism of essential elements, the activity of enzymes, respiration and water regime, in addition to its influence on the growth and development of plants. According to Maxymiec (2007) heavy metal toxicity is one of the main forms of abiotic stress, which leads to effects on health risks in plants. The effect on plants, according to the author, may be a result of their direct toxic effects on membranes and photosynthetic apparatus, causing inhibition of the synthesis of organic substances and stimulating aging plants. It is proved that the replacement of the central metal atom as Mg in the chlorophyll molecule, with heavy metals (Cu, Cd, Ni, Zn, Pb), represents an important mechanism of damage to the plant (Prasad 1998).

All plants need an adequate supply of macro- and micro-elements in order to match their normal physiological and biochemical function. Besides basic mineral nutrients

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(N, P and K), some other elements (Mg, Fe, Zn, B) are considered to be essential for plant metabolic processes because they are cofactors and/or activators of many metabolic enzymes (Bergmann 1992, Marschner 1995). The nutrients mentioned above are required for vine life cycle too, from budburst to leaf senescence, and generally they limit grape production in world viticulture (Mullins et al. 1992).

Both nutrient deficiencies and excessive supply can both lead to physiological disorders. Nutrient deficiencies occur when plants cannot reach sufficient availability of nutrients for their basic metabolism in the surrounding environment, while in case of abundance of minerals, especially trace metals (e.g. Zn, Cu, Mg), sometimes toxicity phenomena can occur (He et al. 2005). Nutrient deficiency affects the physiology of different plant tissues, but normally leaf chlorosis is the most common symptom that can be observed (Bergmann 1992). Less mobile nutrients (e.g. Fe and Zn) are less available in new leaf tissues and shoot tips causing deficiency symptoms visible in the terminal younger part of the canopy. As opposite, mobile nutrients (e.g. Mg and K) can rapidly reach new tissues being less available for older leaves that normally highlight chlorosis in case of deficiency (Bergmann 1992, Marschner 1995).

Soil quality is an indicator of environmental quality and provides a comprehensive overview of the conditions of many features and processes that are in an ecosystem. In industrialized countries, heavy metals are one of the problems for the environment, because many of them are stable in soil for hundreds or even thousands of years (Bislimi et al. 2013).

## **MATERIAL AND METHODS**

Center for Research - Development for Plant Culture on Sands Dăbuleni operates mainly in the sandy soils of southern Oltenia, coarse-textured soils, colloidal clay content below 12% and low humus (0.1 to 1.6%). Sandy soils have low natural fertility, being poorly supplied with nitrogen and phosphorus and medium supplied with potassium.

Samples vine leaves were collected in June 2014, a total of 6 samples of leaves for Pandur variety; notated with P1 to P6. Leaf analysis was made up selecting eight-to-twelve leaves per sample (2-3 leaves x vine for each plant). After sampling, the blades were separated from the petioles and were washed up with tap water first and deionised water thereafter, in order to remove dust and other residues on the leaf surface.

Soil sample was analyzed in terms of agrochemical characteristics in accordance with the methodology cited in the literature (Paulette 2007).

For analyzing samples of vine leaves was used the following equipment: mass spectrometer with inductively coupled plasma, ICP-MS, Perkin - Elmer Elan 9000, flame atomic absorption spectrometer Avanta PM, Milestone microwave digestion system. Calibration standards were made from stock solutions ICP-MS multi element calibration STD 3, monoelement standard solutions 1000 ppm K, nitric acid 65% puris p.a. (Fluka), hydrogen peroxide 30% p.a. (Merck) and ultrapure water, grade 1 according to ISO 3696: 1987. For mineralization of solid samples there was used a Milestone microwave digestion system. Amount of about 0.5 g of sample, weighed accurately 0.0001g, 5 mL nitric acid 65%; 2 mL H<sub>2</sub>O<sub>2</sub>, 1 mL ultrapure water were placed in Teflon vessels and were subjected to a heat treatment under pressure program: heating to 180°C with a gradient of 4.5 / min and held for 20 minutes at 180°C. After cooling, liquid samples were transferred into flasks and were brought to volume of 50 mL using ultrapure water and analyzed according to the specific procedures of the two spectrometric instruments. Control sample (Blank) was composed of 5 mL 65% nitric acid; 2 mL H<sub>2</sub>O<sub>2</sub>, 1 mL ultrapure water being processed under the same conditions as the samples analyzed.

There have been analyzed 19 elements B; Mn; Zn; Li; Se; Ca; Cr; Cu; Fe; Mg; Na; Al; As; Cd; Cs; Ni; Pb; Sr - using the ICP-MS instrument and K content was determined by flame atomic absorption spectrometer using as excitation source hollow cathode lamp for potassium.

## RESULTS AND DISCUSSIONS

Soil type is very important for the stability and incorporation of heavy metals in the food chain. The most important factors affecting the dynamics of heavy metals in soil and also in mobility and access to plants are: soil pH value, organic composition and moisture of the soil. Increasing soil acidity occur a number of problems in plant nutrition. Excessive presence of the hydrogen ions  $H^+$ , activate the aluminum and iron ions, which in large quantities act as toxic to plants, blocking the supply with phosphorus and other elements.

At the soil profile from Dăbuleni, on depth 0-29 cm, soil reaction is moderately acidic, pH ranging from 5.27 to 5.64, the nitrogen supply is decreased, the total nitrogen content is below 0.10% (0.021 to 0.016%), and the rate of nitrogen is between 0.54 to 0.39, P and K supply is very low.

On the depth of 29-50 cm and 50-80 cm pH increases less than the depth of 0-29 cm, soil reaction is slightly acid, being good for the development of the vine. The N supply of soil is weak, and for P and K is very weak, the contents of P and K on the depth are lower (0.8 to 1.8 ppm P and 34-37 ppm K) as compared to 3, 1 to 6.2 ppm for P and 37-41 ppm for K, values which are recorded on depth 0-29 cm.

Table 1

Agrochemical analysis of soil

Depth of sampling (cm)	pH in distilled water, ratio of 1:2.5	CaCO <sub>3</sub> (%)	Humus (%)	Total nitrogen (N%)	Nitrogen index	Mobile phosphorus (ppm)	Mobile potassium (ppm)	Degree of base saturation (%)
0-15	5.27	0.00	0.96	0.021	0.54	6.2	41	56.2
15-29	5.64	0.00	0.66	0.016	0.39	3.1	37	59.3
29-50	6.01	0.00	0.42	0.014	0.28	1.8	34	66.6
50-80	6.05	0.00	0.24	0.012	0.16	0.8	37	67.4
80-96	5.91	0.00	0.72	0.018	0.44	5.5	37	61.3
96-111	5.25	0.00	1.03	0.023	0.58	10.8	38	56.5
111-137	5.58	0.00	1.26	0.026	0.75	3.8	34	59.3
137-165	5.84	0.00	0.48	0.012	0.31	2.6	34	65.3
165-190	6.06	0.00	0.24	0.007	0.16	0.9	30	68.0

On the depths of 96-111 cm, 111-137 cm and 137-165 cm soil reaction is moderately acid, only on depths of 80-96 cm and 165-190 cm is slightly acidic. The supply of soil with N, P and K on all of these depths is very low. Optimum pH values for plant nutrition are considered between 6.0 and 6.5 domain where the solubility and availability of soil nutrients is optimal.

Psamosoils are generally low fertility soils, nutrient content, total nitrogen, phosphorus and mobile potassium is low, lower for low and medium humus soils and higher for medium and strong humus soil.

The average values of heavy metals concentrations in the leaves of Pandur vine variety are presented in table 2.

Potassium content recorded values between 9349.03 (P4) -10,744.27 (P5) mg/kg in the 6 samples of the variety Pandur (P1-P6) indicating a normal nutritional status in this cation. Potassium providing is normal, this will not adversely affect absorption of Mg, Na and B cations. Potassium is absorbed in the form of K monovalent cation and travels through the phloem in the plant. This is responsible for the activity of more than 60 enzymes involved in photosynthesis and in the transport and storage of substances in reserve organs and confers resistance to pests and diseases.

Magnesium recorded values between 2833.98 mg/kg (P4) and 3393.92 mg/kg (P1) indicating a poor nutritional state, this being due to the moderately acidic pH of the soil and the presence of Ca, Mn, NH<sub>4</sub> cations in soil solution which affects the absorption of Mg. Mg is absorbed in the form of Mg<sup>2+</sup> and is vital in the process of photosynthesis, favors the absorption and translocation of phosphorus, maintaining acid-base balance of the cell, favors the production of protein and carbohydrate metabolism. At grapevine is important the value of K/Mg ratio, which for healthy plants is 2.5 and deficiency state, is 1.5.

Table 2

Heavy metal concentrations determined at Pandur variety

Element	P1 ( mg/kg)	P2 ( mg/kg)	P3 ( mg/kg)	P4 ( mg/kg)	P5 ( mg/kg)	P6 ( mg/kg)
B	15.39	20.26	22.69	21.19	25.4	24.51
Mn	128.56	110.81	166.35	171.37	109.43	146.33
Zn	17.73	15.59	15.77	19.9	17.07	14.26
Li	0	0	0.13	0	0	0.07
Se	0.43	0.2	0.42	0.15	0.15	0.19
Ca	8432.87	6785.18	9215.27	7514.82	7113.33	10080.92
Cr	4.25	3.83	3.68	5.03	3.43	4.47
Cu	2.50	3.18	3.93	3.29	4.47	5.93
Fe	235.06	222.72	311.48	221.39	168.56	238.57
Mg	3393.92	3347.89	3609.51	2833.98	3143.91	3050.27
Na	20.43	31.79	42.88	27.46	73.54	66.6
Al	95.76	111.45	173.49	97.7	44.87	93.08
As	0.36	0.09	0.27	0.09	0.07	0.02
Cd	0.03	0.006	0.01	0.008	0.02	0.004
Cs	0.01	0.01	0.01	0.01	0.01	0.01
Ni	2.77	2	3.77	3.78	3.48	5.06
Pb	0.69	0.61	0.82	0.52	0.78	0.69
Sr	35.27	24.43	35.05	33.13	29.38	45.07
K	9559.92	10385.83	9775.29	9349.03	10744.27	9994.54

Calcium content of samples P2 and P5 recorded the lowest values 6785.18 mg/kg, respectively 7113.33 mg/kg indicating a calcium deficient nutritional status. At these samples (P2 and P5) were recorded elevated K contents (10385.83 mg/kg and 10744.27 mg/kg) and is normal that the values of Ca content to be smaller, due to the phenomenon of

ionic antagonism of these two cations. Ca content of the other samples ranged from 8432 mg/kg at P1 and 10,080.92 at P6, indicating a poor nutritional status of plants.

Calcium is a basic element in plant metabolism, which can not grow and develop without it. It is necessary for maintaining cellular plasma in an active and stable condition, for maintaining acid-base balance by neutralizing organic acids that are in excess, participates in the formation of cell walls and contributes to the development of the root system.

Lead and cadmium are on the list of the most dangerous metals that cause acute and chronic environmental contamination. Lead registers values between 168.56 mg/kg (P5) and 311.48 mg/kg (P3) values characteristic of a normal nutritional status, as high mobility and bioavailability of lead is in the acid pH range. Lead is involved in the synthesis of chlorophyll, carbohydrates, cellular respiration, and chemical reduction of nitrate and sulphate and nitrogen assimilation.

The boron content at P1 recorded the lowest value of 15.39 mg/kg which indicates a poor nutritional status. For the other five samples content increased and ranged between 20.26 mg/kg (P2) and 25.4 mg/kg (P5) and fits in a normal nutritional state of boron. Values recorded are normal for soils of our country, because boron is rather poorly represented in soils as a result of participation to the formation of sparingly soluble compounds with Al and Fe and retention in the organic matter and clay minerals.

Usually copper is an essential element for the plant growing. This microelement reaches into the composition of many enzymes in plants, but the interval of positive actions being limited it may become very toxic in high concentrations. Copper recorded the lowest value at P1 2.5 mg/kg and the highest value at P6 - 5.93 mg/kg, these values indicating a poor nutritional status. Low values recorded for copper indicate that the acidic pH of the soil affects the absorption of copper in the plant. Copper is necessary for photosynthesis and is part of some metalloproteins and some enzyme having a role in redox processes.

Zinc content recorded values between 15.59 mg/kg (P2) and 19.9 mg/kg (P4), indicating a poor nutritional status. At the time of sampling the plant does not show signs of zinc deficiency that occurs on top of annual increases through greenish-yellow discoloration in spots all over parenchyma, the veins with narrow bands of parenchyma near them remain green. Zinc participates in the activation of enzymes that catalyze the synthesis of tryptophan and heteroauxin. Zinc is a good nutrient for plants, which help the chlorophyll formation in leaves.

Manganese content registered high values in all six samples from 110.81 mg/kg (P2) to 171.37 mg/kg (P4), indicating a state of excessive nutrition with this element. Manganese contents are higher because this element is, in plants, in amounts higher than other micronutrients, especially in leaves. Manganese participate in physiological processes that provides conversion of  $Fe^{2+}$  to  $Fe^{3+}$  and activates enzyme systems.

Cadmium is a non-essential nutrient, and together with selenium, cesium, silver, lead and mercury, doesn't have yet a well-established biological role, being poisons for plants depending to the concentrations. Regarding those, metal concentrations in the leaves of vines have the following abundance: Sr>Pb>As>Se>Cs>Cd>Li.

## CONCLUSIONS

Leaf analysis is widely recognized as the most reliable laboratory method to determine the nutritional status in grapevines. Plants can accumulate a significant quantity of heavy metals. Recent researches demonstrated that plant tissues accumulate a quantity of heavy metals directly proportional with the gradual addition of metal in the soil, but

accumulation rates depend on the soil characteristics, on plant species, plant age, hydro climatic conditions, and type of tillage.

As a conclusion, monitoring of heavy metal distribution data in soil and plant samples are very useful for becoming main references or guidelines in order to monitor and avoid environmental pollution become worst in terms of quality of soil and also safety level for vegetables to be consumed.

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**RESEARCH REGARDING THE ABUNDANCE ON SOME FISH SPECIES  
OF COMMUNITY IMPORTANCE IN THE SITE NORDUL GORJULUI DE  
VEST**

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**Keywords:** *anthropogenic, impact, fish species*

**ABSTRACT**

*In the context of river alteration, ecologists are asked to develop tools for the assessment of river integrity. Fish are known to be good bioindicators of the ecological condition of rivers. In order to define accurate a site of high conservation, during 2013 - 2014 in the the site of community importance "Nordul Gorjului de Vest", there have been made some researches regarding the anthropogenic impact on some fish species of community importance listed in the IUCN Red List of Threatened Species: *Barbus meridionalis* Risso, *Gobio uranoscopus* Agassiz, *Cottus gobio* L.*

**INTRODUCTION**

Fish communities are an excellent indicator of biological integrity due to their continuous exposure to ambient water conditions, and because they display a variety of responses to environmental disturbance, such as habitat alterations, organic enrichment, chemical toxicity, and thermal fluctuations. Therefore, they are an integral part of any bio-monitoring program.

Although the Mediterranean barbel has a relatively large range, its distribution is extremely fragmented. It is therefore vulnerable to the effects of habitat loss and degradation, and river damming and water extraction also threaten this species. The Mediterranean barbel is very sensitive to the pollution of its habitat from urban, agricultural and industrial sewages. Increasing water temperatures due to global climate change may also cause increased mortality.

*Gobio uranoscopus*. However, it is sensitive to organic pollution, and sedimentation may have occurred because of the building of damns. It is likely to react adversely to human disturbance to its habitat. Although its populations appear to be declining slowly, this is gradual and does not warrant a higher level categorization (Freyhof & Kottelat 2008).

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The bullhead (*Cottus gobio*) is a freshwater fish that is widely distributed in Europe, mainly in rivers. It is a member of the Cottidae family, a type of sculpin.

The bullhead is a small demersal fish that lives both in cold, clear, fast-flowing small streams and in middle-sized rivers. It also occurs on gravelly shores of cold lakes. Further, it thrives in diluted brackish water of the Northern Baltic Sea (Froese & Pauly 2005).

### MATERIALS AND METHODS

The specimens were sampled in August and September using electrical fishing equipment. There were established four sampling stations and each of them was sampled three times, starting downstream. The size of the sampling areas varied between 120 m<sup>2</sup> and 260 m<sup>2</sup>, and with depths from 20 to 100 cm (Zippin 1958, Bohlin et al. 1989).

### RESULTS AND DISCUSSION

Between 2013 - 2014 in the site Nordul Gorjului de Vest, we performed research on the conservation status of some fish species of Community importance under the IUCN Red List: *Barbus meridionalis* Risso, *Gobio uranoscopus* Agassiz, *Cottus gobio* L.

In this research we focused to identify key factors and potential threat for these species.

*Barbus meridionalis* Risso species live in mountainous and hilly regions waters (downstream of the trout), located at an altitude of 400-200 m. This species prefer cooler waters without waterfalls, well-oxygenated, rocky and sandy bottom.

*B. meridionalis* has a large but fragmented distribution with an area of occupancy of nearly 2,000 km<sup>2</sup> as it is present in only parts of streams and not the main rivers, it is also threatened by hybridisation with other *Barbus* species, in addition it has undergone a population decline of nearly 30% (Crivelli 2006).



Photo 1. *Barbus meridionalis*, fished on the Porcu stream (orig.)

*Gobio uranoscopus* Agassiz, species can be found in mountain rivers and hills, locating it in the fords, where the water has a speed of 70-115 cm / s and the substrate is predominantly rock. There are cases where these species reach up to lowland areas, but can be found only in areas with rapid water.

Reproduction takes place from May to June, eggs are deposited on rocks. The food consists of periphyton and reophile invertebrates.



Photo 2. *Gobio uranoscopus*

*Cottus gobio* L. species is present in almost all over the mountain rivers of Europe, as well in Romania, where can be found in the company of trout. It is a reophile species, spending most of their time under rocks, feeding mainly on invertebrates. Reproduction is from March to April.



Photo 3. *Cottus gobio* L., collected from Șușița verde stream

According to our results (table 1) in the site Nordul Gorjului de Vest, *Barbus meridionalis* was the most abundant species compared to other species studied.

*Gobio uranoscopus* recorded lower values than *Barbus meridionalis* except on Motru Sec and Pârâul Racilor where the two species were recorded with approximately equal values.

From our results *Cottus gobo* was recorded with the lowest effective compared to the other studied species.

Table 1

The abundance of the fish species of community importance  
from the site Nordul Gorjului de Vest

River	Species		
	<i>Barbus meridionalis</i> %	<i>Gobio uranoscopus</i> %	<i>Cottus gobio</i> %
Porcu	55,5	38,2	6,3
Susita verde	57,2	39,5	3,3
Susita seaca	59,8	38,7	1,5
Harabor	61,2	37,6	1,2
Sambotin	75,8	23,7	0,5
Cartiu	80,5	19,2	0,3
Tismana	55,9	37,7	6,4
Jales	57,6	35,2	7,2
Bistrita	54,6	36,5	8,9
Plescioara	58,7	37,9	3,4
Motru sec	48,5	46,8	4,7
Paraul racilor	48,7	45,6	5,7

### CONCLUSIONS

Based on our research we can conclude that in the site of community importance Nordul Gorjului de Vest the three fish species of community importance are affected by anthropogenic activity.

Potential threat factors: poaching, extraction of granular materials (sand, gravel, etc.) of small beds of rivers, pollution of watercourses, multiply dams, decreased river flow through capture, contribute to habitat degradation, may thus endanger populations of these endangered species.

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**SOIL DEGRADATION OF OLT COUNTY DUE TO NATURAL  
OR ANTHROPOGENIC CAUSES**

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**Keywords:** *erosion, soil, degradation.*

**ABSTRACT**

*Erosion occurs under the action of water or wind and is favored by the irrational exploitation of the land in the areas with medium and large slope inclination. Irrational use of land takes many different aspects including: the removal of natural vegetation on slopes, plots orientation on greatest slope line and agricultural works executed from the hill into the valley, or parallel to the dominant wind direction, which promotes erosion, etc.*

*Olt County soils are subject to the following degradation processes: water erosion affecting 30,820 ha (6.73%) of the agricultural area of the county, erosion 4,800 ha, landslides 511 ha, collapse shores 563 ha, gleyzation 41,730 ha, stagnogleyzation 64,611 ha, floodability 4,600 ha, the phenomenon of desertification, 112,426 ha.*

**INTRODUCTION**

The soil degradation means „to reduce the current capacity and/or potential of the soil to produce goods and quantitative and qualitative services due to one or more processes of degradation. Lal and Stewart (1990) showed, as already mentioned, that „land degradation is a generic expression, vague, but which it relates, in most of the publications, to the decline in soil productivity by negative changes in the nutrient state and organic matter, structural attributes, the concentration of electrolytes and toxic chemicals”.

Degraded surfaces in the world are estimated at 1,965 million hectares out of the 8,735 million hectares of farmland, forest and meadows and about 305 million ha are seriously affected by various forms of degradation and the other 910 million ha are moderately affected (Oldermann 1990). It is noticed that cultivated soil surface decreases continuously. Thus, in 1990 the world's arable land per inhabitant was 0.3 ha, 0.25 ha in 2000 and to 2050 is assumed to be 0.15 ha. Now, soil degradation has become a very serious global problem, requiring new scientific and politico-economic approaches, based on a deeper understanding of the phenomenon.

Since 2002 in Communication „Towards a Thematic Strategy on Soil Protection (COM 179/2002)” The European Commission has specified eight main negative processes seriously affecting soil erosion, organic matter decline, soil contamination, soil salinisation, compaction, expansion of various types of construction, flooding and landslides.

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In our country, the most widespread soil degradation processes are anthropogenic or secondary compaction (44%), natural or primary compaction in depth (14%), erosion (47%), drought (48%), temporary excess of water (25%), small reserves of humus (50%), low accessible phosphorus content (42%) and acidity (23%).

### **MATERIALS AND METHODS**

Olt County soil degradation has been studied using field observations, observations that consisted in the following steps:

- registration and location of the land subject to pedological observations,
- numbering and coding of soil profiles described,
- determination of geographic coordinates, geo-referential positioning,
- the usage way of the land,
- existing land improvement arrangements and the stage of their execution,
- establishing soil type,
- establish physico-geographical unit / subunit and surface lithology,
- characterization of the topography and terrain relief by establishing the category of relief (plain, wavy, uneven) and their classification in codes,
- characterization of the climate of the area by temperature and moisture regimes and their classification in codes,
- characterization of zones and subzones of vegetation and their encoding,
- site characterization, soil profile description,
- establishment of altitude, slope, shape and exposition of the versant,
- characterization of soil drainage, surface leakage and possible ponding, groundwater's depth and soil permeability;
- determination of type and degree of erosion and landslides categories.

Through all of these steps carried out and recorded we could determine the soil gradation grade (Munteanu & Florea 2009).

### **RESULTS AND DISCUSSIONS**

Erosion occurs under the action of water or wind and is favored by the irrational exploitation of the land in the areas with medium and large slope inclination. Irrational use of land takes many different aspects including: the removal of natural vegetation on slopes, plots orientation on greatest slope line and agricultural works executed from the hill into the valley, or parallel to the dominant wind direction, which promotes erosion, etc.

Water erosion affects approximately 6.73% of the agricultural area from Olt county, mainly on land located on the slopes of the valleys that cross the Piedmont Plateau (Băleasa, Gearmătăului, Horezu, Olteț, Beica, Barla, Olt, Cungrea, Tesla, Plapcea, Vedea and Vedița) where it manifests greater rhythm and intensity due to high energy relief and abundant rainfall.

Lands situated along the slopes that cross Boianului Plain (Dârjov, Iminog, Călmățui, Dorofei) and those that cross the field Leu-Rotunda (Tesluiului of Resca, Caracal, Frăsinetu) are affected by erosion of weaker intensity, due to the lower energy of the relief and to smaller amounts of precipitation.

Damages caused by surface erosion refers to decreasing soil fertility, crop reduction, reduced arable land, worsening the hydrological regime of the area, silting of lakes, especially when this phenomenon is associated with deep erosion (Mocanu et al. 2011).

Table 1

Status of agricultural land degraded by surface erosion  
March 31, 2012

M.U.	Affected area	Weak	Moderate	Strong	Very strong	Excessive
Ha.	30,820	12,937	7,179	3,843	5,307	1,554
%	100.00	41.98	23.29	12.47	17.22	5.04

Deep erosion affects approx. 0.23% of the agricultural area of the county and is manifested in the form of runoffs, gullies, ravines, mainly affecting strongly inclined slopes in hilly areas of the county, and those in the transition zone between the Piedmont Plateau and the Romanian Plain, process sustained also by unconsolidated sedimentary lithologic formations.

Table 2

Status of land degradation through erosion in depth  
March 31, 2012

M.U.	Affected area	Runoffs	Gullies	Ogase	Ravines
Ha.	1,016	74	202	505	235
%	100.00	7.28	19.88	49.70	23.14

Wind erosion is manifested in the south-west of the Olt county, in Dăbuleni Plain, on lands of wind blown sands, covering an area of approx. 3,800 ha, representing about 0.87% of the agricultural area of the county.

In this area, advancement of sands solidification is prevented by the frequency and intensity of winds, which favors the mobilization of the transport and deposition of sand in the form of dunes.

Soil degradation by landslides and shore collapses

Landslides affects lands situated on the moderately-strong tilted slopes in the north of the county; the phenomenon is favored by deforestation of woody vegetation or grasses and abundant rainfall. It occupies approx. 0.13% of the agricultural lands.

The main cause of this phenomenon is the parent material of the soil, which has a fine texture (containing 30% clay, predominantly gonflable) with the ability to greatly increase their volume by moistening.

The high content of clay minerals with expandable network on the alternation background of wet and dry seasons causes contraction-gonflable processes that produce structural aggregates sliding over one another, resulting in polished surfaces and oblique slip with 10-600 tilt the vertical plane.

Shore collapses are found more on the abrupt shores of the Olt (Slatina-Milcov) and Oltețului (Pârșcoveni-Osica) in areas where river waters with high flow rate eroded the shores base.

Table 3

Status of land degradation by landslides or shore collapses  
March 31, 2012

M.U.	Affected area	In furrows	In steps	In waves	Collapses
Ha.	563	485	40	25	13
%	100.00	86.15	7.10	4.44	2.31



Figure 1. Collapse of shore in Fălcoiu locality

### Soil degradation by excess moisture

#### Gleyzation

Phreatic excess moisture can be found in meadow areas, terraces, low areas, where groundwater is deeper than 1-2 m, causing ground moistening and thereby favoring manifestation of gleyzation processes. It manifests on approx. 9.00% of the agricultural area of the Olt county.

Strong intensity gleyzation processes deteriorate due to aerohidric regime of these soils, plants are feeling the negative effects of anaerobic conditions from the seed germination, the roots grow weak and superficial penetrate into soil, plants accumulate toxic products, so that plants produce debilitating and decreased production (Gibbs & Reid 1988).

Table 4

Status of soil degradation through gleyzation  
March 31, 2012

M.U.	Affected area	Weak	Moderate	Strong	Very strong	Excessive
Ha.	41,730	15,498	13,185	5,302	5,239	2,506
%	100.00	37.14	31.60	12.71	12.55	6.00

#### Stagnogleyization

Prolonged stagnation of water at the soil surface causes stagnogleyization processes that occur in approximately 14.7% of the agricultural area. This causes a strong reduction of iron and manganese compounds and their migration on the soil profile, forming horizons with maximum accumulation of iron and manganese sesquioxides, which are toxic to plants.

Stagnant water from the upper soil profile prevents renewal of air in the soil, thus increasing the concentration of carbon dioxide in the soil and a lack of oxygen to adversely affect normal growth and development of plants, the obtained productions being quantitatively and qualitatively inferior.

Strong and very strong stagnogleyized soils have the optimum working field short (workability and trafficability), meaning that they have a relatively large period of time that you can not work and circulate.



Table 5

Status of land degradation through stagnogleyztion  
March 31, 2012

M.U.	Affected area	Weak	Moderate	Strong	Very strong	Excesive
Ha.	64,611	42,872	18,267	2,655	710	107
%	100.00	66.35	28.27	4.11	1.10	0.17

#### Floodability

This negative phenomenon generally occurs in floodplains of rivers undammed and on the valley crossing interfluves high fields during periods of abundant precipitation. It manifests approx. on 1.00 % of the surface. Meadows present specific conditions in terms of hydrology and hydro-geology.

In terms of hydrology, there can be found frequently flooded areas (annually or every few years), periodically flooded areas (at intervals of time) and unflooded areas.

From the hydrogeological point of view, plains being low relief units, located near watercourses, often have mineralized or demineralized underground water near the surface or shallow depths.

In flood conditions, both in the meadows and in the valleys that cross interfluviatile fields, soil formation is weak due to overflows that hinder the development of new vegetation by depositing alluvium. At the same time crops are fully or partially destroyed.

Table 6

Land degradation situation by the outpouring of water courses or leakage on the slopes  
March 31, 2012

M.U.	Affected area	Rare	Frequently	Very frequently
Ha.	4,600	3,114	809	83
%	100.00	77.73	20.19	2.08

#### Soil degradation by salinization

Soil salinization occurs on approximately 0.23% of the agricultural area. The main cause of salinization is mineralized groundwater located at shallow depths that through its evaporation from the soil surface, during periods of drought, causes deposition of soluble salts. The presence of salts in the soil solution prevents the development of vegetation and determines a low degree of coverage. The activity of soil microorganisms is much diminished or even inhibited.

Table 7

Situation of land degradation by salinization  
March 31, 2012

M.U.	Affected area	Weak	Moderate	Strong
Ha.	1,000	600	21	379
%	100.00	60.00	2.10	37.90

#### Land degradation from semi-arid area as a intensifying drought factor

Considering the values of air temperature and mean annual rainfall, semi-arid area

in Olt county stretches from the north of the Danube and to on the alignment localities Rotunda - Studina - Rusanesti. In this area the average annual temperature is between 11-12°C and the annual amount of temperatures equal to or greater than 0°C is about 4,200°C; average annual rainfall has values between 475 and 525 mm.

Land and soil degradation in the south and especially the southwest of Olt county, has the effect of emphasizing the drought soil and the air, over time favoring desertification.

One of the main causes of intensifying drought in the southern and south-western part of the county is the deforestation of protection forestry bands and bushwoods, irrational destruction of vegetation by grazing and by failing irrigation system.

Wind erosion has the effect of amplifying droughts in areas with sandy soils and crust acts as a factor intensifying droughts in areas with silty and silty-sandy soils.

Through changes produced in soil moisture regime, drought is the most important factor in increasing the degradation processes, especially in conditions of poor farming practices or improper use of land.

In the perspective of climate warming in the southern area of the county, in the context of global climate change, the relationship drought - pedogenesis - soil and land degradation is particularly important for the protection and conservation of soil.

### CONCLUSIONS

Olt County soils are subject to the following degradation processes:

- water erosion affecting 30,820 ha (6.73%) of the agricultural area of the county, affecting the slopes of the valleys Piedmont Plateau, Plains Boianului and Leu -Rotunda;
- erosion caused by water into the depth: 1,016 ha (0.23%);
- erosion by wind: 3,800 ha (0.87%);
- landslides 511 ha (0.11%);
- collapse shores 563 ha (0.17%);
- gleyzation 41,730 ha (9%);
- stagnogleyzation 64,611 ha (14.7%);
- floodability 4,600 ha (0.9%);
- the phenomenon of desertification, 112,426 ha, mainly in the south, due to prolonged drought and deforestation of protective curtains.

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## **OLT COUNTY SOILS GROUPING AFTER QUALITY CLASSES**

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**Keywords:** *soil, quality classes, bonitation.*

### **ABSTRACT**

*The quality of the land fund is determined by the limiting factors of agricultural production, of which the most important are: soil (physical, hydro-physical and agrochemical properties), topography (size slope and its orientation, or the presence of landslides), groundwater (depth, the content of soluble salts); climate (temperature and precipitation), etc.*

*Depending on the quality classes, Olt County soils are divided as follows: class I, 10,235 hectares representing 2.37%, class II, 129,405 ha representing 29.82%, class III, 219,828 hectares representing 50.66%, class IV, 47,234 ha representing 10.88%, class V, 27,201 ha representing 6.27%.*

### **INTRODUCTION**

Bonitation is a complex operation of deep knowledge of the conditions of growth and fruiting of plants and determining the degree of suitability of these conditions for each use in part, through a system of indicators that allow quantification techniques in bonitation notes (Negrea et al. 2009).

The objective of agricultural land bonitation is the land that was considered since ancient times, support for plant and animal production, a mean of production which has been assessed, valued, and classified.

Land use is an area of major importance lately growing in the world, but in our country a higher recovery of soil resources. There are now a multitude of problems that must solve land management problems whose complexity and variety is continuously growing (Mihalache & Ilie 2012).

The natural resources consist of the total sum of the resources existing in nature: soil, water, air, flora, fauna, solar energy, etc. They are extracted from their natural medium and transformed under certain technological, economical and social conditions into goods whose usage implies their direct consumption.

The manner of use of these resources must be complex and coordinated so that many goals can be obtained simultaneously and they harmonize with the exigencies of the environment protection (Hartia 1978, Canarache & Teaci 1980, Teaci 1980, Carstea 1995, Florea et al. 1987).

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## MATERIALS AND METHODS

Quality index for natural conditions is based on environmentally homogeneous land unit (EHL). Territorial unit of land (pedotop) is the concrete form in which a relatively homogeneous area appears in a region in terms of the entire attributes specific, natural factors manifesting relatively uniform (Pavel, 2008).

Factors groups, according to which is made the determination of bonitation note, are:

1. climatic factors (characterized by mean annual temperature and mean annual precipitation, corrected in accordance with slope and exhibition).
2. relief (to which the focus is on the slope and exhibition).
3. groundwater and stagnant water (characterized by depth and intensity).
4. soil with the following qualities: useful edaphic volume, texture, content, and reserves of humus, soil reaction, total porosity, gley or pseudo-gley state, state of salinization or sodiunisation.

To calculate the bonitation notes in natural conditions there were used a series of indices, called indicators and coefficients of bonitation, in number of 19, and for the potentiation of bonitation notes are used coefficients of potentiation.

These factors were grouped into three categories: climatic factors, soil influencing factors and factors affecting the land.

## RESULTS AND DISCUSSIONS

The quality of the land fund is determined by the limiting factors of agricultural production, of which the most important are: soil (physical, hydro-physical and agrochemical properties), topography (size slope and its orientation, or the presence of landslides), groundwater (depth, the content of soluble salts); climate (temperature and precipitation), etc.

Summarizing the results of the pedological studies from the archive of OJSPA Olt at the end of 2012, the situation of framing the agricultural land in quality classes is presented according to the table below:

Table 1  
Agricultural land quality classes (after the bonitation note under natural regime)

Categories of use	Total area Ha.	Quality classes									
		I		II		III		IV		V	
		Ha.	%	Ha.	%	Ha.	%	Ha.	%	Ha.	%
Arable	388.603	10027	2,58	121399	31,24	195817	50,39	38744	9,97	22616	5,82
Pasture	32.339	x	x	5420	16,76	15888	49,13	6979	21,58	4052	12,53
Meadows	528	x	x	X	X	232	43,95	296	56,05	x	X
Vineyards	4.949	208	4,19	1296	26,19	2989	60,39	381	7,71	75	1,52
Orchards	7.484	x	x	1290	17,24	4902	65,50	834	11,14	458	6,12
Agricultural	433.903	10235	2,37	129405	29,82	219828	50,66	47234	10,88	27201	6,27

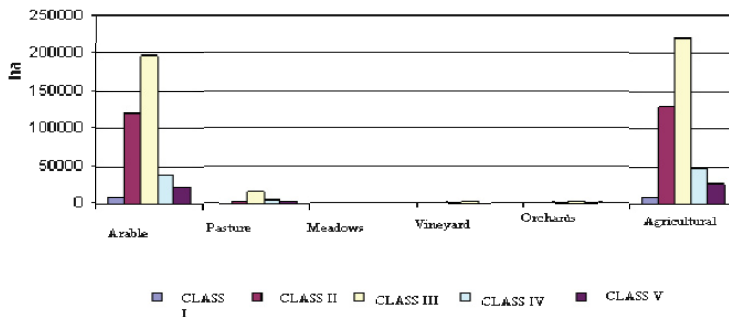


Figure 1. Classification of land quality classes and categories of use

This classification is made according to the Methodology of elaboration the pedological studies, Vol. I-III (ICPA Bucharest 1987) and other normative acts updated by Order MAAP 223/2002.

Bonitation system was designed in a 100-point scale divided into classes of favorability of land for different crops or agricultural use as follows:

**Class I** with notes between 81-100 points - includes lands without limitations or restrictions, with very fertile soils, deep, medium texture, free from decay phenomena, located on flat or very slightly inclined surfaces, with favorable climatic conditions for cultures. It occupies 2.37% of the agricultural area of the Olt County and is represented in particular by the chernozems (typical, cambic and argic), alluvisols and cambosols, wet groundwater or weak gleyed, weak acidic or neutral reaction. On these grounds it is necessary to apply only agrotechnics corresponding to assortment of plants that are cultivated.

**Class II** with notes between 61-80 bonitation points, includes land with limitations or poor restrictions, with deep soils, with medium or medium fine texture, good or medium permeability, poor affected by stagnant excess moisture, located on flat or slightly inclined surfaces, with favorable climatic conditions for crops.

It occupies approx. 30% of the agricultural area of the county and it requires for obtaining large productions, works to prevent temporary excess moisture and application of organic fertilizer every 3 - 4 years. It is principally represented by preluvisols (mollic or typical), chernozems (cambic and argic), mollic cambosols and mollic unflooded alluvisols.

**Class III** with notes between 41-60 points, includes land with limitations or moderate restrictions, occupying approx. 51% of the agricultural area of the county, with medium fertile soils, deep and moderately deep, medium texture, medium-coarse or fine texture, moderately affected by degradation phenomena, located on flat or slightly sloping surfaces, with moderate climatic conditions favorable for crops. It is principally represented by the vertisols, preluvisols (typical or vertice), luvisols and rarely flooded alluvisols. Requires organo-mineral fertilizer works and at soils with pH below 5.8 is needed an application of calcareous amendments, according to agrochemical indices.

**Class IV** with notes between 21-40 points, includes land with severe restrictions, with poor fertile soils, often skeletal of any texture (coarse to fine) affected by degradation phenomena located on flat or moderately inclined surfaces. Are represented mainly by plano-soils, psamosols, erodosols, gleiosols, stagnosols, gleyic alluvisols, floodable and takes approx. 11% of the agricultural area of the Olt county.

For using such lands as arable is necessary to apply a set of specific ameliorative measures: drainage, application of amendments, specific agricultural technology, and the cultivation of a variety of suitable plants.

Class V with notes between 1-20 points, includes lands with very severe restrictions, with weak fertile soils unsuitable for agricultural use, very strongly affected by degradation phenomena and takes approx. 6% of the agricultural area of Olt County. It is represented mainly by regosols, solonetz, marshy gleiosols and very frequently floodable alluvials. These areas require special protection measures through afforestation, restricting grazing, dams, regularization of river beds etc.

Comparing the quality of agricultural land in Romania for 2012 (Proceedings of the XX National Conference for Soil Science, Craiova, 20 to 26 August 2010, vol I page 44) with soil quality in Olt County at 31.12.2005, situation is as follows:

Table 2  
Comparison with respect to quality of agricultural land from Olt County towards Romania (% of surface)

Categories of use	Quality classes									
	I		II		III		IV		V	
	Romania	Olt	Romania	Olt	Romania	Olt	Romania	Olt	Romania	Olt
Arable	8,77	2,6	28,47	31,2	36,48	50,4	19,13	10,0	7,15	5,8
Meadows	1,17	-	6,84	16,4	26,13	49,0	40,58	22,3	25,58	12,3
Vineyard	3,37	4,2	20,86	26,2	32,56	60,4	34,00	7,7	9,21	1,5
Orchards	0,44	-	9,39	17,2	31,49	65,5	44,58	11,2	14,10	6,1

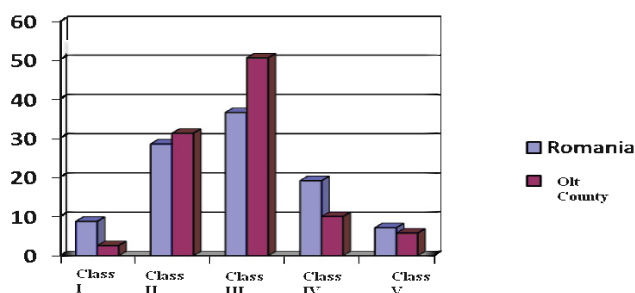


Figure 2. Distribution of arable land in the country as compared to Olt County

Bonitation note (in natural conditions) distinguishes quality of agricultural land in terms of potential production for agricultural use and, within the use, distinguishes them in terms of cultivated species.

Table 3  
Note of bonitation (average weighted) under natural conditions in the agricultural area in Olt county - The categories of use - 31.12.2012

Categories of use	Quality classes and bonitation note											
	I		II		III		IV		V		Average per county	
	ha	NB	ha	NB	Ha	NB	ha	NB	Ha	NB	CL	NB
Arable	10068	87	122024	68	196826	57	38929	36	22722	19	III-	57
Pasture	X	x	5200	71	15241	49	6693	37	3888	17	III-	46
Meadows	X	x	x	X	280	58	357	38	X	x	III-	47
Vineyard	320	81	1998	65	4608	50	588	35	116	18	III-	54
Orchards	X	x	1260	63	4786	52	814	34	447	17	III-	50
Agricultural	10388	*	130482	*	221741	*	47381	*	27173	*	III-	*

If we make a comparison of average bonitation note (Teaci, 1980) and Olt county's average bonitation note, it is noted that in the case of arable use category, Olt County pedological offer is more generous compared to the national average.

Table 4  
Comparison with respect to size of bonitation notes in natural scheme soils of Olt County, compared to the national average for different categories of use

Categories of use	România 1976*	Olt County 2000**	Olt County 2012
Arabil	45	57	57
Pastures	31	33	46
Orchards	20	38	50
Vineyards	35	48	54

The higher bonitation notes from 2012 to pastures, orchards and vineyards is explained by the relocation of these land use categories more favorable, as well as by removing of lands from agricultural use in the lower classes (according to Law no. 18/91).

Table 5  
Ecological favorability for different crops in the Olt County

Agricultural crop	Favorability class	% of arable land
Mixed	II	61
Sunflower	II	50
Wheat or barley	II	47
Soybeans	II	45
Corn grain	II	43

To quantify the ability of agricultural land subject to bonitation, from the conducted researches and investigations on the yields obtained by farmers, was conventionally established the agricultural equivalent of a point of bonitation, considering that there was applied a medium culture technology.

Given the evolution of agricultural technology, the equivalent in products of the bonitation notes evolved as follows:

Table 6  
The evolution of the value of a bonitation point in kg of product to the main crop plants

Crop plants	Considered steps				
	1955*	1965*	1975*	1985**	Lg. 16/94
Wheat	45	50	60	60	40
Corn	50	65	75	80	52
Sunflower	20	25	30	32	16
Autumn potatoes	250	300	400	450	200
Sugar beet	350	400	500	500	280

Value of the bonitation notes can be used to calculate the average production per hectare for each crop in relation to the technology that can be provided at that time.

Analyzing the average yields per hectare, obtained in 2012, for the main crops, it

appears that they are in relative agreement with potential soil fertility expressed by note of bonitation for arable (57 points) and using the point of bonitation in accordance with Law No 16/94 as follows:

Agricultural crops	Average production -kg/ha-	
	estimated	Achieved*
Wheat grains	2.280	2.564
Barley grains	3.508	2.192
Corn grains	2.964	2.482
Sunflower	912	1.499

\* data from the Statistical Breviary of Olt County - Agricultural chapter; 2012

Production capacity of soils, represented by the yields obtained, constitutes the basic criterion for determining the sale price of lands for land tax assessment in evaluating agricultural land and establishing a fair charging fee in assessing land for loans and guidance to purchasers of land.

### CONCLUSIONS

Depending on the quality classes, Olt County soils are divided as follows:

- class I, 10,235 hectares representing 2.37%;
- class II, 129,405 ha representing 29.82%;
- class III, 219,828 hectares representing 50.66%;
- class IV, 47,234 ha representing 10.88%;
- class V, 27,201 ha representing 6.27%;

It is noted that most areas fall within the classes III (50.66%) and II (29.82%).

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**THE BIODEGRADATION OF ALGAE SPECIES *CHLORELLA VULGARIS*  
AND OREGANO FOLIAR TISSUES IN SOIL DURING INCUBATION,  
AND THEIR EFFECTS ON SOIL CHEMICAL PROPERTIES**

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*Keywords: algae, oregano, soil chemical properties, soil organic matter biodegradation*

**ABSTRACT**

*The present work was carried out in vitro (incubation experiment), and the effect of 5 g well milled algal biomass, mixed with 50 g of soil, poor in organic matter content was studied; in a parallel experiment, for comparison purposes, 5g of oregano foliar tissues were also applied in the same soil. All treatments were incubated for 15 weeks at a constant temperature of 28°C and after the incubation period, suitable chemical analyses were performed. The results showed that the algae are subjected too much greater biodegradation, in comparison to the foliar tissue of oregano. Algae as compared with the oregano, increased the soil salinity, promoted nitrification and elevated the available contents of P and Zn; oregano foliar tissues as compared with the algae, increased the content of samples in available Mn, and both materials increased exchangeable K forms.*

**INTRODUCTION**

The addition of various materials in the soil, affects largely its composition as well as the biological activity of microflora, which in turn determines the biochemical status of soil fertility. Particular attention is paid to the biochemical function of the soil, when applying organic fertilizer of different types and origins (Chouliaras, 1994; Chouliaras et al., 1998; Gougoulis et al., 2013), because organic materials dominate microbial nutrition.

The additions of plant residues contributes to the maintenance of soil fertility, while the addition of plant essential oils, protects against plant pathogens (Gravanis et al., 2004).

According to previous work, it was found that the presence of foliar tissues of oregano, act a slowing effect on degradation of organic fertilizer, while the presence of basil, accelerate it (Gravanis et al., 2005; Chouliaras et al., 2007). Similar studies by comparing oregano with Neem (*Azadirachta indica A. juss*) on the biodegradation of organic matter, already composted of organic fertilizer, have shown that adding 2g Neem or

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0.4 g oregano at 100 g soil, the organic matter is biodegraded about 25% by the addition of Neem, and 5% by adding oregano (Gougoulis et al., 2010; Gougoulis et al., 2012).

The role of algae in pedogenesis, it was always underlined (In Duchoufour, 1994); today's algae species are largely exploited to many industrial sectors, and their role as byproducts or as primary materials, should be evaluated for their recycling on agricultural production. In this work the biodegradation in soil of algal species *Chlorella Vulgaris* was studied in vitro, in comparison with the biodegradation of oregano, and the consequences of that biodegradation on chemical properties of soil were approached.

### MATERIAL AND METHODS

**Algae Cultivation:** The algal specie *Chlorella vulgaris* have been obtained from Experimental Phycology and Culture Collection of Algae at the University of Goettingen Germany (EPSAG). They have been kept at 4°C and each vial was used within 3 months. The growth medium was of the following constitution suggested by (Andersen et al., 1991).

For *Chlorella vulgaris* each 50 liter growth medium contains: 0.2g KNO<sub>3</sub> /L, 0.02 g K<sub>2</sub>HPO<sub>4</sub> /L, 0.02 g MgSO<sub>4</sub>. 7H<sub>2</sub>O /L, 30 ml of soil extract /L and 5 ml/L, of solution containing the following micronutrients: (1 mg ZnSO<sub>4</sub>.7H<sub>2</sub>O, 2 mg MnSO<sub>4</sub>.4H<sub>2</sub>O, 10 mg H<sub>3</sub>BO<sub>3</sub>, 1 mg Co(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O, 1 mg MoO<sub>4</sub>.2H<sub>2</sub>O, 0.005 mg CuSO<sub>4</sub>.5H<sub>2</sub>O, 700 mg FeSO<sub>4</sub>.7H<sub>2</sub>O and 800 mg EDTA)/L.

The bioreactors were rectangular Teflon vessels of (30 x 35 x 60) cm. The experiment was carried out in a greenhouse of TEI Thessaly and had a duration of 82 days. Two bioreactors were exposed to exactly the same conditions such as light, orientation, stirring and temperature, and were determined natural fluctuation of growth media pH, and of electrical conductivity. Mass concentrations on a dry basis (mg dry algal mass/liter of growth medium) were determined from the volume of medium and the weight of algal mass after evaporation of the water and drying.

Throughout the course of the crop, the electrical conductivity ranged from 0.517 dS m<sup>-1</sup> to 0.730 dS m<sup>-1</sup>, the pH from 9 to 10.69 and the concentration of algae after 68 days was stabilized at 600 mg / L of growth medium (figure 1). From 100 liters of nutrient solution we received 31.82 g dry matter *Chlorella vulgaris* (namely 318.2 mg/L).

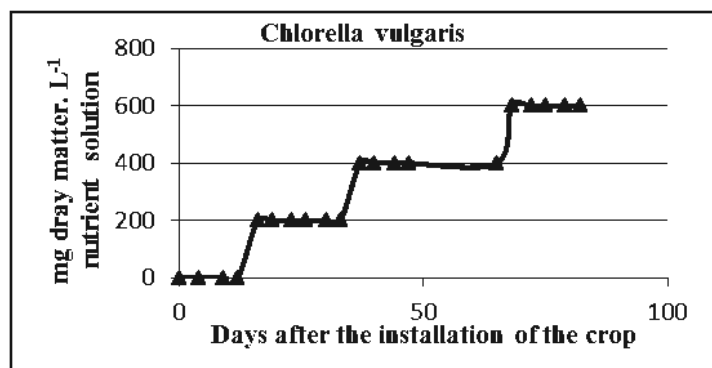


Figure 1. Variation dry algal mass of growth medium during culture

**Incubation experiment:** In the present work, 5 g algae were applied in 50 g of soil, poor in organic matter content, (Table 1) and its effect, after an experiment of incubation of 15 weeks in temperature 28°C, was studied. Also 5 g oregano foliar tissues

were applied into 50 g of the same soil, in a parallel experiment. In the incubator, the treatments were prepared in four replicates and kept at 28°C for a period of 15 weeks.

Table 1

Chemical properties of soil samples, oregano foliar tissues and algae species  
*Chlorella vulgaris*

Property	Soil	Species of Algae ( <i>Chlorella vulgaris</i> ) Air dried material	Greek Oregano ( <i>Origanum vulgare</i> ) Air dried material
Texture	Sandy Loam		
pH (1part : 5parts H <sub>2</sub> O)	7.83		
Electrical conductivity, extract (1part substrate:5parts H <sub>2</sub> O) dS m <sup>-1</sup>	0.28		
Organic matter (%)	0.57		
CaCO <sub>3</sub> (%)	8.60		
N -total (g kg <sup>-1</sup> )	1.54	22.4	19.6
N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	27.7		
N-NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	99.1		
Exchangeable-K (mg kg <sup>-1</sup> )	276.9		
K-Total (g kg <sup>-1</sup> )	5.30	6.7	15.3
Exchangeable-Na (mg kg <sup>-1</sup> )	211.6		
Na-Total (mg kg <sup>-1</sup> )	619	2100	2760
CEC (cmol kg <sup>-1</sup> )	20.6		
P -Olsen (mg kg <sup>-1</sup> )	11.2		
P-organic (mg kg <sup>-1</sup> )	53.2		
P-inorganic (mg kg <sup>-1</sup> )	198.3		
P -Total (mg kg <sup>-1</sup> )	251.5	6700	3600
Cu -DTPA (mg kg <sup>-1</sup> )	0.98		
Zn -DTPA (mg kg <sup>-1</sup> )	0.42		
Mn -DTPA (mg kg <sup>-1</sup> )	5.41		
Cu -Total (mg kg <sup>-1</sup> )	67.4	54.4	130
Zn -Total (mg kg <sup>-1</sup> )	67.1	213.2	54
Mn -Total (mg kg <sup>-1</sup> )	595.1	6.37	66.54
Fe-Total (g kg <sup>-1</sup> )	18.0	4.99	6.88
Ca- Total (g kg <sup>-1</sup> )	34.4	4.0	4.2
Mg-Total (mg kg <sup>-1</sup> )	7940	96.1	3343
Ni- Total (mg kg <sup>-1</sup> )	201.8	14.65	33
Pb- Total (mg kg <sup>-1</sup> )	<0.02	<0.02	<0.02
Cr- Total (mg kg <sup>-1</sup> )	<0.06	<0.06	<0.06
Cd- Total (mg kg <sup>-1</sup> )	<0.02	<0.02	<0.02

During the first three weeks of the incubation period, the moisture was maintained at two-thirds of field capacity, but for the next three weeks the soils were left to dry. This process was repeated until the end of the incubation period according to Wu and Brookes 2005; they reported that the alternation of drying and rewetting soil samples enhances

mineralization of both soil biomass organic matter and non-biomass organic matter. Soil aeration and regulation of moisture enhance the growth and metabolic activity of aerobic soil microorganism (Chouliaras and Jacquin 1976). At the end of the incubation period, soil samples were analyzed.

**Methods of analyses:** Samples were analyzed using the following methods which are referred by (Page et al., 1982 and Hesse et al., 1972).

Organic matter was analyzed by chemical oxidation with 1 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and titration of the remaining reagent with 0.5 mol L<sup>-1</sup> FeSO<sub>4</sub>.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol L<sup>-1</sup> CaCl<sub>2</sub> and estimated by distillation in the presence of MgO and Devarda's alloy, respectively.

Available P forms (Olsen P) was extracted with 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> and measured by spectroscopy.

Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> and all forms were measured by spectroscopy.

Exchangeable forms of potassium and sodium were extracted with 1 mol L<sup>-1</sup> CH<sub>3</sub>COONH<sub>4</sub> and measured by flame Photometer (Essex, UK).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol L<sup>-1</sup> + CaCl<sub>2</sub> 0.01 mol L<sup>-1</sup> + triethanolamine 0.1 mol L<sup>-1</sup>) and measured by atomic absorption.

For the determination of total metals Mn, Cu and Zn, 1 g of material, digestion at 350 °C + 10 ml HNO<sub>3</sub> + 5 ml HClO<sub>4</sub>. According to the method described by (Allen et al., 1974) and Varian (1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture.

The experimental design was completely randomized with three treatments (Soil, Oregano tissues and species of algae *Chlorella vulgaris*) and four replications. Data analysis was made using the MINITAB (Ryan et al., 2005) statistical package. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

## RESULTS AND DISCUSSIONS

The results of the laboratory experiment (figure 2) showed that algal organic matter added to the soil, was subjected to strong biodegradation (about 60%), in comparison with the oregano foliar tissues which, after a long incubation period, was found to be very resistant. Electrical conductivity was increased by the addition of algae due to the greater biodegradation of organic matter, and to ions liberation, while it was not changed by the addition of oregano foliar tissues. The decrease in the value of pH was greater by the addition of algae when compared to the corresponding one of oregano.

Nitrate forms content (figure 2), was increased by the addition of algae, while it wasn't changed, by the addition of oregano. This means that algal biomass promotes the nitrification process in the soil. Ammonium content either by the addition of algae or by the addition of oregano, was not showed significantly differences. The available forms of P were increased by algae or oregano treatments. Moreover the greatest increase was observed when algae were added. The P-Organic was increased by the addition of algae, while it was not changed by the addition of oregano; then P-organic biosynthesis, is more promoted by algae presence.

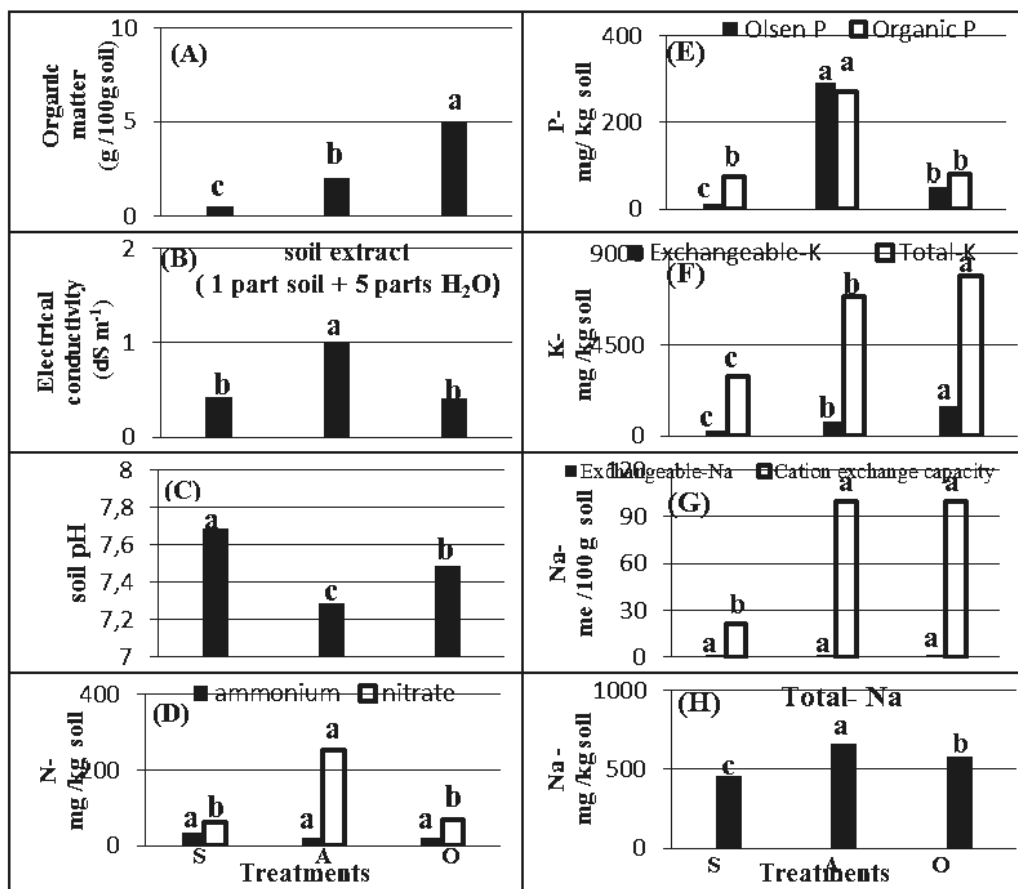


Figure 2. Effect of algae and oregano on soil content in (A) organic matter; (B) electrical conductivity; (C) pH; (D) N- ammonium and N-nitrate; (E) P-available and P-organic; (F) exchangeable K and total K; (G) exchangeable Na and cation exchange capacity; (H) total Na. (Bar values with the same letter on the top are not significantly different according to Tukey test ( $P > 0.05$ )); S, control (soil); A, algal 5 g per 50 g of soil; O, oregano 5 g per 50 g of soil.

Total Na or total K contents were increased by the addition of algae and of oregano. Moreover the greatest increase of total Na was observed where algae was added, while the greatest increase of total K was observed when oregano was added; this remark is explained by the higher content of oregano in K. Also the available forms of exchangeable-K were increased by algae or oregano treatments, while the greatest increase, was also observed where oregano was added; the exchangeable Na forms (figure 2), either by the addition of algae or by the addition of oregano, were not significantly different, while the cation exchange capacity was increased by algae or oregano treatments; these results showed that there was no risk of sodicity for soil.

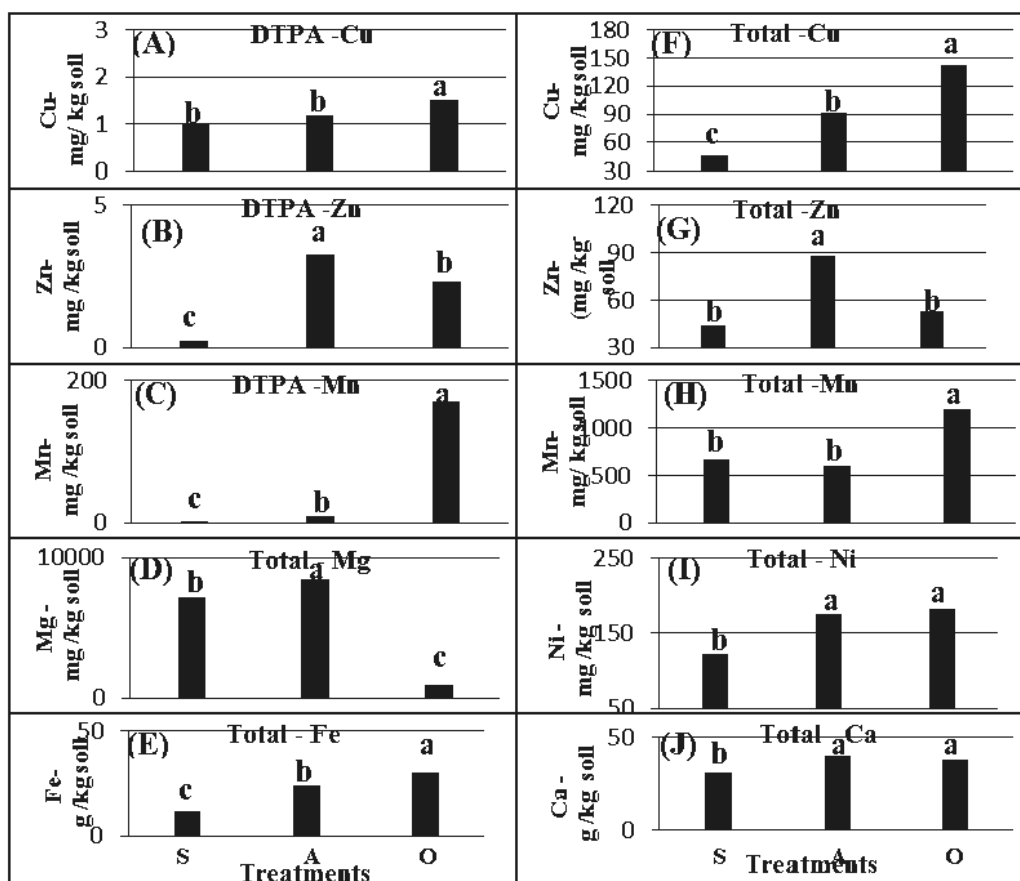


Figure 3. Effect of algal and oregano on soil content in (A) available Cu; (B) available Zn; (C) available Mn; (D) total Mg; (E) total Fe; (F) total Cu; (G) total Zn; (H) total Mn; (I) total Ni; (J) total Ca. (Bar values with the same letter on the top are not significantly different according to Tukey test ( $P > 0.05$ )); S, control (soil); A, algal 5 g per 50 g of soil; O, oregano 5 g per 50 g of soil.

