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THE TROPHIC SPECTRUM OF A *BOMBINA VARIEGATA* POPULATION FROM  
ARGEȘ COUNTY (NUCȘOARA LOCALITY)

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**KEY WORDS:** *Bombina variegata*, feeding, prey category

**ABSTRACT**

*This study was realised at the end of 2009 and aimed to analyze the trophic spectrum of a Bombina variegata population. The individuals populate a habitat situated in the hydrographic basin of Râu Doamnei, at approximately 630 m altitude, near Nucșoara locality, Argeș County. The most important preys were the Diptera Nematocera, respectively the Hymenoptera Formicidae, followed by Arachnida Araneae and Coleoptera, which registered lower values. In comparison to the males, the females consumed large preys that were rich in nutritive substances (Coleoptera, Gastropoda, Lumbricidae, Lepidoptera larvae). Capturing these preys involves a minimum effort, thus the females trying to save their energetic resources. There were not registered any stomachs without content. Most of the consumed preys were terrestrial. Beside the invertebrate preys, we also encountered the consumption of vegetal parts, shed skin and mineral elements.*

**INTRODUCTION**

On overall look upon the entire amphibian group has highlighted that almost a third of the species are threatened with extinction (Alford and Richards, 1999; Blaustein and Kiesecker, 2002). Amphibians have populated Earth for over 300 millions years, but in the last two decades the number of extinctions has raised in an alarming manner, approximately 40% of the populations having decreased. This fact indicates that the number of extinct and threatened species will probably continue to grow (Stuart et al., 2004). Most certainly the most important factors in their decline are the destruction, alteration and fragmentation of their habitats (Marsh and Trenham, 2001). However, it has been recorded that amphibians are also decreasing in the protected and unexplored areas (Lips, 2000). Amphibians are important components of the ecosystems, due to the fact that they direct the energy from the invertebrates, especially from the detritovore and phytophagous ones, to the upper trophic levels, thus playing a special role in the trophic dynamic (Blaustein et al., 1994).

*Bombina variegata* (Linnaeus 1758) is a common, unpretentious species, its area including central and southern Europe, the species ranging from 100 (Madej, 1964) to 1900 m altitude (Cabela et al., 2001). Knowing the food composition of the amphibians offers specific clues regarding the conditions provided by the occupied habitat (Perry and Pianka,

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1997), thus the frogs can be considered as important indicators of the environmental conditions (Bellocq et al., 2000). There are recent data regarding the trophic spectrum of this species in Romania (Sas et al., 2005; Ghiurcă and Zaharia, 2005; Szeplaki et al., 2006; Dimancea et al., 2008; Ferenți and Covaciu-Marcov, 2009). There are no data regarding the composition of the trophic spectrum of this species from the studied region. Therefore, we wish to add new information or to complete the present studies that refer to the trophic spectrum of *Bombina variegata* species.

## MATERIALS AND METHODS

The studied *B. variegata* population belongs to a region that has a piedmont character, from Argeş County (Nucşoara locality – hydrographic basin of Râu Doamnei). The habitat is situated at an altitude of 630 metres, being represented by a bogging area formed from the artificial damming of the main course of Râu Doamnei.

Our study took place in august 2009, when we captured 35 individuals, from which 14 were males and 21 females. The water level allowed their capture directly by hand, afterwards having collected the stomach contents of the frogs. In this respect we used the stomach flushing method (Sole et al., 2005), technique which allows the study of the amphibians' feeding without having any negative effect upon the individuals. It was tried to reduce as much as possible the time between the capturing of the animals and the performing of the stomach flushing due to fact that amphibians digest their food in a short period (Cadwell, 1996), fact that can modify our results. The content was preserved in a 4% solution of formaldehyde and stored in air tight test tubes. The analysis of the contents was realised at the magnifying glass, while the scientific literature was used for determining the taxonomic affiliation of the prey categories. The prey taxa were mostly determined until the order, family and where it was possible, genus level.

We analysed different parameters, such as: the feeding activity rate, the taxonomic affiliation of the consumed prey, the feeding intensity, the origin of the preys identified in the stomach contents of the individuals, as well as the amount and frequency with which these were consumed. The obtained results were discussed regarding the differences that appear between the sexes.

## RESULTS AND DISCUSSIONS

Following the analysis of the stomach contents of the 35 individuals, there were identified 161 preys, which were grouped in 31 taxonomic categories. The identified preys mostly belong to the insect class. In this case, there was not registered any empty stomachs, thus the rate of the feeding activity being of 100%. During the period when the study took place, the temperatures were relatively high, although the habitat is situated in the Middle Carpathians. However, it provides good feeding conditions to the analysed population.

We also identified vegetal fragments, shed skin and mineral elements in the stomach contents, beside the animal preys. It can be noticed that there is a difference regarding the consumption of vegetal debris between the two sexes: thus, 42.85 % of the males consumed vegetal parts, while the females consumed more frequently these elements, registering 57.14% (Table 1). These values can be associated with the feeding intensity, which is different between the two sexes, the females feeding more intensely than the males. The toads' consumption of vegetal parts cannot be regarded as an active one, due to the fact that they are carnivores (Cogălniceanu et al., 2000), only their larvae consume plants, especially algae (Jenssen, 1967). The consumption of vegetal parts is considered to

be accidental, being swallowed together with the followed mobile prey (Stebbins and Cohen, 1995).

Shed skin can also be considered as being swallowed together with the mobile preys, or accidentally. Some authors consider that, due to their rich-protein content, their consumption can be interpreted as an aspect related to the recycling of the epidermal proteins (Weldon et al., 1993). Differences between the two sexes can be noticed regarding the shed-skin consumption, thus the females having consumed twice as much than the males (Table no. 1). In comparison with other studies (Peter et al., 2006; Toth et al., 2007; Ferentî and Covaciu-Marcov, 2009), their percentage is relatively low. It can be said that the habitat displays a varied trophic offer, rich in nutritive substances, thus the frogs do not have to supplement their food through the consumption of shed-skin. Its consumption in a more active manner in the case of the females, can represent an additional source of obtaining energy.

Table 1

The frequency of the vegetal parts, shed skin and mineral elements in the stomach contents; The feeding intensity (the average and maximum number of preys/individual); The origin of the preys (terrestrial or aquatic medium)

	<b>Males</b>	<b>Females</b>	<b>Total</b>
Vegetal parts (%)	42.85	57.14	51.42
Shed skin (%)	14.28	28.57	22.85
Mineral elements (%)	14.28	19.04	17.14
Average no. of preys/ individual	4.35	4.76	4.6
Maximum no. of preys/individual	9	11	11
(%) Terrestrial preys	88.53	89	88.82
(%) Aquatic preys	11.47	11	11.18

Regarding the mineral parts, their consumption is considered to be accidental in the scientific literature, these elements having no nutritive value. The difference between the two sexes was not very high, the females registering a value of 17.14%, while the males, 14.28%. The presence of the minerals could be explained by the type of the habitat, which is a bogging area of a mountainous river, with its specific characteristics, thus a rocky, sandy substratum. The probability of swallowing small pebbles in cases like these is quite high, these being ingested together with the mobile prey. As the individuals move more actively, they bring about the pebbles from the substratum. We can observe from the analysis of the three elements, that the females registered on every occasion higher values than the males. Thus, it can be deduced that the females hunt more actively than the males, attacking every prey that appeared in their sight.

We analysed the average and maximum number of preys/individual in order to establish the intensity with which the frogs have fed. These parameters are influenced by the size of the consumed preys. Concerning the average number of preys/individual, this is very close at both sexes: 4.35 preys/individual registered the males and 4.76 preys/individual the females. These values are low, in comparison to other studies, but the consumed preys are also of large size (Coleoptera, Gastropoda, Araneae, Lumbricidae, Gammaridae), thus the individuals did not have to abundantly consume these preys in order to satisfy their energetic needs. Close values at both sexes were also registered in the case of the maximum number of preys/individual (Table 1). Even if the values are low, it cannot

be stated that the habitat does not provide rich trophic resources, but, the larger preys practically replace the smaller ones. The high thermal values registered at the time of study, probably participated in the decrease of the feeding intensity. The fact is well known that high thermal values have a negative effect on the amphibians' feeding (Aszalos et al., 2005).

In the case of both sexes, the majority of the preys come from the terrestrial medium (Table 1). The identified terrestrial preys, represented by flying insects and some spiders, can originate from the grass from the perimeter of the puddle or of the water mass, the habitat providing a relatively rich amphibious vegetation. On the other hand, they can also come from the terrestrial environment, the individuals hunting in this medium.

From the 161 animal preys, at some taxa there was identified the stage of ontogenetic development, thus separating the larvae from the adults. This fact is a relevant one, because it is considered that the larvae of the holometabolic insects have a higher nutritive value (Brooks et al., 1996).

In the case of the males, the most important amount is held by the Diptera - Nematocera (16.39%), followed by the Formicidae, which registered 14.75%. The following important taxa in the trophic spectrum of the males are the Araneae, Crustacea Gammaridae, Homoptera - Cicadinea. Regarding the amount of the preys registered by the females, the higher value is also held by Diptera - Nematocera, but in a lower percentage, 13%, followed afterwards by the Hymenoptera - Formicidae, which also registered a lower percentage than the males. However, the two categories hold first place within the trophic spectrum of both sexes.

The high amount of the Diptera - Nematocera highlights the fact that these represent an important category for the habitat, occupying an important place in the frogs' nutrition (Low et al., 1990). The Nematocera adults are winged-preys, of terrestrial origin, which could be captured from the water surface, the frogs using the "sit-and-wait" hunting strategy. The higher number of Nematocera individuals in comparison to the Brachycera (1.63%) can be explained through the fact that the nematocerans mostly lay their eggs in the water, thus being connected to the aquatic environment, which eases their capture. High amount values of the Nematocera were registered at other recent studies regarding the feeding of this species (Ferenți and Covaciu-Marcov, 2009).

The high amount of the Hymenoptera Formicidae indicates the fact that the trophic offer of the aquatic habitat does not fully satisfy the energetic needs of the frogs, which leave the aquatic medium in order to hunt in the terrestrial one. Ants are abundantly present due to the fact that they live in large groups (colonies), thus making it easier to be captured. Some authors sustain that the skin toxicity resulted through the alkaloid content is enlarged by the ant consumption (David et al., 2008), being probably connected to the formic acid that they produce.

Spiders, cicadas and Gammaridae are preys that have a variable size and have different origins regarding their environment, thus it can be stated that the males did not select their food, feeding with everything that comes in sight. The high amount of the cicadas can be explained through the fact that they are warm-loving insects, their presence being connected to the ecological conditions, situation which is confirmed by the relatively high thermal values registered during the time of study.

A connection can be observed between the values of the amount registered in the case of the females and in that of the males, the prey taxa with high values being the same for both sexes. Thus, it can be stated that there are no important differences between the sexes regarding the amount. In the case of the females, it was noticed the consumption in low values of Diplopoda, Dermaptera, Plecoptera larvae, Lepidoptera and Brachycera, the

consumption of the larvae being higher than in case of the males. This fact is easily understood because the females need an extra amount of energy, thus they accumulate nutritive substances for the hibernation period and afterwards for the reproduction one. The fact is known that larvae have a higher content in lipids, being thus more nutritive (Brooks et al., 1996), their consumption being an aspect of energetic economy.

Table 2  
The amount and the frequency of the prey taxa; [L.] – larvae; (aq) – aquatic; (t) – terrestrial

Prey category	Amount (%)			Frequency (%)		
	Male	Female	Total	Male	Female	Total
Oligochaeta -Lumbricidae	3.27	6	4.96	14.28	14.28	14.28
Gastropoda (aq.)	-	3	1.86	-	9.52	5.71
Gastropoda (t)	1.64	6	4.34	7.14	19.04	14.28
Gastropoda - Limacidae	3.27	3	3.1	14.28	14.28	14.28
Arachnida - Araneae	8.19	5	6.21	35.71	14.28	22.85
Crustacea-Gammaridae	8.19	4	5.59	28.57	14.28	20
Crustacea-Isopoda (t)	-	2	1.24	-	9.52	5.71
Diplopoda	-	1	0.62	-	4.76	2.85
Chilopoda	1.64	-	0.62	7.14	-	2.85
Collembola	3.27	3	3.1	7.14	9.52	8.57
Plecoptera (aq) (L.)	-	1	0.62	-	4.76	2.85
Orthoptera	1.64	5	3.72	7.14	19.04	14.28
Dermoptera	-	1	0.62	-	4.76	2.85
Homoptera - Cicadinea	8.19	4	5.59	14.28	19.04	17.14
Homoptere - Aphidinea	-	2	1.24	21.42	4.76	2.85
Heteroptera	4.91	5	4.96	14.28	23.8	22.85
Coleoptera	3.27	7	5.59	7.14	28.57	22.85
Coleoptera - Dytiscidae (aq)(L.)	3.27	3	3.1	14.28	14.28	14.28
Coleoptera – Curculionidae	1.64	2	1.86	7.14	9.52	8.57
Coleoptera - Chrysomelidae	1.64	-	0.62	7.14	-	2.85
Coleoptera - Carabidae	1.64	4	3.1	-	14.28	11.42
Coleoptera – Staphilinidae	-	2	1.24	7.14	9.52	5.71
Neuroptera	1.64	-	0.62	-	-	2.85
Lepidoptera (L.)	3.27	2	2.48	7.14	9.52	8.57
Diptera Nematocera	16.39	13	14.28	35.71	33.33	34.28
Diptera Nematocera - Typulidae	1.64	-	0.62	7.14	-	2.85
Diptera Brachycera	1.64	-	0.62	7.14	-	2.85
Diptera Brachycera (L.)	1.64	2	1.86	7.14	9.52	8.57
Hymenoptera	1.64	2	1.86	7.14	4.76	5.71
Hymenoptera- Formicidae	14.75	12	13.04	28.57	38.09	34.28
Mecoptera-Panorpidae	1.64	-	0.62	7.14	-	2.85

The more intense feeding of the frogs towards autumn, especially with larger preys (Lumbricidae, Gastropoda, Araneae, Coleoptera, etc.), or more rich in nutritive substances, such as larvae, is closely related to their life cycle, because frogs must stock up the necessary energy for surviving the hibernation period (Holenweg and Reyer, 2000). The consumption of preys that are richer in nutritive substances once with the arrival of autumn has been recorded at other recent studies (Yu et al., 2009; Mollov and Stojanoja, 2010).

Concerning the frequency of the prey taxa, there are certain differences regarding the sex, but on the other hand, there are also differences with regards to the amount values. This fact is due to the large number of preys consumed from a taxon, which determines its high amount, but not its frequency. The males most frequently consumed Diptera Nematocera, together with Arachnida Araneae. It can be noticed that the Nematocera occupy first place regarding the amount and frequency of consumption. On the other hand, the high amount of the Formicidae does not coincide in this case with their frequency, these being surpassed by the Araneae.

The high frequency of the spiders can be explained through the fact that they are easily captured, both from the grass from the perimeter of the puddle, and from the terrestrial environment, representing preys with a low mobility.

Another important taxa frequently encountered is represented by the Coleoptera. All of the consumed bugs from the performed study make up a percentage of about 50% in the stomach contents of the males. Thus, these frogs are very useful through the fact that they consumed some insects that are harmful to the crops, such as the Curculionidae and Chrysomelidae (Sas et al., 2003). Their high frequency indicates their accessibility in the habitat, but the fact that the high frequency does not coincide with the amount, is the result of the large dimensions of these insects and their high energetic level, thus the frogs did not consume them in large numbers.

The females most frequently consumed Formicidae (38.09%) and Nematocera (33.33%), their high values being in accordance with their high amount. Totalising all of the families belonging to the Coleoptera order, results in a very important frequency of over 50%, taking into account that the amount value is quite low, 15%. The explanation for this difference between the two parameters is the fact that the females did not have to consume these insects in large quantities, because of their large dimensions, thus their amount value is low. It has been noticed the low consumption of the Plecoptera larvae in the trophic spectrum of the females. Their presence in the habitat from Nucșoara is very important, these being indicators of clean waters (Ferenți and Covaciu-Marcov, 2009).

The presence in the males' stomach contents of large –sized insects is very interesting. According to the scientific literature, these usually use the «active foraging» hunting strategy (Perry and Pianka, 1997), thus feeding with smaller preys and having the possibility to capture a wider range of preys. However, in the case of our study, both males and females consumed large preys, which highlight the “sit-and-wait” feeding technique. This is easily understood in the case of the females, due to the fact that they capture easily accessible preys at the time when they enter their sight, thus saving up energy.

These aspects connected to the hunting manner of the frogs are very important, due to the fact that the way in which a species uses its food resources indicates its chances of survival (Cuello et al., 2006).

## CONCLUSIONS

There are no major differences registered between the two sexes regarding the origin of the prey or of the feeding intensity, the values being very similar.

In the case of both sexes, the most well represented taxa, regarding both the amount and frequency of consumption, are the Diptera Nematocera, together with the Hymenoptera Formicidae, followed by the Arachnida Araneae and Coleoptera. Concerning the females, it was observed the consumption in important percentages of the Coleoptera, Plecoptera larvae, Lepidoptera and Brachycera, in comparison to the males. The consumption of these preys, especially of the larvae, is easy to understand, these having a

high nutritive value. Thus the frogs deposit the nutritive substances for the hibernation period, and they also dispose of enough energy during the reproduction period. It is interesting that both males and females use the same hunting technique, "sit-and-wait", which is easily understood in the case of the females, which capture easily accessible preys, thus saving energy. It can be stated that the analysed habitat provides favourable conditions for feeding and for the development of the population taken into account.

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## NEW ASPECTS IN THE TECHNOLOGY OF POMEGRANATE CULTURE IN GREECE

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**KEY WORDS:** pomegranate, crown, variety, production

### SUMMARY

*Pomegranate is a species of perspective for the modern fruit growing in Greece. The study of the varieties led to the conclusion that the foreign varieties can be capitalized with good prices for the internal market and for export.*

*The culture technology adopts new measures in order to make the culture more efficient and to obtain fruits of high quality. The types of plant management system with many strains lead to a good precocity (the bearing of the strains) and good productions as compared to the one strain plant management. The vegetative growth is slightly smaller at plants with many strains, but the ramification capacity is higher.*

### INTRODUCTION

Pomegranate is one of the oldest fruit species cultivated in Greece and use for consumption as fresh fruit (Hoza and Plisiotis, 2010), or processed as spice or medical herb (Lionakis and Lidakis, 2003). Traditionally, the plant management meant the shape of a bush with many strains forming in the area of parcel (Drogoudi, Tsipouridis and Pantazis, 2003).

New solutions are being searched for in the technology of the pomegranate culture in order to obtain quality fruits and constant production over time (Drogoudi, Tsipouridis and Pantazis, 2007). The present research presents the partial results obtained at pomegranate plants managed with different strains that formed on a short trunk.

### MATERIAL AND METHOD

The experiment was conducted in 2008-2009 in Pomegranate village, in Larisa region, Greece. The plantation was founded in 2007, the distance between the plants was 6/2 m and the variety used was Wonderful. The plant management consisted in different strains, each type of management being a variant, as it follows:

V1 – plants with one strain

V2 – plants with 2 strains

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V3 – plants with 3 strains

V4 – plants with 4 strains

Annually, the plants were properly cared by applying irrigation located through two pipelines placed on every side of the row, the formation cutting of the crown and fertilization with 50 kg of nitrogen applied in two phases in the first part of the year.

Observations regarding the growth and fructification capacity of the plants were made.

### THE RESULTS OF RESEARCHES

From the observations made, a different reaction of the plants was registered from the vegetative growths point of view, depending on the type of plant management (Table 1).

Table 1

The growth capacity of pomegranate plants in the third year

Variant	No. of strains	The thickness of strains	The capacity to garnish(m)		
			Semi scaffold branches	Annual short branches	Annual long branches
V1	1	4,5	9,2	45,6	19,6
V2	2	4,1	7,1	41,2	18,5
V3	3	3,9	7,3	41,8	18,9
V4	4	3,2	6,4	39,4	16,3
Average	2,5	3,9	7,5	42	18,3

The plant management with many strains influences their thickness, in the present situation the thickness of the strains decreased as their number increased, from 4,5 cm to 3,2 cm.

The garnish capacity was influenced by the plant management, but also by the conducted cutting works. Through the cutting works, the number of semi scaffold branches was rare, between 9,2 pieces/m at V1 and 6,4 pieces/m at V4, to ensure a proper lightning of the plant.

The pomegranate is a plant with high ramification capacity trough the issue of annual growths and from the adventitious buds from the cutting points. The number of annual branches left after the green cutting had values between 45,6 pieces/ml of strain at V1 and 39,4 pieces/ml of strain at V4.

The decrease in the number of branches at plants managed with many strains was compensated by the additional strains as compared to V1, so that plants managed with many strains had a higher degree of garnish.

The capacity to issue anticipated shoots was influenced by the type of plant management, the plants with many strains forming less anticipates (Table 2). The shoots from the upper part of the crown formed less anticipates than those from the lower part.

Table 2

## The capacity to issue anticipates on long branches

Variant	The length of the main branch (cm)	Number of anticipates (piece)	The length of the anticipates (cm)
V1	98,3	11,1	42,1
V2	99,6	9,6	42,5
V3	94,5	9,1	40,4
V4	85,2	8,4	35,6
Average	94,4	9,5	40,1

The capacity to issue anticipates was correlated with the degree of garnish of the plant. Plants with many ramifications formed less, smaller anticipates and those with a lower degree of garnish formed more, longer anticipates.

The number of anticipates had values between 8,9 pieces/branch at V4 and 11,1 pieces/branch at V1. The length of the anticipates was between 42,1 cm at V1 and 35,6 cm at V4. In practice, it is recommended that a great part of anticipates to be cut in order to ensure a proper growth and coloring of fruits. In classical technology, no green cuts are made and the fruits have weak colors and are smaller.

The fructification capacity was good in the third year since planting, the number of formed flowers being higher than the one necessary for the production. In order to ensure a balanced production, correlated with the age of the plant, the flowers were rare, only the central ones from the branch tips were kept, the side ones being removed.

The production obtained in the third year had values between 14 and 22 t/ha (Table 3), which ensure the low return of the culture from this year.

The first year of production is influenced by the number of branches in the crown. At the first two variants the crown is not yet formed, while at V4 the fruit fence is almost finished.

The capacity to form flowers was higher at plants with man ramifications, where the capacity of vegetative growth was lower. It was also observed at this species the inverse correlation that exists between growth and fructification.

Table 3

## The production capacity

Variant	Outgrowth branches	Average number of flowers/branch	% rarity	Production t/ha
V1	38,4	2,7	60,2	14,2
V2	37,2	3,1	65,1	18,6
V3	36,1	3,2	66,0	22,5
V4	37,4	3,9	73,1	24,0
Average	37,3	3,2	66,1	19,8

## CONCLUSIONS

From the present research, the following conclusions can be drawn:

- the pomegranate plants react positively at management in the shape of a tree with many strains, the growth and ramification capacity being influenced by their number;
- the management with 3- strains leads to a decrease in the length of the annual growths and in the number of anticipates, and the management with 1-2 strains stimulates the growth of the branches;
- the bearing of the plants was better with the 3-4 strains management, the production having values higher than 20 t/ha in the third year since planting;
- It is recommended to continue observing the orchard in the following years.

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THE CHARACTERISTICS OF APPLE CULTURE IN SERGAIA VALLEY,  
SYRIA

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**KEY WORDS:** *irrigation, altitude, crown shape, cuts, production*

**SUMMARY**

*The modern apple culture in Sergaia Valley is relatively recent due to the low tradition of this species in Syria. As the interest to expand the apple culture in Arab countries, especially in the 1970s, increased, the Golden delicious and Starking delicious varieties were introduced on certain valleys that offered favorable conditions. These varieties give good results in the low region of the valley with irrigation and without irrigation only at an altitude higher than 1500 m.*

**INTRODUCTION**

The apple culture in Syria is relatively recent due to the less proper conditions from the majority of regions. However, there are some regions, at a higher altitude, where classical apple culture begun in the last 30 years. Among these there is Sergaia Valley, where apple is cultivated on approximately 13000 ha, from which approximately 8000 ha with irrigation and 5000 ha without irrigation.

Sergaia Valley is situated at the foot of AEAH-NSoul Mountains, with a height of 2364 m high, and during the spring the currents that come from the mountains can affect the blooming trees. In this region, the cultures of apple can be found until the altitude of 2200 m

**MATERIAL AND METHOD**

The observations and measurements were made in Sergaia Valley, where the apple has optimum conditions of culture, favorable soil and irrigation conditions. The plantations are usually classical, the planting distances being 7-8 m/6-7 m. The plant management consists of interrupted pyramid with 7-8 main branches and with a short trunk of 30-40 cm. The shape of the trees is globular.

The soil is maintained as a field with specific works to eliminate the weeds and irrigated at the foot of the slopes and not irrigated at an altitude higher than 1500 m. annually, the soil is fertilized with organic and chemical fertilizers.

The fructification cuts are made in early spring; they are limited to thinning the semi scaffold branches and lightning the crown. No green cuts or thinning the fruits are made. The varieties used were *Golden delicious*, *Starking delicious* and *Sugary*, the local variety. The age of the trees was 18 years.

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## THE RESULTS OF RESEARCHES

The ongoing of the phonological phases of the varieties was influenced by the culture area. At higher altitudes, the opening of the buds was delayed with two weeks, compared to the lower part of the valley (Table 1).

Table 1  
The phenology of the outgrowth organs of some apple tree varieties  
(Average data 2008-2009)

Area	Variety	The opening of the buds	The beginning of blooming	The pick of the blooming	The end of the blooming	The fruit maturation
The foot of the slope	Golden delicious	6 IV	21 IV	24 IV	30 IV	20 IX
	Starking delicious	2 IV	15 IV	20 IV	28 IV	30 IX
	Sugary	25 III	13 IV	19 IV	25 IV	25 VII
1500 m	Golden delicious	18 IV	2 V	5 V	13 V	10 X
	Starking delicious	14 IV	30 IV	3 V	11 V	15 X
	Sugary	9 IV	24 IV	29 IV	4 V	22 VIII

A small offset was recorded between varieties, the earliest one being the local variety at which the buds opened ten days earlier than the modern varieties and matured in the second half of July. From the two foreign varieties, the *Golden delicious* variety is earlier than the other one, both regarding the blooming period and the maturation period.

The tree growth is slightly influenced by the variety, being more influenced by the area (Table 2).

Table 2  
Growth capacity of tree trunk for some apple tree varieties

Area	Variety	Trunk diameter (cm)			Difference
		2008	2009	Increase in growth	
The foot of slope	Golden delicious	24.3	25.6	1.30	M7
	Starking delicious	22.1	23.3	1.20	-
	Sugary	26.4	28.1	1.70	xx
1500 m	Golden delicious	18.9	19.8	0.90	00
	Starking delicious	18.1	19.2	1.10	0
	Sugary	22.5	23.9	1.40	-

DL 5% = 0, 15 cm

DL 1% = 0, 27 cm

DL 0,1% = 0, 45 cm

At the foot of the slope, the *Golden delicious* and *Starking delicious* had closed increases in growth, 1,2-1,3 cm/year, while the local variety had a higher increase of 1,7 cm, being more vigorous.

At higher altitudes, the growth rhythm was slower for all varieties, a lower increase being recorded for the *Golden delicious* variety, under 1 cm/year. Statistically, as compared to the *Golden delicious* variety as the control, the local variety was distinct significant for the first area and the *Golden delicious* and *Starking delicious* were distinct significant and significant negative respectively for the second area. The higher altitude ensured less favorable conditions and because of the lack of water, the trees not being irrigated, the water probably did not have a proper distribution during the vegetation period.

The production capacity was different between varieties. The local variety, producing small fruits, had a production lower than the foreign varieties, which behaved well (Table 3).

The comparison of the values obtained from the two varieties and at the two altitudes shows that the area from the foot of the slope is better from a productive point of view, the values of the production being between 12,65 t/ha for *Sugary* and 18,85 t/ha for *Golden delicious*.

Table 3

The production capacity of some apple tree varieties (t/ha) (Zabadani, 2009)

Area	Variety	2008	2009	Average	Significance
The foot of the slope	Golden delicious	18.5	19.2	18.85	Mt
	Starking delicious	17.4	16.8	17.1	0
	Sugary	12.1	13.2	12.65	000
1500 m	Golden delicious	15.6	16.1	15.85	0
	Starking delicious	12.1	12.3	12.2	000
	Sugary	12.4	13.2	12.8	000

DL 5% = 1.25 t/ha

DL 1% = 3.49 t/ha

DL 0, 1% = 5.60 t/ha

At the altitude of 1500 m, the production decreases with about 3 t/ha for *Golden delicious* and *Starking delicious* varieties and is relatively the same for the local variety, which proved to be well adapted to the local conditions. Statistically, comparing the production from the two areas with the production obtained from the *Golden delicious* variety at the foot of the slope, it can be observed that the values are lower, from significant to highly significant negative.

The economic efficiency of the apple culture shows that good results can be obtained if the production cost of fruits is less than 20 lire/kg (Table 4).

The total expenses are higher for the irrigated culture, but the economic results are comparable for the two systems of culture during 2008-2009, period in which the plantation without irrigation had enough water from the upper area, especially water resulted from the melting of snow.

Table 4

The economic efficiency of apple culture (*Golden delicious* variety) in Syria  
syrian lire

<b>Expenses</b>	<b>Irrigated</b>	<b>Not irrigated</b>
Mechanical soil works	5280	3180
Manual soil works	8000	6220
Anti hail protection	15000	15000
Cuttings	22500	21673
Fertilization	18824	18150
Transport and sorting	18500	16900
Packing	5800	5600
Plant health protection	32010	32010
Irrigation	54226	-
Indirect expenses	31976	25500
Total expenses	217916	147453
Amortization 15%	32687	22117.9
Total cost	250603.4	169570.9
Average production	18850	15850
Average cost	13.29	10.69
Average capitalization price	25.00	25.00
Total revenue	471250	396250
Net revenue	220646.6	226679.1

### CONCLUSIONS

From the conducted research, several conclusions can be drawn, being the followings:

- In Sergaia Valley, Syria, the apple culture can be realized both with irrigation on the valley and without irrigation at higher altitudes where the water contribution is relatively enough for the growing and fructification of apple trees;
- The *Golden delicious* and *Starking delicious* varieties well adapted in the analyzed area, the productions being proper for the two systems of culture;
- Comparing the two systems of culture, with and without irrigation, the production was higher in the irrigated plantation, the maturation of fruits was slightly earlier and the trees had longer growths.

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## RESEARCH REGARDING THE MANAGEMENT OF TOMATO PLANT IN UNHEATED GREENHOUSES

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**KEY WORDS:** tomato, management, support

### SUMMARY

*The research regards the possibility of tomato plant management in protected system, in other ways than the traditional ones, in order to better capitalize the environmental conditions, especially the light. In this respect, a tomato culture was founded in unheated greenhouses, at densities between 3,3 pl/m<sup>2</sup> and 4 pl/m<sup>2</sup> and with a plant management consisting of a single strain, oblique for V2 and V3 and alternatively, in V shape along the row, and vertical for the control variant. It was observed that the plants reacted well at this management system, this being measured by the vegetative growth, but also by the fructification capacity.*

*The plants with oblique management system had a similar height with the plant of the control variant, and the number of flowers and fruits from the inflorescence was slightly lower than for the control. It was visible that the percentage of fruits formed from the flowers had higher values at the oblique managed variants. The same tendency was observed at the average fruit weight and fruit production. This fact demonstrates that this type of plant management is favorable for the tomato plants, better capitalizing the light.*

### INTRODUCTION

The tomato plant management in different systems of culture represents a special research conducted in order to ensure an efficient penetration of the light to the plants (Verkerk, 1955).

The management system influences both the vegetative growth of the plants and the fructification capacity (Maniutiu, 2002). Also, it is a mandatory work for the system of culture in protected and early spaces. The health of the plants and the fruits quality are by far improved in supported cultures compared to the unsupported ones.

The tomato plant management in protected areas usually consists of a single vertical strain, but there also is the possibility of plant management consisting of two strains that come either from two side shoots from the base of the strain, or from the main strain and from the first side shoot from its base. For a better capitalization of the lighting conditions, the plant management can consist of a single strain, but oblique and alternatively, as they form a shape similar to the letter V along the row (Papadopoulos and Pararajasingham, 1997; Hoza, 2008).

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## MATERIAL AND METHOD

The research was conducted in the teaching field of the Faculty of Horticulture, Bucharest, in 2009, in unheated greenhouses.

The used biological material was the *Cristal F1* hybrid. The planting in the unheated greenhouse was made in the III<sup>rd</sup> decade of April, because the temperature conditions during the night did not allow earlier planting due to the danger of lower temperatures, under the resistance limit of the plants.

The experimental variants were the following:

- V1 (control): 80 cm between the rows, and between the plants on a row the distance of 35 cm, resulting a density of 3, 5 plants/m<sup>2</sup>.
- V2: 100 cm between the rows, and between the plants on a row 30 cm, resulting a density of 3, 3 plants/m<sup>2</sup>.
- V3: 100 cm between the rows, and between the plants on a row 25 cm, with a density of 4 plants/m<sup>2</sup>.

The V2 and V3 variants were managed in V shape, perpendicular on the row, alternatively.

During the vegetation period, both general and special maintenance works were applied, specific to the tomato cultures.

During the research, a series of observations and measurements were made regarding the vegetative growth of tomato plants measured by height, number of leaves between inflorescences and the distance to the first inflorescence. Also, the fructification capacity was recorded by analyzing the number of flowers of each inflorescence, the data being recorded from the first five inflorescences, the number of formed fruits and the forming percentage of fruits from each inflorescence. When harvesting, the average fruit weight was determined and the production per unit of area was estimated.

## THE RESULTS OF RESEARCH

The height of the plants to the fifth inflorescence was slightly different between the variants which represented different densities of plants and oblique strain management and the control variant with a single vertical strain and regular planting distances, varying between 136 cm for V2 and 160 cm for the control, indicator that was influenced by the distance between the inflorescences (Table 1).

Regarding the distance to the first inflorescence, it had values between 31,5 cm for the second variant and 36,9 cm for the control. This indicator was influenced by the conditions of culture, but especially by the variety or hybrid. The number of leaves between the inflorescences was between 1,9 for V2 and 2,1 for the control and V3, the distance between the inflorescences varying between 25,9 cm for V2 and 31 cm for the control (Table 1).

Table 1

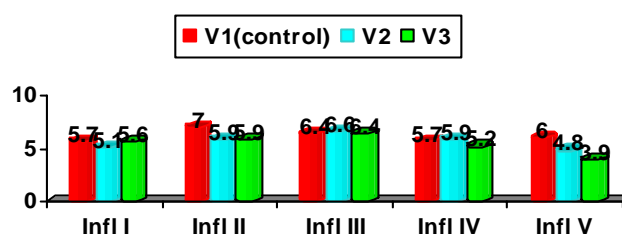
Biometric indicators of tomato plants

Variant	Distance to the first inflorescence (cm)	Number of leaves between inflorescences	Distance between inflorescences (cm)	Plant height ( cm )	Significance
V1(control)	36,9	2,1	31	160	Mt
V2	31,5	1,9	25,9	136	00
V3	33,7	2,1	28,4	145	0

DL 5% = 13,8cm  
DL 1% = 22,84cm  
DL 0,1% = 42,76cm

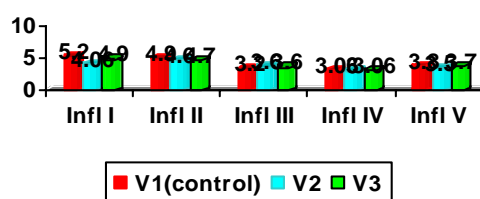
Regarding the average number of flowers in an inflorescence, it can be observed that the results are similar, with small differences between the control and the other two variants, due to the air currents that were stronger for the second and third variants compared to the control. For the control, the results obtained were 5,7 flowers for inflorescences I and IV and 7 for the second one. For the second variant, the results were between 4,8 for inflorescence V and 6,6 for inflorescence III. For the third variant, the results were between 3,9 for inflorescence V and 6,4 for inflorescence III (fig. 1)

**Fig.1 Number of flowers in inflorescences**



The number of fruits in an inflorescence was influenced by the type of tomato plant management, but also by the planting distances, different from the standard ones. This way, it can be observed that the differences in results are very small between the control and the other two variants. Compared to the control variant, where a total value of 19 fruits/plant was obtained, the other two variants had lower values, between 0,5 and 1,3 fruits/plant (fig. 2)

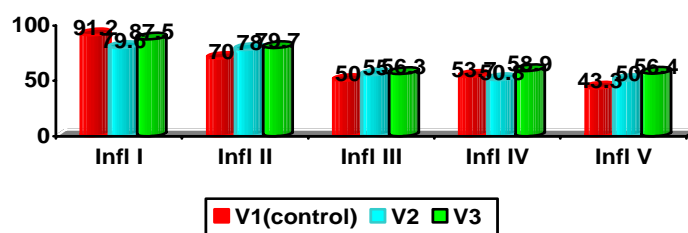
**Fig.2 Number of fruits in inflorescences**



The percentage of fruit that formed from the flowers shows that the planting distances that were chosen for each variant and the V shape plant management of the second and third variants, as compared to the vertical plant management of the control, have led to better results regarding the production. For the control variant, a percentage between 43,3% for inflorescence V and 91,2% for inflorescence I was recorded. For the second variant, the percentage was between 50% for inflorescence V and 79,6% for

inflorescence I. The third variant had a fruit forming percentage between 56,2% for inflorescence III and 87,5% for inflorescence I (fig. 3)

**Fig. 3 Fruit forming percentage**



The fruit production had different values from one variant to another (Table 2). The average fruit weight had values between 85,5 g for the control and 98,5 g for the third variant. The production measured in kg/plant had values between 1,6 kg for the control and 1,7 kg for the second and third variants. Regarding the production measured in kg/m<sup>2</sup>, the values were between 5,6 kg for the control and 6,8 kg for the third variant. The difference from the control show an increase of only 1% for the second variant, and a significant 21% increase for the third variant compared to the control.