The addition of algae or oregano increased the total-Ca; total-Mg (Figure 3) was increased by the addition of algae, but the oregano addition decreased it. The addition of algae or oregano increased both the Zn-available and Mn-available forms (Figure-3); the greatest increase of Zn-available was observed where algae was added, while the greatest increase of Mn-available was observed when oregano was added; also total - Mn was increased by the addition of oregano, while it was not changed by the addition of algae.

The addition of algae or oregano increased both the total-Fe and the total-Cu; the higher increase was observed when oregano was added. The Cu-available forms were increased when oregano was added, while it was not changed by the addition of algae. The total- Zn was increased by the addition of algae, while it was not changed by the addition of oregano. Finally, the total-Ni content increased both by algae and oregano addition.

## CONCLUSIONS

The results of the laboratory experiment, concerning the addition of algae to soil, showed that algal organic matter was subjected to a strong biodegradation by edaphic micro-flora, after a long incubation period. The algae as compared with the oregano foliar tissues, decreased the pH of amended soil, but on the contrary, the algae increased salinity of samples, in N-NO<sub>3</sub> forms, and in available contents of P and Zn; oregano foliar tissues as compared with the algae, increased the content of samples in available Mn, and both materials increased exchangeable K forms. The ammonium contents and exchangeable Na of samples after the addition of algae or oregano, no statistically significant differences showed.

These results, confirm the role of algae as organic soil amendment, and very probably is an important component for composting products. In an upcoming work, the effect of algae, in combination with organic manure, to the chemical properties of soil will be reported. The effect of algae on composting the organic fertilizer will also be reported.

## ACKNOWLEDGMENT

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## STUDY ON BIODIVERSITY *CENTAUREA CYANUS* FLOWERS AND FRUITS

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**Keywords:** *Centaurea cyanus*, capitula, false-ligulae petals, achenes

### ABSTRACT

Between the degree of control of a weed and biodiversity which expresses the field of culture there is a direct link (Aldrich, 1984; Chirilă et Pintilie, 1989). Because lately, *Centaurea cyanus* L. Species became resistant to herbicide treatment was considered as good a biodiversity study to prove it. Research has shown, however, that weed has a specific biodiversity analyzed area. The capitula have lengths and widths between 8-14/4-7 mm. They dominated the 11/6 mm. False-ligulae petals pieces were 6-12 number and 9-17 mm length. They dominated with with 8 petals and 12 mm length. Achenes in a capitulum were number between 5-27, with maximum 10-14 pieces. Achenes sizes were between 2.9-4.0 mm length and 1.2-2.1 mm thickness. They dominated were achenes with 3.2-3.4/1.4-1.7 mm. The correlations between these characters were specific and expressed also the level of weed *C.cyanus* biodiversity from winter wheat crop, luvic soil area.

### INTRODUCTION

Species *Centaurea cyanus* L. pro syn. *C.segetum* Baumg (CENCY code Bayer), is one of the common weeds (Prodan & Buia, 1958; Skrzypczak et al, 1995) in our winter crops: wheat, barley, rye, and triticale. Lately there has been a more obvious its infestation in fields due to the fact that some of the herbicides used no longer fully combat. It is thus possible that some ecotypes of weeds, especially from wheat, have become resistant to chemical treatments. Given the new recommendations, which could meet biodiversity studies to promote integrated weed management (IWM) (Chirilă, 2001) can return to the effective control of the weed. From what we know up to now, a specific weeds in a crop which exhibits obvious morphological biodiversity will be able to control easier, regardless of the method used.

The literature provides some average values of flowers and fruit size *C.cyanus* (Aliotta, 1987; Anghel et al, 1975) without highlights a clear variation, distinct ecotypes of concrete of a complex culture. The head of the plant have the length and width dimensions between 10-14/6-8 mm, without specifying the number of false-ligulae petals external and their length (Bressler, 1998, Bown, 1995, Păunescu & Crivineanu, 1998). Achene fruit size

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is generally 3-4 mm length/ width 1.5-2.0 mm. In general the head of *C.cyanus* contain two types of flowers (Bianco, 1989, Bianco et al., 2001).

Some are ligulae, tubular, large, blue, being sterile. The flowers in the center are short, tubular, purple and fertile. Spring flowers occurs during the month June and may be extended depending on the growing season until autumn - september (Chirilă, 1987; Popovici et al, 1994).

In this study measurements were performed the head/capitula size and were analyzed false-ligulae flowers at flowering in June. Determinations and measurements were conducted in the achenes full ripening thereof.

### MATERIAL AND METHODS

The measurements were made during the 2012-2014 *C.cyanus* plants of winter wheat crop. The four (4) sole were placed on the VI terrace of Argeş River, south of the town Pitesti. The heads are analyzed in full bloom in June, and the fruits formed prior to release. At flowering capitula were measured: length (height) of the stalk up in corolla and width in the widest part. False-ligulae petals were counted on each capitulum and length was measured. Achene fruit type were measured number of capitulum, the length (height) and width of the central portion.

Plants were chosen by following the zig-zag fireplaces. Thus we analyzed a total of 200 flower heads of all four sole grown winter wheat. Morphological characteristics measured were then analyzed by drawing polygons by the frequency (%). Among the main characters were established the correlations. Values are presented as an average over the three years of culture and the statistical calculation using Excel.

### RESULTS AND DISCUSSIONS

Variability in the length and thickness of *C.cyanus* capitula. Capitula analyzed showed lengths between 8 and 14 mm- fig.1. The highest frequencies were 11 mm (40%), followed by the 10 mm (35%) and 12 mm (21%). Extreme lengths: from 8 to 14 mm accounted for 1% of the total. Capitula width or thickness ranged from 4 to 7 mm. The highest frequency was 6 mm (65%), followed by the 7 mm (27%). Capitula of 4 mm thickness have been only about 1 %.

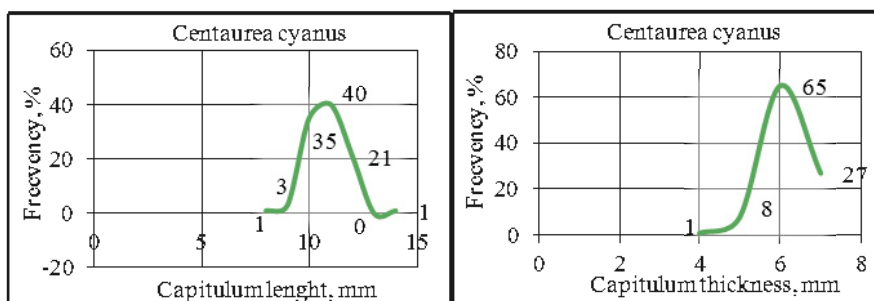


Figure 1. The frequency of capitula length and thickness, *Centaurea cyanus* weed

The correlation between the length and the thickness of heads obtained showing a state the positive ( $r = 0.227$ ), showing that the *C.cyanus* plants can form flower heads more bigger, depending on the existing culture conditions (Bilteanu, 1988)- fig. 2.

**Variability in the number and length of false-ligulae petals.** Fals-ligulae petals included between 6 and 12 pieces- fig. 3. Highest frequency they had 8 capitula petals (48%), followed by the 9 petals (29%). Extremes represented by 1 % from total.

The correlation obtained between the number and length of petals was positive, distinctly significant ( $r = 0.262^{**}$ ). Capitula of *C.cyanus* with more false-ligulae petals can be longer, which clear could give a more obvious observation for pollinating insects (Kulpa, 1988), figure 4.

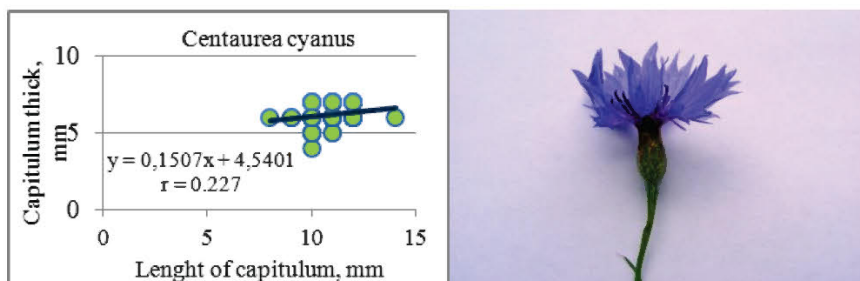


Figure 2. Correlation between length and thickness capitula of *Centaurea cyanus* weed

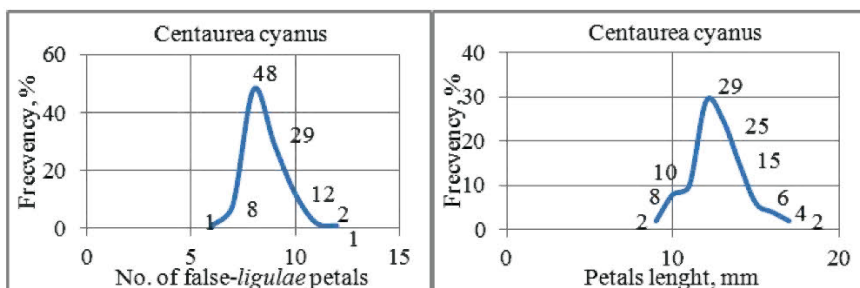


Figure 3. The frequency of false-ligulae petals number and length, *Centaurea cyanus* weed

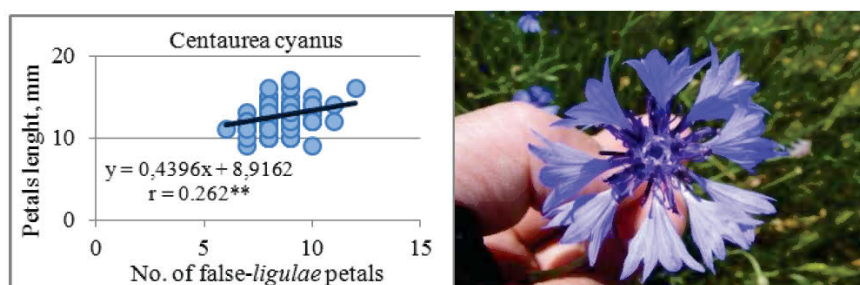


Figure 4. Correlation between number and length petals of *Centaurea cyanus* weed

**Achenes scale variability.** Number of achenes in a capitulum, grouped classes, were found with the highest frequency from 10 to 14 parts (41%), followed by those with 19 to 19 parts (31%). Reduced frequency obtained class with 25-27 pieces (2%) - fig.5. Achenes length classes' grouped frequency ranged from 2.9 - 4.0 mm. The frequency of the dominant class was 3.2-3.4 mm (32%), followed by one with 2.8-3.1 mm (28%) and 3.5-3.7 mm (26%).

The correlation obtained between the number of achenes and their length was less significantly negative ( $r = -0.007$ ), suggesting that the fruits fewer like number can be little longer - fig. 6.

The thickness (width) of achenes was in variability between 1.2 and 2.1 mm- fig. 7. Classes with higher frequency were 1.4-1.5 mm (36%) and 1.6-1.7 mm (35%). The 2.0-2.1 mm class represented only 1% of the total.

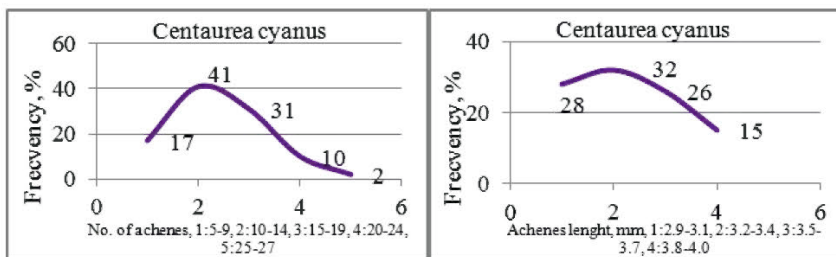


Figure 5. The frequency of achenes: number and length, *Centaurea cyanus* weed

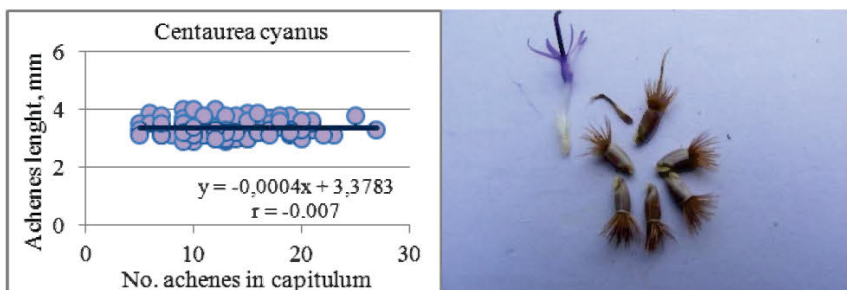


Figure 6. Correlation between number and length achenes of *Centaurea cyanus* weed

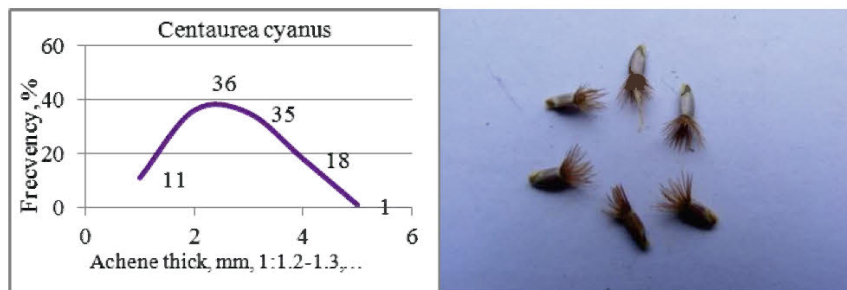


Figure 7. The frequency of achenes number and width, *Centaurea cyanus* weed

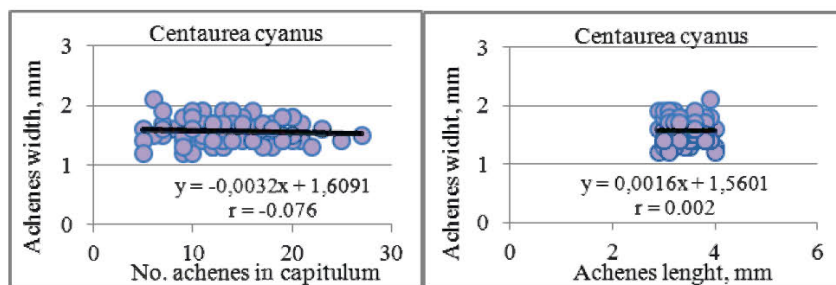


Figure 8. Correlations between number and width and length and width achenes

Table 1

The structure and distribution of analysis values from *Centaurea cyanus* flowers and fruits

Flowers/ capitula		Radial petals		Fruits/ achenes		
Length,mm, %	Thick,mm, %	Number, %,	Length,mm, %	No/capitula, %	Length,mm, %	Width,mm, %
8 1%	4 1%	6 1%	9 2%	5-9 17%	2.9-3.1	1.2-1.3
9 3%	5 8%	7 8%	10 8%	<b>10-14 41%</b>	28%	11%
<b>10 35%</b>	<b>6 65%</b>	<b>8 48%</b>	11 10%	<b>15-19 31%</b>	<b>3.2-3.4</b>	<b>1.4-1.5</b>
<b>11 40%</b>	7 27%	9 29%	<b>12 29%</b>	20-24 10%	<b>32%</b>	<b>36%</b>
12 21%		10 12%	<b>13 25%</b>	25-27 2%	3.5-3.7	<b>1.6-1.7</b>
13 0%		11 2%	14 15%		26%	<b>35%</b>
14 1%		12 1%	15 6%		3.8-4.0	1.8-1.9
			16 4%		15%	18%
			17 2%			2.0-2.1
						1%

Established correlation between the number of achenes with their thickness was negative ( $r = -0.076$ ), suggesting that a few achenes they will be more thick- fig. 8. Between length and width of achenes was obtained a positive correlation, but close to 0 ( $r = 0.002$ ), which indicates a lower dependency between the two characters.

### CONCLUSIONS

One of the common and important weed species from winter wheat crop is the *Centaurea cyanus* L. Its spreading occurs frequent years, and lately it has been more evident. Fighting weeds is more difficult, than plant exhibiting herbicide resistance phenomena. From studies of biodiversity and the degree of control of weed species was found a positive correlation.

One such study included determinations of morphological characters, especially reproductive parts: flowers and fruits. Were analyzed and determined surrendered weed size: length and thickness. Were counted and measured the false-ligulae petals, which are important in the pollination with insects. Mature fruits formed were analyzed in terms of the number and dimensions: length and width.

Variability data obtained are considered to be important for the expression of weed ecotypes present in winter wheat grown in the southern area white luvisol. They express a certain trend exists between morphological characters of this species of weed (plant)- table 1.

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## STUDY ON GREENHOUSE TOMATO CROP IN STONE WOOL SUBSTRATE, UNDER CONVENTIONAL HYDROPONICS AND CLOSED SYSTEM USING GEOTHERMAL ENERGY

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*Keywords: closed hydroponic systems, geothermic, nutrient solution, stone wool substrate, tomato*

### ABSTRACT

*In this work, the cultivation of hydroponic tomato in stone wool substrate in two greenhouses of TEI Thessaly is evaluated. One greenhouse is of conventional type (open system) and the second one is a closed system type using shallow geothermic with heat pumps. The thickness and the height of plants, the number of leaves and inflorescences, the total water consumption and the production were recorded. In conventional greenhouse, the growth and the thickness of plants were lower as compared with the geothermal greenhouse, throughout the duration of the growing season. Also, the number of inflorescences was less in conventional greenhouse as compared with the geothermal one, throughout the duration of the growing season. The water consumption in the geothermal was 1680 m<sup>3</sup>/ha, while in the conventional greenhouse was 5000 m<sup>3</sup>/ha. Finally, the crop production in the geothermal greenhouse was of better quality and reached 286 t/ha, while in the conventional greenhouse was 199 t/ha.*

### INTRODUCTION

The development of hydroponic cultivation (Manios et al. 1995, Inden et al. 2004) is based on modern distribution systems of nutrient solution (Sonneveld et al. 1999, Zekki et al. 1996).

Greenhouses are one of the largest low-enthalpy energy consumers in agriculture. Geothermal energy is derived by absorption of solar radiation and is stored from the earth's surface, up to the depth 200 m and a temperature of 10-18°C. The use of geothermal resource in combination with an existing fossil fuel system succeeds peak heating (Rafferty, 1997).

Geothermal energy reduces greenhouse gases (Bloomfield et al., 2003). The advantages of using geothermal energy are economical (Popovski, 1998), particularly in cold climates, and the good correlation between low-enthalpy geothermal resources and greenhouse production area.

The environmental thermal energy is used by heat pumps, which are now an important tool for energy savings and reduction of polluting fuels (Papageorgakis, 1992).

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The purpose of this study was to compare the efficiency and water savings of a geothermal greenhouse with a conventional one where tomato was cultivated.

### MATERIAL AND METHODS

**Description of facilities:** The work was carried out in two greenhouses of TEI of Thessaly, one (A) of closed type with standard water recycling system, which was based on shallow geothermal energy, and a second one (B) of conventional type. The greenhouse dimensions, the ventilation systems and the coverage materials were identical.

Furthermore, the geothermal greenhouse has a special metallic conduit that recycles part of the air in the greenhouse environment (Photo: 1).

The energy requirements of the geothermal greenhouse for cooling, heating and converting the water vapor into water, covered by closed circuit exchangers (Photo:2) that are constructed adjacent to the greenhouses at a depth of 100 meters.



Photo: 1.The air dehumidification system; 2. The ground heat exchangers (plastic tubes); 3. Heat pump; 4. Placement of the secondary tubes; 5. System preparation of the nutrient solution



For the air conditioning of greenhouses there is the optimal heating and cooling system. Two heat pumps are used (Photo: 3) both for heating, or one for heating and one for cooling.

For cooling the space, the existence of forced air recirculation units is required, aiming at water savings, while for heating either using the same (cooling) units or the water pipe network. Each unit of forced air recirculation controls the flow rate of air in the greenhouse, the crossing or not of the hot/cold water system and the on/off function.

The automation is applied by a standard remote thermostat, with the possibility of separation or not, into zones which require heating/cooling. However, in the space are located and measurement systems of relative humidity. The data of relative humidity are used in air conditioning.

The overall flow of water within the greenhouse is controlled by modern automation which continuously adjusts the flow in the system.

**Crop:** Tomato was cultivated during the period from 10/11/2013 to 20/03/2014 in an area of 115 m<sup>2</sup> for each greenhouse, with substrate stone wool slabs that were placed in double rows, with a distance of plants in each slab of 30 cm. Also, secondary pipes were placed in a specially designed table, in order to reduce heat loss to the environment and to heat the plants in the root zone (photo: 4).

The nutrient solution production unit has three stock solutions that are in three different barrels (photo: 5). The necessary amount of solution from each barrel is delivered out automatically by creating the final nutrient solution after regulating the EC and pH values in a programmable unit. According to the directives of the supplier, for tomato it is recommended a nutrient solution with pH: 6 and electrical conductivity EC: 2 dS m<sup>-1</sup>.

The content of the three parent nutrient solutions for the first and second crop were:

A Barrel: (Ca<sup>2+</sup> = 507, NO<sub>3</sub><sup>-</sup> = 680.6, K<sup>+</sup> = 116.8, NH<sub>4</sub><sup>+</sup> = 55, Fe<sup>2+</sup> = 1.8) me/L H<sub>2</sub>O.

B Barrel: (Mg<sup>2+</sup> = 195, K<sup>+</sup> = 644.2, H<sup>+</sup> = 286, SO<sub>4</sub><sup>2-</sup> = 580, NO<sub>3</sub><sup>-</sup> = 115.2, PO<sub>4</sub><sup>3-</sup> = 430) me/L H<sub>2</sub>O.

C Barrel: 6.6 mL concentrated HNO<sub>3</sub> / L H<sub>2</sub>O.

Each watering had duration of 3 min. and the inflow of the nutrient solution was 58.9 mL/min. The first 70 days after the crop establishment, watering was applied three times per day, the next 40 days 4 times per day and the next 20 days 3 times per day.

During of the growing season, the width and the height of plants, the number of leaves and inflorescences were measured once per week. Eight plants of each greenhouse were selected for measurements. The four of those were always the same, while the other four were selected randomly.

## RESULTS AND DISCUSSIONS

From figure 1 we observe that the growth of plants is lower in conventional greenhouse as compared with the geothermal greenhouse, during the entire growing season.

From figure 2 is revealed that the number of leaves was lower in the conventional greenhouse as compared with the geothermal one, for the first 50 days of the growing season but after wards we observed variations.

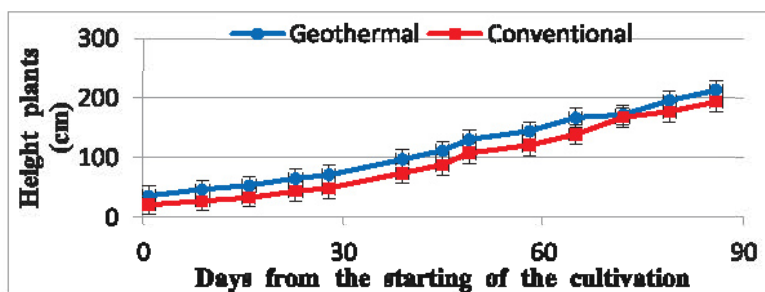


Figure 1. Average height value of tomato in conventional and geothermal greenhouse

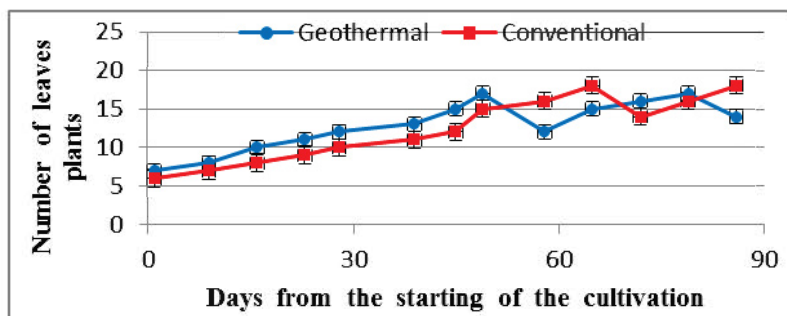


Figure 2. Average number of leaves plants in conventional and geothermal greenhouse

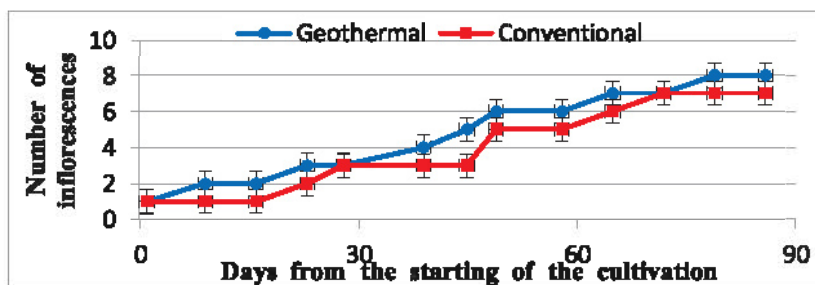


Figure 3. Average number of inflorescences in conventional and geothermal greenhouse

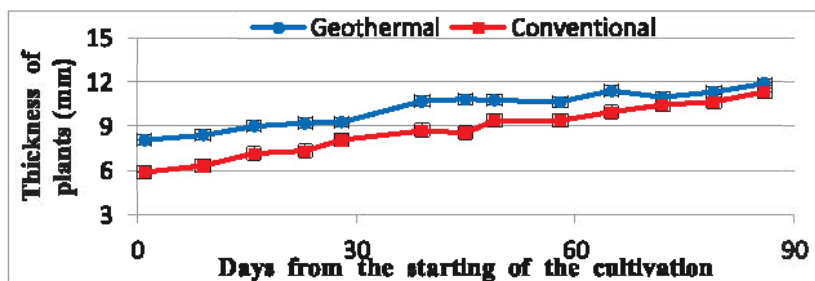


Figure 4. Average thickness of plants in conventional and geothermal greenhouse

In addition, in Figure 3, we observe that the number of inflorescences is lower in the conventional greenhouse compared to the geothermal, throughout the duration of the growing season, with a smooth growth path on both greenhouses.

Also, from Figure 4 we observe that the mean thickness of the plant is the higher in geothermal greenhouse as compared with the conventional one, throughout the course of the growing season, with a smooth growth path on both greenhouses.

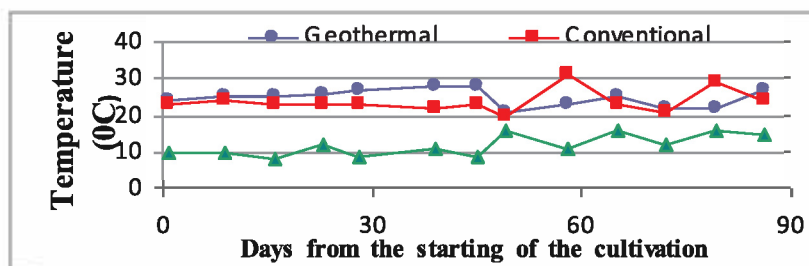


Figure5. Maximum temperature outdoor, and outdoor of both greenhouses

Finally, Figure 5 presents the temperature values as recorded in the outer and the inner space of the greenhouses during the growing season.

The collection of fruits from each plant took place from three heights (30, 70 and 100 cm), with three tomato fruit from each plant height. From each greenhouse 72 tomato fruits were collected. The average weight of tomato, in geothermal greenhouse (A) was  $238 \pm 17$  g, while in the conventional greenhouse (B) was  $189 \pm 14$ g.

The total production for the geothermal greenhouse was 286 t/ha and with better quality fruit, while for the conventional greenhouse was 199 t/ha.

The water consumption for the geothermal greenhouse was 1680 m<sup>3</sup>/ha, while in the conventional greenhouse was 5000 m<sup>3</sup>/ha.

## CONCLUSIONS

The results show that the tomato cultivation in the closed hydroponically system resulted in about 20.9% higher yield compared to the conventional one while a water saving of about 67% was achieved.

The hydroponic cultivation is suitable in soils poor in physical properties, gives full control of nutrition and irrigation while contributes to the protection of the environment.

It is a promised selection for a producer to invest in closed hydroponic greenhouses, due to water saving, better and higher production and environmental protection.

## ACKNOWLEDGEMENT

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## **CHEMICAL PROPERTIES OF A SANDY LOAM SOIL, AMENDED WITH LIQUID WASTE FROM BIOGAS PRODUCTION, IN VITRO**

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*Keywords: soil chemical properties; soil organic matter biodegradation; biogas liquid waste*

### **ABSTRACT**

*The present work was carried out in vitro (incubation experiment), and the effect of 211.8 g of biogas wastewater, mixed with 50 g of soil, poor in organic matter content was studied. All treatments were incubated for 15 weeks at a constant temperature of 28°C and after the incubation period, suitable chemical analyses were performed. The results showed that the biogas wastewater is subjected to a high percentage of organic matter biodegradation, about 50%. The biogas wastewater increased the soil salinity and elevated the available contents of P and K. Also, increased the content of samples in available Mn, Cu, Zn and prevented nitrification.*

### **INTRODUCTION**

The waste from biogas production plants could be applicable as composted material, while the raw waste could have beneficial effects concerning time and cost, (Lopez-Pineiro et al., 2008; 2010). The beneficial effects of these amendments are related to soil organic matter increase (Carbournell et al., 2010).

The organic matter is a soil fraction regulating the biological activity of soils, so a satisfactory content in organic material, dominates the soil fertility (Chouliaras et al., 1998; Gougoulas et al., 2010).

In a previous work, with incubation experiment, solid and water soluble olive oil mill waste, at different mixtures with the soil, showed that the organic content of water soluble olive oil mill waste, is subjected to high rate of biodegradation but solid waste, showed a strong resistance to biodegradation (Gougoulas et al., 2013).

Anaerobic digestion of energy crops and wastes is of increasing interest in order to reduce the greenhouse gas emissions, and to produce biogas and digestate. Production of biogas provides methane that can be used for replacement of fossil fuels, while digestate is produced both by acidogenesis and methanogenesis. The digestate from anaerobic fermentation is a valuable fertilizer due to the increased availability of nitrogen and the better short-term fertilization effect (Weiland, 2010).

The aim of this work was to examine the effects of chemical properties on soil, caused by the nature of the water soluble-waste of biogas production, in vitro.

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## MATERIAL AND METHODS

In this study, 211.8 g of liquid waste, containing 1.25 g of organic matter, were added to 50 g of air-dried, light-textured soil respectively, which was poor in organic matter. All materials (soil and waste) were obtained from the region of Larissa (Greece, Table 1).

More over the waste came out from a new biogas plant, with a production capacity of 0.5 MWatts of electrical power. The mixture used in the digester, at the time of sampling, consisted from pig manure, dairy cows manure and whey. The feeding of the digester is continuous and a certain amount of waste is produced every day. The waste is collected in a concrete tank and after that goes to solid – liquid separator and from there the liquids goes to lagoons. In the concrete tank there is a mixer that mixes all the content before they go daily to the separator. 30 samples of 0.5 L each were taken every 2 minutes from the pipe line that goes from the tank to the separator. All the samples were mixed together and from those 2 samples of 4 L each went to the laboratory.

In the incubator, the treatments were prepared in triplicates and kept at 28 °C for a period of 15 weeks. During the first three weeks of the incubation period, the moisture was maintained at two-thirds of field capacity, but for the next three weeks the soils were left to dry. This process was repeated until the end of the incubation period. According to Wu and Brookes, 2005, the alternation of drying and rewetting soil samples enhances mineralization of both soil biomass organic matter and non-biomass organic matter.

At the end of the incubation period, soil samples were analyzed using the following methods which are referred by Page et al., 1982 and Hesse et al., 1972.

### Methods of analysis:

Organic matter was analyzed by chemical oxidation with 1 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and titration of the remaining reagent with 0.5 mol L<sup>-1</sup> FeSO<sub>4</sub>.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol L<sup>-1</sup> CaCl<sub>2</sub> and estimated by distillation in the presence of MgO and Devarda's alloy, respectively.

Available P forms (Olsen P) was extracted with 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> and measured by spectroscopy.

Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> and all forms were measured by spectroscopy.

Exchangeable forms of potassium were extracted with 1 mol L<sup>-1</sup> CH<sub>3</sub>COONH<sub>4</sub> and measured by flame Photometer (Essex, UK).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol L<sup>-1</sup> + CaCl<sub>2</sub> 0.01 mol L<sup>-1</sup> + triethanolamine 0.1 mol L<sup>-1</sup>) and measured by atomic absorption.

For the determination of total metals Mn, Cu and Zn, 1 g of wet material, was digested at 350 °C + 10 ml HNO<sub>3</sub> + 5 ml HClO<sub>4</sub>. According to the method described by Allen et al., 1974, and Varian, 1989, the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture.

The experiment was repeated and the completely randomized design with four replications was used. Tukey's procedures were used to detect and separate the mean treatment differences at P = 0.05. Statistical analyses were performed by the statistical program MINITAB (Ryan et al., 2005).

Table 1

## Chemical properties of soil samples and biogas wastewater

Property	Soil	Biogas wastewater (wet basis)
Texture	Sandy Loam	-
pH	7.83 (1part soil:5parts H <sub>2</sub> O)	7.43 (raw waste)
Electrical conductivity, extract (dS m <sup>-1</sup> )	0.28 (1part soil : 5 parts H <sub>2</sub> O)	19.62 (raw waste)
Organic matter (%)	0.57	0.59
CaCO <sub>3</sub> (%)	8,60	-
N -total (g kg <sup>-1</sup> )	1.54	1.49
N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	27,7	-
N-NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	99,1	-
Exchangeable-K (mg kg <sup>-1</sup> )	276,9	-
K-Total (g kg <sup>-1</sup> )	5.3	0.384
Exchangeable-Na (mg kg <sup>-1</sup> )	211,6	-
Na-Total (mg kg <sup>-1</sup> )	619	1639.3
CEC (cmol kg <sup>-1</sup> )	20.6	-
P -Olsen (mg kg <sup>-1</sup> )	11.2	-
P-organic (mg kg <sup>-1</sup> )	53.2	-
P-inorganic (mg kg <sup>-1</sup> )	198.3	-
P -Total (mg kg <sup>-1</sup> )	251.5	392.3
Cu -DTPA (mg kg <sup>-1</sup> )	0.98	-
Zn -DTPA (mg kg <sup>-1</sup> )	0.42	-
Mn -DTPA (mg kg <sup>-1</sup> )	5.41	-
Cu -Total (mg kg <sup>-1</sup> )	67.4	0.72
Zn -Total (mg kg <sup>-1</sup> )	67.1	2.73
Mn -Total (mg kg <sup>-1</sup> )	595.1	0.95
Fe-Total (mg kg <sup>-1</sup> )	18000	9.9
Ca- Total (g kg <sup>-1</sup> )	34.4	0.185
Mg-Total (mg kg <sup>-1</sup> )	7940	54
Ni- Total (mg kg <sup>-1</sup> )	201.8	0.03
Pb- Total (mg kg <sup>-1</sup> )	<0.02	<0.02
Cr- Total (mg kg <sup>-1</sup> )	<0.06	<0.06
Cd- Total (mg kg <sup>-1</sup> )	<0.02	<0.02
Moisture (%)		98.66

**RESULTS AND DISCUSSIONS**

The results of the laboratory experiment (figure 1) showed that samples amended with soluble waste, organic contents of soil treated at the end of the incubation period proved a high percentage of organic matter biodegradation, about >50% (Figure 1). A

significant increase of salinity and alkalinity was revealed with of liquid waste applications to soil, while the increase of exchangeable Na cause sodification risk for soil.

With the application of biogas wastewater in the soil at the end of the incubation period, the  $\text{NO}_3^-$  content was reduced, the  $\text{NH}_4^+$  content showed no statistically significant differences, while the  $\text{NO}_3^-$  content is higher in comparison with  $\text{NH}_4^+$ . A significant increase for P organic synthesis was revealed with the applications of biogas wastewater to soil, as well and available forms of P and K. Also, with the application of biogas wastewater in the soil, was increased pH of the soil, the total Na content and total K content.

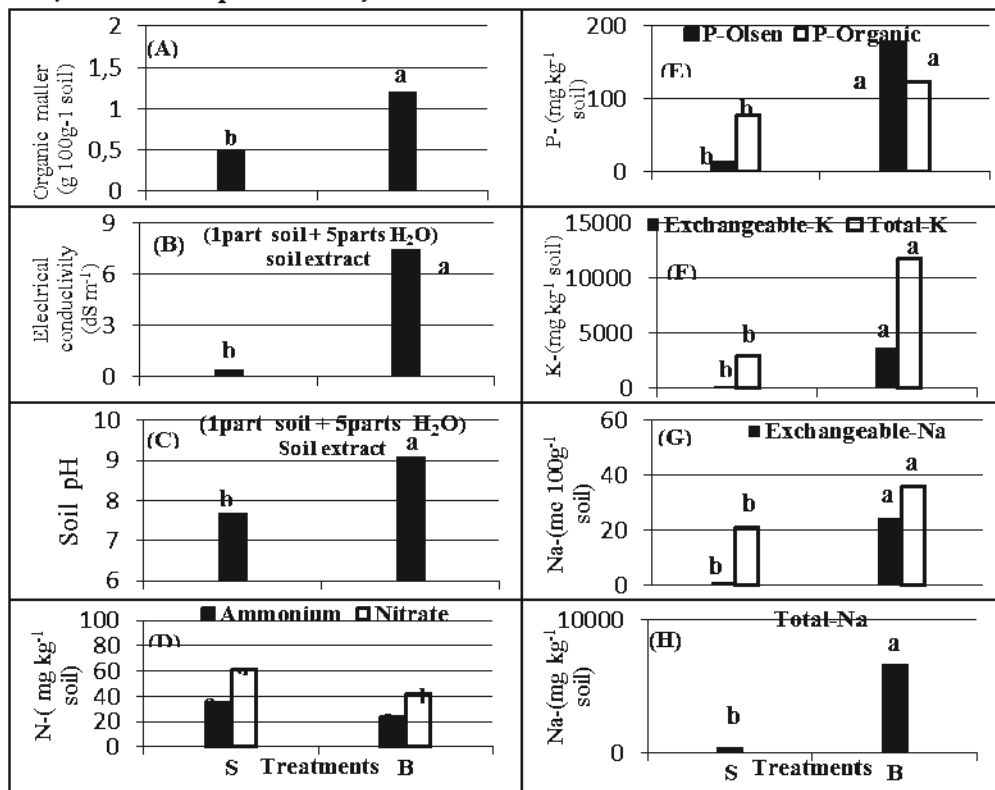


Figure 1. Effect of olive mill wastewater at the end of the incubation time. (A) Soil organic matter, (B) water extract electrical conductivity; (C) soil pH; (D) soil mineral forms of N, ammonium, and nitrate; (E) organic and available P; (F) available K and total K; (G) exchangeable Na and cation exchange capacity; (H) total Na (Bar values with the same letter on the top are not significantly different according to Tukey test ( $P > 0.05$ )). S, control (soil); B, biogas wastewater 211.8 g per 50 g of soil.

The addition of biogas wastewater in the soil, at the end of the incubation period, was observed a significant elevation in available forms for Cu, Mn, and Zn (Figure 2). Also, with the addition of the soluble waste in soil, were increased the total Fe content, the total Mn content and the total Ni content. Contrary, there was reduced the total Mg content, the total Cu content, and the total Ca content. Finally, the total Zn content showed no statistically significant differences, with the addition of biogas wastewater in the soil, at the end of the incubation period.



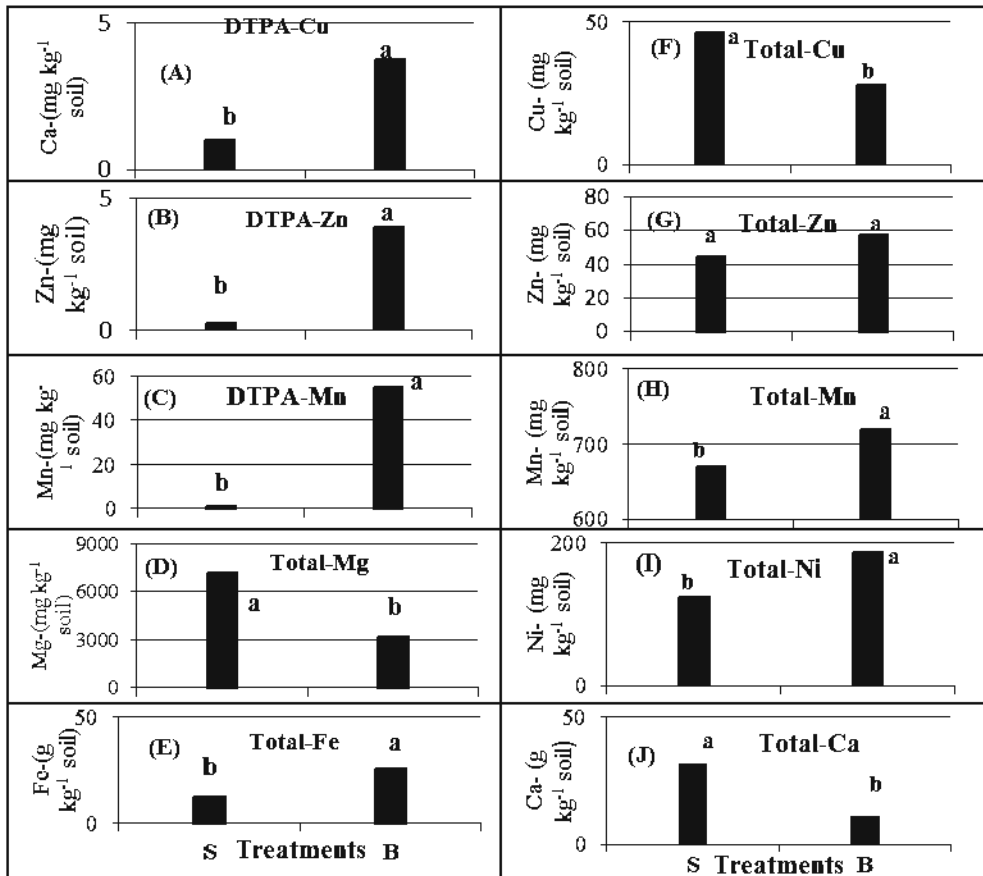


Figure 2. Effect of biogas wastewater added to soil on total and available forms. (A) available Cu; (B) available Zn; (C) available Mn; (D) total Mg; (E) total Fe; (F) total Cu; (G) total Zn; (H) total Mn; (I) total Ni; (J) total Ca. (Bar values with the same letter on the top are not significantly different according to Tukey's test ( $P > 0.05$ )). S, control (soil); B, biogas wastewater 211.8 g per 50 g of soil.

### CONCLUSIONS

The results of the laboratory experiment, concerning the addition of biogas wastewater to soil, showed that biogas wastewater organic matter was subjected to a strong biodegradation by edaphic micro-flora, after a long incubation period.

The biogas wastewater, increased salinity of samples, in available contents of P, K, Cu, Zn, Mn, exchangeable Na forms, and pH of soil, but on the contrary, decreased in N-NO<sub>3</sub> forms. The ammonium contents of samples after the addition of wastewater, no statistically significant differences showed.

These results, confirm that biogas wastewater, very probably is an important component for composting products. In an upcoming work, the effect of biogas wastewater, in combination with organic manure, to the chemical properties of soil will be reported.

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**STUDY ON THE RESISTANCE OF GRAPEVINE VARIETIES AND  
HYBRIDS TO PHYLLOXERA (*DAKTULOSPHEIRA VITIFOLIAE* FITCH  
*HEMIPTERA: PHYLLOXERIDAE*)**

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**Keywords:** vine, varieties, hybrids, phylloxera, response.

**ABSTRACT**

*The response of 4 interspecific hybrids, 6 table grapes varieties and 19 wine varieties to the root form of grape phylloxera (*Daktulosphaira vitifoliae*) was studied under laboratory conditions. The results allowed their classification in two groups: susceptible - Hybrid V 29/27, Hybrid V 29/7 and varieties Storgozia, Brestovitz, Plevin, Palieri, Sauvignon Blanc, Chardonnay, Colombard, Viognier, Petit Verdot, Rubin, Malbec, Sangiovese, Carmenere, Marselan, Super Ran Bolgar, Misket Kailashki, Misket Hamburg, Pinot Blanc, Petit Manseng and Pinot Noir; highly susceptible - Hybrid V 5/20, Hybrid V 39/80, and Cabernet Franc, Syrah, Super Ran Bolgar, Italy, Misket Ottonel and Chenin. The lowest numbers of tuberosity were formed on the roots of Storgozia variety and the greatest number in Hybrid V 39/80.*

**INTRODUCTION**

Phylloxera invasion in Europe set a new viticulture, both technologically and economically. American species of vines resistant to phylloxera were introduced, on which European vines were grafted for the effective control of that great disaster. That laid down the beginning of interspecies hybridization in vine for selection of new varieties with increased resistance to phylloxera.

Numerous and various studies were carried out worldwide on vine resistance to phylloxera. The results of such investigations had been analyzed by Götz (1956), Husfeld (1963), Boubals (1966), Strebler (1975), Nedov (1977), Chebotar (1978), etc. In Bulgaria, the first studies to establish the resistance to phylloxera of newly bred interspecies varieties and hybrids at the Institute of Viticulture and Enology were undertaken by Valchev, et al. (1989). Lyubenova et al., (2011), Ivanov et al. (2013) continued the researches on the resistance to phylloxera, as they included the newly-selected interspecies table and wine grapevine varieties.

The objective of the study was to establish the response of some newly-selected and introduced in Bulgaria grapevine varieties and hybrids to the root form of grape phylloxera.

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## MATERIAL AND METHODS

In 2011-2013 a study was carried out to find out the response to the root form of phylloxera of 31 experimental variants, including:

4 interspecies hybrids – Hybrid V 5/20, Hybrid V 29/7, Hybrid V 29/27, Hybrid V 39/80;

25 grapevine varieties:

- white wine varieties - Misket Kailashki, Sauvignon Blanc, Misket Ottonel, Chardonnay, Colombard, Viognier, Pinot Blanc (=Bianco), Petit Manseng, Chenin;

- red wine varieties – Storgozia, Cyrah, Pinot Noir, Rubin, Malbec, Sangiovese, Carmenere, Cabernet Franc, Marselan, Petit Verdot;

- table grapes varieties - Super Ran Bolgar, Brestovitz, Plevan, Palieri, Italy, Misket Hamburg,

2 grapevine rootstocks – Rupestris du Lot and Berlandieri x Riparia CO4 – controls for resistance.

The study was carried out under laboratory conditions in a growth chamber. Pouget (1975) methodology was used, that distinguished five classes of resistance to phylloxera - immune class, highly resistant, resistant, susceptible and highly susceptible.

## RESULTS AND DISCUSSIONS

The study data revealed that the investigated variants had different response to the root phylloxera, allowing them to be divided into three groups by Pouget methodology (table 1).

The controls in the trial - Rupestris du Lot and Berlandieri x Riparia CO4 referred to the highly resistant to root phylloxera class. Fixed phylloxera was not observed on the roots of the rootstocks, in contrast to the vine varieties. To the fifth day of the inoculation the majority of young phylloxera larvae had died, and those that had penetrated into the root did not cause tuberosity formation. Root phylloxera could not feed on the roots of the one-year old vine rootstocks and moved to the young roots and the callus formed on the notches. Phylloxera on the roots caused thickening - nodosities (fig. 1).

Susceptible to root phylloxera were 19 of the studied variants. They were Hybrid V 29/27, Hybrid V 29/7 and varieties Storgozia, Brestovitz, Plevan, Palieri, Sauvignon Blanc, Chardonnay, Colombard, Viognier, Petit Verdot, Rubin, Malbec, Sangiovese, Carmenere, Marselan, Super Ran Bolgar, Misket Kailashki, Misket Hamburg, Pinot Blanc, Petit Manseng and Pinot Noir.

As a result of the artificial inoculation, their one-year old roots were covered with a great number of tuberosities. On 8 cm long root, 15.73 pc were accounted on the average. Phylloxera population was growing rapidly due to the great fertility of adult females. Single root necroses were observed due to the excessive development of tuberosities.

Varieties and hybrids of this group showed a wide range of susceptibility to phylloxera. For the three-year period, the limit rates were within the range from 5 to 23.5 pc. In two varieties (Storgozia and Hybrid V 29/27) less than 10 tuberosities were recorded while in Rubin, Misket Hamburg, Sauvignon Blanc and Super Ran Bolgar - over 20 pc. The smallest number of tuberosities (5) was formed on the roots of Storgozia variety.

Highly susceptible to root phylloxera were the hybrids V 5/20 and V 39/80, as well as the varieties Cabernet Franc, Syrah, Super Ran Bolgar, Italy, Misket Ottonel and Chenin. On their roots phylloxera larvae were fixed as soon as they went out of the leaf. They were grouped into strips and completely covered large surfaces of each root. After 10-12 days, the first mature females began to lay large numbers of eggs (fig. 2), as after their hatching, the number of phylloxera increased.

Table 1

Response of the investigated variants to root form of grape phylloxera  
(*Daktulosphaira vitifoliae*)

No.	Variant	Pouget Class	Average number of tuberosities per 8 cm root
1	Rupestris du Lot (control)	highly resistant	0
2	Berlandiery x Riparia CO4 (control)	highly resistant	0
3	Hybrid V 29/27	susceptible	9
4	Hybrid V 29/7	susceptible	12
5	Hybrid V 5/20	highly susceptible	26
6	Hybrid V 39/80	highly susceptible	29
7	Storgozia	susceptible	5
8	Brestovitza	susceptible	15.37
9	Pleven	susceptible	17.90
10	Sauvignon Blanc	susceptible	23.50
11	Palieri	susceptible	13.27
12	Chardonnay	susceptible	13.67
13	Colombard	susceptible	15.93
14	Viognier	susceptible	12.27
15	Petit Verdot	susceptible	19.67
16	Marselan	susceptible	12.43
17	Rubin	susceptible	20.30
18	Malbec	susceptible	15.23
19	Sangiovese	susceptible	12.13
20	Carmenere	susceptible	13.83
21	Cabernet Franc	highly susceptible	25.63
22	Syrah	highly susceptible	25.40
23	Super Ran Bolgar	susceptible	23.97
24	Italy	highly susceptible	26.10
25	Misket Ottonel	highly susceptible	25.13
26	Misket Kailashki	susceptible	12.93
27	Misket Hamburg	susceptible	21.30
28	Pinot Blanc	susceptible	18.30
29	Petit Manseng	susceptible	18.97
30	Chenin	highly susceptible	25.67
31	Pinot Noir	susceptible	19.20



Figure 1. Nodositie in roots of Rupestris du Lot rootstock.



Figure 2. Significant phylloxera population on Hybrid V 39/80 roots.

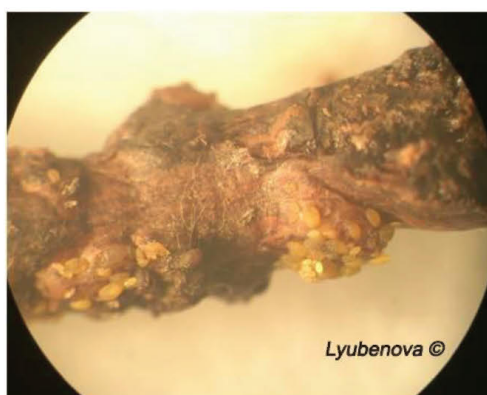


Figure 3. Voluminous tuberosities in Misket Ottonel variety - highly susceptible to root phylloxera class.



Figure 4. Great necrosis on root of Chenin variety - highly susceptible to root phylloxera class.

All stages of development were observed, except the sexual forms. At the end of the first week, the nodosities formed on the young roots become necrotic. The one-year old roots were covered with average 26.13 tuberosities, which, unlike the former class, were merged (fig. 3). A greater number of tuberosities were accounted in Hybrid V 39/80 - 29 pc. After the twentieth day a part of the roots died as a result of the ongoing severe necrosis of the tissues (fig. 4).

The obtained results on susceptibility of Storgozia and Misket Hamburg varieties to root phylloxera confirmed the data of Valchev et al. (1989) and Lyubenova and Ivanov (2011), respectively.

#### CONCLUSIONS

- Neither one of the studied varieties and hybrids was resistant to root form of grape phylloxera.
- Highly susceptible were the hybrids V 5/20 and V 39/80, and Cabernet Franc, Syrah, Super Ran Bolgar, Italy, Misket Ottonel and Chenin varieties.
- Susceptible to root phylloxera were Hybrid V 29/27, Hybrid V 29/7 and the varieties Storgozia, Brestovitza, Pleven, Palieri, Sauvignon Blanc, Chardonnay, Colombard, Viognier, Petit Verdot, Rubin, Malbec, Sangiovese, Carmenere, Marselan, Super Ran Bolgar, Misket Kailashki, Misket Hamburg, Pinot Blanc, Petit Manseng and Pinot Noir.
- The smallest number of tuberosities was formed on the roots of Storgozia variety.

#### ACKNOWLEDGMENT

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## DYNAMICS OF SOME PHYSIOLOGICAL PROCESSES AND OF A CHLOROPHYLL INDEX IN THE PROTECTED CULTURE OF CORNICHON CUCUMBER

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*Keywords: cornichon, greenhouses, foliar fertilizers, physiological processes, chlorophyll*

### ABSTRACT

*This study presents the main results obtained in Isalnița greenhouses complex regarding the influence of certain foliar fertilizers on the chlorophyll index but also the influence of different microclimate factors on the main physiological processes. The data indicate the positive influence of Cropmax, which also assures a higher exploitation of the available water and nutritious resources compared to the butte and the options based on Bionat and Folibor (prior submitted data).*

*The recording of microclimate factors, simultaneously with the intensity of some physiological processes, highlighted their influence and daylight dynamics when using a fertilizing irrigation for Mathilde hybrid.*

### INTRODUCTION

The cucumbers are cultivated for their fruit which are really appreciated in the food service, when reaching technological maturity, for their properties and diverse nutritious content, as well as for their therapeutic effects. In the vegetable basin of Oltenia, maybe the most important one in the country, the production of cucumbers has grown dramatically since 1990.

During the last years, because in some greenhouses, the culture productivity can be assured only by cold cultivation, the culture which allowed the possibility of using the greenhouses in two culture cycles was the Cornichon cucumber.

This was possible because the Cornichon cucumber culture has a shorter vegetation period, assuring larger quantities of production for a consumption which spreads over a long period of time, granting this culture a high economic value. Consequently, this species needs a strict application of technology, among which the fertilizing irrigation and bio-stimulators treatment may assure the economic efficiency of the culture (Butnariu et al. 1993; Ciofu R. et al. 2003).

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## MATERIAL AND METHODS

The registration of the intensity of certain physiological processes in agreement with the climatic factors involved using advanced nondestructive methods which imply the use of some instruments (e.g. Lci) equipped with analysis microchamber with a total surface of 6.25 cm<sup>2</sup>. The method rests on noticing the differences between the intake air composition so as not to be affected by the plant microclimate (approx. 3 – 4 m height) and the circulated air from the analysis chamber (Burzo et al. 2000). The variation of the chlorophyll index was studied depending on the hybrid and the stem segment (basal, middle and apical). The displayed data represent an average of 10 findings for each case, findings performed after applying the last treatment.

Determining the chlorophyll index was based on its property to absorb and reflect a bright radiation with known wave length (700 nm, respectively 840 nm), spread by the equipment used for this purpose (CM 1000).

## RESULTS AND DISCUSSIONS

In figure 1 it can be noticed that all options registered higher values of chlorophyll index compared to the butte.

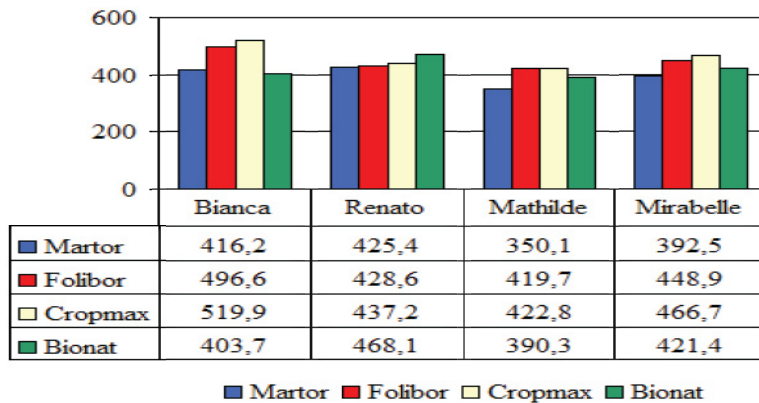


Figure 1. Evolution of chlorophyll index depending on the option

Moreover, option no. 3 is the one that assures in most of the cases a higher index of chlorophyll, while Folibor is the one that assured better results compared to Bionat. As the determination does not also assure a quantitative dimension, the only thing we can appreciate is the product quality and their property to influence more or less the quantity of synthesized chlorophyll from the leaves. In figure 2 it can be noticed the chlorophyll index variation depending on where the leaf is located on the stem.

It is noticed that all options registered higher values than the butte. Furthermore, the lowest index of chlorophyll was registered at the level of the lower leaves, while the middle segment registered the highest values. On the other hand, this result is due to the influence of the used product and also to the maturity of the leaves. At this stage, it is also recorded the highest content of chlorophyll. The apical segment continues to be characterized by lower values, specific to the younger leaves still growing, nevertheless superior to the ones located at the base of the plant.

The efficiency with which the trophic resources are used depends greatly on the weather favorability; it conditions the optimum development of the principles of anabolic processes.

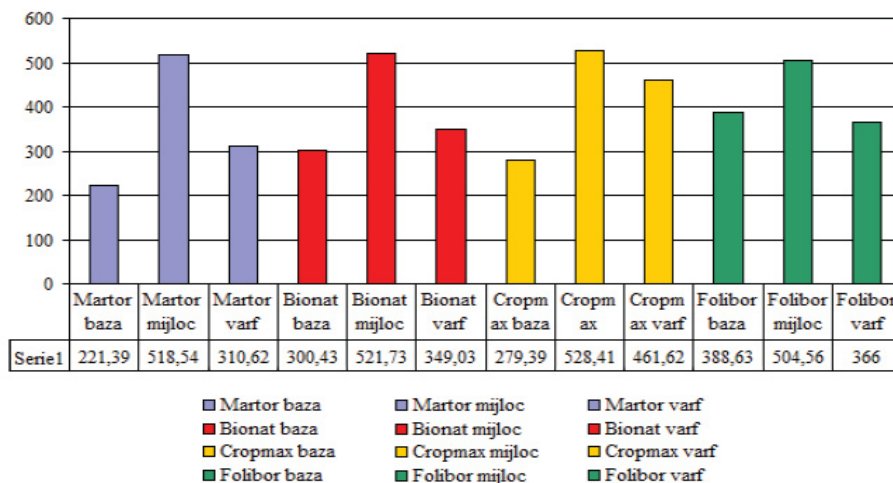


Figure 2. Evolution of chlorophyll index depending on the option and stem segment

Their investigation was performed during two phenophases, namely the seeding and the flowering-fruiting, by using a nondestructive technique. Consequently to all observations, it was concluded that the photosynthesis and perspiration were directly influenced by the radiations which form the solar spectrum. Thus, during seeding, it could be noted that the photosynthesis presents two maximum values, one in the morning and the other one, lower, close to the noon hours.

A decrease of this phenomenon evolution was noted at noon. This ascertaining is also supported by the high temperature value which threatens the smooth development of chlorophyll assimilation (figure 3).

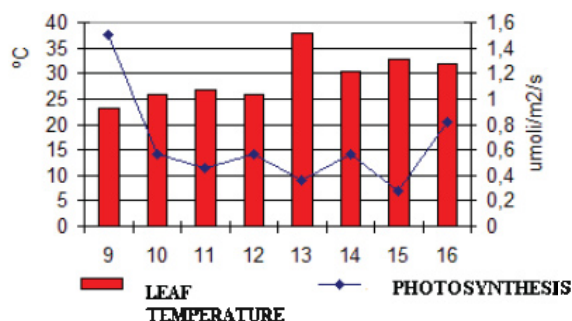


Figure 3. Daylight evolution of photosynthesis depending on the leaf temperature

Following the dynamics of the perspiration process, it can be noted that in general the evolution of this phenomenon is similar to the one registered for photosynthesis. The morning weather determines the materialization of this process at high intensity, being followed by a gradual decrease until around 3 or 4 p.m., when the resulted values create the premises of a hydric stress. This seems a transitory situation as the tissues turgescence can be recovered during the following hours. This fact has been suggested by the revival of photosynthesis which has a higher value after 4 o'clock p.m. compared to the values noted at 3 (figure 4).

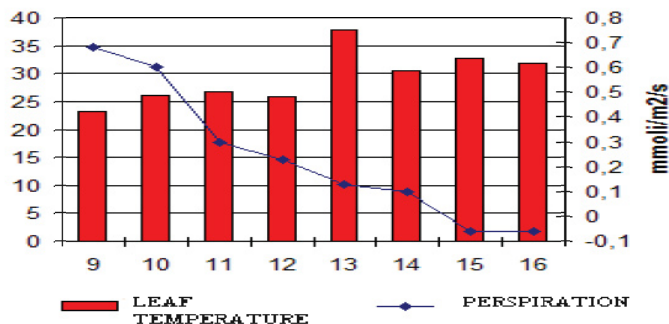


Figure 4. Daylight evolution of perspiration depending on the leaf temperature

The influence of the photosynthetically active radiation over the two studied phenomena is presented in figure 5 and figure 6.

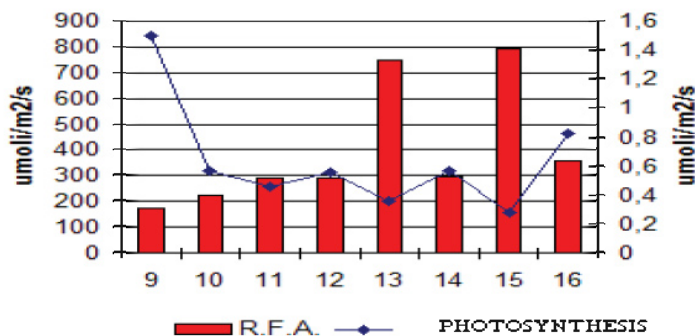


Figure 5. Daylight evolution of photosynthesis depending on the photosynthetically active radiation

The photosynthetically active radiation is represented by bright radiation with a wave length of 400 - 700 nm. This triggers the electronic transportation which culminates in the fixation of carbon dioxide and the synthesis of the first organic substances.

This factor does not act alone. It is associated with other factors too, among which the caloric effect due to the infrared radiations that is part of the solar spectrum.

The results highlight the fact that the intensity of photosynthesis remains at almost constant values when the incidental photosynthetically active radiation is between 200 and 400 μmol/m<sup>2</sup>/s. Reaching these high values, close to 800 μmol/m<sup>2</sup>/s has not led to

significant changes of photosynthesis values, given that at the level of the chloroplast there is a blockage due to the limitative factors.

The action of the high intensity of the photosynthetically active radiation could not stimulate the phenomenon in the case of perspiration either (figure 6). It remains inhibited because of other factors (caloric component of the solar spectrum radiations, etc.).

The dynamics of the two phenomena in the flowering-fruiting phenophase registers an evolution similar to the one recorded during seeding. Thus, the intensity of this photosynthesis phenomenon is more intense in the morning than during the seeding phenophase. In addition to that, the values characteristic to the decrease registered at noon hours are superior to those registered in the previous phenophase (figure 7).

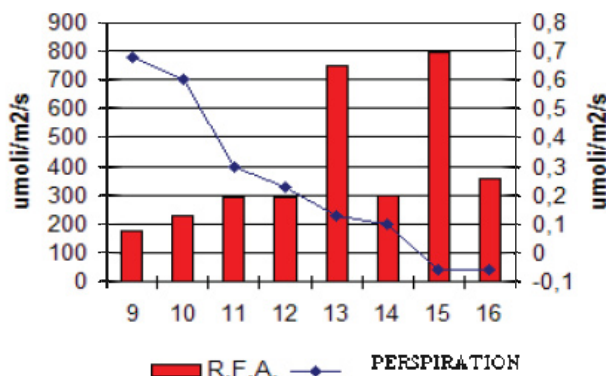


Figure 6. Daylight evolution of perspiration depending on the photosynthetically active radiation

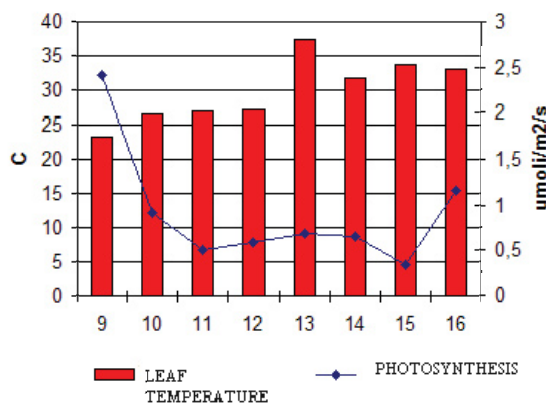


Figure 7. Daylight evolution of photosynthesis depending on the leaf temperature

This can be explained by the age of the leaves whose mature chloroplasts convert with high efficiency the received energy.

The maximum values of the perspiration are around 9 and 10 o'clock. After that, during the next hours it decreases almost up to zero, value registered at noon hours.

Regarding the influence that the photosynthetically active radiation exercised on the two phenomena, it is observed that, in general, they remain unchanged as the radiation increases its intensity, noting that in the morning the assimilation and perspiration values are high, due to radiations of 100 – 200  $\mu\text{mol}/\text{m}^2/\text{s}$ .

For similar values of the photosynthetically active radiation, the intensity of photosynthesis and perspiration decreases at 11 o'clock compared to 10 o'clock. Thus, one can draw the conclusion that the metabolic activity of the plant is influenced by a series of factors which mutually interact.

### CONCLUSIONS

All options registered higher values of chlorophyll compared to the butte;

Option no. 3 (the treatment with Cropmax) is the one that assures in most cases a superior index of a chlorophyll, while Folibor ensures better results than Bionat;

The lowest index of chlorophyll is registered in the base leaves of the plant, while the middle segment registered the highest values;

The photosynthesis and perspiration are directly influenced by the radiations that form the solar spectrum; the lowest intensity of these processes is registered at noon.

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## **THE EXCESSIVELY RAINY SPRING OF THE YEAR 2014 IN OLTENIA**

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**Keywords:** *excessive precipitation, flood, crop damage, waves of abundant rain.*

### **ABSTRACT**

*In Oltenia, the warm winter 2013-2014 was followed by a warm and excessively rainy spring. The spring arrival was an extremely early one. March was warm and rainy overall. April and May were thermally normal and excessively rainy. The quantities of precipitation registered during the spring 2014 varied between 299.8 l/m<sup>2</sup> in Voineasa and 516 l/m<sup>2</sup> in Apa Neagră, while the percentage values of the annual quantities went from 36.2% in Parâng to 70.9% in Craiova. This type of spring led to excellent conditions for the development of crops, but also of some diseases and pests specific to a wet and warm type of climate. The analysis of the climatic conditions of the spring 2014 in the South-West of Romania is of great use to specialists, doctoral and master students as well as all those interested in the evolution of climate.*

### **INTRODUCTION**

In Oltenia, the spring of 2014 was characterized by a weather evolution of exceptional variability, with rapid transitions from an exceedingly rainy to a normally pluviometric weather as well as from warm to cool periods. Five abundant waves of rainfall with pouring intervals were specific to the pluviometric regime of this spring. These extremely rainy intervals were: 2-6 March, 15-19 April, 23-24 April, 3-5 and 13-15 May, while local quantitatively significant rainfall was also registered in the interval 29-30 May. The analysis of the climatic conditions of the spring 2014 in the South-West of Romania is a continuation of the extended studies on the regional climate variability (Bogdan et al. 2011, Marinică et al. 2012 a and b).

### **MATERIAL AND METHODS**

In our analysis of this interval we used the databases of CMR Oltenia (the Regional Meteorological Center), ANM Bucharest (National Meteorological Administration), the maps created by the Agro-meteorology Laboratory (ANM), the maps provided by the Laboratory of Climate Monitoring, synoptic maps, data provided by the satellite and radar images archives, as well as the facilities provided by Microsoft Office.

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## RESULTS AND DISCUSSIONS

*Indexes of spring arrival in 2014.* The winter 2013-2014 was warm in Oltenia as well as in most of Romania, marked by long intervals of warm weather. After the warm and rainy autumn, in the end of November a short very early winter episode was registered, which was followed by an interval of 55 days of warm weather.

*Indexes of spring arrival* in 2014 were comprised between 341.9°C in Voineasa, an intra-mountainous depression, and 560.0°C in Dr. Tr. Severin. In the Western extremity, the deviations from the multi-annual average values of this index were comprised between 163.3°C in Calafat and 256.3°C in Drăgășani, leading to classifications of the spring arrival ranging from early in Calafat and exceedingly early for most of the meteorological stations in Oltenia.

*The mean spring arrival index* calculated for the entire region was 460.7°C, and its percentage deviation from the normal average was of 80.1%, thus confirming an excessively early spring arrival for the entire region.

*In March 2014, monthly temperature averages* were comprised between 6.1°C in Voineasa and 10.2°C in Dr. Tr. Severin. *Daily temperature averages for the entire region* were positive. The sums of positive daily average temperatures were comprised between 189.6°C in Voineasa and 316.5°C in Dr. Tr. Severin, and their percentage deviations from the spring arrival indexes were comprised between 55.3% in Apa Neagră and 59.5% in Bechet and Caracal.

*The average of these sums for the entire region* was 264.4°C representing 57.4% of the average spring arrival index calculated for the entire region.

This shows that the sums of daily average temperatures in March had the most significant contribution to the value of the spring arrival indexes, thus confirming that the *thermal regime of this month is essential for the spring arrival conditions.*

*Climatic conditions in March 2014 - Thermal regime in March.* *Monthly temperature averages* were comprised between 6.1°C in Voineasa and 10.2°C in Dr. Tr. Severin, and their deviations from the multi-annual means were comprised between 3.7°C in Voineasa and Băilești and 4.8°C in Drăgășani and Polovragi, which lead to the classification of warm for the thermal time type at all meteorological stations in Oltenia.

*Monthly thermal average in March*, calculated for the entire region was 8.3°C, and its deviation from the normal mean was 4.1°C, *almost double the value, which was the case for the monthly means of all the meteorological stations*, which confirms the general classification of warm month.

These extremely high deviations of the monthly temperature averages in March were the ones which led to the classification of warm spring, since April and May were thermally normal months as our analysis will show.

*The lowest daily temperature averages* were registered on March 10th and were comprised between 3.9°C in Bechet and 6.5°C in Râmnicu Vâlcea, the daily average for the entire region being 4.4°C.

*Most of the monthly minimum air temperature values* were registered on March 11th and were comprised between -3.5°C in Voineasa and 0.4°C in Drăgășani, their average for the entire region being -1.6°C.

*The maximum monthly air temperature values* were comprised between 20.5°C in Polovragi and 24.9°C in Calafat; their deviation for the entire region was 22.0°C.

*Monthly minimum values of ground surface temperature* were comprised between -3.8°C in Băilești and 0.0°C in Slatina, most of them being registered on the 11th and 12<sup>th</sup> of March.

*Monthly maximum values of ground surface temperature* were comprised between 22.8°C in Slatina registered on 31 March and 42.5°C in Băilești on 29 March.

The extremely warm weather with high temperatures at ground surface led to the continuation of the vegetation phases of plants from agricultural crops and especially those which were highly reactive to temperature increase (rapeseed, apricot tree, wheat, etc.).



*The blossom of trees began extremely early*, on 18 March 2014, when the first blossomed almond trees and apricot trees appeared.

**Pluviometric regime in March 2014.** *Monthly quantities of precipitations* were comprised between 28.7 l/m<sup>2</sup> at Voineasa and 107.8 l/m<sup>2</sup> at Apa Neagră, and the percentage deviations from the multiannual means were comprised between 32.9% at Băcleș and 208.3% in Craiova, and in the mountainous area they were negative.

Therefore, the classifications of pluviometric time type in March were comprised between very rainy at Băcleș in Mehedinți Hills and exceedingly rainy in most of the region.

*Monthly quantities of precipitation mean* calculated for the entire region was 73.6 l/m<sup>2</sup>, and its percentage deviation from the multiannual mean was 75.5%, thus confirming the classification of exceedingly rainy month.

*The rainiest period* was the interval 2-6 March 2014, when the daily precipitation means calculated for the entire region were comprised between 5.7 l/m<sup>2</sup> on 4 March and 19.1 l/m<sup>2</sup> on 6 March, thus registering the *first wave of abundant rains in this spring*.

*The sums of daily quantities* of precipitation registered in this interval of 5 rainy days were comprised between 54.8 l/m<sup>2</sup> in Rm. Vâlcea and 88.5 l/m<sup>2</sup> in Craiova, which represent percentages from the multiannual monthly means comprised between 115.3% at Băcleș (Mehedinți County) and 281.0% in Craiova in Oltenia central part.

On extended surfaces, crops have been flooded, and humidity excess in the ground cause water bogging in crops and grasslands.

Consequently, *water ground reserve, in autumn wheat crop*, in Oltenia in the end of March was comprised between almost satisfactory and almost optimum. In the ground layer of 0-20 cm land, water reserve in Oltenia was comprised between almost optimum and optimum.

***Climatic conditions in April 2014 - Air and ground thermal regime in April.***

*Monthly temperature means* were comprised between 8.6°C at Voineasa in intramountainous depression and 13.0°C in Dr. Tr. Severin in the extreme west of the region, and their deviations from the multiannual means were positive in the entire region with one single exception -0.1°C in Polovragi Subcarpathian Depression. These fell within -0.1°C at Polovragi and 1.1°C in Dr. Tr. Severin and Rm. Vâlcea leading to the classification of normal pluviometric time type in most of the region excepting some restricted areas where there was a warmish month in: Dr. Tr. Severin, Tg. Jiu and Rm. Vâlcea and in the mountainous area.

*Monthly temperature mean* for the entire region was 11.0°C, and its deviation from the normal was 0.6°C, which enables the classification of normal thermal month for the entire Oltenia.

*Monthly minimum thermal temperature values* in the air were comprised between -0.8°C at Voineasa registered on 13 April and 4.5°C at Bechet registered on 3 April, and their mean for the entire region was 1.8°C.

*Monthly maximum temperature values* were registered on 23 April, with one single exception (Bechet) and were comprised between 21.9°C at Polovragi and 25.0°C at Calafat, and the maximum temperature mean for the entire region was 23.1°C.

*At ground surface level the minimum temperature values* were comprised between -1.2°C at Tg. Logrești registered on 14 April and 5.4°C at Calafat on 11 April, and their mean for the entire region was 1.9°C.

*Monthly maximum temperature values at ground surface* were comprised between 25.6°C in Slatina and 47.3°C in Dr. Tr. Severin, most of them being registered on 4 April, and their mean for the entire region was 25.6°C, lower with 10.0°C than the mean of March.

**Pluviometric regime in April.** *Monthly quantities of precipitation* were comprised between 122.9 l/m<sup>2</sup> at Voineasa and 197.4 l/m<sup>2</sup> at Apa Neagră, and their percentage

deviations from the multiannual means were comprised between 82.9% at Voineasa and 277.6% at Drăgășani which lead to the classification of pluviometric time type of exceedingly rainy at all meteorological stations in Oltenia, excepting the mountainous area where at Parâng April was a very rainy month.

*The mean of the quantities of precipitation for the entire Oltenia region* was 135.8 l/m<sup>2</sup>, and its percentage deviation from the normal mean was 140.3%, which confirm the classification of exceedingly rainy month for the entire region.

In April 2014 *stow intervals of exceedingly rainy weather* were registered: 15-19 April and 23-24 April, of which the *rainiest and longest was the interval 15-19 April* in which the daily means of precipitation for the entire region were comprised between 7.5 l/m<sup>2</sup> on 17 April and 28.8 l/m<sup>2</sup> on 16 April.

*The quantities of precipitation registered* in this interval of 5 days were comprised between 46.4 l/m<sup>2</sup> at Parâng in the mountainous area and 101.8 l/m<sup>2</sup> at Tg. Logrești in the area of Oltenia hills. These quantities are percentage ratios of normal monthly quantities comprised between 53.8% at Parâng and 239.9% at Drăgășani. For the intervals of 24 hours the maximum quantity of precipitation registered during this wave of rainfalls was of 56.2 l/m<sup>2</sup> at Filiași, in the interval 16 April at 9AM- 17 April at 9AM.

*Floods and damages* of households and crops were registered in all the counties of Oltenia. At the level of the entire country, a number of 86 villages and communes have been flooded and 248 persons have been evacuated in the counties with hydrological warnings (the report presented by the Ministry of Interior and vice-premier Gabriel Oprea).

*Ground water reserve, in the crop of autumn wheat*, in Oltenia in the end of April was almost optimum in the entire region.

#### ***Climatic conditions in May 2014 - Air and ground thermal regime in May***

*Air temperature monthly means* were comprised between 12.1°C at Voineasa and 17.0 °C at Bechet, and their deviations from the normal values were comprised between - 1.1 °C in Craiova and Slatina and 0.3 °C in Rm. Vâlcea and Parâng, leading to the classification of normal thermal time according to Hellmann criterion in most part of Oltenia excepting restricted areas in Craiova and Slatina where it was cool.

*Air temperature monthly mean* in May 2014, calculated for the entire region was 15.0°C, and its deviation from the multiannual mean was -0.4°C which confirms the classification of normal thermal month for the entire region.

*The monthly minimum values of the air temperature* were comprised between 2.0°C in Subcarpathians at Apa Neagră and 7.2°C in the extreme south of region in Dr. Tr. Severin, and their mean was 5.0°C.

*Monthly maximum values of air temperature* were comprised between 25.8°C at Polovragi and 29.7°C at Calafat, and their mean was 27.8°C. Most of the monthly maximum temperatures were registered in the last five days of the month, on 26 May and some of them in the hilly area on 23 April.

*The warmest day of May* was registered on 26 May with the mean for the entire region of 20.3°C. Days with average close values were on 23 with the mean of 19.1°C, on 27 May with the mean of 19.2°C and on 28 May with the mean of 19.6°C.

*Weather gradual warming in May* has a benefic aspect because it leads to plants progressive development. Due to this thing in the end of June plants reach a development stage close to the normal of the specific calendar period. Significant thermal variations which occur in summer lead to and finalize their development.

*At ground surface monthly minimum temperature values* were registered in the first half of the month and were comprised between 1.4°C at Polovragi registered on 13 May and 8.1°C at Calafat on 7 May, and their mean was 5.3°C.

*Monthly maximum thermal values at the ground surface* were comprised in the last decade of the month between 34.2°C in Slatina registered on 7 May and 60.0°C at Băilești registered on 26 May.

***Pluviometric regime in May.*** *Monthly quantities of precipitation* registered in May were comprised between 86.2 l/m<sup>2</sup> at Băilești in Oltenia Plain and 211.2 l/m<sup>2</sup> in Subcarpathian Depression at Apa Neagră, and their mean for the entire region was 142.4 l/m<sup>2</sup>, almost double compared to the mean in March, exceeding with 6.6 l/m<sup>2</sup> that of March. May is thus the rainiest month of the spring of 2014.

*The percentage deviations of the monthly quantities of precipitation* registered in May in Oltenia were comprised between 23.0% at Băilești and 157.4% at Drăgășani in Olt Couloir, which lead to the classification of pluviometric time type of exceedingly rainy (ER) at all meteorological stations in Oltenia, excepting a restricted area at Băilești where it was rainy.

*The percentage deviation of the monthly mean* from the normal was of 77.9%, which confirms that May was exceedingly rainy in the entire region.

*Intervals with abundant rains* were 2-5 April with daily means for the entire region comprised between 4.4 l/m<sup>2</sup> on 5 May and 19.3 l/m<sup>2</sup> on 3 May and 13-15 May with daily means for the entire region comprised between 8.1 l/m<sup>2</sup> on 13 May and 54.4 l/m<sup>2</sup> on 14 May, but the significant quantities of precipitation were registered also in the interval 29-30 May.

*Daily mean for the entire region* was of 54.4 l/m<sup>2</sup> almost double compared to that of the mean of the rainiest day in April (16 April 2014), classifies the date of 14 April 2014 as being *the rainiest day registered in Oltenia after 2005*, not only in this spring. *The maximum quantity of precipitation registered in 24 hours*, on 14 April, was of 86 l/m<sup>2</sup> at Spineni, in Olt County, a value which represents *the daily pluviometric record* of the spring of 2014, and daily precipitation values  $\geq 50.0$  l/m<sup>2</sup> were registered on restricted area in this day.

*Climatic phenomena* registered in May were: rains under the form of rain showers, hails, pouring rains, thunders, thunderstorms and weather cooling, wind gusts under the form of rain storm.

*Ground water reserve* in the end of May in autumn wheat crop (ground layer of 0-100 cm) was almost satisfactory in the south of the region, optimum and almost optimum in most part of the region.

*In corn crop, water reserve in the ground layer of 0-50 cm* was optimum and almost close to optimum in the entire region.

***Overall climatic conditions of the spring 2014 - Overall thermal regime of the spring 2014.***

*Seasonal temperature means* were comprised between 8.9°C at Voineasa and 13.3 °C in Dr. Tr. Severin, and the deviations from the normal were comprised between 1.0 °C at Băilești, Craiova and Slatina and 1.9 °C in Rm. Vâlcea, leading to a classification of warm spring for the entire region.

***Overall pluviometric regime of the spring 2014.***

*Seasonal quantities of precipitations* were comprised between 299.8 l/m<sup>2</sup> at Voineasa and 516.4 l/m<sup>2</sup> at Apa Neagră, and their deviations from the normal were comprised between 99.2% at Voineasa and 178.8% in Craiova leading to a classification of excessively rainy spring for the entire region.

*Seasonal mean for the entire Oltenia* was of 351.8 l/m<sup>2</sup>, and its percentage deviation from the normal was 97.2% which confirms that the spring of 2014 was excessively rainy in the entire region.

*The spatial-temporal prevalence of the rainy weather* was of 97.8%. Warm winter, early spring arrival, warm and excessively rainy spring created very good climatic

conditions for the development of some pests which damaged crops, fruit trees and vineyards, and caused the repeat of the treatments, thus increasing production costs.

*The synoptic causes of torrential rains in the spring of 2014:* the rain has been produced by the evolution of strong Mediterranean Cyclones, whose trajectory was located over the south of Romania.

## CONCLUSIONS

In the spring of 2014, the spring arrival was excessively early, and warm weather predominated in an interval of 77 days during winter (85.6% of time) and during all spring.

Indexes of spring arrival were far higher than the normal values, with positive deviations comprised between 163.3°C at Calafat and 256.3 °C at Drăgășani.

The general characteristic of warm spring was highly caused by high deviations of air temperature registered in March.

Series of diseases and pests specific to warm and moist weather damaged agricultural and vegetable crops, fruit trees and vineyard, which needed repeated treatments, thus increasing the production costs.

Compared to the annual normal values, the precipitation registered in the spring of 2014 are percentage reports comprised between 38.7% at Voineasa and 70.9% in Craiova, and the general mean for the entire region represents 52.1% of the normal annual mean.

The rainiest spring month was May. Six waves of abundant rains were registered, and there were also periods of pouring rains.

Rains affected a large part of Europe, not only Oltenia and were caused by the evolution of some strong Mediterranean Cyclones above the Balkan Peninsula.

The rainy period correlates at the level of global teleconnections with the negative phase of North-Atlantic Oscillation.

The maintenance of the negative North-Atlantic Oscillation correlates with a strong El Nino observes in Pacific Ocean in this spring.

As a consequence of the abundant rains with intervals of pouring rains, repeated flood occurred on cultivated farmlands, grasslands and households which caused significant damages, forcing the Romanian Government to take special measures for indemnify the effected people and to make territory arrangements in some areas.

Warm and rainy weather led the strong development of crops and consequently in crops of wheat, barley, rye, alfalfa, hay, plants fell on extended areas.

In this spring, rainy periods associated with warm weather showed *an exceptional climatic variability* which kept crops and other plants under significant water stress which impacted them greatly.

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## PHYTOSOCIOLOGICAL STUDY CONCERNING HABITATS WITH *LIGULARIA SIBIRICA* (L.) CASS. FROM HARMAN MARSH

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**Keywords:** *Ligularia sibirica*, plant associations, Harman

### ABSTRACT

*This paper presents the phytosociological research performed during June-August 2014 on Ligularia sibirica (L.) Cass, which allowed the identification of the representative association of Orchido-Schoenetum nigricantis Oberdorfer 1957 and the cenotic species analysis of the identified association from Harman marsh.*

### INTRODUCTION

The glacial relict *Ligularia sibirica* (L.) Cass. which belongs to the family *Compositae*, has migrated in the glaciation periods from Asia to Europe, where it found favorable conditions of existence, following the depression wetlands, meadows, swamps, grasslands and wetlands of alpine, continental, boreal and mediterranean bioregions. In what may regard the environmental conditions, *Ligularia sibirica* (L.) Cass. prefers sunny places and wet organic soils. The activities and uncontrolled grazing and drainage works carried out in excess are the main causes which determine the extinction of this species through habitat destruction.

At European level the legislation that protects this taxon is the Habitats Directive 92/43 / EEC of May 1992 through Annexes II and IV, and the Berne Convention by Annex I (Popescu, 2012), strictly protected flora species. At this moment there are not known enough information about *Ligularia sibirica* (L.) Cass. fitting the IUCN Data Deficient category. Most areas with *Ligularia sibirica* (L.) Cass. (ROSCI1758) are included in 71 Natura 2000 sites, which gives it a high degree of protection. The 32 sites of community importance in Romania, which protect the relict *Ligularia sibirica* (L.) Cass., are in the continental and alpine bioregions, in the bogs and marshes habitats like: 7110\*- active raised bogs, 7120 – degraded raised bogs still capable of natural regeneration, 7140 – transition mires and quaking bogs, 7210 – alkaline fens with *Cladium mariscus* and *Caricion davallianae* species, 7230 - alkaline fens, 91D0\* - bog woodland (Doniță et al. 2005).

Harman Marsh Natural Reserve was declared protected area on 6 March 2000 and corresponds to the fourth category of IUCN, Habitat/Species Management Area. Natura 2000 network associates Harman Swamp with Lempes Castle Hill through the site of

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Lempes Castle Hill-Harman Swamp ROSCI0055. Being located on the eastern boundary of Tara Barsei, the eutrophic marsh from Harman allowed the relict species survival due to abiotic conditions. Thereby from geological view the sedimentary rocks of the Cretaceous period, substrates of gravel, loamy banks and clays are predominant. The existence of relict species like *Ligularia sibirica* (L.) Cass. depended more on hydrological conditions, the hydrological network being formed by Paraul Husbor, Paraul de sub Coasta and Valea Morilor. Harman marsh's climate is continental. Currently the marsh has an area of 4.5 ha, of which 2 ha are fenced and 2.5 are considered buffer zone. The site is administered by the Carpathian Wildlife Foundation and the Forestry Department of Brasov.

## MATERIAL AND METHODS

To analyze the marsh's vegetation from Harman were used the methods developed by J. Braun-Blanquet - Zurich-Montpellier school of phytosociology, adapted to the vegetation particularities of Romania by Al. Borza (Cristea, 2004). The association of plants was used as syntaxonomical basic unit. For the analysis of association and habitats were used: *Flora Europaea* (Tutin et al. 1964-1980), *Prodrome des associations vegetales des Carpates du sud-est* (Coldea, 1991), *Les associations vegetales de Roumanie* (Coldea, 1997), *Habitatele din România* (Doniță et al. 2005), *Mlaștinile de turbă din Republica Populară Română* (Pop, 1960), *Breviar Fitocenologic* (Sanda et al. 2006).

## RESULTS AND DISCUSSIONS

*Ligularia sibirica* was identified in Harman marsh during June-August 2014, by making 5 surveys (Table 1) in eu-mesotrophic southeastern Carpathian marshes habitat with *Schoenus nigricans*, also known as alkaline marshes (Brînzan et al. 2013). On the marsh was identified the *Orchido-Schoenetum nigricantis* Oberd. 1957 association (Syn. *Schoenetum nigricantis* (Pop et al. 1962); *Schoeneto-Armerietum barcensis*, an association that belongs to the order *Tofieldietalia* Prsg. ap. Oberd. 1949, of *Scheuczerio-Caricetea nigrae* Tx.1937 class (Coldea, 1991, 1997). Important cenoses with *Schoenus nigricans* are spread on certain regions of Romania territory, like: Gilau Mountains, Feleacului Hill- Cluj, Brasov county (Harman and Prejmer) and Valcele (Western Carpathians) (Sanda et al. 2006). In what may regard the floristic composition, the habitat is dominated by tall herbaceous layer with a significant number of relict species.

Based on this association, in Romania, there were carried out surveys made by Emil Pop in 1962 at Valea Morii, Csűrös-Káptalan in 1965 at Valcele and Parascan with Danciu in 1976 at Stupini, Brasov County. At the Harman marsh the association *Orchido-Schoenetum nigricans* Oberd. 1957 – *armerietosum barcensis* Morariu 1964, was described by Morariu in 1964 (em. Coldea 1977). The *Schoenus nigricans* coenoses grow on flat terrains sometimes low pitched, with basic substrate (pH = 7,5-8,1). The soils are marshy, gley sols with poor organic substances (13,1-31,4 %), but with a high calcium carbonate percentage (1-42%). Brasov coenoses grow on eutrophic peats with excess moisture.

The floristic composition of this association, has the characteristic species *Schoenus nigricans*, *Carex davalliana*, *Sesleria uliginosa* and *Eriophorum latifolium* along with other species like: *Orchis incarnata*, *Molinia caerulea*, *Carex appropinquata*, *Mentha longifolia*, *Gymnadenia conopsea*, *Valeriana simplicifolia*, *Carex flava*, *Carex panicea*, *Parnassia palustris*, *Epipactis palustris*, *Carex lepidocarpa*, *Swertia perennis*, *Carex hostiana*, *Polygala amarella*, *Primula farinosa*, *Tofieldia calyculata*, *Sesleria uliginosa*, *Eleocharis quinqueflora*, *Eriophorum angustifolium*, *Juncus articulatus*, *Triglochin palustre*, *Ligularia sibirica*, *Phragmites australis*, *Carex paniculata*, *Carex caespitosa*, *Eleocharis palustris*, *Crepis paludosa*, *Caltha palustris*, *Scirpus sylvaticus*, *Cirsium*

*rivulare*, *Lythrum salicaria*, *Equisetum palustre*, *Succisa pratensis*, *Deschampsia caespitosa*, *Linum catharticum*, *Angelica sylvestris*, *Sanguisorba officinalis*, *Lysimachia vulgaris*, *Galium uliginosum*, *Lychnis flos-cuculi*, *Trollius europaeus*, *Cirsium palustre*, *Juncus conglomeratus*, *Galium boreale*, *Briza media*, *Leucanthemum vulgare*, *Festuca rubra*, *Cardamine pratensis*, *Plantago lanceolata*, *Potentilla erecta*, *Polygala vulgaris*, *Eupatorium cannabinum*, *Equisetum ramosissimum*, *Equisetum arvense*, *Cladium mariscus*, *Sesleria uliginosa*, *Agrostis canina* *Juncus castanea*. The conservative value of the association is a very high, if in the habitat are presented *Ligularia sibirica* and *Liparis loeselii*.

The *Orchido-Schoenetum nigricatis* association species were analyzed from coenotic aspect. In what may regard the bioforms (Figure 1), can be observed the high percentage of hemicryptophytes (73%) followed by geophytes (19%).

The biggest percentage of floristic elements (Figure 2) is held by Eurasian species (43%), followed by Circumpolar species (20%) and Cosmopolitan species (11%).

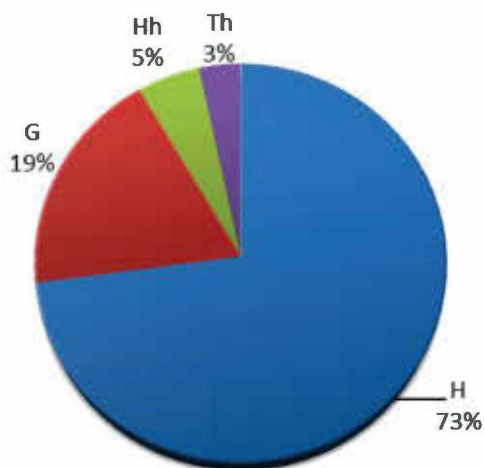


Figure 1. Bioforms spectrum of *Orchido-Schoenetum nigricantis* Oberd. 1974 association

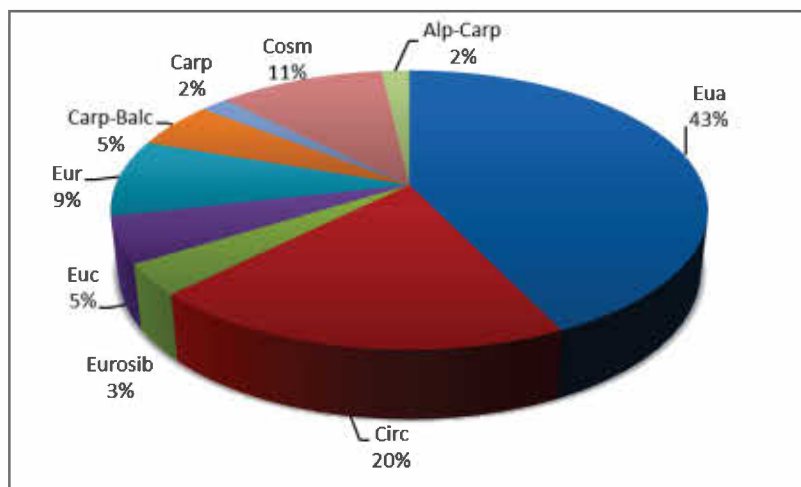


Figure 2. Floristic elements of *Orchido-Schoenetum nigricantis* Oberd. 1974 association

Depending on the moisture preferences of *Orchido-Scoenetum nigricantis* Oberd. 1974 association species, can be noticed the dominance of mesohygrophilic species, followed by hygrophilic species. Regarding the temperature factor the most species are micro-mesotherms, and according to the soil reaction, the majority of species are amphy-tolerant species, followed by acid-neutrophilous, and low-acid neutrophilous species (Figure 3).

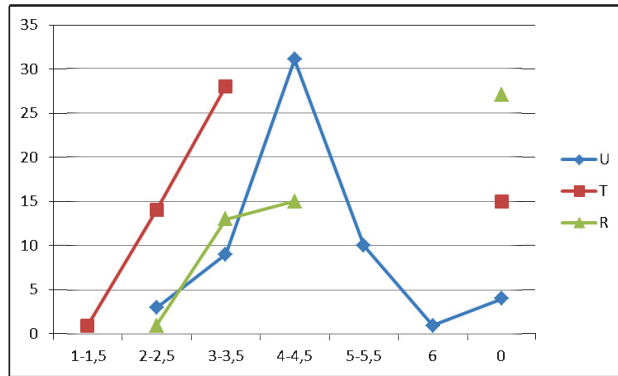


Figure 3. Analysis of ecological indicators of *Orchido-Scoenetum nigricantis* Oberd. 1974 association

Table 1

*Orchido-Scoenetum nigricantis* Oberd. 1974

Relevées	1	2	3	4	5	
Altitude (m s.m.)	518	520	525	522	519	
Exposition	NE	NE	NE	NE	NE	
Slope (°)	7	5	0	3	6	
Surface (m <sup>2</sup> )	20	10	15	20	15	
Coverage of the herbaceous layer (%)	100	100	100	100	100	K
<b>Char. Ass.</b>						
<i>Schoenus nigricans</i>	4	3	2	4	5	V
<i>Carex davalliana</i>	+	+	-	-	+	III
<i>Eriophorum latifolium</i>	-	+	-	+	+	III
<i>Orchis incarnata</i>	1	+	1	1	-	IV
<b>Diff. sous-ass.</b>						
<i>Molinia caerulea</i>	+	+	-	+	+	IV
<i>Carex appropinquata</i>	+	-	+	-	+	III
<i>Mentha longifolia</i>	-	+	+	+	-	III
<i>Gymnadenia conopsea</i>	-	-	+	-	-	I
<b>Diff. carpat.</b>						
<i>Valeriana simplicifolia</i>	-	-	-	-	+	I
<b>Tofieldietalia</b>						
<i>Carex flava</i>	-	+	+	-	+	III
<i>Carex panicea</i>	+	-	-	-	+	II
<i>Parnassia palustris</i>	1	+	+	-	+	III
<i>Epipactis palustris</i>	+	+	-	-	+	III
<i>Carex lepidocarpa</i>	-	-	+	+	+	III
<i>Swertia perennis</i>	-	-	-	+	+	II
<i>Bryum pseudotriquetrum</i>	-	-	-	-	+	I
<i>Carex hostiana</i>	+	+	-	+	-	III
<i>Polygala amarella</i>	+	-	-	-	+	II
<i>Primula farinosa</i>	-	-	+	-	-	I



<i>Campylium stellatum</i>	+	+	+	+	+	V
<i>Camptothecium nitens</i>	-	+	-	-	+	II
<i>Tofieldia calyculata</i>	-	-	-	+	+	II
<i>Sesleria uliginosa</i>	+	+		+	-	III
<i>Drepanocladus intermedius</i>	+	+	-	-	-	II
<i>Eleocharis quinqueflora</i>	-	-	+	+	\	II
<b>Scheuchzerio-Caricetea nigrae</b>						
<i>Eriophorum angustifolium</i>	-	+	-	-	-	I
<i>Juncus articulatus</i>	-	-	-	-	+	I
<i>Triglochin palustre</i>	+		+	+	-	III
<i>Ligularia sibirica</i>	+	+	+	+	+	V
<b>Magnocaricion</b>						
<i>Acrocladium cuspidatum</i>	-	+	+	-	+	III
<i>Phragmites australis</i>	+	+	+	+	+	V
<i>Carex paniculata</i>	-	-	+	-	-	I
<i>Carex caespitosa</i>	-	-	-	-	+	I
<i>Eleocharis palustris</i>	+	-	-	-	-	I
<b>Calthion</b>						
<i>Crepis paludosa</i>	-	+	-	+	-	II
<i>Caltha palustris</i>	-	-	+	-	-	I
<i>Scirpus sylvaticus</i>	+	-	-	-	+	II
<i>Cirsium rivulare</i>	-	+	+	-	+	III
<i>Lythrum salicaria</i>	+	+	+	+	.	IV
<b>Molinion et Molinietalia</b>						
<i>Equisetum palustre</i>	-	-	+	-	+	II
<i>Succisa pratensis</i>	+	+	+	+	+	V
<i>Deschampsia caespitosa</i>	+	+	+	+	-	IV
<i>Linum catharticum</i>	-	+		+	+	III
<i>Angelica sylvestris</i>	-	-	+	-	-	I
<i>Sanguisorba officinalis</i>	+	+	-	+	+	IV
<i>Lysimachia vulgaris</i>	+	+	-	+	-	III
<i>Galium uliginosum</i>	-	-	+	-	+	II
<i>Lychnis flos-cuculi</i>	-	-	+	-	+	II
<i>Trollius europaeus</i>	-	-	-	-	+	I
<i>Cirsium palustre</i>	+	-	-	-	-	I
<i>Juncus conglomeratus</i>	-	+	+	+	-	III
<i>Galium boreale</i>	-	-	-	-	+	I
<b>Arrhenatheretalia</b>						
<i>Briza media</i>	+	+	-	+	+	IV
<i>Leucanthemum vulgare</i>		+	+	-	+	III
<i>Festuca rubra</i>	+	+	-	-	-	II
<i>Cardamine pratensis</i>	-	-	-	+	-	I
<i>Plantago lanceolata</i>	-	+	-	-	-	I
<i>Fissidens adianthoides</i>	-	-	-	-	+	I
<b>Variae syntaxa</b>						
<i>Potentilla erecta</i>	+	+	-	+	-	III
<i>Polygala vulgaris</i>	-	-	-	-	+	I
<i>Eupatorium cannabinum</i>	-	-	+	+	+	III
<i>Equisetum ramosissimum</i>	-	+	-	-	.	I
<i>Equisetum arvense</i>	-	-	+	+	.	II
<i>Cladium mariscus</i>	1	+	+	-	+	IV
<i>Agrostis canina</i>	-	+	-	+	-	II
<i>Juncus castanea</i>	+	+	-	-	-	II

## CONCLUSIONS

*Ligularia sibirica* (L.) Cass can be found at Harman marsh in *Schoenus nigricans* coenoses, *Orchido-Schoenetum nigricantis* Oberd. 1974 association, which grow on eutrophic peats with moisture excess, rich in calcium carbonate, on a flat terrain or low slopping land, with sedimentary rocks and limestones, from the Cretaceous geological period.

Edaphic factors of the *Orchido-Schoenetum nigricantis* association, species, demonstrate the predominance of mesohygrophilic species, micro-mesotherms and amphytolerant species. The hemicryptophytes species are predominant from the bioforms analysis, and the Eurasian floristic elements are prevalent in the studied area.

The Harman marsh holds a good conservative capacity of the *Ligularia sibirica* glacial relict, but in the same time is unstable due to soil drainage.

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## INTEGRATED CONTROL - IMPORTANT LINK IN CHILDREN FOOD SAFETY OBSERVATIONS ON INTEGRATED CONTROL OF *ERIOSOMA LANIGERUM* PEST

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**Keywords:** *Woolly apple aphid, entomophagous, cultivar, insecticides*

### ABSTRACT

*In apple orchards, the woolly apple aphid - Eriosoma lanigerum - populations are limited by numerous entomophagous parasites of which an important percentage are Chrysopa Carnea, Coccinella sp., Aphelinus mali and Forficula auricularia. Integrated control scheme should include the use of resistant or tolerant cultivars to woolly aphid attack (Generous and Romus 3), the use of cultivars that present a high parasitism rate (James Grives, Florina and Pionier). Selective insecticide treatments (such as Calypso 480 SC, Reldan 40 EC and 48 EC Pirinex) will be applied to protect useful fauna.*

### INTRODUCTION

Pesticides have a harmful effect on the human body, an amount of pesticide absorbed can be risky and may cause negative effects. Even though for most adult foods with pesticides do not present major health risks, the situation changes in the cases of children and pregnant women. For these categories of consumers a better care is recommended in terms of fruit and vegetable consumption.

Infants and children are more sensitive on the toxic effects of pesticides than adults because their bodies are developing, the liver does not have maximum capacity to neutralize the toxins and the kidneys can not remove harmful substances from the body, as in the adults' cases. Infants may also be exposed to more pesticide than adults because they take more breaths per minute and have more skin surface relative to their body weight. Children often spend more time closer to the ground, touching baseboards and lawns where pesticides may have been applied. Children often eat and drink more, relative to their body weight, than adults, which can lead to a higher dose of pesticide residue per kilogram of body weight. Babies that crawl on treated carpeting may have a greater potential to dislodge pesticide residue onto their skin or breathe in pesticide-laden dust.

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For this reason, it is important to minimize the exposure of children and infants to pesticides. A way to minimize pesticide remanence is the integrated control of diseases and pests.

The purpose of this paper is to find ways to control one of the most dangerous pests of apple - *Eriosoma lanigerum* - using alternative methods to those chemical ones.

### MATERIAL AND METHODS

Observations were effected during the years 2011, 2012 and 2013, in the apple orchards from the experimental field of the Research Institute for Fruit Growing Pitești - Mărăcineni – Argeș, where large populations of *Eriosoma lanigerum* aphid were found. The behavior of apple cultivars to the attack of *Eriosoma lanigerum* pest was observed in September and October 2013 on the Farm Production Parcel containing eight cultivars of apple trees. The planting distance for M9 was 1.5 – 4 m and for MM 106 2.5 - 5 m. The observation period was September - October 2013. The following indicators were calculated to evaluate the attack: the attack frequency, the attack intensity and the attack level.

The role of biotic factors in population dynamics was established by recording the mortality of the insect at different stages on different dates. To establish the natural parasitism, colonies were sampled and for the parasites identification, samples were isolated in separate tubes and stored in the laboratory until their flight.

To observe the selectivity of insecticides against the useful fauna, 7 products from different groups were tested and compared with untreated control in a period of 3 years.

### RESULTS AND DISCUSSIONS

An important role in regulating the populations of *Eriosoma lanigerum* has the presence of entomophagous parasites which, under certain conditions, can reduce the woolly apple aphid reserve up to 98%.

In laboratory studies conducted by Mueller et al. (1992), *Aphelinus mali* parasites *Eriosoma lanigerum* in all stages of development, but prefer the last age larvae. Regarding the rate of parasitism, that was inversely proportional to the colony size, small colonies having a higher proportion of parasitized insects. In Europe, *Forficula auricularia* was an important supplementary biological control agent against *Eriosoma lanigerum* (Mueller et al. 1988). But predators were not effective against subterranean aphid populations. To control root-dwelling populations of *Eriosoma lanigerum*, entomopathogenic nematodes were used, the method being described by Brown et al. (1992).

In total, 73 species of predators were recorded as natural enemies of the pest worldwide (Asante 1997). Species of the families *Coccinellidae*, *Miridae*, *Chrysopidae*, and *Forficulidae* were noticed as the most common predators. The most common coccinellid in colonies is *Coccinella transversoguttata*. However, *Coccinellides* are a main cause of the reduction of *Eriosoma lanigerum* population in early summer in Europe (Kozár et al. 1979). Also in Europe, *Chrysopa nigricornis* Burm., *Deraeocoris brevis* Uhler and *Metasyrphus fumipennis* Thom. (*Syrphidae*) become abundant in summer and early autumn. In West Virginia, the parasitism rate by *Aphelinus mali* increased in July and August, when predators of the *Syrphidae* family were found also in large number. European earwigs *Forficula auricularia* L., also reduced woolly aphid populations on mature trees (Ravensburg 1981, Mueller et al. 1988), but this species was unable to suppress aphid populations in Washington, unless earwigs populations were very large (Carroll et al. 1985).

From table 1 it results that of the six analyzed apple trees cultivars, James Grives, Florina and Pionier cultivars had the highest parasitism rate and Starkrimson cultivar had the lowest one.

Table 2 presents the results regarding the identified parasites for *Eriosoma lanigerum* pest. Thus, during the 3 years of observations, 43.54% of aphids were parasitized by *Chrysopa carnea* - Neuroptera, *Chrisopidae* - (parasitism rate ranging between 40.25% in 2013 and 50.00% in 2011). 21.29% of aphids were parasitized by *Aphelinus mali* - Hymenoptera, *Aphelinidae* (with parasitism rates between 7.03 in 2011 and 26.82 in 2013). 17.00% of aphids were parasitized by *Coccinella septempunctata* and *Coccinella 14 punctata* - Coleoptera, *Coccinellidae* (the lowest parasitism rate was 10.11 in 2013 and the highest one 21.50 in 2012). 10.20% of aphids were parasitized by *Forficula auricularia* - Dermaptera, *Forficulidae* (with values between 6.23 in 2012 and 16.33 in 2013). According to the annual climatic conditions, there was an average parasitism rate of 88.23% in 2012 and 94.36% in 2011.

Regarding the dynamics of entomophages evolution (table 3), the observations made at the Research Institute for Fruit Growing Pitești – Mărăcineni had shown that parasitism rate was in late July 7.97%, at the end of August 31.57% and at the end of September 93.51%. Data from the literature show that parasitism rate of *Eriosoma lanigerum* pest is inversely proportional to the colony size, smaller colonies having a higher degree of parasitized insects (Muller et al. 1992). The results of our observations do not confirm this aspect, the parasitism rate being not influenced by the colony size.

Regarding the behavior of some apple trees cultivars on woolly apple aphid attack (table 4) we observe that the strongest attack was on Pionier, James Grieves and Idared and the weakest one on Generous and Romus 3 cultivars. From the analysis of these data we can see that rootstock does not affect tree resistance to the attack of *Eriosoma lanigerum*.

In order to obtain rich and good quality crops in the apple orchards from Mărăcineni fruit growing area, 12 summer treatments are necessary, 5 of them being applied to control the woolly apple aphid. From the data analysis we observe that the best selectivity against the useful fauna had the following insecticides: Calypso 480 SC, Reldan 40 EC and 48 EC Pirinex. Thus, the following treatments can be applied: Calypso 480 SC - 0.02% in the second decade of June, the second decade of July and the first decade of August (treatment I, II, and IV); Reldan 40 EC- 0.15% - at the end of June (treatment III); Pirinex 48 EC - 0.2% in the third decade of August for the hibernating reserve reduction (treatment V).

Table 1

Viability of *Eriosoma lanigerum* pest  
in an orchard with six cultivars of apple trees after harvest - 08.10.2013

Crt. no.	Cultivar	Rootstock	No. of observed colonies	Total no. of aphids	of which:			
					Alive		Parasitized	
					No.	%	No.	%
1	James Grives	MM 106	10	205	0	-	205	100
2	Idared	MM 106	10	478	38	7.94	440	92.05
3	Florina	MM 106	10	260	0	-	260	100.0
4	Pionier	MM 106	10	61	0	-	61	100.0
5	Prima	MM 106	10	518	188	36.29	330	63.70
6	Starkrimson	MM 106	10	554	355	64.07	199	35.92

Table 2

Identified parasites of *Eriosoma lanigerum* pest  
at the Research Institute for Fruit Growing Pitești - Mărăcineni - Argeș

Year	Total parasitism rate (%)			<i>Chrysopa carnea</i> (%)			<i>Aphelinus mali</i> (%)			<i>Forficula auricularia</i> (%)			<i>Coccinella sp.</i> (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
	94.36	88.23	93.51	50.00	40.28	40.25	7.03	20.2	26.82	7.13	6.23	16.33	20.20	21.5	10.1
Average	92.01			43.54			21.29			10.20			17.00		

Table 3

Woolly apple aphid *Eriosoma lanigerum* parasitism on apple cultivar Pioneer in the year 2013  
at the Research Institute for Fruit Growing Pitești - Mărăcineni - Argeș

Observation date	Total number of analysed colonies	No. of observations	Alive aphids		Parasitized aphids						Predators observed in colonies	
			No.	% of total	Alive		Hatched		Dead			
					No.	%	No.	%	No.	%		
28.07.2013	8	1,798	1,627	92.02	80	4.52	61	3.45	0	-	-	- <i>Crysope carnea</i> - <i>Coccinella sp.</i> - <i>Aphelinus mali</i> - <i>Forficula auricularia</i>
28.08.2013	8	2,115	1,447	68.41	214	10.11	454	21.46	0	-	-	
28.09.2013	8	3,832	280	7.30	160	4.17	3,392	88.51	32	0.83	-	

Table 4

The presence of woolly aphid *Eriosoma lanigerum* in apple orchards at the Research Institute for Fruit Growing Pitești - Mărăcini - Argeș

Crt. no.	Cultivar	Rootstock	No. of observed apple trees	Gradul de atac (%)
1.	Prima	MM 106	61	100.00
2.	Prima	M 9	113	90.26
3.	Florina	MM 106	100	70.00
4.	Florina	M 9	39	84.61
5.	Pionier	M 9	100	100.00
6.	Pionier	MM 106	100	100.00
7.	Generos	M 9	100	0
8.	Generos	MM 106	100	0
9.	Romus 3	M 9	100	0
10.	Romus 3	MM 106	100	0
11.	Idared	M 9	100	100.00
12.	Idared	MM 106	100	100.00
1	James Grieves	MM 106	100	100.00
2	Idared	MM 106	100	75.00
3	Florina	MM 106	100	30.00
4	Starkrimson	MM 106	100	25.00

Table 5

Observations on the selectivity of some insecticides against the useful fauna

Crt. no.	Comercial name of product	Active ingredient	Average 2011-2013
1	CALYPSO 480 SC	tiacloprid 480g/l	71.3
2	RELDAN 40 EC	clorpirifos metil 400 g/l	70.3
3	PIRINEX 48 EC	Clorpirifos 450 g/l	63.2
4	SUMI ALPHA 2,5 CE	esfenvalerat	46.3
5	FIFANON 50 EC	malation 500l/l	40.3
6	FASTAC 10 EC-RV	alfa-cipermetrin 100 g / l	39.5
7	MAVRIX 2 F	tau-fluvalinat 240 g/l	21.4
8	Untreated control	-	100

### CONCLUSIONS

In Mărăcini fruit growing area 4 entomophagus that reduce *Eriosoma lanigerum* populations were identified, of which *Crysopa carnea* presented the highest average parasitism rate.

Regarding the parasitism on different apple cultivars, the highest parasitism rate presented James Grives, Florina and Pionier cultivars.

The apple cultivars resistant to woolly aphid attack were Generos and Romus 3.

Rootstocks do not affect tree resistance to *Eriosoma lanigerum* attack.

A good selectivity for useful fauna presented the insecticides Calypso 480 SC, Reldan 40 EC and 48 EC Pirinex.

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## OBSERVATIONS REGARDING THE AGRONOMICAL VALUE AND THE USE OF MAIZE HYBRIDS IN DÂMBOVIȚA'S ECOSYSTEMS

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*Keywords: maize, hybrids, production, fertilization*

### ABSTRACT

*In the experiments that were set up in the experimental fields of the Cultivars Testing Center Târgoviște for maize crop, the observations were made during the years 2009 - 2012 and had the objective of contributing to sustainable development technology as an alternative to conventional agricultural technology. 11 hybrids were analyzed both in terms of chemical fertilization and organic fertilizer conditions. Given the different elements of productivity after analyzing the results, if fertilizing with manure, in the pedoclimate conditions of Dâmbovița county, it is recommended to cultivate SCM 7714, SCM 7769, EGZ 8301, EGZ 9501, EGZ 9595, 7482 KXA and KXA8481 hybrids.*

### INTRODUCTION

The maize is situated on the third place as importance from the world's crop (Muntean et. al. 1995) as the grains of this plant are used in the human diet, industry and animal feed (Bîlteanu 1998). In Romania, maize is the main crop, taking first place in size (on average 30% of arable land), and as in production (total and average per hectare). Therefore, the production and economic efficiency of maize crop are a matter of national interest (Sacra et al. 2007).

Maize productivity, same as any other crop, is the result of the interaction between all factors involved one way or another in the vegetal production. For a better use of farm lands and of natural resources for maize, in order to reach a profitable production, a sensible setting of areas for cultivated hybrids, depending on climate resources and biological needs of hybrids, is required (Rusu et al. 2006).

The approached research regarding the improvement of the production technologies for maize seeds were oriented to specific requirements in order to obtain seeds with varietal purity, quality indices and high health status, and also achieve high yields of seeds/ha so that the activity to be effective (Sarca & Oprea 2007).

Research on maize production shows that of 14 Romanian hybrids, created by the I.N.C.D.A. Fundulea, studied under irrigated and non-irrigated conditions during 2001 - 2004, were identified with good behavior, both irrigated and non-irrigated: Paltin, Partizan,

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Campion, Rapsodia, Danubiu, Faur, Olt and Fundulea 376, suggesting a high capacity to adapt them in the years with different climatic conditions (Șerban 2010).

Other research shows the behavior of commercial maize hybrids under different water stress conditions associated with heat stress and the economic analysis. The results of the mass of 1000 grains have revealed that the application of an additional water maize crop brings a significant weight gain. The differences are between 4-62g in the four tested variants. Regarding the yield of grains/cob, meaning grain weight based on the weight of a cob without grains, the measured data from researching the eight commercial maize hybrids in four different variants of irrigation, have values between 80-84%; the differences between hybrids and between variants are significant from this point of view (Melut et al. 2011).

Regarding maize productivity elements in the pure culture and in soybean intercropping can see that, in pure crop the formed cobs had an average length of 22 cm, with 6.5 rows of grains in average and 594 grains/cob. Grain yield was 81% and the MMB was calculated to be 310 g.

In the soybean intercropping, the cobs formed reached 21 cm in length, and have noted that a number of 16 rows of grains and an average of 560 grains/cob. It was observed that the length of the cob was close to the one of the pure culture. In the soybean intercropping the maize gave an average yield of 80% beans and the calculated MMB was 300 g.

The maize production of intercropped crop was slightly lower with 104.8 kg/ha compared to the pure culture with 1964.2 kg/ha (Popescu & Roman 2008).

Regarding the influence of works on the yields of maize, it was observed that plowing to 30 cm resulted in achieving the highest yields (+ 10%) and soil tillage with chisel and disc harrow yielded some minuses 11% and 20% statistically (Jitäreanu & Răus 2007).

## **MATERIAL AND METHODS**

The experiments were set up in the experimental maize fields of the Cultivars Testing Center Târgoviște on the medium hybrids in the south and west of the country - Group 400-500.

Parameters of the experimental plot:

- Settlement method - random blocks, coded with the number 1;
- Altitude - 262 m;
- Soil type - brown luvic, coded with number 9;
- Rehearsal number - 4 + 1, for calculating the production 4 repetitions and one repetition for biometric measurements;
- Plants/ha - 55.000
- Plot surface (sqm) - 18,2
- Prior plant - wheat

Elements of technology:

The sowing was done for all variants on the same day, when it was 9°C at a soil depth of 6 cm for 3 consecutive days, namely: 13.05.2009; 05.05.2010; 19.05.2011 and 07.05.2012.

After harvest, the resulting cobs were weighed and were noted in kilos, with one decimal. The grain yield is the ratio between the grains weight and the total weight of the cobs. It is determined for the total amount of cobs resulted from one repetition. The determination is performed at harvest immediately after weighing the cobs.

1000 grain weight (MMB = TKW) was determined as:

- seeds are randomly counted and were grouped by 10, then were grouped by 100 and then by 500
- the two 500 samples are weighed separately and the results are added together.

It is obtained the mass of 1000 seeds.

The hectoliter mass (MHL) is determined immediately after harvest for each hybrid. An amount of seeds were weighed filling a cylindrical vessel with a volume of 1 liter. The balance hectoliter was used. For each sample two determinations were made.

## RESULTS AND DISCUSSIONS

The climatic conditions of 2009, the experiences with maize, where chemical fertilising has been done, all hybrids had similar production. It ranged between 20.75 kg/plot (or 11.40 t/ha), the hybrid EFG5209 26,08 kg/plot (or 14.33 t/ha) KXA8481 hybrid. From the viewpoint of production, 9 of the analyzed hybrids, excess the production of the F322 blank test (table 1).

Production data, chemical fertilization, 2009

Table 1

Crt. no.	Cultivar	Production on parcel (Kg)				Yield (%)	MMB (g)	MHL (kg)
		R1	R2	R3	R4			
1	Fundulea 322	23.6	22.5	22.9	23.7	83	381	71
2	CSM 7717	26.2	24.4	24.0	24.1	83	329	68
3	CSM 7769	25.9	24.8	23.7	24.6	85	358	69
4	EF 5209	20.5	20.8	21.3	20.4	81	332	70
5	EGZ 8301	24.1	23.0	24.2	21.9	87	356	70
6	EGZ 9501	23.2	22.6	23.5	21.8	80	367	70
7	EGZ 9505	22.2	23.5	22.4	23.4	84	313	70
8	KXA 7482	26.6	26.2	25.7	24.4	76	389	70
9	KXA 8481	27.6	26.8	24.6	25.3	82	356	68
10	KXA 9483	26.8	24.2	24.7	25.1	82	336	70
11	NJ5481	24.4	24.2	23.7	24.6	84	395	70

The yield, respectively, the ratio of grain weight and total weight of cobs, ranged between 76 and 87%. The highest yield was recorded in the hybrid KXA 7482. 1000 grain weight ranged between 313 g and 395 g. It is found that the hybrid NJ 5481, had the greatest weight. The hectoliter weight fluctuated between 68 and 71 kg, the 10 hybrids having at least the same value as the hybrid blank test.

In 2010, in terms of chemical fertilization, the results are shown in table 2. Basic fertilization was made with 80 kg N/ha P<sub>2</sub>O<sub>5</sub> - 80 Kg/ha as a phasial fertilizer N 40 kg/ha. The production of hybrids ranged from 16.63 kg/plot (9.14 t/ha) with the EGZ 9505 hybrid and 20.53 kg/plot (11, 28 t/ha) with the KXA 7482 hybrid. It is found from experience, that all hybrids have lower production than the blank test. The yield ranged between 84% and 87%, all 10 hybrids exceeded the hybrid test blank percent. 1000 grain weight ranged between 240 g - with the CSM 7717 hybrid and 327g - with the KXA 8481 hybrid. Hectoliter weight fluctuated between 69 and 73 kg. The highest value equal to the blank test was recorded with the NJ 5481 hybrid.

The climatic conditions of 2011 (table 3) in which it was fertilized with manure (40 t/ha), high yields were obtained compared with the average yield per hectare for maize,

but all hybrids, including the blank test, were production values were lower than in previous years, when chemical fertilising was made. The yields ranged between 16.33 kg/plot (8.97 t/ha) for the SCM 7717 hybrid and 21.38 kg/plot (11.75 t/ha) for the EGZ 9505 hybrid. Most hybrids have achieved production equal to or greater than the hybrid blank test. The yield values were equal to the hybrid KXA 9483 blank test, the remaining hybrids showing a higher yield than of the F 322 hybrid. 1000 grain weight ranged from 314 g to 360 g for the SCM 7717 and 7482 KXA hybrids. The hectoliter weight fluctuated between 71 and 73 kg.

Table 2

Production data, chemical fertilization, 2010

Crt. no.	Cultivar	Production on parcel (Kg)				Yield (%)	MMB (g)	MHL (kg)
		R1	R2	R3	R4			
1	Fundulea 322	22.4	23.2	21.2	22.8	80	322	73
2	CSM 7717	18.5	19.6	20.8	18.9	85	240	70
3	CSM 7769	18.0	18.8	18.5	17.3	86	271	72
4	EF 5209	18.0	18.6	18.3	17.4	86	310	69
5	EGZ 8301	18.8	18.3	19.6	18.2	87	270	72
6	EGZ 9501	18.1	18.9	18.0	19.4	86	279	71
7	EGZ 9505	15.8	16.6	18.5	15.6	84	241	70
8	KXA 7482	20.5	21.9	20.8	18.9	84	351	71
9	KXA 8481	19.4	20.0	18.5	19.0	84	327	72
10	KXA 9483	17.8	17.5	18.8	19.0	84	304	70
11	NJ5481	18.7	19.2	20.0	18.1	87	307	73

Table 3

Production data, organic fertilization, 2011

Crt. no.	Cultivar	Production on parcel (Kg)				Yield (%)	MMB (g)	MHL (kg)
		R1	R2	R3	R4			
1	Fundulea 322	18.3	18.6	19.1	17.8	80	354	73
2	CSM 7717	16.6	16.3	15.3	17.1	83	314	71
3	CSM 7769	17.1	19.4	17.9	18.3	84	331	71
4	EF 5209	19.8	21.0	19.5	20.7	83	364	72
5	EGZ 8301	19.2	19.0	17.8	18.4	86	331	72
6	EGZ 9501	19.2	22.3	21.5	22.5	84	364	72
7	EGZ 9505	19.8	18.2	17.9	17.9	84	333	72
8	KXA 7482	19.1	22.8	19.9	20.7	82	360	72
9	KXA 8481	17.8	20.4	18.5	19.1	83	332	71
10	KXA 9483	17.1	17.8	16.5	17.2	80	338	71
11	NJ5481	19.2	20.8	19.7	21.1	86	354	73

In 2012, the results were obtained after fertilization with manure (Table 4). The lowest production was recorded with the 8301 EGZ hybrid, or 8.48 kg/plot (4.66 t/ha) and the largest with the KXA 9483 hybrid, 13.5 kg/plot (7.42 t/ha). The yield ranged between 81% (EGZ 9505 hybrid) and 87% (NJ 5481 hybrid). 1,000 grain weight ranged from 310g (the EGZ KXA 9501 and 8481 hybrids) and 378g (SCM 1717 hybrid). Hectoliter weight ranged from 68 kg (EF 5209) and 73 kg (EGZ 9505).