Table 2

Fruit production

Variant	Average weight (g)	PRODUCTION				
		Significance	Kg/plant	Kg/m <sup>2</sup>	Difference from V1 (%)	Significance
V1 (control)	85,5	Mt	1,6	5,6	100	Mt
V2	98,5	xxx	1,7	5,7	1	N
V3	94	xxx	1,7	6,8	21	xxx

DL 1% = 0,65g  
DL 5% = 1,08g  
DL 0,1% = 2,02g

DL 1% = 0,34kg  
DL 5% = 0,57kg  
DL 0,1% = 1,07kg

## CONCLUSIONS

From the research conducted about the tomato culture founded at different planting distances and a different type of plant management, the following conclusions can be drawn:

- The V shape plant management system, with a single oblique strain positively influenced the production for the second and third variants, as compared to the control, where the production was lower;

- The number of flowers was higher at the control, because it was protected against the air currents, unlike the second and third variants which suffered from the environmental factors;
- The number of fruits in an inflorescence was influenced by the light and variety, the results obtained being similar, with small differences between the second and third variants and the control variant;
- Regarding the percentage of fruits that formed from flowers, it had the highest value for the second and third variants, which can be explained by single strain plant management, alternatively, in V shape, the light being better capitalized, and the number of plants per unit of area was higher than for the control variant;
- The estimated production for the second variant, as compared to the control, had an increase in value of only 1%, but the third variant presented an increase of 21%. This is due to the V shape plant management system, but also to the capitalization of light which positively influenced the production for the second and third variant.

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## PROLACTINOMA: CLINICAL AND EPIDEMIOLOGIC ASPECTS

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**KEY WORDS:** *pituitary adenoma, hyperprolactinemia, galactorrhea*

### ABSTRACT

*Hyperprolactinemia represents the excessive production of prolactin (PRL) by the lactotropa cells, being the most common affection of the anterior pituitary. The causes of the increase of PRL production are numerous, including the adenoma secreting PRL or the prolactinoma, the most common type of pituitary secreting tumor (represents about 39% of them), followed by GH secreting adenomas (acromegaly) and ACTH secreting tumors (Cushing).*

*The goal of the study is to study the incidence and clinical and paraclinical characteristics of the pituitary PRL secreting adenomas in Dolj county.*

### INTRODUCTION

The discovery of the prolactinoma (PRL) in 1930 by Riddle and co-workers was followed by the first clinical observations of a syndrome which associated amenorrhea with galactorrhea.

In the following 20 years 3 distinct clinical syndromes were described:

1. Chiari-Frommel syndrome – amenorrhea, galactorrhea and low levels of urinary gonadotropins appeared postpartum.

2. Ahumada – Argonz – del Castillo syndrome – non puerperal amenorrhea, galactorrhea și nivelele de gonadotrofine urinare scăzute, fără evidența unei tumori hipofizare pe șaua turcească standard (1959).

3. Sindromul Forbes – Henneman – Groszold – Albrith – amenoree nonpuerperală, galactoree and low levels of urinary gonadotropins in association with the cromofob pituitary adenoma (1954) (Ambrosi, 1997).

Hyperprolactinemia (HPRL) = the excessive production of PRL by the lactotropa cells, is the most common affection of the anterior pituitary. The causes of the increase of PRL production are numerous, including the adenoma secreting PRL or the prolactinoma, the most common type of pituitary secreting tumor (represents about 39% of them), followed by GH secreting adenomas (acromegaly) and ACTH secreting tumors (Cushing). The ACTH secreting adenomas leading to Nelson syndrome, TSH, gonadotropins or subunits are rare. (Bistriceanu, 2000)

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The prolactinomas are 4,5 – 5 times more frequent at women than at men: microprolactinomas (adenomas with the diameter smaller than 10 mm) are rare at men (7 – 15%), at women representing 58 – 64% of the prolactinomas.

In literature a rapport of 20/1 in favour of women for microprolactinomas and of 1/1 for macroprolactinomas is mentioned. (Dumitrache, 1997)

The medium age of diagnostic is of 33 years at women and 49 years at men.

The etiology of the excessive secretion of PRL is multiple and implies functional disturbances generated by stress, physical effort, hormones, medication, organic hypothalamic-pituitary causes, PRL secreting or non secreting tumors, but which stop the circulation of blood through port veins, as well as distance organic lesions which generate hyperprolactinemia through reflex mechanisms.

## **MATERIALS AND METHODS**

In the studied lot I had 27 prolactinomas which were studied clinically and paraclinically. The clinical exam was based on history, endocrinologic, neurologic and eye exams (visual acuity and visual field). The paraclinical exam implied hormone determinations made through Eclesys 1010 dosing system produced by Roche Diagnostics company, held by the Clinical Laboratory of the County Emergency Hospital of Craiova, together with the sets of Kits suitable for the determination of hFSH, hLH (LH specific human), hPRL, hGH, hTSH, ACTH. This uses as a working method the method of electrochemiluminescence.

The paraclinical exam comprised also imagistic methods:

1. Skull radiography of profile centered on the Turkish saddle is the method of morphological screening in the diagnostic suspicion of pituitary adenoma and represents a routine investigation. I practiced it automatically at each internation of a patient with the diagnostic of pituitary adenoma, in order to compare the successive images and to obtain a first imagistic investigation concerning the evolution of the tumor.

2. The CT was done in the Medical Radiology and Imagistic Section of the County Emergency Hospital of Craiova. This investigation is irreplaceable in exploring the intracranial formations (100 times more precise the conventional radiologic image) and allows the evaluation of the pituitary tumor and of the extension level.

3. The nuclear MRI was performed at a number of selected cases, at which the CT was not conclusive. The used MRI protocols consisted of T1 sections in the coronary and sagittal planes, pre-and post administration of Gadolinium, with a thickness of the sections up to 2 mm. Occasionally T2 incidences were performed.

## **RESULTS AND DISCUSSIONS**

In the studied lot the PRL determination presented a wide range of values. The confirmation of the fact that the pituitary adenomas were prolactinomas was done based on a unic criteria, that is that the PRL must be higher than 637  $\mu\text{UI/ml}$  (the dosing of the prolactin was done at a laboratory which had normal values between 127-637  $\mu\text{UI/ml}$ , although literature includes a series of opinion divergences regarding the validity of this minimum value of PRL as unique criteria of diagnostic of a prolactinoma when the pituitary adenoma was already imagistic detected through MRI).

The serum prolactin was dosed at 27 patients, the medium value registered of the prolactin was of 2229,19  $\mu\text{UI/ml}$ , with limits between 386  $\mu\text{UI/ml}$  and 9360  $\mu\text{UI/ml}$  (the value of 386  $\mu\text{UI/ml}$  was under treatment with bromocriptin). In the statistic calculation

was not included the value of 124200  $\mu\text{UI/ml}$ , met at a patient with macroprolactinoma with diameters of 32,7/28/32,2 mm, with left cavernous sinus invasion, with intraparasellar dislocation especially on the left side and with minimum suprasellar extension. This value was not included in the study because of the enormous difference from the other values of PRL from the studied lot. Although the diagnostic technics have improved a lot (CT, MRI, hormone determinations), the historical period of the pituitary adenomas is not shorter compared to that from the older studies. Women complain more generally of poor condition, headache, fatigue.. At men, the clinical manifestations of hyperprolactinemia draw attention at older ages, being put in relation with a higher frequency of the macroprolactinomas. (Aerts, 1998)

The clinical manifestations of HPRL, appear in variable rate in prolactinoma.

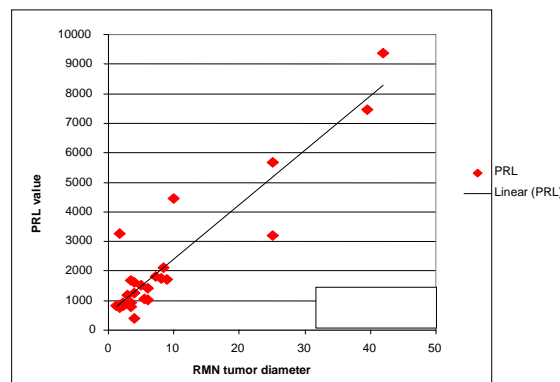
At women appear:

- Secondary amenorrhea – in 70% of the cases it can be the only manifestation;
- Primary amenorrhea – 6%
- Regular menses 15%
- galactorrhea 57%
- galactorrhea with regular menses 6%
- infertility 94%
- infertility with regular menses and luteal phase abnormalities 4%

At men appear:

- impotence and decrease of the libido 91%
- galactorrhea and/or gynecomasty 14%
- visual defects 41%
- fatness 69%
- apathy 63%
- headaches 63%.

In the case of tumors with supra/parasellar extension (macroadenomas) appears the neurologic syndrome determined through the compression of the nearby structures.



Graphic 1. The correlation between the dimensions of the tumor and the PRL level

From the Graphic 1 we can draw the conclusion that there is a direct correlation between the dimensions of the RMN measured tumor and the secretion of prolactin (the bigger the diameter of the tumor is, the bigger is the quantity of hormone), because the right



slope of the regression is clearly upward. This fact is underlined also statistically, the Pearson correlation coefficient  $r$  having the value of 0,923, which far surpasses the materiality of  $\pm 0.381$  of  $r$  for 27 subjects (25 degrees of freedom).

In the studied lot were 27 prolactinoma cases, out of which 5 macroprolactinomas (PRL higher than 4468 microUI/ml), that is 18,5%.

Taking this correlation into account, I underlined a higher incidence of microprolactinomas at women, compared to men, and a higher incidence of macroprolactinomas at men. This aspect confirms the dates existent in literature, which registered a higher incidence of macroprolactinomas at men, the major causes being late presentation to the doctor or minimising the sexual dynamic disorders.

In the subplot of the patients with prolactinoma, I had the following pathologic changes (Table 1):

- systolic HTA at 3 patients (11,1%) and systolo - diastolic HTA at 1 patient (3,7%);
- ventricular shape –AV-bigger than 80 beats/min at 10 patients;
- no patient had the glycemia over 110 mg/dl;
- cholesterol over the superior limit (over 200 mg /dl) was present at 9 patients (33,3%);
- triglycerides over the maximum limit of 150 mg /dl were met at 5 patients (18,5%);
- 18,5%, respectively 5 patients had macroprolactinoma (one of the diameters of the tumor bigger than 10 mm), while 22 patients (81,5%) had microprolactinoma.

Table 1

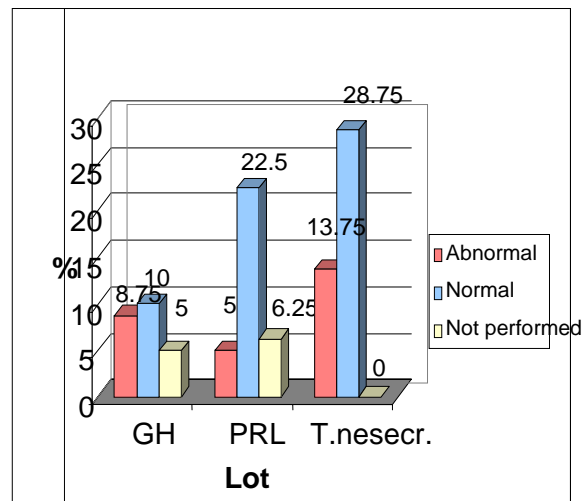
Pathological changes at patients with pituitary adenoma

PRL	TS>140	TD>90	AV>80	GLIC>110	COL>200	TRI>150	RMN>10
NO	24	26	17	27	18	22	22
YES	3	1	10	0	9	5	5

Although in literature is mentioned the appearance of high glycemias in the group of PRL secreting adenomas, there are no reliable data on the direct involvement of PRL in the pathogenesis of diabetes (Groot, 1995; Kadioglu, 1998). Although, in hyperprolactinemia resistance to insulin was documented, explaining the increase of secretion of this. The hypothesis that, in normal conditions, PRL is not diabetogen was issued, this effect being showed only at subjects with malfunction of  $\beta$  pancreatic cells. In the present study no patient with prolactinoma had glycemias over the maximum limit. Also the prolactin modifies the lipidic metabolism, but this effect does not correlate with the degree of overweight, patients with dyslipidemia being met in the present study too (9 patients with hypercholesterolemia and 5 patients with hypertriglyceridemia).

Visual disorders appear through compression and distension of optic nerves, especially at prolactinomas with suprasellar extension. More than 50% of the men have visual disorders, while, at women, these exist only in 25% of the cases, and this because of higher frequency of macroprolactinomas and of the suprasellar extension at men. The most frequent visual disorders are:

- the optic chiasma syndrome, manifested by bitemporal hemianopsy (defect of the external half of the visual field); homonymous hemianopsy, produced by compression of fibers which cross and do not cross on the same part of the chiasma; blindness – by compressing the optic nerve uni- or bilateral; central scotom or temporal or full blindness of an eye, with temporal defect in the other; decrease of the visual acuity;



Graphic 2. Analyse of the visual field

-at the exam of the fundus: discoloration and then atrophy of the optic nerve head.

In the lot of the studied 80 pituitary tumors, in the case of PRL secreting tumors, the affecting of the visual field was met at only 5% of prolactinomas, because of the high percent of the microprolactinomas.



Figure 1. Radiography of Turkish saddle at patient with macroprolactinoma

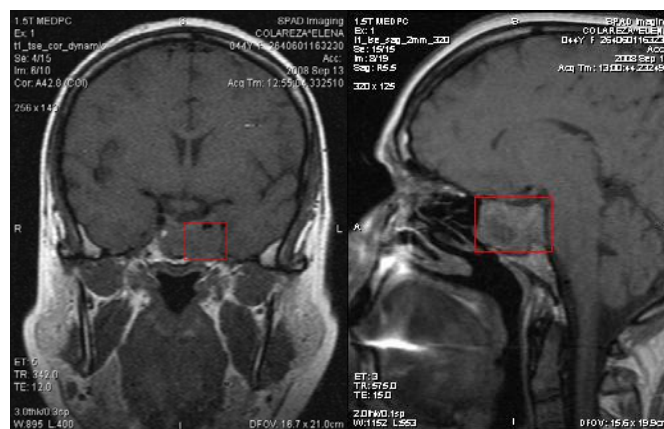


Figure 2. MRI image - sagittal and coronary section at patient with macroprolactinoma with cavernous sinus invasion

Natural history of development and progression of the prolactinoma is not known precisely, but it is known that most of these tumors develop slowly, in years. Autopsy studies showed that 23 – 27% of the persons had pituitary microadenomas, most of them not having antemortem evidences of endocrine malfunctions and about 40% of the tumors are positive for PRL in immunohistochemistry tests.

Microprolactinomas which evolve become macroprolactinomas and extend especially in the suprasellar tank, then invade and destroy the base of the brain. The invasive growth characterises 7 – 31% of the prolactinomas and appear only after long periods of growth of the tumor (Paoletta, 1998).

The diagnostic of progressive prolactinoma is placed based on comparing the serial radiographies of Turkish saddle, which show a growth in time of the tumor.

The complications of the prolactinoma can be classified in:

1. complications determined by the dimensions of the tumor;
2. complications determined by the HPRL syndrome.

1. Microprolactinoma is an intrasellar tumor, so no visual defects appear. Anyhow, headache appears more frequently (50%) than at normal subjects (27%).

A large tumor, which extends beyond the limits of the Turkish saddle, is often accompanied by headaches and visual defects. Disorders of the visual field appear and can last even after the treatment. A complication which appears more rarely is blindness.

Lateral extension into cavernous sinus can cause poate cauza deterioration of oculomotor functions in which the cranian nerves III, IV and V are implied and the affectation of the ophtalmic branches (IV) and/or maxilary (V2) of the V nerve. In the present study I had a patient with macroprolactinoma with invasion in the left cavernous sinus (see the above MRI image), but without the deterioration of the oculomotor function!

The tumor can determine by compression the damage of the hypothalamic cores, consecutively can appear: insipidus diabetes, endocrine disorders, dietary adjustment disorders, thermoregulation disorders, sleep disorders, vegetative disorders. Very rarely, the inferior evolution of the tumor towards the sphenoid sinus, can lead to communication with the exterior and to appearance of licvoree and meningeal infections.

Occasionally, the large tumors can extend in the temporal lob, having as complication temporal epilepsy.

The patients with big tumors present a risk to compromise the other functions of the adenopituitary by compressing the normal pituitary tissue, isolated or combined hormonal deficiencies of GH, ACTH, LH, FSH, TSH being produced (Ferrari, 1997).

Also, there is the possibility of spontaneous stagnation of the evolution of a pituitary adenoma, by necrobiosis tumor and subsequent calcification, without manifesting with clinical visible signs.

2. Complications determined by hyperprolactinemic syndrome - Involution of the genital tract – frequently irreversible – as well as gonadal insufficiency is because of the affectation of pulsed release of gonadotropins LH and FSH.

Among the complications generated by chronic HPRL, was included also the decrease of the bone density. At men the decrease of the HPRL and the restoration of the gonadal function are accompanied by an increase of the bone density in the cortical bone and a very small change in the density of the vertebral bone.

At the amenorrhoeic women with HPRL, the mineral bone content is low compared to the one of the amenorrhoeic women but with normal PRL and with the one of the eugonadal women, which suggests that PRL could have a direct effect on the bone metabolism.

Conclusions:

- prolactinoma has a relatively high frequency among the affections which produce disorders of the gonadic function, holding, because of this, an important role in determining endocrine infertility;
- PRL dosing and radiography of Turkish saddle are absolutely necessary exams in screening diagnostic of prolactinomas;
- Investigation of the patients with disorders of the menstrual rhythm, with or without galactorrhea, is necessary before beginning a treatment for these symptoms (Bistriceanu, 2000).

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STUDIES UPON THE INFLUENCE OF MANUAL THINNING OF GENEROS  
APPLES IN CONDITIONS OF TIMISOARA

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**KEY WORDS:** *Generos, apples, manual thinning, weight, production*

**ABSTRACT**

*Fruit thinning is one of the most efficient methods of controlling fruits qualities, being done in different development stages of fruits and by different means. Manual thinning is normally done after the physiological fall of fruits in order to improve the physical and chemical features of fruits. It is recommended to leave only one fruit in the fruit group, so that there will be a distance of 12 to 20 cm between the fruits and to have enough space for nutrition. This operation has to be done very carefully and there are always removed the injured and damaged fruits, leaving the healthy ones on the branches. In this article there is presented the impact of manual thinning upon Generos apple variety cultivated in conditions of Timisoara, concerning the fruits' weight and production. Thinning was done in June, after the physiological fall of apples, leaving only one or two apples in the group. Out of the collected data we can say that the manual fruit thinning definitely has an impact upon fruits' qualities (weight), but it does not influence too much the fruit production.*

**INTRODUCTION**

Fruit rate-setting can be done by different methods, such as: fruiting pruning, reduction and thinning, having the goals of reducing the number of fruits left on the tress in order to obtain quality fruits and not large productions and also of reducing the alternation phenomenon that is very frequent in apple tree culture (Baciu, 2005).

Apple thinning can be done in different ways: chemical, mechanical or manual, and can be applied in different fruiting phenophases: to mixed buds, flowers or fruits (Gonda, 2003).

Manual thinning can be done in different stages of fruiting development. It can be applied early in the season to mixed buds, flowers or fruits that have a diameter of 1 cm (Baciu, 2005). This early thinning has a good impact upon the quality of fruits, but it also stimulates the buds differentiation, having an indirect influence upon alternation (Gonda, 2003). When manual thinning is done sometime in June, after the physiological fall of apples when they have 3-4 cm diameter, it has a good influence upon fruits' physical and chemical qualities, but a very small impact upon buds differentiation (Iordanescu et al., 2009). While doing the manual thinning there are taken away the small, damaged, attacked and inadequate apples and there are left the healthy and large ones in the group (Iordanescu et al., 2009). Normally one leaves only one apple in the group at a distance of 10-20 cm between them (Iordanescu, 2008; Iordanescu et al., 2009).

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## MATERIAL AND METHODS

The experiment was made in the Didactic Plantation of the Fruit Tree Culture Department in our University, in the specific ecological and pedological conditions of the area, during the years 2008 and 2009.

The biological material consisted in Generos apple trees variety established in 1997, which were grafted on M106, planted at the distances of 4x2 m and given a free palmed crown.

The research purpose consisted in observing the impact of apples manual thinning of different intensities upon the productivity of fruits.

The experimental variants were:

- V1 – 50% thinned fruits
- V2 – 25% thinned fruits
- V3 – 30% thinned fruits
- V4 – 40% thinned fruits
- V5 – not thinned (witness)

The collected data was statistically calculated and interpreted, using the variance analyses method.

## RESULTS AND DISCUSSIONS

Of all the results obtained in the years 2008 and 2009, in this paper work we will present two parameters: apples' average weight (g) and average production (kg/tree).

Table 1

Generos apples' average weight (g) in 2008

Variant	Average value (g)	Relative value (%)	Difference to the witness	Significance
V <sub>1</sub> –50% thinned	166.50	137.60	45.5	XXX
V <sub>2</sub> –25% thinned	105.20	86.94	- 15.80	000
V <sub>3</sub> –30% thinned	140.30	115.95	19.30	XXX
V <sub>4</sub> –40% thinned	154.30	127.52	33.30	XXX
V <sub>5</sub> – not thinned	121.00	100.00	0.00	ct.

DL 5% = 4.48

DL 1% = 6.51

DL 0.1% = 9.77

In 2008, the highest average weight for Generos variety of apples was registered in variant 1 – 166.50g, being very significant positive than the control variant which had 121.00 g.

Variants 3 and 4 with an average weight of 140.30 g, respectively 154.30 g surpassed the value of the control variant and were also very significant positive than it.

The lowest average weight of fruits was obtained in variant 2, whit only 105.20 g, lower than the average weight obtained in the control variant, reason why the differences to it were very significant negative.

In 2009, the highest average weight was obtained in variant 1 – 188.50 g, being very significant positive than the control variant – 171.40 g.

A good average weight was also obtained in variant 4, of 182.20 g, being followed by variant 2 where the average weight was of 178.00 g. both of these variants had very significant positive differences to the control variant.

In variant 3, where there were 30% thinned apples, the average weight (176.60g) was also higher than the value obtained in the control variant, but it registered distinct significant positive differences than the control.

Table 2

Generos apples' average weight (g) in 2009

Variant	Average value (g)	Relative value (%)	Difference to the witness	Significance
V <sub>1</sub> –50% thinned	188.50	109.97	17.10	XXX
V <sub>2</sub> –25% thinned	178.00	103.85	6.60	XXX
V <sub>3</sub> –30% thinned	176.60	103.03	5.20	XX
V <sub>4</sub> –40% thinned	182.20	106.30	10.80	XXX
V <sub>5</sub> – not thinned	171.40	100.00	0.00	ct.

DL 5% = 2.86

DL 1% = 4.16

DL 0.1% = 6.24

Table 3

Generos' variety average production (kg/tree) in 2008

Variant	Average value (kg/tree)	Relative value (%)	Difference to the witness	Significance
V <sub>1</sub> –50% thinned	19.64	83.22	- 3.96	0
V <sub>2</sub> –25% thinned	18.41	78.00	- 5.19	0
V <sub>3</sub> –30% thinned	23.15	98.09	- 0.45	-
V <sub>4</sub> –40% thinned	21.91	92.83	- 1.69	-
V <sub>5</sub> – not thinned	23.60	100.00	0.00	mt

DL 5% = 3.67

DL 1% = 5.33

DL 0.1% = 8.00

Concerning the average production obtained in 2008, in variant 5 - the control, we obtained the highest value of 23.60 kg/tree, which is why the other variants did not register positive significations. Variants 1 and 2 had average productions lower than the control that is why they had significant negative differences. In fact in these variants the average productions obtained were under 20 kg/tree, being surpassed by variants 3 and 4.

In variant 3, where 30% of apples were thinned, the average production was of 23.15 kg/tree, while in variant 4 the average production was of 21.91 kg/tree. Both of these variants were not statistically assured because the values were close to the one of the control variant.

Table 4

Generos' variety average production (kg/tree) in 2009

Variant	Average value (kg/tree)	Relative value (%)	Difference to the witness	Significance
V <sub>1</sub> –50% thinned	45.30	127.49	9.77	XXX
V <sub>2</sub> –25% thinned	38.50	108.35	2.97	XXX
V <sub>3</sub> –30% thinned	43.30	121.86	7.77	XXX
V <sub>4</sub> –40% thinned	39.40	110.89	3.87	XXX
V <sub>5</sub> – not thinned	35.53	100.00	0.00	mt.

DL 5% = 0.84

DL 1% = 1.23

DL 0.1% = 1.84

In 2009, Generos variety gave the highest average production in variant 1 – 50% thinned fruits, of 45.30 kg/tree, followed by variant 3 (43.30 kg/tree), variant 4 with 39.40 kg/tree and variant 2 with 38.50 kg/tree.

All of the variants where thinning was done gave higher average productions than the control (35.53 kg/tree) being very significant positive than it.

Concerning the average weight of apples and the average productions, we can say that 2009 was a very good year for apple culture, while in 2008 all the values were lower.

## CONCLUSIONS

By comparing the two studied years we can observe big differences concerning fruits' weight and the production obtained per tree, meaning that in 2009 the values were higher than in 2008. This can be explained by the fact that a constant thinning from one year to another, not just by manual thinning of fruits, but also by good pruning, has the effect of a better buds' differentiation and it assures a sufficient nutritive space for fruits, so that they become more attractive and of a higher quality, with an increase of productions.

In variant 5 – the control variant not thinned, the number of fruits left on the trees was higher, the apples had a lower weight, but the production was higher than in the thinned variants. On the trees where manual thinning was done, the apples weighted more, they were more attractive, but the production was lower than in the control variant.

In the case of Generos apple tree variety we could notice that on the variants where the thinning was more severe and the apples had higher weights, the production registered negative significations to the witness.

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**ASSESSMENT OF THE HUMAN IMPACT ON THE LANDSCAPE OF THE  
DANUBE FLOODPLAIN, IN DROBETA-TURNU SEVERIN – BECHET SECTOR**

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**KEY WORDS:** *Danube Floodplain, human impact, landscape changes, naturality*

**ABSTRACT**

*The human interventions in the Danube Floodplain are of great diversity and they altered the balance and the metabolism of natural ecosystems. In numerous cases, this type of intervention, under its multiple forms, led to irreversible changes of the natural environmental characteristics.*

*The present paper aims at analysing the nature and intensity of the human influence in the landscape transformation. Special attention has been paid to the important hydrological arrangement works and to their correlation with the extension of the agricultural fields, to the natural vegetation changes and to the evolution of the system of settlements, as well as that of the transportation network.*

*The dysfunctions induced by the human factor at the level of the landscape were analysed by using a series of indices concerning the human stress and the environmental changes.*

**INTRODUCTION**

The floodplain sector under analysis is located in south-west Romania and it covers a distance of about 260 km between the urban settlements of Drobeta-Turnu Severin and Bechet; its width is variable, going from a few tens of meters to more than 14 km (e.g. near Ciuperceni and Cârna). The minor landforms are heterogeneous, but, generally, there is to be noticed the longitudinal disposition in stripes: the marginal bank near the riverbed (the most elevated part), the median floodplain (partially swampy) and the low stripe of depressions (former swamps and lakes), regularly located towards the limit of the terraces (Antipa, 1910).

The strategic, natural and economic importance of the Danube Floodplain explains the development of human settlements and activities since ancient times and represents the framework of the important landscape changes registered in the region. Despite this exceptional value, the approaches towards the valorisation of its geographic individuality, the analysis of its natural, demographic and economic aspects in the framework of the sustainable development are relatively few. The present paper aims at analysing the nature and intensity of the human influence in the landscape changes, starting from the basic transformations occurred during the last two centuries and emphasizing those that took place during the second part of the 20<sup>th</sup> century, as well as the change of the tendency in the transition period.

**DATA AND METHODS**

For the analysis of the fundamental landscape transformations within the Danube Floodplain, a GIS database was realised, starting with cartographic materials from the 18<sup>th</sup>

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and the 19<sup>th</sup> centuries (Specht, 1790; Szatmari, 1864), continuing with different generations of topographical maps (1912, 1955-1960, 1975-1985) and with the 21<sup>st</sup> century orthophotos.

The transformations and the dysfunctions induced by the human factor were analysed by means of a series of synthetic indicators that represent quantitative values resulted from proportions of statistical data (Dumitraşcu, 2006); starting from the classical formulas, certain adjustments were made for the floodplain sector under analysis. The following indices were taken into account: the agricultural density, the human stress on the environment through arable use, through surfaces occupied by orchards or by vineyards, through built-up areas; the naturalness index; the environmental change index. The assessment was conducted at the level of the territorial-administrative units (TAUs) that include surfaces of the Danube Floodplain, in the Drobeta-Turnu Severin – Bechet sector. The data concerns the transition period, being presented the situation at the level of the years 1992, 2002, 2007.

### RESULTS AND DISCUSSIONS

The interaction of the natural processes within the floodplain environment leads to its evolution, but the intensity and manifestation forms of the human activity mark this. Although few settlements developed in the floodplain proper, the natural and strategic particularities of this unit generated early transformations of the landscape. At the end of the 18<sup>th</sup> century, the natural pastures and hay fields dominated the Danube Floodplain. The only human marks in the fluvial corridor were represented by the access roads to the river or by the reed fences in the Danube ponds – empiric fisheries as compared to the present ones. The sheep breeding starts to decline in the second half of the 19<sup>th</sup> century, the cultivation of cereals being favoured especially after the Adrianopole Peace (1829) (*Geografia Văii Dunării Româneşti*, 1969; Tomescu, 1998). After the Second World War, the most extensive works started in the Danube Floodplain, the man-induced landscape transformations having sometimes alarming effects (Figure 1).

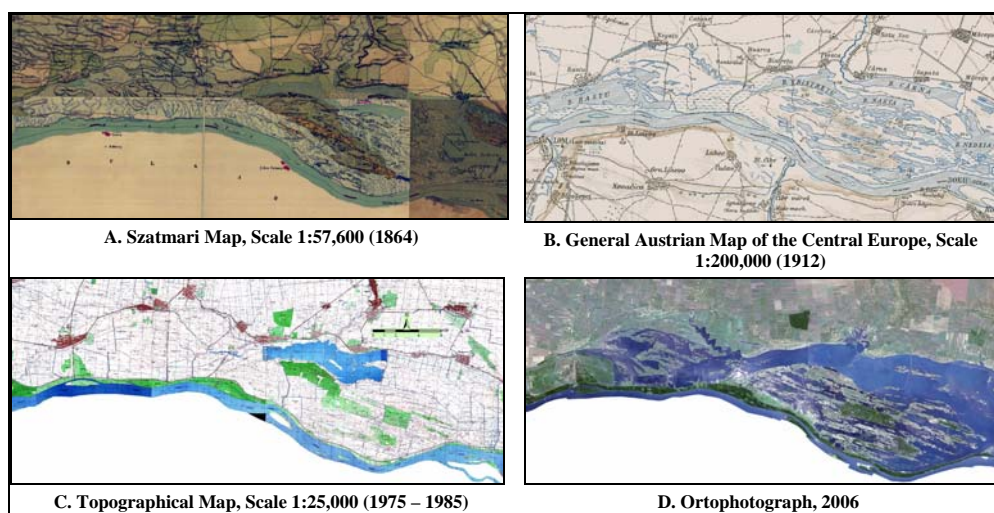


Figure 1. Rast – Bistret Area. Landscape Changes Mirrored in the Cartographic Documents from the Last Two Centuries

The human stress within the Danube Floodplain takes numerous forms and represents the main responsible for the state of the environment. The most important transformations that marked the natural landscape of the floodplain concern the achievement of hydroelectric dams, of the transportation network, the development of

human settlements and economic activities, the hydrological improvement works (longitudinal and partitioning dams, drainage or irrigation canals, rice paddies, levelling of the sand dunes), the appearance of fisheries, the new types of land use; there are also to be noticed other changes induced by human activity (the secondary soil salinization, the covering with ash heaps, the restructuring of the terrestrial and aquatic biocenoses etc.) It may be concluded that, from the viewpoint of the agricultural use, the floodplain undergone the most severe transformations (Pătroescu et al., 2000).

One of the relevant methods that leads to the assessment of the type and dimension of the anthropic intervention within a territorial unit is represented by the computation of synthetic indexes that help evaluate the degree of landscape change (Gergel et al., 2002; Turner et al., 2001).

**The agricultural density** offers an image of the population's repartition related to the agricultural terrain resources. The dominant values of this index (Fig. 2) range between 30 and 100 inhab./100 ha agricultural surface (18 of the 25 TAUs under study), the minimum of 31.17 inhab./100 ha being registered in Măceșu de Jos. These low values are explained by the prevalently rural character of the sector, with relatively few inhabitants that mostly activate in the primary sector and extended agricultural surfaces.

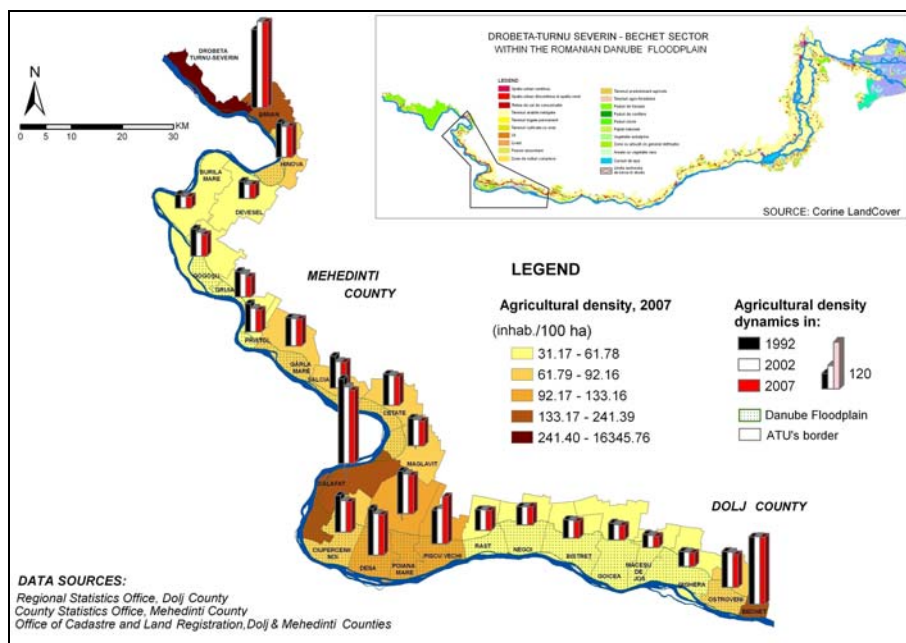


Figure 2. Agricultural Density in the Sector under Analysis

The higher values are registered in the three urban settlements (Drobeta-Turnu Severin – maximum of 16345.76 inhab./100 ha, Calafat and Bechet), as well as in the nearby communes (Șimian) or in the large TAUs (Poiana Mare – 11,848 inhabitants in 2007). There are to be added certain rural units where important surfaces are still covered by forest or are inappropriate for agriculture (Desa, Piscu Vechi).

**The human stress on the environment** is a synthetic indicator of its quality and of the degree of its transformation, as effect of the anthropic intervention. Taking into consideration the fact that in the floodplain sector under analysis the rural space and the agricultural activities are prevailing, it is necessary to analyse the interactions among these

activities and the various types of land use with the elements of the natural environment (Dale et al., 2000; O'Neill et al., 1997). The human stress upon the environment was more accentuated as the above-mentioned activities included surfaces on which the spontaneous vegetation was replaced with various cultures. The arable fields hold important shares (about 40% of the surface under analysis), even more important values being registered at the level of Hinova, Gogoşu, Rast, Negoî, Măceşu de Jos, Gighera, Ostroveni and Bechet communes, where the natural vegetation was replaced by agricultural crops that were mostly irrigated before 1989, but which are realized in non-irrigated system at present.

*The human stress through arable use* (the proportion between the arable surface and the population of the TAUs on study) registers the lowest values (Fig. 3) in the three urban centres, where important surfaces are occupied by constructions, industrial infrastructure, recreation areas etc. (minimum at Drobeta-Turnu Severin – 0.004), while high values are noticed in Rast – Gighera sub-sector (maximum at Măceşu de Jos – 2.84). The somewhat lower values registered in Ciupercenii Noi – Piscu Vechi sub-sector are explained by the extension of the sandy terrains or of those that preserve more extended forest surfaces. The dynamics of the index undergoes a slight decrease between 1992 and 2007 in the eastern sector, fact which underlines the relation between the arable surfaces and the destruction of the once extensive irrigation systems in the Danube Floodplain. The inclusion of certain surfaces - formerly occupied by more demanding crops - in the category of arable terrains might explain the augmentations that are sometimes noticed.

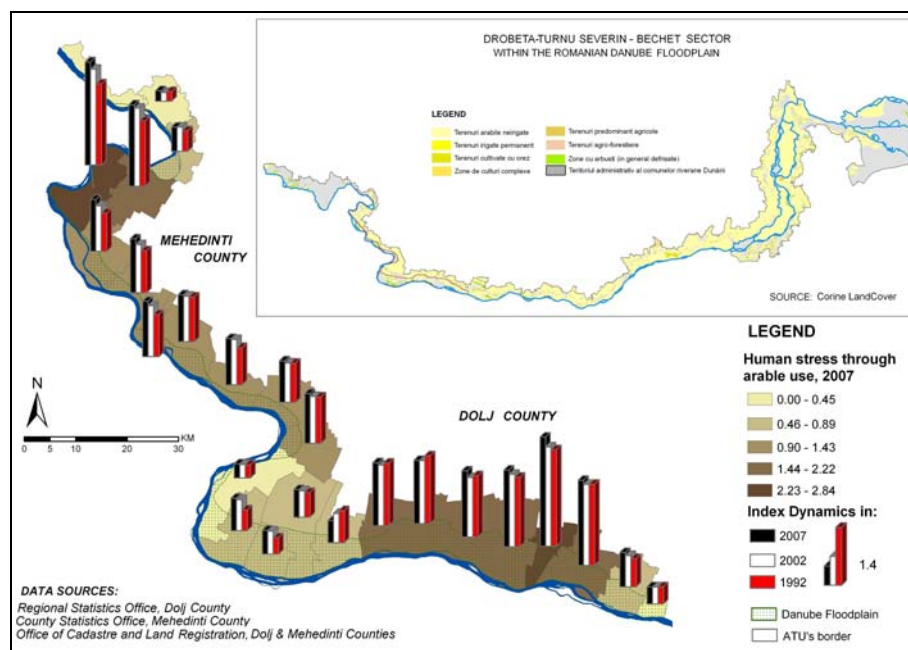


Figure 3. Human Stress on the Environment through Arable Use in the Sector under Analysis

*The human stress through orchards* is less significant in the Danube Floodplain (the orchards hold about 0.05% of the surface, being mostly located in the northern part, on the Danube terraces). In 2007, most of the TAUs registered extremely low values, under 0.004 (Maglavit, Ciupercenii Noi, Rast, Bechet and others). The quasi-generalised drop of this type of stress is explained by the changes appeared in the ownership form after 1989, the

difficulties connected with the maintenance, the production costs and the obstacles faced in the market, all leading to abandonment from the part of small orchard owners.