In terms of average yields (figure 1), it appears that under chemical fertilization, they were higher than in the experiences with manure. In the case of chemical fertilization, the average was between 10.67 t/ha for EF 5209 hybrid and 12.71 t/ha for KXA 7482 hybrid. When fertilization with manure the average production had values between 7.35 t/ha for EGZ 9505 hybrid and 9.05 t/ha for KXA 7482 hybrid.

Table 4

Production data, organic fertilization, 2012

Crt. no.	Cultivar	Production on parcel (Kg)				Yield (%)	MMB (g)	MHL (kg)
		R1	R2	R3	R4			
1	Fundulea 322	10,4	10,9	10,2	10,6	83	350	70
2	CSM 7717	10,5	10,6	10,7	10,9	86	378	70
3	CSM 7769	10,3	10,9	10,7	10,6	86	312	72
4	EF 5209	11,2	11,3	11,9	11,4	85	333	68
5	EGZ 8301	8,3	8,4	8,7	8,5	84	320	72
6	EGZ 9501	8,3	8,9	8,7	8,6	85	310	72
7	EGZ 9505	8,9	8,3	8,1	7,9	81	360	73
8	KXA 7482	12,3	12,8	11,9	12,1	85	362	69
9	KXA 8481	10,3	10,3	9,8	10,1	85	310	72
10	KXA 9483	13,5	13,2	13,1	14,2	85	375	70
11	NJ5481	10,8	10,7	10,2	11,3	87	348	72

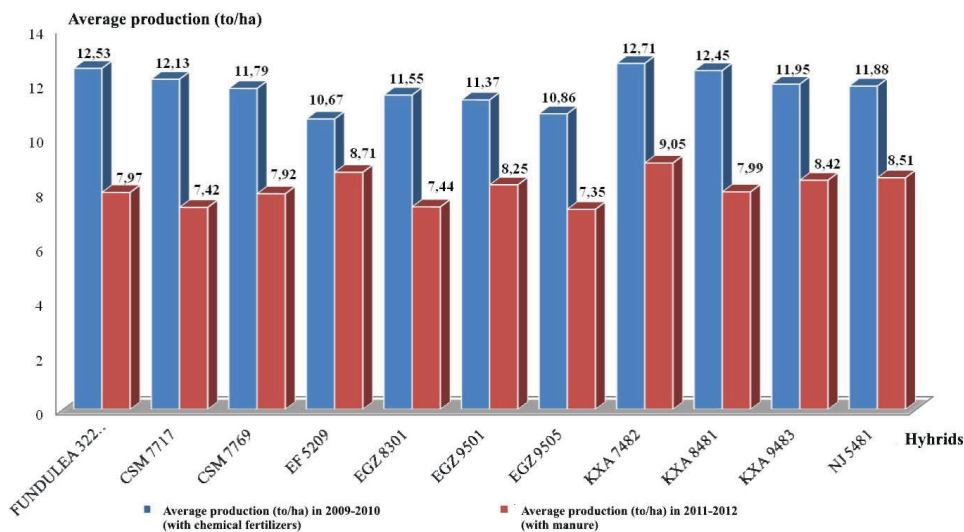


Figure 1. Average productions in 2009-2010 and in 2011-2012

Production of hybrids for fertilization with manure is high compared to the average production of maize in Romania, so the hybrids with the highest productive potential can be successfully used to achieve ecological culture technologies.

In figure 2 we plotted the average yield for the 10 maize hybrids, namely the ratio of grain weight and total weight of cobs. It is noted that in all hybrids, the average yield

exceeds the average value of the hybrid test blank the hybrid F 322. Exception is KXA 7482, that shows a 1.5% lower than the test blank under organic fertilization.

The highest yield stands for EGZ 8301 hybrid - 87% for chemical fertilization and the NJ 5481 hybrid to 85.5% for manure fertilization. It is noted that there are significant differences between the two systems yield fertilization.

1,000 grain weight is a very important quality index as from seed larger and heavier, with the higher MMB, vigorous plants are produced with high productive potential.

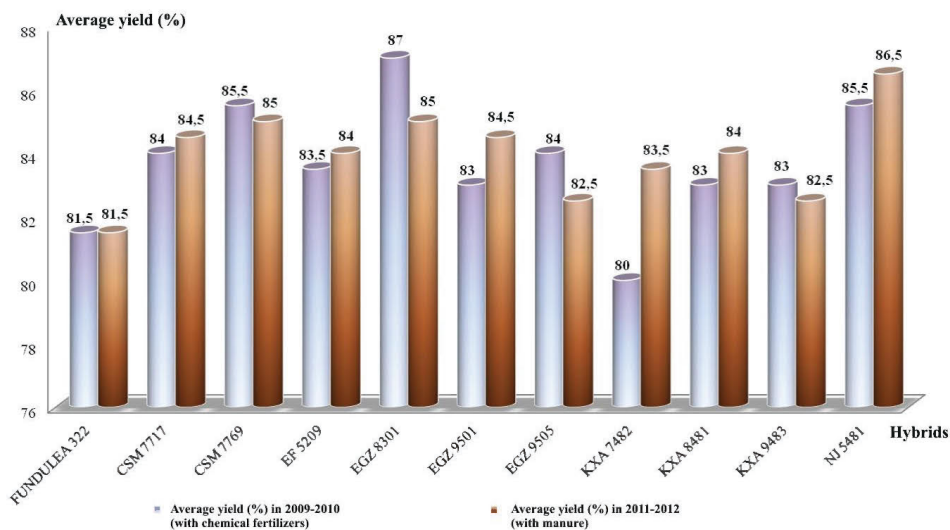


Figure 2. Average yield of maize hybrids

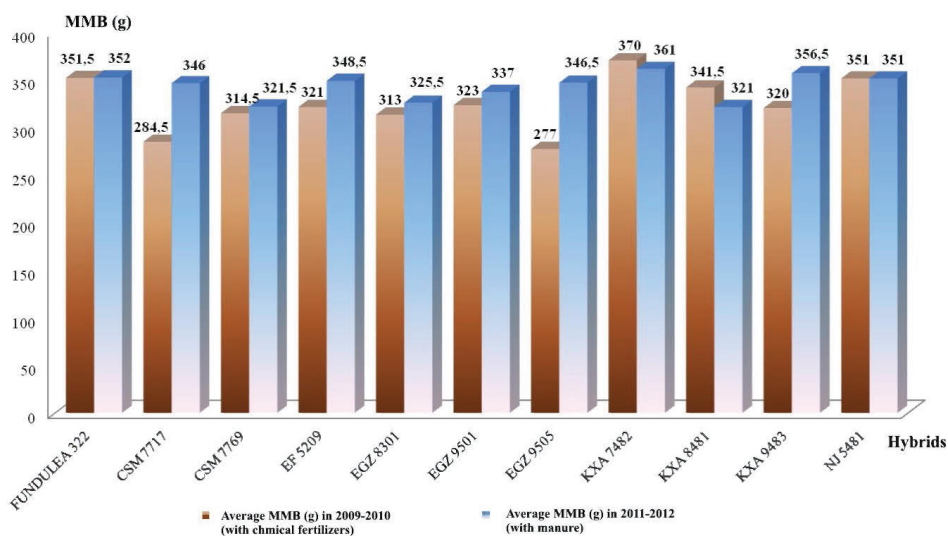


Figure 3. 1,000 grain weight (MMB) of the maize hybrids

From figure 3, it is observed that the KXA 7482 hybrid has the highest value of MMB, in both experimental variants, respectively 370 g (chemical fertilizer) and 361 g (fertilization with manure). The lowest value was recorded in the EGZ 9505 hybrid (277 g) - Chemical fertilising and KXA 8481 (321) - organic fertilization.

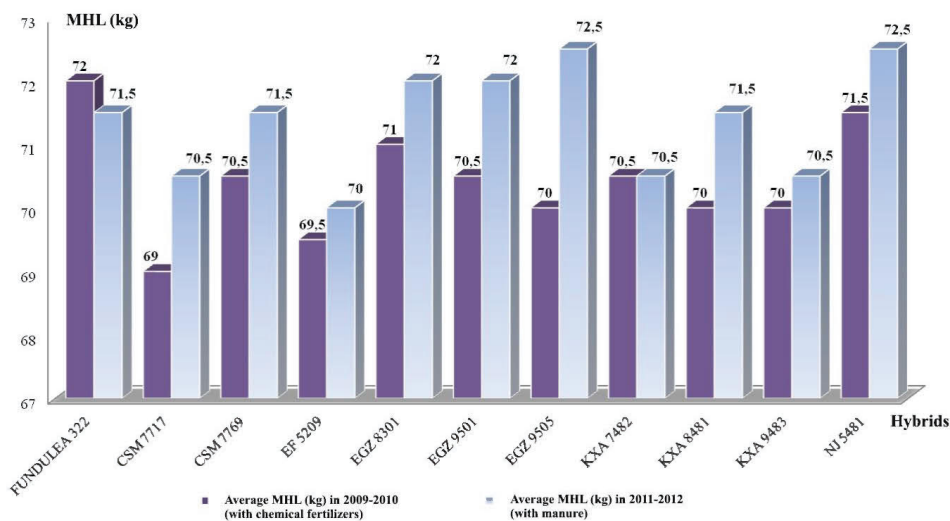


Figure 4. Hectoliter mass (MHL) of the maize hybrids

From the analysis results for the hectolitre mass (figure 4), it appears that in most hybrids the values obtained when fertilising with manure are higher than chemically fertilized variants. The highest values were obtained for the hybrids EGZ 9505, NJ 5481, EGZ EGZ 8301 and 8301, for the variant with organic fertilizer. For the version in which the fertilizer used were chemical, it noted the NJ EGZ 5481 and 8301 hybrids, but their values are smaller than the hybrid blank test.

## CONCLUSIONS

In terms of average yields and in terms of chemical fertilizer, they were higher than with the experiences with manure. For chemical fertilization, the average was between 10.67 t/ha for the EF 5209 hybrid and 12.71 t/ha for the KXA 7482 hybrid. If fertilising with manure the average production had values between 7.35 t/ha for the EGZ 9505 hybrid and 9.05 t/ha for the KXA 7482 hybrid.

Production of hybrids for fertilization with manure is high compared to the average production of maize in Romania, so the hybrids with the highest productive potential can be successfully used to achieve ecological culture technologies.

The average yield for the 10 maize hybrids exceeds the average of F 322, except for the KXA 7482 hybrid. It is noted that there are no significant differences in yield between the two systems of fertilization.

The KXA 7482 hybrid has the highest value of MMB, in both experimental variants. The lowest value was recorded in the EGZ 9505 hybrid (277 g) - chemical fertilization and KXA 8481 (321) - organic fertilization.

Regarding the hectoliter mass is noted that most hybrids values obtained by fertilising with manure are higher than chemically fertilized variants.

After analyzing the results, where fertilising with manure, in pedoclimatic conditions of Dâmbovița county, it is recommended to use SCM 7714, SCM 7769, EGZ 8301, EGZ 9501, EGZ 9595, 7482 and KXA8481 KXA hybrids.

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**SCREENING FOR TRANSFUSION-TRANSMISSIBLE INFECTIONS  
(SYPHILIS AND HIV INFECTION) AT DONORS OF THE REGIONAL  
CENTRE OF BLOOD TRANSFUSION OF CRAIOVA IN 2013**

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**Keywords:** *transfusion, blood, infections, syphilis, HIV*

**ABSTRACT**

*Transfusion activity is one of the extremely important parts of the medical act since blood transfusion represents a vital intervention for many patients. This is the reason why it is obvious that we should pay special attention and should be extremely exigent when selecting the donors or the ones to be. The donors' medical control and selection should prevent all risks, both for the donor and for the beneficiaries of this biological product. Therefore, the medical control is based on three exams (the clinical one, the laboratory one and the epidemiological one), by means of which we could settle the contraindications of blood donation. These contraindications may be: absolute (from causes that permanently exclude the individual from donation, such as hepatitis, syphilis, tuberculosis, stomach resection, mitral disease, operated and cured cancer, etc.), relative and temporary.*

**INTRODUCTION**

Screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible. Effective screening for evidence of the presence of the most common and dangerous TTIs can reduce the risk of transmission to very low levels (Dodd 2007).

Screening for the following four infections that are transmissible by transfusion is recommended as mandatory for the provision of a safe blood supply. These infections can cause chronic disease with possible serious consequences and present the greatest infection risk to recipients of transfusion: human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum* (syphilis) (WHO 2010).

**The human immunodeficiency virus (HIV)** is a retrovirus, an enveloped RNA virus, which is transmissible by the parenteral route. It is found in blood and other body fluids. Once in the bloodstream, the virus primarily infects and replicates in lymphocytes. The viral nucleic acid persists by integrating into the host cell DNA.

As HIV can be present in the bloodstream in high concentrations and is stable at the temperatures at which blood and individual blood components are stored, the virus may be present in any donated blood from an HIV-infected individual.

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Infectivity estimates for the transfusion of infected blood products are much higher (around 95%) than for other modes of HIV transmission owing to the much larger viral dose per exposure than for other routes (Baggaley et al. 2006).

Screening donations for both antibody and antigen will identify the vast majority of donations from infected donors (Laperche et al. 2000).

**Syphilis** is caused by the bacterium *Treponema pallidum*. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the bacteria spread throughout the body. A primary lesion, chancre, usually occurs about three weeks after exposure, although the duration may be shorter in cases of transfusion-transmitted infection where the organism enters the bloodstream directly. Syphilis is endemic in many parts of the world.

While *Treponema pallidum* may be found in the bloodstream, levels are variable, even in acute primary syphilis, and the bacteraemia is often short-lived.

Treponemal serology is relatively complex with different profiles seen at different stages of infection and depending on whether treatment has been given. Infection with the four major types of pathogenic treponemes cannot be distinguished by serological screening because the major immunodominant epitopes are so similar that the antibodies produced are detected by any specific antibody assay for syphilis (WHO 2010).

## MATERIALS AND METHODS

Laboratory screening of donated blood is the step that determines whether or not a donation is non-reactive for specific markers of infection and is therefore safe to release for clinical or manufacturing use.

Various assay systems with differing sensitivities and specificities are available for blood screening (WHO 2010).

Test kits:

-Screening kit for the detection of HIV p24 antigen and antibodies to HIV-1 and HIV-2 in human serum/plasma by enzyme immunoassay (<http://www.bio-rad.com/>)

-Kit for the qualitative and semi-quantitative detection of antibody to *Treponema pallidum* in human serum or plasma by Enzyme-Linked Immunoassay (<http://www.bio-rad.com/>)

**The Genscreen™ ultra HIV Ag-Ab** is a qualitative enzyme immunoassay kit for the detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) (Simon et al. 1998) and HIV-2 in human serum or plasma. This kit can be used for both HIV Ag and HIV Ab screening.

The Genscreen™ ULTRA HIV Ag-Ab is an enzyme immunoassay based on the principle of the sandwich technique for the detection of HIV antigen and of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma.

The sampled blood is totally tested for HIV, but there is still the possibility for some donors to be HIV seropositive and this might not be spotlighted by the tests since they are accomplished during the “immunologic window” (the time starting with the HIV infection moment until the anti-HIV specific antibodies show up, when the tests are negative even if the HIV virus is in the body) (<http://www.bio-rad.com/>).

**Syphilis EIA II Total antibody** use three recombinant antigens in a sandwich test to produce a test that is both highly specific and sensitive (Larsen et al.1995). The antigens will detect *Treponema.pallidum* - specific IgG, IgM, and IgA; enabling the test to detect antibodies during all stages of infection (Norris 1993). All reagents except the Wash solution are supplied ready to use and colour-coded, and the procedure uses undiluted samples

and standard volumes for ease of both manual and automated use. The assay can be used with both serum and plasma (<http://www.bio-rad.com/>).

All test kits and reagents should be stored and transported under controlled conditions. The blood transfusion service should ensure that reliable cold chain systems are in place in each screening laboratory to assure compliance at all times (WHO 2002). Appropriate temperature-controlled storage equipment which conforms to defined specifications should be made available for normal maximum stocks of all test kits and reagents (WHO 2005).

Serum or plasma (collected into EDTA, sodium citrate or heparin) may be used. Particles in the sample should be removed by centrifugation prior to testing. The samples should be stored at + 2°C -8°C if testing is to be carried out within 7 days of stored at - 20°C for a longer period (WHO 2010).

## RESULTS AND DISCUSSIONS

This paper is based on a study containing a number of 3830 donors (2691 males and 1131 females) (Table 1), donors who were tested in 2013 at the Regional Centre of Blood Transfusion of Craiova.

Table 1  
Donors at the first donation at the Regional Centre of Blood Transfusion of Craiova (tested for anti-HIV and VDRL-TPHA), in 2013

Age group	Total tested		anti-HIV				VDRL-TPHA			
	Male	Female	Male		Female		Male		Female	
			poz	neg	poz	neg	poz	neg	poz	neg
20-24 years old	537	236	-	537	1	235	1	536	3	233
25-34 years old	929	337	1	928	-	337	8	921	8	329
35-44 years old	924	383	-	924	-	383	22	902	12	371
45-54 years old	290	180	-	290	-	180	9	281	2	178
55-64 years old	11	3	-	11	-	3	-	11	-	3
TOTAL	2691	1139	1	2690	1	1138	40	2651	25	1114

The donors were divided in age groups (20-24 years old, 25-34 years old, 35-44 years old, 45-54 years old, 55-64 years old), but also on sex groups (males and females) and they were tested for double transmission infections - sexual transmission and blood transmission, such as: syphilis and HIV infection.

The obtained results show that the most frequent infection among the ones with double transmission - sexual transmission and blood transmission, was syphilis and, not least, the infection with the human immunodeficiency virus, which has finally found among donors, after more than 20 years.

Regarding the HIV virus, from a total of 3830 tested donors, only 2 (1 male, 1 female) were positive (Figure 1, Figure 2) and this is a situation found after more than 20 years among donors.

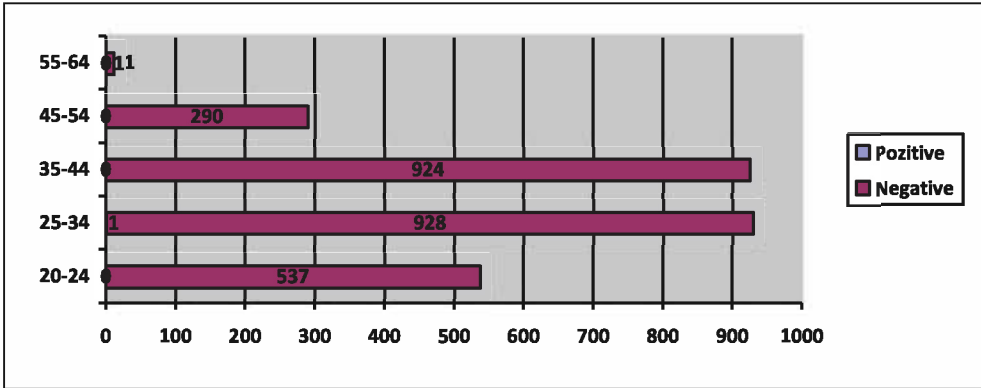


Figure 1. Detection of anti-HIV antibodies at male donors

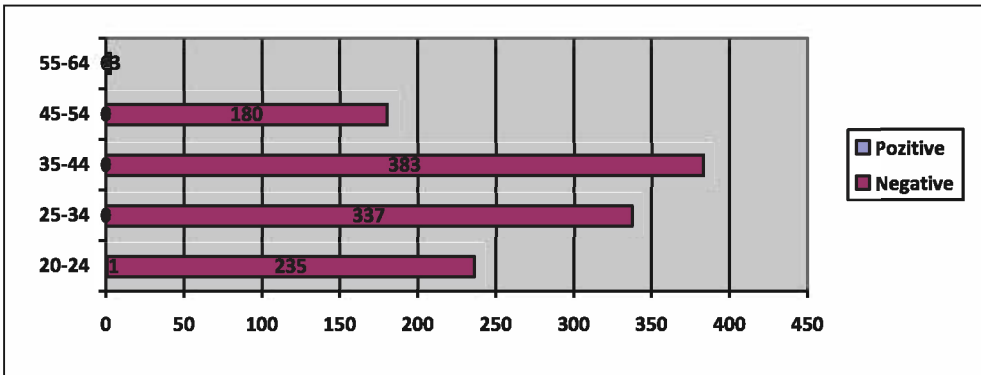


Figure 2. Detection of anti-HIV antibodies at female donors

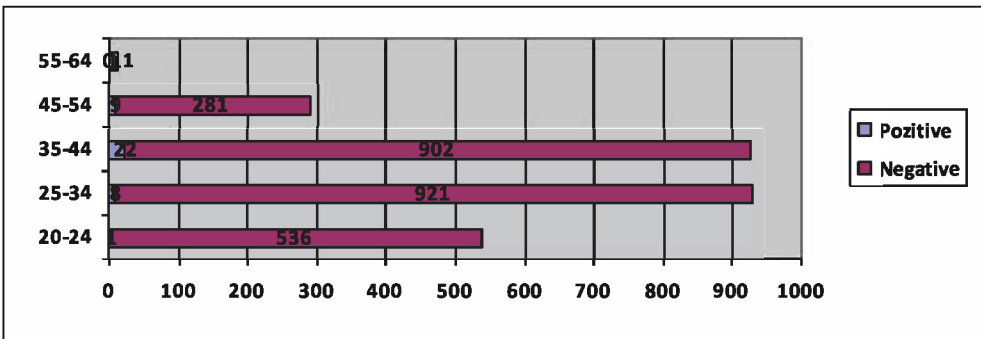


Figure 3. Detection of VDRL-TPHA antibodies at male donors

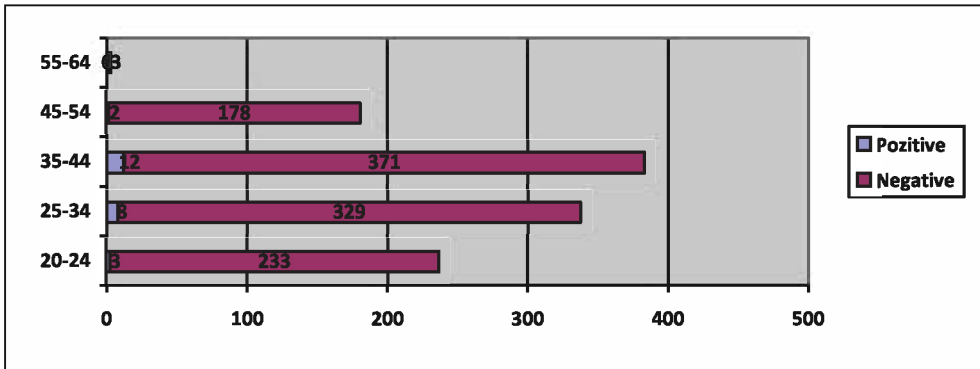


Figure 4. Detection of VDRL-TPHA antibodies at female donors

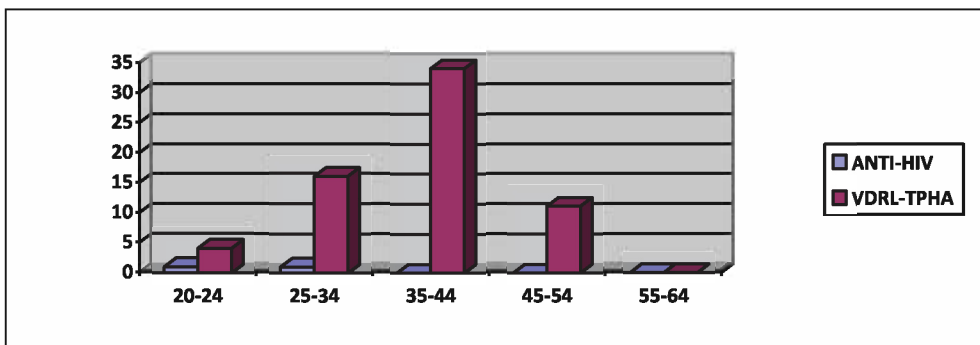


Figure 5. Prevalence of anti-HIV antibodies and VDRL-TPHA antibodies on age groups, at tested donors

From a total of 3830 donors tested for VDRL-TPHA, only 65 donors were positive (40 male, 25 female), and the rest of 3765, were negative (Figure 3, Figure 4).

Considering the age groups, the results show that the weigh both for men and women was obtained by the subjects aged between 25 and 54 (Figure 5).

### CONCLUSIONS

In order to avoid the problems related to the transmission of different diseases associated to the blood donor or to the corresponding blood, it is imposed to keep a number as high as possible of trusty donors, to reasonably use the blood transfusions as “back-up”, to promote self-transfusion and also to bring new compulsory screening techniques for the donors, knowing the fact that this therapeutic act, the blood transfusion, is also the bearer of a certain risk degree.

The adoption of screening strategies appropriate to the needs, infrastructure and resources of each country can contribute significantly to improvements in blood safety. In countries where effective blood screening programmes have been implemented, the risk of transmission of TTIs has been reduced dramatically over the last 20 years (Pomper et al. 2003, Maresch et al. 2008).

In Romania, the HIV/AIDS infection has been a major problem of public health, with high social and economical impact. Actually, Romania was the first country in Central and Eastern Europe that announced the AIDS Supervision Centre of Paris of the WHO

regarding the diagnosing of an AIDS case in 1985. The HIV infection cases at children have been signalled since 1989, with notification, starting with 1990.

In 2000, HIV/AIDS was declared a national priority of public health and this measure was followed by the launch of the national program of universal access to HIV/AIDS cares and treatment.

In our country, the incidence of syphilis has been increasing during the last years (7,1 % in 1986, 19,8% in 1989, 32,3% in 1998, 35,9% in 1999, 44,3% in 2000, 52% in 2001, 58,53% in 2002 and 56% in 2003). Syphilis is sexually transmitted in 96-98 % of the cases and the transmission is extragenital in 2-4 % of the cases, by blood transfusions, transplacental from the mother to the fetus, by a direct contact with the secretions containing treponemas. The indirect transmission by contaminated object is hypothetical. There is no immunity to syphilis, so any person can get sick if they receive a certain number of pathogen *Treponema pallidum* (Pătrașcu 2012).

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**SCREENING FOR TRANSFUSION - TRANSMISSIBLE INFECTIONS  
(HEPATITIS B, HEPATITIS C) AT DONORS OF THE REGIONAL  
CENTRE OF BLOOD TRANSFUSION OF CRAIOVA IN 2013**

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**Keywords:** *transfusion, infections, hepatitis B, hepatitis C*

**ABSTRACT**

*Blood transfusion is a life-saving intervention that has an essential role in patient management within health care systems.*

*The provision of safe and efficacious blood and blood components for transfusion involves a number of processes, from the selection of blood donors and the collection, processing and testing of blood donations to the testing of patient samples, the issue of compatible blood and its administration to the patient. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of bloodborne infections. Screening of donated blood for transfusion-transmissible infections (TTIs) represents one element of strategies for blood safety and availability.*

**INTRODUCTION**

Screening of donated blood for TTIs represents one element of strategies for blood safety and availability.

As large volumes of blood or blood components are given to patients during transfusion therapy, even a blood unit with a low viral load may cause infection in the recipient. It is imperative that blood transfusion services have effective screening systems to detect, segregate and remove reactive blood donations and all components derived from these donations from the quarantined useable stock. Only non-reactive blood and blood components should be released for clinical or manufacturing use.

Screening of all blood donations should be mandatory for the following infections and using the following markers: HIV-1 and HIV-2- screening for either a combination of HIV antigen-antibody or HIV antibodies, hepatitis B - screening for hepatitis B surface antigen (HBsAg), hepatitis C: screening for either a combination of HCV antigen antibody or HCV antibodies, syphilis (*Treponema pallidum*) - screening for specific treponemal antibodies.

All blood donations should be screened for at least one suitable serological marker for each of these four infections. Screening for additional markers for these infections and for other transfusion-transmissible infectious agents could then be considered, depending on the residual risk, logistics and level of resources available (WHO 2010).

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**Hepatitis B virus (HBV)** is a member of the hepadnavirus group and is an enveloped DNA virus. HBV is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes. HBV is endemic globally and hyper-endemic in parts of the world. It is difficult to determine the total number of cases of transfusion-transmitted HBV globally.

The use of unscreened HBV-infected blood and blood products will result in the transmission of HBV in the vast majority of cases. In general, the earlier in life that HBV is acquired, the more likely the individual is to develop chronic infection which then has a higher probability of progressing to cirrhosis and hepatocellular carcinoma (WHO 2010).

**Hepatitis C virus (HCV)** is a member of the flavivirus group and is an enveloped RNA virus. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes, resulting in a similar picture to that seen with HBV infection. Seroreversion has been seen in numbers of individuals who have resolved their infections. The loss of circulating antibody may leave no readily detectable evidence of previous infection (Lefrère et al. 2004).

While HCV is present in the bloodstream, the levels of the virus itself are variable. In recently infected individuals, virus is normally present. However, only around 70% of chronically infected individuals are viraemic and the length of time that viraemia persists is not fully understood. Nonetheless, it is expected that most HCV infected donations would contain virus and thus be infectious (WHO 2010).

## MATERIALS AND METHODS

Laboratory screening of donated blood is the step that determines whether or not a donation is non-reactive for specific markers of infection and is therefore safe to release for clinical or manufacturing use. Various assay systems with differing sensitivities and specificities are available for blood screening. However, the efficacy of screening depends on their correct use in laboratories that are appropriately resourced and staffed and that have well-maintained quality systems (WHO 2010).

### Test kits:

-Kit for the detection of the surface antigen of the hepatitis B in human serum or plasma by the enzyme immunoassay technique (<http://www.bio-rad.com/>)

-Screening kit for the detection of HCV (hepatitis C virus) infection in human serum/plasma by enzyme immunoassay (<http://www.bio-rad.com/>)

**Monolisa™ HBs Ag ULTRA** assay is a one step enzyme immunoassay technique of the "sandwich" type for the detection of the surface antigen of the hepatitis B virus (HBs Ag) in the human serum or plasma. The detection of HBs Ag in the serum indicates an infection caused by the hepatitis B virus. It is the first marker to appear and may be observed 2 or 3 weeks before the clinical and biological symptoms of the disease. Its period of presence may be very short (a few days) or very long (several years). HBs Ag persisting beyond 6 months in the serum denotes "chronic hepatitis" (Fields et al. 1983).

Because of the existence of numerous asymptomatic chronic carriers, hepatitis B represents an important transfusion hazard and the prevention of the transmission is based upon the detection of the HBs Ag at the time of each blood donation (<http://www.bio-rad.com/>).

**Monolisa™ HCV Ag-Ab ULTRA** assay is an enzyme immunoassay (<http://www.bio-rad.com/>) for the detection of HCV infection, based on the detection of capsid antigen and antibodies associated with an infection by hepatitis C virus in patient serum or plasma (Lambert et al. 2004).



All test kits and reagents should be stored and transported under controlled conditions. The blood transfusion service should ensure that reliable cold chain systems are in place in each screening laboratory to assure compliance at all times (WHO 2002). Appropriate temperature-controlled storage equipment which conforms to defined specifications should be made available for normal maximum stocks of all test kits and reagents (WHO, 2005). Serum or plasma (collected into EDTA, sodium citrate or heparin) may be used. Particles in the sample should be removed by centrifugation prior to testing. The samples should be stored at + 2°C - 8°C if testing is to be carried out within 7 days of stored at - 20°C for a longer period (WHO 2010).

## RESULTS AND DISCUSSIONS

This paper is based on a study containing a number of 3830 donors (2691 males and 1131 females), donors who were tested in 2013 at the Regional Centre of Blood Transfusion of Craiova (Table 1).

Table 1

Donors at the first donation at the Regional Centre of Blood Transfusion of Craiova  
(tested for Ag HBs and anti-HCV), in 2013

Age group	Total tested		Ag HBs				Anti-HCV			
	Male	Female	Male		Female		Male		Female	
			poz	Neg	poz	neg	poz	neg	poz	neg
20-24 years old	537	236	19	518	7	229	3	534	1	235
25-34 years old	929	337	40	889	14	323	3	926	2	335
35-44 years old	924	383	26	898	9	374	4	920	10	373
45-54 years old	290	180	16	274	3	177	7	283	9	171
55-64 years old	11	3	-	11	-	3	-	11	-	3
TOTAL	2691	1139	101	2590	33	1106	17	2674	22	1117

The donors were divided in age groups (20-24 years old, 25-34 years old, 35-44 years old, 45-54 years old, 55-64 years old), but also on sex groups (males and females) and they were tested for double transmission infections - sexual transmission and blood transmission, such as: hepatitis B and hepatitis C.

The obtained results show that the most frequent infection among the ones with double transmission: sexual transmission and blood transmission, is hepatitis B. The disease incidence has been permanently increasing since 2008 (156 cases) to 2012 (158 cases), and the top was reached in 2009 – 242 cases and in 2010 – 207 cases. The other double transmission diseases were hepatitis C with a higher incidence among women.

From a total of 3830 donors tested for Ag HBs, only 134 donors were positive (101 male, 33 female), and the rest of 3696, were negative (Figure 1, Figure 2).

From a total of 3830 donors tested for anti-HCV, only 39 donors were positive (17 male, 22 female), and the rest of 3791, were negative (Figure 3, Figure 4).

Considering the age groups, the results show that the weigh both for men and women was obtained by the subjects aged between 20 and 54 (Figure 5).

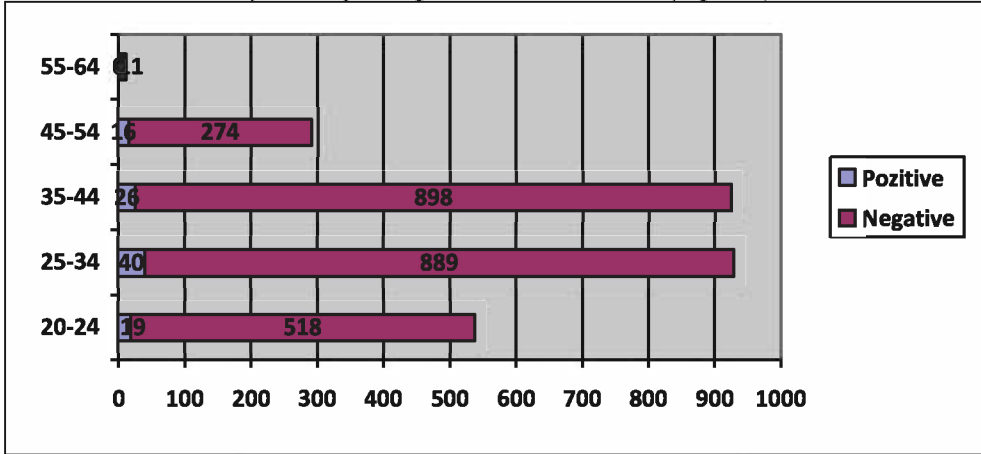


Figure 1. Detection of Ag HBs at male donors

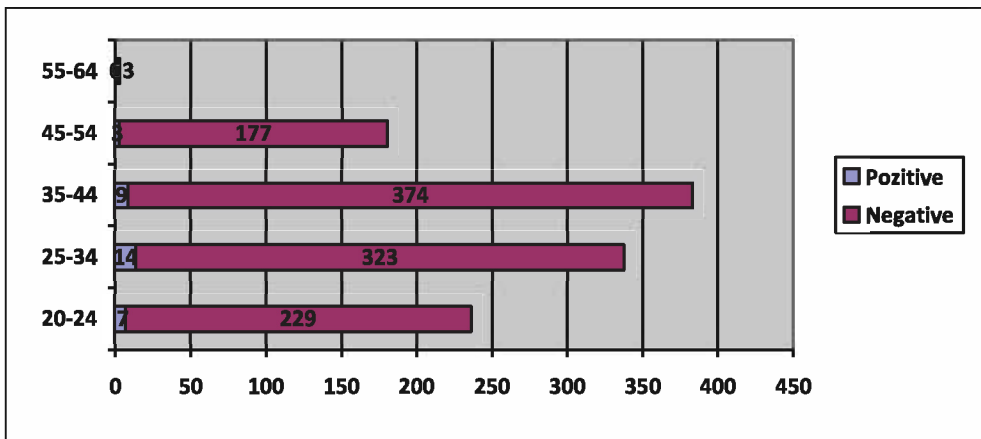


Figure 2. Detection of Ag HBs at female donors

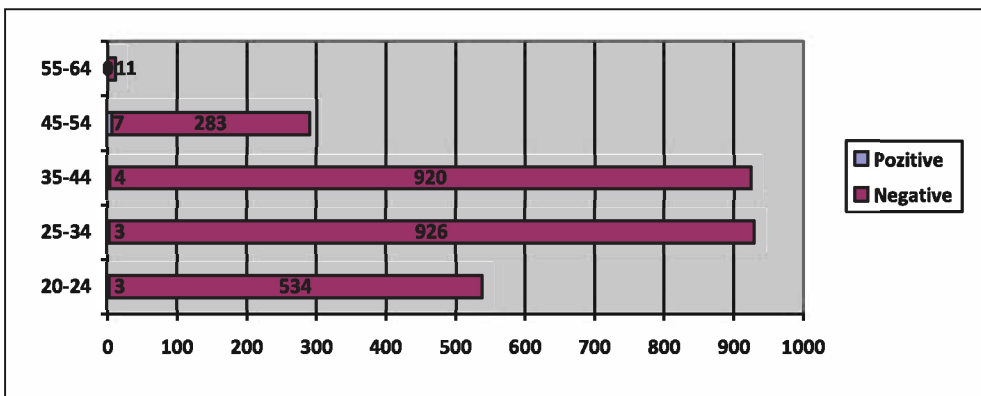


Figure 3. Detection of anti-HCV antibodies at male donors

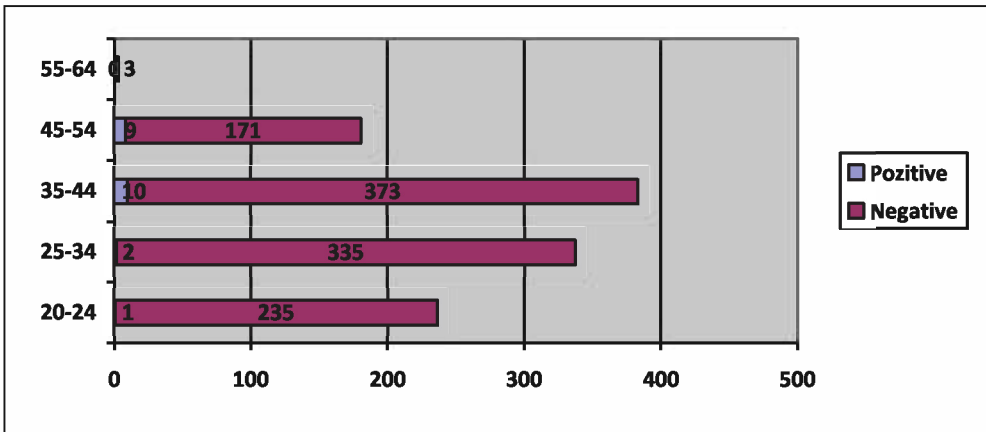


Figure 4. Detection of anti-HCV antibodies at female donors

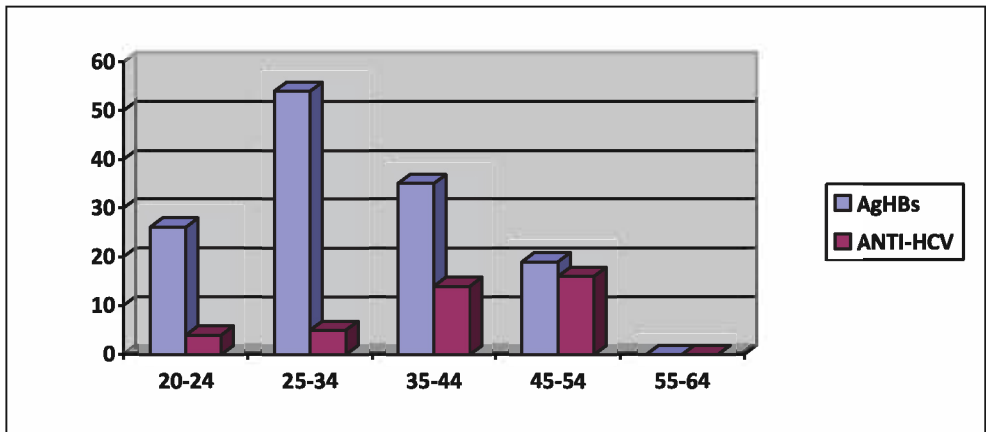


Figure 5. Prevalence of Ag HBs and anti-HCV antibodies on age groups, at tested donors

## CONCLUSIONS

In order to avoid the problems related to the transmission of different diseases associated to the blood donor or to the corresponding blood, it is imposed to keep a number as high as possible of trusty donors, to reasonably use the blood transfusions as “back-up”, to promote self-transfusion and also to bring new compulsory screening techniques for the donors, knowing the fact that this therapeutic act, the blood transfusion, is also the bearer of a certain risk degree.

The blood donors are frequently tested for the presence of the surface antigen of hepatitis B virus (Ag HBs) so, the incidence of the post-transfusion hepatitis B depends on a series of factors such as the prevalence and the strategy of the donors’ testing: In the areas with low prevalence, the estimation was 1-4/1000000 transfused persons (Dodd 2007), and in the regions with high prevalence it is considerably higher – about 1 to 20000.

There are different strategies for screening donors. Most of the countries use Ag HBs for screening. Other countries, such as the USA, recommend both Ag HBs and Ac anti HBc for screening. As a routine, the screening for determining Ac anti HBc is still controversial because the specificity is low and the patients cured from hepatitis B will be

excluded. The screening of the sample pools or even of the individual samples may be improved by using the detection techniques for the viral nucleic acids. Fortunately, the incidence of the infections with these viruses has decreased considerably in most of the world areas (Pătrașcu 2012).

In case of the co-infection VHC and VHB, the hepatitis C virus is usually predominant as it may lead to lower levels of the transaminases and of ADN VHB (Jardi 2001).

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## MONITORING PEST *LYMANTRIA DISPAR* L. IN THE PITEȘTI FORESTRY IN ARGEȘ COUNTY

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*Keywords: monitoring, defoliating insects*

### ABSTRACT

*Defoliating insects are a constant concern for researchers, both nationally and internationally, because it causes damage to the fruit crops and forests. In this situation, researchers have to take concrete steps leading to the restoration of forest and fruit crops.*

*The success of these actions depends on agro-technical and protection measures against various pests.*

### INTRODUCTION

Protection of forest ecosystems and orchards has to be done through a set of measures which are designed to ensure good plant health status of forests and orchards. This is possible only through knowledge of harmful agents and their mode of action (Ionescu 1962, Teodorescu et al. 2003).

An important group of pests is represented by the defoliating insects that at different stages of their development damage and consume whole leaves of trees. Among these hairy caterpillars of the oak-*Lymantria dispar* L.

Their attack is frequently followed by others species of insects or parasitic fungi, thus physiologically weakened trees becoming more vulnerable (Iancu 1999, Colbert & Fekedulegn 2001, Simionescu et al. 2003, Oltean 2005).

In deciduous forests, especially in oaks, from time to time there has been registered high value of the *Lymantria dispar* L. populations requiring a constant concern to limit the negative effects produced by these species (Milescu et al. 1997).

During 1950-2000 there has been recorded significant attack values of this pest, the area infested ranging between 20-30%, mostly in the forests of Muntenia and Oltenia Romanian Plain.

### MATERIALS AND METHODS

Research on monitoring species *Lymantria dispar* L. were made in Arges County, in two stationary: Armeneasa in 2011-2012, in several types of forests where there has been reported the presence of this pest.

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Research has been done in the area shown on several types of forests were the pest has been identified. Grouping them by age classes leads to the following conclusions: forests under the age of 20 years representing 32% from all the forests, those aged 21-40 years-28%; 81-100 years old, 19% between 101-120 years - 21% (table 1).

Table 1

Grouping by age classes of forests

the studied area	Age <20 years	Age 21-40 years	Age 41-80 years	Age 81-100 years	Age 101-120 years
HA 141	32%	0%	28%	19%	21%

The evolution of *Lymantria* populations has been studied in the oaks forests and orchards by surveys on the stages of egg, larva and pupa and by direct counting. For each parcel of the two stationary there has been made observation on ten trees diagonally displayed.

In the forests the counting has been made on 50 oak trees daily.

## RESULTS AND DISCUSSIONS

*Lymantria dispar* wintering in the egg stage. Embryonic development occurs 20-25 days after egg laying. Caterpillars' hatching occurs in the second half of April - beginning of May, depending on the evolution of air temperature. At first, the caterpillars are on "mirror" deposit and then go on the stem in the treetops, where they feed on leaves. Larval stage lasts two to three months of age larvae of 5-6, which can be determined by measuring the width of the cephalic capsule.

The transformation into pupa occurs in late June and early July, between 2-3 leaves weakly bound with silk thread or withdrawn in bark crevices, branch insertion. The duration of the pupa stage is 2-3 weeks, after which the butterfly occurs. The duration of a generation is one year, *Lymantria dispar* L. is a polyphagous insect, which attack oaks, hornbeam, elm, acacia and fruit trees (Tudor 1968).

Mortality factors are represented by parasitoids, predators and viral epizootic. The most important role is played by the parasitoids oophagous, predators oophagous, pupae and animal parasitoids and viral epizootic VPN.

As shown, *Lymantria dispar* L. is one of the pests with a high potential for breeding and also with a high degree of discomfort. Coefficient of increasing population density has different values from one year to another. At the start multiplying mass density is generally uneven, because the last year of the numerical growth phase and especially during the eruption to flatten. In recent decades, in many cases, by applying chemical or biological treatments, usually in growth phase numerical gradation stopped before completing all phases.

Sometimes, during the period when it is signalized a graduation of *Lymantria dispar* L. in a certain area, there is abnormal jump in density from a low level of infestation to a high and very high intensity. This is due to the potential for propagation of the insect.

The presence of the defoliators can be reported in all stages of development, as adults, eggs, larvae and pupae. Instead infested areas *Lymantria dispar* L. detection is performed routinely after laying egg or after caterpillars. An area is considered infested if

there is more than one deposit to 50 trees. In egg stage, detection can be carried out from September to April next year. For statistical and forecasting detection work is done in September-October (table 2 and table 3).

Table 2

The phenogram of *Lymantria dispar* L. in 2011

Stage / Month	I	II	III	12IV	V	25VI	23VII	15VIII	10IX	X	XI	XII
Egg	O	O	O	O								
Larva				L	L	L						
Caterpillar						P	P					
Adult							A	A	A			
Egg								O	O	O	O	O

Legend: O-Egg; L-larva; P- Caterpillar; A-adult

As seen from above phenogram in 2011 egg stage lasted from August 15th until April 12th, the larval stage from April 12 to June 25, and the stern between 25 June and 23 August. All of these table shows that flight and mating butterflies occur during July 23 to September 10.

Table 3

The phenogram of *Lymantria dispar* L. in 2012

Stage / month	I	II	III	15IV	V	30VI	27VII	13VIII	1IX	X	XI	XII
Egg	O	O	O	O								
Larva				L	L	L						
Caterpillar						P	P					
Adult							A	A	A			
Egg								O	O	O	O	O

Legend: O-Egg; L-larva; P- Caterpillar; A-adult

Compared with the previous year in 2012 the egg stage lasts from the beginning of the year on April 15, the larva from 15 April to 30 June and caterpillar's pupation runs from June 30 to July 27. Flight and mating occur between 27 July and 1 September. Evolution of *Lymantria* populations has been studied in the forests of oaks and orchards by surveys and by direct counting.

From our observations it was found that mating and flight of *Lymantria dispar* L. coincides with late July-early September, flying taking place at different times due to weather conditions (figure1).

In the observations were taken into account the actual number of pupa found in the study area, not the larvae or pupae. The counting has been made during July to September, when the insects reach maturity period.

The count has been made daily on 50 oak trees. The data has been recorded in a table by day, compared to the two years of research (table 4).

Table 4

Number of eggs culster per ha of *Lymantria dispar* L. in Armeneasa forest

Dates	Year	
	2011	2012
21VII		
23VII		
25VII		0
27VII		0,2
29VII		0,7
31VII		1
1VIII		1,2
3VIII		2
5VIII		2,5
7VIII		3
9VIII		3
11VIII		3
13VIII	0	4
15VIII	0,2	5
17VIII	0,8	4,8
20VIII	1	3
23VIII	1	2,5
25VIII	1	1,9
27VIII	1,5	1,2
29VIII	2	1
31VIII	2,5	0,8
1IX	3	1,2
3IX	2,5	0
5IX	2,3	
7IX	1,2	
9IX	0,2	





Figure1. *Lymantria dispar* L. female laying eggs-original

Analyzing the data recorded in the same period, over two consecutive years, we found that due to different climatic conditions (higher temperatures) *Lymantria dispar* L. flight began in 2011 on August 15 and ended on September 7, while next year the flight began on July 27 and ended on September 1.

Due to the fact that in 2012 during June-August temperatures were higher than the previous year the flight and mating start two weeks earlier than the normal range (figure 2 and figure 3).

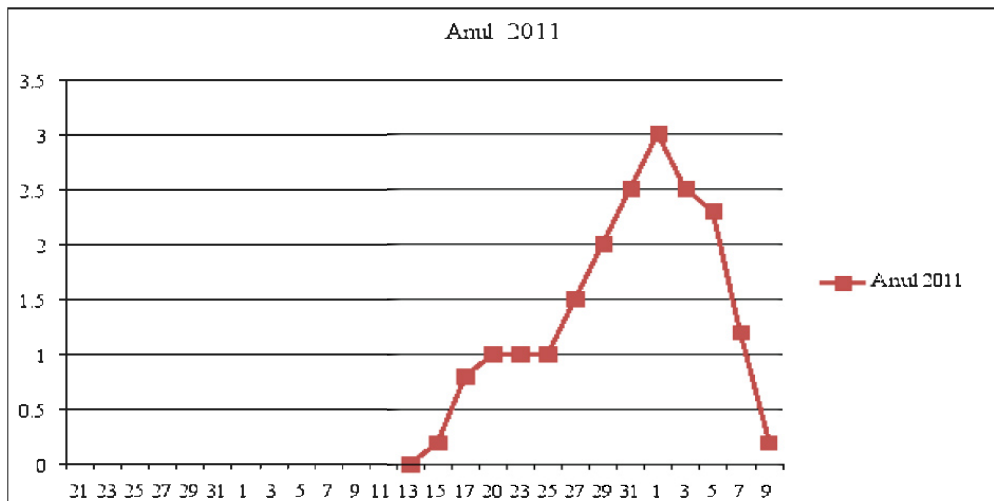


Figure 2. The evolution of eggs cluster for *Lymantria dispar* L. in 2011

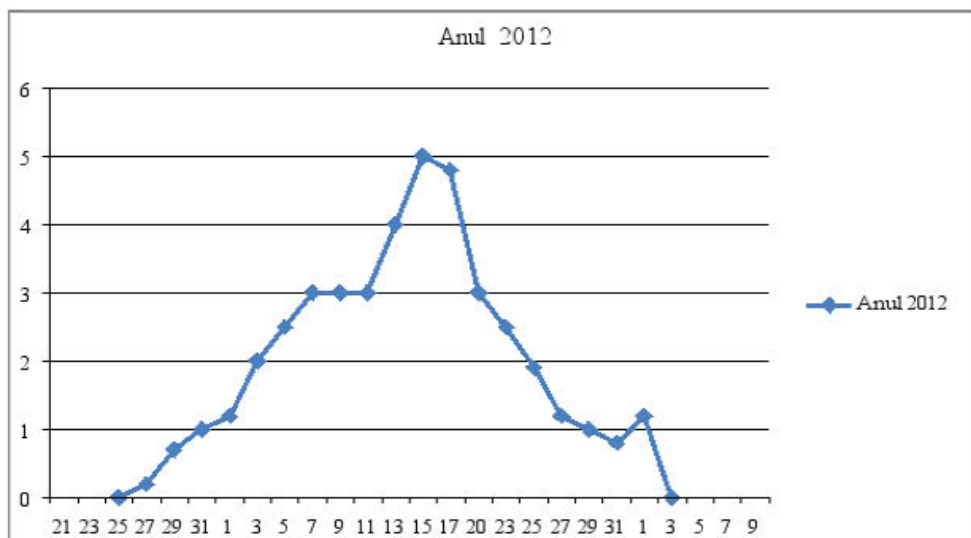


Figure 3. The evolution of eggs cluster for *Lymantria dispar* L. in 2012

The study was conducted from June 21 to August 9, depending on the average number of deposits on the surface (ha).

Due to the fact, that in 2012 during June-August temperatures was higher than the previous year the flight and mating start two weeks earlier than the normal range (table 5).

Table 5

Monthly average temperatures in 2011 and 2012

Month	Year 2011	Year 2012
January	-0,4	-2,1
February	-0,5	-6,6
March	4,7	6,1
April	10,5	13,9
May	15,3	19,8
June	19,8	27,8
July	26,8	32,7
August	26,4	27,3
September	23,8	18,7
October	11,2	12,9
November	3,6	7,3
December	2,7	-1,1

Also during this year there has been recorded a higher frequency of the attacks made by *Dermestride* species (figure 4) on the eggs which necessarily leads to a reassessment of the data for recalculation in spring 2013.



Figure 4. *Dermestride* attack on the *Lymantria dispar* L. clock - original

The Dermestride species play an important role (over 25%) in all outbreaks and crisis erupting, regardless the type of forest. They parasitize caterpillars of the first three larval stages with a very little impact in the first two stages of gradation, but during the crisis phases occurs differentiate between them (Rosca et al. 2011).

Parasitoids whose larval stages parasitize larva in last stages (the last three ages) and pupae are the most important segment from the complex of entomophagous insects attached to *Lymantria dispar* L. (Naidoo & Lechowicz 2001, Tobin et al. 2013).

### CONCLUSIONS

In the studied area it come out that the presence of *Lymantria dispar* L. was closely linked to climatic conditions and particularly air temperature.

The data has been collected from on a sample of 50 trees.

The increasing temperature has conducted to a shift directly to the period of one to two weeks.

Number of adults and intensity of the attack was limited by the presence of the Dermestride species.

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RESEARCH REGARDING THE MONITORING OF THE SPECIES  
*TORTRIX VIRIDANA* L. WITHIN PITEȘTI FOREST DISTRICT, ARGEȘ  
COUNTY

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**Keywords:** *Tortrix viridana* L., monitoring, pheromone traps, prognosis

ABSTRACT

*The green oak leaf roller (Tortrix viridana L.) is a very dangerous pest that causes the most frequent defoliation in deciduous stands. Monitoring is often used to survey the populations of green oak leaf roller. For adult stage, monitoring is done using pheromone traps of Atravir type. In case of Colibași stationary sampling site, Argeș County, the number of catches varied from year to year depending on the evolution of the registered climatic conditions. In 2013, the percentage of defoliation recorded in the six stationary sampling sites was 3% within Pitești forest district.*

INTRODUCTION

Presently, disturbances to the normal state of health are noticed within forests, due to the proliferation of certain species of defoliating insects that produce regular gradations followed by total or partial defoliation.

It is necessary to use some means of controlling the propagation of insects in order to restore the ecological balance. Thus, it is necessary to know in detail the evolution of the population dynamics in order to reduce as much as possible the impact upon the environment (Simionescu et al. 2003).

In our country, there have been developed a number of detection and prognosis methods. The great drawback is that the study period coincided with the widespread use of organochlorine non-selective and highly toxic insecticides.

MATERIALS AND METHODS

In order to monitor the adult defoliators, it is used the synthetic sexual pheromone Atravir mounted in the middle of plastic panels (30 x 45 cm) coated with adhesive on one side, called pheromone traps (Milescu et al. 1997).

The traps were placed in Colibași stationary sampling site, in the oak forests, where oaks species represents more than 30% of the composition. The presence of *Tortrix viridana* L. pest was monitored by checkpoints established by placing 2-3 pheromone panels.

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In the stands of even aged trees (relatively uniform), it was chosen a single point of control consisting in three panels. The panels were placed at the edge of the forest, within it, and close to a clearing (sunny place), being chosen trees with larger diameter that were not covered by seedlings; they were numbered as the pheromone panels.

In the middle of the traps, there is placed the pheromone bait. This is manufactured from a rubber plug similar to that used for penicillin bottles, the pheromone being placed in the convex side of the plug. The bait is fixed carefully in order not to penetrate the middle part of the plug, which would decrease the surface covered by the pheromone, and is protected from the rain with a folded cardboard.

The pheromone traps are placed before the flight of *Tortrix viridana* L. butterflies (Ghizdavu & Oprean 1987, Oltean 2005, Simionescu 1988).

After 2 or 3 days, the butterflies caught in the traps are verified and counted and, then, the traps are cleaned.

### RESULTS AND DISCUSSIONS

In the period 2011- 2013, within Pitești Forest District, Colibași stationary sampling site, the species *Tortrix viridana* developed a single generation per year. Hibernation state occurs in the egg stage, on the thin branches of the upper part of the crown, at the base of the stem, in the small holes present on branches or at the bifurcation of the thin branches.

The results obtained in 2011 referring to the number of catches made by pheromone traps within Colibași stationary sampling site are rendered in Table 1.

Table 1

The number of *Tortrix viridana* L. catches per trap within Colibași stationary sampling site in 2011

Date	Average air temperature (°C)	Air humidity (average)	Rainfall amount (sum)	No. of catches/trap
May 22	17.8	73	0	0
May 24	18.6	72	0	0
May 26	18.0	69	0	1
May 29	20.0	67	0	2
June 1	18.5	82	7	3
June 4	21.3	67	0	5
June 7	20.4	61	0	4
June 10	16.7	86	2.6	3
June 13	19.2	81	7.2	3
June 15	18.9	79	0	1
June 17	22.1	65	0	0
June 20	19.7	58	18	0
Total				22

Within Colibași stationary sampling site, the flight of the adults started on the 26<sup>th</sup> of May, when the first butterflies were also caught into the pheromone traps; the total number of caught adults was 22 in 2011.

The flight of the moths lasted for 20 days (between the 26<sup>th</sup> of May and the 15<sup>th</sup> of June), the maximum number of caught butterflies per day being 5, on the 4<sup>th</sup> of June (fig. 1). The flight of the adults stopped on the 15<sup>th</sup> of June.

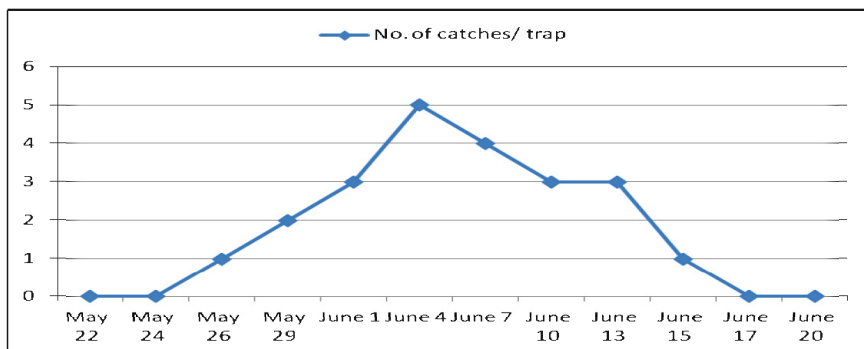


Figure 1. The curve of *Tortrix viridana* L. flight in 2011, Colibași

In 2012, the flight of the butterflies started on the same date, namely the 25<sup>th</sup> of May (Table 2).

Table 2

The number of catches of *Tortrix viridana* L. per trap within Colibași stationary sampling site in 2012

Date	Average air temperature (°C)	Air humidity (average)	Rainfall amount (sum)	No. of catches/ trap
May 25	16.5	80	1.2	0
May 27	13.1	93	2.0	0
May 29	14.0	80	2.0	1
May 31	16.8	83	3.0	1
June 3	19.4	74	0	3
June 7	17.8	68	0	2
June 10	24.3	67	0	4
June 13	23.9	59	0	3
June 16	22.3	55	0	3
June 18	24.2	58	0	1
June 20	25.7	53	0	1
June 22	26.6	50	0	0
June 24	19.8	89	16.8	0
Total				20

The total number of adults caught in 2012 was 20, 2 samples less than 2011.

The flight of the butterflies lasted for a period of 23 days (from the 29<sup>th</sup> of May till the 20<sup>th</sup> of June), the maximum number of caught butterflies, 4, being registered on the 13<sup>th</sup> of June (fig. 2). The flight of the adults stopped on the 20<sup>th</sup> of June, just as it occurred in case of Colibași stationary sampling site.

The results obtained in 2013, within Colibași stationary sampling site prove that the flight of the butterflies started on the 18<sup>th</sup> of May, 11 days earlier than in 2012, being influenced by the climatic conditions; on the same date, there were also caught the first butterflies (Table 3).

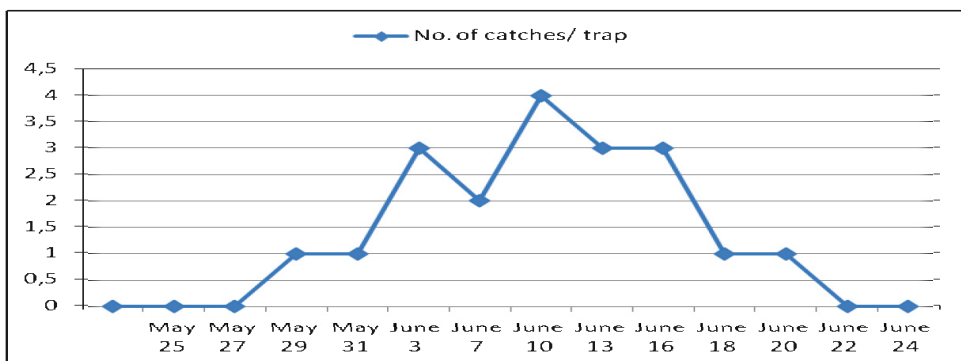


Figure 2. The curve of *Tortrix viridana* L. flight in 2012, Colibași

Table 3  
The number of catches of *Tortrix viridana* L. per trap within Colibași stationary sampling site in 2013

Date	Average air temperature (°C)	Air humidity (average)	Rainfall amount (sum)	No. of catches/ trap
May 14	15.9	76	2.3	0
May 16	17.1	59	0	0
May 18	19.9	64	4.8	1
May 21	21.0	52	0	2
May 24	17.0	69	0	2
May 27	13.3	63	0	2
May 30	16.8	76	24.4	1
June 2	15.5	72	0	1
June 5	15.8	77	28.4	2
June 8	17.7	82	0	3
June 11	19.0	78	7.2	2
June 15	20.5	78	0	1
June 19	25.4	68	0	1
June 22	26.0	65	0	0
June 25	23.0	74	0	0
June 27	17.1	91	16.4	0
June 29	20.4	67	0	0
Total				18

The total number of adults caught in 2013 was 18 specimens, 2 specimens less than in 2012 and 4 less than in 2011.

The flight of the butterflies started on the 18<sup>th</sup> of May and ended on the 19<sup>th</sup> of June; there was caught a maximum number of 4 specimens on the 8<sup>th</sup> of June (fig. 3).

*Tortrix viridana* L. produces defoliations only in oak stands, especially pedunculate oak, pubescent oak, Hungarian oak and sessile oak. In case of Turkish oak, even if infestation is registered, defoliation does not occur. Most of the attacks occur in the upper third of the canopy (Gliga et al. 2013).

Infestation by *Tortrix viridana* L. is often associated with that of the winter moth and sometimes with that of *Lymantria dispar* L. or other species. The detection and



prognosis of *Tortrix viridana* L. defoliators can be performed in all stages of the insect development. The number of caught butterflies sets the infestation intensity, as it is rendered in the following table (Table 4):

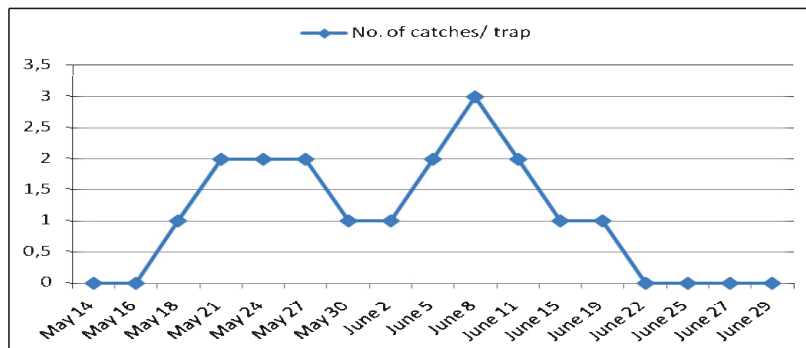


Figure 3. The curve of *Tortrix viridana* L. flight in 2013, Colibași

Table 4

Intensity of the infestation according to the caught butterflies  
(Simionescu et al. 2003)

Defoliation degree (%)	Number of butterflies
10-20%	≤50
21-25%	51-75
26-35%	76-120
36-57%	121-200
58-70%	>200

Defoliations are considered weak up to 25%, medium to 50% and strong between 50-70%. In the forests where the average number of butterflies caught per panel exceeds 50 specimens, egg analyses are performed.

In order to achieve an accurate prognosis, eggs were sent for analyses at the Station of Research, Development and Experimentation in Production Craiova.

The analyses for prognosis were achieved on eggs. We determined the average percentage of young branches with eggs by random sequence in the months between September and December. There were collected 1.2-1.5 m long branches, one at the top, one from the middle and one at the bottom, and the intensity results from the number of living eggs per checked buds (Table 5).

Table 5

Analysis bulletin no. 36 (S.C.D.E.P. Craiova, 2013)

Sample site	No. of analysed trees	No. of analysed branches	% of probable defoliation
Cant. 33 Ruginoasa	3	18	3
UP VI Valea Mare-ua. 72, 24	3	18	3
UP. II Trivale- ua.78b, 43	3	18	3
UP. II Trivale- ua.83b, 33b	3	18	2
UP.Trivale- ua.3,4	3	18	9
UP.Trivale- ua 7c, 50b, 57a	3	18	2

The results from the analyses performed on 18 branches from a number of three trees show that the percentage of probable defoliation was 3% within the six studied stationary sampling sites from Pitești Forest District.

To determine the sex index, mortality and fecundity, there are studied 150 pupae or pupal exuviae from 5 trees with average defoliation from the area of study. The period of analysis was the last part of May-beginning of June.

At the same time, the prognosis for adult stage is achieved when butterflies are caught in pheromone traps.

*Tortrix viridana* L., also called the green oak leaf roller, is parasitized by more than 50 species of hymenoptera (*Pimpla instigator*, *Meteorus cinctellus*), as well as by some species of Tachinidae (*Actia pilipennis*, *Actia crassicornis*).

The places attacked by *Tortrix viridana* L. can be noticed from the distance as the canopy of trees is no longer thick, the annual growth is affected and fruits destroyed.

Control is done by dusting or spraying contact or ingestion insecticide over the tree canopy, during the development of caterpillars. It is preferable that this operation to be performed by plane, because the pest attacks first the top of the canopy on large areas.

### CONCLUSIONS

In the studied area it comes out that the presence of *Tortrix viridana* L. was closely linked to climatic conditions and particularly temperature and humidity.

In order to monitor the adult defoliators, it is used the synthetic sexual pheromone Atravir.

As a conclusion of the objectives of the study, we highlight the integrated control, which is based on the combination between silvicultural measures and physico-mechanical, biological and chemical procedures, the last ones as reduced as possible, this method being completed by schemes on groups of pests and forest formations.

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**EFFECT OF DIFFERENT CONCENTRATIONS OF MACRONUTRIENTS  
ON THE GROWTH RATE AND CULTIVATION OF ALGAE SPECIE  
*CHLORELLA VULGARIS***

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Dimitrios Kantas<sup>2</sup>, Serafeim Papadopoulos<sup>2</sup>, Athanasios Kokkalis<sup>4</sup>, Lampros Ntoufas<sup>5</sup>

**Keywords:** growth rate, macronutrient restriction, microalgae, photobioreactor, sufficiency of macronutrients

**ABSTRACT**

*Algal culture represents a new opportunity for producing significant biofuel quantities in the future. In this study the dependence of the rate of growth of a algal species *Chlorella vulgaris*, on the concentrations of inorganic nitrogen, phosphate ions and potassium was examined. In the first set of experiments, the concentrations of inorganic nitrogen was: (0, 122.65, 590.15 and 1057.65) mg/L, in the second, the concentrations of phosphate ions was: (0, 10.91, 420.91 and 830.91) mg/L and in the third, potassium concentrations were: (0, 86.3, 652.1 and 1218) mg/L. The absorbance at 665 nm and 500 nm was measured daily as a function of time and was used as a measure of cell density. For inorganic nitrogen, phosphate ions and potassium, the optimum growth rate was measured at a solution concentration of 590.15 mg/L, 10.91 mg/L and 652.1 mg/L respectively and a maximum absorbance (at  $\lambda=665$  nm) reading equal to 1.530 after 8 days, 0.588 after 20 days and 1.036 after 22 days respectively. In the second experiment, in four photobioreactors, *Chlorella vulgaris* was grown, under identical conditions but the concentration of inorganic nitrogen in each culture-photobioreactor was different, while P and K were fixed.*

**INTRODUCTION**

Large scale microalgae cultivation is a potential new source of renewable energy (biofuel), owing to the high oil content (Gouveia et al. 2011, Hossain et al. 2008; Li et al. 2008, Schenk et al., 2008). Additionally, some algae strains favor high biomass production and other high lipid concentration (Yoo et al. 2010). Also, after the oil extraction, the algal mass, since it contains substantial quantities of proteins, carbohydrates, micronutrients, can be used either as an animal feed, or as additive to agricultural soil.

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Compared with conventional terrestrial crops (Singh et al. 2010, Chakradhar et al. 2008), the cultivation of microalgae has fast growth rate, does not need the application of insecticides and pesticides and can be conducted in photobioreactors (open ponds or closed).

In previous work (Spiliotis et al. 2014) it was found that, natural fluctuations in the pH occurs during a 3-month culture period for the wild algal species *Chlorella vulgaris*, *Botryococcusbraunii*, *Nannochloropsisoculata* and *Dunaliellasalina* was in the range of 9-10.7, 9.8-10.8, 7.7-8.3 and 7.3-8.3, respectively. It was also found that, the growth media pH significantly affects the growth rate of the microalgal species *Chlorella vulgaris*. From the five pH: 3, 5, 7, 9 and 11 studied, optimum growth occurred at pH 9.

Nutrients, and especially *nitrogen* presence in the cultivation process, significantly affects both growth rate and oil content of the algal biomass (Lardon et al. 2009, Solovchenko et al. 2008). All available nitrogen is utilized for the synthesis of essential enzymes and cellular structures while nitrogen starvation promotes lipid accumulation. Also, some researchers found that nitrate concentration favours biomass productivity while ammonium concentration results in oil content enhancement (Chen et al. 2011, Linet et al. 2011). An increase in lipid production was observed, under phosphorus shortage conditions, which is attributed to the breakdown of cell membrane of the phospholipids into neutral lipids in order to obtain phosphorus (Raetz et al. 2009).

In this work, the dependence of the rate of growth as a function of different concentrations nitrogen, phosphorus and potassium of nutrient solution is studied. Also, the total production on a dry basis (g dry algal mass/ 50 liter of growth medium) was determined, for four different concentrations of nitrogen.

## MATERIALS AND METHODS

The algal specie *Chlorella vulgaris*, have been obtained from Experimental Phycology and Culture Collection of Algae at the University of Goettingen Germany (EPSAG). They have been kept at 4°C and each vial was used within 3 months.

The growth medium was of the following constitution suggested by (Andersen et al. 1991). For *Chlorella vulgaris* each 50 liter growth medium contains: 0.2g KNO<sub>3</sub>/L, 0.02 g K<sub>2</sub>HPO<sub>4</sub> /L, 0.02 g MgSO<sub>4</sub>. 7H<sub>2</sub>O /L, 30 ml of soil extract /L and 5 ml/L, of solution containing the following micronutrients: (1 mg ZnSO<sub>4</sub>.7H<sub>2</sub>O, 2 mg MnSO<sub>4</sub>.4H<sub>2</sub>O, 10 mg H<sub>3</sub>BO<sub>3</sub>, 1 mgCo(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O, 1mgMoO<sub>4</sub>.2H<sub>2</sub>O, 0.005mgCuSO<sub>4</sub>.5H<sub>2</sub>O, 700mg FeSO<sub>4</sub>.7H<sub>2</sub>O and 800 mg EDTA)/L.

**First experiment:** The bioreactors were 1000 ml pyrex flasks, the same specie of algae, namely *Chlorella vulgaris*, was grown. All experiments were carried out in a greenhouse and bioreactors were exposed to exactly the same conditions such as light, orientation, stirring and temperature. Absorbances were measured with the use of a spectroscopy UV/Vis instrument. The absorbance readings were used as a measure of cell density.

### a) Different concentrations inorganic nitrogen of growth medium

In the first bioreactor the growth medium had a nutrient content suggested by Andersen with concentration N-NO<sub>3</sub> = 122.65 mg/L. In the second bioreactor the growth medium had a content suggested by Andersen, but with concentration inorganic nitrogen = 0. In the third bioreactor the growth medium had a nutrient content suggested by Andersen, but with concentration of inorganic nitrogen = 590.15 mg, namely N-NO<sub>3</sub> = 122.65 mg/L + 467.5 N-NH<sub>4</sub><sup>+</sup>, with adding CH<sub>3</sub>COONH<sub>4</sub> and in the fourth bioreactor the growth medium had a content suggested by Andersen, but with concentration of inorganic nitrogen = 1057.65 mg/L, namely N-NO<sub>3</sub> = 122.65 mg/L + 935 N-NH<sub>4</sub><sup>+</sup>, with adding CH<sub>3</sub>COONH<sub>4</sub>.

### **b) Different concentrations PO<sub>4</sub><sup>3-</sup> of growth medium**

In the first bioreactor the growth medium had a nutrient content suggested by Andersen with concentration PO<sub>4</sub><sup>3-</sup> = 10.91 mg/L. In the second bioreactor the growth medium had a nutrient content suggested by Andersen, but with concentration PO<sub>4</sub><sup>3-</sup> = 0. In the third bioreactor the growth medium had a content suggested by Andersen, but with concentration of PO<sub>4</sub><sup>3-</sup> = 420.91 mg/L, namely adding 410 mg/L PO<sub>4</sub><sup>3-</sup> with solution H<sub>3</sub>PO<sub>4</sub> and in the fourth bioreactor the growth medium had a nutrient content suggested by Andersen, but with concentration of PO<sub>4</sub><sup>3-</sup> = 830.91 mg/L, namely adding 820 mg/L PO<sub>4</sub><sup>3-</sup> with solution H<sub>3</sub>PO<sub>4</sub>.

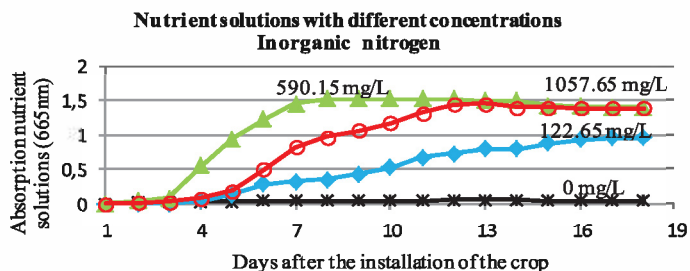
### **c) Different concentrations K of growth medium**

In the first bioreactor the growth medium had a nutrient content suggested by Andersen with concentration K = 86.3 mg/L. In the second bioreactor the growth medium had a content suggested by Andersen, but with concentration K = 0. In the third bioreactor the growth medium had a content suggested by Andersen, but with concentration K = 652.1 mg/L, namely adding 565.8 mg/L K with K<sub>2</sub>CO<sub>3</sub> and in the fourth bioreactor the growth medium had a content suggested by Andersen, but with concentration K = 1218.1 mg/L, namely adding 1131.8 mg/L K with K<sub>2</sub>CO<sub>3</sub>.

**Second experiment:** The photobioreactors were rectangular Teflon vessels of (30 x 35 x 60) cm. The same specie of algae *Chlorella vulgaris*, was grown in all four vessels. All four photobioreactors were exposed to exactly the same conditions such as light, orientation, stirring and temperature. In the first photobioreactor the growth medium (control) had a nutrient content suggested by Andersen et al., with concentration N-NO<sub>3</sub> = 122.65 mg/L. In the second, third and fourth photobioreactor in the growth medium all the elements have had the same nutrient content with the first, but the N concentration varied. In the second, the concentration of nitrogen were 0, in the third photobioreactor, the concentration of inorganic nitrogen were 590.15 mg/L, namely N-NO<sub>3</sub> = 122.65 mg/L + 467.5 N-NH<sub>4</sub><sup>+</sup>, with adding CH<sub>3</sub>COONH<sub>4</sub> and in the fourth the concentration of inorganic nitrogen were 1057.65, namely N-NO<sub>3</sub> = 122.65 mg/L + 935 N-NH<sub>4</sub><sup>+</sup>, with adding CH<sub>3</sub>COONH<sub>4</sub>. The experiment was carried out in a greenhouse of TEI Thessaly and had a duration 82 days and the growth medium in each vessel was 50 liters.

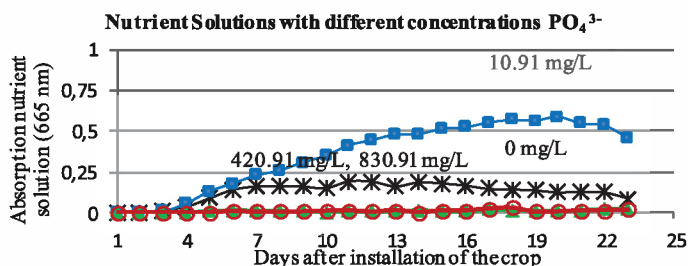
## **RESULTS AND DISCUSSION**

Figure 1 shows the variation of the absorbance of the growth media during the cultivation (18 days) of *Chlorella vulgaris* versus the cultivation time in days. *Chlorella vulgaris* was grown, at four different concentrations of inorganic nitrogen namely, 0 mg/L, 122.65 mg/L, 590.15 mg/L and 1057.65 mg/L, under identical conditions of natural light, temperature and stirring. Absorbance was taken in both 500 nm and 665 nm and was not significantly different. The absorbance (665nm) after 10 days of cultivation at concentrations of inorganic nitrogen of 0 mg/L, 122.65 mg/L, 590.15 mg/L and 1057.65 mg/L, was 0.045, 0.529, 1.530 and 1.182 respectively, while after 18 days of cultivation the absorbance was 0.039, 0.959, 1.392 and 1.392 respectively. The delay phase, at concentrations of inorganic nitrogen of 0 mg/L, 122.65 mg/L, 590.15 mg/L and 1057.65 mg/L, was 18, 4, 2 and 3 days respectively, after inoculation. *Chlorella vulgaris* growth, at concentrations of inorganic nitrogen of 0 mg/L, 122.65 mg/L, 590.15 mg/L and 1057.65 mg/L, ceases about 0, 17, 8, and 12 days respectively, after inoculation. During the cultivation of *Chlorella vulgaris* in a temperature range from 15°C to 29°C, it was found that the maximum growth rate occurred for nutrient solution with a concentration of inorganic nitrogen equal to 590.15 mg/L (N-NO<sub>3</sub><sup>-</sup> = 122.65 mg/L + N-NH<sub>4</sub><sup>+</sup> = 467.5 mg/L).



**Figure 1.** Increase in the absorbance readings of the growth media during the cultivation of *Chlorella vulgaris* under identical conditions at concentration of inorganic nitrogen 0 mg/L, 122.65 mg/L, 590.15 mg/L and 1057.65 mg/L.

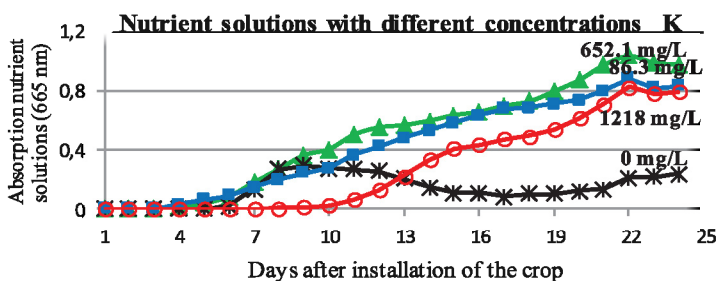
Figure 2 shows the variation of the absorbance of the growth media during the cultivation (23 days) of *Chlorella vulgaris* versus the cultivation time in days. *Chlorella vulgaris* was grown at four different concentrations of phosphate ions namely, 0 mg/L, 10.91 mg/L, 420.91 mg/L and 830.91 mg/L, under identical conditions of natural light, temperature and stirring. Absorbance was taken in both 500 nm and 665 nm and was not significantly different. The absorbance (665nm) after 10 days of cultivation at concentrations of phosphate ions 0 mg/L, 10.91 mg/L, 420.91 mg/L and 830.91 mg/L, was 0.149, 0.350, 0.011 and 0.011 respectively, while after 20 days of cultivation the absorbance was 0.123, 0.588, 0.007 and 0.007 respectively. The delay phase, at concentrations of phosphate ions of 0 mg/L, 10.91 mg/L, 420.91 mg/L and 830.91 mg/L, was 4, 3, 22 and 22 days respectively, after inoculation. *Chlorella vulgaris* growth, at concentrations of phosphate ions of 0 mg/L, 10.91 mg/L, 420.91 mg/L and 830.91 mg/L, ceases about 7, 18, 0, and 0 days respectively, after inoculation. During the cultivation of *Chlorella vulgaris* in a temperature range from 12°C to 29°C, it was found that the maximum growth rate occurred for nutrient solution with a concentration of phosphate ions equal to 10.91 mg/L.



**Figure 2.** Increase in the absorbance readings of the growth media during the cultivation of *Chlorella vulgaris* under identical conditions at concentration of phosphate ions 0 mg/L, 10.91 mg/L, 420.91 mg/L and 830.91 mg/L.

Figure 3 shows the variation of the absorbance of the growth media during the cultivation (24 days) of *Chlorella vulgaris* versus the cultivation time in days. *Chlorella vulgaris* was grown, at four different concentrations of potassium namely, 0 mg/L, 86.3 mg/L, 652.1 mg/L and 1218 mg/L, under identical conditions of natural light, temperature and stirring. Absorbance was taken in both 500 nm and 665 nm and was not significantly

different. The absorbance (665 nm) after 10 days of cultivation at concentrations of potassium of 0 mg/L, 86.3 mg/L, 652.1 mg/L and 1218 mg/L, was 0.280, 0.280, 0.407 and 0.025 respectively, while after 20 days of cultivation the absorbance was 0.126, 0.738, 0.873 and 0.616 respectively. The delay phase, at concentrations of potassium of 0 mg/L, 86.3 mg/L, 652.1 mg/L and 1218 mg/L, was 6, 3, 3 and 9 days respectively, after inoculation. *Chlorella vulgaris* growth, at concentrations of potassium of 0 mg/L, 86.3 mg/L, 652.1 mg/L and 1218 mg/L, ceases about 8, 22, 22 and 22 days respectively, after inoculation. During the cultivation of *Chlorella vulgaris* in a temperature range from 14°C to 29°C, it was found that the maximum growth rate occurred for nutrient solution with a concentration of potassium equal to 652.1 mg/L.



**Figure 3.** Increase in the absorbance readings of the growth media during the cultivation of *Chlorella vulgaris* under identical conditions at concentration of potassium 0 mg/L, 86.3 mg/L, 652.1 mg/L and 1218 mg/L

Additionally, during the 82 days culture period for the algae specie *Chlorella vulgaris*, with growth medium of 50 liters and concentrations of inorganic nitrogen, of 0 mg/L or 122.65 mg/L or 590.15 mg/L and 1057.65 mg/L, the total production on a dry basis algae was 5.88g, 15.91 g, 16.88g and 13.80g respectively.

### CONCLUSIONS

The growth medianutrient concentration significantly affects the growth rate of the microalgal species *Chlorella vulgaris*. From the four concentrations of inorganic nitrogen studied, 0 mg/L, 122.65 mg/L, 590.15 mg/L and 1057.65 mg/L, optimum growth occurred at a concentration of inorganic nitrogen equal to 590.15, namely  $\text{N-NO}_3^- = 122.65 \text{ mg/L} + 935 \text{ N-NH}_4^+$ , and slower growth in nitrogen deficient media. From the four concentrations of phosphate ions studied, 0 mg/L, 10.91 mg/L, 420.91 mg/L and 830.91 mg/L, optimum growth occurred at a concentration phosphate ions 10.91 mg/L, and slower growth inabundance of phosphate ions. Also, from the four concentrations of potassium studied, 0 mg/L, 86.3 mg/L, 652.1 mg/L and 1218 mg/L, optimum growth occurred at a concentration of potassium equal to 652.1 mg/L, and slower growth in potassium deficient media.

During the 82 days culture period for the algae specie *Chlorella vulgaris*, with growth medium of 50 liters and concentrations of inorganic nitrogen, of 0 mg/L or 122.65 mg/L or 590.15 mg/L and 1057.65 mg/L, the maximum production on a dry basis of algalbiomass was 16.88g for a concentration of inorganic nitrogen equal to 590.15 mg/L, and the smallest production on a dry basis algae was 5.88g for a concentration of inorganic nitrogen equal to 0 mg/L.

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SITE OF VASCULAR FLORA TÂRNOVU MARE - LATORIȚA VÂLCEA - ROMANIA

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*Keywords: flora, Latorița, Târnovu Mare, Romania*

**ABSTRACT**

*This paper presents the results of the research conducted by our flora Tarnovu Mare - Latorița site. Following this study there have been identified 278 taxa degrees zoological, ecology and belonging to cenotic different.*

*The analysis of floristic inventory remarked the high number of Carpathian species (22 Balkan and Romanian 10). Besides floral rarities identified in the investigated area (*Alyssum montanum*, *Silene vulgaris* subsp. *glareosa*, *Rumex scutatus*, *Kohlruschia saxifraga*, *Silene saxifraga* subsp. *petraea*, *Athamanta turbith* subsp. *hungarica*) there been found glacial relict flora of Romania (*Ligularia sibirica*) or protected taxa as natural monuments (*Larix decidua* subsp. *carpatica*).*

**INTRODUCTION**

Investigated area belongs administratively to Vâlcea county and of the Capatana Mountain (north side) (Popescu 1977). It has an area of 1366 ha and a difference of altitude of 1,180 m (Natura 2000).

The Tarnovu mountain is caught in a pincers between Latorița River and its major tributary the Repedea river (Natura 2000).

The Latorita Valley takes place along the River Latorița. This is the main tributary of the river Lotru. The course is formed in the southwestern mountains Latorita by uniting Upper Latorita, Middle and Lower Latorita. It flows in Lotru, south to Voineasa, a place called Gura Latoritei (Ujvári 1972).

The most course of the river flows in the mountainous region.

Up to Perimanu Lake this valley separates the Latorita Mountains from the Parâng Mountains. From this lake it receives the right tributaries downstream from the Căpățâna Mountains as: streams Curmatura, Hotaru Huluzu, Crucea, Răgălia, Pietrii, Borgeana Mare, Duminecii, Pepinierii, Deep Creek and several other slope streams (Ploaie 2012).

On the route of this river there was built the power system Lotru - Ciunget that changed substantially the flow of the two rivers.

These mountains have attracted the attention of botanists from ancient times. Many years ago, the botanists Bielz, Krauss, Hergotta and Klotz (Ploaie 2012) made researches and collected plants. As a proof there are the herbarium sheets which are at the

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Museum of Natural Sciences in Sibiu. Subsequently, the data in this part appear sporadically in the literature (Grințescu 1953, Buia 1959, Buia & Maloș 1963, Popescu et al. 2001).

## MATERIALS AND METHODS

The floristic survey of the site started from a previous documentary on its location and previous floristic studies. The next step consisted of field trips to observe, collect and photograph plants which were found.

Determination and systematic classification of taxa was made using literature (Beldie 1977, 1979, Ciocârlan 2009, Sârbu et al. 2013) and coenotic membership by Sanda et al. (1983). The data obtained were compared with those from other points from the Căpățâni Mountain (Buia 1959, Ciurchea 1962, Popescu et al. 2003).

For each taxon there were listed bioforms, geoelemental and coenotic membership. Authors' abbreviations were made by Brummitt & Powell (1992).

## RESULTS AND DISCUSSIONS

As a result of the research there was made a floristic inventory with the following taxonomy: Pteridophyta, Lycopodiopsida, Lycopodiaceae: *Lycopodium annotinum* L. - Ch., Circ. *Piceetalia excelsae*; *L. clavatum* L. - Ch., Cosm. Car. *Piceetalia excelsae*; *L. selago* L. - Ch., Cosm. Car. *Piceetalia excelsae*; Selaginellaceae: *Selaginella helvetica* (L.) Spring - Ch., Euras. *Seslerion*; Equisetopsida, Equisetaceae: *Equisetum hyemale* L. - G., Circ. Car. *Alno-Ulmion*; Hypolepidaceae: *Pteridium aquilinum* (L.) Kuhn - G., Cosm. Car. *Quercetalia pubescenti-petraeae*; Aspleniaceae: *Asplenium adiantum-nigrum* L. - H., Circ. Car. *Vaccinio-Piceion*; *A. ramosum* L. - H., Circ. Car. *Vaccinio-Piceion*; *A. ruta-muraria* L. - H., Euras. Car. *Asplenetalia rutae-murariae*; *A. scolopendrium* L. G., Circ. Car. *Tilio-Acerion*; *A. trichomanes* L. subsp. *quadrivalens* D.E. Mey. - H., Cosm. Car. *Asplenetalia trichomanis*; Polypodiaceae: *Polypodium vulgare* L. - G., Circ. Car. *Asplenetalia trichomanis*; Athyriaceae: *Athyrium filix-femina* (L.) Roth - H., Cosm. *Alno-Ulmion*, *Adenostyletalia*; *Cystopteris fragilis* (L.) Bernh. - H., Cosm. Car. *Asplenetalia trichomanis*, *Dryopteris expansa* (C. Presl) Fraser-Jenkins & Jermy - H., Circ. *Vaccinio-Piceion*; *D. filix-mas* (L.) Schott - H., Euras. Car. *Fagetalia sylvaticae*, *Gymnocarpium dryopteris* (L.) Newman - G., Circ. *Symphyto-Fagion*, *Vaccinio-Piceion*; *G. robertianum* (Hoffm.) Newman - G., Circ. *Fagetalia sylvaticae*, car. *Thlaspietalia rotundifolii*; *Matteuccia struthiopteris* (L.) Tod. - H., Circ. Car. *Alno-Ulmion*; *Polystichum braunii* (Spenn.) Fée - H., Circ. Car. *Symphyto-Fagion*; *P. setiferum* (Forskål) Woy. - H., Cosm. *Symphyto-Fagion*; Spermatophyta, Pinopsida, Pinaceae: *Abies alba* Mill. - Ph., Centr. Eur.-Mont. Car. *Symphyto-Fagion*; *Larix decidua* Mill. subsp. *carpatica* (Dom.) Šiman (Buia 1959) - Ph., Alp. *Symphyto-Fagion*; *Picea abies* (L.) Karst. - Ph., Centr. Eur. and N. Car. *Vaccinio-Piceion*; Cupressaceae: *Juniperus communis* L. var. *intermedia* (Schur) Sanio - Ph., Circ. *Junipero-Bruckenthalion*; *J. sibirica* Lodd. in Burgsd. Ph., Arct.-Alp. *Junipero-Bruckenthalion*; Magnoliophytina (*Angiospermae*), Magnoliopsida, Ranunculaceae: *Aconitum anthora* L. - H., Euras., *Seslerietalia*; *A. moldavicum* Hacq. - H., End. Carp. *Symphyto-Fagion* (Popescu & Păun 1973); *A. variegatum* L. subsp. *paniculatum* (Arcang.) Greuter et Burdet - H. Alp. Carp. *Adenostyletalia*; *A. tauricum* Wulf. - H., Alp.-Carp. *Adenostylion alliariae*; *Anemone nemorosa* L. - G., Circ. *Fagetalia sylvaticae*; *A. ranunculoides* L. - G., Eur. *Fagetalia sylvaticae*; *Caltha palustris* L. - H., Circ. *Alno-Ulmion*, car. *Calthion*; *Helleborus odoratus* Waldst. & Kit. - H., Balc. *Symphyto-Fagion*; *Hepatica nobilis* Mill. - H., Circ. *Fagetalia sylvaticae*; *Pulsatilla alba* Rchb. - H., Alp. Carp. *Potentillo-Nardion*, *Vaccinio-Piceetalia*; *Ranunculus montanus* Willd. subsp.

*pseudomontanus* (Schur) Ciocârlan - H., Carp.-Balc. *Juncetea trifidi*; *R. répens* L. - H., Euras. *Alno-Ulmion*, car. *Agropyro-Rumicion*; *Thalictrum aquilegifolium* L. - H., Eur. *Filipendulo-Petasition*, *Adenostylin alliariae*; Fumariaceae: *Corýdalis solida* (L.) Clairv. - G., Eur. Car. *Fagetalia sylvaticae*; Ulmaceae: *Úlmus glábra* Huds. em. Moss - Ph., Euras. *Fagetalia sylvaticae*, car. *Alno-Ulmion*; Urticaceae: *Urtica diòica* L. - H., Cosm. *Fagetalia sylvaticae*, *Galio-Alliarion*; *Parietária officinális* L. - H., Medit.-Submedit. *Arction lappae*; Fagaceae: *Fáguş sylvática* L. - Ph., Eur. Centr.-Atl.; *Fagetalia sylvaticae*, car. *Symphyto-Fagion*; Betulaceae: *Álnus alnobétula* (Ehrh.) C. Koch - Ph., Alp. Eur. *Adenostylin alliariae*; *Á. incána* (L.) Moench - Ph., Eur. car. *Alno-Ulmion*; *Bétula péndula* Roth - Ph., Euras. *Vaccinio-Piceion*, *Sambuco-Salicion*, Corylaceae: *Corylus avellána* L. - Ph., Eur. Car. *Querco-Fagetea*; *Cárpinus bétulus* L. - Ph., Centr. Eur. Car. *Carpinion betuli*; Caryophyllaceae: *Arenária biflora* L. - Ch., Euras.-Arct.-Alp.-Eur. *Seslerietalia*; *A. serpyllifolia* L. - T., Circ. *Alyso-Sedion*; *Cerástium arvénse* L. subsp. *calcícola* (Schur) Borza - T., Balc. *Seslerietalia*, *Thlaspietea rotundifolii*; *C. fontanum* Baumg. Ch.-H., Cosm. *Potentillo-Nardion*; *Diánthus spiculifolius* Schur - H.(Ch.), Carp. Rom. *Seslerietalia*; *Gypsophila murális* L. - T., Euras. *Stellarietea mediae*; *Kohlráuschia saxifraga* (L.) Dandy - Ch., Pont.-Medit. *Festuco saxatilis-Seslerion bielzii*; *Minuártia setacea* (Thuill.) Hayek - Ch., Centr. Eur. Balc. Pont. *Stipo - Festucetalia pallentis*; *Moehringia trinervia* (L.) Clairv. - T.-H., Euras. *Querco-Fagetea*; *Sagína procúbens* L. - H.(Ch.), Circ. *Arrhenatheretalia*; *S. saginoídes* (L.) Karst. - H.(Ch.), Circ.-Arct.-Alp. *Cardamino-Montion*; *Siléne arméria* L. - T.-HT., Eur. Centr. Car. *Festuco-Sedetalia*; *S. latifolia* Poir. subsp. *álba* (Mill.) Greuter & Burdet - T., H. Euras. *Alno-Ulmion*; *S. nútans* L. subsp. *dúbia* (Herb.) Zapal. H., - Carp. Rom. *Seslerietalia*, *Asplenetalia*; *S. saxifrága* L. subsp. *petraea* (Waldst.& Kit.) Guşuleac - H., Balc. Car. *Micromerion banaticum*; *S. vulgáris* (Moench) Garcke subsp. *glareosa* (Jordan) Marsden-Jones - Turrill - H., Centr. Eur. (mont.) *Thlaspietea rotundifolii*; Polygonaceae: *Rúmex obtusifolius* L. subsp. *tránsiens* (Simonk.) Rech. f. - H., Centr. Eur. *Epilobietalia angustifolii*; *Rumex scutatus* L. - H., Alp. Eur. *Thlaspietea rotundifolii*; Crassulaceae: *Jovibárba heuffelii* (Schott) A. & D. Löve - Ch., Carp.-Balc. *Asplenetalia trichomanis*, *Seslerio-Festucion*; *Sédum alpêtre* Vill. - Ch., Alp. Eur. *Juncetea trifidi*; *S. annuum* L. - T.-HT., Euras.-Arct.-Alp.-Eur. *Sedo-Scleranthion*, *Asplenetalia trichomanis*; *S. hispánicum* L. - T.-HT., Medit. *Asplenetalia trichomanis*; Saxifragaceae: *Chrysosplénium alternifolium* L. - H., Circ. *Alno-Ulmion*, *Betulo-Adenostyletea*; *Parnássia palústris* L. - H., Circ. *Caricetalia davallianae*; *Saxifraga cuneifolia* L. subsp. *robústa* D.A. Webb - Ch., Alp. Eur. *Symphyto-Fagion*; *S. margináta* Sternb. - Ch., Carp. Balc. *Seslerion rigidae*; *S. paniculáta* Mill. - Ch., Euras.-Arct.-Alp.-Eur. *Thlaspietea rotundifolii*, car. *Asplenetalia rutae-murariae*; *S. rotundifolia* L. - H., Eur. Centr. (Mont.). *Adenostylin alliariae*; *S. stelláris* L. subsp. *robústa* (Engl.) Gremli - Ch., Euras.-Arct.-Alp.-Eur. *Montio-Cardaminetea*; Rosaceae: *Alchemilla connívens* Buser - H., Eur. (Mont.). *Potentillo-Nardion*; *Fragária véşca* L. - H., Euras. *Epilobietalia angustifolii*; *Géum montánium* L. - H., Alp. Eur. *Potentillo-Nardion*; *Potentilla erecta* (L.) Ráusch. - H., Euras. *Molinio-Arrhenatheretea*; *P. réptans* L. - H., Euras. *Agropyro-Rumicion*; *P. ternáta* C. Koch - H., Carp.-Balc. Car. *Potentillo-Nardion*; *P. thuringiaca* Bernh. ex Link - H., Centr. Eur. *Symphyto-Fagion*; *Rosa pendulína* L. - Ph., Centr. Eur. (Mont.), *Adenostyletalia alliariae*; *Rúbus idaéus* L. - Ph., Circ. *Epilobietalia angustifolii*; *Sorbus aucupária* L. - Ph., Eur. *Vaccinio-Piceetea*; *Spiréa chamaedrífolia* L. Ph., Euras. *Asplenetalia trichomanis*, *Thlaspietea rotundifolii*; Fabaceae: *Cytisus nigricans* L. - Ph., Centr. Eur. and SE. *Stipo-Festucetalia pallentis*, *Geranion sanguinei*; *Genísta tinctoria* L. subsp. *oligosperma* (Andrae) Borza. Ch., Carp. Rom. *Junipero-Bruckenthalion*; *Láthyruş vérnus* (L.) Bernh. - G., Euras. Car. *Fagetalia sylvaticae*; *Lotus corniculátus* L. - H., Euras. *Molinio-*

*Arrhenatheretea*; *Trifolium répens* L. - H., Euras. *Cynosurion cristati*, *Plantaginetea majoris*; Onagráceae (Oenotheráceae): *Epilobium angustifolium* L. - H., Circ. Car. *Epilobietalia angustifolii*; *E. montánum* L. - H., Euras. Car. *Fagetalia sylvaticae*; *E. nútans* A.F.W. Schmidt - H., Alp. Eur. *Cardamini-Montion*; Thymeleáceae: *Dáphne mezeréum* L. - Ph., Euras. Car. *Fagetalia sylvaticae*; Santaláceae: *Thesium alpinum* L. - H., Eur. (Mont.). Car. *Seslerietalia*; Euphorbiáceae: *Euphorbia amygdaloides* L. - Ch., Centr. Eur.-Subatl.-Submedit. Car. *Fagetalia sylvaticae*; *E. salicifolia* Host - H., Pont.-Pan. *Origanetalia vulgaris*; *E. stricta* L. - T., Eur. Cont. *Alno-Ulmion*, car. *Senecion fluviatilis*; *Mercurialis perénnis* L. - G.(H.), Eur. *Fagetalia sylvaticae*; Aceráceae: *Acer pseudoplátanus* L. - Ph., Centr. Eur. *Quercu-Fagetea*; Oxalidáceae: *Oxalis acetosélla* L. - H.(G.), Circ. *Fagetalia sylvaticae*, *Vaccinio-Piceetea*; Geraniáceae: *Geranium robertianum* L. - T.-HT., Cosm. *Alno-Ulmion*, *Thlaspietalia rotundifolii*, car. *Fagetalia sylvaticae*; Balsamináceae: *Impatiens noli-tángere* L. - T., Euras. *Alno-Ulmion*, *Fagetalia sylvaticae*; Polygaláceae: *Polýgala amára* L. subsp. *brachýptera* (Chodat) Hayek - H.(Ch.), Carp. *Bromo-Festucion pallentis*; Apiáceae (Umbelliferae): *Angélica archangélica* L. - HT.-H., Euras. Bor. *Filipendulo-Petasition*, *Adenostyletalia*; *A. sylvéstris* L. - HT.-H., Euras. *Molinio-Arrhenatheretea*, *Alno-Ulmion*; *Athamánta turbith* (L.) Brot. subsp. *hungárica* (Borbás) Tutin - H., Carp. Rom. *Seslerietalia*, (Popescu et al. 2003); *Bupleúrum falcátum* L. - H., Alp.-Eur. *Geranion sanguinei*; *Chaerophýllum aromáticum* L. - H., Centr. Eur. *Fagetalia sylvaticae*; *C. hirsútum* L. - H., Centr. Eur. (Mont.). *Filipendulo-Petasition*, *Adenostyletalia*; *Cnidium silaifolium* (Jacq.) Simonk. - H., Medit. *Seslerio-Festucion*; *Laserpítium latifolium* L. - H., Eur. (Mont.), *Origanetalia vulgaris*; *Séseli libanotis* (L.) Koch - HT.-H., Euras. Cont. *Seslerietalia*, *Geranion sanguinei*; Hypericáceae: *Hypéricum maculátum* Crantz - H., Euras. Car. *Nardetalia strictae*; Violáceae: *Viola biflora* L. - H., Circ. Car. *Adenostyletalia*; *V. declináta* Waldst. & Kit. - H., Carp.-Balc. *Potentillo-Nardion*; *V. tricolor* L. subsp. *tricolor* (incl. var. *lutéa* Peterm.) - T.-HT., Euras. *Molinio-Arrhenatheretea*; Cistaceae: *Helianthemum nummularium* (L.) Mill. subsp. *obscurum* (Čelak.) Holub - Ch., Centr. Eur. *Seslerietalia*; Tamaricáceae: *Myricária germánica* (L.) Desv. - Ph., Eur., As. SV. *Salicion elaeagni*; Brassicáceae (Cruciferae): *Alyssum montanum* L. H., Centr. Eur. Medit. *Stipo-Festucetalia pallentis*; *Arabis hirsúta* (L.) Scop. s. str. - HT.-H., Euras.-Medit. *Festuco-Brometea*; *A. turríta* L. - HT.-H., Centr. Eur.-Medit. *Quercetea pubescenti-petraeae*; *Capsélla búrsa-pastoris* (L.) Medik. - T.-HT., Cosm. *Chenopodietalia*; *Cardámine amára* L. - H., Euras. Car. *Alno-Ulmion*; *C. hirsúta* L. - T.-HT., Euras. *Galio-Alliarion*; *C. impatiens* L. - HT., Euras. Car. *Fagetalia sylvaticae*; *Cardaminopsis arenosa* (L.) Hayek - T.-HT., H., Centr. Eur. *Sedo-Scleranthetea*; *Dentária glandulosa* Waldst. & Kit. - G., Carp. Rom. *Symphyto-Fagion*; *Erysimum comatum* Pančic. HT., Pont. *Seslerio-Festucion pallentis*, *Thlaspietalia rotundifolii*; *Peltária alliécea* Jacq. - H., Alp.-Carp.-Balc. Car. *Peltarion*; *Roríppa sylvéstris* (L.) Besser - H., Euras. *Agropyro-Rumicion*; *Thláspi kovatsii* Heuff. - T., Carp. Balc. *Papavero-Thymion pulcherrimi*; Salicáceae: *Salix elaeagnos* Scop. - Ph., Centr. Eur. *Salicion elaeagni*; Ericáceae: *Bruckenthália spiculifolia* (Salisb.) Rchb. - Ph.(Ch.), Carp. Balc. Car. *Junipero-Bruckenthalion*; *Vaccínium myrtillus* L. - Ch. (Ph.), Circ. Car. *Piceetalia excelsae*; *V. vitis-idéa* L. - Ch.(Ph.), Circ. Car. *Piceetalia excelsae*; Pyroláceae: *Orthília secúnda* (L.) House - Ch., Circ. Car. *Piceetalia excelsae*; Primuláceae: *Lysimachía nummulária* L. - Ch., Euras., Am. de N. *Calthion*, *Alno-Ulmion*; *Primula véris* L. subsp. *columnae* (Ten.) Maire & Petitm. - H., Medit. *Seslerion rigidae*; *Soldanélla hungárica* Simonk. subsp. *máior* (Neilr.) S. Pawl. - H., Alp.-Carp.-Balc. Car. *Vaccinio-Piceion*; Gentianáceae: *Gentiána asclepiádea* L. - H., Centr. Eur. (Mont.) *Adenostyletalia alliariae*, car. *Symphyto-Fagion*; Convolvuláceae: *Calystégia sépium* (L.) R. Br. - G.(H.), Euras. *Calystegion*, *Salicion*;

Boraginácea: *Échium vulgáre* L. - HT., Euras. *Festuco-Brometea*; *Myosotis scorpioides* L. - H., Euras. Car. *Calthion*; *Pulmonária rúbra* Schott - H., Carp.-Balc. *Vaccinio-Piceion*; Lamiácea (Labiatae): *Galeopsis speciosa* Mill. T., Euras. *Epilobietalia angustifolii*; *Lámium álbum* L. - H., Euras. *Galio-Alliarion*; *L. galeobdolon* (L.) L. - H.(Ch.), Centr. Eur. *Fagetalia sylvaticae*; *L. maculátum* L. subsp. *cupreum* (Schott, Nyman / Kotschy) Hadač - H.(Ch.), Euras. *Alno-Ulmion*; *Népeta núda* L. - H.(Ch.), Euras. Cont. Car. *Festucetalia valesiaca*; *Origanum vulgáre* L. - H., Euras. *Thlaspietalia rotundifolii*; *Prunélla vulgáris* L. - H., Cosm. *Molinio-Arrhenatheretea*; *Sálvia glutinosa* L. - H., Euras. (Mont.). Car. *Fagetalia sylvaticae*; *Stáchys officinális* (L.) Trev. - H., Euras. Car. *Molinion caeruleae*, *Origanetalia vulgaris*; *S. sylvática* L. - H., Euras. *Fagetalia sylvaticae*, *Alno-Ulmion*, *Filipendulo-Petasition*; *Thýmus balcánus* Borbás - Ch., Carp. Balc. *Festucetalia valesiaca*; *T. comosus* Heuff. ex Griseb. - Ch., Carp. Rom. *Seslerio-Festucion* (Popescu et al. 2003); Plantaginácea: *Plantágo lanceoláta* L. - H., Euras. *Festuco-Brometea*; *P. májor* L. - H., Euras. *Plantaginetea majoris*; Scrophulariácea: *Digitális grandiflora* Mill. - H., Centr. Eur. *Symphyto-Fagion*; *Euphrásia salisburgénsis* Funck - T., Alp. Eur. *Seslerietalia*; *E. stricta* D. Wolff ex J. F. Lehm. - T., Eur. *Arrhenatheretalia*, *Festuco-Brometea*; *Linária vulgáris* Mill. - H., Euras. *Epilobiete a angustifolii*; *Rhinánthus angustifolius* C.C. Gmel. - T., Euras. *Molinio-Arrhenatheretea*; *Scrophulária nodosa* L. - H., Euras. *Epilobiete a angustifolii*; *S. scopolii* Hoppe - H., Pont.-Pan.-Balc. *Fagetalia sylvaticae*, *Galio-Alliarion*; *Verbáscum lychnítis* L. - HT., Eur. *Geranion sanguinei*; *V. nígrum* L. subsp. *abietinum* (Borbás) I. K. Ferguson - H., Daco-Balc. *Arction lappae*; *Veronica bachofénii* Heuff. - H., Carp.-Balc.-Anat. *Asplenion septentrionalis*; *V. montána* L. - Ch., Eur. *Alno-Ulmion*; *V. officinális* L. - Ch., Euras. *Potentillo-Nardion*; *V. urticifolia* Jacq. - H., Centr. Eur.-Submedit. (Mont.). *Symphyto-Fagion*; Campanulácea: *Campánula abietína* Griseb. - H., Carp.-Balc. *Piceetalia excelsae*; *C. pátula* L. - HT., Eur. *Arrhenatheretalia*; *C. rapunculoídes* L. - H., Euras. *Querco-Fagetea*; *C. serráta* (Kit.) Hendrych - H., Carp. Rom. *Potentillo-Nardion*, *Junipero-Bruckenthalion*; *C. trachélium* L. - H., Euras. (Submedit.). *Fagetalia sylvaticae*; *Phyteuma confusum* A. Kern. - H., Alp.-Carp.-Balc. *Potentillo-Nardion*; *Symphyandra wanneri* (Rochel) Heuff. - H., Carp.-Balc. *Silenion larchenfeldiana*; Rubiácea: *Cruciáta glábra* (L.) Ehrend. - H., Euras. *Querco-Fagetea*, *Alno-Ulmion*; *Gálium álbum* Mill. - H., Eur. *Seslerio-Festucion*, *Thlaspietalia rotundifolii*; *G. flavescens* Borbás - H., Carp.-Balc. *Festucetalia rupicolae*; *G. palústre* L. subsp. *palústre* - H., Circ. *Molinio-Arrhenatheretea*; Caprifoliácea: *Sambúcus racemosa* L. - Ph., Circ. Car. *Sambuco-Salicion*; Valerianácea: *Valeriana montána* L. - H., Alp.-Eur. *Adenostyletalia alliariae*, *Asplenietalia*; Asterácea (Compositae), *Tubuliflorae (Asteroideae)*: *Achilléa dístans* Waldst. & Kit. ex Willd. - H., Alp.-Carp.-Balc. *Potentillo-Nardion*, *Adenostylion alliariae*; *Adenostyles alliariae* (Gouan) A. Kern. var. *kernerii* (Simonk.) G. Beck - H., Alp. Eur. Car. *Adenostyletalia*; *Antennária dioica* (L.) Gaertn. - Ch.(H.), Euras. Car. *Nardetalia strictae*; *Ánthemis tinctoria* L. subsp. *fussii* (Griseb. Et Scenk) Beldie - H., Carp. Balc. *Festucetalia valesiaca*; *Árctium mínus* (Hill) Bernh. - HT., Carp. Balc. Car. *Arction lappae*; *Artemísia absínthium* L. - H.(Ch.), Euras. Car. *Arction lappae*; *Áster améllus* L. - H., Eur. Cont. *Festucetalia valesiaca*; *Béllis perénnis* L. - H., Eur. *Cynosurion cristati*; *Cárduus acanthoídes* L. - HT., Eur. Car. *Onopordion acanthii*; *C. kernerii* Simonk. - H., Carp. Balc. *Seslerietalia*; *C. personáta* (L.) Jacq. - H., Centr. Eur. (Mont.), *Filipendulo-Petasition*, *Alno-Ulmion*; *Carlína acaúlis* L. - H., Centr. Eur.-Medit. (Mont.). *Potentillo-Nardion*; *Centauréa apiculáta* Ledeb. subsp. *spinulosa* (Roch.) Dostal. - H., Centr. și SE. Eur. *Festucetalia valesiaca*; *C. nervosa* Willd. - H., Alp. Eur. *Potentillo-Nardion*; *Círsium erisitháles* (Jacq.) Scop. - H., Centr. Eur. (Mont.). *Fagetalia sylvaticae*, *Filipendulo-Petasition*; *C. oleráceum* (L.) Scop. - H., Euras. *Filipendulo-*

*Petasition*, *Alno-Ulmion*, car. *Calthion*; *C. waldsteinii* Rouy - H., Alp.-Carp. *Filipendulo-Petasition*; *Doronicum austriacum* Jacq. - G., Eur. (Mont.). *Filipendulo-Petasition*, car. *Adenostylion alliariae*; *Erigeron ácris* L. - HT.-H., Circ. *Festuco-Brometea*; *E. ánnuus* (L.) Pers. - T., HT., H., Adv. (Am. de N); *Alno-Ulmion*; *Gnaphalium sylvaticum* L. - H., Circ. Car. *Epilobietea angustifolii*; *Homogyne alpina* (L.) Cass. - H., Alp. Eur. Car. *Vaccinio-Piceion*; *Leucánthemum rotundifolium* (Willd.) DC. Non Opiz - H., Carp., Jugosl. Centr. *Adenostylion alliariae*, *Symphyto-Fagion*, *Chrysanthemo-Piceion*; *Ligulária sibirica* (L.) Cass. - H., Euras. Bor. *Magnocaricion elatae*; *Petasites álbus* (L.) P. Gaertn. - G., Euras. *Symphyto-Fagion*, *Alno-Ulmion*; *Senécio ovátus* (P. Gaertn., E. Mey. & Scherb.) Willd. - H., Centr. Eur.-Submedit. *Fagetalia sylvaticae*, *Epilobietea angustifolii*; *S. rupéstris* Waldst. & Kit. - HT.-H., Centr. Eur. and S. (Mont.). *Thlaspietea rotundifolii*; *S. subalpinus* Koch - H., Alp.-Carp.-Balc. *Adenostylion alliariae*, *Alno-Ulmion*; *Telékia speciosa* (Schreb.) Baumg. - H., Carp.-Balc.-Cauc.-Anat. *Alno-Ulmion*, *Filipendulo-Petasition*; *Tussilágo fárfara* L. - G., Euras. *Arction lappae*, *Filipendulo-Petasition*; *Liguliflorae (Cichorioideae)*: *Hierácium aurantiacum* L. subsp. *carpathicola* Naegeli & Peter. - H., Euras.-Arct.-Alp.-Eur. *Potentillo-Nardion*; *H. murorum* L. - H., Eur. *Querco-Fagetea*, *Piceetalia excelsae*; *H. transsylvanicum* Heuff. - H., Carp. Balc. *Fagetalia sylvaticae*, *Piceetalia excelsae*; *H. umbellátum* L. - H., Circ. *Origanetalia vulgaris*; *Leontodon autumnális* L. - H., Euras., *Cynosurion*; *Mycélis murális* (L.) Dumort. - H., Eur. Car. *Epilobietea angustifolii*; *Prenánthes purpúrea* L. - G., Centr. Eur. (Mont.). *Adenostylion alliariae*; *Sonchus arvensis* L. - G., Euras. Car. *Chenopodietalia*; *Taráxacum officinále* Web. ex Wiigg. - H., Euras. *Plantaginetea majoris*; Liliopsida (Monocotyledonatae), Liliáceae: *Gágea lútea* (L.) Ker.-Gawl. - G., Euras. *Carpinion betuli*; *G. mínima* (L.) Ker.-Gawl. - G., Euras. Cont. *Carpinion betuli*, *Lílium mártagon* L. - G., Euras. *Fagetalia sylvaticae*; *Polygonatum latifolium* (Jacq.) Desf. - G., Pont.-Pan.-Balc. *Querco-Fagetea*; *P. verticillátum* (L.) All. - G., Euras. *Betulo-Adenostyletea*; *Scilla bifolia* L. subsp. *drunénsis* Speta - G., Centr. Eur. și Sudică. *Querco-Fagetea*; *Verátrum álbum* L. subsp. *lobeliánium* (Bernh.) Arcang. - H., Euras. Car. *Betulo-Adenostyletea*; Alliáceae: *Allium senescens* L. subsp. *montanum* (Fries) Holub - G., Centr. Eur. Submedit. (mont.), *Seslerio-Festucion pallentis*; Amaryllidáceae: *Galánthus nivális* L. - G., Centr. Eur.-Submedit. *Querco-Fagetea*; Iridáceae: *Crocus vérnus* (L.) Hill - G., Carp.-Balc. *Potentillo-Nardion*; Orchidáceae: *Cephalanthera longifolia* (L.) Fritsch - G., Eur. Car. *Querco-Fagetea*; *Dactylorhiza maculáta* (L.) Soo - G., Centr. Eur. și N. *Alno-Ulmion*; *Epipáctis atrorubens* (Hoffm.) Besser - G., Euras. *Abieti-Piceion*; *Platanthera bifolia* (L.) Rich. - G., Euras. *Symphyto-Fagion*; Juncáceae: *Júncus articulátus* L. em. Richt. - H., Circ. *Agropyro-Rumicion*; *J. bufonius* L. - T., Cosm. Car. *Isoëto-Nanojuncetea*; *J. conglomerátus* L. em. Leers - H., Circ. Car. *Molinietalia caeruleae*; *J. effúsus* L. - H., Cosm. Car. *Molinietalia caeruleae*; *J. inflexus* L. - H., Euras. Car. *Agropyro-Rumicion*; *J. trifidus* L. - H., Circ. Arct. Alp. *Juncetea trifidi*; *Lúzula campéstris* (L.) DC. - H., Circ. *Arrhenatheretalia*; *L. luzuloídes* (Lam.) Dandy & Wilmott - H., Centr. Eur. *Symphyto-Fagion*; *L. sylvática* (Huds.) Gaudin - H., Atl.-Medit.-Centr. Eur. *Piceetalia excelsae*; Cyperáceae: *Cárex digitáta* L. - H., Euras. Car. *Fagetalia sylvaticae*; *C. dístans* L. - H., Euras. (Subatl.-Submedit.). *Molinio-Arhenatheretea*; *C. ovális* Gooden. - H., Circ. *Nardetalia strictae*; *C. remota* L. - H., Circ. Car. *Caricion remotae*, *Alno-Ulmion*; *C. sempérvirens* Vill. - H., Alp. Eur. *Seslerietalia*; *C. sylvática* Huds. - H.(HH.), Circ. Car. *Fagetalia sylvaticae*; *C. vulpína* L. - H., Euras. *Agrostion stoloniferae*; *Eriophorum latifolium* Hoppe - H., Circ. *Caricetalia davallianae*; Poáceae (Gramineae): *Agrostis capilláris* L. - H.(G.), Circ. *Molinio-Arhenatheretea*; *A. rupéstris* All. - H., Alp. Eur. *Potentillo-Nardion*; *Anthoxánthum odorátum* L. - H., Euras. *Molinio-Arhenatheretea*; *Avémula versicolor* (Vill.) M. Lainz -

H., Alp.-Eur. *Potentillo-Nardion*; *Calamagrostis arundinacea* (L.) Roth - H., Euras. Cont. *Epilobion angustifolii*; *Cynosurus cristatus* L. - H., Eur. Car. *Cynosurion cristati*; *Dactylois glomerata* L. - H., Euras. Car. *Molinio-Arrhenatheretea*; *Deschampsia caespitosa* (L.) P. Beauv. - H., Cosm. Car. *Molinietalia caeruleae*; *D. flexuosa* (L.) Trin. - H., Circ. *Piceetalia excelsae*, *Junipero-Bruckenthalion*; *Elymus caninus* (L.) L. - H., Circ. *Alno-Ulmion*; *Festuca gigantea* (L.) Vill. - H., Euras. *Fagetalia sylvaticae*, car. *Alno-Ulmion*; *F. rubra* L. - H., Circ. *Molinio-Arrhenatheretea*; *F. rupicola* Heuff. subsp. *saxatilis* (Schur) Rauschert - H., Carp. Rom. *Festucetalia valesiacae*; *Koeleria macrantha* (Ledeb.) J.A. & J.H. Schult. subsp. *transsilvanica* (Schur) A. Nyár. H., Carp. Rom. *Seslerion bielzii* (Popescu et al. 2003); *Melica ciliata* L. - H., Centr. Eur.-Medit. *Thlaspietalia rotundifolii*, *Seslerio-Festucion pallentis*; *Nardus stricta* L. - H., Euras. (Circ.). *Nardetalia strictae*; *Phleum alpinum* L. - H., Circ. Alp. *Nardetalia strictae*; *Poa annua* L. - T.-H., Cosm. *Polygonion avicularis*; *Sesleria rigida* Heuff. - H., Carp. Balc. *Seslerietalia*.

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### CONCLUSIONS

From the analysis of the determined floristic species one can draw several conclusions:

- the presence of large numbers of phanerophytes and chamephytes (both in number of species and as individuals) demonstrates a good environment for forests and thickets in this site.
- 1st place occupied by hemicriptophytes is justified by the presence of these species in all habitats in the investigated area (grasslands, forests, rocks, wet places).
- systematic units identified by us on this site show the existence of similarities between it and the chain -Vânturarița - Buila limestone.

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**STUDY OF HYGROPHILOUS VEGETATION FROM TÂRNOVU MARE-LATORIȚA, VÂLCEA - ROMANIA**

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*Keywords: habitat, hygrophilous, Târnovu Mare-Latorița, Romania*

**ABSTRACT**

*Based on a detailed floristic study in this site a study of vegetation was conducted too. Identifying the places along the river Latorița hygrophilic taxon glacial *Ligularia sibirica*, we considered useful to undertake a detailed study of this type of vegetation. Of the three habitat types listed in the standard Natura 2000 (6430 Communities from tall grass hygrophilous fringe of the plains, to mountain and alpine, 3220 Herbaceous vegetation on mountain rivers and 3240 Woody vegetation with *Salix eleagnos* along mountain rivers) we can say that the first two have the best representation. They are stationed along the river Latorița and its tributaries.*

**INTRODUCTION**

The natural vegetation of the investigated surface currently occupies considerable areas as human factor, which always shortened its spread in the past, through deforestation and grubbing and produced changes in floristic composition, structure and dynamics of woody and herbaceous associations by the irrational exploitation of forests and overgrazing of grasslands, has been minimized.

Târnovu Massif has developed limestone relief on cretaceous and paleogene conglomerate (Ploaie 2012) in the mountain massifs surrounding of metamorphic origin, which creates specific conditions for the development of a large number of habitats of forest, meadows, thickets, riparian vegetation, cliff vegetation and debris. The site includes Latorița forest nature reservation.

**MATERIALS AND METHODS**

The working method consisted of field trips at regular intervals to capture all the plants that grow in the phytocenosis and have a floristic inventory completeness.

Identification and description of the habitats were based on their characteristic phytocoenoses (Doniță et al. 2005). The name of the associations was made by the provisions of the Code of phytosociological nomenclature (Barkman et al. 1986).