*The human stress through vineyards* shows more important values (as the vineries hold 2.8% of the surface under analysis, having relatively favourable conditions of development: S and SW exposition, sandy fields etc.). Although the changes do not show significant changes between 1992 and 2007, it must be noticed the slight decrease of the human stress through this type of culture. There are also situations in which the new owners restored the old vineries or set up new ones, but this is an unsystematic and disparate phenomenon at the level of the floodplain, the grape-vine being cultivated on small surfaces and often intermingled with other crops.

*The human stress through built-up surfaces.* The surfaces occupied by civil constructions, industrial or trade units, transportation infrastructure etc. have a share of about 2% of the floodplain sector on study (Figure 4). Higher stress values are registered at the level of the rural settlements Măceșu de Jos (0.27), Gighera, Burila Mare (over 0.23), an important aspect being the small number of inhabitants to which this type of surface is reported. The lowest values are characteristic to the urban centers (Drobeta-Turnu Severin – 0.01, Calafat, Bechet – under 0.07), as well as to Șimian, Hinova, Gârla Mare communes (under 0.06). In most of the TAUs on study, there is to be remarked a significant increase of this stress, especially after 2002.

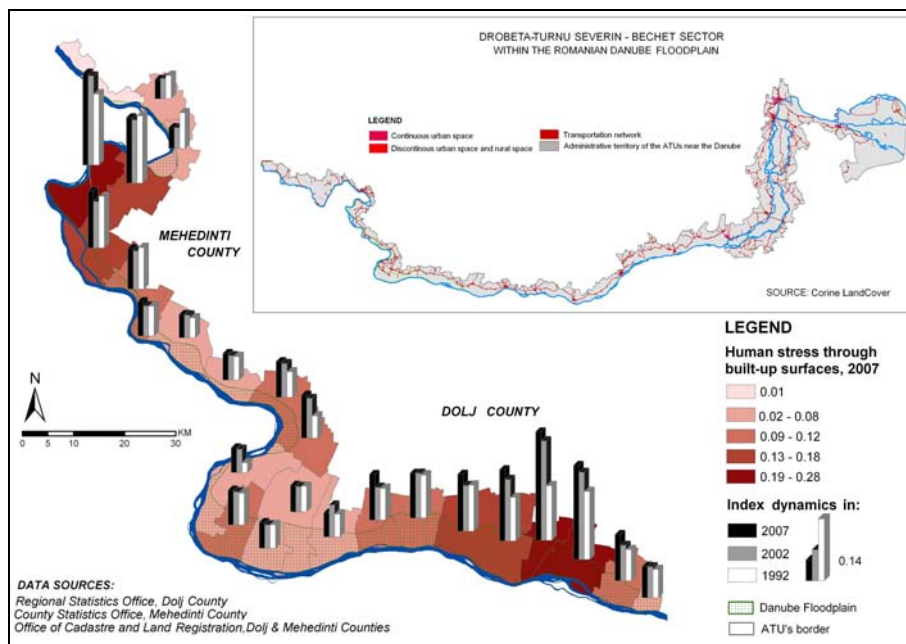


Figure 4. Human Stress through Built-up Surfaces within the Sector under Analysis

*The naturality index* is computed as the part of the forested surface in the total analysed surface. The forests cover 18.7% of the surface of the floodplain sector on study. The index values show strongly altered conditions of the natural landscape, the following classes being delineated: values under 3 (landscapes with totally affected balance: Cetate – minimum of 2.44, Pristol, Negoii), values between 3 and 9 (landscapes with seriously affected balance: Salcia, Bistreț, Gruia, Rast), values between 9 and 15 (landscapes with moderately affected landscape: Măceșu de Jos, Devesel, Maglavit, Calafat), values between 15 and 23 (landscapes with slightly affected balance: Gighera, Șimian, Gogoșu), values above 23



(landscapes with very small changes of the balance: Hinova – maximum of 43.0, Piscu Vechi, Desa). More that one third of these TAUs are characterized by strongly and very strongly altered balance of the landscape. At the antipole, there are those units that maintain parts of the formerly great forests and, especially, those that undergone afforestation works.

The fact that the forests specific to the floodplain are not differentiated from the surfaces sometimes afforested with allochthonous species (*Robinia pseudoacacia*, *Pinus nigra*, *Pinus sylvestris*, *Pseudotsuga menziesii*, *Populus canadensis*) is a drawback. On the other hand, the index is representative if the forest is considered as a natural surface, not radically altered by anthropic intervention and is the forest represented the initial formation (Dumitraşcu, 2006). All this being taken into account, it is difficult to assess the landscape naturality and the part of the anthropic intervention only by evaluating the part of the present forest in the total surface.

The index may undergo corrections for the floodplain sector on study. The modified naturality index (Fig. 5) is given by the part of the forest and meadow in the total analysed surface. Under the conditions presented by the newly computed index, seven of the TAUs on study show strongly and moderately altered balance of the landscape (Cetate – 8.77, Pristol, Salcia, Rast, Bistreţ, Bechet), at the antipole being located nine units with slightly altered landscape (Hinova – 54.21, Gogoşu, Ciupercenii Noi, Desa, Şimian and others). The dynamics of the index shows an augmentation of the values between 1992 and 2007, mostly due to afforestation and to the exit of certain surfaces from the agricultural framework after 1990.

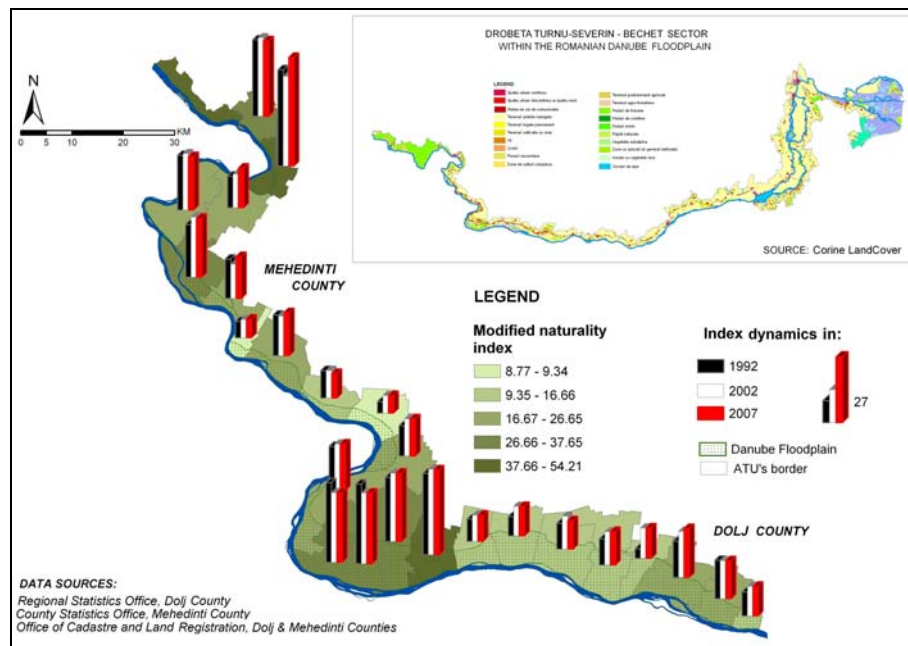


Figure 5. Naturality Index Modified for the Sector under Analysis

**The environmental change index** (according to the formula used by Marusycyak, in 1988 and by Malgorzata Pietrzak, in 1998, for the assessment of the human impact on the Polish landscape), is given by the ratio between the surface of the forests and meadows and the built-up surface. In Romania, this index was used by Manea (2001) in the assessment of the anthropic transformation of the landscape in *Portile de Fier* Nature Park, by Manea and Pătru (2004) for the Buzau Valley and by Dumitraşcu (2006) for the

evaluation of the landscape changes in the Oltenian Plain. In 2007, the minimum value was reached at Bechet (1.37), while the maximum was registered in Hinova commune (25.97).

For the Danube Floodplain, the proportion between the forest and aquatic surfaces, on the one hand, and the agricultural and built-up surfaces, on the other seems to be more significant. The amendment brought to the initial formula is based on the consideration that the agro-ecosystems represent human-modified entities of the landscape, which are not capable of self-regulation and are subordinated to the interests of the human society. In this case, the formula becomes:  $Modified\ E.Ch.I = (forestS.+meadowS.+aquaticS.)/(built-upS.+arableS.+vineriesS.+orchardsS.)$ . The surfaces occupied by the transportation network are included both in the initial and in the modified formulas (Fig. 6). The results were comprised in the following specific classes: 1.68 – 1.12: landscape with relatively stable ecological balance; 1.11 – 0.65: landscape with slightly affected ecological balance; 0.64 – 0.41: landscape at the limit of the ecological balance; 0.40 – 0.29: landscape with seriously altered ecological balance; 0.28 – 0.20: very strongly altered landscape.

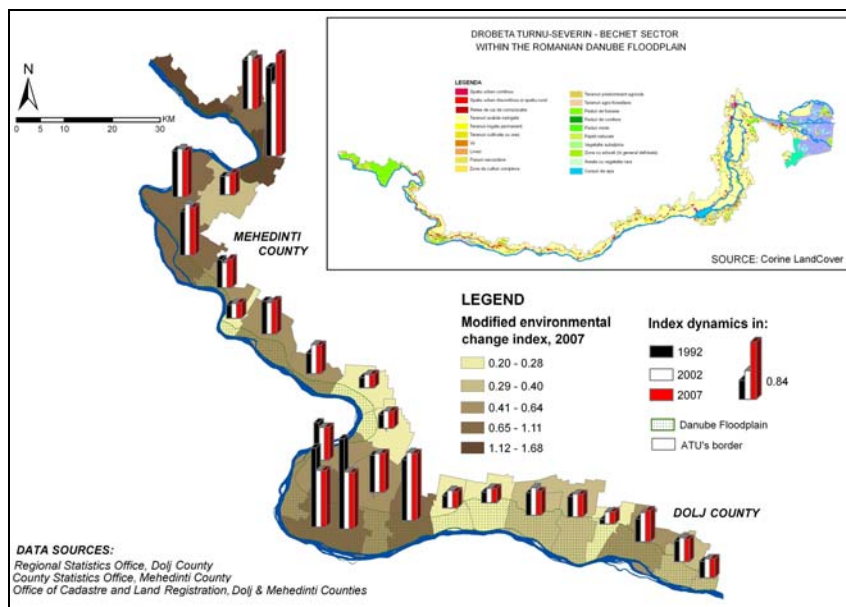


Figure 6. Environmental Change Index Modified for the Sector under Analysis

## CONCLUSIONS

During the last decade, the regional development became a major research preoccupation for the scientific community, as well as a debate topic for the local and central authorities, the entrepreneurs and the large public. The natural capital of the Danube Floodplain holds a productive capacity that must be known through its functional cells, in order to avoid the degradation under the human impact and to favour the sustainable valorisation of its support capacity. The guarantee of the durable social-economic development in the Danube Floodplain is based on accurate knowledge concerning the ecological sustainability, the integrity of ecosystems, the support capacity of the environment, the regional and local ecological balance of the ecosystems (*Redimensionarea ecologică și economică pe sectorul românesc al Luncii Dunării*, 2008).

The anthropic actions, initially destined to increase the arable surface and to reduce the flood danger, proved to have negative impact, through the drop of the fish quantities or the disappearance of some valuable species; they also led to the destruction of extended surfaces formerly covered with specific vegetation with great biological and landscape value or to the change of the microclimate, with the alternation of the drought/dryness and moisture excess phenomena, to the accentuation of the erosion etc. The old floodplain landscape remains visible in few natural units, but it starts to take shape once again in the conditions offered by the abandonment and by the protectionist measures that characterise the post-revolutionary period. There must be remarked that the current structure of the terrains is not the most appropriate to the local natural conditions; in many sectors, it does not represent the optimal category (Freeman et al., 2003). Thus, despite the drainage works, numerous areas, usually depressions, former ponds or lakes register moisture excess, which alter the agricultural production of the surfaces turned into arable land. It is necessary to revise these structures by closely considering the geographic particularities.

The local restoration and functioning of the landscape that is characteristic to this unit must be related with a series of measures that concern the bettering of all environmental components, from the ecologic support and the biotic sub-system to the demographic component and the related activities, with a strong emphasis on the educational and awareness actions for the population.

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PARTIAL RESULTS ON MICOFLORA FROM CERNA DE OLTET BASIN  
(VALCEA)

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**KEY WORDS:** *myxomycete, host plant, Cerna de Oltet*

**ABSTRACT**

*In this paper there are mentioned 51 myxomycete species systematically assigned in 7 classes, 13 orders and 16 families, parasites on host-plants from 25 botanical families, contributing in some measure to the formation of an image on microflora from Cerna de Oltet Basin, area not covered in the study of mycology from Romania.*

**INTRODUCTION**

Concerns in mycology, resulting the diversity of fungi which represent a major component of biodiversity had many researchers, an evidence being the worldwide identification of 345 000 taxons (Kirk et al., 2001).

For our country, Bontea Vera 1985, 1986, has made the inventory of 8724 species currently being raised over 9000 (Tanase and Șesan, 2006). Regarding the microflora on the area under study (Cerna de Oltet Basin) of the bibliographic data it results the lack of a study, except the identification of some more common species of macromycetes (Răduțoiu, 2002).

So hereupon the purpose of our research is, first of all, to fill an empty hole on the mycological map of Oltenia and to make additions to the knowledge of mushrooms, and this way, being able to make some environmental considerations to this group of organisms, which are basic components in establishing the natural ecosystems. In addition, when choosing the area was envisaged that Cerna de Oltet Basin which covers an area of about 750 km<sup>2</sup>, is rich in karst phenomena, which offers equally varied station and microclimatic conditions, which can thus infer that they hide surprises and news, which have hitherto remained outside the attention of mycology.

In terms of natural vegetation within the study, it currently occupies relatively small areas due to the anthropogenic factor, the greatest importance in the economy and landscape of the regions being the forests and grasslands.

Flora, which enables the study of micromycetes after the floristic division made by Borza Al. 1957, belongs to the Euro-Siberian area, the central European East Carpathians area, the Southern Carpathians district. The floristic list of the area established by Răduțoiu D. 2006, informs about 1394 assigned taxons: 1 - Chlorophyta, 7 - Lichenophyta, 122 - Bryophyta; 31 - Pteridophyta and 1233 - Spermatophyta with 11 - to Pinophytina and 1222 to Magnoliophytina.

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In this first note, regarding the micoflora from Cerna de Oltet Basin, we present 51 species of parasitic fungi collected in 2009, by which we are trying to bring an image on the micoflora from the area under study.

## **MATERIALS AND METHODS**

The mycological material which makes the object of this paper was collected during field trips, while making observations on the complex host-parasite – environment plant.

Micromycetes determination was made directly on the substrate on which it grows in nature, using a magnifying glass and optical microscope revising at the same time the mycological herbarium of the discipline of Phytopathology, of University of Craiova and reference works produced by local and foreign experts with concerns in this area (Bontea Vera 1985, 1986, Cummins and Hiratsuka, 2003; Eliade 1990, Ellis 1971, Isaac, 1992; Parvu, 2007, Radulescu et al., 1973).

The list of the identified species was made in order with the systematic kingdoms: Chromysta and Fungi, in accordance with the present system of classification of fungi, as the literature data (Kirk et al., 2001). Within each family the genders and species have been arranged alphabetically with indication of the host plants (also alphabetically epigraphs), locality and date of the collection (in chronological order).

## **RESULTS AND DISCUSSIONS**

This paper contains the epitome of the species determined and identified by the indication of the host plant, the material being in the mycological herbarium of the discipline of Phytopathology from the University of Craiova.

To a first analysis it results that the region researched, there were very well represented the fungi in the phylum Ascomycota: 24 species (47.05%) and anamorphic fungi 12 species (23.5%).

A smaller representation had the Oomycetes: five species (9.8%), while the attack of the phylum of fungi Chytridiomycota was not ascertained.

Among families, as number of species they are in decreasing order: Erysiphaceae: 13 species (25.4%), Sphaeropsidaceae: 9 species (17.6%), the remaining families, including 1-5 species of fungi.

The 51 identified taxons, belonging to 27 types of fungi, of which, the best represented type as number of species: Septoria: 7 species, Erysiphe: 6 species, the remaining types included 1-4 species (Table 1).

Regarding the polyphagia, most species were found in the area and the research period, on a single host-plant.

The identified micromycetes have as host-plant, species from 25 botanical families (Table 2), of which more commonly contested are: Rosaceae, Asteraceae and Poaceae, which have 8-12 species attacked by fungi.

Due to the fact that the areas planted in this area were limited, most of the identified micromycetes are parasits on spontaneous plants, so, of the total number of micromycetes, only a few species are parasitic on crop plants to which they also produce serious diseases.

The micromycetes dynamics, as the expression of the trophic conditions, namely the existence of host-plants and those of humidity and temperature, takes place throughout the year when they meet the conditions auspicious to fungi development.

Table 1

Partial results on the parasite species identified in Cerna de Oltet Basin

Class	Order	Family	Nr. of	
			Genus	Species
Oomyces	Peronosporales	Albuginaceae	1	2
		Peronosporaceae	3	3
Taphrinomycetes	Taphrinales	Taphrinaceae	1	1
Ascomycetes	Erysiphales	Erysiphaceae	5	13
	Phyllachorales	Phyllachoraceae	1	1
	Diaporthales	Gnomoniaceae	1	1
	Mycosphaerellales	Mycosphaerellaceae	1	3
	Pleosporales	Venturiaceae	1	2
	Helotiales	Dermateaceae	2	3
Ustilaginomycetes	Tilletiales	Tilletiaceae	1	1
	Ustilaginales	Ustilaginaceae	1	1
Uredinomycetes	Uredinales	Melampsoraceae	1	1
		Phragmidiaceae	1	2
		Pucciniaceae	2	5
Hyphomycetes	Hyphomycetales	Dematiaceae	3	3
Coelomycetes	Sphaeropsidales	Sphaeropsidaceae	2	9

Table 2

Botanical family of host plants

Nr crt.	Botanical family of host plants	Number of plant species parasitized
1	Ranunculaceae	1
2	Moraceae	1
3	Juglandaceae	1
4	Fagaceae	2
5	Caryophyllaceae	1
6	Amaranthaceae	1
7	Polygonaceae	1
8	Rosaceae	12
9	Fabaceae	4
10	Cornaceae	1
11	Euphorbiaceae	1
12	Apiaceae	2
13	Salicaceae	2
14	Cucurbitaceae	1
15	Primulaceae	1
16	Oleaceae	1
17	Solanaceae	1
18	Convolvulaceae	1
19	Boraginaceae	1

20	Lamiaceae	2
21	Plantaginaceae	2
22	Caprifoliaceae	1
23	Dipsacaceae	2
24	Asteraceae	8
25	Poaceae	8

#### Epitome of the determined and identified species

##### Albuginaceae

*Albugo bliti* Kze., the leaves *Amaranthus retroflexus* L., Lădești, 14. 07. 2009.

*Albugo tragoponis* DC ex J.F. Gray, the leaves *Xeranthemum cylindraceum*, Oteteliș, 14. 07.2009.

##### Peronosporaceae

*Bremia sonchi* K. Sawada the leaves *Sonchus arvensis* L., Lădești, 14. 07. 2009.

*Peronospora alta* Fuck., the leaves *Plantago major* L., Lădești, 14. 07. 2009.

*Plasmopara viticola* (B.et C.) Berl. et de Toni, the leaves *Vitis vinifera* L., Lădești, 14. 07. 2009.

##### Taphrinaceae

*Taphrina pruni* Tul., on fruit *Prunus spinosa* L., Lădești, 14. 07. 2009.

##### Erysiphaceae

*Blumeria graminis* f sp. *secale* (sin. *Erysiphe graminis* f. sp. *secale*) on leaves and ears *Secale cereale* L., Lădești, 14. 07. 2009.

*Erysiphe cichoracearum* D.C. ex Mérat. the leaves *Sonchus arvensis* L., Roești 14. 07.2009.

*Erysiphe convolvuli* D.C. ex. St. Am., the leaves *Convolvulus arvensis* L., Lădești, 14. 07. 2009.

*Erysiphe galeopsidis* D.C. ex. Mérat. the leaves *Betonica officinalis* L. (sin. *Stachys officinalis* (L.) Trev.) Lădești, 14. 07. 2009.

*Erysiphe graminis* D.C. ex. Mérat. the leaves *Brachypodium sylvaticum* (Huds.), Oteteliș 14.07.2009.

*Erysiphe polygoni* D.C. ex. St. Am., the leaves *Rumex crispus*, Oteteliș 14.07.2009.

*Erysiphe trifolii* Grev. the leaves *Trifolium pratense* L., Oteteliș 14.07.2009, the leaves *Melilotus officinalis* Lam., Oteteliș 14.07.2009.

*Microsphaera alphitoides* Griff. et Maubl., the leaves *Quercus dalechampii* Ten., Roești, 14. 07. 2009, the leaves *Quercus robur* Willd., Lădești, 14. 07. 2009.

*Sphaerotheca alchemillae* (Grev.) L. Junell the leaves *Agrimonia eupatoria* L., Oteteliș 14.07.2009.

*Sphaerotheca euphorbiae* (Cast.) Salm., the leaves *Euphorbia virgata* Waldst. et Kit., Lădești, 14. 07. 2009.

*Sphaerotheca fuliginea* (Schlecht ex. Fr.) Poll the leaves *Cucurbita pepo*, Lădești, 14. 07. 2009.

*Uncinula adunca* (Wallr. ex. Fr.) Lév., the leaves *Salix fragilis* L., Lădești, 14. 07. 2009.

*Uncinula prunastri* (D.C. ex Mérat) Sacc. the leaves *Prunus spinosa* L., Lădești, 14. 07. 2009.

##### Phyllacoraceae

*Polystigma rubrum* (Pers) D.C. the leaves *Prunus domestica* L., Lădești, 14. 07. 2009; the leaves de *Prunus spinosa* L., Lădești, 14. 07. 2009.

Valsaceae (sin Gnomoniaceae)

*Gnomonia leptostyla* (Fr.) Ces. et de Not., the leaves *Juglans regia* L., Roești și Lădești, 14. 07. 2009.

Mycosphaerellaceae

*Mycosphaerella fragariae* (Tul.) Lindau the leaves *Fragaria ananassa* (Weston) Decne. et Naudin, Lădești, 14. 07. 2009.

*Mycosphaerella mori* (Fuck.) Lind., the leaves *Morus alba* L., Giulești 14.07.2009.

*Mycosphaerella sentina* (Fuck.) Schroet. the leaves *Pyrus pyrausta* (L.) Burgsd., Giulești 14.07.2009.

Venturiaceae

*Venturia inaequalis* (Cooke) G. Winter., on leaves and fruit *Malus domestica*.

*Venturia pirina* Aderh. On leaves and fruit *Pyrus pyrausta* (L.) Burgsd., Giulești 14.07.2009.

Dermateaceae

*Blumeriella jaapii* (Rehm.) Arx (sin *Coccomyces hiemalis* Higg.) the leaves *Cerasus avium* (L.) Moench., Giulești 14.07.2009.

*Diplocarpon rosae* Wolf. (sin. *Fabraea rosae*) the leaves *Rosa canina* L., Giulești 14.07.2009.

*Diplocarpon soraueri* (Kleb.) Nannf pe frunze de *Cydonia oblonga* Mill., Giulești 14.07.2009.

Tilletiaceae

*Tilletia caries* (D.C.) Tul., (sin *Tilletia tritici* (Bjerk.) Winter, attack the fruit *Triticum aestivum* L., Oteteliș, 14.07.2009.

Ustilaginaceae

*Ustilago avenae* (Pers.) Rostr. the inflorescences of *Avena sativa* L., Giulești 14.07.2009.

Melampsonaceae

*Melampsona larici – tremulae* Kleb. the leaves *Populus alba* L., Lădești, 14.07..2009.

Phragmidiaceae

*Phragmidium disciflorum* (Tode) James, (sin. *Phragmidium mucronatum* (Pers.) Schlecht the leaves *Rosa canina* L., Oteteliș, 14.07.2009.

*Phragmidium violaceum* (Schultz.) Wint. pe frunze de *Rubus candicans* Weihe et Rchb. Oteteliș și Lădești, 14.07.2009.

Pucciniaceae

*Puccinia agrostidis* Plowr. on *Agrostis capillaris* L., Giulești 14.07.2009.

*Puccinia coronifera* Kleb. the leaves *Bromus inermis* Leysser, Roești 14.07.2009.

*Puccinia dispersa* Erikss. et Henn., the leaves on stems *Secale cereale* L., Lădești 14.07.2009.

*Uromyces bäumlerianus* Bub. the leaves *Melilotus officinalis* Lom., 14.07.2009.

*Uromyces lathyri* Guyot the leaves *Lathyrus tuberosus*, Lădești 14.07.2009.

Dematiaceae

*Alternaria trititica* Prasada et Prabhu the leaves *Triticum aestivum* L., Lădești 14.07.2009.

*Cladosporium herbarum* (Pers.) Link., the aboveground organs of *Triticum aestivum* L., Oteteliș 14.07.2009, on *Secale cereale* L., Lădești 14.07.2009.

*Stigmina carpophila* (Lev.) Ellis (sin. *Coryneum beijerinckii* Oudem.) the leaves *Prunus domestica* L., Giulești 14.07.2009; the leaves *Cerasus avium* (L.) Moench., Giulești 14.07.2009.

#### Sphaeropsidaceae

*Ascochyta dipsaci* Bub. the leaves *Dipsacus fullonum* L., Oteteliș 14.07.2009.

*Ascochyta viciae* Libert. the leaves *Vicia sepium* L., Giulești 14.07.2009.

*Septoria calaminthae* Massal., the leaves *Calamintha menthifolia* Host., Oteteliș, 14.07.2009.

*Septoria convolvuli* Desm., the leaves *Convolvulus arvensis* L., Lădești 14.07.2009.

*Septoria cornicola* Desm. the leaves *Cornus sanguinea* L., Giulești 14.07.2009.

*Septoria gei* Rob et Desm., the leaves *Geum urbanum* L., Oteteliș, 14.07.2009.

*Septoria levistici* West., the leaves *Levisticum officinale* Koch., Lădești 14.07.2009.

*Septoria lycopersici* Speg., the leaves *Lycopersicum esculentum* Mill., Lădești 14.07.2009.

*Septoria scabiosicola* Desm., the leaves *Cephalaria transylvanica* (L.) Roemer et Schuller., Oteteliș 14.07.2009.

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SEROLOGICAL DETECTION OF PLUM POX VIRUS AND PRUNE DWARF  
VIRUS AT SEVERAL PLUM CULTIVARS FROM S.D. BANU MĂRĂCINE

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**KEY WORDS:** Plum, PPV, PDV, substrate, optical density

**ABSTRACT**

*From the fourteen plum genotypes taken under observation, eleven were determined positive for the presence of Plum pox virus. The values obtained at 405 nm shown no viral infection in case of the genotypes: Record, Valor and Dambovită. Also, for five were determined both positive and negative results: Tuleu Timpuriu (1,018 and 0,478), Pitestean (1,405 and 0,681), Valcean (1,889 and 0,771), Ialomita (2,775 and 0,772) and Stanley (1,428 and 0,508). Exist the possibility that the concentration of the viral protein in the samples tissues be very low, and the serological method is not sensitive enough to render evident. Thus, tests will be made to see the exact cause of this variation of the results. Regarding the Prune dwarf virus, none of the plum genotypes had a positive result.*

**INTRODUCTION**

Depending on the species, frequent mixed viral infections were reported in the Mediterranean stone fruit industry in variable percentages from 8 to 20% (Myrta et.al, 2003). The expressions of symptoms depends on climate, virus isolate, host species and cultivar, nutrient supply and the age of the plant at the time of infection (Vaškova et.al., 2000). Also it is known that all viruses may produce general systemic symptoms similar to those caused by environmental factors such as nutritonal disorders, thus it is possible visually to mistaken the virus symptomatology to one of the nutritonal disorders (Narayananasamy, 2001).

Many of the pathogens may be latent and cannot be detected by visual assessment, therefore it is recommended that all plants be extensively tested (Diekmann and Putter, 1996). Availability of diagnostic methods based on viral coat protein provides greater flexibility, increased sensitivity and specificity for rapid diagnosis of viruses in disease surveys, epidemiological studies, plant quarantine and seed certification, and breeding programs (Naidu and Hughes, 2001).

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The aim of this study is to establish the presence or absence of two viruses, Plum pox virus and Prune dwarf virus at several plum genotypes through the rapid serological method Double Antibody Enzyme Linked Immunosorbent Assay (DAS-ELISA).

## MATERIAL AND METHODS

Samples were collected in June 2010 from 14 plum genotypes at S.D. Banu, respectively: Centenar, Tuleu gras, Tita, Record, Valor, Tuleu Timpuriu, Pitestean, Valcean, Silvia, Dâmbovita, Diana, Renclod Althan, Ialomița and Stanley. The samples consisted in symptomatic and asymptomatic leaves. For each genotype were sampled two trees.

The method DAS-ELISA (Clark and Adams, 1977) was applied corresponding to the work protocols received with the reagents kits from Bioreba (Switzerland) (Figure 1,2,3,4).

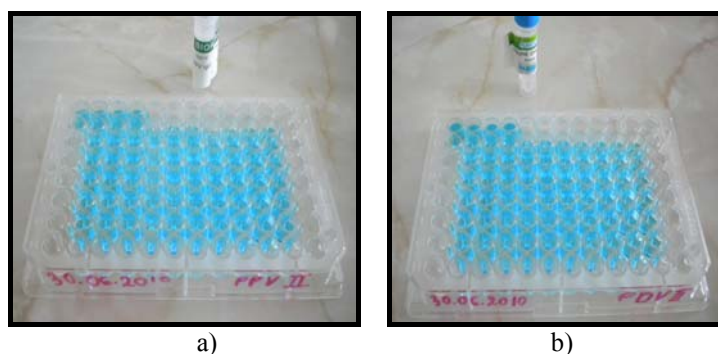


Figure 1. The distribution of the anticorp sustrate: a) PPV; b) PDV

The readings were made chromatically at 405nm with Stat Fax 3200, the values obtained representing the optical density of the protein coat protein. Values higher than 1,000 signifie an infection with the virus in question.

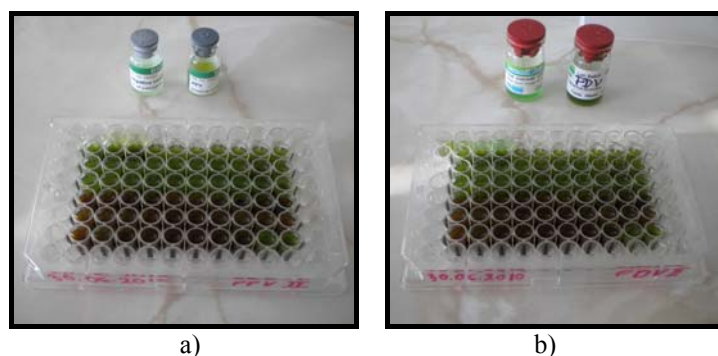


Figure 2. The distribution of the plant extracts, positive and negative controls: a) PPV; b) PDV



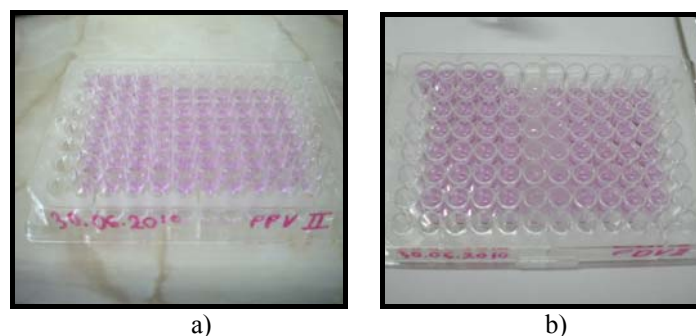


Figure 3. The distribution of the conjugate substrate: a) PPV; b) PDV

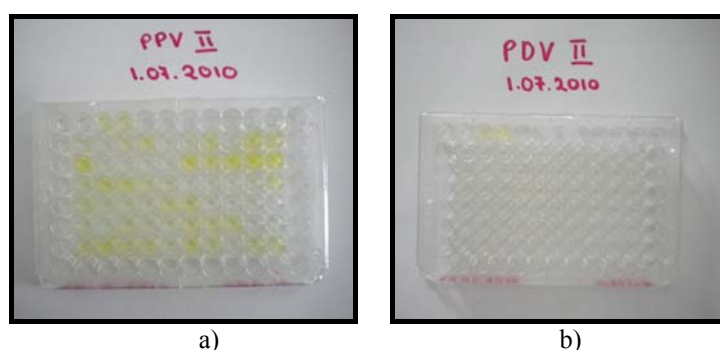


Figure 4. Colorimetric reaction of the positive controls and plant extracts at 1-3 minutes after adding the reaction substrates with pNPP: a) PPV; b) PDV.

## RESULTS AND DISCUSSIONS

Following the readings at 405 nm, Prune dwarf virus (PDV) wasn't serologically noted in any of the samples tested (Tabel 1). Also, it is seen in Figure 4 (b) that only the two positive controls (wells A3 and A4) had a colorimetric reaction. The presence of the viral protein in the analyzed tissue samples, at the end of the serological assay, can be easily observed visually at 1-3 minutes after the distribution of reaction substrates with pNPP.

Tabel 1  
The serological method DAS-ELISA for the detection of Plum pox virus (PPV) and Prune dwarf virus (PDV) in plum genotypes

Distribution of samples on the microtiter plates	Sample code.	Genotype	Optical density			
			PPV II		PDV II	
E2	91	Centenar 1	3,239	+	0,437	-
E3	92	Centenar 2	1,584	+	0,420	-
E4	93	Tuleu gras 1	1,677	+	0,417	-
E5	94	Tuleu gras 2	1,534	+	0,444	-
E6	95	Tita 1	3,239	+	0,425	-
E7	96	Tita 2	3,239	+	0,411	-
E8	97	Record 1	0,528	-	0,415	-

E9	98	Record 2	0,413	-	0,418	-
E10	99	Valor 1	0,563	-	0,411	-
E11	100	Valor 2	0,466	-	0,417	-
F2	101	Tuleu timpuriu 1	1,018	+	0,426	-
F3	102	Tuleu timpuriu 2	0,478	-	0,427	-
F4	103	Pitestean 1	1,405	+	0,417	-
F5	104	Pitestean2	0,681	-	0,442	-
F6	105	Valcean 1	0,771	-	0,436	-
F7	106	Valcean 2	1,889	+	0,413	-
F8	107	Silvia 1	1,482	+	0,419	-
F9	108	Silvia 2	2,171	+	0,411	-
F10	109	Dambovita 1	0,415	-	0,418	-
F11	110	Dambovita 2	0,465	-	0,419	-
G2	111	Diana 1	2,114	+	0,402	-
G3	112	Diana 2	3,231	+	0,405	-
G4	113	Renclod Althan 1	1,790	+	0,412	-
G5	114	Renclod Althan 2	1,973	+	0,410	-
G6	115	Ialomita 1	0,772	-	0,419	-
G7	116	Ialomita 2	2,575	+	0,406	-
G8	117	Stanley 1	1,428	+	0,427	-
G9	118	Stanley 2	0,508	-	0,410	-
A3 / A4		Positive controls	3,241/3,241		1,124/1,180	
A1 / A2		Negative controls	0,460/0,475		0,420/0,434	

Note: + =viral infection; - = not infected

Regarding the Plum pox virus, a viral infection was established for eleven of the plum genotypes in question, only three were determined not to be infected: Record, Valor and Dambovita. Both samples collected from different plants were found not positive for PPV. In case of the genotypes positive for PPV, for four of them one of the sample was positive, one was negative: Tuleu Timpuriu (1,018 and 0,478), Pitestean (1,405 and 0,681), Valcean (1,889 and 0,771), Ialomita (2,775 and 0,772) and Stanley (1,428 and 0,508). It is known that the virus appears to be unevenly distributed through the plant and this combined with seasonal fluctuations of viral concentrations can cause chronically infected trees to appear healthy when tested by Elisa (Mekuria et. al., 2003; Viršček Marn and Mavrič, 2005). Thus, the different results indicate that another test must be done in order to establish the phytosanitary state of the genotypes mentioned above. In figure 6 is presented the visual attack of the disease on different cultivars leaves: Tita, Tuleu gras and Renclod Althan.



Figure 6. PPV Symptoms on different plum genotypes

## CONCLUSIONS

Through DAS-ELISA, Prune dwarf virus wasn't determined in any of the samples tested. Regarding the infection with Plum pox virus was established that in case of 5 genotypes both samples were positive: Centenar, Tita, Silvia, Renclod Althan, Tuleu gras and Diana. In case of the genotypes Stanley, Ialomita, Pitestean, Tuleu timpuriu and Valcean, one sample was found infected and one not infected. Another test will be performed to see the real phytosanitary state of the plants, because it is well known that the serological assay gives negative false results.