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The data obtained were compared with those in the literature dealing with this type of vegetation (Beldie & Dihoru 1967, Borza 1934, Nýaradý 1958, Oroian 1998, Popescu 1974, Răduțoiu 2008, Sanda et al., 2001, Mihăilescu 2001, Sanda 2002).

## RESULTS AND DISCUSSIONS

Vegetation that characterizes the three habitats is strictly linked to water, either near rivulets, or in marshy places.

### **6430 Communities from tall grass hygrophilous fringe of the plains, to mountain and alpine**

This type of habitat meets the site ROSCI0239 “Târnovu Mare - Latorița” both vegetation along mountain valleys and on the basis of shady beech forest, spruce and mixed beech timber.

The key feature of these sites is the presence of very high humidity at the ground level. The latter is part of the skeletal or sandy categories.

As recognition of this species habitat we can mention their presence in site ROSCI0239 “Târnovu Mare - Latorița”: *Ligularia sibirica* (Fig. 1), *Lysimachia vulgaris*, *Stachys sylvatica*, *Adenostyles alliariae*, *Valeriana officinalis*, *Telekia speciosa*, *Petasites albus*, etc.



Figure 1. *Ligularia sibirica* from Târnovu Mare – Latorița (orig.)

### *Telekia speciosae - Petasitetum hybridi* Morariu 1967 (Table 1)

It appears on wet soils, shady, tree line at the edge of beech forest and in beech mixed with resin at the lower end of the site.

These plant groups develop the site after sliding occurring especially in winter, when avalanches that occur frequently in this site, weather phenomena that destroy forest vegetation in these places where soils have moderate humidity. Witness stand high crosses in memory of those surprised by the avalanche. This summer we can say that due to heavy rainfall there occurred powerful avalanche which seriously altered the composition of phytocenosis of the site.

Species recognition printed in a specific area characteristic to Romanian Carpathians has considerable coverage, thanks to the large leaf system of the two taxa giving the name of the association.

In the structure of the class association stands *Epilobietea* species such as: *Fragaria vesca*, *Rubus idaeus*, *Digitalis grandiflora*, *Galeopsis speciosa*, *Gnaphalium sylvaticum*.

Table 1

*Telekia speciosae – Petasitetum hybridi* Morariu 1967

Releve number	1	2	3	4	5
Altitude (m)	800	850	900	950	1100
Exposure	N	N	N	N	N
Inclination (°)	10	15	10	10	10
Vegetation covering (%)	100	100	100	100	100
Releve area (m <sup>2</sup> )	100	100	100	100	100
Characteristic species of the association					
<i>Telekia speciosa</i>	1	1	1	+	4
<i>Petasites hybridus</i>	4	4	4	5	4
Petasition & Convolvuletalia					
<i>Carduus personata</i>	+	+	+	+	+
<i>Cirsium erisithales</i>	+	-	+	+	-
<i>Myosoton aquaticum</i>	+	-	+	-	+
<i>Calystegia sepium</i>	-	-	+	+	-
Galio-Urticetea					
<i>Salvia glutinosa</i>	+	+	+	+	-
<i>Urtica dioica</i>	+	+	+	-	+
<i>Chaerophyllum hirsutum</i>	-	+	+	-	+
<i>Chamerion angustifolium</i>	-	+	+	-	-
<i>Galeopsis speciosa</i>	-	+	-	-	-
<i>Aegopodium podagraria</i>	+	-	-	-	-
Epilobietea angustifolii					
<i>Digitalis grandiflora</i>	+	+	+	-	-
<i>Gnaphalium sylvaticum</i>	+	+	+	-	-
<i>Fragaria vesca</i>	-	+	-	+	-
<i>Rubus idaeus</i>	+	-	+	-	-
Adenostylion					
<i>Heracleum sphondylium</i>	+	-	+	+	-
<i>Thalictrum aquilegiifolium</i>	-	-	+	-	+
Variaesyntaxa					
<i>Lysimachia nummularia</i>	+	+	+	-	+
<i>Urtica dioica</i>	+	+	+	-	+
<i>Campanula patula</i>	+	-	+	-	+
<i>Cirsium arvense</i>	+	-	+	-	+

<i>Tussilago farfara</i>	+	-	+	+	-
<i>Erigeron annuus</i>	+	-	+	+	-
<i>Rhinanthus rumelicus</i>	+	-	+	+	-
<i>Aegopodium podagraria</i>	+	+	+	-	-
<i>Geranium robertianum</i>	+	+	-	+	-
<i>Lotus corniculatus</i>	+	+	+	-	-
<i>Rumex sanguineus</i>	-	-	+	-	+
<i>Ranunculus repens</i>	-	+	+	-	-
<i>Circaea lutetiana</i>	-	+	-	+	-
<i>Lysimachia vulgaris</i>	-	-	+	-	+
<i>Scrophularia nodosa</i>	-	-	+	+	-
<i>Leucanthemum vulgare</i>	-	-	+	-	+
<i>Campanula rapunculoides</i>	+	-	-	-	+
<i>Salvia verticillata</i>	-	+	+	-	-
<i>Clinopodium vulgare</i>	-	+	+	-	-
<i>Mentha longifolia</i>	+	+	-	-	-
<i>Ajuga reptans</i>	+	-	+	-	-
<i>Cirsium oleraceum</i>	-	+	+	-	-
<i>Ligularia sibirica</i>	-	-	-	+	-

Species present in one survey: 1. *Knautia arvensis*; 2. *Valeriana officinalis*; 4. *Alchemilla connivens*.

Place and date of performing the surveys: 1.3. On both sides of the river Latorița (18.03.2014, 27.04.2014, 24.06.2014); 2. Boroșeana stream banks (27.04.2014, 24.06.2014); 4. Brook Fratoșteanu (beech creek) (27.04.2014, 24.06.2014); 5. Brook Adâncu (27.04.2014, 24.06.2014).

### 3220 Herbaceous vegetation on mountain rivers

This habitat vegetation grows along cold streams site. The climax stage of these places is highly provided by oxygenated waters where oxygen comes from the atmosphere.

Due to the low amplitude of water temperature variation of fontina groups that belong to this habitat have a high coenotic homeostasis.

The floristic compositions of this phytocenosis are few vascular species, but their small number is offset by muscinal layer well developed.

*Cardamino amarae - Chrysosplenietum alternifolii* Maas 1959 (Table 2)

The places where the two species that give the name of the association (*Chrysosplenium alternifolium* and *Cardamine amara*) form compact phytocenosis are common around springs located in *Larix* forests, spruce stands of beech and resinous mixture of the protected area.

Along with increasing altitude, appear elements from *Adenostyletalia alliariae*. They are mezohygrophilous and even hygrophilous.

Place and date of the reports: 1, 2, 3 Next streams slope near the river Latorița (18.03.2014, 27.04.2014, 24.06.2014); 4, 5. Brook Boroșeana in the beech forests of mixed conifers (27.04.2014, 24.06.2014); 6. Brook Martorul lui Duminica (27.04.2014, 24.06.2014).

Table 2

*Cardamino amarae – Chrysosplenietum alternifolii* Maas 1959

Releve number	1	2	3	4	5	6
Altitude (m)	750	900	700	1100	850	700
Exposure	NE	E	NE	N	E	NV
Inclination (°)	10	10	15	10	5	10
Vegetation covering (%)	80	80	85	80	85	80
Releve area (m <sup>2</sup> )	4	4	4	4	4	4
Characteristic species of the association						
<i>Cardamine amara</i>	+	+	+	+	+	+
<i>Chrysosplenium alternifolium</i>	4	4	5	4	5	4
Caricion remotae						
<i>Philonotis fontana</i>	+	1	+	1	+	+
<i>Carex remota</i>	-	+	-	+	-	-
Montio-Cardaminetalia						
<i>Caltha palustris</i>	+	+	+	+	-	+
<i>Saxifraga stellaris</i> subsp. <i>robusta</i>	+	+	-	-	+	-
Adenostyletalia alliariae						
<i>Doronicum austriacum</i>	+	+	+	+	-	+
<i>Chaerophyllum hirsutum</i>	+	+	+	-	+	+
<i>Stellaria nemorum</i>	+	-	+	+	+	-
Variaesyntaxa						
<i>Impatiens noli-tangere</i>	-	+	-	-	+	+
<i>Ranunculus repens</i>	+	-	-	+	+	-
<i>Myosotis scorpioides</i>	+	-	+	-	-	+
<i>Juncus effusus</i>	-	-	+	-	+	-
<i>Poa trivialis</i>	-	-	+	-	-	-

**3240 Woody vegetation with *Salix eleagnos* along mountain rivers**

Unlike central and northern Europe where the habitat is stuck to the superior floor of fir-tree, in river valleys, in the investigated area there was identified in the forests of spruce and mixed beech tree on the river Latorița and very little on brook Borgeana.

Floods and flash floods this year have sometimes led to the disappearance of these thickets of willow, which means that we can say that at the moment this habitat area is extremely small.

**ACKNOWLEDGMENT**

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**CONCLUSIONS**

In the site Tarnovu Mare Latorița, best represented is the vegetation within association *Telekio speciosae - Petasitetum hybridae* 1967 Morariu. Human influence on these sites is minimal.

The factors that contribute to the floristic hydrophilic modification composition of vegetation of this site are consequences of floods during the year. Sometimes they have catastrophic consequences.

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**COMPATIBILITY BETWEEN BIOLOGICAL AND CHEMICAL  
PLANT PROTECTION PRODUCTS FOR SUPPRESSING  
BACTERIAL ROT OF POTATO TUBERS**

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*Keywords: phytosanitary product compatibility*

**ABSTRACT**

*Bacterial soft rot of potato tubers caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) is an important pathogen that can produce great economic damages. Considering consumer interest for organic food, we evaluated some biocontrol microbial strains that can reduce Pcc growth. Three biocontrol bacterial strains of *Bacillus* spp. were in vitro evaluated as antagonistic against this phytopathogen. Taking into consideration that copper excessive application can lead to pollution with this mineral, we figured an organic strategy to reduce Pcc attack by combing biocontrol bacteria and low doses of copper based products. In order to achieve this, we evaluated in vitro compatibility between the biocontrol strains, found to be antagonistic to Pcc, with two commercial pesticides based on copper hydroxide. Results revealed that the tested biocontrol strains were compatible with the copper based products at the concentration recommended for use to protect potato crop.*

**INTRODUCTION**

Over the last three decades, there is an increased interest into reducing chemical pesticide use for food and feed production. Public concern regarding human health and environmental protection determined the agricultural research to provide different strategy to reduce pesticide use in agriculture. Several studies described the potential use of combined chemical and biological treatments for plant protection (Senthilvel et al. 2004, Dean et al. 2006, Anand et al. 2007, Devi & Prakasam 2013).

Considering the importance of potato (*Solanum tuberosum*) in Romania (Chiru et al. 2009), we focused our attention on *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*), which is a phytopathogenic bacteria that infects potato tubers. This pathogen is causing bacterial soft rot of potato tubers, producing important economic damages. Several agro-technical, physical, chemical and biological methods are reviewed as control strategies (Czajkowski et al. 2011). However, there are no efficient bactericides against this pathogen or any other bacterial species causing soft rot (James et al. 2009, Baz et al. 2012).

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The present study aims to analyze the *in vitro* effect of some previously selected biocontrol bacterial strains against *P. carotovorum* subsp. *carotovorum* (abbreviated Pcc) and their compatibility with two copper based commercial pesticides.

## MATERIAL AND METHODS

### Pcc isolation and characterisation

*Pectobacterium carotovorum* subsp. *carotovorum* strain C1.7 was isolated from an infected potato tuber of Verdi cultivar (figure 1), using Noble & Graham selective medium based on salicin-sodium taurocholate-bromthymol blue (Noble & Graham 1956). The pathogenicity of the strain was revealed on potato slices and confirmed Koch's postulate. Bacterial identification was carried out using the Biolog GEN III microbial identification system (Biolog Inc., USA).



Figure 1. Potato tuber with bacterial soft rot.

### Biocontrol bacterial strains

Three bacterial strains were used in this study, *Bacillus pumilus* OS15 and *Bacillus amyloliquefaciens* OS17 and BW. These strains are mentioned as good biocontrol bacteria against different soil borne fungal pathogens (Constantinescu et al. 2010, Dinu et al. 2012, Siciua et al. 2012).

### Preparation of bacterial cultures

All bacterial strains were stored in Luria-Bertani medium containing 30% glycerol (1:1, v/v) and maintained by cryopreservation -80°C. Routinely, there were grown on Luria Bertani agar medium at 28°C. However, for the experimental trials, the bacterial strains were multiplied in LB broth, and incubated at 28°C and 150 rpm orbital shaking. The Pcc strain C1.7 was grown over night, while the biocontrol strains were grown for 48h in order to produce endospores and different metabolic compounds. All bacterial strains were adjusted and tested at the concentration 10<sup>8</sup> cfu/ml concentration.

### Antagonistic assay

The antagonistic activity of the *Bacillus* sp. strains OS15, OS17 and BW was performed *in vitro* against *Pectobacterium carotovorum* subsp. *carotovorum* C1.7. Petri plates with LB-agar were inoculated with 100µl Pcc suspension, uniformly spread on the medium surface with a Drigalski spatula. Subsequently, three spots of 10µl of *Bacillus* sp. inoculum at 10<sup>8</sup>cfu/ml were placed in every plate. Pcc control plates were similarly prepared, using sterile distilled water instead of bacteria inoculum. *Bacillus* sp. control



plates were also prepared by placing the bacteria inoculum directly on LB agar. All plates were incubated at 28°C, and the inhibition halo was measured, if the biocontrol bacteria of *Bacillus* sp. suppressed the growth of the pathogenic Pcc C1.7 strain.

### Commercial pesticides

Two commercial pesticides based on copper hydroxide were used in this study. First product, formulated as wettable powder (WP), contained 20% copper hydroxide as active compound. We tested this in five concentrations: 20%, 10%, 5%, 2.5% and 1%. The second pesticide, formulated as concentrated solution (CS) contained 488g/L copper hydroxide. This was tested at the initial concentration, and at the concentration recommended to be used for potato crop protection.

### Compatibility of biocontrol bacteria with copper based pesticides

The compatibility assay was performed *in vitro*, in Petri dishes with LB-agar. The plates were covered with 100µl bacterial inoculum of *Bacillus* sp. Then, three sterile cotton plugs of 6 mm in diameter were placed on the surface of each plate already inoculated with bacteria. On these cotton plugs we added 10µl pesticide at the concentration to be tested. Control plates were similarly prepared, using sterile distilled water instead of pesticide. All plates were incubated at 28°C for 72h and analysed in order to evaluate bacteria compatibility with the pesticide. Bacterial strain sensitivity to pesticides was appreciated as using the following index: 0 – when no bacterial growth was developed, index 1 – when the inhibition zone was greater than 5 mm, index 2 – when the inhibition zone was less than 5 mm, and index 3 – when no inhibition halo was present and the bacterial growth was not affected by the pesticide (Constantinescu et al. 2014). Each experimental variant had three replicates, and the experiment was performed three times.

## RESULTS AND DISCUSSIONS

### Characterisation of Pcc C1.7 strain

*Pectobacterium carotovorum* subsp. *carotovorum* C1.7 strain was isolated from an infected potato tuber having clear bacterial soft rot symptoms (figure 1). The physiological profile of the strain was revealed using the Biolog GEN III system. According to the identification system this bacteria is able to consume several sugars such as D-trehalose, D-cellobiose, gentiobiose, sucrose, D-raffinose, stachyose $\alpha$ -D-lactose, D-melibiose,  $\beta$ -methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine,  $\alpha$ -D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, D-mannitol, and grew in 1-4% NaCl (figure 2).

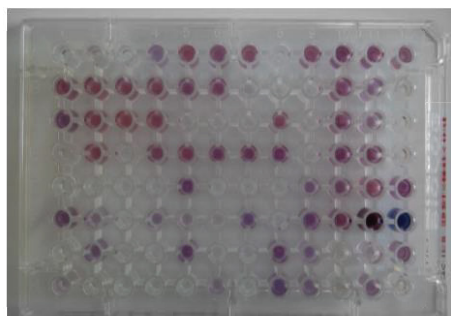


Figure 2. The physiological profile of *Pectobacterium carotovorum* subsp. *carotovorum* C1.7 strain on Biolog GEN III MicroPlate after 22h of incubation at 33°C.

The pathogenic characteristic of Pcc C1.7 strain was revealed at 24–48h after inoculation on potato tuber slices (figure 3). This bacterial strain was able to develop soft rot on 75.96% of the surface of the inoculated potato slices, as we determined using the Assess 2.0 Image Analysis Software for plant disease quantification.

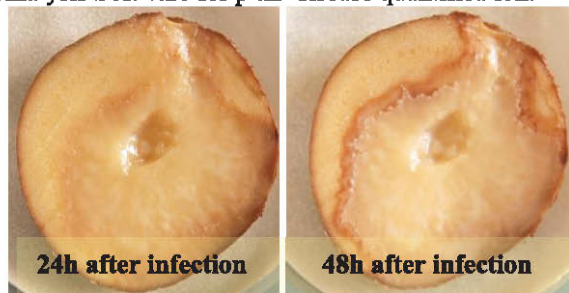


Figure 3. Bacterial soft rot induced on potato slices at different infection times

#### Antagonistic activity against Pcc

Pcc growth inhibition was visually appreciated according to Manka and Manka method (1992), as follows: ++++ = very strong inhibition of the pathogenic growth; +++ = strong inhibition; ++ = moderate inhibition; + = slight inhibition; – = no inhibition of the pathogenic growth. Pcc growth was slightly inhibited in the presence of *Bacillus* sp. biocontrol strains (table 1).

Table 1  
Antagonistic activity against Pcc (after 3 days of incubation at 28°C)

Biocontrol strain	Colony diameter of <i>Bacillus</i> sp. strains	Clear inhibition zone	Pcc growth inhibition (Manka&Manka)
<i>Bacillus pumilus</i> OS15	1.0 mm	0.8 mm	+
<i>B. amyloliquefaciens</i> OS17	1.3 mm	0.5 mm	+
<i>B. amyloliquefaciens</i> BW	1.3 mm	0.5 mm	+
Sterile distilled water	–	0 mm	–

The inhibition area where Pcc was not able to grow was similarly big at any biocontrol treatment. However, the clear inhibition zone of Pcc were smaller when *Bacillus amyloliquefaciens* BW and OS17 strains were used, comparing with clear zone induced by *B.pumilus* OS15 (figure 4). When colony diameter was measured, it was noticed that *B.amyloliquefaciens* BW and OS17 strains had a larger diameter of 1.6 times than *B.pumilus* OS15 strain, because BW and OS17 strains have a better colonization capacity than OS15 strain.

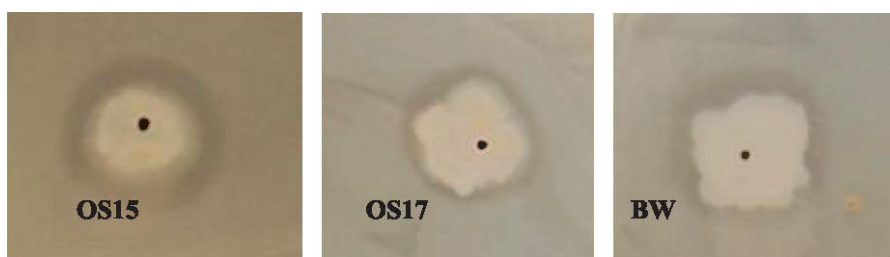


Figure 4. Inhibitory activity of *Bacillus* sp. strains against *Pectobacterium carotovorum*

### ***Bacillus* sp. compatibility with copper based pesticides**

The compatibility assay revealed that the biocontrol *Bacillus* sp. strains can not survive at undiluted or high concentrations of copper based pesticides (table 2).

Table 2.

Compatibility of biocontrol bacteria with copper based pesticides

Pesticide active substance	Copper hydroxide 488g/L		Copper hydroxide 20%				
	30%	0.25 %	20%	10%	5%	2.5%	1%
Biocontrol bacteria	Compatibility index						
<i>Bacillus pumilus</i> OS15	1	3	1	1	2	2	3
<i>B. amyloliquefaciens</i> OS17	2	3	1	1	2	2	3
<i>B. amyloliquefaciens</i> BW	1	3	1	2	2	2	3

where: index 1 = inhibition zone  $\geq$  5 mm; index 2 = inhibition zone < 5 mm; index 3 = no inhibition zone.

When we analyzed the compatibility of our *Bacillus* sp. biocontrol strains with the pesticide having copper hydroxide 488g/L we noticed that at 30% concentration in metallic copper all bacteria strains suffered growth inhibition. However, at the recommended dose for application in potato crop, the pesticide did not affect the growth of any biocontrol bacteria tested (figure 5).

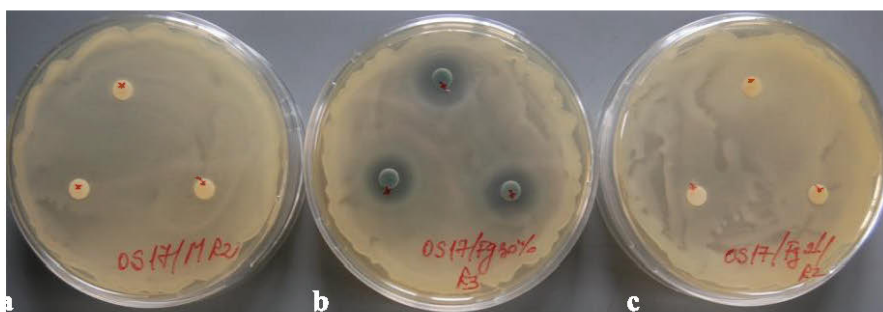


Figure 5. Bacterial growth in the presence of copper hydroxide 488g/L. a- *Bacillus amyloliquefaciens* OS17 control plate, b- Growth inhibition of *B.a.* OS17 in the presence of 30% copper based pesticide, c- no inhibition of the biocontrol *B.a.* OS17 at the recommended dose of copper based pesticide

The other copper based pesticide formulated as wettable powder was tested in five different concentrations: 20%, 10%, 5%, 2.5% and 1%. The first two concentrations, 20% and 10% respectively, generated an inhibition zone bigger than 5mm length against all tested biocontrol strains. At the concentrations of 5% and 2.5 % the inhibition zone decreased at less than 5mm in length. The only concentration that did not affect the growth of our biocontrol strains was 1% pesticide concentration.

### **CONCLUSIONS**

The biocontrol strains *Bacillus pumilus* OS15, *Bacillus amyloliquefaciens* OS17 and BW showed a reduced antagonistic activity against the highly phytopathogenic strain *Pectobacterium carotovorum* subsp. *carotovorum* C1.7.

Biocontrol *Bacillus* sp. strains analysed were compatible with copper based pesticide at 1% concentration of copper hydroxide or at the recommended dose for potato crop protection (0.25%).

This study suggest that combining copper based pesticides with the microbiological means to reduce the phytopathogenic attacks could offer a better protection than the microbial means alone, and could decrease the amounts of copper applied in conventional treatments.

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## MOLECULAR FINGERPRINTING OF A ROMANIAN *BACILLUS* SPP. POPULATION USEFUL IN BIOCONTROL

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**Keywords:** *biocontrol bacteria, RAPD, molecular fingerprinting*

### ABSTRACT

*The main approach for biocontrol bacteria impact evaluation on the environment, after their application in agriculture, assumes molecular fingerprinting of the bacterial strains used as agro-inoculants. In these regards, the aim of the present study was to achieve comparative analysis by randomly amplified polymorphic DNA (RAPD) – PCR. This technique allows the identification of strains genetic diversity. Therefore, we subjected to our study ten oligonucleotide primers and eight biocontrol bacterial strains, formerly identified as B. amyloliquefaciens, B. subtilis, and B. pumilus, with the BIOLOG Microbial Identification System using GEN III technique. Results showed molecular variability within the analyzed bacterial populations. Using the Treecon software we were able to generate a molecular dendrogram for the analyzed biocontrol strains of Bacillus spp.*

### INTRODUCTION

The biocontrol bacteria confer great attributes in plant health by reducing pests and diseases attack. Additionally, they trigger great environmental benefits by reducing chemical pesticide needs for plant protection, decreasing the rate of potential residues and, in some cases, promoting plant growth.

*Bacillus subtilis* is well-studied bacterial species ubiquitously spread in nature (Pignatelli et al. 2009) and widely exploited for biotechnological and industrial applications (Rooney et al. 2009). *Bacillus subtilis* strains and their closely related species are frequently mentioned for the biological control of plant pathogens. Their advantages include the capacity to secrete broad spectrum active metabolites (Awais et al. 2010, Sicuiu 2012); rapidly adapt in various environmental conditions (van Dijl 2012) and form highly resistant endospores, easy to be formulated (Schisler et al. 2004). Moreover, they can compete with other microbes that could adversely affect the plants; they can activate host defense system in order for plants to resist at potential pathogenic attacks, can stimulate plant growth, and increase the availability of some mineral nutrients (Nagorska et al. 2007).

Along with *Bacillus subtilis* there have been described several closely related species with high degree of biochemical and genetic similarities, named as members of 'Bacillus subtilis-spectrum' (Gordon 1973), 'B. subtilis species complex' (Rooney et al.

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2009) or ‘*Bacillus subtilis* - group’ (Jeyaram et al. 2011). These species include *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus axarquiensis*, *B. licheniformis*, *B. malacitensis*, *Bacillus mojaviensis*, *Bacillus pumilus*, *Bacillus sonorensis*, *Bacillus tequilensis*, *Bacillus vallismortis* and *Bacillus velezensis* (Ash et al. 1991, Gatson et al. 2006, Wang et al. 2007).

Studying the genetic diversity within microbial populations became a global practice in determining genetic variation between biocontrol microbial agents (El-Hamshary et al. 2012). To investigate the similarities and diversity within the *B. subtilis* species complex different approaches were used over the time, the most accurate being the molecular techniques. The information generating through these analysis methods confer the possibility to develop species and strains specific markers. The microbial diversity of environmental samples could be evaluated by various molecular methods, like Temperature Gradient Gel Electrophoresis (TGGE), and Denaturing Gradient Gel Electrophoresis (DGGE), Single-Strand Conformation Polymorphism (SSCP), Thermal Restriction Fragment Length Poly-morphism (T-RFLP), Length heterogeneity PCR (LH-PCR), Automated Ribosomal Intergenic Spacer Analysis (ARISA) techniques (Rastogi & Sani 2011). Different other analysis methods of DNA polymorphism are the Random Amplified Polymorphic DNA (RAPD), also known as Arbitrary Primed PCR (AR-PCR), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Amplified Ribosomal DNA Restriction Analysis (ARDRA), Repetitive Sequence-Based PCRs (rep-PCRs), and the analysis of short DNA sequences of no more than six nucleotides called Microsatellites (Medlin & Töbe 2011, Gowdaman et al. 2014).

The purpose of this study was to investigate the molecular diversity within a Romanian *Bacillus* sp. biocontrol population, in order to identify molecular markers for strain specific differentiation within the soil microbiota, after its application as agroinoculants for plant protection.

## MATERIAL AND METHODS

### **Bacterial biocontrol strains**

In this study we analyzed eight bacterial strains (Us.a2, OS15, OS17, BW, Icpc, 83.2s) isolated from plant rhizosphere and agricultural soil from the South and South-East of Romania. These strains were previously selected for their beneficial characteristics in plant protection (Constantinescu et al. 2010, Siciua 2012). Other two strains, *Bacillus subtilis* ATCC6633 and *B. subtilis* ATCC11774 were used as reference for the molecular fingerprinting.

### **Bacterial identifying procedure**

The bacterial strains were identified using the Biolog Microbial ID Semi-Automated System, based on the GEN III technique, according to the manufacturer protocol B, for spore-forming Gram-positive bacilli. This technology is based on the redox chemistry and reveals the strain ability to metabolize major classes of biochemicals and other physiological properties such as pH, salt and lactic acid tolerance, and chemical sensitivity. The biochemical profile of each bacterial strain was analyzed with the MicroLog™ 3 Software (version 5.2.2.) and the phenotypic fingerprint obtained was compared with the GEN III Database (version 2.6.1.) that generated the identification.

### **Bacterial genomic DNA extraction**

For DNA extraction fresh bacterial cultures were used and the nucleic acids were purified with a Chelex® resin technique. For this reason all tested bacterial strains were grown for 18h in aerated Luria Bertani broth medium, at 150 rpm orbital shaking and 28°C. One hundred microliters of bacterial culture were transferred in thin-wall tubes and

centrifuged at 13.000 rpm for 1min. The harvested bacterial biomass was subjected to DNA extraction using Chelex®100 resin (Bio-Rad Laboratories, Hercules, CA). We added 100µl of 20% Chelex®100 suspension (pH 9-11) over the bacterial sediment and vortex vigorously. Tubes were than centrifuged at 16.000×g for 20sec. at 4°C, and incubated for 30 min. at 56°C into a thermal-cycler machine. The previous vortex and centrifugation steps were repeated and the samples were than incubated for 10 min. at 98°C. The last centrifugation step was 16.000×g for 3 min. at 4°C. The DNA solution was aspirated and transferred in new tubes. For PCR the DNA samples were diluted 1:10 in TE buffer.

#### **RAPD-PCR**

RAPD analysis was performed with 10 primers with arbitrary nucleotide sequence (table 1). The RAPD-PCR was made in 25 µl reaction volume containing 0.2mM dNTPs; 2.5mM MgCl<sub>2</sub>; 1µM primer; 1U *Taq* polymerase (Promega), 1X *Taq* reaction buffer and 40ng ADN template. PCR conditions included an initial DNA denaturation step of 3 min. at 94°C, 36 cycles of 94°C for 1 min., 36°C for 1 min. and 72°C for 2 min., and a final extension at 72°C for 10 min.

Table 1

Randomly Amplified Polymorphic DNA primers

No.	Primer name	Primer sequence (5'-3')
1	OPA-11	CAATCGCCGT
2	OPB 12	CCTTGACGCA
3	OPB-17	AGGGAACGAG
4	OPE-02	GGTGCGGGAA
5	OPE-03	CCAGATGCAC
6	OPE-10	CACCAGGTGA
7	OPE-20	AACGGTGACC
8	OPM-07	CCGTGACTCA
9	OPM-11	GTCCACTGTG
10	OPT-14	TCACCTCCTG

The PCR products were separated in 1.5% agarose gel and visualized by staining with ethidium bromide. The electrophoretic patterns were revealed in UV light with an UVP transilluminator, and their images were captured with the BioDoc-It™ Imaging System (from UVP). To determine the genetic variability of the *Bacillus* spp. biocontrol strains, the electrophoretic profiles obtained with the RAPD analysis were manually rendered as a binary matrix. The raw data were analysed with the Treecon software (version 1.3b for Windows) using the UPGMA method (Unweighted Pair-Group Method using arithmetic Averages) based on Nei-Li coefficient and maximum Bootstrap values conferred by the program.

## **RESULTS AND DISCUSSIONS**

### **Bacterial identification**

The identification results obtained with the Biolog GEN III technique showed that the bacterial strains Us.a2 and Ipc belong to *Bacillus subtilis* species, OS17 and BW belong to *Bacillus amyloliquefaciens* and OS15 and 83.2s belong to *Bacillus pumilus*. Considering this identification procedure, all the biocontrol bacterial strains analyzed belong to *Bacillus subtilis* group (Jeyaram et al. 2011) also known as 'B. subtilis species complex' (Rooney et al. 2009).

**Molecular fingerprinting of the selected *Bacillus* spp. biocontrol stains**

The RAPD technique was chosen to assess the genetic variability among the eight biocontrol strains of *Bacillus* sp. using oligonucleotide primers. Five different operons, OPA, OPB, OPE, OPM and OPT, were chosen for their ability to generate informative patterns rich of polymorphic bands. The RAPD profiles of the eight strains were compared, and differences between the strains were observed with each primer. After analyzing the RAPD profiles obtained with all ten selected primers, the amplified DNA fragments were scored and 201 polymorphic bands were obtained (table 2), representing 51% of the total amplified bands. The RAPD markers obtained had a length of 100 to 1500bp.

Table 2

The number of DNA polymorphic bands in the selected *Bacillus* spp. biocontrol strains amplified with RAPD primers.

Primer name	OPA-11	OPB-12	OPB-17	OPE-02	OPE-03	OPE-10	OPE-20	OPM-07	OPM-11	OPT-14
Bacteria strain	Number of fragments scored of polymorphic loci									
ATCC6633	5	6	4	12	4	4	7	4	6	3
ATCC11774	4	4	5	8	0	6	0	3	8	4
Us.a2	5	3	3	9	6	6	5	4	4	4
Icpc	5	2	6	9	4	4	9	4	8	4
BW	7	4	8	7	9	7	5	4	4	5
OS17	4	4	6	5	6	7	8	4	3	1
OS15	5	5	2	5	2	4	6	4	3	5
83.2s	5	5	6	4	5	3	4	4	2	4
Total polymorphic bands	25	20	21	27	13	20	24	16	21	14
	201									

When analyzing the variability within the binary matrixes generated by each tested primer (as single) and the total, we noticed that sometimes one primer could be sufficient to score a genetic distance among the strains. According to our results, the primers that generated the highest number of polymorphic bands were OPE-02, OPA-11 and OPE-20, best results being obtained with OPE-02 primer (27 polymorphic amplicons).

The genetic variability of the *Bacillus* spp biocontrol strains was rendered as a binary matrix. The raw data were analysed with the Treecon software (version 1.3b for Windows) which generated a dendrogram using the UPGMA clustering analysis (Unweighted Pair-Group Method using arithmetic Averages) based on Nei-Li genetic distance estimation method. Reproducibility of the groupings below each node of the dendrogram was confirmed by the analysis of 1000 multiple data sets of bootstrapping. The obtained dendrogram complied with the taxonomic relationship between the identified species (figure 1).



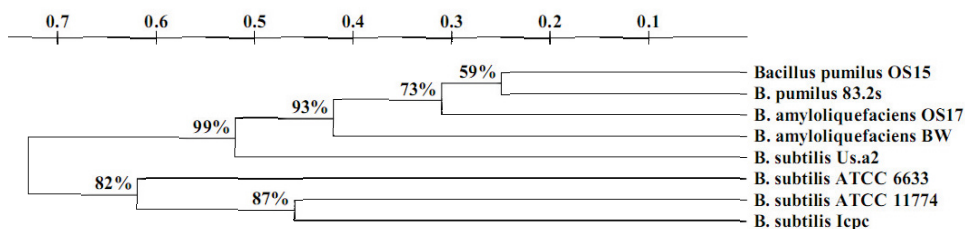


Figure 1. Dendrogram describing the genetic variability of eight biocontrol strains from *Bacillus subtilis* group, based on Nei and Li genetic distance estimation method. The grouping was carried out by the unweighted pair-group method using arithmetic averages (UPGMA) using the Treecon software on the RAPD data obtained with 10 oligonucleotide primers: OPA11, OPB12, OPB17, OPE02, OPE03, OPE10, OPE20, OPM07, OPM11, OPT14. The percentages mentioned at nodes represent the proportion of 1000 bootstrap samples in which a particular clade was found.

According to the dendrogram, the analyzed strains could be enlisted in two groups, one group including *Bacillus pumilus* OS15 and 83.2s strains, *Bacillus amyloliquefaciens* OS17 and BW strains and *B. subtilis* Us.a2 strain, and the other group with *Bacillus subtilis* ATCC6633, ATCC 11774 and Icp strains, suggesting increased intragroup diversity. Moreover, clear intraspecific differences for *B.subtilis* strains were detected.

### CONCLUSIONS

Genetic diversity among the biocontrol strains belonging to *Bacillus subtilis* species complex was assessed by RAPD technique. The eight primers used were able to generate a high number of polymorphic bands that allowed the construction of a dendrogram with two distinct groups.

When analyzing the variability within the binary matrixes generated by each tested primer (as single) and compared with the total, we noticed that the primers OPE-02, OPA-11 and OPE-20 tested alone, could be sufficient to score a genetic distance among the strains. Among the tested primers, the highest number of DNA fragments was obtained by OPE-02 primer.

A precise identification and characterization of the biocontrol strains could lead to the possibility of analyzing the survival and chance to spread after their release in the environment, with a great value in understanding the behavior of biocontrol agents in nature. In these regard, our research is in progress to develop strain-specific markers for a biocontrol strains of *Bacillus subtilis* species complex analyzed in these experiments.

### ACKNOWLEDGMENT

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**BENEFICIAL SPECIES FROM THE APPLE ORCHARDS  
„BANU MARACINE”**

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*Keywords: beneficial species, predators species*

**ABSTRACT**

*From our observation regarding the beneficial species from the „Banu Maracine” apple orchards, we have identified a number of 41 beneficial species. Most of the insect species identified belonged to the Coleoptera order (7 species), followed by Diptera order (6 species), Hymenoptera order (4 species), Neteroptera (2 species).*

*Vertebrates beneficial species from Aves class were represented by 12 species and those from the Mamalia class by 3 species. Amphibia class was represented by 1 specie and Reptilia class by 3 species.*

**INTRODUCTION**

The apple orchard from Banu Maracine hosts a serie of harmful animal species (invertebrate –insects and vertebrate – birds and rodents), for controlling these species annually there are applied a series of agrotechnical and chemical methods. These measures affect the whole trophic chain, which include as well the parasites and predators that feed on the harmful species mentioned before.

Classical biological control (natural enemy introductions) has long served as a paradigm for the role of predators and parasitoids in insect herbivore population dynamics, and it is widely held that there is no fundamental difference between successful biological control and the action of native natural enemies 'natural control' (Hawkins 1999).

There is an adjustment of harmful populations, natural control, where internal factors of these populations intervene (endogenous factors) as well external factors (exogenous).

The endogenous factors are represented by the population polymorphism, which imply the differentiation of individuals from these populations, on special categories phenotypically and genotypically differentiated by the interrelationships between individuals of different morpheme and the functions performed by these morphemes.

Populations exogenous factors are as for other organisms, represented by abiotic factors: physical, chemical, mechanical and biotic ones: predators, parasites, pathogens, also called "natural enemies", antagonists, as well food and competition (Toncea 2011)

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In this paper we have propose to inventory the beneficial species present in „Banu Maracine” apple orchards ecosystem.

### MATERIALS AND METHODS

Experiments were conducted during May 2013 - July 2014 in „Banu Maracine” apple orchards ecosystem.

To determine the structure of the beneficial insects species were made collection of material using various means and methods: directly by hand from plants or soil, frame metric, soil surveys and soil surface collected with entomological net, collection with sticky traps for flying insects, visual inspection and binocular for Aves class.

For as little impact on the ecosystem we have preferred to capture images with the camera than to capture live specimens were subsequently removed from their natural environment.

After collecting, the biological material was analyzed and determined with the binocular magnifier glass using the Identification Manual (Panin 1951, Chatened du Gaetan 1990, Chinery 1998, Godeanu 2002, Zoltán 2010, Bruun 2011).

### RESULTS AND DISCUSSIONS

There has been identified a number of 41 beneficial species (table no. 1), belonging to 6 classes: ARACHNIDA, INSECTA, AVES, MAMALIA, AMPHIBIA and REPTILIA.

Table 1

Beneficial species identified during research

Crt. no.	Class/Order	Species
1	ARACHNIDA/ACARI	<i>Typhlodromus spp.</i>
2		<i>Amblyseius spp.</i>
1	INSECTA/ORTHOPTERA	<i>Mantis religiosa L.</i>
1	INSECTA/NEUROPTERA	<i>Crisopa carnea Ste.</i>
2		<i>Crisopa perla Steph.</i>
1	INSECTA/HYMENOPTERA	<i>Scolia flavifrons Fabr.</i>
2		<i>Vespa crabro L.</i>
3		<i>Vespa germanica L.</i>
4		<i>Vespa vulgaris L.</i>
1	INSECTA/COLEOPTERA	<i>Adalia decempunctata L.</i>
2		<i>Adalia bipunctata L.</i>
3		<i>Coccinella 7 punctata L</i>
4		<i>Carabus cancelatus L.</i>
5		<i>Carabus ulrichi L.</i>
6		<i>Carabus violaceus L.</i>
7		<i>Calosoma sycophanta L.</i>
1	INSECTA/DIPTERA	<i>Syrphus ribesii L.</i>
2		<i>Syrphus torvus L.</i>
3		<i>Episyrphus balteatus De Geer</i>
4		<i>Metasyrphus corollae F.</i>
5		<i>Scaeva albmaculata Macq.</i>
6		<i>Scaeva pyrastris L.</i>

Crt. no.	Class/Order	Species
1	AVES/	<i>Parus minor</i>
2		<i>Parus caeruleus</i>
3		<i>Parus major</i>
4		<i>Picus viridis</i>
5		<i>Picus picus</i>
6		<i>Athene noctua</i>
7		<i>Upupa epops</i>
8		<i>Cuculus canorus</i>
9		<i>Hirundo rustica</i>
10		<i>Falco tinnunculus</i>
11		<i>Buteo buteo</i>
12		<i>Phasianus colchicus</i>
1	MAMALIA/	<i>Myotis myotis</i>
2		<i>Talpa europaea</i>
3		<i>Ermacurus europaeus</i>
1	AMPHIBIA/	<i>Bufo bufo</i>
1	REPTILIA/	<i>Lacerta agilis</i>
2		<i>Lacerta viridis</i>
3		<i>Natrix natrix</i>

As it can be observed most beneficial insect species identified belonged to the Order Coleoptera (7 species) followed by the Diptera (6 species) and Hymenoptera (4 species).

Beneficial vertebrates from Aves class were represented by 12 species and those of Mammalia class by 3 species. Amphibia class was represented by 1 specie and Reptilia class by 3 species.

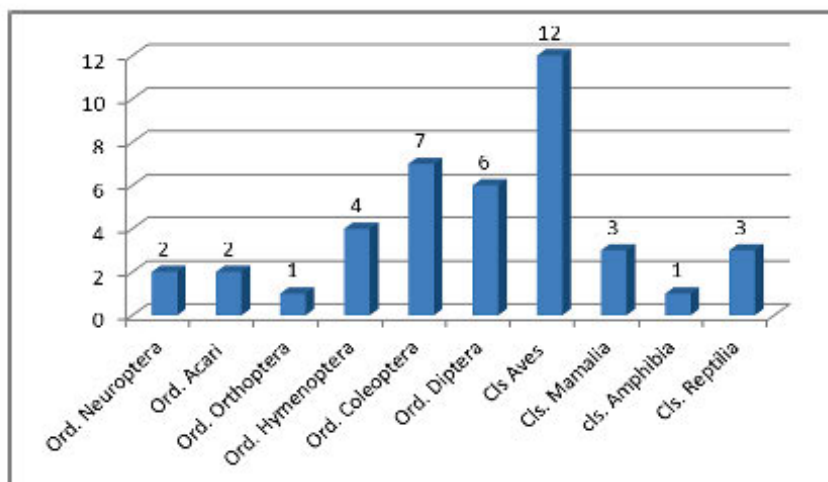


Figure 1. Structure of beneficial species identified during research



Figure 2. Beneficial species identified during research

We have to mention that some harmful species under certain conditions (larval growth, raising fledgling, etc.) can consume different harmful organism. Such is carwig - *Forficula auricularia*, *Agriotes spp.* adult stage which feeds on small insects and, of corvids and house sparrow that during the fledgeling feeding consumes harmful insects.

In anthropogenic agroecosystems human intervention through chemical methods to limit the pest attacks, resulting in serious disruption of beneficial organisms activity having a negative influence, due to direct exposure to pesticide action and indirect action due to the active substance ingested with the prey.

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## OBSERVATIONS ON THE SOME HARMFUL SPECIES OF APPLE ORCHARDS

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*Keywords:* pests, Tortricidae, moths

### ABSTRACT

*This paper presents the observations which were made in the period 2011-2013 in a apple orchard belonging SCD Fălticeni of varieties: Jonathan, Golden Delicious and Starkrimson.*

*We have used two variables: the "chemical treatment" lot and to "without chemical treatments" lot. It was watched the proportion of vegetative and flowering attack on buds in two variants in the 3 years of observations produced by species of carnation leaf-rollers larvae that attack fruit trees buds.*

### INTRODUCTION

A separate group of lepidopteran defoliators consists of some species of the Tortricidae family, whose caterpillars are building with silk threads, each feeding a shelter consisting of leaves or buds and shoots components (Diaconu 1997a, 1997b, Diaconu et al. 2004).

Tortricidae family is one of the largest families microlepidoptere with over 5,000 species recorded mainly in temperate and tropical regions, their number is much higher because the tropical equatorial region are poorly studied (Brown 1991).

The present paper presents the results of research on some species of leaf-rollers carnation that attacks the buds of apple orchards. The predominant species was *Adoxophyes* genus and especially *Adoxophyes orana* species.

### MATERIAL AND METHODS

The research was conducted in apple orchards of the Fruit Growing Research and Development Station Fălticeni in 2011-2013, the species of carnation leaf-rollers (Lepidoptera, Tortricidae) using the methods: determining the level of attack in the bud, nonselective collection biological material, identifying, analyzing and increasing actual laboratory carnation leaf-rollers species resulting from collections, identifying pests and pest related species, determining the level of attack on fruit determination of key harmful hibernating reserve (Tălmăciu et al. 2010). There were studied two groups of apple (chemical and unchemical treated).

The chemical lot is represented by the fruit tree plantation on the farm "Dumbrava Centre" established in 1990 with an area of 53 ha, the predominant varieties are Jonathan, Golden Delicious and Starkrimson to 90% and the remaining 10% as Ionared varieties, Florina, Rădășeni, Fălticeni to and untreated with an area of 0.5 ha is represented all the three varieties (Jonathan, Golden Delicious and Starkrimson).

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The resort also has a collection teaching Pomology very old varieties such as Costești, Domnești, Pătul, Renet, Astrahan, Agapia, London, Mutsu, Pătlașele ș.a. Vegetative buds attacked curve with the growth of their components consumed by the caterpillars and those colors often can not be opened with the ascension inflorescence take a form more or less spherical. Establishment of the attack was carried out buds of apple orchards in Fălticeni during the 3 years of research in the months April-May of flowering period.

The method consists in analyzing the vegetative and floral buds in the crown of trees being studied three varieties: Jonathan, Golden Delicious and Starkrimson with a time control of five trees for each variety. Every tree we randomly selected one branch on each side of him and I looked at each bud individually, thus establishing the percentage attacked buds and not attacked (Artenie et al. 2007).

The species most commonly encountered was *Adoxophyes orana*, so that data about how pest, the attack by the assortment of varieties etc. to refer this species.

### RESULTS AND DISCUSSIONS

The level of attack buds of apple orchards in the SCDP Fălticeni in the two groups (chemical and unchemical treated) was performed on the following dates, namely: in 2011 on 09.05 in 2012 on 08.05 and 12.05 in 2013. The chemical group of the S.C.D.P. Fălticeni in 2011 (Table 1) the bud attack was made on 09 May, hence the middle attack Jonathan and Golden Delicious varieties both flower buds and vegetative those limits between 10 and 13 % shoots attacked and weak registered variety Starkrimson a 4% attack buds and 5% in the vegetation.

Table .1  
Level attack buds in apple orchards of the SCDP Fălticeni in 2011 – chemical lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
09-05-2011	1	Jonathan	3+4	0+1	6+5	0+0
	2		9+7	0+2	2+4	1+1
	3		9+7	1+0	6+2	1+0
	4		6+2	0+1	3+7	0+1
	5		2+6	0+1	9+5	1+1
	Total		55	6	49	6
	%		90	10	89	11
	1	Golden delicious	0+1	1+0	10+9	1+1
	2		2+1	0+1	6+8	0+1
	3		1+2	1+0	7+3	0+2
	4		4+9	0+1	7+6	1+0
	5		3+4	0+0	3+6	1+0
	Total		27	4	65	7
	%		87	13	90	10
1	Starkrimson	3+1	0+0	0+8	0+1	
2		1+4	1+0	5+0	0+1	
3		1+0	0+0	5+8	0+0	
4		1+0	0+0	6+0	0+0	
5		4+10	0+0	2+2	0+0	
Total		25	1	36	2	
%		96	4	95	5	



In 2012, the attack was carried out on 08.05, (Table 2) limits ranging between 10% and 16% bud attacked thus registering all three varieties middle attack both flower buds and in the vegetative buds.

Table 2

Level attack buds in apple orchards of the SCDP Fälticeni 2012 - chemical lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
08-05-2012	1	Jonathan	0+3	0+1	9+4	0+0
	2		9+4	0+0	2+1	0+0
	3		5+3	2+0	10+6	3+3
	4		6+0	2+0	7+8	0+3
	5		5+0	0+1	10+0	2+0
	Total		35	6	57	11
	%		85	15	84	16
	1	Golden delicious	2+5	0+2	6+2	2+1
	2		0+6	0+1	15+2	3+0
	3		3+7	0+3	12+3	1+0
4	12+5		3+0	2+5	0+1	
5	0+8		0+0	10+12	1+3	
Total		48	9	69	12	
%		84	16	85	15	
1	Starkrimson	1+1	0+0	6+8	2+0	
2		6+3	1+0	5+5	0+0	
3		3+0	1+0	12+7	2+0	
4		2+3	0+0	2+1	0+1	
5		3+4	0+1	15+3	3+0	
Total		26	3	64	8	
%		90	10	89	11	

Year 2013 is represented by a weak attack to medium, being performed on 12.05 limits ranging between 4% attacked bud Jonathan variety, 4% vegetative bud Starkrimson and 12% vegetative bud attacked the Golden Delicious (Table 3).

In the untreated lot attack was made on the same dates as in the chemical situation years as follows: in 2011, there was strong attack Jonathan varieties with 25% at flower bud and 23% vegetative bud attack and Golden delicious flower bud attack by 40% and 20% vegetative bud variety Starkrimson recording medium with 14% attack flower bud and respectively vegetative bud attacked it was 20% (Table 4).

Year 2012 registered strong attack flower buds all three varieties, namely 30% bud attacked the variety Jonathan, Golden Delicious 50% to 26% in the middle variety Starkrimson and attack the vegetative buds with a 18% attack bud Jonathan, Golden Delicious 16% to 20% in Starkrimson (Table 5).

Table 3

Level attack buds in apple orchards of the SCDP Fälticeni 2013 - chemical lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
12-05-2013	1	Jonathan	1+3	0+0	9+3	0+0
	2		4+2	0+0	8+10	0+1
	3		1+3	1+0	12+4	3+0
	4		2+3	0+0	9+8	0+1
	5		1+3	0+0	7+3	0+0
	Total		23	1	73	5
	%		96	4	94	6
	1	Golden delicious	2+1	0+0	7+3	0+1
	2		3+1	1+0	12+4	4+0
	3		3+1	0+1	9+5	2+0
	4		4+2	0+0	6+4	1+0
	5		3+4	1+0	12+9	0+2
	Total		24	3	71	10
	%		89	11	92	12
	1	Starkrimson	1+3	1+0	4+6	0+0
2	4+2		0+0	7+8	0+0	
3	1+2		0+0	8+11	0+1	
4	2+2		1+0	6+3	0+0	
5	2+3		0+0	13+8	1+1	
Total		22	2	74	3	
%		92	8	96	4	

Table 4

Level attack buds in apple orchards of the SCDP Fälticeni in 2011 – untreated lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
09-05-2011	1	Jonathan	3+0	2+0	10+8	4+2
	2		0+0	0+0	12+11	8+1
	3		0+4	0+2	3+7	1+2
	4		6+8	0+0	9+8	3+0
	5		1+2	3+1	11+7	2+2
	Total		24	8	86	25
	%		75	25	77	23
	1	Golden delicious	0+0	2+0	16+11	4+2
	2		0+1	0+0	6+9	3+2
	3		0+2	0+1	10+2	1+0
	4		2+2	2+0	7+21	0+4
	5		2+0	0+1	4+3	2+4
	1	Starkrimson	0+1	1+0	2+8	3+2
	2		1+3	1+0	3+7	0+2
	3		2+0	0+0	5+6	2+0
4	1+0		0+1	4+3	1+0	
5	7+4		0+0	4+2	0+1	
Total		19	3	44	11	

Table 5

Level attack buds in apple orchards of the SCDP Fälticeni 2012 – untreated lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
08-05-2012	1	Jonathan	3+1	1+0	12+7	1+2
	2		0+1	1+0	16+17	3+2
	3		0+2	0+1	4+6	2+2
	4		0+3	1+0	12+1	2+2
	5		4+0	0+2	11+7	3+2
	Total		14	6	93	21
	%		70	30	82	18
	1	Golden delicious	0+0	2+0	16+12	4+1
	2		0+1	0+1	7+4	3+2
	3		0+1	1+0	0+3	2+0
	4		3+0	0+0	20+11	2+1
	5		0+0	0+1	4+8	0+1
	Total		5	5	85	16
	%		50	50	84	16
	1	Starkrimson	0+0	0+1	2+11	1+2
	2		2+2	1+0	2+7	0+2
	3		2+1	0+2	7+6	0+0
	4		1+1	0+0	3+3	2+1
	5		6+5	1+2	4+6	2+3
	Total		20	7	51	13
%		74	26	80	20	

Table 6

Level attack buds in apple orchards of the SCDP Fälticeni 2013 – untreated lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
12-05-2013	1	Jonathan	0+1	1+0	9+6	2+0
	2		0+0	1+0	11+9	3+0
	3		1+2	0+0	11+6	2+1
	4		1+1	2+0	11+4	1+2
	5		2+1	0+0	8+12	2+2
	Total		9	4	87	15
	%		70	30	85	15
	1	Golden delicious	1+0	0+1	13+9	1+2
	2		0+0	0+0	8+4	0+2
	3		1+1	0+0	8+3	2+3
	4		2+0	0+1	17+9	1+3
	5		0+0	0+0	7+10	0+4
	Total		5	2	88	18
	%		70	30	83	17
	1	Starkrimson	1+0	0+0	7+10	2+2
	2		1+2	1+1	4+11	0+2
	3		2+2	0+1	4+6	2+0
	4		0+1	2+1	5+3	1+1
	5		2+1	0+0	6+8	1+1
	Total		12	6	64	12
%		67	33	84	16	

Year 2013 limits the level of attack flower buds are 30% bud attack Jonathan and Golden Delicious varieties and 33% bud attacked the Starkrimson resulting in strong attack and the vegetative buds joined middle attack with a range between 15% bud vegetative attacked Jonathan, Golden Delicious and 17% to 16% vegetative bud attacked the Starkrimson (table 6).

### CONCLUSIONS

Apple varieties studied to establish the level of attack are Jonathan, Golden Delicious and Starkrimson and limits the level of attack are weak attack (up to 10% appeal bodies), middle attack (10-20% appeal bodies), strong attack (over 20% organs attacked).

After determining the level of review of the species of carnation leaf-rollers, the buds in 2011-2013 the situation is as follows:

- The chemical Lot, there is middle attack Jonathan and Golden Delicious varieties both flower buds and vegetative those in 2011, 2012 attack middle and low to medium in 2013;

- In the untreated Lot in 2011 to S.C.D.P. Fălticeni recorded strong attack Jonathan and Golden Delicious varieties and variety Starkrimson middle attack in 2012 that 2013 registered strong attack flower buds in all three varieties and attack the middle vegetative buds.

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CONTRIBUTIONS TO THE KNOWLEDGE OF HARMFUL SPECIES OF  
APPLE ORCHARD ACCORDING TO TECHNOLOGY FOR THE  
CONTROL AND THE ASSORTMENT OF GROWN VARIETIES

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**Keywords:** buds, control, defoliators

**ABSTRACT**

*This paper presents the period 2011-2013 a fruit tree apple plantation at the varieties: Jonathan, Golden Delicious and Starkrimson, in stationary "V. Adamache" belonging to Didactic Station from Iasi, were the observations were made on attack the Adoxophyes orana larvae produced on vegetative and flowering buds .Observations were made on the following dates: 04.05. in 2011, 3.05. in 2012 and 8.05. 2013*

**INTRODUCTION**

In the last years thanks to great damage caused by representatives of the Lepidoptera order, attracted the attention of specialists, being done research on the prevalence, description, biology, and mode pest prevention and control of these pests in orchards (Bovey 1966, Pătrășcanu 1968, Tălmăciu et al. 2002, 2007).

The most important harmful species which belong to the Lepidoptera order and cause serious damage to the apple orchard are: *Spilosoma ocellana* F., *Hedia nubiferana* Haw., *Aporia crataegi* L., *Malacosoma neustria* L., *Operophtera brumata* L., *Hibernia defoliaria* Cl., *Hyphantria cunea* Drury, *Hyponomeuta malinella* Zell., *Leucoma salicis* L., *Leucoptera scitella* Zell., *Stigmella malella* Stt., *Lyonetia clerckella* L., *Phyllonorycter blancardella* F *Phyllonorycter corylifoliella* Hb., *Recurvaria nanella* Hb., *Calisto denticulella* Thumb *Anarsia lineatella* Z., ), *Cydia pomonella* L., *Adoxophyes reticulana* Fisch ( Rogojanu & Perju 1979).

This paper aims to bring new contributions to knowledge of moth attack that damages the buds and in particular on their species of the genus *Adoxophyes*.

**MATERIAL AND METHODS**

Biological material in research during the 3-year study was the apple orchards of stationary teaching farm "Vasile Adamachi" Iasi belonging to Agricultural University of Iasi, the two experimental lots, namely a group of chemically treated and a lot where no chemical treatments were applied. In each of the two stationary predominant varieties are Jonathan, Golden Delicious and Starkrimson with good to very good resistance to pests and pathogens.

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For identification and characterization of species of carnation leaf-rollers in apple Orchards, was applied the method of nonselective collection of biological material.

The principle of this method is in a non-selective collection of caterpillars and a pupa of foliofage-rollers from the crown of the tree not collected those species whose larvae live in nests or demolishes the blade leaves. Collection of larva stage (fig.1) was performed with leaves that feed or shelter where they were feeding and pupae with pupae shelter, taking care to be with last exuvie larvae (Diaconu, 1998).

The material was collected in bags, thick nylon cloth, after which they were placed in metal see vendors in order to provide ventilation and not to be damaged during transportation. For longer periods of transportation, and added fresh leaf feeding mites. Each bag was introduced by a label noted locality, date of collection, mode of operation of the plantation (chemical or unchemical treated) host plant phenology, etc (Cârdei et al. 2006).

The samples are collected when parasitoids show signs of having parasites isolated in small tubes or tubes.

Samples were collected throughout the growing season, but most often repeated in the months from March to July, when most foliofage-rollers are active larval stage or pupa.



Figure 1. Nonselective method collection of biological material a, b (original)

Following the application of non-selective collection method follows the evolution of the main species of carnation leaf-rollers, but especially evolutionary *Adoxophyes orana* (Fv R.), who after genus *Cydia* is the most important pest in apple orchards.

## RESULTS AND DISCUSSIONS

The two lots (chemical and unchemical treated) farm belonging teaching "Vasile Adamachi" Iasi area of 1 ha and 0.5 ha, being the predominant varieties: Jonathan, Golden Delicious and Starkrimson. The resort also has a collection teaching Pomology ancient varieties such as Costești, Domnești, Pătul, Renet, Astrahan, Agapia, London, Mutsu, Pătlașele, et all.

Vegetative buds attacked curve with the development of their components consumed by the caterpillars, and the colors (fig. 2) often can not be opened with the ascension inflorescence take a form more or less spherical.

Establishment of the attack was carried out buds of apple orchards on the farm "Vasile Adamachi" Iasi, Iasi during the 3 years of research in the months April-May of flowering period.



Figure 2. Attack of carnation leaf-rollers in the flowering buds a, b (original)

At the teaching farm "Vasile Adamachi" Iasi (Figure 3.) the level shoots attack was carried out in two lots of apple, a chemical group and one where no treatments were performed.

In 2011 the attack was carried out on 04.05, in 2012 03.05 2013 08.05 respectively, the data is the same for both lots.



Figure 3. Collection of biological material from "Vasile Adamachi" Iasi stationary a, b (original)

The chemical lot in 2011 to attack the buds was performed on 4.5 (Table 1), hence the weak attack at the variety Jonathan buds with a 4% attack buds and medium Golden Delicious and Starkrimson 14% and 17% bud attack. The vegetative buds has been weak attack in all varieties, the highest percentage of attacked buds registered the variety Jonathan (7%).

In 2012, following the attack on the level of 03.05 (Table 2.) was registered buds weak attack at variety Jonathan the percentage is 7%, middle 13% for Golden Delicious and strong buds attacked Starkrimson attack is 20%.

For vegetative buds Jonathan middle attack is 11% attack and weak shoots varieties Golden Delicious and Starkrimson.