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RESEARCHES ABOUT THE MANGANESE REGIME FROM AGRICULTURAL  
ECO-MEDIUM IMPROVED BY THE SEWAGE SLUDGE

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**KEY WORDS** : maize, wheat, soybeans, Mn, sludge

ABSTRACT

*Mn is another chemical element within the heavy metals category which in reduced quantities can benefit plants feeding process, yet while in excess can induce specific toxicity phenomena. For observing the Mn content trend in agricultural plants, progressive doses of sludge were used: 0, t.ha<sup>-1</sup>, 5 t.ha<sup>-1</sup>, 10 t.ha<sup>-1</sup>, 25 t.ha<sup>-1</sup> and 50 t.ha<sup>-1</sup>, together with doses of chemical fertilizers: 0, ½ and 1/1 of the specific plants need. By using these fertilizers some statistically covered increases were observed compared to the sample in respect to mobile Mn forms in case of all cultivated plants. Field plants absorbed Mn<sup>2+</sup> in specific quantities. Correlations obtained between plants biomass and the Mn contents show evident increases for total biomass: maize r=0,772\*\*\*, and soybeans r=0,311, and insignificant decreases for grains. Such researches are required since they emphasize most of the aspects related to the nutrition of each specific agriculture plants.*

INTRODUCTION

In its current state, the luvisol contains Mn in appreciable quantities, having its origin in the decomposition of ferromagnetic rocks. After iron (Fe) and aluminum (Al), manganese (Mn) represents the most abundant chemical constituent in rocks making up the earth shell (Davidescu and Davidescu, 1981). Soil contains transformed Mn under different forms associated with the mineral and organic part. The most common are Mn oxides and hydroxides. They can originate from the parental material or alteration process. Both Mn crystalline and amorphous state exist under more forms, including ferromanganese balls or concretions. Their forming has at their base the alternation of oxidation and reduction processes. Dominant forms of Mn in the ferromagnetic concretions composition (balls) are represented by Mn<sub>2</sub>O<sub>3</sub>.nH<sub>2</sub>O (Bajescu and Chiriac, 1984). In order to become accessible for plants, Mn oxides and hybrids need to be reduced to Mn<sup>2+</sup> ions. Between Mn<sup>2+</sup> from soil solution, Mn<sup>2+</sup> changeable and superior oxides of Mn there is a dynamic balance controlled by the complexity of the reduction conditions (redox). Accessibility of Mn<sup>2+</sup> depends of several factors, among which the most important are: pH, microbiological activity, organic matter (OM) and soil's humidity regime. The last researches proved that the availability of Mn<sup>2+</sup> occurs in soils with pH contained between 5 and 6. Mn<sup>2+</sup> is thus present mainly in

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acid soils, while on neutral soils manganese is under a trivalent form ( $\text{Mn}^{3+}$ ) as  $\text{Mn}_2\text{O}_3$ , and in alkaline ones (pH over 8) under tetravalent form ( $\text{Mn}^{4+}$ ) in an inert oxide,  $\text{MnO}_2$  (Tisdale and Nelson, 1975). Luvisol having as characteristic the acid environment (Taylor et al. 1964) favors the reduction processes following which manganese is in bivalent form -  $\text{Mn}^{2+}$ , available for plants absorption (Baize, 1988). The specific microbial activity here is reduced, leading to a true conservation of accessible  $\text{Mn}^{2+}$  forms. OM influences the mobility of  $\text{Mn}^{2+}$  both by lower affinity compared with other heavy metals, being thus permanently available and by the specific decomposition degree. Due to unfavorable drainage the reduced forms of Mn are predominant by stimulation of bacteria which decompose OM using Mn oxides as  $\text{O}_2$  source. It is such noted that Luvisol specific to Pitesti research center contains Mn accessible to plants in relatively higher concentrations. A safe source of OM for local agriculture is represented by the digested sludge (Bruce and Newman, 1992) from Pitesti Wastewater Treatment Plant. Being qualitatively comparable with manure (Mihalache et al. 2006) sludge such processed represents a new source both for macro-nutrients for agricultural plants: nitrogen, phosphorus, potassium, calcium etc., but also for micro-nutrients among which manganese is in concentrations close to the ones of the soil.

## MATERIAL AND METHODS

In the period 2004- 2007 a complex experiment was initiated. During this experiment plants were cultivated by the structure: 1.- maize, 2.- winter wheat, 3.- soybeans and 4.- winter wheat. In normal cultivation technologies these plants were fertilized with different doses of organic- mineral. Thus, these doses were applied to sewage sludge: 0  $\text{t.ha}^{-1}$ , 5  $\text{t.ha}^{-1}$ , 10  $\text{t.ha}^{-1}$ , 25  $\text{t.ha}^{-1}$  and 50  $\text{t.ha}^{-1}$ . The sewage sludge suffered an anaerobic digesting followed by dewatering within Pitesti Wastewater Treatment Plant. Chemical fertilizers were differentiated on three levels: unfertilized, needs to  $\frac{1}{2}$  of normal and total doses (1/1). Plants have received such  $\text{N}_{50}\text{P}_{50}$ /maize,  $\text{N}_{60}\text{P}_{40}$ /wheat,  $\text{N}_{30}\text{P}_{30}$ /soybeans and  $\text{N}_{40}\text{P}_{40}$ /wheat for doses  $\frac{1}{2}$  and  $\text{N}_{120}\text{P}_{80}$ /maize,  $\text{N}_{120}\text{P}_{80}$ /wheat,  $\text{N}_{60}\text{P}_{60}$ /soybeans and  $\text{N}_{80}\text{P}_{80}$ /wheat for the 1/1 doses. Sludge doses were applied in the same quantities in the first two years- from maize and wheat in year two, following that soybeans and wheat in the past year to receive their residual effect. The experiment with the lot divided had the A factor- sludge doses and the B factor- chemical fertilizers doses. Each variant had a surface of 100  $\text{m}^2$  each and was rehearsed (replicated) for three times. Leaves samples were taken during flowering period: at maize the leaves located at cob level, at winter wheat the last 3 leaves including the standard leaf and the soybeans the leaves in the central area of the plant but also with bean- pods in formation process. Soil samples were collected with the agrochemical sampling device of arable horizon 0-20 cm, between flowering to maturity period. Chemical analysis were performed according to the latest European standards and methodologies: Mn/ leaves and grains- SR ISO 11047-99, Mn- mobile forms of ground- SR ISO 14870-99, both over sludge an-aerobically digested and over soil and plants. The data were statistically processed by analysis of the variant (Anova test) and with the help of correlations and regressions.

## RESULTS AND DISCUSSIONS

i) Influence of sludge doses and chemical fertilizers over mobile forms of manganese from cultivated soil. Soils had and currently have modifications of their Mn content (Taylor et al., 1964). Periodical investigations demonstrated Mn values contained within 280 and 3200 ppm total forms and approximately 300 ppm mobile forms (Davidescu and Davidescu, 1981). A global average figure would be situated between 950 and 1000

ppm manganese (Bajescu and Chiriac, 1984). Of all agricultural soils the luvisol in Pitesti research facility has  $Mn^{2+}$  contents under this average figure, yet with annual variations due to numerous cultivation factors, eco-environment factors, weather conditions factors etc. The 4 cultivation years in the experiment emphasized this fluctuation of soil's contents, especially in terms of mobile forms of manganese – figure 1. The entire experiment demonstrates that by applying the sludge and chemical fertilizers increased by  $7.93 \text{ mg.kg}^{-1}$  first year, with  $2.91 \text{ mg.kg}^{-1}$  in year two, fell  $7.59 \text{ mg.kg}^{-1}$  third year and increased by  $5.3 \text{ mg.kg}^{-1}$  last year, for each complex dose applied. The data demonstrate moderate effect of sludge on mobile Mn accumulation in the soil. The mobile of Mn concentrations between  $400$  and  $600 \text{ mg.kg}^{-1} \text{ d.w.}$  are normal for this type of soil.

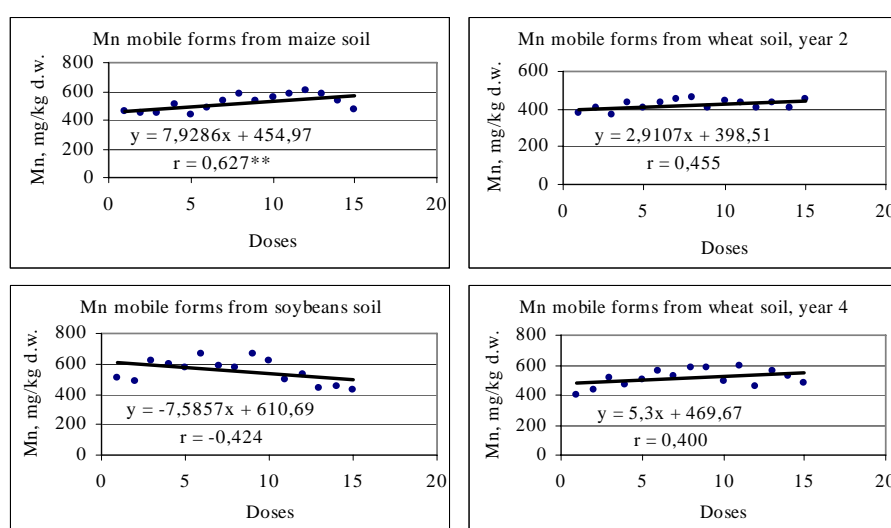


Figure 1. The evolution of mobile forms of Mn from soil improved by sewage sludge.

ii) Influence of experimental factors upon Mn contents in leaves and grains. Given the parameters of the cultivation soil, in which both total and mobile forms were situated at sufficiently high values, it was expected the absorption of  $Mn^{2+}$  to occur in the same manner. Specialized literature is rather vague, without presenting clear cases of Mn contents in different cultivation plants. In turn, one thing is certain: in small quantities, of about 30-50 ppm Mn, there is ensured a micro-nutrient indispensable for the plants' life (Hera et al., 1988). Manganese is required by plants and contributes to the normal physiology of all tissues in development, while the analyzed leaves – irrespective of their stage, show this content in the chemical micro-element Mn.

The luvisol here ensure sufficiently the minimal required quantity – as feeding factor for plants, irrespective of the species. In the same time, the high values of  $Mn^{2+}$  in soil contributes to the occurrence of phyto-toxicity phenomena (Ionescu et al., 2008). In this case there is of a great importance the analyses of plants in several moment of plant's development, and the chemical element Mn becomes the heavy metal hazardous for both plants' life and the entire food chain (Capatina and Simonescu, 2007). However, both Mn and other chemical elements, mineral salts etc., are absorbed selective by each plant species, and the clayey-humic complex activates through complex moderation of plants'

feeding process (Aggelides and Londra, 2000). For supporting the process of proving the way Mn was absorbed by plants, there are given the experimental data obtained within the present research. Regarding the Mn contents both in leaves and beans of the three cultivation plants there are noticed evident differences.

Maize had at blooming time values of 4 and 7 mg.kg<sup>-1</sup> d.w. Mn in its leaves. Thus maize is a plant which from the absorption point of view represents a species quite selective due to the relatively low contents by the leaves during blooming periods. Experimental factors increased the Mn contents in leaves at significant levels compared to the blank test, while the average value of Mn on the entire experiment was situated at 6.0 mg.kg<sup>-1</sup> d.w.- figure 2. Mn from maize grains show decreasing and demonstrate that plant had no need of this micronutrient.

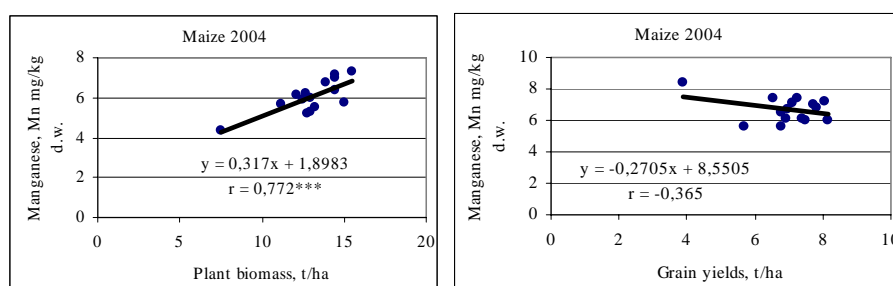


Figure 2. Correlations between total biomass and grains, and Mn contents, in maize plants.

Manganese concentrations in wheat leaves were framed theoretically (as right correlation) around 130 mg.kg<sup>-1</sup> d.w., while the kernels were deposited between 100 and 94 mg.kg<sup>-1</sup> d.w.- figure 3. Wheat has provided large concentrations of Mn since the young stages.

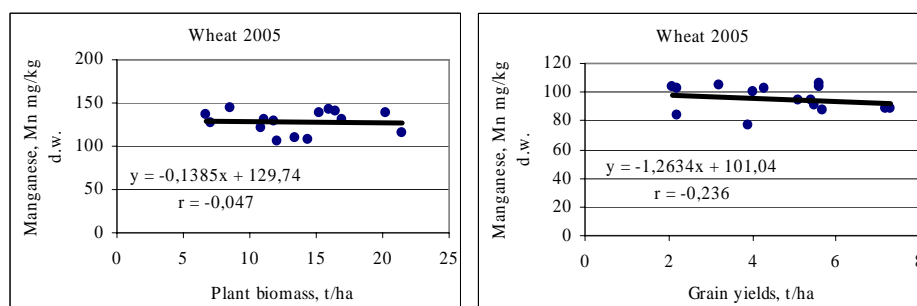


Figure 3. Correlations between total biomass and kernels, and Mn contents, in wheat plants.

Soybean plants were contained in the leaves at flowering increasing concentration of Mn. The function shows the correlation increases between 160 and 200 mg.kg<sup>-1</sup> d.w. Stage of maturity soybeans have much lower concentrations of Mn content. The regression show declines between 42 and 39 mg.kg<sup>-1</sup> d.w.- figure 4.

Winter wheat last year had similar decreases in both Mn concentrations of leaves at flowering and kernels at maturity- figure 5. The regression shows that at flowering

period falls between 130 and 110 mg.kg<sup>-1</sup> d.w. Mn, while kernels regression enrolled between 78 and 60 mg.kg<sup>-1</sup> d.w. Mn. Comparing the two years the wheat crop, the highest Mn concentrations were obtained in second year, and the decrease in the concentration of Mn in plants were obtained in the last year.

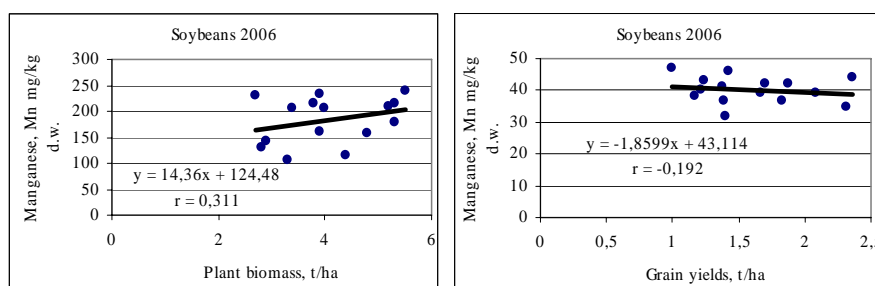


Figure 4. Correlations between total biomass and kernels, and Mn contents, in soybeans.

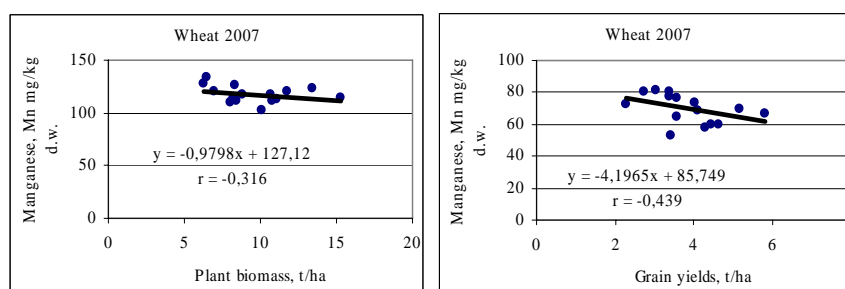


Figure 5. Correlations between total biomass and kernels, and Mn contents, in wheat plants.

Expressing the Mn<sup>2+</sup> concentrations according to performed determinations (table 1) show differences compared to the forecasts presented by specialized literature. In the case of experiments performed in concrete cultivation conditions obtained data emphasize the specificity of the plant with its characteristics in the absorption and usage of manganese (as Mn<sup>2+</sup>) during vegetation. Multi-annual observations did not prove major phyto-toxicity phenomena due to higher but also fluctuating Mn concentrations in soil. Sludge processed has a contribution in the case of this heavy metal, under the limits recommended by INCPA and does not influence its absorption over the needs.

Table 1  
Manganese concentrations expressing- Mn<sup>2+</sup> mg.kg<sup>-1</sup> dry weight, in cultivation plants

Cultivated plant	Value required in development phase (after YARA)	Determinations performed on cultivation plants	
		At blooming – in leaves	At maturity – in grains
Maize	40	6	7
Winter wheat	35	130	90
Soybeans	30	180	40
Winter wheat	35	120	70



## CONCLUSIONS

By using processed sludge (anaerobically digested and dewatered) there occurs an improvement of the luvisoil's eco-environment: it increases the organic matter content, increases the nitrogen supply and the pH values. In the same time, there occurs a completion of soil's contents in  $Mn^{2+}$ .

Soil analysis in the 4 experimenting years show a slight improvement of the mobile Mn forms according to studied factors. Mobile forms of Mn were significantly influenced positively compared to the blank tests, and the average values obtained – between 422 and 550  $mg.kg^{-1}$  d.w. show increases with possible negative influence upon the absorption of the heavy metals by the plants.

Sludge and the chemical fertilizers significantly modified the Mn content in case of leaves. Soybean contained the least Mn in leaves, with possible positive influence on behalf of sludge and mineral fertilizers. Winter wheat contained Mn over 100  $mg.kg^{-1}$  d.w. in leaves and between 70 and 95  $mg.kg^{-1}$  d.w. in kernels, and maize between 6 and 7  $mg.kg^{-1}$  d.w. both in leaves and grains.

Study of correlation between both total biomass and grains showed that only the maize absorbed Mn in direct and evident relation with biomass, soybeans show a positive trend, and winter wheat show in general negative relations. Regarding grains, Mn was no longer necessary, the development status showing decreases in relation with production levels.

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STUDY OF THE ANTHOCYANIN POTENTIAL OF “NOVAC” AND “NEGRU DE  
DRAGASANI” GRAPES CULTIVATED IN VARIOUS CENTRES OF  
DRAGASANI VINEYARDS

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**KEY WORDS:** variety, anthocyanins, extractability, technological reserve, pigments

**ABSTRACT**

*Under the ecological conditions especially favourable of Dragasani vineyard for viticulture, the inland varieties Novac and Negru de Dragasani, besides the higher glucidic potential dispose of a remarkable capacity of biosynthesizing anthocyanin and storing them in the grapes. The chromatic structures of anthocyanin extracts represented by the yellow, red and blue pigments are balanced, attractive and correspond completely to current exigencies. They are sized by the values of the colouring intensity, but especially by the qualitative chromatic indicators: the colour tonality and proportions of flavin.*

**INTRODUCTION**

Since a few decades back, Dragasani vineyard has also been distinguished by red wines, some of them of high quality, obtained especially from Cabernet Sauvignon and Merlot. The interest for the red wines in the vineyard, along with those semi-flavored and flavored ones has considerably boosted after the oenologist, Phd. Stefan TEODORESCU, (1977), established the oeno-climatic ability in the area, the level of this synthesis climatic indicator being just a little below the one specific for the vineyard of Orevita – Vanju Mare, but with something more than the values stated for the vineyards of Samburesti and Valea Calugareasca.

During the last decade, the high degree of favorability of the vineyard for red wines has been proven through high level scientific works (Ionica, 2006; Nicolaescu, 2007; Bacanu, 2009; Cocos, 2010).

An actual possibility for increasing the quantities of superior quality red wines is represented by the extension of domestic breeds, such as Novac and Negru de Dragasani, whose anthocyanic potential in grapes is represented in the data of this paperwork here.

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## MATERIAL AND METHODS

During the wine-growing years of 2007, 2008 and 2009, distinct depending on the climatic aspect, according to the official methods of O.I.V., when ripe, the full maturity and technological maturity have been established according to the level of the main chromatic components of anthocyanin compounds in the grapes of the breeds Novac and Negru de Dragasani cultivated in three representative centers of Dragasani vineyard. Dealul Olt – Sutești, Zlătărei – Strejești and Dealul Banului – Cârlogani.

The chromatic elements aimed have been: the total contents of anthocyanins (mg/kg grapes), extractability of anthocyanins (%), technological reserve (mg), participation in the complex of the three categories of pigments (%), the chromatic conditions of anthocyanin extracts.

For each breed and wine-growing centre, the quantification of various chromatic parameters has been done based on analysing an important number of tests run in various points of the plantations. In this paperwork, the results from the technological maturity of grapes are presented, from the aforementioned centers.

## RESULTS AND DISCUSSIONS

Table 1

Contents of anthocyanins, extractability of anthocyanins and technological reserves on grapes (2007-2009)

Wine-growing center	Breed	Anthocyanins, mg/kg grapes		Extractability of anthocyanins, %		Technological reserve, mg	
		L V	M	L V	M	L V	M
Olt – Sutești Hill	Novac	1380-1430	1408	52,5-55,1	53,6	745-760	752
	Negru de Drăgășani	1402-1450	1422	53,5-55,4	54,10	752-780	766
Zlătărei – Strejești	Novac	1396-1425	1412	53,0-54,8	54,15	740-768	751
	Negru de Drăgășani	1420-1432	1426	53,9-55,1	54,20	748-775	761
Banului - Cârlogani Hill	Novac	1395-1465	1445	52,9-54,8	53,70	753-782	770

The domestic breed, Novac (table 1), highlights an exceptional anthocyanin potential in all these three centres. This potential is not materialized only through the very important contents of colouring matter in grapes (included, as averages, between 1408 mg/kg grapes and 1445 mg/kg grapes), as well as in high proportions of the extractability contained throughout the years and centres, between 52.5% and 55.1%, variability also appearing in the averages ranging between 53.6 and 54.8 for all centres targeted and production years.

For the breed Negru de Dragasani, the analysis of the data of the two vine-growing centres emphasises the exceptional capacity of the breed to synthesise and store in grapes significant contents of anthocyanin matter, even during those less favourable years, creating averages over 1420mg/kg grapes.

Table 2

Percentage participation of different categories of pigments in the anthocyanic compound from the grapes of Novac breed (2007-2009)

Wine-growing center	Yeallow pigments, %		Red pigments, %		Blue pigments, %	
	L V	M	L V	M	L V	M
Olt – Sutești Hill	29,0-30,9	29,80	61,9-63,4	62,40	7,30-8,90	7,80
Zlătărei – Strejești	28,1-30,2	29,30	61,4-63,2	62,35	8,05-8,90	8,35
Banului – Cârlogani Hill	28,20-30,8	29,6	61,0-63,1	62,45	7,50-9,00	7,95

The breed is also listed with an exceptional ability of releasing an important proportion of anthocyanins (during primary winemaking), the extractability exceeding 54% as an average. The contents of anthocyanins in grapes and proportions of their extractability determine as technological reserves (that may reach into the wine) to be especially advantageous, exceeding 760mg/kg grapes.

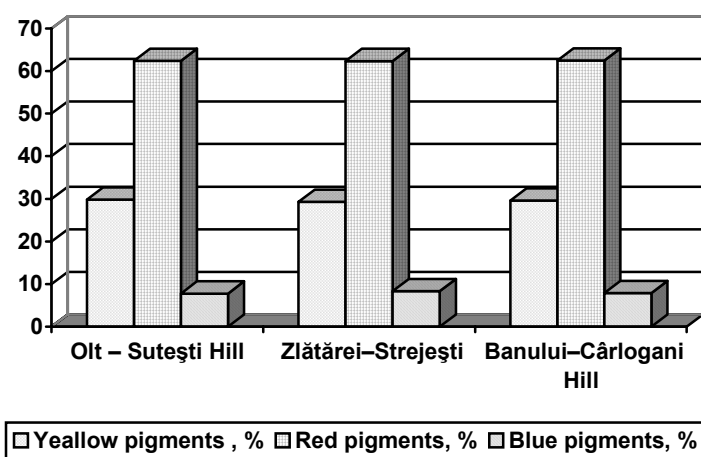


Figure 1. Percentage participation of different categories of pigments in the anthocyanic compound from the grapes of Novac breeds.

In the chromatic structures (table 2), the practically balanced participation of red components in the anthocyanic compound is remarkable, which is also in significant proportions (62.3-62.45).

This continuance by wine-growing centres is set forth by the variability of the other two components (yellow and blue), with average values between 29.3% and 29.8% for the yellow pigments and between 7.8% and 8.35% for the blue pigments. Thusly: for lower values of the yellow component correspond higher values of the blue component and vice-versa (Fig.1).

Table 3

The chromatic qualities of anthocyanic extracts from the grapes of Novac breed  
(2007-2009)

Wine-growing center	Colouring intensity, Ic		Colour tones, Tc		Flavin cations, dA%	
	L V	M	L V	M	L V	M
Olt – Sutești Hill	3,05-3,15	3,09	0,465-0,488	0,478	67,9-69,50	68,66
Zlătărei – Strejești	2,98-3,10	3,05	0,460-0,481	0,470	68,10-69,60	68,90
Banului – Cârlogani Hill	3,10-3,20	3,14	0,446-0,510	0,474	69,0-70,6	70,01

The chromatic qualities of anthocyanic extracts, quantified in table 3, highlight the outstanding richness of anthocyanins in grapes (given by the colouring intensity, ranging between 3.05 and 3.14), as well as superior quality of the anthocyanic compound, defined by the levels of the colour tones (ranging from 0.470 and 0.478), but especially by the values of the chromatic synthesis indicator – flavin, with limits of oscillation throughout the centres and years of production, ranging from 67.90% and 70.60%, by reaching some averages located between 68.66% (Olt – Sutești Hill) and 70.01 (Banului – Carlogani Hill).

Table 4

Percentage participation of different categories of pigments in the anthocyanic compound  
from the grapes of Negru de Drăgășani breed (2007-2009)

Wine-growing center	Yeallow pigments, %		Red pigments, %		Blue pigments, %	
	L V	M	L V	M	L V	M
Olt – Sutești Hill	29,05-30,90	29,55	61,00-61,90	61,40	8,80-9,55	9,05
Zlătărei – Strejești	29,20-31,05	30,60	60,05-60,40	60,25	8,80-9,45	9,15

For the centres, the chromatic structures of the anthocyanic compounds indicate certain differences in values, between all categories of pigments, especially for the averages during the experimental cycle (table 4). The difference in addition for the yellow pigments in favour for the centre of Zlatarei – Strejesti and of 1.15% for the red pigments in favour for the centre of Dealul Olt – Slutesti explains the influences exercised by the wine-growing area onto the complex anthocyanic of grapes (Fig.2).

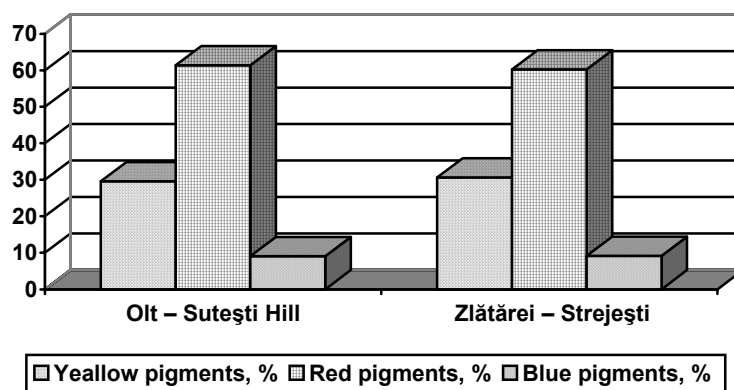


Figure 2. Percentage participation of different categories of pigments in the anthocyanic compound from the grapes of Negru de Drăgășani breed.

The anthocyanine potential Novac de Dragasani, quantitatively as well as qualitatively, arises from the data in table 5.

Table 5

The chromatic qualities of anthocyanic extracts from the grapes of Negru de Drăgășani breed (2007-2009)

Wine-growing center	Colouring intensity, Ic		Colour tones, Tc		Flavin cations, dA%	
	L V	M	L V	M	L V	M
Olt – Sutești Hill	2,90-3,22	3,10	0,470-0,490	0,481	67,60-69,50	68,70
Zlătărei – Strejești	2,85-3,18	3,04	0,492-0,525	0,508	66,95-68,70	68,15

The values of the colouring intensity (3.04-3.10) indicate a significant richness of the colouring matter in grapes, those of tonality (0.481-0.508), an advantageous relation between the yellow pigments and the red ones, and those registered by the flavin cations, at a higher qualitative level of the anthocyanic chromatic compound, in favour of the red expressive hue.

From the data of table 6, it results eloquently that the anthocyanic potential of Novac and Negru de Dragasani is in overwhelming proportions, compared to that of the famous breed for high quality red wines – Cabernet Sauvignon, cultivated in the same area of Dragasani vineyard.

Table 6

The anthocyanic potential of Novac and Negru de Drăgășani breeds and elements of chromatic structure compared to those belonging to Cabernet Sauvignon grapes from the vineyard (2007-2009)

Breeds	Anthocyanins, mg/kg grapes	Extractability of anthocyanins, %	Technological reserve, mg	Pigments participation, %			Chromatic qualities of anthocyanic extracts		
				Yellow pigments, %	Red pigments, %	Blue pigments, %	Colouring intensity, Ic	Colour tones, Tc	Flavin cations dA%
Cabernet Sauvignon	1445	50,10	72,99	29,25	62,70	8,05	3,15	0,467	69,90
Novac	1422	53,82	757,7	29,57	62,40	8,03	3,09	0,474	69,19
Negru de Drăgășani	1424	54,15	763,5	30,08	60,83	9,10	3,07	0,494	68,43

The larger technological reserves for the two domestic breeds are due to a larger extractability of the colouring matter, although the content of anthocyanins in the Cabernet Sauvignon grapes is represented by an extra.

### CONCLUSIONS

Besides a higher glucidic potential, the domestic breeds Novac and Negru de Drăgășani, cultivated in the Dragasani vineyard also have an ability to biosynthesise and store the anthocyanic colouring matter into the grapes. The content of anthocyanins, constant of over 1400 mg/kg grapes indicates a chromatic structure, conferred by the three categories of pigments, with the red component being dominant (61-62%) and without excess of violet or blue hues. The contents of anthocyanins in grapes, the proportions of their extractability (approx. 54%) and the sizes of the technological reserves guarantee positive evolutions of the chromatic features specific to red wines, the first elements of sensory feature, which reasonable consumers come into contact with.

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RESEARCH ON SOME TECHNOLOGICAL DEFINING SEQUENCE  
FOR CULTURE OF SWEET POTATOES (*IPOMOEA BATATAS* POIR.)

Muşat Cosmin<sup>1</sup>

**KEY WORDS:** Sweet potatoes, forcing roots, substrates, rooting cuttings, varieties

**ABSTRACT**

*The results presented show some sweet technology issues defining culture. Keeping the roots over winter required propagation material is difficult and losses are 6-56%, depending on variety. The best method of producing cuttings used as material for the establishment of cultures were forcing medium-sized parent roots in soil in greenhouse and rooting cuttings in water. Within a month, made a forced root formed shoots at up to 13 varieties and eight from Victoria IANB Crux, with a maximum length of 68-77 cm. By successive potting-up, made from a root forced to obtain a variable number of cuttings (variety 38 and 47 Victoria IANB Crux). Dimensions of the establishment cuttings root system were superior crop varieties and variations Victoria IANB rooting in water.*

**INTRODUCTION**

This paper presents partial results of research on sweet potato (*Ipomoea batatas* Poir.). As a vegetable plant, is valued for nutritional value of sweet roots (high in carbohydrates, protein, starch, mineral salts of potassium, calcium, phosphorus, magnesium, various organic acids, B-carotene, vitamin E, C and A), which they are used in obtaining various dishes: smoothies, soups, desserts, French fries, dessert, etc. (Ciofu et al., 1987; Chaux et al., 1995; Ciofu et al., 2003).

Sweet for many countries is undernourished in tropical areas, a basic food source, representing the 7th world agricultural crop, with an average annual production of 138 million tonnes. Grown on all continents, 95 countries, with an area of over 9.5 million ha, the highest in China (FAO Statistics, 2006).

Importance of research and setting goals, lies in these considerations: is a sweet little known plant in Romania, and culture expansion is an opportunity to diversify current vegetable assortment; national bibliographic information is very small and are only international, unfit for specific climatic conditions of our country is absolutely necessary to resolve unclear issues on some sequences of technological problems.

One of them is due to the particular that, having removed origin, sweet seeds do not form the conditions of our country and therefore can grow only through vegetative propagation using cuttings obtained from shoots grown on roots made forced (Maier, 1969; Ciofu, 2005).

Research objectives contained in this work were: to specify the behavior of certain varieties of sweet potatoes in storage roots, knowing that it is difficult (Homer et al., 1967;

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Niculescu and Ciofu, 1994); establishing appropriate substrate forcing the parent roots, finding methods for rooting cuttings, lighter and efficient than those recommended in the literature (Maier, 1969; Ciofu and Roşu, 1997).

### **MATERIAL AND METHODS**

Results were obtained in 2008, in greenhouse and field research inside U.A.S.V.M. Bucharest. To achieve these objectives were conducted three experiments on: methods of forcing the roots, methods of rooting of cuttings, the influence of variety and propagating material on the productive potential of sweet in terms of southern Romania.

The biological material studied was the roots of two varieties of sweet potatoes Romanian Victoria IANB and Crux. Roots harvested in the previous autumn, were kept 96 days in the dark room at 8-10°C, with newspapers stacked in crates type P. Practiced technology experiences presented the following features:

- forcing the roots was made on February 27, the hot gases in the ground (holes spaced 50 / 40 cm depth of 10-15 cm), or in large pots (30/30/20 cm) filled with peat substrate nutritive, RS II and BIOLAN. After planting the soil was wet and black foil mulch was perforated;

- cuttings production started after 40-50 days of forcing roots (May 7). Cuttings from 10-15cm tall, slightly based, at least 3 eyes (after Ciofu 2003) were deployed in stages by the roots and were rooted in pots made of water (V1) or pots filled with peat RS II (V2);

- the establishment of crops, land was prepared as for roots, and 8-10 days before planting, it - has shaped the bilons 30-50 cm wide, 25-28 cm high and 70cm apart;

- planting was done on June 19 in succession after the early crop of spinach, lettuce, onions and green garlic.

- were applied current care work and special work of haulm lift off the ground to avoid the formation of adventitious roots (Maier, 1969).

- harvesting roots was made on November 12, before falling frosts.

During the research, have made numerous observations and measurements of the intended objectives and experimental variants, using specific methods of work namely:

- registration of greenhouse microclimate conditions during storage and forcing the roots and cuttings during;

- morphometric measurements of forcing phase (number, length and mass in 10-rooted cuttings made forced, for each variant) and cuttings (length, mass and volume of root system);

### **RESULTS AND DISCUSSIONS**

During the period of forcing the roots and the rooting of cuttings, there have been major factors in the dynamics of greenhouse microclimate. Of data analysis revealed that in March, temperatures ranged between 13 and 22.8°C, and in April increased gradually, especially in the third decade of the month, when at noon ranged from 27.3 - 47°C. The humidity was a reverse trend, decreasing values as the increasing temperatures (from 78-80% in the morning until noon 32-38%). Light intensity was adequate plant growth, with an average of 20,000 Lux. The soil was slightly alkaline pH (7.2 to 8.0), indicating an excess of values required by the plants best sweet potatoes (from 5.6 to 6.5 after Chaux et al., 1995).

Roots of sweet potatoes on the retention results are shown in Table 1. Roots harvested in 2007, on November 23 were put to hold by 20 Kg of each species in the study, resulting in different numbers of roots (lower in Crux variety, characterized by higher average root mass). After a retention period of 96 days before settlement roots for

tampering determinations were performed to analyze the behavior of the two Romanian varieties of sweet potatoes and highlight differences between them.

The resulting data show that the initial amount placed in storage roots for each variety, after about 100 days, the variety Victoria IANB was kept in good conditions, a quantity of two times higher than that of the variety Crux. In the number of roots, differences between varieties are slightly smaller share of well-preserved roots were approximately 1.6 times higher in Victoria IANB variety.

Table 1

Influence of varietal resistance to storage roots of seet potatoes

Variety	November 23, 2007		February 27, 2008				Losses %	
	Put in storage roots		Roots obtained after 96 days storage				Root mass	Root number
	masa (kg)	nr.	masa (kg)	%	nr.	%		
Victoria IANB	20, 000	138	18,830	94,15	135	97,83	5,85	2,12
Crux	20, 000	125	8,846	44,23	78	62,40	55,77	37,60

The results show the superiority of the variety Victoria IANB to keep material losses, expressed in mass and number of roots were very low, approximately 10 and 18 times lower than those recorded in variety Crux.

After our findings, causes loss of material during storage, large roots were rotting and excessive dehydration of small roots, as 50-70 g. explains, why in both varieties, the percentage loss was greater in the mass than roots of numbers.

Fungal rots attacks occurred as wet as dry rots. The presence of wet rots increased stratification and substrate humidity because after about three months of storage, especially the variety Crux, there was a resumption of physiological activity, 26% of the roots are 4-6 mm long, the eyes .

Showing the influence of forcing refers to the variety of roots and shoots of the substrate used on the formation cuttings are derived from the establishment of cultures.

Data presented in Table 2 shows that in terms of training dynamics of shoots, first fall variations in soil greenhouse forcing, where the first shoots emerged from V6 - variety Crux, on March 21 (after 3 weeks of forcing ). In these variants, and recorded the highest percentage of rooted shoots that have sprung up within a month. (Of the 10 roots planted in each variant, have sprung all the variety Victoria and 80% at Crux).

Forcing variations in peat RS II were ranked second (60%) in both varieties and was in last place Biolan substrate (40% roots Victoria IANB east and 60% at Crux). It was noted that in four pots (40%) in the substrate surface was black mulch sheet were formed which prevented the emergence plant fungi.

The average weight of roots made forced arised not influenced the overall dynamics of shoots, the differences are small variations in this aspect. Should therefore be noted that the influence of substrate variety and forcing are more important.

Sequence differences between the versions is kept in the average number of shoots formed on a root and size. The first place was in V6 (Crux variety), which roots in the soil for forcing in approximately 30 days after emergence, were obtained most numerous and vigorous shoots.

Shoots emerged from the period May 15 to June 13 were carried out successively, the establishment crop cuttings. During this period, there were differences in the dynamic variations of the number and mass produced cuttings. Figure 1 shows how the variety of forcing the roots die and have influenced the formation cuttings.

Table 2

Substratum influence of forcing and the variety of training shoots

Variant - Kind  / date	Substrate of forcing	Root mass	Emergence date (% of roots)	Total number shoots/ root	Long shoots cm	
					min.	max.
		Feb 27	21.03 – 21.04		May 10	
V1-Victoria	RS II	247,0	24 March (10 %) 27 March (20 %) 21 April (30 %)	3	30,5	77
V2- Crux		259,4	24 March (20 %) 27 March (20 %) 21 April (20 %)	10	31,0	54,6
V3-Victoria	Biolan	256,8	24 March (10%) 27 March (10%) 21 April (20 %)	4	9,5	33,5
V4- Crux		260,0	24 March (10 %) 27 March (20 %) 21 April (20 %)	7	39,75	46,3
V5-Victoria	Greenhouse Soil	260,6	24 March (20 %) 27 March (40 %) 21 April (40 %)	8	26,8	42,6
V6- Crux		254,2	21 March (20%) 27 March (30 %) 21 April (30 %)	13	40,3	68,1

On top were placed variations of forcing the roots in soil (V5 and V6), which throughout the period of cuttings were allowed to obtain a larger number of cuttings compared with other variants. Within one month of cuttings, the V6 (variety Crux, forcing the ground) to make a total of 47 cuttings, 1.2 times higher compared with the variety Victoria IANB (V5) and 3.5 times the V4 (variety Crux, forcing the Biolan).

In the mean mass of cuttings, differences between the two varieties were small, while the same variety, there were significant differences in favor of forcing variations in soil (4 grams and 5 grams Victoria IANB Crux compared with peat type Biolan).

These results demonstrates superiority over Victoria IANB Crux variety and method of forcing in soil, compared with substrate composed of peat.

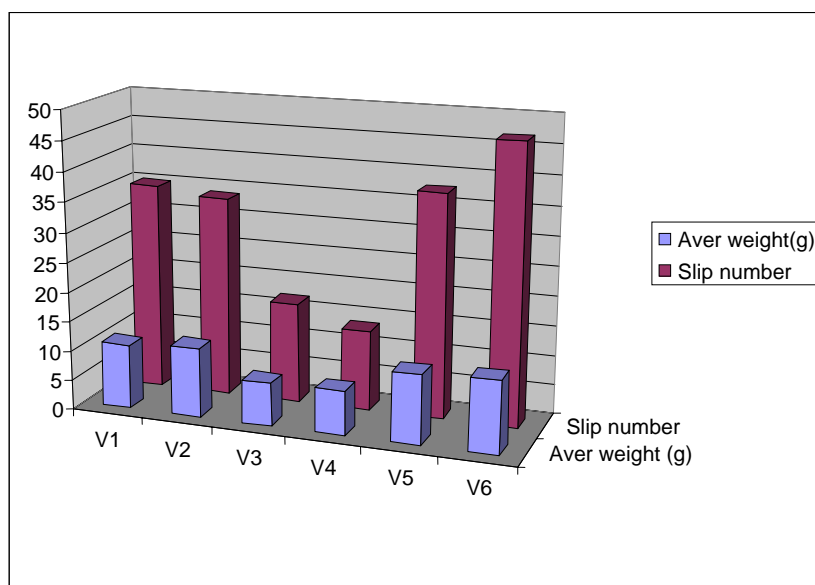


Fig. 1 Influence of substrate variety and forcing the roots cuttings of sweet potatoes on training (June 13, 2008)

We appreciate that soil offers better conditions for growth of shoots from which the cuttings, especially as regards ensuring a more constant level of humidity and an unlimited volume of the root layer.

Rooting cuttings is a technological sequence for which some authors recommend expensive methods such as using wooden boxes filled with soil mixture (Maier 1969), planting in the mixed layer or the greenhouse soil (Ciofu et al., 2003) or planting in warm shelter, specially designed (Homer and William, 1967), the purpose of simplifying and streamlining the sequences in our research we tested the method proposed by Ciofu (2005). for rooting in water, with a variation in substrate nutrient peat cuttings.

Table 3 presents results on the influence of two methods of rooting of cuttings on the development of their root system.

Table 3

Influence of substrate on rooting cuttings root system

Date	Variety (kind)	Substrate	Root length (cm)	Root volume cm <sup>3</sup>	Mass (g)
June 13	V1- Victoria	water	10,7	0,10	-
	V2- Victoria	peat	5,8	0,08	-
	V3- Crux	water	6,2	0,09	-
	V4- Crux	peat	3,9	0,08	-
June 24	V1- Victoria	water	15,6	0,15	2,0
	V2- Victoria	peat	7,0	0,12	3,0
	V3- Crux	water	7,3	0,14	1,22
	V4- Crux	peat	6,0	0,11	2,3

Note that in general, cuttings of the variety Victoria IANB have a force greater than the variety Crux root system. For both species, root length and root volume were higher variations rooted in water, while the mass of root system had higher values rooted in peat variants.

### CONCLUSIONS

*Ipomoea batatas* (sweet potato) does not form seeds in the conditions of our country and therefore can grow only through vegetative propagation using cuttings obtained from shoots grown on roots made forced.

Keeping the roots over winter for use as propagation material is difficult and specific variety. After about 100 days were kept in good condition, 94% of the roots of the variety Victoria IANB and only 44% of Crux variety.

Substrate variety and forcing the roots and the rooting of cuttings, vegetative propagation potential influence on sweet potatoes.

Within a month, the root made a forced shoots were formed at 7-13 and 3-8 at the Victoria variety Crux IANB and their length can reach 68-77 cm. In both species, using as substrate greenhouse soil, peat gave superior results.

From a root called forced by successive potting-up, can get up to 47 cuttings of the variety and 38 from Victoria IANB Crux, with a mean weight of about 12g, if the soil is made.greenhouse.forcing.

The variety Victoria IANB were rooted cuttings obtained larger compared Crux, rooting in water favoring root growth of both varieties.

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**PHYSIOLOGICAL MODIFICATIONS PRODUCED BY  
DIPLOCARPON EARLIANUM (ELLIS & EVERH.) F.A. WOLF  
IN FRAGARIA X ANANASSA DUCH.**

Nicolae I.<sup>1\*</sup>

**KEY WORDS:** attacked plants, healthy plants, physiological processes, strawberry.

**ABSTRACT**

*The research regarding physiological modifications produced by Diplocarpon earlianum (Ellis & Everh.) F.A. Wolf. have been made on Real strawberry variety cultivated in climatic conditions of Oltenia region, Romania. In the attacked plants one can observe the diurnal dynamics of the photosynthesis and of transpiration presents a minimum in the morning, a maximum after lunch and a minimum toward the evening, in connection with the climatic conditions, with pathogen specific variations. At the attacked plants one can also observe a decrease of chlorophyll content as result of the blockage biosynthesis of the chlorophyllian pigment. One can also observe a decrease of total water content as a result of the malfunctioning of closing and opening mechanisms of the stomates, which is manifested by the withering and premature drying of the plants.*

**INTRODUCTION**

*Fragaria* genus, belonging to *Rosaceae* family, including over 20 species and subspecies with widespread geographical (Jungnickel, 1988).

The U.S.A. is the world's leading strawberry producer with California producing approximately 80% of the U.S. crop. Japan ranks second in the world followed by Poland, Italy, U.S.S.R., France, Mexico and Spain (Hancock et al., 1991).

*Diplocarpon earlianum* (Ellis & Everh.) F.A. Wolf. is destructive to numerous cultivars of strawberry in temperate, subtropical and tropical regions. The fungus attacks the leaves, petioles, runners, pedicels, and calyxes of strawberry plants.

When plants are attacked by pathogens, production decreases and can lead, in some cases to affect the harvest. Losses from negligible to severe, depending on numerous factors, cultivar susceptibility, type of cropping system, and weather conditions.

There was no significant dependence between yield and single fruit weight and the scale of infection in the year of appearance of the disease, but an adverse effect of the pathogen was observed in the following year (Hortynski et al., 1994).

We note that in literature there are relatively few studies on the physiology of plants attacked by pathogens and research on some physiological modifications produced by *Diplocarpon earlianum* on *Fragaria ananassa* are not mentioned.

The net photosynthetic activity is subjected to seasonal changes and to diurnal changes which are mainly influenced by the stage of shoot development, the leaf ageing,

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the accumulation of hormones and of carbohydrates in the leaves, as well as the by the fluctuations of light, leaf temperature, air temperature and humidity (Lakso, 1985).

The diurnal dynamics of transpiration has a maximum at noon and a minimum during the night when cuticle transpiration occurs. Interception of surface solar radiation is correlated with the amount of heat retained by the leaves, and therefore the intensity of transpiration (Burzo et al., 1999).

Intensity of transpiration process commensurate increases with that of photosynthesis, both processes are dependent on solar radiation intensity (Bignami & Natali, 1992).

### MATERIAL AND METHODS

The research regarding physiological modifications produced by *Diplocarpon earlianum* have been made on *Real* strawberry varieties cultivated in climatic conditions of Oltenia region, Romania.

*Fragaria ananassa* is a perennial plant, with adventitious roots, developing from buds located near the young leaves. The stalk is short (rhizome) and the bifurcation branches. Strawberry leaves are large, trifoliate, toothed, glossy or pubescent. Flower is organized by type 5. Sepals are green, arranged in two rows - one on each line separator 5. Petals, placed behind sepals have formed white corolla. The center flower is the total of 20 stamens and numerous pistils. Fruit flower receptacle results in thickening (false fruit). The fruits are achena located on the surface padded.

Romanian *Real* variety (1998) is a variety resistant to frost. The plant is vigorous, with late flowering, fruit is high (14-15 g), conical, red, uniform, shiny, sour sweet taste without intense flavor. Production: 19-29 t/ha.

The estimation of the attack was made using the calculation formulae (Săvescu & Rafailă 1978).

The intensity of the photosynthesis and transpiration was established by a non-destructive method with the analyzer LCI (Ultra Compact Photosynthesis Measurement System) and results obtained were graphically represented and statistically interpreted. The total water contents and that of dry substance were determined by the help of the gravimetric method. The contents of the chlorophyllian pigments were estimated with the help of the Minolta SPAD 502 chlorophyll meter.

### RESULTS AND DISCUSSIONS

Strawberry leaf scorch is caused by the fungus *Diplocarpon earlianum* (Ellis & Everh.) F.A. Wolf. (1924), is the most common leaf disease.

Symptoms of strawberry leaf scorch consist of numerous small, irregular, purplish spots or "blotches" that develop on the upper surface of leaves. The centers of the blotches become brownish. Blotches may coalesce until they nearly cover the leaflet, which then appears purplish to reddish to brown (Figure 1).

The fungus shows subcuticular mycelium that develops, forming stroma with acervuli in that form conidiophore and conidia bicelulare, hyaline, ovoid (Figure 2).

*Diplocarpon earlianum* most frequently infects strawberry leaves at any stage of development. The fungus produces spore forming structures in the spring on both surfaces of dead leaves. These structures produce spores abundantly in midsummer. In the presence of free water these spores can germinate and infect the plant. Older and middle-aged leaves are infected more easily than young ones.

In cross section made by strawberry leaf attacked by pathogen is observed that the upper skin loss occurs due to transverse walls attacked tissue necrosis in the right areas,

palisade parenchyma cells have reddish brown colour due to the disappearance of chloroplast and lower epidermis and part of the parenchyma grows less incomplete (Figure 3).



Figure 1. *Diplocarpon earliarum* in the strawberry leaves (Original).



Figure 2. *Diplocarpon earliarum* - bicelulare conidia (oc. 10 x ob. 40) - Original.

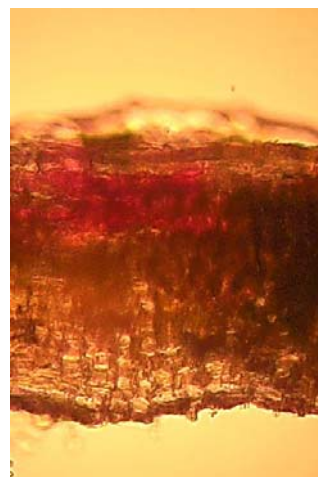


Figure 3. The section transversal in strawberry leaf attacked by *Diplocarpon earliarum*

The intensity of the physiological processes at strawberry plants was established, according to the frequency, the intensity and the degree of attack, but also by the climatic conditions, on July 10<sup>th</sup> 2009. The estimation of the attack produced by the *Diplocarpon earliarum* at strawberry plants is presented in Figure 4.

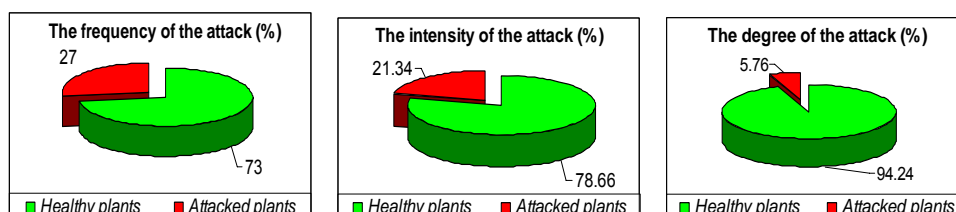


Figure 4. The estimate of the attack produced by *Diplocarpon earliarum* in the strawberry plants.

The diurnal dynamics of photosynthesis and transpiration in the attacked plants is similar to that in healthy plants but the recorded values are lower as a result of the reduction of the assimilation surface, the cover of stomata by the mycelium of the fungus, but also by malfunctioning of stomata closing and opening mechanisms (Figure 5 and Figure 6).

The diurnal dynamics of photosynthesis and transpiration depend on the light radiation received by leaves, which are dependent on the position of the leaves on plants.

At the strawberry plants one can observe an increase of the photosynthetic active radiations presented on the surface of the leaves morning (9 a.m.) when one can record values of 1246  $\mu\text{mol} / \text{m}^2 / \text{s}$  for the healthy plants and of 1214  $\mu\text{mol} / \text{m}^2 / \text{s}$  for the attacked plants, their growth up until after lunch (1 p.m.) when one record 1564  $\mu\text{mol} / \text{m}^2 /$



s for the healthy plants and  $1510 \mu\text{mol} / \text{m}^2 / \text{s}$  for the attacked plants, and towards evening (5 p.m.) one can notice a gradual decrease, recording values of  $1365 \mu\text{mol} / \text{m}^2 / \text{s}$  for the healthy plants and of  $1348 \mu\text{mol} / \text{m}^2 / \text{s}$  for the attacked plants.

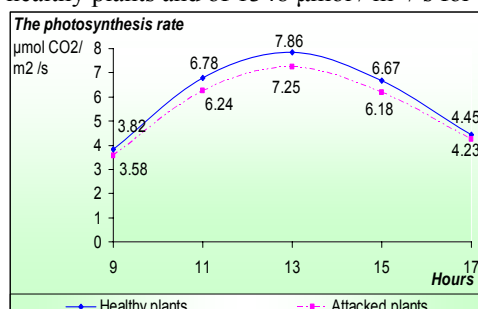


Figure 5. The diurnal dynamics of photosynthesis at the strawberry plants.

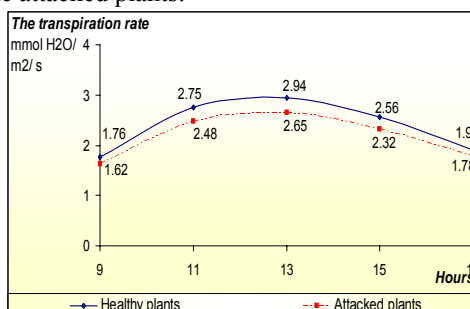


Figure 6. The diurnal dynamics of transpiration at the strawberry plants.

In the *Fragaria ananassa* a very strong association was established between the physiological processes (photosynthesis rate and transpiration rate) and photosynthetic active radiations, leaf temperature and stomatal conductance, Pearson correlation coefficient ( $r$ ) presenting different values in attacked plants, compared with healthy plants.

Linear regression shows a positive correlation between the photosynthesis rate and photosynthetic active radiations (the coefficient of determination  $R^2$  was 0.95 for the healthy plants and 0.91 for the attacked plants) and between the transpiration rate and photosynthetic active radiations (the coefficient of determination  $R^2$  was 0.91 for the healthy plants and 0.87 for the attacked plants) - Figure 7 and Figure 8.

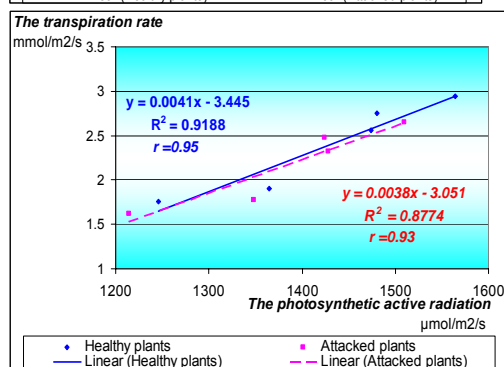
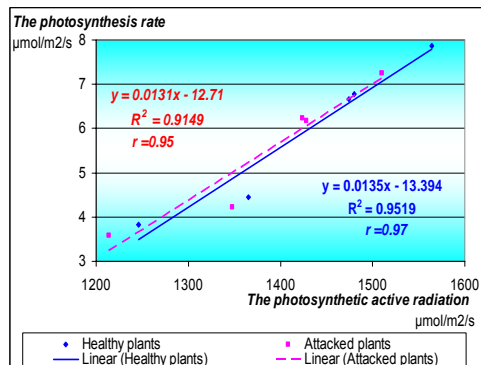


Figure 7. The correlation between the photosynthesis rate and the photosynthetic active radiation at the strawberry plants.

Figure 8. The correlation between the transpiration rate and the photosynthetic active radiation at the strawberry plants.

The photosynthesis and transpiration depend on the temperature. One can observe an increase of leaf temperature in the morning (9 a.m.) when values of 28.6 °C are recorded in the healthy plants and 28.6 °C in the plants attacked, the increase of the temperature up until after lunch (1p.m) when one record 33.2 °C in the healthy plants and 33.3 °C in the attacked plants and towards the evening (5 p.m) the decrease of the temperature, recording values of 31.5 °C in the healthy plants and 31.7 °C in the attacked plants.

Linear regression shows a positive correlation between the photosynthesis rate and of the leaf temperature (the coefficient of determination  $R^2$  was 0.79 for the healthy plants and 0.78 for the attacked plants) and between the transpiration rate and of the leaf temperature (the coefficient of determination  $R^2$  was 0.76 for the healthy plants and 0.75 for the attacked plants) - Figure 9 and Figure 10.

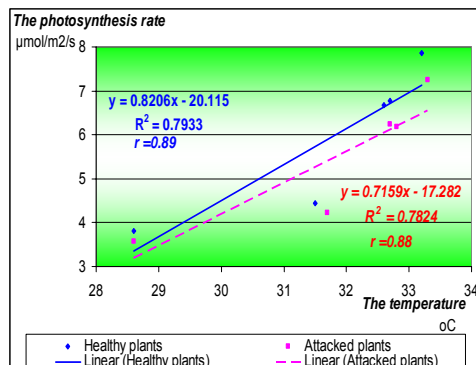


Figure 9. The correlation between the photosynthesis rate and the leaf temperature at strawberry plants.

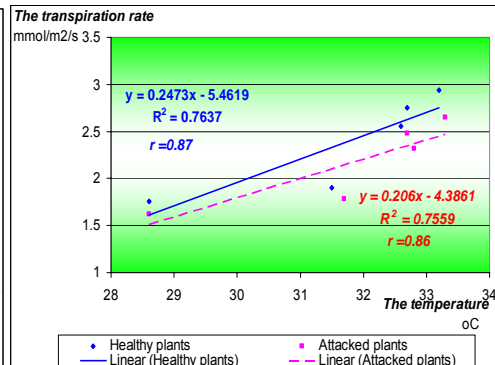


Figure 10. The correlation between the transpiration rate and the leaf temperature at the strawberry plants.

Stomatal conductance varies throughout the day. So during the day one can observe an increase of the stomatal conductance starting with the early hours of the morning (9 a.m.) when one can record values of  $0.13 \mu\text{mol} / \text{m}^2 / \text{s}$  in the healthy plants and  $0.15 \mu\text{mol} / \text{m}^2 / \text{s}$  in the attacked plants, the increase of the stomatal conductance up until after lunch (1p.m) when one can record values of  $0.21 \mu\text{mol} / \text{m}^2 / \text{s}$  in the healthy plants and  $0.23 \mu\text{mol} / \text{m}^2 / \text{s}$  in the attacked plants and towards the evening (5 p.m) the gradual decrease of the stomatal conductance when one can record values of  $0.14 \mu\text{mol} / \text{m}^2 / \text{s}$  in the healthy plants and  $0.16 \mu\text{mol} / \text{m}^2 / \text{s}$  in the attacked plants.

Linear regression shows a positive correlation between the photosynthesis rate and of the stomatal conductance (the coefficient of determination  $R^2$  was 0.95 for the healthy plants and 0.87 for the attacked plants) and between the transpiration rate and of the stomatal conductance (the coefficient of determination  $R^2$  was 0.94 for the healthy plants and 0.84 for the attacked plants) - Figure 11 and Figure 12.

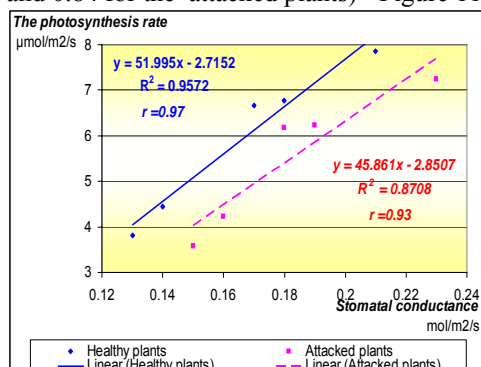


Figure 11. The correlation between the photosynthesis rate and the conductance stomatal at the strawberry plants.

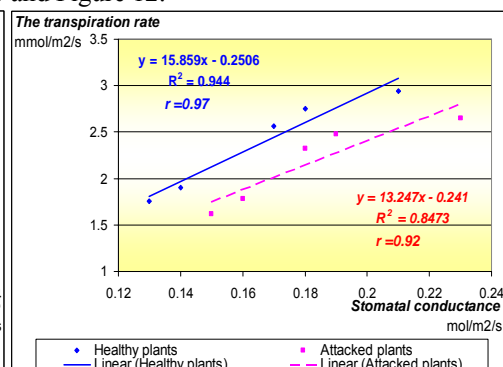


Figure 12. The correlation between the transpiration rate and the conductance stomatal at the strawberry plants.

At the attacked plants there can be seen a decrease of the total water content by 1.94 % and an increase of the dry substance content by 2.83 %, which is manifested by the decrease of the cellular turgor, the withering and premature drying of the plants (Figure 13).

The plants attacked present a decrease of the chlorophyll content by 14.52 % as a result of the blockage of its biosynthesis and the deterioration of the chlorophyll (Figure 14).

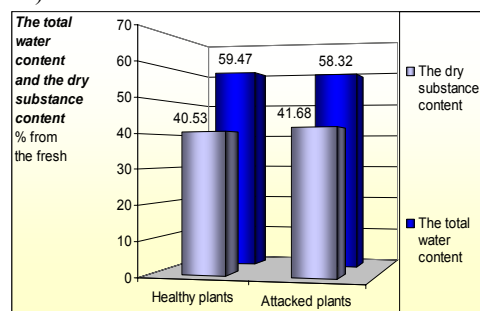


Fig. 13. The water content and the dry substance content at the strawberry plants.

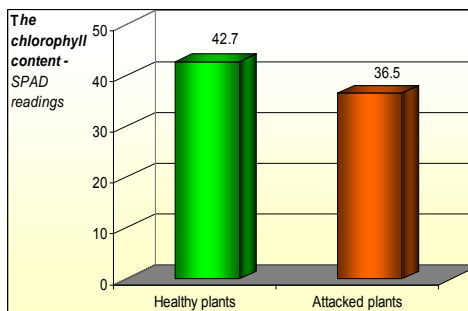


Fig. 14. The chlorophyll content at the strawberry plants.

## CONCLUSIONS

The intensity of the physiological processes at the strawberry plants varies according to the frequency, the intensity and the degree of attack, but also by the climatic conditions. At the analyzed attacked plants one can observe that photosynthesis and transpiration presents a minimum in the morning, a maximum after lunch and a minimum toward the evening, but the recorded values are small in comparison with healthy plants.

The increase of the photosynthesis and transpiration is positive correlate with the increase of the photosynthetic active radiations, temperature leaf and stomatal conductance, but shows variations in the attacked plants as a result of several structural modifications produced by pathogen. In the attacked plants one can observe a decrease of the chlorophyll content because of the intensification of the chlorophylls and deterioration of the chloroplasts. The physiological modifications produced in the attacked plants determine several metabolic unbalances, with implications on the growth and development plants.

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PHYSIOLOGICAL MODIFICATIONS PRODUCED BY  
*PHRAGMIDIUM MUCRONATUM* (PERS.) SCHLTDL. IN *ROSA CANINA* L.

Nicolae I.<sup>1\*</sup>

**KEY WORDS:** attacked plants, healthy plants, pathogen, physiological processes.

**ABSTRACT**

The research regarding physiological modifications produced by *Phragmidium mucronatum* (Pers.) Schltdl. have been made on *Rosa canina* cultivated in Botanical Garden "Al. Buia" of Craiova. In the attacked plants one can observe the diurnal dynamics of the photosynthesis and of transpiration presents a minimum in the morning, a maximum after lunch and a minimum toward the evening, in connection with the climatic conditions, with pathogen specific variations. At the attacked plants one can also observe a decrease of chlorophyll content as result of the blockage biosynthesis of the chlorophyllian pigments. One can also observe a decrease of total water content as a result of malfunctioning of stomata closing and opening mechanisms, which determines hidric unbalances, with implications on the growth and development of plants.

**INTRODUCTION**

*Rosa canina* L. is a native plant species in Europe, northwest Africa and western Asia. It is a light-loving species, which grows on the hills, from roadsides and forests as it is less demanding from rocky, poor ground. It withstands very low humidity and low temperatures. It grows in plains and hill regions.

*Phragmidium mucronatum* (Pers.) Schltdl. is a truly parasite species. This species produces the disease known as rust rose, as reported on cultivated varieties and hybrids of roses, but also on wild species of the *Rosa* genus (Tănase and Şesan, 2006)

Nine different species of *Phragmidium* have been reported to cause rust on roses (Horst, 1983) and four of them were reported for dogroses in Europe so far (Gäumann 1959; Ritz *et al.* 2005).

Rust infects the tissue through stomatal openings (Horst, 1983), implying that the density of stomata on the leaves might have an influence on the severity of the infection.

We note that in literature there are relatively few studies on the physiology of plants attacked by pathogens and research on some physiological modifications produced by *Phragmidium mucronatum* on *Rosa canina* are not mentioned.

At the attacked plants, there is a decrease in the intensity of photosynthesis as a result of the occurrence of chlorate spots (chlorosis) in leaf and the degradation of chloroplast and the covering of stomatitis by fungus mycelium (Nicolae, 2010).

The net photosynthetic activity is subjected to seasonal changes and to diurnal changes which are mainly influenced by the stage of shoot development, leaf ageing,

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hormones and carbohydrates accumulation in leaves, as well as by light intensity fluctuations, leaf temperature, air temperature and humidity (Lakso, 1985).

### MATERIAL AND METHODS

The research regarding physiological modifications produced by *Phragmidium mucronatum* have been made on *Rosa canina* cultivated in Botanical Garden "Al. Buia" of Craiova.

*Rosa canina* is a ligneous arbustus, with a height ranging between 1 and 5 meters. The leaves are pennate, with oval or elliptic folioles, having the stipes well visible at the base of the petiole. The color of the flowers are pink, sometimes white, placed 2-3 in the top branches. Each flower consists of 5 sepals, 5 petals, numerous stamens arranged in a receptacle, which mature, become fleshy and red. In this receptacle, called improper fruit, are numerous achenes, which is in fact the real fruit.

The estimation of the attack was made using the calculation formulae (Săvescu and Rafailă, 1978).

The intensity of the photosynthesis and transpiration was established by a non-destructive method with the analyzer LCi (Ultra Compact Photosynthesis Measurement System) and results obtained were graphically represented and statistically interpreted. The total water contents and of dry substance were determined by the help of the gravimetric method. The contents of the chlorophyllian pigments were estimates by the help of the Minolta SPAD 502 chlorophyll meter.

### RESULTS AND DISCUSSIONS

The rust is evident on the green parts of plants: the tongue of leaves, stems, young branches, peduncle floral buds and fruit in particular. On the surface of the attacked organs spring dusty orange pustules from which is aecia with aeciospores (Mitrea, 2006).

In a more advanced stage of the disease on leaves, spots of discoloration appear; they are well-determined and may confluate. Corresponding spots on the underside of the leaves form small pustules, orange, powdery, representing the uredinia of fungus (Figure 1 and Figure 2).

In autumn on the bottom of the leaves there appear the telia of the fungus in the shape of pustules of dark colour. The strong rust attack causes drying and early fall of leaves, drying of branches, the fall of the non-opening of buds.

*Phragmidium mucronatum* (Pers.) Schltdl. presents picnidia which is formed on green plant organs, arranged in small groups, within them on sporophore filaments forming small pinospores having a thin, colourless membrane. Aecia is orange colour, while inside aeciospores spherical form.

Uredinia are formed only on the underside of leaves and are surrounded by a corona of paraphyses clubbed and curved inwards. The urediniospores are spherical or nearly spherical, ellipsoidal or oval.

The telia is formed in the same places with urediniospores, have dark colour and are pulverous. The teliospores are oval or spindle elongated, multicellular (6-9 cells) rounded at the basis, and on the apical side they are narrow and finished by a colourless papilla. The membrane is dark brown, and the pedicel is colourless, dilated at the basis (Figure 3).

The germination of the telia results in a promycelium, on which basidiospores are formed and they ensure the first infections on the young leaves. The disease is favoured by rainy periods combined with higher temperatures during the day and by misty nights.

The intensity of the physiological processes was established, according to the frequency, the intensity and the degree of attack, but also by the climatic conditions, on August 26<sup>th</sup> 2009.



Figure 1. *Rosa canina* attacked by *Phragmidium mucronatum* (Original).



Figure 2. *Rosa canina* attacked by *Phragmidium mucronatum* - leaves with uredinia (Original).

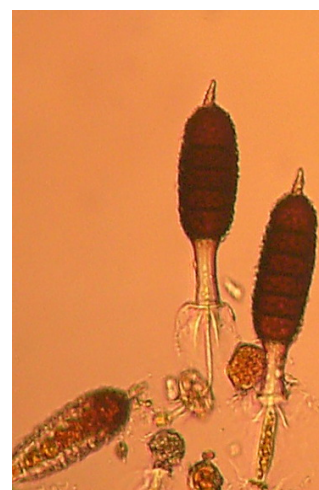


Figure 3. *Phragmidium mucronatum* - teliospores and urediniospores (oc 10 x ob 40) - Original

The estimation of the attack produced by the *Phragmidium mucronatum* in the *Rosa canina* is presented in Figure 4.

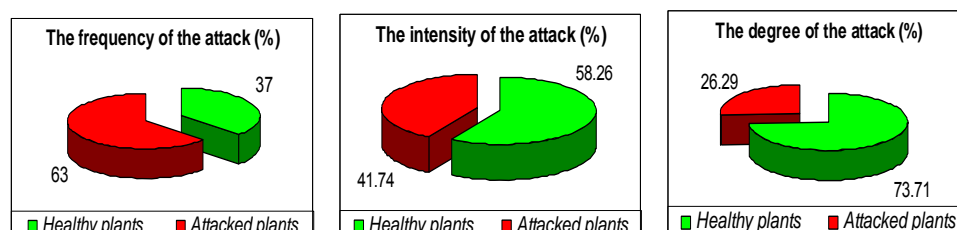


Figure 4. The estimate of the attack produced by *Phragmidium mucronatum* in the *Rosa canina*.

The diurnal dynamics of photosynthesis in the attacked plants is similar to that in healthy plants but the recorded values are lower in comparison with these as a result of the reduction of the assimilation surface through the deterioration of the chlorophyll. The dynamics of transpiration in the attacked plants is similar to that in healthy plants, but the recorded values are lower as a result of the reduction of the transpiration surface and the malfunctioning of the stomatic apparatus (Figure 5 and Figure 6).

The diurnal dynamics of photosynthesis and transpiration depend on the light radiation received by leaves, which are dependent on the position of the leaves on plants.

At the *Rosa canina* plants one can observe an increase of the photosynthetic active radiations presented on the surface of the leaves in the morning (9 a.m.) when one can record values of  $1082 \mu\text{mol} / \text{m}^2 / \text{s}$  for the healthy plants and of  $1054 \mu\text{mol} / \text{m}^2 / \text{s}$  for the attacked plants, their growth up until after lunch (1 p.m.) when one record  $1490 \mu\text{mol} / \text{m}^2 /$



s for the healthy plants and  $1458 \mu\text{mol} / \text{m}^2 / \text{s}$  for the attacked plants, and towards evening (5 p.m.) one can notice a gradual decrease, recording values of  $1345 \mu\text{mol} / \text{m}^2 / \text{s}$  for the healthy plants and of  $1327 \mu\text{mol} / \text{m}^2 / \text{s}$  for the attacked plants.

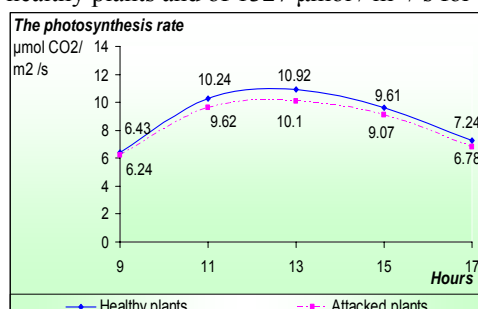


Figure 5. The diurnal dynamics of photosynthesis at the *Rosa canina*.

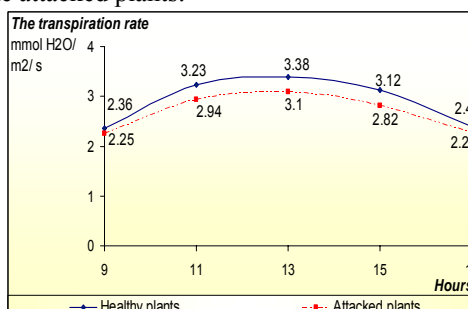


Figure 6. The diurnal dynamics of transpiration at the *Rosa canina*.

In the leaves of *Rosa canina* a very strong association was established between the physiological processes (photosynthesis rate and transpiration rate) and photosynthetic active radiations, leaf temperature and stomatal conductance, Pearson correlation coefficient ( $r$ ) presenting different values in attacked plants, compared with healthy plants.

Linear regression made show a positive correlation between the photosynthesis rate and photosynthetic active radiations (the coefficient of determination  $R^2$  was 0.79 for the healthy plants and 0.71 for the attacked plants) and between the transpiration rate and photosynthetic active radiations (the coefficient of determination  $R^2$  was 0.70 for the healthy plants and 0.62 for the attacked plants) - Figure 7 and Figure 8.

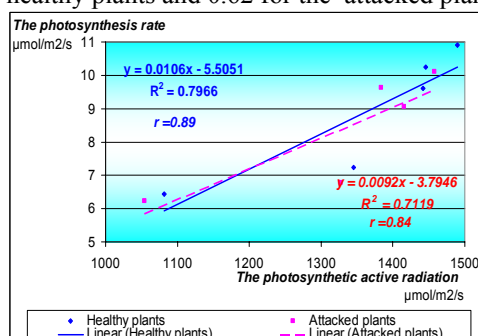


Fig. 7. The correlation between the photosynthesis rate and the photosynthetic active radiation at the *Rosa canina*.

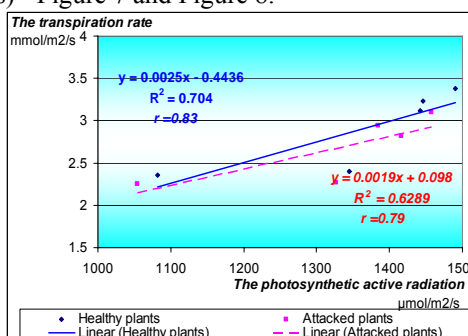


Fig. 8. The correlation between the transpiration rate and the photosynthetic active radiation at the *Rosa canina*.

The intensity of photosynthesis and transpiration depends on the temperature. At the *Rosa canina* plants one can observe an increase of the leaf temperature morning (9 a.m.) when values of  $26.2^\circ\text{C}$  are recorded in the healthy plants and  $26.3^\circ\text{C}$  in the plants attacked, the increase of the temperature up until after lunch (1p.m) when one record  $31.6^\circ\text{C}$  in the healthy plants and  $31.8^\circ\text{C}$  in the attacked plants and towards the evening (5 p.m) the

gradual decrease of the temperature, recording values of 29 °C in the healthy plants and 29.3 °C in the plants attacked.

Linear regression shows a positive correlation between the photosynthesis rate and of the leaf temperature (the coefficient of determination  $R^2$  was 0.67 for the healthy plants and 0.61 for the attacked plants) and between the transpiration rate and of the leaf temperature (the coefficient of determination  $R^2$  was 0.60 for the healthy plants and 0.56 for the attacked plants) - Figure 9 and Figure 10.