Table 1

Level attack buds in apple orchards of the teaching farm "Vasile Adamachi" Iasi  
in 2011 - Chemical lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
04-05-2011	1	Jonathan	2+0	0+0	5+12	0+1
	2		8+4	1+0	2+0	0+0
	3		1+6	0+1	9+4	0+0
	4		3+7	0+0	8+12	1+0
	5		8+5	0+0	2+3	0+2
	Total		44	2	57	4
	%		96	4	93	7
	1	Golden delicious	3+2	0+0	16+12	0+2
	2		0+1	0+1	8+9	0+0
	3		1+0	0+0	6+6	0+1
	4		2+1	0+1	2+9	1+0
	5		0+2	0+0	9+7	0+0
	Total		12	2	84	4
	%		86	14	95	5
	1	Starkrimson	0+0	0+0	11+13	1+1
	2		6+1	0+0	3+17	0+2
	3		1+1	0+1	16+9	0+0
4	0+0		0+0	11+3	0+0	
5	1+0		1+0	10+6	0+0	
Total		10	2	99	4	
%		83	17	96	4	

Table 2

Level attack buds in apple orchards of the farm "Vasile Adamachi" Iasi in 2011 - Chemical  
lot - Chemical lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
03-05-2012	1	Jonathan	7+9	1+1	2+3	0+1
	2		7+2	0+0	4+3	1+0
	3		7+6	1+0	2+3	0+1
	4		2+4	0+0	3+11	0+2
	5		1+7	0+1	9+2	0+0
	Total		52	4	42	5
	%		93	7	89	11
	1	Golden delicious	4+5	1+0	12+3	1+0
	2		1+1	0+1	26+18	4+2
	3		2+9	0+2	13+3	1+0
	4		0+2	1+0	18+12	1+0
	5		12+4	1+0	3+12	0+1
	Total		40	6	120	10
	%		87	13	92	8
	1	Starkrimson	1+4	0+1	6+3	0+1
	2		0+3	0+1	8+11	0+2
	3		0+1	0+0	8+12	1+1
4	1+0		0+0	4+12	0+0	
5	2+0		1+0	9+11	0+1	
Total		12	3	101	6	
%		80	20	94	6	



Table 3

Level attack buds in apple orchards of the teaching farm "Vasile Adamachi" Iasi  
in 2013 - Chemical lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
08-05-2013	1	Jonathan	4+6	0+1	2+4	0+0
	2		3+2	0+0	5+6	0+2
	3		6+3	0+0	4+4	0+0
	4		2+1	0+0	7+10	1+2
	5		1+3	0+0	8+7	2+0
	Total		26	1	57	7
	%		96	4	89	11
	1	Golden delicious	2+0	1+0	10+11	2+0
	2		2+1	0+0	16+21	0+3
	3		2+0	0+0	7+3	0+0
	4		2+2	0+0	12+6	1+0
	5		1+4	0+1	13+9	0+0
	Total		16	2	108	6
	%		89	11	95	5
	1	Starkrimson	1+3	0+2	6+5	0+0
2	1+2		0+0	8+10	0+3	
3	1+3		0+0	6+12	0+3	
4	1+1		1+0	7+4	1+0	
5	1+0		1+0	10+8	2+0	
Total		14	4	76	9	
%		78	22	89	11	

Table 4

Level attack buds in apple orchards of the teaching farm "Vasile Adamachi" Iasi  
in 2011 – untreated lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
04-05-2011	1	Jonathan	3+5	0+1	14+2	5+0
	2		6+2	3+0	3+5	0+2
	3		7+5	0+0	2+5	0+1
	4		4+8	0+2	2+3	0+0
	5		5+7	1+1	5+2	1+1
	Total		52	9	43	10
	%		85	15	81	19
	1	Golden delicious	0+0	0+0	12+15	0+6
	2		2+3	0+1	7+1	2+0
	3		0+0	0+0	11+6	3+0
	4		0+1	0+1	8+6	1+0
	5		0+0	0+0	5+12	1+2
	Total		6	2	83	15
	%		75	25	85	15
	1	Starkrimson	1+0	1+0	6+8	0+2
2	1+1		0+0	4+7	0+2	
3	0+0		0+0	9+10	1+3	
4	0+1		0+1	11+2	2+0	
5	1+0		0+0	3+13	1+1	
Total		5	2	73	12	
%		71	29	86	14	

Table 5

Level attack buds in apple orchards of the teaching farm "Vasile Adamachi" Iasi  
in 2012 – untreated lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
03-05-2012	1	Jonathan	3+6	0+2	3+2	2+0
	2		7+5	1+0	8+2	2+1
	3		4+8	1+3	8+12	0+4
	4		9+6	0+0	11+10	2+1
	5		9+4	2+1	2+6	1+0
	Total		61	10	64	13
	%		86	14	83	17
	1	Golden delicious	2+2	1+1	3+3	1+2
	2		2+2	1+0	4+12	0+2
	3		1+1	2+1	11+8	1+3
	4		2+3	1+0	7+8	0+1
	5		3+3	1+1	4+6	1+2
	Total		21	9	66	13
	%		70	30	84	16
	1	Starkrimson	2+1	1+1	8+6	2+0
	2		0+2	1+0	4+5	0+1
	3		1+2	0+0	10+9	4+1
	4		3+1	0+0	11+7	3+1
	5		2+1	1+1	4+8	0+2
	Total		15	5	72	14
%		75	25	84	16	

Table 6

Level attack buds in apple orchards of the farm teaching "Vasile Adamachi"  
in 2013 – untreated lot

Data of analysis	No. trees	Variety	Buds analyzed			
			Floral buds		Vegetative buds	
			Not attacked	Attacked	Not attacked	Attacked
08-05-2013	1	Jonathan	3+2	0+1	6+4	0+0
	2		3+1	1+0	10+6	3+2
	3		1+3	0+1	8+14	1+0
	4		3+6	2+0	10+11	2+1
	5		7+5	1+0	3+6	0+1
	Total		34	6	78	10
	%		85	15	89	11
	1	Golden delicious	2+3	0+1	4+6	1+0
	2		3+2	1+2	7+14	0+2
	3		5+1	2+0	7+10	1+1
	4		5+4	0+1	7+9	0+2
	5		2+2	1+1	4+6	3+0
	Total		29	9	74	10
	%		76	24	88	12
	1	Starkrimson	1+2	0+2	9+6	2+0
	2		2+0	0+0	7+6	1+0
	3		0+2	0+0	11+10	3+0
	4		1+3	1+1	11+12	2+3
	5		0+1	0+0	9+10	1+2
	Total		12	4	91	14
%		75	25	87	13	

Following the attack the level of buds in 2013 on 08.05 (Table 3), the resulting buds weak attack variety Jonathan (4%), attack the middle to Golden Delicious (11%) and strong attack variety Starkrimson (22%).

The vegetative buds registered varieties middle attack Jonathan and Starkrimson percentage was 11% in both attack and weak attack shoots cultivar Golden Delicious 5% level of attack.

At the teaching farm untreated lot "Vasile Adamachi" Iasi in 2011 to attack the buds is performed on 04.05 (Table 4) hence the middle attack and strong buds 15% attack variety Jonathan, 25% and 29% respectively Starkrimson attack on Golden Delicious.

The vegetative buds middle attack is no variety not exceeding 20% buds attacked.

In 2012 were registered for buds values the resulting from the variety Jonathan middle attack (14% buds attacked), strong attack from the other two varieties values were 30% and 25% for Golden Delicious and Starkrimson.

The vegetative buds is recorded middle attack, the attack within the 17% variety Jonathan attacked buds and 16% for Golden Delicious and Starkrimson. Level attack is made on 03.05. (Table 5).

In 2013, establishing the level of attack takes place on 08.05 (Table 6), recorded values of middle and high attack buds (15% buds attacked Jonathan, 24% buds attacked Golden Delicious and to 25% for Starkrimson) and middle attack from vegetative buds by between 10-20% buds attacked.

## CONCLUSIONS

After determining the level of attack of the species of carnation leaf-rollers, the buds in 2011-2013 the situation is as follows:

1. The chemical lot belonging to teaching farm "Vasile Adamachi" Iasi in 2011 resulting from weak attack buds variety for Jonathan, Golden Delicious and Starkrimson middle and the vegetative buds was recorded weak attack in all varieties, in 2012 there was weak attack the variety buds Jonathan, Golden Delicious and powerful medium to Starkrimson vegetative buds and the attack is middle to Jonathan and Golden Delicious varieties and poor Starkrimson, but in 2013 resulting in weak attack buds variety Jonathan, Golden Delicious middle Starkrimson powerful variety and the vegetative buds registered varieties middle attack Starkrimson Jonathan and Golden Delicious variety and weak;

2. In the treated lot in 2011 resulting medium and strong attack at flowering buds and vegetative buds in the middle attack is no variety not exceeding 20% buds attacked, in 2012 is recorded the variety Jonathan middle attack and strong in the other two varieties in flowering buds and from vegetative buds is recorded middle attack, attack values are in 2013 middle and high middle from flowering buds and vegetative buds attack.

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## A FIRST APPROACH TO FIRE MANAGEMENT OF PROTECTED AREAS IN GREECE

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**Keywords:** *protected areas, fire, management body, Greece*

### ABSTRACT

*The protected areas are very important for maintaining the biodiversity of an area, while showing a keen interest for the scientific research, the forest recreation and the environmental education. The fire protection of a protected area (P.A.) is an essential preventive measure. The management of fire in national parks requires adequate material and technical infrastructure, trained staff and the use of new technologies and satellite data.*

*The purpose of this research is to study and record the risk management of fire in the protected areas of Greece, particularly in those with Management Body (MB), with the method of questionnaire. Recorded differences in infrastructure and fire protection measures between national parks in Greece. The National Park of Samaria uses modern telematic means for fire prevention, while others are at a satisfactory level and try for the best.*

### INTRODUCTION

The leading cause of shrinkage and degradation of the natural and human environment (forests, agricultural crops, villages, crafts, plants, etc.) are the fires. The main enemy of the forests in Greece, which is a Mediterranean country, are the forest fires. Both wildfires and grazing are the main cause of bare vegetation, on Greek mountains. Because of the fires in Greece, which is a mountainous country with forests mainly on steep slopes, rain has generated leaching of soil, so often that the first signs of desertification are already obvious.

The institution of Protected Areas is a guarantee for the protection and preservation of natural heritage and is an indicator of increased environmental awareness of society. The establishment of protected areas is an urgent need for the conservation and protection, environmental, scientific, cultural and recreation values of these areas (Efthimiou 2014). According to IUCN (1994), protected areas are: "land or water devoted to the protection and maintenance of biological diversity, and of natural and cultural resources and their preservation is guaranteed by law or other effective means".

The ecological awareness and environmental education can be accomplished successfully in protected areas which offer the greatest variety of topics and experiential

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participation and education in shaping these essential ecological awareness successfully participants.

An effective measure in this direction is the establishment of protected areas with mild human activities and also controlled and directed traffic and promotion of natural resources (Efthimiou 2000, Efthimiou 2014).

The effect of fire may be favourable or unfavourable, depending on the type, intensity, frequency of recurrence, the composition of ecosystems and the effective of other factors, notably grazing (Papanastasis 1978). In some terrestrial ecosystems, fire is an important ecological factor and key trends. It appears that these natural fires are occurred almost at regular intervals, with frequent cause of thunders.

According to Konstantinidis & Gatzogiannis (2001), fires, although partially or completely, destroys a specific ecosystem each time have reversible action and they are not consist, not a factor retrogressive succession, as the plant species which constitutes the ecosystems have developed mechanisms to adapt to environments which are struck by fire recur.

The environmental impact of fires due to the microclimate and the change in the biological processes of ecosystems with the consequent change in vegetation composition have been extensively investigated in the last decades (Valeo et al. 2003, Timoney & Wein 1991). The high ground temperatures and increased nutrient availability after a bushfire create favorable growth conditions of the populations of specific species (Espirito-Santo et al. 1993, Kazanis & Arianoutsou 1996, Hanes 1971, Arianoutsou & Thanos 1996).

In a wildfire where indicates complete destruction of vegetation, many forest species can not be installed immediately after the fire, as well as shade trees such as silver fir (Efthimiou et al. 2014) with their development is not favoured. Besides, their development depends on the management practices and the ability to effectively protection of the burned area, mainly across the grass and prevention of new fires at short intervals.

Objective problem to restore forest vegetation after a fire is the exact knowledge of the mechanisms of recovery and adaptation of certain plants in metapyriko environment, but also the sensitivity of other plant and animal species that have not many times, the ability to adapt, even after from slightly different climatic conditions in places of development (Cramer & Steffen 1997, Pereira & Chaves 1995).

However, the situation of Mediterranean forests characterized particularly critical, as many of them adjacent to urban or tourist areas and under heavy pressure from the change of land use (Lekakis 1993).

The responsibility for the management and protection of Greek national forests belong to "General Development and Protection of Forests and Natural Environment" of the Ministry of Environment and Climate Change. The competent 'Forest Department of Environment, National Parks and Forest Recreation "directs and supervises national local Forest Service, which is responsible for the protection, organization and management of forests and forest National Park status.

In Greece, the management authorities of protected areas are the Management Bodies (M.B.) and the local Forest Services. The responsibility of firefighting in the National Parks belong to the Fire Brigade. For the successful implementation of the Management Plan by Management Entities is necessary to select the appropriately trained staff and in some cases it is helped by the work of the Civil Service, such as guarding, policing and surveillance of the area, from voluntary groups local residents or the wider region, provided that they have previously trained to respond to specific tasks.

Therefore, for effective management of forest ecosystems and forest fires, it is necessary to be accompanied by long-term knowledge of all issues related to forest fires (climate, vegetation, soil, terrain, etc.), which are vary locally.

By the laws 2742/1999 and 3044/2002 are established the creation of the Bodies Management of the Protected Areas. In our country, the issues of protection and conservation of nature are constantly being improved, constantly needs while ensuring modern logistics and technology infrastructure. The collection and analysis of data in a fire, it is imperative and important information for the design of prevention and suppression of potential fires, and rehabilitation of burned areas. Moreover, the collection, processing and analysis of fire incidents are not confined to the national level, but extends on a larger scale through international organizations such as the European Union.

Those concerning the management of forest fuel material (controlled burning) in many countries (U.S.A., Canada, Australia and much less in Europe) in recent years using a controlled fire under certain strict conditions (such as weather) for the destruction of understorey without loss of high forest (Vorisis, 2000).

In Figure 1 is given all the burned areas (in 0,1 hecter) in Greek territory for the years 2011 to 2013. In total there have counted the burned areas on landfills and residues resulting crops.

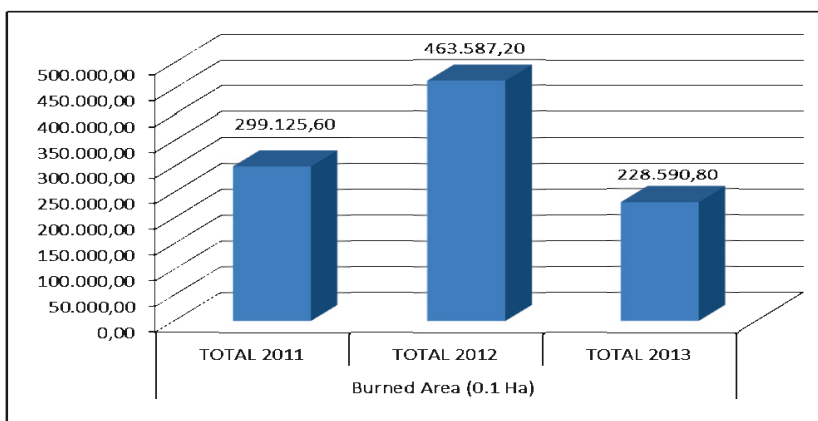


Figure 1. Total burned area (0.1 Ha) for the years 2011-2013 ([www.fireservice.gr](http://www.fireservice.gr))

Figure 2 shows the total burnt area (0.1 hecter) in land use for the years 2011 to 2013. In total there have not counted the burned areas on landfills and residues resulting crops.

### MATERIAL AND METHODS

For the realization of this research statistics used by the Fire Service, while logistic substructure was recorded and means fire in the protected areas of our country, with greater emphasis on those that are managed by the Management Bodies (M.B.). To achieve this objective questionnaire was sent to 28 management bodies currently operating in Greece. The survey was conducted from December 2011 to April 2012, and were sent electronically by email, a total of 28 questionnaires to all Protected Areas Management Bodies were taken and all completed questionnaires respectively.

## RESULTS AND DISCUSSIONS

### A. Fire risk management globally

An essential tool for the prevention of fire in a forest or in a National Park is the management of biomass - fuel in them. Controlled fires used as a management tool. In the United States used to manage the planned fuel controlled fires (prescribed fires) as a preventive tool in combination with other techniques (mainly mechanical) to reduce the risk of fire (firebreaks, forest cleaning, etc.). Meanwhile, UK States have concluded border agreements with Canada and Mexico to assist each other in case of major fire and exchange of firefighting equipment, training and technology.

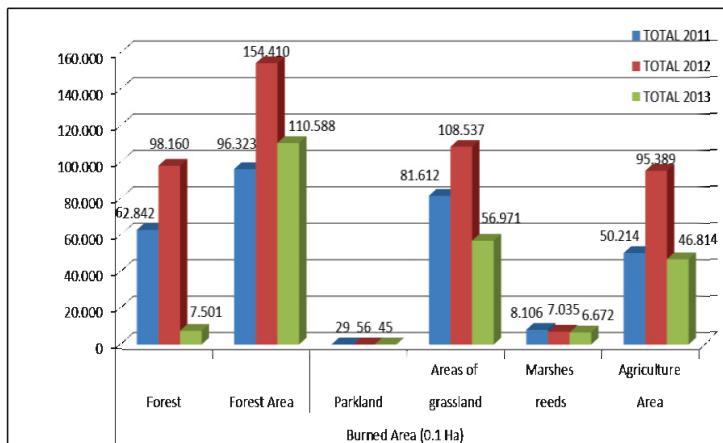


Figure 2. Total burned area (0.1 Ha) in land use for the years 2011-2013 (www.fireservice.gr)

In Canada the traditional use of fire for forest management and the ever increasing awareness of the public for the role of forest fires has guided to the selection of a strategic forest management which does not exclude even the beneficial ecological role of fire. On the other hand, in some cases there are efforts to control forest fires when they act in low priority areas or remote areas. In these areas, fires are considered an essential part of the natural process. In National Parks management includes scheduled fires to reduce fuel and preventive measures such as monitoring and early warning fire (N.K.U.A., 2010).

In Australia the organization and management of fires varies by region. Usually local firefighting agencies (Fire Services) undertake to protect people and property. Forestry services assume a degree of responsibility for forest and non-residential areas and prevention activities. The period 1998-2009 was significantly strengthened the control as regards the question of forest fires ("Bushfires") in Australia. In the years 2000-2010 there were significant fires in Australian continent, which led to the planning and management of forest fires and, in a series of improvements and revisions. According to Gill et al. (2001) and Moore (2006) statistics that retain the services of Australia on fires pose considerable problems and inaccuracies (Tsiolis 2012). The overall design of the management of woodlands and fire strategy includes three types of prevention: a) Prevention of ignition, b) Preventing spread, c) Prevention to reduce losses and impacts. Preventing the spread includes the management of fuel, reducing actions of the woodland. One of the main actions are planned fires and their use as a measure to reduce the fuel significantly supported by the authorities and the legal framework.



## **B. Fire Risk Management in Greece**

By establishing of the Greek state (1830), the Forest Service had the responsibility for firefighting. By the law 2612/1998 the firefighting passed to the Fire Service. When the status of firefighting has changed and passed from the Forest Fire Department to the Fire Service, along with the equipment (vehicles), many forest wardens have transferred to the Fire Service, which gradually staffed with Foresters.

Following are the essential elements for the infrastructure created in the protected areas of Greece and particular those whose managed by Management Body (M.B). In other protected areas without Management Body, are managed by the relevant public services (Forest and Fire Services).

By the collection of the questionnaires at 28 Management Bodies of the country, in which all responded, there were important and interesting information regarding the infrastructure for the prevention and suppression of fire risk. There are protected areas while two thirds of these (75%) had drafted a plan of protection or safekeeping while only 10.7%, have drawn fire protection study (Tsiolis 2012).

Regarding the use of new technologies, the use of satellite data is recovered from eighteen (18) M.B. that is (64.3%) while the use of forest fire management models, used only by the Management Body of National Park of Samaria, which made a study of daily fire risk assessment for the White Mountains, with subsequent management recommendations. The National Park of Samaria have been placed five points around the core of the National Park, forest fire detection cameras, which carry on line and real time image data. Also have installed five (5) weather stations and a plurality of linear and raster data, among which interesting data on fires. The National Park of Delta Nestos Vistonidas, Ismarida, has made an effort to install a smoke detector station at selected points of the park (in collaboration with the Department of Information Management of Technological Educational Institute of Kavala).

Regarding the use of new technologies, are used by four M.B. that is only 14.3%, while the fire management programs, used only by the Management Body of National Park of Olympus. The eight (8) of the 28 M.B., that is about one third of the carrier, said it has no involvement in the management of forest fires. Maps fuel used only by two bodies (7.1%), that is: a) F.D. Olympus National Park and b) the F.D. National Park of Samaria. Information actions for civil matters Prevention - Fire Protection, has produced only one third of the Management bodies.

As regards the activity and extroversion M.B. of Protected Areas, the majority of them talking with carriers for fire safety, while the one third of them works with these Rescue - Fire groups and do joint patrols with others. For example, M.B. of the National Park of Schinias-Marathonas, cooperate in patrolling team of volunteers (Volunteer firefighting N. Makri).

On the direct involvement of M.B. in the management of forest fires (firefighting), found that only one quarter of M.B. be actively involved. Although many M.B. of Protected Areas staffed with personnel in the last five years, there is no record for the number of fires (how many and which occurred, what is the burned area, coordinates fire, etc.). The M.B. of Evros Delta reports that there is a Guarding plan of the area with patrols, fire control and auxiliary support services authorized (Forestry and Fire Service) in their work.

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## BIOLOGICAL AND ECOLOGICAL FEATURES OF THE SAPROXYLIC BEETLES LISTED ON IUCN RED LIST FROM THE SITE OF COMMUNITY IMPORTANCE „NORDUL GORJULUI DE VEST”

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**Keywords:** *saproxylic, beetle species*

### ABSTRACT

*Oak and beech old forests in Romania are under strong anthropogenic pressure. Sites of high conservation value can be efficiently defined by the use of indicator species, but very few studies have identified indicator species for beech forests on a continental scale. In order to define accurately a site of high conservation, during 2013 - 2014 in the site of community importance "Nordul Gorjului de Vest", we conducted researches regarding the saproxylic beetles fauna. The research focused on the biological, ecological features of four species of saproxylic beetle listed in the IUCN Red List of Threatened Species: *Cerambyx cerdo* L., *Lucanus cervus* L., *Osmoderma eremita* Scop., *Rosalia alpina* L.*

### INTRODUCTION

Saproxylic beetles are a functional group of Coleoptera that depend, at some point in their life cycle, on dead or decaying wood or fungi associated with deadwood (Speight 1989). They are associated with both living and dead trees. They prepare the substrate for colonisation by other species (e.g. hymenoptera) and, because of their high degree of specialisation and often specific colonisation sequences, contribute significantly to the complex ecological interrelationships of deadwood-rich forests.

Their differentiated lifestyles, their high species numbers and their sensitivity to changes in their habitat make saproxylic beetles an indicator group in nature conservation and landscape planning (Heinz Bussler et al. 2005).

### MATERIALS AND METHODS

To determine the structure of the xylophage, saproxylic species were made collection of material using various means and methods:

Hand capture: The animals are captured by manually searching woody structures.

Sifting trees humus: Tree humus and decomposed wood are roughly sifted. The remaining material is spread onto a white sheet and searched for beetles, larvae and fragments control (Heinz Bussler et al. 2005).

After collecting, the biological material was analyzed and determined with the binocular magnifier glass using the Identification Manual (Panin 1951, Chatened du Gaetan 1990, Chinery 1998, Godeanu 2002).

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## RESULTS AND DISCUSSION

The studies were carried out within the area administered by the Targu Jiu Forest Authority.

*Cerambyx cerdo* L.  
Kingdom: *Animalia*  
Phylum: Arthropoda  
Class: *Insecta*  
Order: *Coleoptera*  
Family *Cerambycidae*  
Genus *Cerambyx*  
Species *Cerambyx cerdo* L.

*Cerambyx cerdo* L. is a species xylophage, saproxylic species that prefer old forests with deciduous, especially those of oaks. Trees with deadwood have ethological significance, attracting females.

The duration of a generation is usually 3 years, or can be extended to 4 or 5 years if the ecological conditions are difficult. A female lays up to 100 eggs. The oviposition period lasts from June to September. Larvae hatch after 14 days of laying. Larval galleries are irregular, oriented in all directions. The pupal stage takes place during July. Adults are nocturnal and twilight. Flight takes place from May to August.

In the site of community importance "Nordul Gorjului de Vest", the population of great capricorn beetle compared to other species of beetles, is less abundant, being affected due to deterioration of natural habitats. This species can be preserved for a considerable time, by protecting old trees.

*Lucanus cervus* L.  
Kingdom: *Animalia*  
Phylum: Arthropoda  
Class: *Insecta*  
Order: *Coleoptera*  
Family *Lucanidae*  
Genus *Lucanus*  
Species *Lucanus cervus* L.

*Lucanus Cervus* is one of the largest beetle species in Europe. In Romania is a common species, being found in all areas of oak forests.

During the day, adults can be seen on the trunks of oaks and other trees feeding on their sap. The adults fly in dusk, during the period from May to July. Larvae develop in strong roots of old trunks of *Quercus*, *Fagus*.

Individuals of the species *Lucanus cervus* have different colonization capacity, according to sex: the maximum dispersion of females is 1 km, while males can fly up to about 3 km.

In the site of community importance "Nordul Gorjului de Vest", the species was identified in: Pădurea Tismana – Pocruia, Cornetul Pocruiei, Pădurea Răchițeana, Valea Bistricioara, Valea Jaleșului, Cheile Sohodolului, Dobrița. In these locations *Lucanus cervus* L. species compared with other species of beetles was less abundant. For conclusive results this species, should be monitored in consecutive years, at least during the adult flight period (May-July). In addition, monitoring this species could provide information on the status of

the habitats from this protected site, given that the species larvae develop in decaying woody debris in old hollow oaks.

*Rosalia alpina* L.  
Kingdom: *Animalia*  
Phylum: *Arthropoda*  
Class: *Insecta*  
Order: *Coleoptera*  
Family *Cerambycidae*  
Genus *Rosalia*  
Species *Rosalia alpina* L.

*Rosalia alpina* is a xylophage, saproxylic species that lives in the complex climate of beech and conifers, rarely at the oak, especially old beech forests. This species prefer rotten wood and hollow trunks of *Fagus sylvatica*, rarely on the *Acer* and other deciduous species. Females lay eggs in freshly cut tree trunk. The adult can be met from June to September. Adults feed on the sap of trees, leaves and rarely flower. Larvae are xylophage form galleries in the wood of trees.

Period of development takes about 2-3 years. The female lays eggs in bark crevices in sun-exposed areas. Larvae develop in wood old beeches (*Fagus sylvatica*, *F. orientalis*). The larval stage duration is 2-3 years, towards the end of this period occurs pupation during May-June. Adults appear from June to August, depending on weather conditions and altitude.

In the site of community importance "Nordul Gorjului de Vest", the species was identified in: Valea Motrului, Cheile Sohodolului, Valea Porcului, Valea Sâmbotinului. In these locations *Rosalia alpina* L. species compared with other species of beetles was less abundant. For conclusive results this species, should be monitored in consecutive years.

*Osmoderma eremita* Sco.  
Kingdom: *Animalia*  
Phylum: *Arthropoda*  
Class: *Insecta*  
Order: *Coleoptera*  
Family *Scarabeidae*  
Genus *Osmoderma*  
Species *Osmoderma eremita* Sco.

*Osmoderma eremita* is a xylophage, saproxylic species that prefers old deciduous forests where can be found on old and hollow trees. The larvae live in rotten wood of deciduous hollows of different species. Usually, adults appear in June and September.

The hermit beetle can carry out the entire life cycle on a single tree. Life cycle takes three to four years, of which three years last larval stage. Before pupation larvae are building a cocoon of excrement and decaying wood. The adults appear next summer. Adult stage is spread from July to September, the life of an adult is approximately 30 days. The dispersion of this species is limited because adults rarely fly, not more than 100 meters. This is why adults need a stable environment, with a habitat suitable for its requirements.

In the site of community importance "Nordul Gorjului de Vest", the population of the hermit beetle compared to other species of beetles, is less abundant. For conclusive results this species, should be monitored in consecutive years.

## CONCLUSIONS

Based on our research we can conclude that in the site of community importance Nordul Gorjului de Vest the saproxylic beetle species are less abundant comparative with other beetle species. For conclusive results this species, should be monitored in consecutive years, at least during the adult flight period.

In addition, monitoring this species could provide information on the status of the habitats from this protected site, given that the species larvae develop in decaying woody debris in old hollow oaks.

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## THE CHARACTERIZATION OF SOME NEW VARIETIES OF TOMATOES WHICH HAVE BEEN GROWN UNDER ECOLOGICAL CONDITION CERTIFICATED AT INCDBH ȘTEFĂNEȘTI

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**Keywords:** *tomatoes, combating, phytopathogenic agents*

### ABSTRACT

*Tomatoes with all derivatives are one of the most important sources of carotenoids, lycopene, folic acid, ascorbic acid, flavonoids,  $\alpha$  - tocopherol and potassium to the body.*

*Biological material studied was represented by four varieties of tomatoes growing determined Arges 16, Arges 11 Arges 20, Arges 123. Determinations were performed on fruit shape, skin color intensity and anthocyanin content, polyphenol, carotenoid pigments.*

### INTRODUCTION

Tomato crop is one of the most important crops worldwide, representing the second product as consumption in the western countries after the potato (Willcox et al. 2003).

Tomatoes with all derivatives are one of the most important sources of carotenoids, providing an estimated value of 80% of daily intake of lycopene, folic acid, ascorbic acid, flavonoids,  $\alpha$  - tocopherol and potassium (Lenucci et al. 2006).

Many studies have shown the beneficial effect of tomatoes to preventing the consumption chronic diseases such as cancer, cardiovascular disease, due to their chemical composition (vitamins, amino acids, minerals), (Klipstein - Grobush et al. 2000 Giovannucci et al. 2002). The benefit is even greater as the products come from organic culture (Tzortzakis 2007).

### MATERIAL AND METHODS

The experiment was placed in a greenhouse at INCDBH Ștefănești where grown tomato varieties and biotypes created in the institute, and other varieties used as controls in different studies.

The range that worked was the four varieties growing their own creations determined (Figure1, 2, 3 and Figure 4) approved in the time of the experiments. The varieties were grown in organic farming system in depository collection of varieties of tomato germplasm. Tomato crop has been certified organic by an authorized.

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Figure 1. Variety *Argeş 11*



Figure 2. Variety *Argeş 16*



Figure 3 . Variety *Argeş 123*



Figure 4. Variety *Argeş 20*

Obtaining good results in organic tomato culture must meet the following minimum requirements:

- varieties show high biological resistance;
- the seed and seedlings to be produced by a technology officially accepted as organic;
- the soil maintenance to meet the requirements of international law;



-the combat pest acceptable to use only organic products and at least two complementary methods insulation preventive and curative.

The purpose of the research was establishing phenological and biochemical characteristics at the four tomato varieties, possible implications and behavior of specific pathogen attack.

Phenotypic characterization was done by field and laboratory observations, which concerned the type of growth and the characterization of fruit ripening.

Physico-chemical characterization of the fruits was done in laboratory using the following methods:

-The dry matter content was determined by refractometer and expressed in g / 100 g fresh material;

-Total water was determined by the PIS N 21 (test procedure) according to standard methodology and expressed in g / 100 g fresh material;

- Ash content was determined by the calcination at 650<sup>0</sup> C;

- The content of polyphenols, flavonoids was determined by colorimetric method;

- Determination of anthocyanins was performed by the spectrophotometric method.

### RESULTS AND DISCUSSIONS

At the varieties under analysis we have determined growth, late to very late period of maturing, and are suitable for both fresh consumption and for processing. Regarding phenotypic characterization of fruit was found different form of each variety, the average weight for varieties Argeş 16 and Argeş 20, and slightly lower for varieties Argeş 11 and Argeş 123 (Table 1).

Table 1

Characteristics of tomato varieties with determined growing grown in the organic greenhouse at I.N.C.D.B.H.

Variety name	Type of growth	Fruit characteristics					The period of maturing
		Look	No. fruit / inflorescence	Weight	firmness	fruit color	
<i>Argeş 16</i>	Determined	elongate	5-12	180g	Strongly to very strongly	red	Late to very late
<i>Argeş 11</i>	Determined	elongate	5-16	150g	Strongly to very strongly	red	Late to very late
<i>Argeş 20</i>	Determined	slightly elongated	4-12	180g	Firmly	red	Late to very late
<i>Argeş 123</i>	Determined	globular	3-5	120g	Firmly	red living	Late to very late

Table 2

## Physico-chemical characteristics at four tomato varieties

Variety	The dry matter content g/ 100 g fresh material	The total water g/ 100 g fres material	Total acidity g/ 100g fresh material	Ash g/ 100 g fresh material	Polyphenols g/ 100g fresh	Flavonoids Toda g/ 100 g fresh material	Anthocyanins g/ 100 g fresh material
Argeş 16	3.5	95.16	3.8	9.5	412.2	19.6	136.61
Argeş 11	3.4	94.92	3.5	7.1	435.5	22.6	227.65
Argeş 20	3.8	95.49	3.1	8.5	387.8	19.3	125.23
Argeş 123	4.1	95.12	3.3	8.9	398.3	19.4	228.5

Fruit firmness and color is easy variable from one variety to another, but the differences observed between the number of fruits per inflorescence, highlighting the variety *Arges 123* with the lowest number of fruits per inflorescence, the lowest average weight, but most intensely stained pulp.

Physico-chemical analyzes (table 2) have revealed differences by variety.

In terms of the quantity of dry matter was obtained almost the same values at *Arges 11*, *Arges 16* and *Arges 20* varieties, and a higher value at the variety *Arges 123*.

Determination of dry matter quantity is important quality characteristic when tomato fruits are subjected to processes of industrialization, as with total water gives consistency pulp and high efficiency processing.

Total acidity values are close to the 4 varieties, the differences are insignificant.

The acidity plays an important role in the quality of the product, taking part directly in the formation of the taste and a very good indicator to determine the freshness, but also contribute to the resistance to the attack of pathogen of tomato ranging from bacteria.

The amount of ash recorded at the four varieties of tomato is variable, the highest value being observed at the *Arges 16* variety, and the lowest recorded at the variety *Arges 11*. Ash content is an important quality characteristic, expressing the amount of minerals and mineral impurities of the analyte.

Polyphenols content had values close to varieties *Arges 16*, *Arges 11*, and slightly lower for varieties and *Arges 20*, *Arges 123*.

Polyphenols is one of the most popular classes of secondary metabolites of plants, consisting in ensuring normal growth and development of the plant.

The role of polyphenols is very varied from pigmentation organs to self-defense reactions against pathogens.

The values obtained for the four varieties flavonoids are nearest, the differences are small.

Highest anthocyan content recorded variety *Arges 123*, *Arges 11* and, and the lowest in *Arges 16*. Anthocyan are antioxidants special effect protects plants against UV radiation act as a defense mechanism against pests, have an important role in pollination reproduction. On to consumers, anthocyan have a positive effect due to their property (anti-inflammatory, antioxidant).

1 Varieties obtained at INCDBH Ștefănești, meet all requirements for products intended to be bio. In the year when the experiment was done, the tomato crop has obtained a certificate of compliance for organic products no 2-06168-2012.

Variety *Arges 11* was highlighted by the highest number of fruits per inflorescence and varieties *Arges 16*, *Arges 20* by the great weight of the fruit.

Variety *Arges 11* has the highest polyphenol content while *Arges 123* has the highest content of anthocyan, which gives them an increased tolerance to specific pathogens.

Total acidity ranges from 3.1 g / 100 g fresh material variety *Arges 20* and 3.8 g / 100 g fresh material.

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**BEHAVIOUR OF TOMATO VARIETY COSTATE 21 ATTACKED BY  
*PHYTOPHTHORA INFESTANS* AND *BOTRYTIS CYNAREA***

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**Keywords:** *Botrytis cynerea, Phytophthora infestans, plant protection, tomatoes*

**ABSTRACT**

*In this paper a brief characterization of the variety Costate 21, variety with indeterminate growth approved in 2012 to INCDBH Ștefănești-Argeș. To maintain the required plant health to the potential production of the variety has been used a wide range of chemical synthesis products and biologically active extract, aiming their biological action in combating pathogens *Phytophthora infestans* and *Botrytis cynerea*.*

**INTRODUCTION**

As mentioned Ciofu et al. 2003, horticultural products in general, and ecological, in particular, contribute to the body hydration and mineralization and stimulate of different systems (digestive, muscular, immune).

Tomatoes are the most eaten fresh vegetables in the world and in our country are one of the most important vegetable species; this being due to the fact that they can be eaten fresh and different forms industrialized (Willcox et al. 2003). Chemical composition of tomato fruits is influenced by variety and external factors. It has been shown that they contain vitamins (A, B1, B2, B3, K, and C), minerals iron, magnesium, potassium, amino acids, leucine, tryptophan and significant amount of antioxidants (Thybo et al. 2006, Ciofu et al. 2003). Obtaining great productions and good quality, however, requires accurate and timely implementation of all technologies in the framework of which a special place is occupied by the disease and pest control.

**MATERIAL AND METHODS**

Biological material studied is represented by variety Costate 21, variety with indeterminate growth (Figure 1), approved in 2012, at INCDBH Ștefănești Argeș.

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Figure 1. Variety Costate 21

There were made observations on phenotypic and biochemical characteristics of fruit. Observations were made both in the field and in the laboratory. Fruits were chosen randomly at the beginning, middle and end of the row in ripening stage (Figure 2).

For phenotypic characterization measurements have been made on the shape, appearance, weight and firmness of the fruit.

To determine the biochemical characteristics of fruit were used following laboratory methods:

- Dry matter content was determined by the method spectrophotometric, whose basic principle determining the refractive index of the sample analyzed.

- The water content was determined according to the standards in force, by the oven drying method to constant mass. The principle of the method is to determine the mass loss by heating in an oven at 103°C to constant weight.

- Determination of total acidity by titrimetric method, the principle of the method is to neutralize the sample to be analyzed with the sodium hydroxide alkaline solution.

- Determination of ash was achieved by calcination at 650°C method; the principle of the method is the determination of residues resulting from the calcination of sample for analysis.

To determine the amount of polyphenols, anthocyanins and flavonoids, tomato fruits were washed, mixed and frozen at -20°C.

- Polyphenol content of tomato fruit Costate 21 was determined by colorimetric method using Folin- Ciocalteu reagent.

- Total amount of flavonoids was determined by the same method as polyphenols content.

- The amount of anthocyanins was determined by the spectrophotometric method, UV-Vis spectrophotometry using.

To maintain plant health demonstration batch placed on a uniform field were experienced following treatment:

V<sub>1</sub> – Teldor 500 S C 0.1%, Bravo 500 S C 0.2%,

V<sub>2</sub> – Dithane M 45 0.25%, Calidan 0.2%,

V<sub>3</sub> – Captadin 50 P U 0.2%, Calidan 0.2%,

V<sub>4</sub> – Funguran OH 50 WP 0.4%, Calidan 0.2%,

V<sub>5</sub> – Alcoholic macerate plant (nettle, sumac, fern);

V<sub>6</sub> – Untreated control.



Figure2. Demonstration lot (original)

Notations were made on the biological actions of treatment to combat blight and gray mold by visual inspection in the field and laboratory.

Attack of the pathogen *Phytophthora infestans* on leaves and fungus *Botrytis cinerea* on the fruit was represented in value by frequency (F%), intensity (I%), and degree of attack (GA%).

Efficacy was based on Abbott's formula:

$$Et = (GA\% \text{ of untreated control} - GA\% \text{ in the variant treated} / GA\% \text{ of untreated control}) \times 100$$

## RESULTS AND DISCUSSIONS

After phenotypic observations (table1) it was found that tomato variety Costate 21 has an indeterminate growth during late fruit ripening and the weight is 250g, registering 3-8 fruits per inflorescence.

After the biochemical analysis (Table 2) it was found that the total acidity was 3.5 g / 100 g fresh material, the ash content was 9.5% polyphenols content was 412 g / 100 g fresh material, the amount of anthocyanins is 136.61 g / 100 g fresh material which demonstrates the variety of tomato Costate 21 is suitable for both fresh consumption and for industrialization.

Maintaining under control of pathogenic populations was achieved by prevention methods (destruction of host plants, leaf removal and plants showing strong symptoms of attack), and curative methods as planned above.

Effectiveness of treatments to combat blight on leaves, respectively mold on tomato fruit variety Costate 21 are listed below (Table 3 and Table 4).

Table 1

## Phenotypic characterization of the variety of tomato Costate 21

Name of the variety	Type of growth	Fruit characteristics					Maturation period
		Appearance	Number of fruits / inflorescence	Weight	Firming	Fruit color	
Costate 21	indeterminate	globular	3-8	250g	Firm	red living	late

Table 2

## Biochemical characteristics of tomato fruit variety Costate 21

Name of the variety	The dry matter content g / 100 g fresh material	The total water g / 100 g fresh material	Total acidity g / 100g fresh material	Ash%	Polyphenols g / 100g fresh material	Total Flavonoids g / 100g fresh material	Anthocyanins g / 100 g fresh material
Costate 21	3.5	95.16	3.8	9.5	412.2	19.6	136.61

Table 3

## The effectiveness of treatments to combat blight on tomato leaf at the variety Costate21

Number	Variant	Active substance	Dose or concentration	F %	I %	GA %	Efficiency
1.	Teldor 500 S C 0.1%,+ Bravo 500 S C 0.2%,	Fenhexamid 500 g/l Clorotalanil 500 g/l	0.1% 0.2 %	40	26	10.4	64
2.	Dithane M 45 0.25%,+ Calidan S C 1.5l	Mancozeb 80% Iprodiane 175 g/l	0.25 % 0.2%	40.86	39.9	16.3	41.36
3.	Captadin 50 P U+Calidan S C 1.5l	Captan 50% Iprodiane 175 g/l + Carbendazin 87.5g/l	0.2 % 0.2 %	33.7	34.06	11.4	58.9
4.	Funguran OH 50 WP 0.4%+, Calidan 0.2%,	Copper metal 50% - Copper hydroxide Iprodiane 175 g/l + Carbendazin 87.5g/l	0.4 % 0.2 %	35.5	22.1	7.84	71.8
5.	Alcoholic macerate plant (nettle, sumac, fern)	Alcool 2%		36.8	30.5	11.2	59.7
6	Untreated control	-	-	44.85	62	27.8	



From the dates related Table 3 compared with untreated control shows that all products tested provided a low level attack blight on the leaves, it is between 7.84% (Funguran OH 50 WP 0.4% Calidan SC 1.5 l) and 16.3% (Dithane M 45 0.25% + Calidan SC 1.5l).

The organic variant (alcoholic macerate) the degree of attack was 11.2% close to that in version V3 (Captadin 50 P U + Calidan SC).

Table 4

The efficacy of treatment in combating gray mold on tomato fruit variety Costate 21

Number	Variant	Active substance	Dose or concentration	F %	I %	GA %	Efficiency
1	Teldor 500 S + C Bravo 500 S C	Fenhexamid 500 g/l Clorotalanil 500 g/l	0.1% 0.2%	33,8	27	9.1	75
2.	Dithane M 45 + Calidan S C 1.5l	Mancozeb 80% Iprodiane 175 g/l + carbendazin 87.5g/l	0.25 % 0.2 %	19.08	26.3	5	86.8
3.	Captadin 50 P U + Calidan S C 1.5l	Captan 50% Iprodiane 175 g/l + Carbendazin 87.5g/l	0.2 % 0.2 %	14	30	4.2	88.9
4.	Funguran OH 50 WP + Calidan S C 1.5 l	Metal copper 50% - Copper hydroxide Iprodiane 175 g/l + Carbendazin 87.5g/l	0.4 % 0.2 %	14	26.2	3.6	90
5.	Untreated control	-	-	46.22	82	37.9	
6.	Alcoholic macerate plant (nettle, sumac, fern)	Alcohol 2%		18	26.4	4.75	87.4

Regarding the degree of gray mold attack leaves (Table 4), it is observed that it ranged from 3.6% in the variant treated with Funguran OH 50 WP Calidan SC 1.5 l and 37.9% in the untreated control variant.

The biological efficacy of the products tested in combating fungus *Botrytis cinerea* on tomato fruit variety Costate 21 were recorded range from 75% to 500 Teldor variant S + C Bravo 500 SC and 90% in the variant treated with Funguran OH 50 WP + Calidan Efficacy nearest by the variant treated with Funguran OH 50 WP + Calidan SC were recorded and treated with variant Captadin 50 PU + Calidan SC 1.5 l and to "bio", treated with herbal macerate.

## **CONCLUSIONS**

In climatic conditions specific to experimental lot, the most valuable experimental plant protection scheme against foliar pathogens proved to be variant V4.

The best Plant Protection of fruit was recorded in the V4 variant.

Variant "bio" where it was used plant extract, to protect both plant leaves and fruits compared to V4 variant.

Prolonged droughts during the fruitfulness of tomatoes prevented destructive pathogens evolved from version control (untreated), to which was obtained a 60% lower production.

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## EFFECTS GENERATED BY SOME PESTICIDES APPLIED WITHIN VITICULTURAL EXPLOITATIONS UPON THE GRAPES' MICROFLORA

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*Keywords: pesticides, microflora, grapes*

### ABSTRACT

*In horticulture, the applied pesticides do generate, as their main effects, the inhibiting or even the destruction, mostly focused upon phyto-pathogenic fungi (in the case of fungicides) and upon insects (respectively for insecticides). For the purpose of ensuring the sanitary safety of all aliments, a very important action is the one of exactly establishing the amount of pesticides' residues which might persist in "dwelling" within horticultural products; this is the reason why the continuous monitoring of such substances is necessary. The present study is dedicated to the effect that some pesticides applied in viticulture might have upon phyto-pathogenic fungi, but as well upon the yeast' strains which are able to perform a fermentative activity.*

### INTRODUCTION

Agricultural exploitations have come to be intensified due to the demographical explosion that has been recorded worldwide, as well as due to the continuous increase of the consumers' expectations concerning the quality level of alimentary products. In the domain of vegetal production, this trend has been followed, among other procedures, through the widely extended and intensive use made of pesticides (both of fungicides and insecticides). To these circumstances also comes to join an always stronger resistance proven by the phyto-pathogenic agents and by the detrimental insects towards the substances employed in order to strive against them.

This is the reason why the scientific research carried on in order to obtain new pesticides could only be developed in a tight touch with the research carried on in the respective domains of: microbiology, phyto-pathology and entomology. In agriculture and horticulture, the applied pesticides, through their cumulative actions, not only increase the soil's pollution level but they might as well act upon some micro-organisms which are useful for the fermentation process.

The most among the studies concerning the effect of pesticides were performed upon the microorganisms that dwell within the soil (Roger 1994; Lo 2010; Oliva 2007). Slayicova E. (2003), in Slovakia, does study the effect exerted by pesticides upon the yeast'

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strains isolated from the agricultural soils. Still in Slovakia, Vadkertiova R. (2011) does publish the results of the researches concerning the effects exerted by pesticides upon the leaves and the fruits that were, previously, bearing colonies of yeast' strains. These researches were performed through cooperations with the Chemistry Institute, with the Yeast' Strains' Collection and with the Academy of Sciences from Bratislava. Throughout the world, there are few researches carried on concerning the influence exerted by pesticides upon the useful microflora.

These few are pointing out the effects exerted by such substances especially upon the yeast' strains involved into the alcoholic fermentation of grapes' must or either upon the ones which, respectively, render possible the fermentation of olives. Should the microorganisms which usually lie upon the grapes' bacca's pellicle skin be inhibited, even for a brief delay, this fact might lead to postponements or deviations occurring in the fermentation process which ultimately may cause noxious effects able to deteriorate the final product. The study of the micro-organisms' resistance towards the action of pesticides has not been, until now, able to attend to the progress level corresponding to the elaboration of a taxonomy drawn by genders or species; therefore, this criterion could not yet be the ground of a microorganisms' repertoire.

#### MATERIAL AND METHODS

The studied biological material was represented by grapes belonging to the Cabernet Sauvignon kind, cultivated within the viticultural center of Stârmina, owned by Vinarte S.A.

In order to evaluate the effect of some pesticides upon the grapes' microflora, we have studied both grapes upon which pesticides were applied and untreated ones, the latter constituting the witness sample in respect to which the comparison was made. All clusters were harvested from the same plantation, thereby uniformity being preserved in what concerns the climate conditions and the biological features. Before pesticides were applied, the grapes from the witness sample were carefully isolated so that they could never be in contact with the pesticides.

The samples were harvested immediately after the pesticides were applied at the half of the remanence period. The bags and instruments employed for the harvesting operation were sterile. The samples were immediately transported to the laboratory, where they were submitted to the microbiological analysis. The applied pesticides were: Funguran OH 50 WP combined with Pyrus 400 SC and with Cosavet 80DF.

The product Funguran OH 50 WP is a contact fungicide of which the main effect is targeted towards the fungus *Plasmopara viticola*, which is responsible for the appearance of blight at vine. Pyrus 400 SC is a systemic fungicide the effect of which is targeted towards the *Botrytis cinerea* mildew which is responsible for causing losses in quantity and quality to the grapes' harvest. The active mechanism of this fungicide consists in inhibiting some enzymes that the fungus needs in order to carry on with the grapes' infection process.

Cosavet 80 DF is a fungicide which is administrated as a preventive treatment in order to strive against the mildew's extension. The three pesticides were administrated in a combined way, through an one and only treatment session, executed on the day of August 20-th, that is to say about 40 days before harvest. At the moment of the treatment's administrating, the grapes had already accumulated 150 g/l of sugars, an amount which corresponds to a clear evidence of the fact that they were already populated with the micro-organisms usually involved in the fermentative processes.

In order to evaluate the effects caused by these products upon the grapes' microflora, at the time intervals we have mentioned above we also performed micro-

organisms' isolations directly from the grapes' epidermis (skin). Isolations were performed through the use of sterile sanitation swabs. The surface extent wherefrom isolations were performed was of 1 cm<sup>2</sup>. This area was wiped out with the sanitation swab in three directions: horizontally, vertically and obliquely, without lifting the swab from the investigated surface. The employed culture's environment was one that is usual for fungi, namely YMA. Incubation was performed at 25°C, for a duration of 48 hours. When the incubation time was concluded, the developed colonies were submitted to quantitative and qualitative determinings.

## RESULTS AND DISCUSSIONS

The distribution of microorganisms upon the grape' surface is not uniform in respect to their types neither to their numerical amounts; therefore, some of them may be predominant over the others. Should the applied pesticides act upon the useful microorganisms which, numerically, might be less represented, then, necessarily, some other species would be able to better resist and, therefore, to metabolize the existing substratum.

Due to their intrinsic enzymes' endowment, the micro-organisms do dispose of many such "tool" enzymes through which they are able to biologically deteriorate some among the substances that can be found within pesticides; this action does allow them to survive upon the surfaces of agricultural and horticultural products. G. Gallardo and coll. (2012) have demonstrated that some microorganisms do exist which are, by their own deed, able to deteriorate the pesticides' residues, thereby preventing these latter from accumulating within the fermented products. In our country, analogous researches concerning the biological degradation of pesticides by the natural microorganisms have been performed by a collective of scientists from the "Al.I. Cuza" University in Iasi, in co-operation with the U.A.S.V.M. from Bucharest (Zaharia M., 2013).

From the analysis of the results obtained due to their insemination within an usual environment, we have noticed that, subsequently to the administrated treatment (which involved the three products: Funguran, Cosavet, Pyrus) a large number among the mildews were inhibited, while the yeast' strains have gone on developing themselves very well. Each of the inseminated plates has been submitted to analysis in the matters of quantity and quality; thereby we have established the number of the units wherefrom colonies were formed, as well as the respective types of the micro-organisms which have generated these colonies. For the quantitative analysis, the colonies' counting up was performed through the FG-type reckoning device with which the laboratory of microbiology is endowed.

The qualitative analysis has consisted in establishing the exact number of the existing type-shaped colonies which, afterwards, were submitted to the usual identification tests. Thereby we have established the exact amounts' percentages for each of the identified kinds of colonies. In Table 1 are presented, as a synthesis, the existence ratios of microorganisms upon the skins of the treated and untreated grapes, at 15 days since the treatment was applied. Images 1 and 2 present some macroscopic views of the colonies developed within an usual environment by the microorganisms isolated from the grape' surface, consequently to their insemination.

The applied treatment has reduced a lot the presence of mildews; we have been able to isolate from the grape' surface scarcely few of them only; therefore, it has been proven that a lot of space was left for the yeast' strains to develop upon. From the table's data let us notice the fact that, among the mildews, the kinds of *Alternaria* and *Aspergillus* have been the most sensitive towards the applied treatment, while the species of the *Penicillium* kind have been resisting more. Insofar is concerned the presence of yeast' strains, let us remark that they have successfully resisted to the treatment, this fact

demonstrating that the fermentative activity is not at all endangered by the pesticides applied in viticulture. In the untreated sample, the small amount of yeast' strains may be explained through the fact that the mildews, as they had expansively grown, had consequently inhibited the formers' development. The quantitative analysis has allowed us to establish the respective percentage amounts of yeast' strains and of mildews which were present in the two types of samples-the treated and the untreated ones (figures 1 and 2).

As a conclusion, we may say that the applied treatment did have a very strong effect upon mildews. The second stage of our study had the purpose of determining the amounts of micro-organisms that were present upon the grape' surface after the remanence period was concluded, that is to say 15 days since the treatment was applied.



**Photo 1. Macroscopic views of the colonies by the microorganisms isolated developed within an usual environment from the grape' surface untreated**



**Photo 2. Macroscopic views of the colonies by the microorganisms isolated developed within an usual environment from the grape' surface treated**

Table 1

**Kinds of microorganisms which exist upon the surface of the skins of Cabernet Sauvignon grapes, cultivated in the viticultural center of Stârmina, 10 days after the treatment was applied**

Sample	Kinds of microorganisms								
	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium expansum</i>	<i>Penicillium verucosum</i>	<i>Candida</i>	<i>Debbera</i>	<i>Kloockera</i>	<i>Pichia</i>	<i>Sakharo-Myces</i>
Untreated	+	+	+	+	+	+	+	+	+
Treated	-	-	+	+	-	+	+	+	+

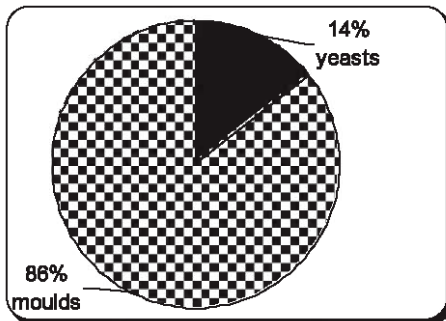


Figure 1. Ratios between yeast' strains and moulds (untreated sample)

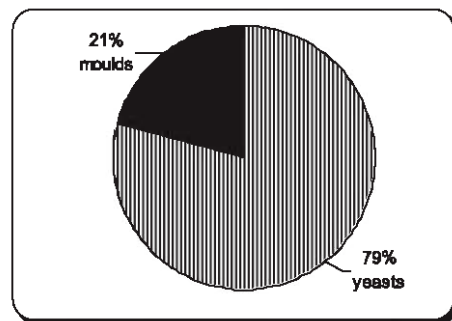


Figure 2. Ratios between yeast' strains and moulds (treated sample)



Photo 3. Macroscopic views of the colonies by the microorganisms isolated developed within an usual environment from the grape' surface untreated – after 15 days



Photo 4. Macroscopic views of the colonies by the microorganisms isolated developed within an usual environment from the grape' surface treated - after 15 days

We must also precise the fact that the concerned interval was highly rich in precipitations, therefore the excessive humidity has especially favoured the development of mildews. Determinings were made in parallel for the treated and the untreated samples. Images 3 and 4 present some macroscopic views of the colonies developed within an usual environment by the microorganisms isolated from the grape' surface.

As we are able to remark, mildews have developed in both cases, but, as far as the treated grapes are concerned, the presence of mildews upon them was reduced, both in the matters of their amounts and in the ones of their quality levels. The results which concern the quality are presented in Table 2.

From the data of this table we can notice that the micro-organisms which were present during the previous stage of our study could as well be found after 15 days. Furthermore, we then found as developed the kind of *Fusarium*, which, initially, was not there at all. The applied treatment was efficient upon the mildew of the *Aspergillus* kind.

Table 2  
Kinds of microorganisms which exist upon the surface of the skins of Cabernet Sauvignon grapes, cultivated in the viticultural center of Stârmina, 15 days after the treatment was applied

Sample	Kinds of microorganisms								
	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium expansum</i>	<i>Penicillium verucosum</i>	<i>Fusarium</i>	<i>Candida</i>	<i>Kloeckera</i>	<i>Pichia</i>	<i>Sakharomyces</i>
Untreated	+	+	+	+	+	+	+	+	+
Treated	-	-	+	+	-	-	+	+	+

Figures 3 and 4 present the ratios between yeast' strains and mildews within the two samples.



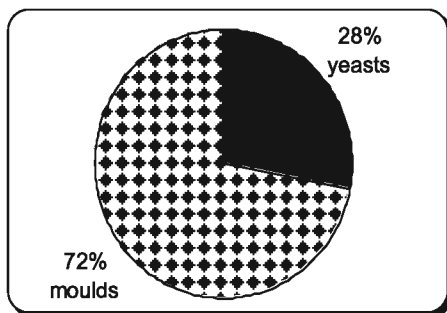


Fig.3. Ratios between yeast' strains and moulds (untreated sample) - after 15 days

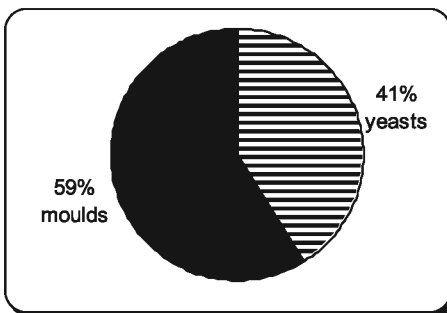


Fig.4. Ratios between yeast' strains and moulds (treated sample) - after 15 days

From the graphs we can notice the fact that, within the untreated sample, the amount of yeast' strains has increased in comparison with the previous stage, while in the case of the treated sample it is the amount of mildews that has grown. This fact can be explained through the decreasing of the product's remanence period and through the abundant level of precipitations.

### CONCLUSIONS

In order to obtain some healthy viticultural harvests, it is imperative to apply a large number of treatments within the vegetables', trees' and viticultural exploitations, treatments which should have effects that ought to be immediate, selective and with a brief remanence. Therefore, inside the finite product, the consumer should no more find the applied pesticides, not even under the form of traces.

The fact that the detrimental items (insects, phyto-pathogenic fungi) have increased their resistance towards the treatments applied versus them does impose a permanent trend of research in the domain of pesticides; thereby, the sortiments circulating on the market are incessantly changing. However, the research in the domain of pesticides' chemistry ought to be compulsorily completed with the research in micro-biology, so that each of the accessory (and explanatory) leaflets should contain the precision made if the respective product is or not effective upon the micro-organisms that are able to perform a fermentative activity.

When the treatment with Funguran, Cosavet, Pyrus, was applied within a viticultural exploitation, the obtained results have demonstrated the fact that the respective combination proves itself to be the most effective upon mildews; insofar the yeast' strains are concerned they keep developing themselves well even after this treatment was applied.

After the conclusion of the remanence period, some among the inhibited mildews have continued to develop. This fact was due to the simultaneously exerted effects of, on one side, the pesticides' brief remanence time and, on the other side, of the excessive humidity that has been recorded during the concerned period.

The applied pesticides had no inhibiting effects upon the yeast' strains that were present upon the grapes; on the contrary, these latters were, indirectly, stimulated in their development, due to the mildews' inhibiting or destruction.

### ACKNOWLEDGMENT

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## SECONDARY DISMETABOLIC AFECTIONS OF OVERWEIGHT AND OBESITY

Carmen Vlădulescu<sup>1</sup>

**Keywords:** obesity, overweight, metabolism.

### ABSTRACT

*Obesity is a public health problem affecting more developed or developing countries. The increasing incidence has features pandemic emergency and requires special attention because of the potential morbidity and mortality associated. Obesity is characterized through a storage of excess body fat. The term defines overweight body weight (mass weight) which corresponds to the height of the person.*

*Overweight and obesity are leading risks for global deaths. Around 3.4 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity.*

### INTRODUCTION

Last decade is characterized by a worrying rise in obesity among both adults and children. Morbidity and mortality associated with obesity has reached alarming levels.

Etiopathologically speaking, obesity is a very complex disease, many factors are involved in determining obesity: genetic factors, environmental factors, metabolic factors, psychological factors, behavioral factors, neuroendocrine factors. The combination of the various risk factors and pathogenic mechanisms in different proportions and fragmentary in each child is affected by excess weight causes obesity to be a highly heterogeneous disease. In terms of pathological hypertrophy and hyperplasia fat explain weight gain (Predu M., 2011).

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health.

Obesity is the most common metabolic disease and is often associated with severe chronic diseases such as cardiovascular disease, diabetes, apnea, etc. It is characterized by an excess of body fat. Normally male body contains a proportion of 15-20% of adipose tissue and the body women a share of 25-30% adipose tissue.

We know two basic models of body fat distribution in obese individuals:

- abdominal obesity (android type) with two components,
- visceral and subcutaneous hips obesity (gynoid).

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The World Health Organization has defined and classified levels of obesity based on BMI - body mass index, calculated by the weight / height as follows:

-grade 1 obesity with body mass index of between 30 to 34.9 kg / m<sup>2</sup>

-grade 2 obesity with body mass index of between 35 to 39.9 kg / m<sup>2</sup>

-grade 3 obesity with body mass index equal to or more than 40 kg / m<sup>2</sup>.

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended.

Globally, there has been:

- an increased intake of energy-dense foods that are high in fat;
- an increase in physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing, distribution, marketing and education (Mincu I., 2001).

WHO has developed the "Global Action Plan for the prevention and control of non-contagious diseases between 2013-2020" which aims to achieve the commitments of the UN Political Declaration on NCDs which was endorsed by Heads of State and Government in September 2011. This action plan aims to build on the WHO Framework Convention on Tobacco Control and the WHO Global Strategy on Diet, Physical Activity and Health. The action plan will contribute to progress on global NCD targets to be attained in 2025, including a 25% relative reduction in premature mortality from NCDs by 2025 and a halting of the global obesity rates to those of 2010.

The diagnosis of obesity is simply based on physical examination. It will measure weight, height, waist circumference, and BMI is calculated. These parameters can be measured at regular medical checks at each medical advice in all specialties and at the request of those concerned about their weight status (Moța M., 1998).

Practical approach to the person with obesity involves among others: screening, diagnosis, assessment and setting goals of care, clinical management of obesity / overweight, complications and comorbidities (THEMES methods) and psychological and environmental optimization (Dincă & Moța., 2010).