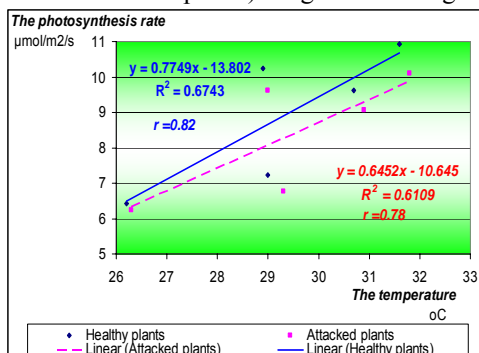


Figure 9. The correlation between the photosynthesis rate and the leaf temperature at *Rosa canina*.

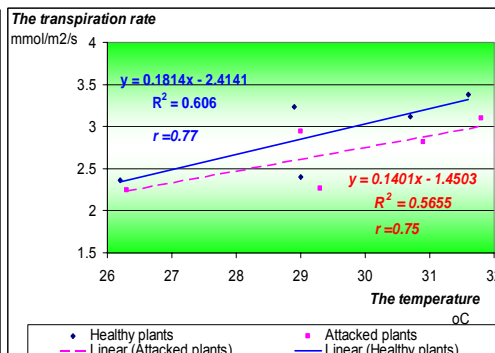


Figure 10. The correlation between the transpiration rate and the leaf temperature at the *Rosa canina*.

The stomatal conductance increases until noon and decreases from then on due to the reduction of the stomata opening level under the influence of high temperature and the decrease of relative air humidity.

One can observe an increase of the stomatal conductance starting with the early hours of the morning (9 a.m.) when one can record values of  $0.14 \mu\text{mol} / \text{m}^2 / \text{s}$  in the healthy plants and  $0.16 \mu\text{mol} / \text{m}^2 / \text{s}$  in the attacked plants, the increase of the stomatal conductance up until after lunch (1p.m) when one can record values of  $0.23 \mu\text{mol} / \text{m}^2 / \text{s}$  in the healthy plants and  $0.25 \mu\text{mol} / \text{m}^2 / \text{s}$  in the attacked plants and towards the evening (5 p.m) the gradual decrease of the stomatal conductance when one can record values of  $0.16 \mu\text{mol} / \text{m}^2 / \text{s}$  in the healthy plants and  $0.18 \mu\text{mol} / \text{m}^2 / \text{s}$  in the attacked plants.

Linear regression shows a positive correlation between the photosynthesis rate and of the stomatal conductance (the coefficient of determination  $R^2$  was 0.90 for the healthy plants and 0.81 for the attacked plants) and between the transpiration rate and of the stomatal conductance (the coefficient of determination ( $R^2$ ) was 0.85 for the healthy plants and 0.81 for the attacked plants) - Figure 11 and Figure 12.

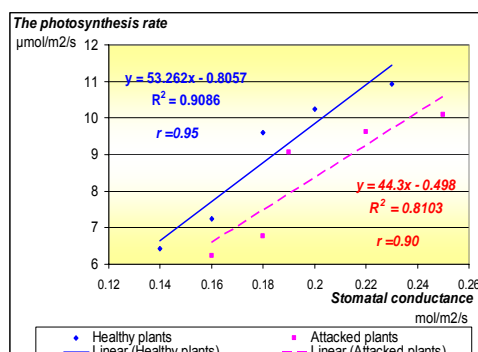


Figure 11. The correlation between the photosynthesis rate and the conductance stomatal at the *Rosa canina*.

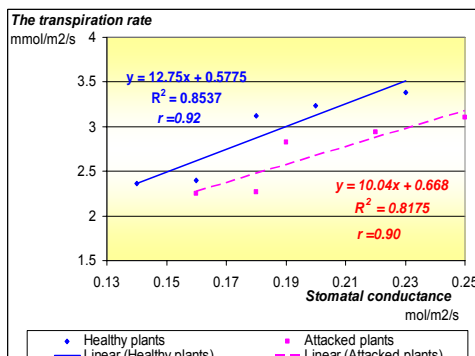


Figure 12. The correlation between the transpiration rate and the conductance stomatal at the *Rosa canina*.

At the attacked plants there can be seen a decrease of the total water content by 1.78 % and an increase of the dry substance content by 3.82 %, as a result of the action of the pathogen and malfunctioning of stomata mechanisms, which is manifested by the decrease of the cellular turgor, the withering and premature drying of the plants (Figure 13).

The plants attacked present a decrease of the chlorophyll content by 14.05 % as a result of the blockage of its biosynthesis and the deterioration of the chlorophyll (Figure 14).

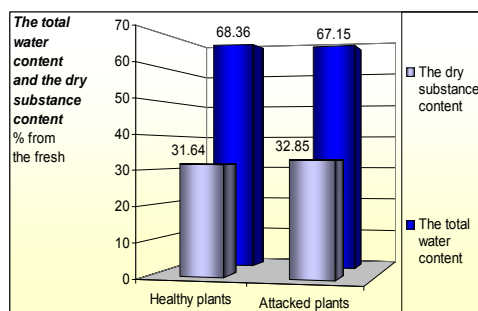


Figure 13. The water content and the dry substance content at the *Rosa canina*.

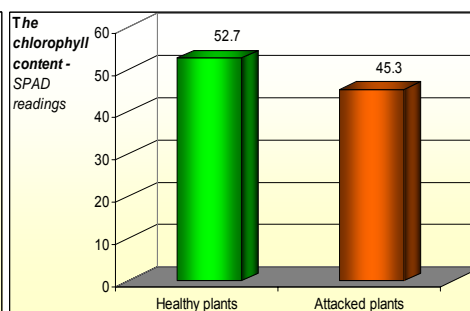


Figure 14. The chlorophyll content at the *Rosa canina*.

## CONCLUSIONS

At the attacked plants one can observe that intensity of the photosynthesis and of transpiration presents a minimum in the morning, a maximum after lunch and a minimum toward the evening, but the recorded values are small in comparison with healthy plants. The increase of the photosynthesis and transpiration is positive correlate with the photosynthetic active radiations, temperature leaf and stomatal conductance, but shows variations in the attacked plants as a result of structural changes produced by pathogen.

In the attacked plants one can observe a decrease of the chlorophyll content and the decrease of the total water content, which determines several hidric and metabolic unbalances, with implications on the growth and development of plants.

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STUDY ON THE DYNAMICS OF LACTIC ACID FERMENTATION IN FRUIT  
AND VEGETABLE JUICE COCKTAILS REALIZED BY *LACTOBACILLUS*  
*ACIDOPHILUS*

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**KEY WORDS:** lactic acid fermentation, vegetable and fruit cocktail, *Lactobacillus acidophilus*, probiotic biomass accumulation, dynamics trend

ABSTRACT

*The lactic acid fermentation occurs in vegetable/fruit cocktail juices (C1 – carrots : apple [3:1] and C2 – carrots : celery : apple (6:1:2)) using Lactobacillus acidophilus strains revealed by the sugar consumption and lactic acid accumulation, accomplished by a low bacterial biomass increase. Its dynamics was analyzed following the evolution of pH, reducing sugar content, lactic acid accumulation and lactic acid bacteria number. Starting with differences in the physico-chemical parameters and even in the amount of bacteria in the inoculum, in the two analysed batches C1 and C2, the fermentation process presented differences all over its development. The lactic acid synthesis and bacteria growth were similar in both cocktails, but sugar concentration was quasi 2-fold higher in C1 and its consumption was different. The evolution trends of almost all parameters were best described by a polynomial equation. Only the pH had a linear development.*

INTRODUCTION

During the closing decades of the 20th century people became more and more interested in food, its composition and role in preservation of human health (Rakin, 2005). The vegetable juices processed by lactic acid fermentation introduce a change in the beverage assortment for their high nutritive value and high contents of vitamins and mineral compounds. For the juices processed by lactic acid fermentation, the content of lactic acid is important from the nutritive point of view. This acid shows disinfecting effects that are caused mainly by its acidity (Károvičová and Kohajdová, 2003).

Lactic acid bacteria are industrially important microbes that are used all over the world in a large variety of industrial food fermentations (Panda, 2007). Their contribution in these fermentation processes consists primarily on the formation of lactic acid from the available carbon source resulting in a rapid acidification of the unprocessed food material, which is a critical parameter in the preservation of these products (Kohajdová and Károvičová, 2004). *Lactobacillus acidophilus* LAB, isolated from the human feces or intestine, is thought to have beneficial effects on health and particularly useful probiotic bacteria like the *Bifidobacterium* group of bacteria (Saito, 2004).

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In most of the cases such commercial cultures do not correspond to autochthonous strains. To get desirable properties of fermented vegetable juices, lactic acid bacteria has to be adapted to the intrinsic characteristics of the raw materials (Di Cagno *et al.*, 2009).

Adding probiotics to juices is more complex than formulating in dairy products where the bacteria can be easily added to other cultures (Kun *et al.* 2008). The standard for some foods with healthy claims for the probiotics addition is a minimum content by  $10^6 - 10^7$  c.f.u. alive probiotic bacteria/g.

The aim of this study is to show (1) if a lactic acid fermentation occurs in vegetable/fruit cocktail juices using *Lactobacillus acidophilus* strains, (2) to analyze the dynamics of the lactic acid fermentation following the evolution of pH, reducing sugar consumption, lactic acid accumulation and bacterial biomass growth and to point out trends in the evolution of these parameters, and (3) to establish the differences existing between cocktails with different substrate composition in their physico-chemical and microbiological parameters during the lactic acid fermentation.

## MATERIALS AND METHODS

**Fermentation substrat.** Fresh carrots, celery and Golden cultivar apples from Austria were bought from the local free vegetable market of the Dambovită County (Romania). Their processing included washing, scrubbing and removing non-edible pieces and then transforming into juices with a domestic juice maker. There were made 2 cocktails: C1 – carrot : apple [3:1] and C2 – carrot : celery : apple (6:1:2). The cocktails were thermal treated at 80°C/10min in order to remove the epiphytic microbiota and than cooled to 40°C.

**Microorganisms inoculum.** An inoculum of Christian Hansen lyophilized pure culture of *Lactobacillus acidophilus* LA-5 strain was used.

**Process performing.** 0.2 g/l lyophilized pure culture was aseptically added in the cocktail juice and vigorous homogenized. 100ml juice, from each experimental batch, was distributed in sterile tubes, covered by cotton stopper and metal folia. Each tube represented a single sample and the experiments were performed in double. The lactic acid fermentation was performed in a thermostat at 37°C during 48h. The anaerobiosis was assured by the metal folia that covered each tube. The samples were investigated during the lactic acid fermentation through chemical and microbiological analysis at 2, 4, 6, 8, 24, and 48 h.

**Assay.** The pH was measured with a HACH pH-meter. Reducing sugars were analyzed using a spectrophotometrical method with 3,5-dinitrosalicylic acid (DNS) after defecation with basic lead acetate. The results were expressed as g glucose/100ml. Titrable acidity, expressed as g lactic acid/100ml, was determined by titration with NaOH 0.1N in presence of phenolphthalein. The microorganisms were counted on MRS plates after incubation at 37°C/48h, and expressed as (log c.f.u./ml).

**Data processing.** The data were processed using the Microsoft Excel computer application. In order to evaluate the dynamics of the lactic acid fermentation process at first there were performed column plots to point out the differences of the analysed parameters between the experimental batches and these differences were recorded. Next, the increasing/decreasing rate of each parameter during the 48 hours of fermentation was calculated. Finally, time depending line plots were used to point out the evolution of each analysed parameter during the investigation period. Best trends of each parameter evolution were pointed out according the highest R squared coefficient.

## RESULTS AND DISCUSSION

In preparing the two batches of vegetable cocktail juice there was kept a constant proportion of 3:1 between carrot and apple. C2 was supplemented with 1 part celery. These proportions were chosen in accordance with the best results of a preliminary sensory analyse.

The initial parameters of the two cocktail juices are presented in Table 1. The differences counted at the beginning between the two juice batches were large (Table 2). The acidity is given most by organic acids like malic acid but the acidity was expressed in terms of lactic acid content by conversion. The sugar content of the substrate was lowered by the celery addition with 1.97 g/100ml. Celery buffered also the higher apple acidity so that the pH increased with 0.17 units and the titrable acidity was lower with 0.018 g/100ml. Even if both batches were inoculated with the same amount of lactic acid bacteria (0.2g/l) the difference between them was relative high ( $27.95 \times 10^{-11}$  c.f.u./ml), maybe because of a different dispersion of the microorganisms that can adhere on the particulate mater from the juice. This fact recommends the using of a homogenised liquid pre-culture of microorganisms as inoculum.

Differences were kept all over the fermentation time. The cause of these differences can be the different substrate composition and different amounts of bacteria in the inoculum. The highest difference was observed in the sugar content.

Table 1

Initial parameters of the cocktail juices

Lactic acid fermentation parameters	Experimental batch	
	C1	C2
pH	5.03	5.2
Reducing sugar content of the substrate [g glucose/100ml]	5.57	3.6
Titrate acidity [g lactic acid/ 100ml]	0.198	0.18
Number of <i>Lactobacillus acidophilus</i> inoculated [log c.f.u./ml]	12.45	10,16

Table 2

The differences between the physico-chemical and microbiological parameters during the lactic acid fermentation of cocktail juices

Time [hours]	Difference C1-C2			
	pH [units]	glucose [g/100 ml]	lactic acid [g/100 ml]	Number <i>L. acidophilus</i> [nr. $\times 10^{-11}$ ]
0	-0.17	1.97	0.018	27.95
2	-0.14	2	0.03	-47.4
4	-0.08	2.7	-0.04	20.3
6	-0.2	2.7	0.07	89
8	-0.18	2.57	0	40.4
24	-0.24	2.63	0.18	14
48	-0.13	2.76	0.069	-3.4

The comparative evolution of the physico-chemical and microbiological parameters is presented in Figure 1 A-D.

As expected, during the lactic acid fermentation process, the acidity related parameters were higher in the probe without celery. All physico-chemical parameters kept a relative constant evolution during the fermentation time, so that there were recorded even

relative constant differences between the two batches. pH decreased and acidity increased constantly during the 48 hours of fermentation, correlated with the constant diminution of the reducing sugar content from the substrate. The number of lactic acid bacteria had a not constant evolution. The fermentation started with a biomass difference that was kept all over the fermentation time. The batch C1, without celery, reached the maximum biomass accumulation after 6 hours of fermentation, while the probe C2, with celery, reached its maximum after 2 hours, and than, after a decrease in the next 6 hours, there started a new biomass accumulation that did not reached the amount counted at 6 hours.

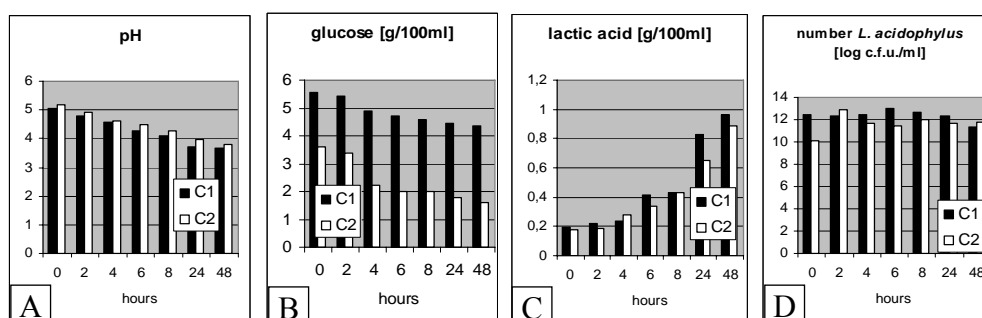


Figure 1. Comparative evolution of the physico-chemical and microbiological parameters during lactic acid fermentation of cocktail juices

Analyzing the increasing/decreasing rate of each parameter during the 48 hours of fermentation, there was observed that even if the initial substrate had different sugar content (the celery juice was lower), the lactic acid accumulation dynamics and pH decline were quite similar in the two analyzed cases. After 48h, a growth of acidity with 384.85% (in C1) and respectively 395.00% (in C2) and a decrease with 26.64% (in C1) and respectively 26.54% (in C2) of pH, in the conditions of a substrate consumption of 21.72% (in C1) and respectively 55.56% (in C2) were recorded.

The number of microorganisms increased by 38.89 times in C2, where the maximum was recorded after 2 hours of fermentation and even a decline at 48 hours of 92.17% in C1, where the maximum was recorded after 6 hours of fermentation. That means an increase with 1 order in C2 (from  $14.4 \times 10^9$  c.f.u./ml to  $56 \times 10^{10}$  c.f.u./ml) and a decline with 1 order in C1 (from  $28.1 \times 10^{11}$  c.f.u./ml to  $22 \times 10^{10}$  c.f.u./ml).

C2 started with lower sugar content in the substrate and a lower amount of bacteria in the inoculum but had a better accommodation rate of the microorganisms and fermentation rate in the first 4 hours. Celery seems to have a stimulating influence on the lactic acid bacteria. The growing of bacteria was sustained until 48 hours being accomplished with a low decrease of glucose and high accumulation of lactic acid.

The evolution trends of the physico-chemical and microbiological parameters of the lactic acid fermentation process in the cocktail juices was analysed on time depending line plots (Figure 2). Best trends of each parameter evolution were pointed out according the highest R squared coefficient ( $R^2$ ). Only the evolution of pH can be good described by a linear evolution, that gave  $R^2$  higher than 0.98 in both cases. The other physico-chemical parameters can be satisfactory described only by a polynomial equation. The linear trendline equation gave  $R^2$  between 0.83 – 0.92. The evolution of the bacteria from the inoculum is more disordered. In the case of C1 batch the  $R^2$  is at the lowest limit for a polynomial trend ( $R^2=0.95$ ) if a polynomial equation order 3 was used, but this in the case



of a total decrease of the microbial biomass of the inoculum. In the C2 batch, the  $R^2$  is not satisfactory for a polynomial trend ( $R^2=0.81$ ) even if a polynomial equation order 4 is used. In this case a low increase of the bacterial biomass was obtained.

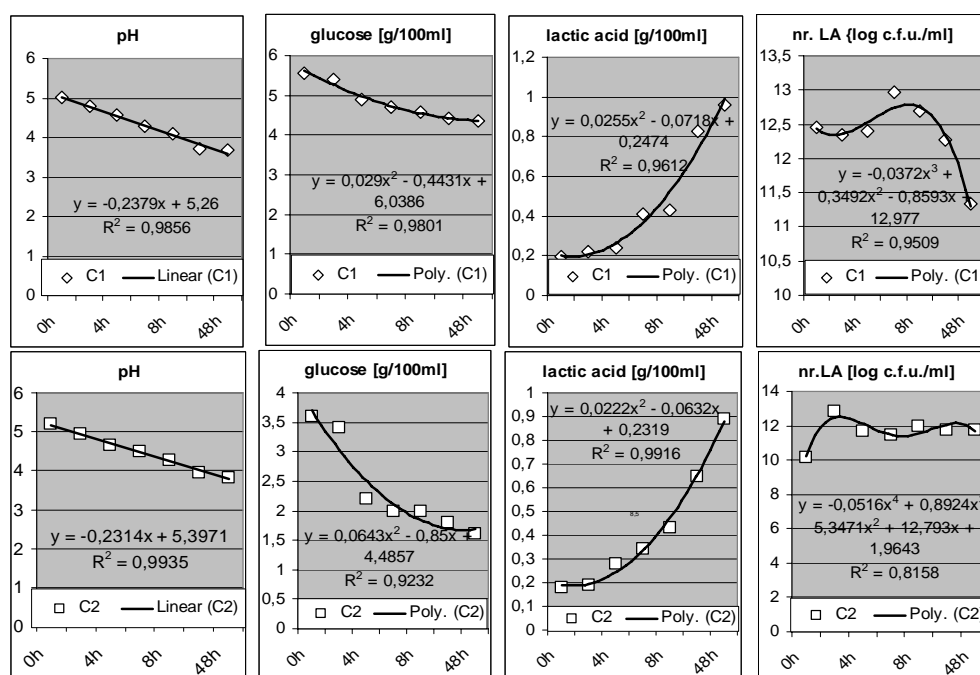


Figure 2. The evolution trends of the physico-chemical and microbiological parameters of the lactic acid fermentation process in the cocktail juices

Multivariate statistical techniques are useful to simplify a large and complicated data with many variables and observations and discover valuable information in such data by finding the structure and patterns of the data and creating new variables [Lee et al. 2009].

Many researchers studied the modelling of different fermentation processes. For example, in the batch alcoholic fermentation the input and desired output sets of data have been scaled (based on minimum and maximum values) and principal component analysis has been used for improving the computation precision and training performance [Cristea et al. 2009].

The interaction between variables is strong as the change of a single parameter may affect all the others [Houde et al. 2004]. In this condition, a good suggestion is to study the correlations between the analysed parameters to point out the influence of each of them on obtaining a good final soft drink with a good conservability, taste and probiotic potential.

## CONCLUSIONS

Using juice cocktails from vegetables and fruits an adequate nutritional substrate

was ensured for *Lactobacillus acidophilus*, so that they can perform a lactic acid fermentation process revealed by the sugar consumption and acidity accumulation, accomplished by a low bacterial biomass increase.

Starting with differences in the physico-chemical and even in the amount of bacteria in the inoculum, in the two analysed batches C1 and C2, the fermentation process presented differences all over its development. The lactic acid synthesis and bacteria growth were similar in both cocktails, but sugar concentration was quasi 2-fold higher in C1 and its consumption was different.

The evolution trends of almost all parameters were best described by a polynomial equation that revealed the implication of many causes in its development. Only the pH had a linear development that sustains the presence of an acidification process.

**Acknowledgments.** The researches were performed in the frame of the Project PN-II-ID-PCE-2008-2 (ID\_1359) Researches concerning the preparation of lactofermented juices with specific probiotic and evaluation through methods specific for intelligent modelation.

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THE DISTRIBUTION OF THE ESTROGEN RECEPTORS IN THE  
HYPERPLASTIC HUMAN ENDOMETRIUM

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**KEY WORDS:** *endometrium, estrogen receptors, immunohistochemistry*

**ABSTRACT**

*Adenocarcinomas of the endometrium are classified into two pathogenetic categories: type I which has hormone-related risk factors, is preceded by estrogen – induced hyperplasia; type II which usually has a background of endometrial atrophy and it is resistant to hormone therapy. Overexpression of the estrogen receptors have been considered to play an important role in tumorigenesis. Estrogen receptor expression is more frequent in type I tumors. The purpose of this study was to evaluate the level of the estrogen receptors in the hyperplastic endometrium and to correlate it with the histopathologic degree of the lesions.*

**INTRODUCTION**

The functioning of the endometrium is hormonally regulated, especially by the estrogen hormones and by the progesterone, which the endometrium concentrates from plasma against a concentration gradient. Usually, the estrogens produce a synchronic increase of the glands, the stroma and the vascularisation of the endometrium and induce the apparition of the progesterone receptors. The excessive estrogenic stimulation, associated with the diminution or lack of progesterone secretion determines an exaggerated and benign proliferation called hyperplasia, which can be an isolated step or can evolve towards an adenocarcinoma.

The acting mechanism of the steroid hormones and the specific answer of a cell to the specific hormonal stimulus are due initially to a macromolecule called receptor which can be found on the cellular level. The steroid hormones have the capacity to passively trans-membraneously enter the target-cell, where they attach to a specific cytoplasmatic receptor which ensures the transport of the steroid on the nuclear level.

On the nuclear level, a specific receptor is activated and thus the steroid-receptor complex acts on the genetic transcription by triggering the synthesis mechanism of proteins specific for every cell (Mylonas et al., 2007).

It has been proven that the so-called “target” organs contain a much larger concentration of steroid receptors compared to other tissues. The target organs (the internal and external genital organs, the hypothalamus, the hypofise, the breast) contain specific

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receptors for every type of steroid: estrogens, progesterone and androgens. On the endometrium, the receptors can be found in much larger quantities than in the myometrium, but their presence has also been signalled in the excretory apparatus (especially in the trigone of bladder and the urethra), the skin and the annexes and in the bones (McCluggage, 2002).

Different areas of the endometrium answer differently to the hormonal stimulation. The ligands, i.e. the estrogens and the progesterone, induce, through the specific receptors, the effects on the various cellular constituents of the endometrium. The hormonal receptors are much more numerous in the endometrium of the fundus of the uterus than in that of the isthmus and better represented in the glands than in the stromal cells (Soslow and Isacson, 2002).

## **MATERIAL AND METHODS**

The material used in the study was represented by 27 endometrial biopsy specimens with a diagnosis of endometrial hyperplasia. The lesions were classified according to the World Health Organization (WHO) classification system of endometrial hyperplasia: simple hyperplasia (20 cases); complex hyperplasia (5 cases) and complex atypical hyperplasia (2 cases). We assessed the all cases with immunohistochemical staining for estrogen receptors. All specimens were fixed in formalin, embedded in paraffin and cut in 3-4- $\mu$ m-thick sections. Endogenous peroxide activity was blocked with 3% H<sub>2</sub>O<sub>2</sub> in distilled water for 10 minutes. A microwave oven was used for antigen retrieval at a frequency of 2450 MHz at level III (800 W) for 5 minutes and at level II (560 W) for 20 minutes.

The section were the washed with distilled water and incubated with monoclonal mouse antiestrogen protein (Dako, ID<sub>5</sub>, code: M 7047, Denmark) at room temperature and 1/100 dilution using the streptavidin-biotin-peroxidase method. Diaminobenzidine was used as chromogen and slides were counterstained with Mayer hematoxylin. For a negative control, normal saline was added in place of the primar antibody.

ER was regarded as positive when staining was more than 10%. All degrees of staining intensity were considered positive.

IP (positivity index) was defined as percentage of cells showing positive nuclear staining per total of 500 counted cells. We examined at least 500 cell nuclei in at least five fields under a grid at 400x magnification (10x ocular and 40x objective).

The hormonal receptors for the estrogen connect the hormones which have effects on the nuclear level. They can be found in the nucleus as well as in the cytoplasm, but have a predominantly nuclear immunomarking. The immunocoloring for both types of proteins is usually present in the mammary acini, which serve as positive control. The nuclear immunocoloring in the normal mammary tissue is heterogeneous and alternates with the phases of the menstrual cycle. The positive reactions to ER outside of the mammary gland also appear in the endometrium, in the endocervical and exocervical epithelium, in the vaginal epithelium, as well as in certain hepatic and thyroidal carcinoma.

## **RESULTS AND DISCUSSIONS**

By using the monoclonal antibodies, receptors for the estrogens have been detected in the epithelial and stromal cells of the endometrium, thus observing that during the menstrual cycle, in the proliferative stage, the majority of the cells have a positive nuclear marking. The receptors for the estrogen (ER) have an extensive connective ability

in the proliferative stage, following which their decrease is noticed, partly as a consequence of the nuclear translocation, partly because of the inhibitory action of the progesterone.

The nuclear marking of the receptors for estrogen diminishes during the secretion stage, the interesting fact being that some epithelial cells and superficial stromal cells, as well as the epithelial basic ones of the glands remain intensely positive. The post-menopause endometrium also has a strong marking of the epithelial and stromal cells. The smooth cells of the myometrium contain receptors for estrogens, which cannot be found in the endothelial cells of the uterine vessels (Mylonas *et al.*, 2005).

For the immunohistochemical study we have used antibodies for the estrogenic, in order to identify their involvement in the mechanism of carcinogenesis, in the determining of the biological behaviour and in the selection of the possible prognostic factors.

The 27 cases of hyperplasia studied, immunohistochemically investigated, have been divided as follows (Table 1).

Table 1

The repartition of the cases

Histologic type	Simple hyperplasia	Complex hyperplasia	Complex atypical hyperplasia
No. of cases	20	5	2
Total	27		

The presence of the estrogenic receptors in the endometrial epithelium, has been proven by the positive black nuclear immunomarking.

All the hyperplastic lesions, no matter their histological type, had estrogenic receptors.

In literature, in the studies made on a large number of cases, the positivity of the estrogenic receptors of the hyperplastic lesions is described, in 86-96% of the cases on the epithelial level and in 100% of the cases on the stromal level (Bircan *et al.*, 2005 ; Lysenko *et al.*, 2004).

For every type of lesion we have calculated the positivity index and we have established an average value for it (Table 2).

Table 2

Estrogen receptors positivity

Histologic type	Simple hyperplasia	Complex hyperplasia	Complex atypical hyperplasia
IP-ER	41%	73%	55%

The analysis of the ER expression for the various types of endometrial hyperplasia has shown that the complex hyperplasia without atypia had the highest level of estrogen receptors, being followed by the complex atypical hyperplasia and then by the simple one without atypia.

In the simple hyperplasia without atypia we have found the lowest values in the group of the hyperplastic lesions, the average value of the IP-ER for the 20 cases of simple hyperplasia studied being of 41% (Figure1, 2).

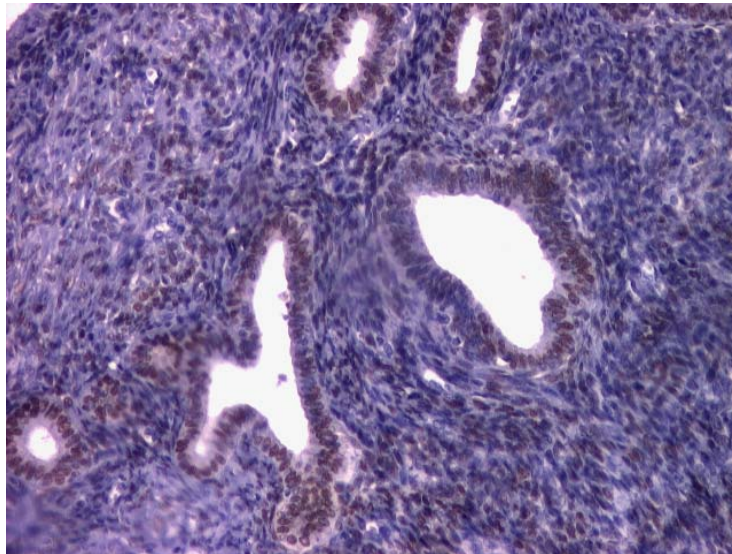


Figure 1. Immunohistochemical staining of ER in simple hyperplasia, X 100

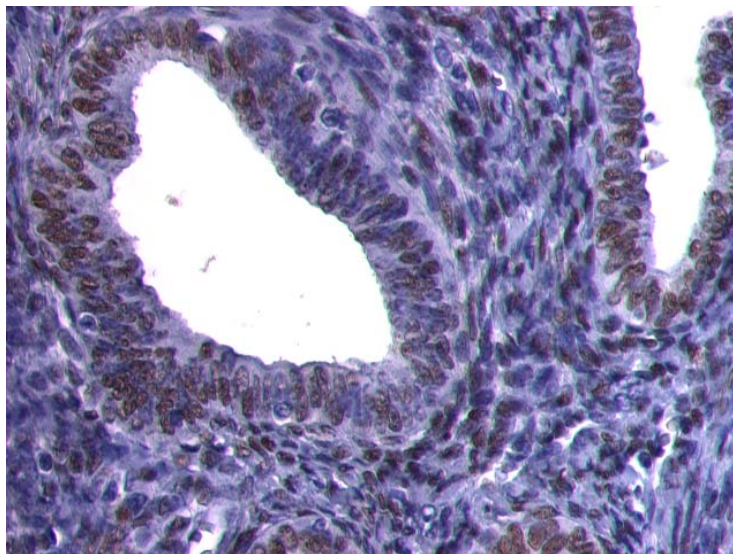


Figure 2. Immunohistochemical staining of ER in simple hyperplasia, X 200

For the complex hyperplasia without atypia, the average value of IP-ER has been the highest of the group of hyperplasia as well as compared with all the hyperplastic lesions analysed. The average value of the IP-ER for the 5 cases of complex atypical hyperplasia has been of 73% (Figure 3, 4).

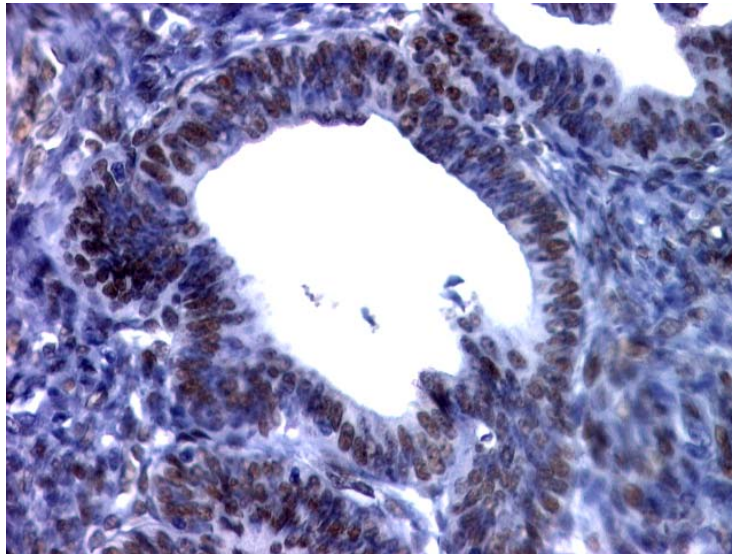


Figure 3. Immunohistochemical staining of ER in complex hyperplasia, X 100

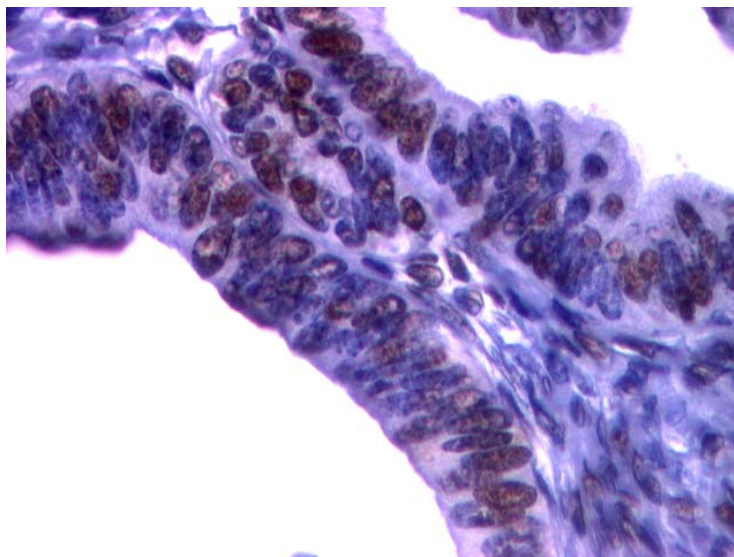


Figure 4. Immunohistochemical staining of ER in complex hyperplasia, X 200

The complex atypical hyperplasia had an average positivity index of 55%, as compared with the complex hyperplasia without atypia.

The literature studies, which have followed the values of the IP-ER comparatively in the normal and hyperplastic endometrium, have noted that the ER expression is lower in the endometrial hyperplasia as compared with the proliferative endometrium (Mitselou et al., 2003 ; Lysenko et al., 2004).



The presence of the cytological atypia makes the hyperplastic lesions be little hormonal-sensitive or not at all. In practice, this translates by resistance to the progestational treatment or by a tendency to recurrence. It is considered that the hyperplastic epithelium with cytological atypia is different from the simple hyperplastic one and is accompanied by genetic changes close to those found in carcinomas (Veral et al., 2002).

## CONCLUSIONS

The estrogen receptors were present in all varieties of endometrial hyperplasia, but their expression was lower as compared with the proliferative endometrium.

The analysis of the ER expression for the various types of endometrial hyperplasia has shown that the complex hyperplasia without atypia had the highest level of estrogen receptors, being followed by the complex hyperplasia with atypia and then by the simple one without atypia.

Immunohistochemistry is a simple and useful method to detect estrogen receptors overexpression in surgical pathology specimens.

The evaluation of estrogen receptor overexpression in endometrial lesions might be useful as a prognostic indicator.

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THE IMPLICATIONS OF THE OBESITY AND FAT DISTRIBUTION  
IN ENDOMETRIAL TUMORIGENESIS

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**KEY WORDS:** *obesity, fat distribution, menopause, endometrial cancer*

ABSTRACT

*Many changes occur in body composition during the mid-life years. There is an increase in weight and a change in fat distribution. In this study we try to estimate the body fat distribution in women with histopathologic diagnosis of endometrial hyperplasia and endometrial cancer.*

INTRODUCTION

Endometrial cancer ranks as the fourth most frequent cancer, among women living in Europe (Bergstrom *et al.*, 2001 ; Bray *et al.*, 2005).

Epidemiological studies have shown that  $\geq 40\%$  of its incidence can be attributed to excess body weight (Information Resources Centre, UK, 2008).

Obesity is an established risk factor for endometrial cancer but the evidence linking risk to height and weight change since age 20 is limited.

Excess weight has metabolic and hormonal consequences. The main theory describing the relationship between endogenous steroid hormones and endometrial cancer risk is known as the unopposed estrogen hypothesis.

The principal mechanism by which hormones are thought to influence cancer risk are their regulatory effects on the balance among cell proliferation, differentiation and apoptosis (Calle and Kaaks, 2004).

Alterations in endogenous hormone metabolism may provide the main links between endometrial cancer risk and excess body weight (Akhmedkhanov *et al.*, 2001).

Previous research has proposed that the distribution of body fat has implications for hormonal profiles and subsequent endometrial cancer risk. The fat located primarily on the upper body (chest, stomach) is named android obesity and it has been associated with increased estradiol and testosterone levels compared with body fat distributed mainly on the lower body (hips, thighs, buttocks). (Weiderpass *et al.*, 2000) This type of fat distribution is named gynoid obesity.

The waist-to-hip ration (WHR) is commonly used measure of the relative degree of upper body weight. Other methods use the waist circumference skin-fold measures, dual

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energy X-ray absorptiometry (DEXA) measures of truncular fat and computed tomography of infra-abdominal adipose tissue (Schouten *et al.*, 2004).

## MATERIAL AND METHODS

The study includes 137 obese women with a histopathologic diagnosis of endometrial hyperplasia and 57 obese women with a diagnosis of endometrial cancer.

Body fat distribution can be estimated using a number of different methods. In this study we used the most common method, which measures an anthropometric index represented by waist-to-hip ration (WHR).

Waist represent the shortest circumference between ribs and iliac crest. Hip represent the longest circumference between iliac crest and thigh. A ratio over 0,85 is correlated to the android obesity and a value under 0,75 is correlated to the ginoid obesity.

## RESULTS AND DISCUSSIONS

The analysis of the fat distribution in obese women with endometrial hyperplasia has shown that android obesity was present in 89 cases (64,96%) and ginoid obesity in 48 cases (35,04%) (Table 1; Figure 1, 2).

Table 1

The repartition of the obese women with endometrial hyperplasia conform to the fat distribution

Fat distribution	Android obesity (abdominal)	Ginoid obesity (gluteal-femoral)	Total
No. of cases	89	48	137
Percentage %	64,96%	35,04%	100%

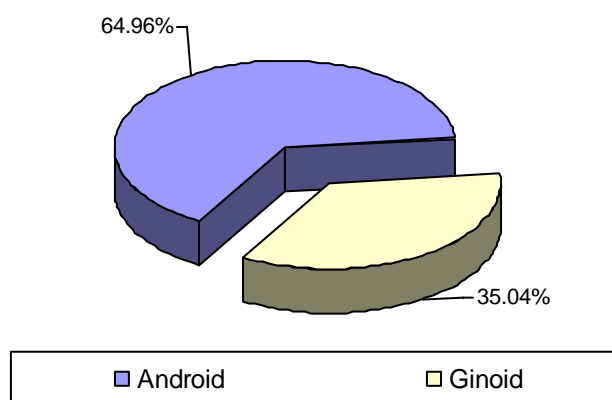


Figure 1. The repartition of the obese women with endometrial hyperplasia conform to the fat distribution



Figure 2. Android obesity (adipose accumulation toward the abdominal area)

The analysis of the fat distribution in obese women with endometrial carcinoma has shown that android obesity was present in 40 cases (70,18%) and ginoid obesity in 17 cases (29,82%) (Table 2; Figure 3).

Table 2

The repartition of the obese women with endometrial carcinoma conform to the fat distribution

Fat distribution	Android obesity (abdominal)	Ginoid obesity (gluteal-femoral)	Total
No. of cases	40	17	57
Percentage %	70,18%	29,82%	100%

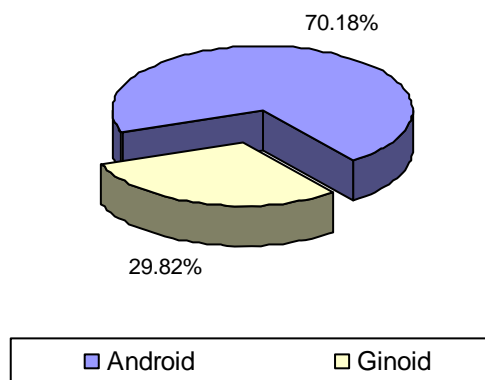


Figure 3. The repartition of the obese women with endometrial carcinoma conform to the fat distribution

The percentage of the cases with android obesity was higher in the group of patients with endometrial cancer as compared to the group of patients with endometrial hyperplasia.

The factors that influence the distribution of body fat are not entirely known.

Sex hormones are implicated in menopause associated changes in body fat distribution.

There are some possible biological explanations for this theory. Estrogens stimulate proliferation of the endometrium whereas progestagens stop cell division and stimulate cell differentiation (Guthrie and Dennerstein, 2001).

Serum levels of sex-hormone binding globulin (SHBG) are decreased in menopause and in increased body weight and the proportion of free estradiol increases. In consequences women with higher body mass index due to fat accumulation may have higher levels of estrogen than those who have less body fat (Trentham-Dietz *et al.*, 2006).

The changing levels of SHBG have an effect on free androgen levels.

In obese postmenopausal women, androgens are converted into estrogens in peripheral fat tissue (Kaaks *et al.*, 2002).

The changing levels of estrogens, free androgens, and SHBG during the menopausal transition may act directly on fat stores or they may modify expression of genes controlling adipocyte differentiation and metabolism. In normal ovulating women, basal lipoprotein lipase (LPL) activity is higher in subcutaneous femoral adipose tissue than in abdominal sites, and this femoral adipose tissue may serve the specialized function of providing a readily utilized fat store in the reproductive years. Femoral fat cells are larger than abdominal fat cells, a difference attributed to high femoral fat LPL activity. After menopause femoral adipocytes lose their higher LPL activity and abdominal LPL activity does not change. This could explain the adipose accumulation toward the abdominal area. Hormone replacement therapy has been reported in some studies to attenuate the menopause related acceleration of central fat accumulation and has also been shown to stimulate LPL activity in femoral region. This suggests that estrogen deficiency may directly influence regional adipose tissue metabolism (Guthrie and Dennerstein, 2001).

It has been shown that the fat distribution is a more important risk factor for endometrial carcinoma than obesity itself, the risk being from 2 to 15 times higher in women with android obesity (Schapira *et al.*, 1991).

Several studies investigated the correlations between the risk of endometrial cancer and height, weight gain since age 20 and loss weight. No relationship with height was found. Apparently, the weight gain since the lowest adult weight is strongly related to increased endometrial cancer risk (Jonsson *et al.*, 2003; Lacey *et al.*, 2007).

Women who reported sustained weight loss, defined as at least 5 years at a weight that is less than their highest weight since age 20 (excluding weight during pregnancy) had a reduced risk. The studies did not observe an association between magnitude of weight loss and endometrial cancer risk (Trentham-Dietz *et al.*, 2006).

Only a few studies have evaluated the risk associated with weight cycling (defined as losing and then regaining 20 pounds/9,072 kg at least 5 times). It seems that women with a history of weight cycling had an increased risk of endometrial cancer (nearly tripled the risk). (Trentham-Dietz *et al.*, 2006; Weiderpass *et al.*, 2000).

The lifestyle interventions maintained over a longer period could be effective in preventing weight gain and reducing waist circumference during a high-risk period (Furberg and Thune, 2003).

## CONCLUSION

The majority of the obese patients with endometrial hyperplasia and endometrial carcinoma have android obesity.

The increase in weight and the change in fat distribution are correlated with the risk of endometrial carcinoma. Studies that examined WHR have reported that women with upper body fat have a 2-15-fold increase in endometrial cancer risk.

Sex hormones are implicated in changes in body fat distribution. The weight gain has an effects on SHGB, estradiol and free androgen levels.

In obese postmenopausal women, androgens are converted into estrogens in peripheral fat tissue.

The reduction in lipoprotein lipase activity in femoral adipose tissue may lead to the increased accumulation of intra-abdominal adipose tissue.

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ASPECTS CONCERNING THE INCIDENCE OF PATHOGENS IN THE SLUDGE  
RESULTED FROM WASTEWATER TREATMENT

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**KEY WORDS:** *pathogen, organic sludge, temperature, coliform bacteria*

**ABSTRACT**

*The premises of this work are mainly assigned for the possibility of revaluation of organic sludge from biological wastewater treatment plants in the agricultural network. The main problem is represented by the microbiological aspects of these new types of fertilisers, which may constitute a danger to human and animal health.*

*This paper advocates for ensuring hygienico-sanitary activated sludge resulted from the treatment plant in town "P" through pre-treatment steps (I, II) inside the bioreactors arrangement of stations, representing a preamble of a complex, large study of the microbiological state of the sludges, for a reconsideration of their assets and their reintroduction in agricultural strategies. This paper aims to highlight the results in stabilization / disinfection of municipal sludge in order to achieve quality conditions that allow their use in agriculture field.*

**INTRODUCTION**

As organic waste sludge from municipal sewage stations contain a number of species of pathogenic bacteria: *Salmonella*, *Listeria*, *Escherichia coli*, *Campylobacter*, *Mycobacteria*, *Clostridia*, *Yersinia* (Bitton, G., 2000; Cadiergues, B., 2002)) it must be proven the hygienic safety of both human and animal digested waste for reuse. Most of them cause zoonoses, being a real danger to human and animal health. Some bacteria show high resistance and can also expand even in the anaerobic sludge digestion tanks.

The Directive 86/278/EEC and the MAPAR Order 344/2004 limit the application of treated organic sludge to the land through the control of heavy metals and priority hazardous substances, without taking into account the load of pathogenic bacteria. U.S. law regarding the use of these treated sludge in agriculture, the EPA Part 503 "Biosolids Rule", controls the content in faecal coliform, indicators of the degree of pathogenicity to  $2 \times 10^6$  CFU / for type B crops (which are not consumed fresh, uncanned) and to 0 CFU / g n.s.u for type A crops (eg. vegetable crops, etc.).

The rate of inactivation of pathogens depends on several factors, temperature being the most important factor in reducing pathogens, but other factors may be involved: time retention, pH, the driving process of fermentation, bacterial species existing in the substrate, their number, available nutrients (Farrah and Bitton, 1983, Kearney et al., 1993.)

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The inactivation of pathogens is also dependent on the initial amount of the pathogens in the biological waste (Strauch,1991).

Since the majority of pathogens is in low concentrations and difficult to detect, faecal streptococci bacteria are used as indicators (*Enterococcus* type). Faecal coliforms are indicators of the existence of common pathogens, such as *Salmonella*, *Listeria*, *Campylobacter* and *Yersinia* (Kearney et al, 1993). Total and faecal coliforms are also used as indicators of the presence of pathogens. „EPA Part 503 Biosolids Rule” only controls the faecal coliforms.

## MATERIAL AND METHODS

The experiments conducted on the active sludge resulted from the town treatment plant "P", were made by steps of treatment (I, II), respecting the regime bioreactors of the station. The microbiological analysis consisted in pointing the indicators regarding the total Number of Bacteria Coliforms /100ml, Number of Faecal Bacteria Coliforms/100ml coliform bacteria (CFU / g n.s.u) and *Escherichia coli*/100ml according to STAS 3001/91 and were evaluated in terms of quality according to STAS 4706/88.

The activated sludge was taken into study in the following alternatives: thermal thresholds of 32°C, 50°C, 70°C in order to establish favorable temperatures to reduce the number of total and faecal coliforms; the duration of monitoring: september 2009 - may 2010. The determination of the number of total coliforms/cm<sup>3</sup>, and of the number of faecal coliforms/cm<sup>3</sup> (mesophilic microorganisms that grow at 35°) was analyzed using STAS 3001/91 through the sowing method on culture media: Levine medium (peptone 10g/liter, lactose 10 g / l iter, di-potassium-hydrogen-phosphate 2 g/liter yellow eosine, 0.4 liter V, methylene blue 0.065 g/liter, agar-agar 13.5 g/liter and is obtained by dissolving these ingredients to warm slightly stirring then autoclaving (15 min. at 121°C).

The analysis of coliforms (**colimetry**) may be a good indicator of hygienic quality, but the determination of the total number of Enterobacteriaceae shows a greater interest. The Enterobacteriaceae and the *Escherichia coli* have termoresistance as the unsporulated pathogens.

Their analysis thus allows the assessment of the effectiveness of heat treatments used during the manufacturing process. *Escherichia coli* is considered a good indicator of fecal contamination (and of assuming the presence of these pathogenic bacteria) for non-thermal products, in some cases being considered a general indicator of hygiene (General Directives for establishing the presumptive number of *Escherichia coli* - the most probable number technique -SR ISO 7251/1996)

## RESULTS

The evaluation of pathogenic potential by determining the titre of mesophilic aerobic bacteria, total coliform and fecal coliform bacterial showed oscillating loads during the study (autumn, winter, spring), according to the data presented in Table 1.

Experiences followed the pathogen reduction efficiencies at temperatures of 50°C and 70°C and retention time of 2 - 12 days, noticing that the thermal thresholds of 50°C and 70°C inhibited the development of the mesophilic aerobic bacteria after only two days. The results of microbiological load are represented in Figure 1.

The results come in favor of establishing the total coliforms and faecal group as normal quality indicators of biologically active EPA sludge obtained from wastewater treatment plants, proving that thermal thresholds of 50°C and 70°C have a good efficiency



in reducing these potentially pathogenic germs.

Table 1

Potentially pathogenic bacterial load dynamics into the biologically active sludge

Analyzed sample	Experimental period	The analyzed bacterial indicators		
		No.Total.Colif. Bact./100ml	No.Colif. Bact./100ml	<i>Escherichia coli</i>
Biological sludge Step I	Sept. 2009	$2,4 \times 10^7$	$7,9 \times 10^6$	++++
	Oct. 2009	$4,4 \times 10^8$	$3,4 \times 10^7$	++++
	Nov. 2009	$3,5 \times 10^8$	$2,4 \times 10^7$	++++
	Ian. 2010	$3,7 \times 10^7$	$2,9 \times 10^6$	++++
	Mart. 2010	$4,4 \times 10^7$	$3,5 \times 10^6$	++++
	May 2010	$5,4 \times 10^8$	$4,4 \times 10^7$	++++
Biological sludge Step II	Sept. 2009	$1,8 \times 10^7$	$1,4 \times 10^6$	+++
	Oct. 2009	$2,7 \times 10^7$	$2,1 \times 10^6$	+++
	Nov. 2009	$2,5 \times 10^7$	$2,1 \times 10^6$	++
	Ian.2010	$2,2 \times 10^7$	$1,9 \times 10^6$	++
	Mart. 2010	$3,7 \times 10^7$	$2,6 \times 10^6$	++
	May 2010	$4,4 \times 10^7$	$3,1 \times 10^6$	+++

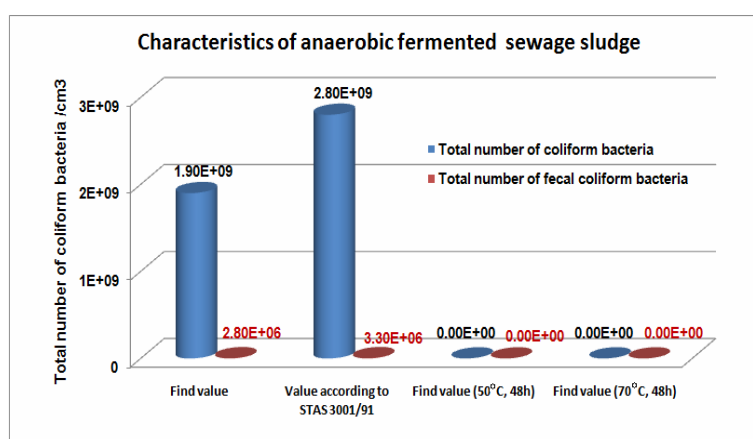


Figure 1. Quantitative estimation of the pathogens in heat stabilized biological sludge.

## CONCLUSIONS

Following the study on fermented sludge resulted from the town treatment plant P, we may draw the following conclusions:

- residual organic sludge from municipal treatment plants contain a number of pathogenic bacteria; some bacteria show high resistance and even develop in the anaerobic digestors and may cause zoonoses, being a real danger to human and animal health;
- microbiological control of the biologically active sludge is mandatory for ensuring the quality of the sludge as a future fertilizing source;

- the number of mezophilic and psychrophilic germs from urban sludge is close in value, only the presence of thermophilic germs' being higher due to the fact that in the urban sludge there is a high microbiological activity caused by the high content of organic matter and the highly pathogenic species of *Escherichia coli* not allowed in the sludge that is applied to the soil is absent;

- having in view the current global crisis, the poor sources of raw materials and energy, the growing price of fertilizers, the principles of sustainable agriculture, it could be considered that the municipal sludge can represent a real fertilizing resources in agriculture, complying with the active legislation and prevalent with the microbiological standards.

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TECHNICAL ASPECTS CONCERNING THE MODERNIZATION OF RADNA-  
VALEA MURESULUI RAILWAY LINE STRETCH

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**KEY WORDS:** railway line, geometrical constraints, curve radius

**ABSTRACT**

*Scope of the study is to improve the existing technical conditions of the railway line stretch Radna-Valea Muresului (38 km length) in order to get the quality of Romanian railway network and the traffic closer to western European standards. Currently, line minimum curve shows radius of 350/400 m, while line speed of 160 km/h requires a minimum radius of 1500 m. Thus there were analyzed all actual and natural geometrical constraints in order to propose the best work improvement combination of railway infrastructure. Particular attention was given to the horizontal curve radius in order to propose their increasing were possible, limiting as much as possible impacts on the existing urban settlements and roads or installation, and limiting stations removals or adaptation. Studied solutions envisage high changes compliant with minimum radius of 800 m (corresponding to 120 km/h speed).*

**INTRODUCTION**

The railway line Radna-Valea Muresului is component of Corridor IV on Romanian territory, respectively railway line Hungarian Border-Curtici-Arad-Sighisoara-Brasov-Bucuresti-Constanța. From historical point of view, the railway line was first built as single line, stretch by stretch, depending on the economic and political interests in the transport field of the Hungarian Government (in the period of this railway execution, this part of Romanian's territory was under Austro - Hungarian Empire) (Belc, 2004)

Thus, Mureș Valley offered proper conditions for transportation on railway to get into the centre of Transylvania. The railway literature registered the fact that design and line construction were realised complying with the German and Austrian technical norms. From morphological point of view, this section is characterised by a mountainous relief, on the right side being placed the Metaliferi and the Zarandului Mountains, and on the left side (south) Poiana Ruscă Mountains. Running along a corridor of 150 -200 m high, these mountains are separated by the Mureș River, following the East-West direction. Mureș corridor has some lateral formations giving it an aspect similar to some depressions. Mureș corridor low hydraulic energy has generated a lot of meanders and marshy areas. Mureș River, that is the collector of the hydro-graphic basin in this area, is collecting the some affluent rivers from the North side (on the right side), which generally have small rates of flows.

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The geologic structure of the Mureş corridor is characterised by meadow and low terrace areas. The fluvial fine deposits (clayey, silt deposits) at their upper parts are followed then by sands in the depth (superior Holocene) gradually passing towards gravel and cobbles (superior Pleistocene), having almost a horizontal position, inclined towards the Mureş river bed.

### MATERIAL AND METHODS

From Radna (km 597+000) to Valea Mureşului (km 559+00), on a length of 38 km, the line interests the narrow gorges of the Mureş River and it is characterized by the physical constraint of Radna-Lipova. Radna is the main railway station on this alignment stretch, being also a railway junction to Timisoara city.

Along this railway stretch there are located the following passenger's transportation points: junction station Radna, Milova station, Corfeni halt, Conop station (that will be transformed in halt), Bârzava station and Bătuţa halt. This paperwork represents a part from a study performed in cooperation with Italferr S.p.A., Italy. The proposed solution considered to be the most appropriate from economical and financial point of view foresees to maintain the existing alignment considering improvements that permit to take the line speed from the eastern entrance of Radna station to Bârzava station to 120 km/h. (Italferr, 2006)

The line speed in Radna station shall be maintained at 90 km/h. The stretch from Bârzava to Bătuţa and km 559, just before Valea Mureşului, shall keep the 100 km/h speed but eliminating the alignment constraint south of Bătuţa. These alignment corrections need some land appropriation, but interesting agricultural area only without taking close the line to the urbanized areas of the villages. In this way no relevant civil works shall be needed on this part of the alignment.

Along this railway line stretch, all level crossings shall be maintained and equipped with automatic controlled systems, with double barriers or only with lights, depending on the roads importance class.

Table 1

Technical characteristics of Radna-Valea Mureşului railway line stretch

Radna-Valea Mureşului stretch	Proposed solution	
Length (m)	37,900	
	Maximum design speed (km/h)	Length (m)
Radna station	90	3,160
Radna-Milova (inclusive)	120	8,000
Milova-Bârzava (inclusive)	120	17,367
Bârzava -Valea Mureşului	100	9,455
	Corresponding length	(%)
160 km/h	0	0%
120 km/h	25,367	66,79%
90-100 km/h	12,615	33,21%

Transformation of these railway stations shall be accompanied by a rela improvement that foresees not only the increase of general service level, but mainly the adoption of a complete information passengers system (megaphones, warning systems) and the construction of new facilities for passengers. All the existent railway structures such as

culverts and bridges located on the railway shall be completely renewed or replaced to be compliant with the requested standards. (Belc and Lucaci, 2004)

## RESULTS AND DISCUSSIONS

Further on we detail railway variants studied on this railway stretch. This part of the alignment interests one of the main physical constrain of the line that is represented by the towns of Radna and Lipova, that practically constitute a united urban centre, just sited at the end of Mureş valley, closing the whole valley and laying on the neighbour hills too. The railway infrastructure runs tortuously through the low density urbanised area of Radna (about 1.000 inhabitants), on the right side of Mureş river, quite parallel to E68 roadway except in the eastern part, between the town of Radna and Milova village where some flat agricultural cultivated areas are crossed. On the left side of the river, there is the town of Lipova. This town is an important tourist site especially for the spas, localised in the south part of the town. It is important to note that this part of the line is interested by important cultural and religious (pilgrimage sites) heritage sites. In fact in the north part of Radna is located the catholic Maria - Radna Abbey (build on 1756, now it is a hospital). The present station is placed in straight and curves alignment. The Timișoara line enters the west end of the station just after the crossing of the Mureş river. Being this part of the alignment very tortuous, it is possible to find a series of narrow curves interesting the station plan and having a minimum radius of 530 m, so permitting a maximum line speed of 90 km/h thus proposing to maintain the current location of the station.

The railway variant, from Radna area, was taken into consideration due to the fact that this part of the alignment interests one of the main physical constrain of the line. In fact, this stretch runs in a part of the Mureş valley that is very narrow, having a width comprised between 500 m and 1 km. Practically, the mountains close the valley on both sides. This stretch of the existing alignment is interested by many narrow curves, having the minimum radius of 470 m. This part of the line runs quite parallel to the existing E68 roadway that links Arad to Deva. There are some critical points particularly where the Mureş river pushes the railway and the E68 close to the hill, therefore the railway is practically the river side (km 591-590). Erosion is particularly evident and railway structure must be continuously protected and maintained to prevent damages coming from the river action. (Peptan et al., 2004)

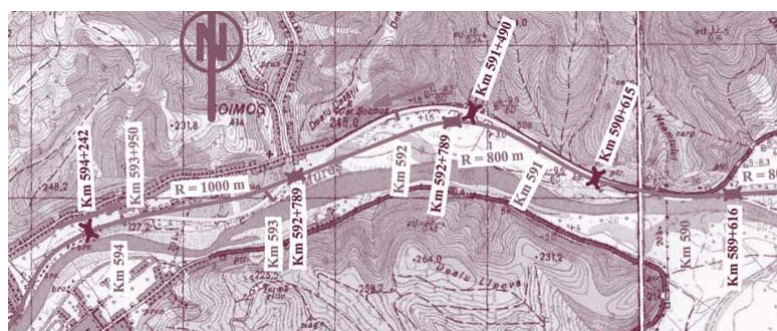


Figure 1. Railway variant proposed in Radna area

Considering the alignment characteristics, the existent geometry has been deeply revised and an alignment for 120 km/h speed is proposed. This alignment does not need tunnelling works and avoid and it minimises the existing interference with the Mureş River.

The re-alignment shall adopt 800 – 1,000 m curve radii and it shall interests a part of the line comprises between km 594+073 and km 588+875, so having a length of 5 km about; the re-alignment shall maintain also a part of the existing line for 1 km about. In detail, three relevant stretches shall be interested by a complete new realisation, they are comprised as follows: between km 594+073 and km 590+429; between km 590+102 and km 589+901; between km 589+331 and km 588+875, so interesting a total length of 4200 m, permitting a shortage of the line of 140 m. From km 594+073 the alignment continues quite straight to cross an open agricultural land back to rural urbanised area but at the same time keeping away the line from the Mureş River nearby km 593. Then the new line shall run between the existing one and the Mureş River up to km 591 about where it becomes quite parallel to the existing line up to km 590+429. (Herman and Ivana, 1999)

After the maintaining of a short existing straight stretch, a short variant must be adopted between km 590+102 and km 589+901 to improve the existing curve radius of 600 m: considering the presence of the Mureş river, the variant has to maintain the same curve vertex so the E68 roadway must be moved away for few meters toward the hills. The hills area is already interested by a former quarry so the environmental impact generated by the variant could be minimised. In this case, a considerable amount of investment has been considered in this case for moving the roadway and for retaining walls toward hills.

After Milova station the line turns northeastward with a narrow curve having a 460 m radius, then it runs straight through an open land south of Odvoş, turning with a narrow curve to join Conop station. On this stretch, considering tge alignment characteristics, the proposed solution is represented by the adoption of alignment variant that permitts the maximum travelling speed of 120 km/h. (Peptan, 2009)



Figure 2. Railway variant proposed in Milova area

The re-alignment consists in the improvement of the curve on the eastern entrance of Milova station, introducing a double curve and moving the alignment in the exterior of existing curve and adopting 800 – 1000 m curve radii. The re-alignment shall affect the stretch comprised between km 586+300 and km 584+736 having a length of 1664 m, generating an increasing of the line length of 100 m about. The re-alignment shall be developed on low embankment crossing flat agricultural open areas, without particular obstacles. This alternative supposes land appropriations affecting only agricultural lands, of about 51000 sqm. (STAS 3192/2, 1990)

Conop variant maintains the exusting line along this stretch, but with some loacal improvements, mainly nearby Conop station. This variant is formed from different sections that permitt the maximum line speed of 120 km/h speed and is based on transformation of Conop station in a simple halt.

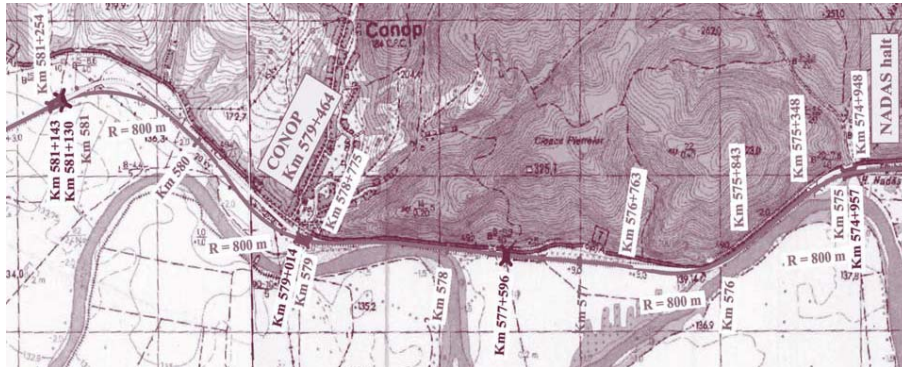


Figure 3. Railway variant proposed in Conop area

The first re-alignment supposes the improvement of western entrance curve of Conop station, from km 581+165 to km 580+200 that presently is equal to 470 m. The length of the new alignment shall be equal to 905 m, permitting a shorting of the line with 60 m. The second re-alignment is connected to the possibility of building a double curve between km 579+961 and km 578+825, moving westward the existing alignment for few meters: this in order to improve up to 800 m the radius of the existing curve located nearby km 579, that presently is equal to 470 m. The moving shall be easily realised on low embankment in an open land avoiding to interfere with the urbanised areas and it shall be 1172 m long, so increasing the line length of 40 m. From Conop station the line runs along a narrow corridor, closed by the Mureș river on the south side and by the mountains on the north side, quite parallel to the E68 roadway. After km 575 the line continues straight, maintaining the same infrastructural characteristics, up to Barzava station. So this stretch, having a length of 8 km, is characterised by 2 different parts, each of them 4 km long, the first one being tortuous up to Nadaș halt, the second one being straight.

The third re-alignment consists in the improvement of the curve sited nearby km 576, now having a radius of 460 m, introducing a double curve ending in the same point of the existing one. The length of the new alignment shall be equal to 1400 m, generating an increasing of the line length of 42 m.

The fourth re-alignment consists in the improvement of the curve sited in correspondence of Nadaș halt, having a radius of 475 m. The vertex of this curve shall be maintained in order to avoid the interference with the close E68 roadway. The length of the re-aligned stretch shall be equal to 437 m generating an un-relevant shortage of the line. This alternative needs relevant land acquisition affecting only agricultural areas, for about 123000 sqm.

In Bârzava station, the main modification refers to the narrow curve located at the western entrance of the station. Bârzava variant consists in the improvement of present radius of 475 m up to 800 m in order to permit a line speed of 120 km. The re-aligned stretch shall be comprised between km 570+359 and km 570+036, measuring 321 m and generating an un-relevant shortage of the line. The proposed solution foresees the alignment improvement through two curves, between km 561+990 and km 561+185 and between km 561+990 and, adopting curves with the radii of 550 m that permits the maximum travelling speed of 100 km/h. These modifications supposes the movement of railway line axis towards centre, occupying the land between the road and the river; in this way, the railway line being closer to the river (Peptan and al., 2004).

The following table synthesizes the proposed modifications for the railway variants.

Table 2

Railway modifications on Radna-Valea Mureşului stretch						
Variant	Location	Initial point (m)	Final point (m)	Radius (m)	Maximum sped (km/h)	New land exploitation
V4	Radna	590+429	594+073	1000	120	Medium
V5	Radna	589+901	590+102	800	120	Reduced
V6	Radna	588+875	589+331	800	120	Reduced
V7	Milova	584+736	586+300	800	120	Medium
V8	Conop	580+200	581+165	800	120	Medium
V9	Conop	578+825	579+961	800	120	Medium
V10	Nădaş	575+831	577+237	800	120	Medium
V11	Nădaş	574+799	575+241	800	120	Reduced
V12	Bărzava	570+036	570+359	800	120	Insignifiant
V13	Bătuţa	561+185	561+990	550	100	Reduced
V13	Bătuţa	560+274	560+668	550	100	Reduced

### CONCLUSIONS

Rehabilitation and improvement of Radna-Valea Mureşului railway stretch was performed in order to comply with the standards set forth by the European Corridors and TEN Corridors, this railway stretch belonging to the IV TEN Corridor, linking the Western part of South Europe to the Black Sea (Costanţa Harbor). Romanian public railway infrastructure rehabilitation offers a more interoperability with the railway system of EU.

The adoption of this railway alternative will increase the compliance with the AGC/AGTC standards, ensuring a travelling speed of 120 km/h on 97% from the studied railway stretch. Also, the national railway company saves 25-35% on the present operating costs and it enhances the capacity to ask in the future increased tariffs because of the better service offered. Another effect is represented by 20% time savings for passengers and goods transported.

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STAS 3192/2–90 Căi ferate normale. Elemente geometrice.



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**CONSIDERATIONS REGARDING THE TRANSPORT CAPACITY OF A  
REHABILITATED RAILWAY LINE STRETCH IN THE CONTEXT OF PUBLIC  
RAILWAY INFRASTRUCTURE DEVELOPMENT**

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**KEY WORDS:** *railway line, line capacity, train speed*

**ABSTRACT**

*The railway line from Hungarian border to Simeria is the busiest railway route of the CFR National Company network because the most important freight and passenger traffic both national and international runs on it. The present practical capacity of the line is between 60 and 210 trains/day, but the present traffic volume is between 10 and 30 tons/year. The maximum speed of circulation is 65-140 km/h for passenger trains and 65 – 95 km/h for freight trains. Thus the railway under study should comply with the AGC and AGCT Agreements and reaching a maximum speed of 160 (passenger trains) and 120 km/h (freight trains). This paperwork has the object of defining the current operational existing conditions of the railway line and the results of its exploitation in the future, in terms of railway capacity compliant with the proposed modifications by its modernisation.*

**INTRODUCTION**

The present railway line Border–Curtici-Arad-Deva-Simeria has a length of 185 km, out of which 176 km are double line. It has 23 sectioning points (19 railway stations and 4 crossing halts). This line ensures, through Arad, Radna, Ilia, Păuliș and Deva stations, the railway connection to Timișoara-Stamora Moravița, Salonta, Oradea, Cluj, Brașov, Sibiu and Filiași. The railway line is sinuous because of the topographical situation, running along the Mures River for its longest part. This paperwork represents a part from a study performed in cooperation with Italferr S.p.A., Italy. The scope of this study is to define the current operational existing conditions of the railway line and the results of its exploitation in the future, in terms of railway capacity, of a railway line rehabilitated and improved and to be compliant with the standards established by European Corridors and TERs. In this way, the railway line that constitutes the object of this study must be compliant with the AGC and AGCT Agreements and to reach a maximum speed of 160 (passenger trains) and 120 km/h (freight trains) (Italferr, 2006).

**MATERIAL AND METHODS**

The railway stretch under study is part of Corridor IV (from Germany to the Black sea) and it can constitute an important link towards Caucasian and Central Asia countries. The present practical capacity of the line is between 60 and 210 trains/day, but the present

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traffic volume is between 10 and 30 tons/year. The maximum speed of circulation is 65-140 km/h for passenger trains and 65-95 km/h for freight trains. The effective travel time for fast trains (without stopping time) is around 11 hours and 16 hours for container trains between Curtici and Constanta. One hour is the average delay due to improper infrastructure conditions. At the base of this study, there were carried out a series of investigation “on field” and verifications on statistical data from the existent database.

Particular attention has been given at the following operational aspects, which will be mainly affected by the line upgrade: (Ianca, 2005)

- Line capacity in terms of trains/day;
- Line and trains speed, divided per traffic category (freight, local passenger, direct trains);
- Technological systems.

On Hungarian Border - Curtici - Simeria railway line there are 23 sectioning posts (19 railway stations and 4 moving halts) and 13 commercial halts (stopping points in the main lines). Considering the railway line divided in five stretches, in the following table the existing sectioning posts and the commercial halts on each stretch are detailed:

Table 1

Sectioning posts and commercial halts, on each stretch for the studied railway line

Stretch		Sectioning posts		Commercial halts
		Stations	Moving halts	
I	Hungarian border-Curtici	1	-	-
II	Curtici - Arad	2	-	-
III	Arad - Radna	5	-	1
IV	Radna - Ilia	8	3	8
V	Ilia - Simeria	3	1	4
TOTAL		19	4	13

The real capacity of existing Hungarian Border-Curtici-Simeria railway line is of 210trains/day, with the exception of Border (Lököshaza)-Curtici where, being a single railway line, its real capacity has been estimated as 52trains/day. The maximum distance between two adjacent stations shall be equal to about 12.6km, along the critical stretch comprised between the stations of Zam and Campuri Surduc. It is important to note that at present the turnouts in Campuri Surduc have been fixed so that it could not be considered as a station; in this way the longest stretch becomes the Zam-Gurasada, about 16.6km long. Concerning line capacity calculation it is important to consider that at present the CFR rules foresee a period of 4 hours for single track line maintenance and a period of 6 hours for double track line maintenance. During these intervals trains are not operating therefore the actual capacity of the line is minor than the theoretical one. It was carried out an alternative calculation of present line capacity, carried out by using the Fiche UIC 405 formula, according to the following parameters, and giving the results expressed at the following pages (UIC 405 formula is now being modified, but it has been taken into consideration because it is still considered as one of the best and international methodology for capacity estimation). The capacity of a line is determined by the most restrictive section between two stations in terms of trip time (the longest section or the section with the minimum speed limit) and by the block sections length. The parameters to be taken into account within the UIC method, as to say the parameters affecting the value of the capacity are listed below:

1. Average speed on the most restrictive section of line [V];

2. Distance between two following main signals (such as, for example, the distance between the starting signal and the home signal of the following station in case the section of line is composed only by a single block section) [D];

3. Average train length [te];

4. Headway between two trains (both for crossing trains and following trains, including the time for setting the routes within the station) [tm]; the headway must be conceived as the necessary lost time between two consecutive trains operated in the same station (for example, the minimum time between the departure of one train and the arrival of a second train in case of following trains) Headway is mainly depending on the signaling and communication system used in the station or in block sections and according to the envisaged typology for the Romanian network, this time has been assigned in this calculation analysis as 1 min and 1 min depending on the direction of the two following trains into the station

5. Distance between distant and main signal [d];

6. Visibility distance of the distant signal [l];

Line capacity was calculated with the following formula:

$$P = \frac{T}{t_{fm} + t_r + t_{zu}}$$

where:

“T” is the total operating time per day;

“tr” is  $0,67 * t_{fm}$ ;

“tzu” is  $0,25 * \text{number of sections}$ ;

“t<sub>fm</sub>” is calculated with the following formula:

$$t_{fm} = \frac{D}{V} + \frac{l + d + t_e}{V} + t_m$$

where:

“D” is the distance between two following main signals;

“V” is the average speed of the section calculated according to the running simulations of the typical freight;

“l” is the visibility distance of the signal;

“d” is the distance between distant and main signal;

“te” is the train length;

“tm” is the lost operating time between two consecutive trains into the same station.

In this calculation, the parameters from table 1 have been assumed as fixed.

Table 2

Parameters assumed as fixed in the calculus of railway line capacity

Main fixed parameters of the Capacity formula (according to UIC 405)	
Total operating hours per day (h)	18,0
Visibility distance (m)	200,0
Distance between alarm and main signal (m)	1700,0
Train average length (m)	600,0
Operating time between two following trains (min)	1,0
Operating time between two crossing trains (min)	1,0
Block sections length (km)	1,7

The average speed on the most critical section has been assumed from the traffic mix as 55 km/h (freight + passenger present speed mix with a percentage of trains calling in every station-local trains). Existing line capacity has been therefore estimated at 270 trains/day (Ianca, 2003).

Hungarian Border - Curtici - Simeria railway line is 185 km long, electrified on its whole length and operated by Automatic Block Line (ABL) installations, this aspect already maximizing line capacity from the point of view of signaling system. (Herman, 2000) In fact, this is corresponding to the braking distance for fast passenger trains plus a security distance depending on the characteristics of the line and of the rolling stock. Passenger and freight trains are trailed with powerful electric locomotives 060 EA that can assure a high traction capacity. This locomotive is characterized by the presence of 6 motorized axis, this assuring a high adherence when re-starting on high gradient slopes. Moreover, this locomotive can pull till 3,000 gross tones over the maximum gradient of 6‰, corresponding, on average, to a train with more than 1900 net tones, 45 wagons (each one long about 16 m). This typology of train can reach the length of 700 m. (Belc & Lucaci, 2001) The maximum tonnages to be pulled as well as the train's traction ways are 650 gross tons for passenger's trains and 3000 gross tons for freight trains. It is important to underline that train speed, currently, is limited by line geometry and not by traction effort of the locomotive. In fact, this kind of locomotive does not have any constraints to pull a passenger train at the speed of 160 km/h on the maximum gradient of this line. For freight trains, the situation is different: they will not fully benefit from line parameters upgrading, because their speed limits will be in any case due to traction capacity of the locomotive and in any case they will not overcome 100-120 km/h in any section of the line.