## **MATERIAL AND METHODS**

Estimating the prevalence of DM and prediabetes in the population of Romania.

Estimating the prevalence of overweight and obesity in the adult population of Romania.

Estimating the prevalence of the chronic kidney disease in the adult population of Romania.

PREDATORR study aims to estimate with reasonable accuracy the actual prevalence of diabetes, the BCR and associated comorbidities (prediabetes, obesity, overweight, dyslipidemia, hyperuricemia, hypertension, metabolic syndrome) in the population.

PREDATORR Study is conducted according to the applicable ICH/GCP standards and WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, approved in *Seoul, Korea, October 2008*.

Since 2009, CEBIS International holds an ISO 9001:2008 Certification for the Development of Clinical Research Projects, Project Management, Monitoring and Consulting in Clinical and Epidemiology Research ([www.predator-study.com](http://www.predator-study.com)).

CEBIS also holds an ISO 27001:2005 Certification regarding the Security and Management of Information implemented at the company level.

The study of the frequency of overweight and obesity and the frequency distribution on the pre-specified subgroups in the adult population was performed covering three administrative regions (South, South-West, Bucharest-Ilfov) of Romania.

Evaluation of the prevalence of different metabolic phenotypes of obesity and overweight in the mentioned population group was observed during two years of research.

### RESULTS AND DISCUSSIONS

The present analysis comprises 972 subjects (569F/ 403M; 55.4 ± 14.1 years) from three administrative regions:

- 38.8% from South region,
- 33.3% from South-West region,
- 27.7% from Bucharest-Ilfov County.

The results of the research are presented in the following figures.

In figure number 1 it is graphically presented the ratio of obesity, as well as the incidence of abdominal obesity.

Final results of the epidemiologic study PREDATORR shows that over 80% of Romania's adult population aged 20-79 years have blood lipid abnormalities, while over 2.6 million Romanian, aged between 20-79 years have hyperuricemia (syndrome characterized by increasing the level of uric acid in the blood).

### BMI AND ABDOMINAL OBESITY CATEGORIES

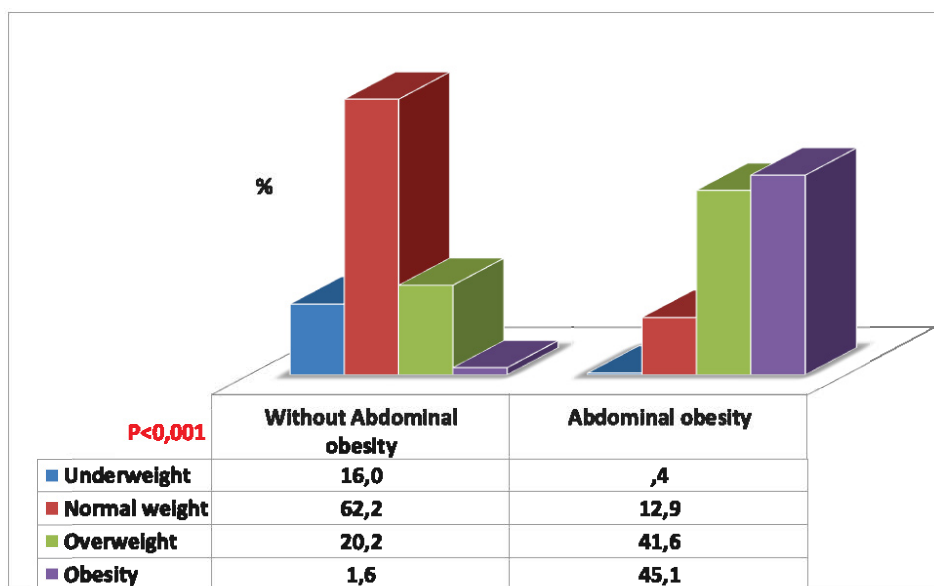


Figure 1. Graphical presentation of BMI and abdominal categories

From the people who have normal weight, 12.9% of them have abdominal obesity, and from the people who have obesity, 45.1% of them have obvious signs of abdominal obesity, and only 1.6% from the subjects does not have abdominal obesity.

In figure number 1, there can be observed that from the underweight people only 0.4 % of the subjects have abdominal obesity.

### Anthropometric parameters distribution on BMI and Abdominal obesity categories

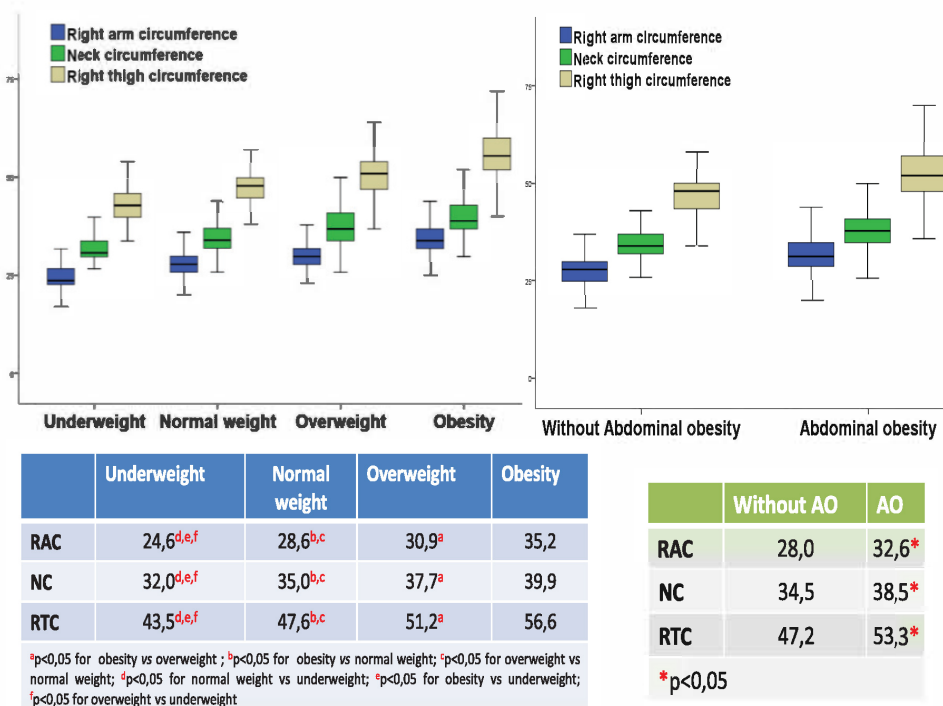


Figure 2. Anthropometrical parameters distribution on BMI and Abdominal obesity categories

The values of the main anthropometric parameters are correlated – right arm circumference, neck circumference and right thigh circumference – in order to show the incidence of obesity, overweight as well as normal weight.

From the subjects who had anthropometric parameters – RAC – within limits, only 32.6% presented abdominal obesity, compared to the subjects with almost normal RTC values, from whom 53.3 % presented abdominal obesity.

The results indicated that there was significant relationship between all metabolic traits and BMI categories

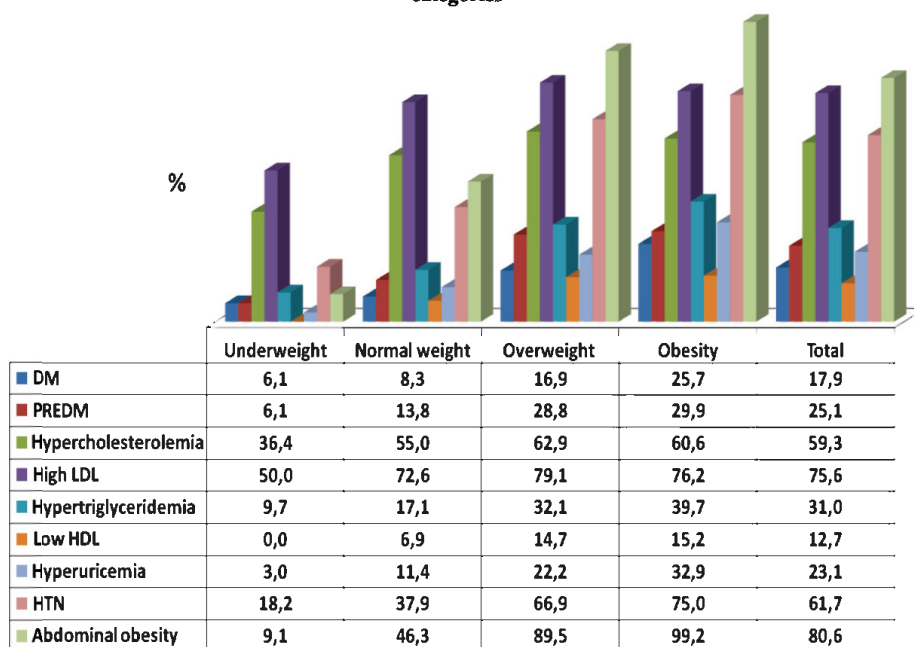


Figure 3. BMI categories and metabolic disorders

In figure number 3 there is presented the ratio between the main metabolic disorders and body weight.

From the subjects who suffer of hypertriglyceridemia 9.7 % of them are underweight, 17.1 % have normal weight and 39.7 % of them have obesity. From the subjects who have high values of LDL, 50% they are underweight, 72.6 % of them have a normal weight and 79.1 % of them are overweight.

From the subjects who have abdominal obesity, 9.1 % of them are underweight, 46.3% of them have a normal weight and 99.2 % of them have obesity.

There is no "quick fix" for obesity. Weight loss programmes take time and commitment, but they work best when people are able to complete the programmes fully and are offered advice about maintaining the weight loss achieved.

Regularly monitoring your weight, setting realistic goals and involving your friends and family with your attempts to lose weight can also help.

Remember that even losing what seems like a small amount of weight (such as 3% or more of your original body weight), and maintaining this for life, can significantly reduce your risk of obesity-related complications like diabetes and heart disease.

## CONCLUSIONS

The results indicated that there was significant relationship between all metabolic traits and BMI categories.

The presented preliminary data indicate a high frequency of overweight and obesity, in the studied regions (South, South-West, Bucharest-Ilfov).

A higher frequency of obesity and overweight was registered after age of 40 years especially in females.

Obesity, overweight and abdominal obesity phenotypes were associated with more pronounced insulin resistance, glucose metabolism impairment, dyslipidemia, arterial hypertension and hyperuricemia.

Studies of the public health in Romania must deal with the detection of metabolic diseases.

For all subjects, their role in the prevalence assessment of a major disease of the present has an outstanding contribution to the improvement of the public health from the perspective of society as a whole.

Providing primary prevention measures is very important in the future.

A more accurate quantification of the prevalence of metabolic disorders is important for a rational allocation of human and financial resources.

For further information, visit the study website: [www.predatorr-study.com](http://www.predatorr-study.com).

#### **ACKNOWLEDGMENT**

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**THE INFLUENCE OF TEMPERATURE ON ACETIC LIMIT GROWTH  
OF BACTERIA IN A WHITE WINE (TYPE) WITH AN ALCOHOL  
CONCENTRATION OF 10.5 VOL%**

Carmen Vlădulescu<sup>1</sup>, Ramona Căpruciu<sup>1</sup>

*Keywords: temperature, acetic bacteria, viable cells, white wine*

**ABSTRACT**

*The optimum temperature for growth of acetic bacteria is in the range of 25 - 30°C (Holt et al. 1994). In general, there were no increases in bacterial population above the 37°C, but some researchers were able to isolate several termo-tolerante strains at 37 - 40°C (Saeki et al. 1997).*

*At lower temperatures, acetic bacteria may still be active, for example in the wine kept at 18°C (Joyeux et al. 1984), but could not be detected at 10°C. As a result, it is difficult to draw a strict temperature range in which these bacteria can develop (Drysdale and Fleet, 1988), the temperatures used during the winemaking process seemed not to affect the growth of acetic bacteria.*

**INTRODUCTION**

Temperature is a determining factor. Vinegary process progressing faster as the temperature is higher; volatile acid alteration is made to the beginning of the two-fold higher than 23°C to 28°C and at 23°C twice as fast than 18°C.

Wine lactic bacteria belonging mezofili grow very well at temperatures between 25 - 35°C. The wine temperature lower limit would seem to be 5°C, but it is most often between 9 - 15°C. The optimum temperature of lactic acid bacteria in wine varies from 20°C to 28°C; upper limit temperature is about 35°C (Teodorescu et al. 1987). Temperature influence over the speed limit than the fermentation temperature is higher as the growth is more rapid at first, but the production of the population and the acid are usually slightly different from 15 - 30°C, and even 35°C.

Hull are generally less sensitive than the bacilli temperature conditions between 15 - 25°C. Action fran of pH - down or the presence of alcohol on the development of lactic acid bacteria at high temperature is more intense than at 15°C. Under heavy malolactic fermentation, the low temperatures of the order of 15°C are most favorable.

A malolactic fermentation primed to 15°C continuous and 12°C often slow or 10°C. At higher temperatures (39°C), lactic acid bacteria are becoming more demanding in terms of nutrition. Nutritional factors needs to be high temperature or low pH, on the other hand to be examined on the basis of a possible deficiency in carbon dioxide. When the atmosphere above the culture is rich in carbon dioxide, the culture grows abundantly at 39°C become less picky about food.

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## MATERIAL AND METHODS

We followed the activity of bacteria in the first 10 days of the start of fermentation.

The material used for the experiments consisted of:

- White wine 10.5 vol% alcohol, pH = 3.5, obtained from grapes coming from vineyards Segarcea center. The thermostat was also an indispensable device of our research.

- Viable cells

Analytical methods were related to:

- Determination of sugar in the grapes
- Determination of the alcoholic strength of wine
- Determination of acetic acid in wine
- Determination of sulfur dioxide dosage microbiological examinations.
- Determination of wine temperature and the environmental temperature.

## RESULTS AND DISCUSSIONS

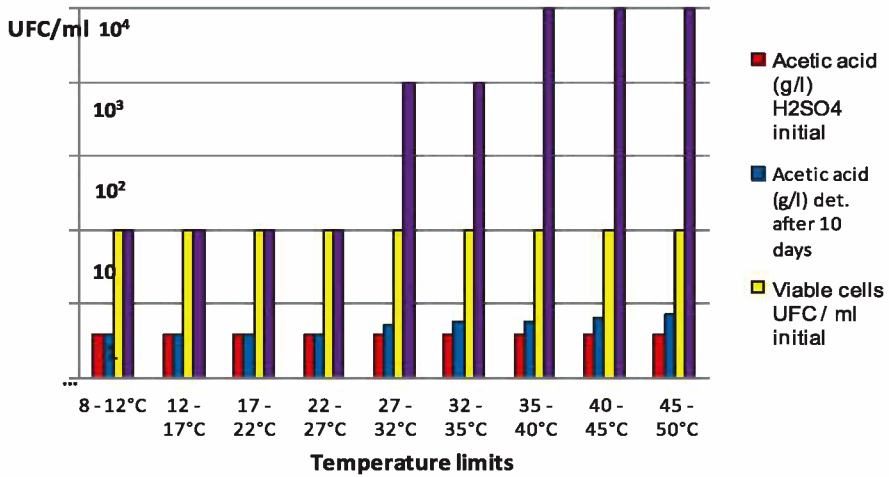
Experiments that used a white wine (range) of an alcoholic strength of 10.5% alcohol by volume, pH 3.5 and 10 mg SO<sub>2</sub> providing free/liter (table 1 and Charts 1 and 2) reveal that when storage temperature is maintained between 8 and 12°C, acetic bacteria population increases and as a result knows there is no record increases of acetic acid content of the wine, as a consequence of metabolic activity as an acetic bacteria that have maintained during the 10 days same load 10 C.F.U / mL of wine (Vlădulescu Carmen, 2010).

Relatively similar results are seen for the storage of wine at temperatures of 12 - 17°C, when acetic bacteria although the number of cells does not increase (remain 10 C.F.U/mL wine) there is still a slight increase in acetic acid content in wine 0.38 g / L (initial) to 0.59 g/L (H<sub>2</sub>SO<sub>4</sub>) by 10 (ten) days.

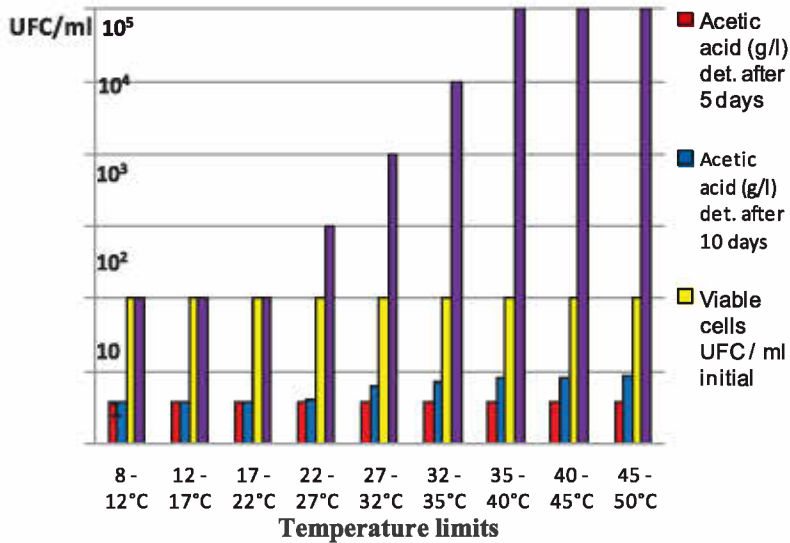
Table 1

The influence of temperature on acetic limit growth of bacteria over a period of 10 days, in a white wine (type) with an alcohol concentration of 10.5 vol% alcohol, pH = 3.5

Temperature limits (°C)	Acetic acid content in wine (g/L) H <sub>2</sub> SO <sub>4</sub>	Initial number of viable bacterial cells UFC/ml	Determinations after .....days			
			5		10	
			Acetic acid (g/L) H <sub>2</sub> SO <sub>4</sub>	The number of viable bacterial cells UFC/mL	Acetic acid (g/L) H <sub>2</sub> SO <sub>4</sub>	The number of viable bacterial cells UFC/mL
8-12	0.38	10	0.38	10	0.38	10
12-17	0.38	10	0.38	10	0.38	10
17-22	0.38	10	0.38	10	0.38	10
22-27	0.38	10	0.38	10	0.38	10
27-32	0.38	10	0.46	10 <sup>2</sup>	0.52	10 <sup>3</sup>
32-35	0.38	10	0.47	10 <sup>2</sup>	0.58	10 <sup>3</sup>
35-40	0.38	10	0.50	10 <sup>3</sup>	0.59	10 <sup>4</sup>
40-45	0.38	10	0.52	10 <sup>4</sup>	0.66	10 <sup>4</sup>
45-50	0.38	10	0.59	10 <sup>4</sup>	0.72	10 <sup>4</sup>



Graphic 1. The influence of temperature on acetic limit the growth of bacteria over a period of 10 days, a white wine (type) with an alcohol concentration of 10.5 vol% alcohol, pH=3.5 and 10 mg / L free SO<sub>2</sub> - Determination after 5 days



Graphic 2. The influence of temperature on acetic limit the growth of bacteria over a period of 10 days, a white wine (type) with an alcohol concentration of 10.5 vol% alcohol, pH=3.5 and 10 mg / L free SO<sub>2</sub> - Determination after 10 days

## CONCLUSIONS

It is important to know, in oenological practice, the optimum growth temperature for the acetic acid bacteria is in the range of 25 - 35°C.

At lower temperatures acetic bacteria are still active and 10°C as well. Therefore it is difficult to draw a strict temperature range in which these bacteria can grow, temperatures used during the wine making process seem not to affect the growth of acetic bacteria.

The acetic growth limit of bacteria is influenced by the temperature. Temperatures of 8-12°C maintained during storage of white wines regardless of their alcohol concentration may maintain vital processes in place - metabolism of acetic bacteria.

Temperatures above 12°C and up to 45 - 50°C stimulate the multiplication of acetic bacteria and increase the metabolic processes by which wines are rich in acetic acid content making them unacceptable for consumption.

In all cases, regardless of alcoholic white wines, maintained at 60 - 65°C for 2 to 10 minutes acetic bacteria cells are inactivated.

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**PLANT DENSITY AND ADD-ON FERTILIZATION EFFECT ON CROP MORPHOMETRICS, CHEMICAL CHARACTERISTICS AND ANTIRADICAL ACTIVITIES OF *MENTHA PIPERITA* UNDER GREEK ENVIRONMENTAL CONDITIONS**

Eleni Wogiatzi<sup>1</sup>, Nikolaos Gougoulias<sup>1</sup>, Alexandros Papachatzis\*<sup>1</sup>, Dimitrios Kaltsas<sup>1</sup>

**Keywords:** *Mentha piperita*; sucker; total phenolics; antiradical activity DPPH; essential oil.

**ABSTRACT**

*In this work, spatial plant density and fertilization level effect were studied on plant height, dry drogue yield, essential oil production, total phenolics content and antioxidant activity of M. piperita leaf. Plant density increased crop height and expand arithmetically suckers production. Fertilized crops with spatial density of 4 plants /m<sup>2</sup> demonstrated greatest average plant height while crops with plant density of 2 plants/ m<sup>2</sup> maximized their sucker production independently of their fertilization scheme. In terms of dry drogue yield and leaf essential oil content comparison between the two seasonal harvests (early June and September), the first harvest revealed higher delivery quantities for both substance categories than second one. Analytically estimated concentration of total phenolics of the Mentha piperita leaf ranged of from 27.37 to 58.43 mg g<sup>-1</sup> and the antiradical activity ranged from 18.01 to 38.73 μmols DPPH g<sup>-1</sup> dry weight. The correlation between total phenolics and antiradical activity appear to be statistically strong (r<sup>2</sup> = 0.9226).*

**INTRODUCTION**

*Mentha piperita*, family *Lamiaceae*, is a hybrid derived from a cross between *Mentha aquatica* and *Mentha spicata*. It is a perennial plant with erect stems which reaches to 80 cm height and develops numerous suckers (Marquard et. al. 2001).

Different tissues and organs of *Mentha* spp. and other related medicinal plants contain provide various chemical and structural composition chemical substances with diverse biological and healing properties (Pokorny et. al. 2001; Wojdylo et. al. 2007). In the last years, many studies were focused on evaluation of polyphenols composition and antioxidant activities of *M. piperita* and of other medicinal plants (Gougoulias N. 2009; 2012a; 2012b).

Polyphenols are well-known as one of the most widely spread secondary metabolites in edible and non-edible plants (Harborne et. al. 1980; Macheix et. al. 1990). Concerning their use, they counteract to strong oxidizers which damage protective biomolecules of biological systems related to inflammatory processes (Halliwell et. al. 2007) and lipid cellular management (Halliwell et. al. 2007).

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The essential oil of the *Mentha piperita* is used in cosmetology and food industry; however, there are no locally established cultivational standards related to environmental conditions, cropping system protocols and season of collection (Dachler et. al. 1999; Schneider et. al. 1999; Teuscher et. al. 1997; Galle-Hoffmann et. al. 1998).

Therefore, the aim of this work was to examine the effect of spatial plant density and fertilization level, in some plant morphologic characteristics, in dry drogue yield in essential oil production, in total phenols content and in antioxidant activity of *M. piperita* leaves.

## MATERIALS AND METHODS

The experiment was conducted at the Technological Education Institute (TEI) of Thessaly, Laboratory of Medicinal and Aromatic Plants, between March and September 2012, for two successive cultivation periods. The experiment had a randomized block design with two plant density level (2 and 4 plants per m<sup>2</sup>), two fertilization level (with and without) and two harvest times (June and September). There were four replicates per treatment combination.

Seedlings of *Mentha piperata* were established in silt loam soil (SiL) which has improved with blond peat moss addition. During growing season 37.5 Kg-N, 37.5 Kg-K<sub>2</sub>O and 37.5 Kg-P<sub>2</sub>O<sub>5</sub> per hectare were incorporated into the soil. Plants were irrigated twice weekly with low salt irrigation water (0.48 dS.m<sup>-1</sup>). Weeds were control mechanically, while insect infestation prevented by applying a solution of 20 g green soap and 5 ml of alcohol per liter of water on the plants.

Plants were harvest at the beginning of blossom period, where done according to (Marquard et. al. 2001) when essential oils concentration is maximized, by cutting plant shoots 10 cm above soil surface. Plant leaves were dried in the dark at room temperature, finely ground and kept at 4°C.

**Preparation of the methanol extracts:** Five hundred (500) mg of the finely ground leaf samples were two rounds treated by 20 ml of 80% aqueous methanol. Samples were incubated for 24 h in the extractant at stirring; the supernatant material was removed. The pellet was re-treated with aqueous methanol for 2 h at stirring at ambient temperature. The extract was gathered after centrifugation/filtration and the volume was made up to 50 ml with aqueous methanol.

**Determination of Total polyphenolics (TP):** Total polyphenolic content was determined with the Folin-Ciocalteu (F-C) reagent according to the method of Singleton and Rossi (Singleton VL, 1965) using the microvariant proposed by Baderschneider et al. (Baderschneider B, 1999) and the results were expressed as gallic acid equivalent (GAE) in mg/g dry weight.

**Determination of antiradical activity (DPPH<sup>•</sup> assay):** The antiradical activity of the methanol extracts was determined on the basis of the method of Brand – Williams (Brand-Williams et. al. 1995) using the stable free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>), as a reagent. The activity was expressed in μmols DPPH<sup>•</sup> / g dry weight.

**Extraction of the essential oil:** Oil was extracted by hydrodistillation using a Clevenger type apparatus. The duration of this procedure was 2 hours. The yield (v/w) of the obtained essential oil expressed as a percentage of absolute dry weight.

**Statistical analysis:** Data were analyzed using the MINITAB (Ryan et. al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using the standard error of differences at the 5% level when significant differences between varieties were found.

## RESULTS AND DISCUSSIONS

Plant height was increased during cultivation periods in all treatments (Figure 1). At harvest time, plant density increased average plant height affected by fertilization level (Table 1). In treated with fertilizer blocks and spatial density of 4 plants /m<sup>2</sup>, plants had greater average height compared to other treatments. No statistical differences were observed in plant height between treatments with or without fertilization treatment.

Similarly Mansoori (Mansoori I., 2014), observed elongation of plant stem at high planting density that might be caused by the competition for space and light interception.

Table 1  
Mean plant height and suckers number of *M. piperita* plant at the first harvest

	Without fertilization		With fertilization	
	Mean plant height	Mean number of suckers / plant	Mean plant height	Mean number of suckers / plant
4 plants / m <sup>2</sup>	59 ± 1.24	2.88 ± 0.18	62.25 ± 0.95	3.75 ± 0.23
2 plants / m <sup>2</sup>	59 ± 1.08	5.44 ± 0.55	57.25 ± 2.69	7.0 ± 0.42

Data represent average means and SE deviation. (n) = 4

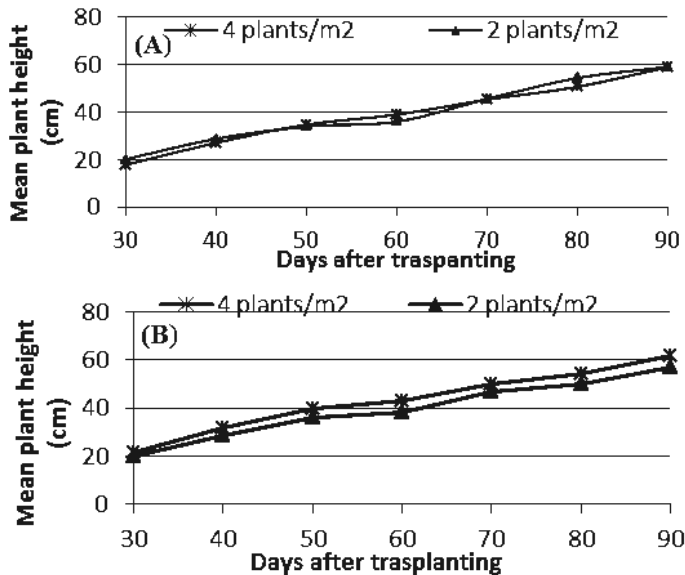


Figure 1. Mean variation in plant height of *M. piperita* according to plant density level: (A) without fertilization, (B) with fertilization.

In both harvest times (June and September) dry drogue yield per plant was greater in fertilized plots with spatial density of 2 plants/m<sup>2</sup> (Table 2). Comparing parametrical yields per harvest time, dry drogue production per plant was higher in the first harvest than in the second one. The above data are in full accordance with Mansoori (Mansoori I, 2014), where peppermint dry biomass show maximum yield in first harvest.

Fresh to dry drogue ratio was 2.8/1. Plant density and fertilization level had no effect in plant leaf essential oil production. However harvesting time significantly affected essential oil content. Maximum essential oil content was obtained at first harvest time. The

better whether conditions from spring to early summer and the relatively short time of re-growth until second harvest contribute to higher oil production at first harvest time (Mansoori 2014; Valtcho et. al. 2010).

Table 2

Dry drogue and essential oils yield of *M. piperita* leaves

Treatments	First harvest		Second harvest	
	Dry mass (g) / plant	Essential oil (ml) / 100 g dry mass	Dry mass (g) / plant	Essential oil (ml) / 100 g dry mass
4 plants / m <sup>2</sup> without fertilization	21,97 ± 1,89	3,2	14,25 ± 1,60	3,0
2 plants /m <sup>2</sup> without fertilization	20,38 ± 4,20	3,1	7,44 ± 1,36	2,8
4 plants / m <sup>2</sup> With fertilization	26,25 ± 1,97	3,2	14,50 ± 1,68	3,1
2 plants / m <sup>2</sup> with fertilization	39 ± 1,68	3,5	16,19 ± 2,56	2,8

Data represent average means and SE deviation. (n)=4

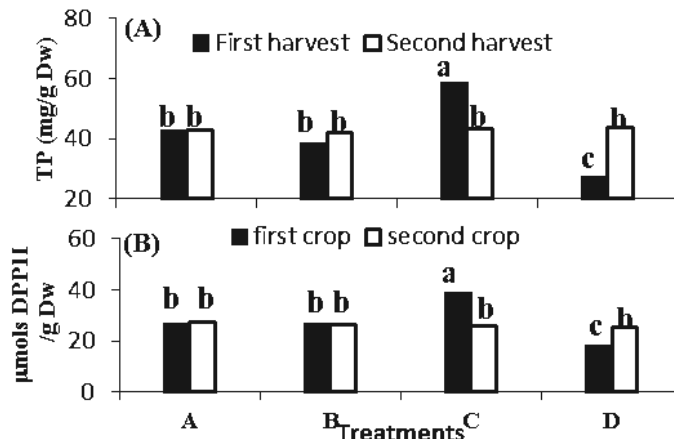


Figure 2. Total phenolic content (A) and antiradical activity DPPH<sup>\*</sup> (B) of *M. piperita* leaf according to plant density and fertilization level. A: 4 plant/m<sup>2</sup> without fertilization; B: 2 plant/m<sup>2</sup> with fertilization; C: 2 plant/m<sup>2</sup> without fertilizer and D: 2 plant/m<sup>2</sup> with fertilization. The letter above each bar gives differences between varieties according to Tukey test (P=0.05).

Differences in the total phenolics content and antiradical activity DPPH<sup>\*</sup> of *M. piperita* leaves were found according to spatial plant density, fertilization level and harvest time (Figure 2). The greatest and lowest total phenolic concentration as well antiradical activity DPPH<sup>\*</sup> was measured at first harvest time, in plants at wide density planting system (2 plants/m<sup>2</sup>), without and with fertilization respectively. Strong correlation ( $r^2= 0.9226$ ) was found between total phenolics content and antioxidant capacity of *M. piperita* (Figure 3).



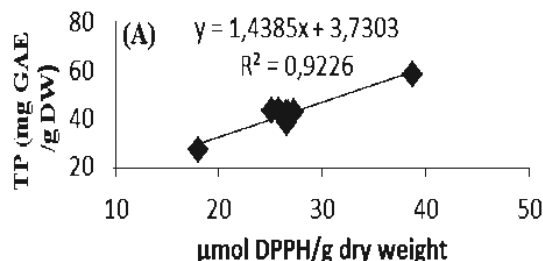


Figure 3. Correlation data analysis between antiradical activity and total phenolic content (GAE) in *M. piperita* leaf

Studies concerning the total content of phenols and antioxidant activity in 5 species of peppermint (*M. piperita* L., *M. pulegium* L., *M. rotundifolia* L., Huds. and *M. spicata*) have shown that the *M. piperita* L., has a higher content of total phenols and antiradical activity DPPH in comparison with the other species of peppermint, using ethanol extract (Nickavar et. al. 2008). Also studies in differently extracts (methanol, ethanol and mixture of methanol: ethanol = 1:1) of the *Mentha piperita* have shown that higher content of total phenols was observed in methanol extract, while greater antiradical activity DPPH was observed in the extract mixture of methanol: ethanol (Farnad et. al. 2014).

### CONCLUSIONS

High plant density and fertilization practice increased the average height of *M. piperita* plants while the low planting density altered positively the number of offshoot produced per plant under the same fertilization scheme. Maximum quantity of dry matter per plant and higher essential oil yield of *M. piperita* leaves were observed in sparsely planting system by adding fertilizers at the first harvest time. Total phenolics content and antiradical activity was observed to have the higher values at the first harvest time in the sparsely planting system without fertilizer supply.

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**EFFECT OF DIFFERENT GROWING TECHNIQUES OF *MENTHA PIPERITA* IN THE CHEMICAL COMPOSITION OF ESSENTIAL OIL**

Eleni Wogiatzi <sup>1</sup>

**Keywords:** *Mentha piperita*, essential oil; menthol; methane; menthofuran

**ABSTRACT**

In this work using GC/MS instrument, spatial plant density and fertilization level effect were studied on active substances content of *Mentha piperita*, between the two seasonal harvests (early June and September). Menthol content of essential oil *M. piperita* the first harvest, ranged of from 35.53 to 40.2%, while during the second harvest, ranged of from 36.77 to 38.9 %. Methane content of essential oil *M. piperita*, ranged of from 27.56 to 33.6% and of from 17.5 to 20.09% respectively. While 1,8-cineole content, ranged of from 5.47 to 5.99% and of from 5.26 to 5.81% respectively. Significant differentiation was observed in Menthofuran, where content during the first harvest, ranged of from 4.6 to 5.3%, while during the second harvest, ranged of from 14.7 to 16.77 %. Finally, Limonene content the first harvest, ranged of from 1.18 to 1.37% and during the second harvest, ranged of from 1.50-1.91 %.

**INTRODUCTION**

*Mentha piperita*, family *Lamiaceae*, is a hybrid derived from a cross between *Mentha aquatica* and *Mentha spicata*. It is a perennial plant with erect stems which reaches to 80 cm height and develops numerous suckers (Marquard et. al. 2001).

The essential oil of the *Mentha piperita* has high antimicrobial activity (Yadegarinia et. al. 2006; Kurita et. al. 1981) and is used in cosmetology and food industry (Marquard et. al. 2001). The composition varies, depending with the variety, season of collection and environmental conditions (Dachler et. al. 1999; Schneider et. al. 1999; Teuscher E. 1997; Galle-Hoffmann et. al. 1998).

Studies from different regions, of the essential oil of *Mentha piperita*, have shown chemical composition different (Iscan et. al. 2002).

Purpose of this work was to examine the effect of spatial plant density and fertilization level, in dry drogue yield, in essential oil yield and in the quality of essential oil particularly in relation to the date of collection.

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## MATERIALS AND METHODS

The experiment was conducted at the Technological Education Institute (TEI) of Thessaly, Laboratory of Medicinal and Aromatic Plants, between March and September 2012, for two successive cultivation periods. The experiment had a randomized block design with two plant density level (2 and 4 plants per m<sup>2</sup>), two fertilization level (with and without) and two harvest times (June and September). There were four replicates per treatment combination.

Seedlings of *Mentha piperata* were established in silt loam soil (SiL) which has improved with blond peat moss addition. During growing season 37.5 Kg-N, 37.5 Kg-K<sub>2</sub>O and 37.5 Kg-P<sub>2</sub>O<sub>5</sub> per hectare incorporated into the soil.

Plants were harvest at the beginning of blossom period, where done according to (Marquard, et. al. 2001) when essential oils concentration is maximized, by cutting plant shoots 10 cm above soil surface. Plant leaves were dried in the dark at room temperature, finely ground and kept at 4°C.

**Extraction of the essential oil.** Oil was extracted by hydrodistillation using a Clevenger type apparatus. The duration of this procedure was 2 hours. The yield (v/w) of the obtained essential oil expressed as a percentage of absolute dry weight.

**Essential oils analysis.** The active substances were analyzed using GC/MS instrument (Agilent 7890 type gas chromatograph and Agilent 5975 mass-selective detector). Helium was used as carrier gas at a flow rate of 3 ml min<sup>-1</sup>. Separation of oil substances was done with a DB-WAX capillary column (30 m × 0.25mm; film thickness 0.25 µm). Essential oil solution (1 µl) in methanol was injected using split mode (split ratio 100:1). The temperature program was 50°C per 5min, and then rate of increase 5°C min<sup>-1</sup> from 50°C to 250°C. The identification of the compounds was achieved by comparing the retention times and the mass spectra with those of the standards included in the GCMSD library.

**Statistical analysis.** The experiment was repeated and the completely randomized design with four replications was used. Tukey's procedures were used to detect and separate the mean treatment differences at P= 0.05 ANOVA statistical analyses were performed by the statistical program MINITAB (Ryan, et. al. 2005).

## RESULTS AND DISCUSSIONS

From the results table 1 we observe that the essential oil content of dry drogue is more increased compared with the literature (Marquard, et. al. 2001), because the shoots were removed. Harvesting time significantly affected essential oil content. The maximum essential oil content was obtained at first harvest time, regardless of planting density and fertilization level. However, at first harvest time, maximum essential oil content was obtained, in low planting density with fertilization and minimum essential oil content was obtained, in low planting density without fertilization. While at second harvest time, higher essential oil content was obtained, in high planting density and smaller essential oil content was obtained, in low planting density, regardless of fertilization lever.

From the figure 1 we observe that the Menthol where is due to the characteristic aroma (Marquard et. al. 2001) the essential oil of *Mentha piperita*, fluctuated at high levels in all treatments, regardless of harvesting time. However, the higher Menthol content was observed in essential oil we received, at first harvest time in low planting density without fertilization, while the lowest Menthol content was observed in essential oil we received, at first harvest time in low planting density with fertilization. The Menthan content was higher in essential oil we received, at first harvest time in comparison with essential oil we received at second harvest time, but at first harvest time, in crop low planting density with fertilization was observed more Methane content.

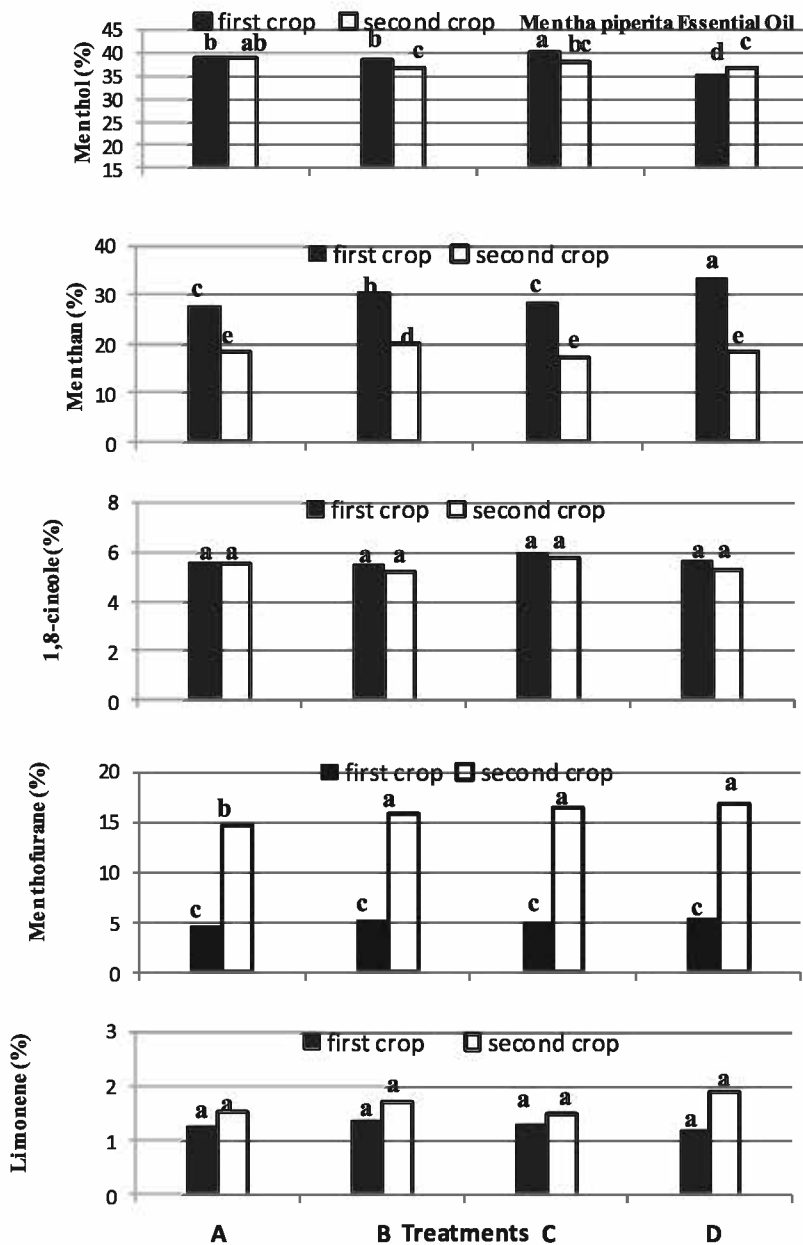


Figure 1. Menthol, Menthan, 1,8 –cineole., Limonene and Menthofurane content of essential oils *M. piperita* . A, 4 plant/m<sup>2</sup> without fertilization; B, 4 plant/m<sup>2</sup> with fertilization; C, 2 plant/m<sup>2</sup> without fertilization; or D, 2 plant/m<sup>2</sup> with fertilization. Columns in each graph with the same letter do not differ significantly according to the Tukey's test (P=0.05)

Table 1

Essential oil yield of the dry drogue *Mentha piperita*\*

Treatments	First harvest June 2012	Second harvest September 2012
	Essential oil mL / 100 g dry mass	Essential oil mL / 100 g dry mass
4 plants / m <sup>2</sup> without fertilization	3.2	3.0
2 plants / m <sup>2</sup> without fertilization	3.1	2.8
4 plants / m <sup>2</sup> with fertilization	3.2	3.1
2 plants / m <sup>2</sup> with fertilization	3.5	2.8

Data represent average means, (n) = 4.

Contrary, the 1,8-cineole and Limonene content in essential oil of *Mentha piperita*, showed no statistically significant difference relative to spatial plant density, fertilization level and the two seasonal harvests (Table 1). The Menthofurane content in essential oil we received, at first harvest time was at normally levels (<5%), while the Menthofurane content in essential oil we received, at second harvest time was at high levels (about 16%) resulting in downgrade of quality.

### CONCLUSIONS

From the results conclude that the Menthol content, in essential oil of *Mentha piperita*, was at high levels in all treatments. Menthol where is due to the characteristic aroma of the essential oil, had the highest content, at first harvest time, in low planting density without fertilization.

Also, the essential oil must be extracted only, at first harvest time, because low content Menthofouran (Marquard et al. 2001; Dachler et al. 1999).

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**THE ARTICHOKE MIKROMANI'S (*CYNARA CARDUNCULUS* VAR. *SCOLYMUS*) AS ONE MEDITERRANEAN NUTRACEUTICAL**

Georgios Zakyntinos<sup>1</sup>, Alexandros Papachatzis<sup>2\*</sup>

**Keywords:** *globe artichoke, nutraceutical, nutrition, phenolics, minerals*

**ABSTRACT**

*Globe artichoke is considered one of the most important vegetable crops in the European and non-European countries of Mediterranean basin. The Mediterranean region is well known for a dietary tradition commonly called 'Mediterranean diet', which is renowned for health benefits based among others on widely consumed foods and beverages. The artichokes have been recognized for the treatment of several ailments and reveal that their edible parts in therapeutical activity. In our case we have the Micromani's artichoke that is a local variety of Micromani area in South region of Peloponnese. In present work was realized a nutritional estimation about vitamins such as "C "and folic acid, minerals, fibers and total phenolics.*

**INTRODUCTION**

Globe artichoke is considered one of the most important vegetable crops in the European countries: Italy (364,871t), Spain (199,100 t), France (42,465 t) and Greece (31,600 t) and non European countries of Mediterranean basin (FAO 2012). Globe artichoke is also cultivated, although to a lesser extent, in the Near East, North Africa, South America, and the United States. Globe artichoke has important nutritional values due to its particularly high content of bioactive phenolic compounds, fibers and minerals. The economic use of the crop is currently mainly focused on the consumption of the edible immature (flower) heads, commonly referred to as 'heads', eaten as a fresh, canned or frozen vegetable, and more recently, demand has been increased because of its reputation as a health food. The Mediterranean region is well known for a dietary tradition commonly called 'Mediterranean diet(s)', which is renowned for health benefits based among others on widely consumed foods and beverages. While the focus of research has mostly been on the more widely used elements of the Mediterranean diets (especially olive oil and red wine), in this paper the focus is on 'local traditional food'. These are grown and produced locally and prepared into dishes, which often represent local specialties. In our case we have the Micromani's artichoke that is a local variety of Micromani area in South region of Peloponnese. The artichoke is a popular vegetable in both Greece and other

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Mediterranean countries (Yerram Raju Behara, 2011) and is known from ancient times for its medicinal properties (Sonnant et al. 2007; Lattanzio et al. 2009). The artichoke Mikromani's is a local variety - population, specific genetic material from Mikromani grown in an area of 650 acres with 50 producers, in Messina prefecture, South region of Peloponnese (Greece). Artichokes are an important crop for the Messina region and the production reaches three million (flower) heads per year. Artichokes are packed frozen or fresh and promoted in supermarkets in domestic market and abroad. Due to the considerable interest in preventive medicine and in the food industry in the development of natural antioxidants from botanical sources, research has been focused on elucidating the composition of the artichoke phenolic fraction from a qualitative and quantitative viewpoint, as well as the mechanisms underlying the therapeutic activity of artichoke extracts. Many studies confirm the popular use of artichoke for the treatment of several ailments and reveal that this therapeutical activity is probably primarily due to the phenolic structure substances, and these may have a function to inhibit free radical-mediated processes. Thus the product called "Micromani's" Artichoke could be processed in an alternative form such as that the dietary supplement or nutraceutical, giving in the product a high added value. In the present study we determined the composition of nutrient ingredients such as are vitamins, minerals and total polyphenols of this local variety artichoke heads and will try to suggest alternative way of product processing.

#### MATERIALS AND METHODS

Five samples of immature (flower) heads were received from nine different cultivated areas so as to have credibility and a representative of sampling. Total forty five (45) samples of fresh artichoke Mikroomani's were collected. The five samples of each one of nine areas homogenized in one sample as representative for each one area. Each of nine samples was mashed and the mashed material was used for determination of Vitamin C by the titration method of DPI, folic acid with the creation extracts by sodium acetate buffer and the following method Gregory et al 1984, total phenolics content was determined by the Folin-Ciocalteu method (Djeridane et al. 2006). The extract (0.2 ml) was pipetted into a 10.0 ml volumetric flask containing 4.0 ml water; next, 0.5 ml Folin-Ciocalteu's reagent and, after 1 min, 2.0 ml 20% aqueous solution of sodium carbonate were added. The volume was made up to 10.0 ml with distilled water. After 30 min, absorbance was measured at 760 nm against the reference solution. The results are averages of five measurements. The total phenolics concentration was calculated from a calibrated curve ( $R^2= 0.9954$ ), using gallic acid as standard (0.001–0.006 mg/ml). The results are expressed as gallic acid equivalent (mgGAE/g). The antioxidant potential was measured by method DPPH. The DPPH assay was performed as described before by (Gordon, Paiva-Martins, & Almeida, 2001) introducing some modifications (Gouveia & Castilho, 2012a). Briefly, 100  $\mu$ L of the methanolic solution (10 mg/mL) was added to 3.5 mL of a 0.06 mM methanol DPPH radical solution (Li X, Wux, and Huangl. 2009). The decrease in absorbance was determined at 516 nm until it reached a plateau (after 30 min), in the dark. The DPPH antioxidant capacity was determined using a Trolox standard curve and results were expressed as  $\mu$ mol Trolox equivalent per 100 g dried plant ( $\mu$ mol eq. Trolox/100 g). The DPPH (1,1-Diphenyl-2-picrylhydrazyl) was obtain from Sigma-Aldrich Chemie GmbH, Germany.

The containing minerals Fe, Se Zn, Cu Mg, Mn, Ca and K were determined by atomic absorption and flame emission spectrometry according to the methods of AOAC (2003). At the end, by AOAC 2009.01 method (*Codex Alimentarius Commission*) was determined the fiber content.



## RESULTS AND DISCUSSIONS

The total dietary fiber fluctuated from 6.15 to 6.47 g, a little more than that of Italian artichoke. Micromani's artichoke had a significant folic acid content, reaching to 72g/100 g (Table 1). The content of total phenols evidenced a relative abundance of phenolic phytochemicals in Micromanis artichoke in fact, nine tested samples yielded average value of  $1789,43 \pm 101.16 - 1932.29 \pm 109.13$  mg/100 g, expressed as gallic acid equivalents (GAE; see Method above mentioned). The antioxidant capacity by DPPH assay of artichoke heads gave quite high measurement values exceeding the  $5X10^3 \mu\text{mol eq. Trolox}/100 \text{ g}$  (Table 2).

Table 1

Average of measurement factors of nine samples of fresh artichoke heads  
in 100 gr fresh weight

Factor measurement	Average
Fibers	6.15-6.47 g
<b>Vitamines In 100 gr fresh weight</b>	
Folic	68-72 $\mu\text{g}$
vitamine C	11.7-13.1 mg
<b>Electrolytes</b>	
K	370-398 mg
<b>Minerals</b>	
Ca	38-44 mg
Cu	0.175-0.231 mg
Fe	1.49-1.58 mg
Mg	72-80 mg
Mn	0.198-0.256 mg
P	73-96 mg
Se	0.22 -0,34 $\mu\text{g}$
Zn	0.49 -0,65mg

Table 2

Antioxidant capacity by DPPH assay of artichoke heads ( $\mu\text{mol eq. Trolox}/100 \text{ g}$ )

Samples	Antioxidant Capacity, $\mu\text{mol eq. Trolox}/100 \text{ g}$
SH1	4290.00
SH2	3789.00
SH3	4110.33
SH4	3458.23
SH5	3876.34
SH6	4200.65
SH7	4000.34
SH8	5020.32
SH9	3893.45

According to various studies, the artichoke flower heads have a high content of vitamin C (10 mg /100 g fresh weight) and minerals (K 360 mg/100 g fw; Ca 50 mg/100 g fw) (Ceccarelli et al. 2010). In present work it was observed a higher content amounts in minerals relative to other studies. Leaves and heads of artichoke have been found to be rich in polyphenolic compounds, fibers and minerals (Lattanzio et al. 2009). In our case of Micromani's artichoke heads the content C of some samples reached the 13 mg/100 g f.w.

## CONCLUSIONS

Nutritional and pharmaceutical properties of artichoke heads are linked to their special chemical composition, which includes high levels of polyphenolic compounds, fibers and minerals. Due to the considerable interest in preventive medicine and in the food industry in the development of natural antioxidants from botanical sources, research has focused on elucidating the composition of the artichoke phenolic fraction from a qualitative and quantitative viewpoint, as well as the mechanisms underlying the therapeutic activity of artichoke extracts. Many studies confirm the popular use of artichoke for the treatment of several ailments and reveal that this therapeutically activity is probably primarily due to the phenolic structure substances, and these may have a function to inhibit free radical-mediated processes. The present work according to results as these were analyzed it could be concerned as high nutrition value product. Thus in our case the product called "Micromani's" artichoke could be processed in an alternative form such as that the dietary supplement or nutraceutical, giving in the product a high added value. In the present study determined the composition of nutrient ingredients such as are vitamins, minerals and total polyphenols of this local variety artichoke heads and suggesting alternative way of product processing.

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**LIPID PROFILE AND ANTIOXIDANT PROPERTIES IN SELECTED  
ECOTYPES FROM SOUTHERN GREECE OF PEAR CACTUS  
(*OPUNTIA FICUS INDICA*)**

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**Keywords:** *Opuntia spp.*, cactus pear, phenolics, lipids profile

**ABSTRACT**

*The lipid profile was determined in selected ecotypes of Cactus pear (Opuntia ficus indica), and this research completed by an approach of antioxidant properties of seeds from Cactus pear fruits. Using the methodology of methyl esters was determined the composition of fatty acids of seeds and in the case of antioxidants properties the known technique of Trolox Equivalent Antioxidant Capacity (TEAC) was used. The trolox evaluation assay was applied in aqueous - organic extracts and in their residues. The seed oil composition of cactus pear fruits was studied at the harvest time. Linoleic acid (70.03%) was the dominant fatty acid, followed by oleic (20.11%) and palmitic (11.86%) acids, respectively. Among cactus pear seeds, polyphenols contribution to antioxidant properties ranged from 37.9% to 42% for TEAC values. According to results the seeds of Cactus pear are a good source of useful lipids and antioxidants.*

**INTRODUCTION**

The cactus pear also known as prickly pear (*Opuntia spp.*) is native of tropical regions of the Americas, where can be found 300 different species. Over time it began to grow in different parts of Europe, especially in the Mediterranean, as well as and in Africa and Australia (Barbera, 1995). Due to the ability of the plant to adapt to different environmental conditions, the prickly pear can be grown in a variety of soils and areas (lowlands, coastal areas, plateaus etc). The cactus pear fruit is an oval, elongated berry of 67-216 g weight. They offer a wide spectrum of colors from white, yellow, orange, red, and purple based on betalains (Stintzing & Carle 2005, 2006) and contain about 85% water, 15% sugar, 0.3% ash and less than 1% protein (Mohamed-Yasseen et al., 1996). The fruits and other parts or plant organs and exploited in various ways (fresh fruits, fresh cladodes, jams, alcoholic beverages, soft drinks etc) and in different sectors such as production of organic fertilizers industry, colours, biogas, medicines, cosmetics, animal feed etc. For this reason there is a growing demand for Prickly pear cactus products are quite auspicious and

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the future of farming (Saenz-Hernandez 1995). In Greece there are no systematic plantations of pear cactus, but there are approximately 100,000 scattered plants occurring mostly in several parts of southern Greece (Lionakis 1994). Polyphenols have received much attention among the various kinds of natural antioxidants. These bioactive compounds exhibit a wide range of physiological properties, such as antioxidant and protective for function human body (Scalbert et al 2005). Polyphenols exist as easily extractable compounds (free) (compounds solubilized by aqueous organic solvents) and as less extractable types (bound) (compounds that remain in their corresponding extraction residues) (Hartzfeld et al. 2002, Kristl et al. 2011). Recent studies have used an alkaline hydrolysis, acid hydrolysis, or enzymatic digestion (Iqbal et al. 2005, Taberero et al. 2011) polyphenols content of plant foods have been less estimated and significant amounts of bioactive compounds remain in the residue from extraction as non extractable polyphenols (Pérez-Jiménez & Torres 2011, Saura-Calixto 2012). Arranz et al. (2010) have concluded, by studying dietary polyphenols in cereals, fruits, vegetables, nuts, and legumes, that non extractable polyphenols are the major part of dietary polyphenols. Studies of non extractable polyphenols are few respect to those on extractable polyphenols (Arranz et al. 2009, Tarascou et al. 2010). There are no studies addressed to the estimation of antioxidant properties in aqueous-organic extracts and residues of Cactus pear seeds. The aim of this research was to determine the seeds lipids profile, to give more information about total polyphenols (extractable – non extractable) and to evaluate the antioxidant properties of seeds from colored ecotypes of Cactus pear, giving so, a scientific signal by these data for consolidation the above mentioned genetic material in a local industrial level.

## MATERIAL AND METHODS

### *Plant material*

Fruits of *Opunia ficus-indica* from native populations of Messinia's coast places were collected. Mature fruits were collected from three different ecotypes from well developed plants with colored flesh. The collected fruits were classified according to flesh color in Red Samples, Purple Samples and Orange Red Samples. Thus a number of 30 randomly selected fruits from each one color samples were washed and drained. Spines and peels of fruits surface were removed under running water. The manipulated fruits were cut in half and introduced into a pulper to separate the seeds from the pulp. Seeds were washed under running water several times on a screen, dried at ambient temperature and weighed.

### *Analytical procedures*

The oils were analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent mass selective detector coupled with an Agilent gas chromatograph. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass units were monitored from 30 to 450 m/z. The oil components were identified by comparison of their retention times and mass spectra with the NIST mass spectral library 23. The chromatographic conditions were identical to those used for GC analysis.

### *Extractions & Antioxidant Properties*

Total phenolic compounds were extracted as described by Durazzo et al. 2013, with minor modifications as follows. Aqueous - organic extracts (extractable polyphenols) and their residues (non-extractable polyphenols) were isolated and studied.

#### *Aqueous-organic extract*

About 3 – 4 g of samples were placed in a test tube and 25 mL of acidic methanol/water (50:50 v/v, pH 2) were added. The tubes were vortexed at room temperature for 3 min, followed by 12h shaking in a water bath at room temperature. The tube was centrifuged at 2500g for 10 min, and the supernatant was recovered. Twenty milliliters of acetone/water (70:30, v/v) were added to residue, then vortexing, shaking and centrifugation were repeated. Both methanolic and acetonic extracts were combined and centrifuged at 3500g for 15 min. The resulting supernatant was transferred into tubes and directly used for the determination of antioxidant capacity.

#### *Residue*

Residues were left in a ventilating and heating apparatus (max temperature 25°C), until dryness. Briefly, 300-400 mg of the residue was mixed with 20 mL of methanol and 2 mL of concentrated sulfuric acid (18 M). The samples were gently stirred for 1 min and were shaken in a water bath at 85°C for 24 h. The samples were then centrifuged (3000g for 10 min), and the supernatant was recovered. After two washings with minimum volumes of distilled water and re-centrifuging as necessary, the final volume was taken up to 50 mL. The tube was centrifuged at 3500 g for 20 min and was transferred into tubes and directly used for the determination of antioxidant capacity.

#### *Antioxidant Properties Determination*

Antioxidant properties have been determined in both aqueous -organic extracts and their residues using the methodology of Trolox Equivalent Antioxidant Capacity (TEAC).

#### *Statistical Analysis*

All analyses were performed in triplicate. Data are presented as mean  $\pm$  Standard Deviation (SD). Statistica for Windows statistical package was used to perform One-Way Analysis of Variance (ANOVA).

## **RESULTS AND DISCUSSION**

Several studies had been reported that prickly pear seeds are considered unexploited source of oil obtained from the seeds and they constitutes 5.7% of dry seeds material. Table 1 presents the fatty acid profil with refer mainly in the amounts of linoleic acid. The amount of linoleic acid in three diferent samples of *Opuntia ficus-indica* oil was higher than that in majority of commonly consumed oils such as corn, soybean and cotton seed and close to that of safflower oil (Swern 1982). In general, the higher level of unsaturation and particularly high level of linoleic acid in conjunction with the absence of linolenic acid, which affect adversely the stability of the oil, indicated that *O. ficus-indica* seeds might be an excellent potential source of oil.

Table 1

Fatty acid profile of the oil extracted from seeds of color flesh fruits

Cactus pear (selected sample of seeds from colored fruits)			
Fatty acids			
Samples	Palmitic acid C16:0	Oleic acid C18:1	Linoleic acid C18:2
Red sample 1	13.3±4.6	19.3±1.4	63.5±11.7ab
Purple sample 2	12.5±6.9	23.7±5.1	62.9±9.5a
Orange-red sample 3	12.9±3.2	18.5±3.2	61.5±6.2ab

\*mean ± SD: Average value of triplicate analyses (±standard deviation)

In Table 2 TEAC values (mmol/kg d.w.) of aqueous-organic extracts (combining two extraction cycles) and the corresponding residues of selected seeds were reported. For colored fruits seeds, TEAC values ranged from  $113.3 \pm 4.6$  to  $125.3 \pm 6.9$  mmol/kg d.w. in aqueous-organic extracts and from  $76.5 \pm 1.5$  to  $85.6 \pm 9.5$  mmol/kg d.w. In present work the hydrolysable polyphenols contribution to antioxidant properties ranged from 37.9 % to 42% for TEAC values; the purple samples exhibited the highest contribution for TEAC values. As reported in literature data, non extractable polyphenols are more abundant than extractable polyphenols in many foodstuffs (Saura-Calixto 2012). High antioxidant capacity of hydrolysable phenolics was found in the residues of aqueous-organic extracts in cereals (Perez-Jimenez & Saura-Calixto 2005) and walnuts (Luthria 2006).

Table 2

Equivalent Antioxidant Capacity (TEAC) values (mmol/kg d.w.) of selected seeds\*

Cactus pear (selected sample of seeds from colored fruits)		
Samples	Aqueous-organic extract	Residue
Red sample 1	113.3±4.6ba	69.5±11.7ab
Purple sample 2	125.3±6.9a	85.6±9.5a
Orange-red sample 3	104.3±3.2c	76.5±6.2ab

\* mean ± S.D.; a–c: Anova, Duncan Test: within samples of each type of seeds, by columns, means followed by different letters are significantly different ( $p < 0.05$ ).

## CONCLUSION

This study demonstrated that cactus seeds could be considered as a source for natural phenolic antioxidants giving information for total polyphenols (extractable – non extractable). The trend towards natural ingredients and products of health promotion is likely to increase. The data of the present work are advocated as evidence likely the cactus pear oil to be a nutraceutical and could be used as a new source of oil from untapped fruits of Pear cactus native population. Pear cactus (*Opuntia ficus-indica*) seeds might be an excellent potential source of oil. In the future, prickly pear cultivation can be expected to increase if there is a demand for its fruits for the production of fruit juices or other industrial products.

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