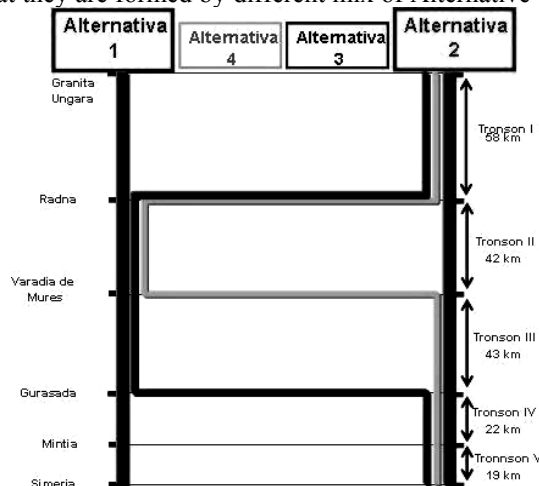
## **RESULTS AND DISCUSSIONS**

Modernisation of the Hungarian Border-Simeria railway line permits a considerable increasing of the line capacity, generally obtained by the combination of the upgrading of the alignment standards and of the technological equipment. On the Hungarian Border-Curtici stretch the increasing of the line capacity is strictly related with line doubling. The proposed alternatives, the speed limit shall be improved as much as possible to reach at least the 120 km/h speed limit, except where the morphology of the areas involves relevant variants with heavy investments (for example Arad area). In order to evaluate the best alternative, both the basic proposed solutions do not modify the railway network in the project area so: Curtici shall remain the border station and Arad, Glogovaț, Radna, Ilia, Mintia and Simeria shall remain as junction station. It is necessary to point out that Mintia shall remain as junction station for the Brad branch that now is not under operation; in the subsequent phase it could be possible to evaluate the re-arrangement of the station lay-out without maintaining this link. (Stafie, 2008)

At present both passenger and freight trains are operated on the line. Some long distance passenger and freight trains are formed and broken down in other Countries or in other main stations and shunting yard of Romania, so they run along the line only. Anyway some trains are formed and broken down in stations of the line, particularly as follows: passenger trains (intercity, express) at Arad, Deva and Simeria; passenger trains (local) at Curtici, Arad, Savarșin, Ilia, Deva and Simeria; freight container trains at Curtici, Arad, Glogovaț and Simeria; direct freight trains at Curtici, Arad, Radna, Mintia, and Simeria and local freight trains at Arad, Radna, Savarșin and Ilia. Therefore trains are formed and broken down in 8 stations of the line; particular care shall be taken in order to avoid removal of these stations or, at least, maintaining these operation functions.

The maximum adopted admissible line speed shall be equal to 160 km/h to be compliant with the AGC standards for passengers train, except otherwise indicated. This limit shall corresponds to the maximum speed for passenger trains but it shall be equal to 120 km/h for freight train except otherwise stated, so it shall be compliant with AGTC standards. Alternative no. 2 shall be fully compliant with AGC standards except in

correspondence of the main stations as stated in the following paragraph. (Muşat & al., 2005) In fact, Alternative 2 envisages the continue speed of 160 km/h except for the areas of Arad, Barzava, and the section between Branisca and Simeria, where train speed will be limited at 120 km/h. Alternative 3 and 4 will be a mix of the preceding speed limits, according to the fact that they are formed by different mix of Alternative 1 and 2, as shown



in the following scheme.

Figure 1: Scheme of the alternatives studied on the railway line from Hungarian border to Simeria

The following table resumes existing and future average service speed (commercial speed) and running time for three train categories. (Peptan, 2009)

Table 3

Values of running time and commercial speed depending on the considered scenario

Scenario	Type of train	Time (min)	Commercial speed (km/h)
Today	Local trains	240	46
	Rapid trains	180	62
	Direct trains	155	72
Alternative no. 1	Local trains	180	62
	Rapid trains	120	93
	Direct trains	90	123
Alternative no. 2	Local trains	200	56
	Rapid trains	130	85
	Direct trains	110	101

Times and speeds mentioned in this table must be considered as indicative for Scenario “Today”, while times of Alternative1 and 2 can be further reduced according to the adoption of a modern system of traffic management (ERTMS). Times and speeds for Alternative 3 and 4, will be comprised between those of Alternative 1 and Alternative 2. In particular Alternative 4, being formed by Alternative 2 for four sections on five, will be very close to the values of Alternative 2, while Alternative 3 will be more similar to Alternative 1. Considering the doubling of the whole line, its theoretical capacity shall be equal to 296trains/day. (Izdrăilă and Herman, 1993) According to this new calculation for the capacity of the upgraded line, capacity on the double track line will increase from 270

train/day to the theoretical value of: and 335 trains/day for Alternative 2, corresponding to a new average speed of 90 km/h (calculated by eliminating stations and halts stops). Therefore, the future line will allow increasing capacity by the 19% with Alternative 1 of ps.

### CONCLUSIONS

The research of the fully compliant alternative has been based on the basic criterion to maintain as much as possible the present existing alignment, due to the fact that some relevant straight stretches are located on it. Another basic criterion to define the alternative alignment could be fixed on the basis of the analysis of the main stations that interest the existing line. It is important to note that some main stations could be considered as key points along the line. These stations are: the border station of Curtici; the junction station of Arad that is the main urban centre of the line; the station of Deva that is the main station interesting Hunedoara province and the junction station of Simeria.

Particularly the station plans of Arad and Deva present some alignment constraints that seriously limit the maximum admissible line speed in correspondence of them. The compliance with the AGC-AGTC parameters involves heavy investments with a complete relocation of these main stations. It could be very difficult to justify this technical proposal, even considering all other negative aspects as the removal of the station from the urban centre, therefore making worse the accessibility to the station for the passengers. Moreover the maintaining of the speed limits would not influence the operation due to the fact that the main part of the passenger train will stop at these stations. Thus maintaining these stations would be another basic criterion to develop the line alternative.

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**PRODUCTIVITY OF THE APPLE ORCHARD DEPENDING OF THE TRAINING  
AND PRUNING OF TREES**

Pesteanu A.<sup>1</sup>

**KEY WORDS:** *Productivity, orchard, varieties, crown formation, pruning.*

**ABSTRACT**

*The experiment was carried out in a commercial orchard "Codru - St" Ltd. founded in 2000 with bench-graftings on rootstock M 9 at the varieties Gala Must, Golden Reinders, Idared and Florina. The distance of plantation between rows is 4.0 m, and between trees in the row is 1.0 m. There was studied the productivity of varieties in dependence on crown formation variants. It was established that the varieties Gala Must, Golden Reinders and Idared, the highest productivity of fruits was obtained in the variants with minimalization of pruning's degree at crown formation and design of 2 provisional branches horizontalized through fixation on row direction – 30.38-35.69 t/ha. For the variety Florina the highest indexes of production were registered by pruning's of transfer at lateral ramifications – 27.06 t/ha.*

**INTRODUCTION**

The experience obtained on the international scale in the fruit growing domain demonstrate that at present the most efficient fruit growing plantations are considered to be the superintensive orchard, planted on cloned rootstocks with a little vigor of growth (Pesteanu, 2008). In recent years the majority of fruit growing plantations were established with cloned rootstocks with an under- and little vigor that correspond to attributed requirements to intensify the pomology (Uselis et al., 2006).

Productivity of apple superintensive orchards depends on variety's biological features, apple tree leading and pruning system. Fruit growing development program in the Republic of Moldova until 2020 provides for the establishment of 100 thousand hectares of modern orchards and increasing global production of fruits to 998 thousand tons (Balan et al., 2008). Around 60% of fruit production and 40% of the fruit-growing plantation area returns to intensive and superintensive apple-tree orchards (Balan et al., 2001).

The main objectives of the superintensive system of apple-tree culture are: entering the economic fructification 2-3 years after planting the orchard; rapidly increasing harvest and considerable capital investment recovery with the first two crops of fruit; high harvest of quality fruit with high economic indicators (Pesteanu, 2005, 2008, 2009; Sadowski et al., 2005; Licznar-Malanczuk, 2006).

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The listed objectives is achieved by using varieties of performance, application of rational training and cutting of trees, the agrotechnical higher level, including irrigation.

## **MATERIALS AND METHODS**

The experimental plot is placed in the orchard “Codru-ST” Ltd. There was established in summer of 2000 a plantation with bench grafts. As objects of research served the apple tree varieties Gala Must, Golden Reinders, Idared and Florina grafted on rootstock M9.

The distance of plantation 4x1 m was established in conformity with present recommendations for apple trees grafted on M9, leaded after the slender spindle system (2,3). In 2000 vegetation grafts reached about 120 cm which allowed initiation of slender spindle crown formation in four variants.

Variant 1. (control) - as recommended in force: the trunk of the trees with 50 - 55 cm and well-developed vertical few zigzag shaped axis; 3-4 frameworks 40 - 50 cm short with inclination angles of about 60° to the vertical; the frameworks and above the axis at intervals of 20 cm are uniformly located radially fruit-bearing branches (fruit branches), horizontal oriented predominantly through lateral transfer side parting cuts, after fructification fruit-bearing branches is to renew the cycle of 3 - 4 years.

Variant 2. Crown bioconstructive base as in the control variant completed with: rational minimizing of the degree of training cuts, placement above the crown of the provisional frameworks alternatively horizontalized fixed on espalier onto the row direction that gradually shortens after fructification stage, transferring them into fruit branches; forced horizontalization of vertically growing scions and branches into the free end of the crown to transfer into fruit branches.

Variant 3. Formation of a crone is made as in variant 2 routing the fruit-bearing branches to horizontal position through lateral transfer side parting cuts.

Variant 4. Formation of a crone basically is made as in variant 2 with the renovation of branches by division fruit-bearing to obtain scions from sleeping buds.

Each variant includes 4 repetition and 8 trees in a row. Between rows are grass and the row strips are loose and herbicide. The fertilization system is on scheduled harvest. Through is drip irrigation.

In the years 2003 – 2008, according to approved methods were studied the production of apples-trees plantation.

## **RESULTS AND DISCUSSIONS**

The apple trees have entered on economic fructification in 2003 from the initiation of crown formation with high harvest in variant two et the Gala Must and Golden Reinders varieties. These varieties had also higher yield of fruit in the years 2004 - 2005. The average harvest from 2003 to 2005 (Table 1) in version 2, the afore mentioned varieties constitute respectively 34.32 t / ha and 35.69 t / ha. The Idared variety's average major harvest of fruits in the years 2003 - 2005, was still in a degree to minimize pruning training and placement above the crown base of two provisional frameworks alternatively horizontalized and attached to espalier to turn, but with absolute value less - 30.38 t/ha.

The Florina variety's average productions of superior fruit were obtained in variant 3 with routing the framework through transfer cuts to side parting - 27.06 t/ha. In other variants, the average fruit yield for the years 2003 - 2005 is greater than control variant, but lower than in variant 2 for Gala Must, Golden Reinders, Idared varieties and



variant 3 for Florina variety. After training the crown in 2005 and entry in full fructification, due to fructification cutting in accordance with the biological particularities of the varieties and adjusting the load of fruit, including hand thinning after falling in June under physiological cross-sectional area of the trunk (5-6 fruits/cm<sup>2</sup>), harvest fruit varieties taken from the study do not differ essentially because they have a high potential productivity (Table 2).

In all varieties significant differences were recorded by year of study. In 2007, with acute drought, particularly air, trees tied sufficient amount of fruit to obtain the planned harvest, but being smaller apples, fruit production had declined compared with 2006. This year reduced the differentiation of floral buds and diminished harvest in 2008.

On average for the years 2006 - 2008 harvest fruit varieties taken from the study is 31.14 - 32.73 t / ha and can be considered satisfactory for the weather conditions during this period. Gala Must, Golden Reinders and Idared varieties assigned to type III fructification, and the Florina variety to the type IV fructification.

On varieties of type III and IV of fructification, during maximum fructification, fruit-bearing branches elongate and due to terminal buds' fructification becomes arched, harvest is moving on the periphery of the crown. To exclude these shortcomings in these varieties is recommended staggered cutting of both fruit-bearing branches and fruit branches.

Table 1

Harvest of apple plantation depending on the training mode of the tree crown, t / ha,

Variants	2003	2004	2005	Average 2003 – 2005
Gala Must variety				
V <sub>1</sub>	22.22	24.00	38.17	28.31
V <sub>2</sub>	28.01	27.82	45.15	34.32
V <sub>3</sub>	25.77	26.07	42.47	31.43
V <sub>4</sub>	25.32	25.90	42.10	31.10
Average	25.33	25.94	42.47	-
Golden Reinders variety				
V <sub>1</sub>	26.65	22.15	40.83	29.87
V <sub>2</sub>	33.07	25.37	48.63	35.65
V <sub>3</sub>	30.10	26.50	46.34	34.31
V <sub>4</sub>	29.30	22.87	44.57	32.24
Average	29.78	24.22	45.09	-
Idared variety				
V <sub>1</sub>	20.75	21.15	33.43	25.11
V <sub>2</sub>	25.32	24.72	41.11	30.38
V <sub>3</sub>	23.65	23.90	37.33	28.29
V <sub>4</sub>	23.02	23.42	37.09	27.84
Average	23.18	23.29	37.24	-
Florina variety				
V <sub>1</sub>	10.90	17.35	37.65	21.96
V <sub>2</sub>	15.57	20.03	39.52	25.04
V <sub>3</sub>	14.42	23.43	43.35	27.06
V <sub>4</sub>	14.13	18.00	36.31	22.81
Average	13.75	19.70	39.20	-

Table 2

Harvest of fruit in the planting of apple-tree with training provisional crown branches (V<sub>2</sub>),  
t / ha

Variety	2006	2007	2008	Average 2006-2008
Gala Must	43.15	30.10	22.48	31.91
Golden Reinders	43.81	30.85	18.97	31.21
Idared	42.72	30.87	24.62	32.73
Florina	39.60	36.47	17.35	31.14
DS 5%	2.22	1.87	1.24	-

Varieties of type III fructification bearing branches are located on the 2-3 years old fruit-bearing branches. The rate-setting of the load with floriferous buds to be carried out by fructification cutting where cyclical replacement of elongated fruit-bearing branches, that permanently fructificated in the crown, form branches of one, two or three years.

Idared variety with premature arched secondary branches under the weight of harvest requires shortening the knot leaving 1-3 bearing branches.

Fruit-bearing branches with horizontal slope form a large amount of fruit branches when they grow free at least two years and only after that can be shortened to a stake or rod for rate-setting of load of fruit.

For Florina variety, type IV fructification, with great force growth, low bud excitability and ability of shoot formation, when annual branches length greater than 50 – 60cm <sup>3</sup>/<sub>4</sub> from the base, remain ungarnished. For more uniform garnishing with spears and annual rods branches are shortened to 1/4 - 1/3 of their length.

To inhibit the vigorous growth during cyclical renewal cutting of fruit-bearing branches, the replacing knot length on the stock of small force growth should be 8-10 cm long and on the rootstock of sub average force growth, 15-20 cm to get 3-4 annual 30 - 40 cm branches.

On Golden Delicious varieties and clones, the Alpinist, Florina, etc., when the branches of fruit-bearing insertion angle of 50 - 70° is excessive, or compete with the developing central axis to not practice their ring suppression, but leaving a knot. The cuttings done at the top of the ring and continue perpendicularly to the bisectrix formed between the suppressed fruit-bearing branch and the tree axis.

On some holes in the crown greedily branches from the area turn into fruit-bearing branches by severe shortening to 4-6 buds to achieve annual increases. Next choose 1-2 lower branches with more favorable location in space to complement the crown and the others are suppressed.

Making the proper cuts of fructification in the superintensive apple-tree orchards allow to maintain the physiological balance between growth and fructification period as long and production is of high quality.

## CONCLUSIONS

Using performance varieties, grafted on M9 rootstocks and led by the improved slender spindle with minimizing the extent of cutting, especially in the biological rest period, the formation of two provisional frameworks horizontalized onto the row direction, forced tilting of some scions and branches of the vertical free space of the crown contribute

significantly to expedite the entry of the trees on economic fructification and considerable capital investment recovery.

The varieties Gala Must, Golden Reinders and Idared, the highest productivity of fruits was obtained in the variants with minimalization of pruning's degree at crown formation and design of 2 provisional branches horizontalized through fixation on row direction – 30.38-35.69 t/ha. For the variety Florina the highest indexes of production were registered by pruning's of transfer at lateral ramifications – 27.06 t/ha

After completion of crown formation, fructification cutting and rational adjustment of the load of fruit trees, applied in accordance with the varieties' biological particularities and superior agrotechnics to obtain high yields of quality fruit in the center of the Republic of Moldova with frequent dry years.

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STUDY REGARDING THE NATURAL VIRUS INFECTIONS IN  
SWEET CHERRY GENE BANK

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**KEY WORDS:** virus, cherry, test

**ABSTRACT**

*The viruses are diseases that produced the important damages in fruit species of Prunus genus. The study conducted at the Research Institute for Fruit Growing, Pitesti shows presence of some viruses in Cherry Romanian National Genebank.*

*The tests have been made on 56 cherry genotypes in natural infection conditions, using leaves as biological material, collected from an orchard 25 years old. TAS-ELISA, DAS-ELISA and AGRISTrip have been used as testing methods, to mark presence of virus infection with ACLSV, ApMV, ArMV, CLRV, PDV, PNRSV, RpRSV, SLRV, TBRV and PPV. Tests show the presence of PPV in 33% from sweet cherry genotypes, TBRV in 30%, RpRSV in 44%, CLRV in 4% and ArMV in 2%, respectively. Presence of PPV infection was confirmed by TAS-ELISA and AGRISTrip test methods, also. Didn't was detected infections with other viruses as ACLSV, ApMV, PDV, PNRSV and SLRV.*

**INTRODUCTION**

The quantity and the quality of the sweet cherry production is not dependent on only the environment factors and the growing technologies. One of the very important factor is represented by viral health.

It is known that sweet cherry is susceptible at around 14 viral diseases. The most important are: *PDV*, *PNRSV*, *CLRV*, *ACLSV*, *ApMV*, *TBRV*, *RpRSV*, *SLRV* and *PPV* that are observed from short time in Romania (Isac, 2000) and only spontaneously (Maxim, 2002).

For cultivars with high sensitivity the fruit weight can be diminish with till 8, 6 % in the case of *PNRSV* and 4, 7 % to *PDV* (Maxim, 1998). *PPV* and *PNRSV* have a negative influence started from fruit binding and until the fruits reach harvesting maturity (Maxim, 2001). Also, the viral infections by a single or more virus influence the fruit chemical composition as the content of soluble carbohydrates, vitamin C, etc. (Maxim and Papp, 2001).

To evaluate the natural viral infection level in the cherry gene bank of RIFG Pitesti, research has been conducted aimed to detecting presence and type of specific cherry viruses.

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## MATERIAL AND METHODS

The samples have been collected from *ex situ* cherry collection. Studied cultivars were: Rubin, Daria, Merton Favorit, Jabonlay, Kordia, Adelone, Poznaska, Mona, NY 6806, Salmo, 2 D 28-28, Chinook, Summit, HY 5690, Bigarreau Oratowsky, Orias, Chavanes, Italia 14, Valera, Durono Nero, Alămii, Ramon Oliva, Vinka, Vista, Lyons, Rainier, Italia 3, Silva, Italia 19, Roz amar de Mărculești, Bigarreau Burlat, Kristin, Amara, Scorospelka, Larian, Izverna, Szegedi Orias, Venus, Roșii de Bistrița, Beriessa, Metropoliskaia Ciornaia, Ponoare, Precoce de la Marca, New Star, Sam, Amar negru de Maxut, Bigarreau Dönissen, Bing, Colina, Vanda, Rainbow, Kordia, Rivan, Van, Germersdorf cultivars.

The tested biological material was represented by leaves collected from plants without exactly diagnosis. The samples tested through DAS-ELISA (Clark & Adams, 1977) method for detecting the virus *PPV* (*Plum pox virus*), *CLRV* (*Cherry leaf roll virus*), *ArMV* (*Arabidopsis mosaic virus*), *ACLSV* (*Apple chlorotic leaf spot virus*), *RpRSV* (*Raspberry ringspot virus*), *SLRV* (*Strawberry latent ring spot virus*), were effectuated with serological reagents from BIOREBA company follow the instructions of the protocols.

TAS-ELISA method was used for detecting the virus: *PPV* (*Plum pox virus*), *PNRSV* (*Prunus necrotic ring spot virus*) and *PDV* (*Prune dwarf virus*) and was effectuated with serological reagents from SEDIAG company.

To detect virus *PPV* (*Plum pox*) was used AGRISTRIP test, marketed by the company BIOREBA.

## RESULTS AND DISCUSSION

Tests carried out revealed the presence of viruses *ArMV*, *CLRV*, *RpRSV*, *TBRV* and *PPV*.

The highest incidence was found in *RpRSV* virus which recorded a rate of 44% out of tested samples. *RpRSV* virus was followed by *PPV* an infection rate which stood at 33% out of tested samples and *TBRV* virus were positive for 30% of samples. *CLRV* and *ArMV* viruses showed a rate of 4% and 2% (Figure 1).

Regarding the susceptible cultivars to detected viruses (Table 1) have been found positive *RpRSV*: Poznaska, Mona, Salmo, 2D 28-28, Chinook, Summit, Bigarreau Oratowsky, Durono Nero, Alămii, Ramon Oliva, Vinka, Lyons, Rainier, Italia 3, Silva, Italia 19, Roz amar de Mărculești, Bigareau Burlat, Kristin, Amara, Izverna, Szegedi Orias, Venus, Roșii de Bistrița, Bigarreau Dönissen. *PPV* virus was detected to: Rubin, Kordia, Adelone, 2 D 28-28, Chinook, Orias, Chavanes, Italia 14, Bigarreau Burlat, Kristin, Izverna, Venus, Roșii de Bistrița, Beriessa, Amar negru de Maxut, Bing, Colina, Rivan cultivars.

Infection with *TBRV* virus presented: Kordia, 2 D 28-28, Chinook, HY 5690, Orias, Chavanes, Italia 14, Vinka, Italia 3, Larian, Szegedi, Orias, Beriessa, Metropoliskaia Ciornaia, Precoce de la Marca, New Star, Sam cultivars.

*CLRV* virus was detected at Rubin and Daria cultivars.

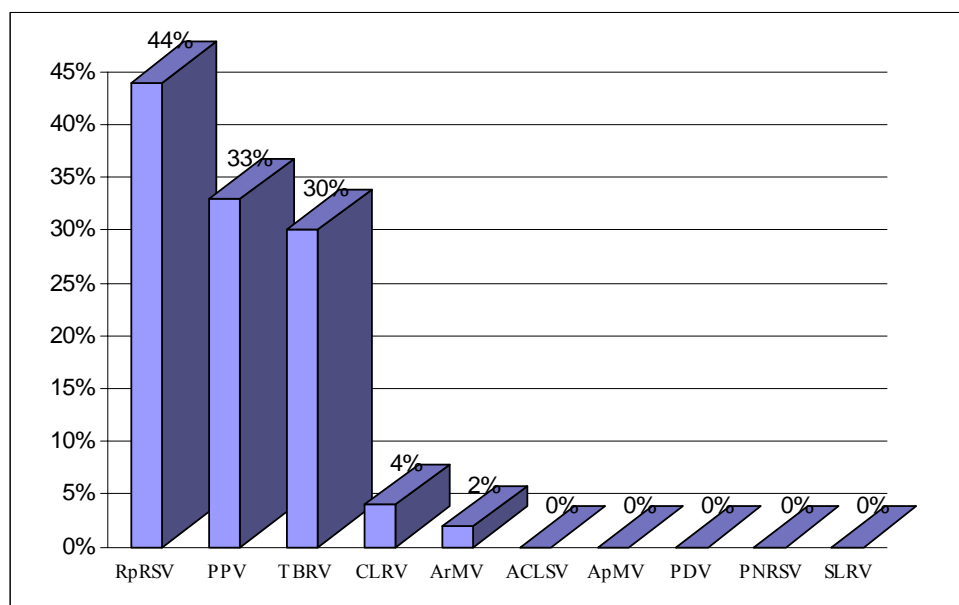


Figure 1. Incidence of tested viruses in cherry genebank from RIFG Pitesti

ArMV infection was detected at Ponoare cultivar.

In our studies were identifying the situations where the viral infection was produced by a many viruses (table 1):

-*RpRSV* + *TBRV* + *PPV*, for: 2D 28-28, Chinook cultivars;

-*CLRV* + *PPV* – Rubin cultivar;

-*TBRV* + *PPV* – Kordia, Oria, Chavanes, Italia 14, Beriessa cultivars;

-*RpRSV* + *TBRV*, to: Vinka, Italia 3 and Seize Geidi Oria cultivars;

-*RpRSV* + *PPV*, to : Bigarreau Burlat, Kristin, Venus, Roşii de Bistriţa cultivars;

With a single virus were found positive: Daria (*CLRV*), Adelone Poznaska, Mona, Salmo, Summit, Bigarreau Oratowsky, Durono Nero, Alâmii, Ramon Oliva, Lyons, Rainier, Silva, Italia 19, Roz amar de Mărculeşti, Amara, Amar negru de Maxut, Bing, Colina, Rivan (*PPV*), HY 5690, Larian, Metropoliskaya Ciornaia, Precoce de la Marco, New Star, Sam (*TBRV*), Bigarreau Dönissen (*RpRSV*), Ponoare (*ArMV*). Didn't present infections: Merton Favorit, Jabonlay, NY 6806, Valera, Vista, Scorospelka, Vanda, Rainbow, Kordia, Van, Germersdorf cultivars. The presence of *Plum pox* virus was put in evidence by the test method AGRISTRIP (figure 2).

Table 1

Viruses detected by ELISA TEST in sweet cherry genebank (significance/ELISA value)

No	Cultivars	ACLSV	ApMV	ArMV	CLRV	PDV	PFRSV	PpRSV	SLRV	TBRV	PPV
0	1										
1.	Rubin	-(0.993)	-(0.506)	-(0.557)	<b>+(0.874)</b>	-(0.854)	-(0.993)	-(0.518)	-(1.100)	-(0.436)	<b>+(2.342)</b>
2.	Daria	-(0.968)	-(0.494)	-(0.569)	<b>+(0.855)</b>	-(0.829)	-(0.968)	-(0.469)	-(1.079)	-(0.415)	-(0.537)
3.	Merton Favorit	-(0.940)	-(0.453)	-(0.618)	-(0.362)	-(0.839)	-(0.475)	-(0.499)	-(1.053)	-(0.469)	-(0.572)
4.	Jabonlay	-(0.946)	-(0.519)	-(0.468)	-(0.351)	-(0.858)	-(0.746)	-(0.501)	-(1.143)	-(0.480)	-(0.608)
5.	Kordia	-(0.938)	-(0.472)	-(0.452)	-(0.466)	-(0.868)	-(0.739)	-(0.477)	-(1.114)	<b>+(1.096)</b>	<b>+(2.000)</b>
6.	Adelone	-(0.944)	-(0.491)	-(0.622)	-(0.483)	-(0.862)	-(0.748)	-(0.575)	-(1.091)	-(0.517)	<b>+(2.050)</b>
7.	Poznańska	-(0.954)	-(0.495)	-(0.631)	-(0.321)	-(0.864)	-(0.754)	<b>+(0.382)</b>	-(1.103)	-(0.428)	-(0.522)
8.	Mona	-(0.947)	-(0.496)	-(0.521)	-(0.434)	-(0.853)	-(0.749)	<b>+(0.565)</b>	-(1.101)	-(0.495)	-(0.615)
9.	My 6806	-(0.775)	-(0.405)	-(0.509)	-(0.441)	-(0.861)	-(0.777)	-(0.460)	-(0.957)	-(0.483)	-(0.600)
10.	Salmo	-(0.776)	-(0.417)	-(0.603)	-(0.418)	-(0.858)	-(0.777)	<b>+(0.592)</b>	-(1.043)	-(0.538)	-(0.587)
11.	2 D 28-28	-(0.781)	-(0.518)	-(0.628)	-(0.450)	-(0.858)	-(0.783)	<b>+(0.551)</b>	-(1.050)	<b>+(1.312)</b>	<b>+(2.032)</b>
12.	Chirook	-(0.767)	-(0.490)	-(0.631)	-(0.599)	-(0.850)	-(0.769)	<b>+(0.523)</b>	-(1.033)	<b>+(1.093)</b>	<b>+(2.013)</b>
13.	Summit	-(0.764)	-(0.446)	-(0.590)	-(0.529)	-(0.847)	-(0.764)	<b>+(0.627)</b>	-(1.067)	-(0.492)	-(0.568)
14.	HY 5690	-(0.779)	-(0.471)	-(0.452)	-(0.506)	-(0.851)	-(0.779)	-(0.492)	-(1.057)	<b>+(1.089)</b>	-(0.573)
15.	Bigareau Oratowsky	-(0.798)	-(0.448)	-(0.483)	-(0.495)	-(0.871)	-(0.795)	<b>+(0.328)</b>	-(1.029)	-(0.594)	-(0.624)
16.	Orlas	-(0.805)	-(0.429)	-(0.428)	-(0.475)	-(0.868)	-(0.801)	-(0.480)	-(1.022)	<b>+(1.999)</b>	<b>+(2.116)</b>
17.	Chavanes	-(0.798)	-(0.453)	-(0.525)	-(0.444)	-(0.863)	-(0.795)	-(0.556)	-(1.016)	<b>+(1.028)</b>	<b>+(2.095)</b>
18.	Italia 14	-(0.856)	-(0.463)	-(0.557)	-(0.415)	-(0.863)	-(0.845)	-(0.535)	-(1.015)	<b>+(1.042)</b>	<b>+(1.941)</b>
19.	Valera	-(0.831)	-(0.462)	-(0.556)	-(0.514)	-(0.861)	-(0.828)	-(0.563)	-(1.017)	-(0.577)	-(0.590)
20.	Durono Nero	-(0.844)	-(0.506)	-(0.468)	-(0.421)	-(0.876)	-(0.844)	<b>+(0.501)</b>	-(1.056)	-(0.605)	-(0.562)
21.	Alâmii	-(0.816)	-(0.473)	-(0.559)	-(0.464)	-(0.895)	-(0.818)	<b>+(0.647)</b>	-(1.126)	-(0.557)	-(0.674)
22.	Ramon Oliva	-(0.820)	-(0.509)	-(0.519)	-(0.554)	-(0.896)	-(0.817)	<b>+(0.473)</b>	-(1.130)	-(0.535)	-(0.637)
23.	Vinka	-(0.809)	-(0.503)	-(0.479)	-(0.572)	-(0.893)	-(0.805)	<b>+(0.638)</b>	-(1.136)	<b>+(1.160)</b>	-(0.578)
24.	Visia	-(0.822)	-(0.536)	-(0.601)	-(0.443)	-(0.886)	-(0.816)	-(0.562)	-(1.123)	-(0.533)	-(0.591)
25.	Lyons	-(0.821)	-(0.479)	-(0.597)	-(0.516)	-(0.899)	-(0.822)	<b>+(0.569)</b>	-(1.139)	-(0.470)	-(0.571)
26.	Rainier	-(0.950)	-(0.474)	-(0.602)	-(0.559)	-(0.887)	-(0.955)	<b>+(0.588)</b>	-(1.126)	-(0.667)	-(0.595)

0	1	2	3	4	5	6	7	8	9	10	11
27.	Italia 3	-(1.009)	-(0.521)	-(0.578)	-(0.523)	-(1.004)	-(0.968)	+(1.571)	-(1.127)	+(1.033)	-(0.452)
28.	Silva	-(0.964)	-(0.473)	-(0.477)	-(0.486)	-(1.005)	-(0.946)	+(1.599)	-(1.139)	-(0.636)	-(0.450)
29.	Italia 19	-(0.989)	-(0.468)	-(0.413)	-(0.449)	-(0.994)	-(0.942)	+(1.552)	-(1.135)	-(0.498)	-(0.439)
30.	Roz amar de Mărculești	-(0.996)	-(0.535)	-(0.564)	-(0.460)	-(0.981)	-(0.940)	+(1.494)	-(1.116)	-(0.509)	-(0.444)
31.	Bigarreau Burlat	-(1.008)	-(0.442)	-(0.550)	-(0.512)	-(0.990)	-(0.988)	+(1.528)	-(1.138)	-(0.464)	+(2.110)
32.	Kristin	-(1.018)	-(0.461)	-(0.547)	-(0.454)	-(0.991)	-(0.998)	+(1.674)	-(1.136)	-(0.539)	+(1.939)
33.	Amara	-(0.993)	-(0.474)	-(0.654)	-(0.433)	-(1.060)	-(0.994)	+(1.595)	-(1.049)	-(0.601)	-(0.495)
34.	Scorospelka	-(0.994)	-(0.429)	-(0.664)	-(0.450)	-(1.069)	-(0.999)	-(0.538)	-(1.036)	-(0.503)	-(0.501)
35.	Larian	-(1.004)	-(0.473)	-(0.471)	-(0.377)	-(1.074)	-(0.988)	-(0.465)	-(1.048)	+(1.557)	-(0.550)
36.	Izverna	-(0.989)	-(0.513)	-(0.537)	-(0.510)	-(1.070)	-(0.994)	+(1.573)	-(1.035)	-(0.477)	+(1.975)
37.	Seize Gedi Onas	-(1.007)	-(0.422)	-(0.521)	-(0.455)	-(1.064)	-(0.980)	+(1.669)	-(1.053)	+(1.011)	-(0.598)
38.	Venus	-(0.993)	-(0.471)	-(0.652)	-(0.564)	-(1.048)	-(0.990)	+(1.663)	-(1.005)	-(0.474)	+(1.956)
39.	Roșii de Bistrița	-(1.009)	-(0.434)	-(0.507)	-(0.409)	-(1.048)	-(0.977)	+(1.493)	-(0.978)	-(0.450)	+(1.956)
40.	Berlesca	-(0.977)	-(0.510)	-(0.528)	-(0.443)	-(1.075)	-(0.997)	-(0.499)	-(1.047)	+(1.102)	+(2.136)
41.	Metropoliskaia Ciornai	-(0.994)	-(0.509)	-(0.706)	-(0.385)	-(1.046)	-(0.956)	-(0.414)	-(0.927)	+(1.047)	-(0.573)
42.	Ponoare	-(0.975)	-(0.504)	+(1.928)	-(0.499)	-(1.067)	-(0.943)	-(0.520)	-(0.970)	-(0.482)	-(0.656)
43.	Preoce de la Marco	-(0.995)	-(0.530)	-(0.597)	-(0.413)	-(1.063)	-(0.960)	-(0.535)	-(0.975)	+(1.054)	-(0.695)
44.	New Star	-(0.997)	-(0.461)	-(0.046)	-(0.454)	-(1.056)	-(0.983)	-(0.466)	-(0.968)	+(1.045)	-(0.605)
45.	Sam	-(0.998)	-(0.493)	-(0.686)	-(0.548)	-(0.975)	-(0.996)	-(0.546)	-(1.036)	+(1.201)	-(0.670)
46.	Amar negru de Maxut	-(0.956)	-(0.476)	-(0.557)	-(0.489)	-(0.873)	-(0.854)	-(0.527)	-(1.027)	-(0.563)	+(1.981)
47.	Bigarreau Dönussen	-(0.941)	-(0.460)	-(0.544)	-(0.574)	-(0.904)	-(0.845)	+(1.621)	-(1.012)	-(0.434)	-(0.559)
48.	Bing	-(0.960)	-(0.442)	-(0.491)	-(0.521)	-(0.887)	-(0.839)	-(0.473)	-(1.041)	-(0.657)	+(2.056)
49.	Colina	-(0.982)	-(0.494)	-(0.725)	-(0.500)	-(1.001)	-(0.948)	-(0.507)	-(1.021)	-(0.461)	+(2.025)
50.	Vanda	-(0.990)	-(0.473)	-(0.565)	-(0.581)	-(0.882)	-(0.854)	-(0.517)	-(1.052)	-(0.447)	-(0.562)
51.	Rainbow	-(1.000)	-(0.412)	-(0.559)	-(0.551)	-(0.903)	-(0.864)	-(0.591)	-(1.123)	-(0.457)	-(0.503)
52.	Kordia	-(0.983)	-(0.467)	-(0.477)	-(0.604)	-(0.974)	-(0.928)	-(0.579)	-(1.216)	-(0.485)	-(0.648)
53.	Rivan	-(0.993)	-(0.491)	-(0.654)	-(0.536)	-(0.923)	-(0.944)	-(0.511)	-(1.121)	-(0.492)	+(2.027)
54.	Van	-(0.894)	-(0.454)	-(0.652)	-(0.555)	-(1.042)	-(0.918)	-(0.550)	-(1.105)	-(0.588)	-(0.677)
55.	Gernersdorf	-(0.921)	-(0.452)	-(0.521)	-(0.577)	-(1.035)	-(0.978)	-(0.492)	-(1.326)	-(0.677)	-(0.682)
	Positive control	3.352	3.390	2.040	3.380	3.388	3.358	2.218	3.326	2.298	2.254
	Negative control	0.972	0.455	0.610	0.360	0.872	0.972	0.500	1.071	0.426	0.395





Figure 2. *PPV* virus detected through AGRISTRIP method to cherry

### CONCLUSIONS

1. Virus with the most highest incidence was *RpRSV* followed by *PPV*, *TBRV* and *CLRV*.
2. The largest share of the group was NEPOVIRUSURI (*ArMV*, *CLRV*, *RpRSV*, *TBRV*) this viruses are transmitted by nematodes and only virus (*PPV*) from POTYVIRUS group which is transmitted by aphids, mites, flies.
3. The most sensitive genotypes were 2 D 28-28 and Chinook , Kordia, Oria, Chavanes, Italy 14, Vinka, Italy 3 Bigarreau Burlat, Kristin, Izverna, Szegedi Oria, Venus, Rosii de Bistrita, Berriesa that were detected as infected by two or three viruses.

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SEROLOGICAL EVALUATION OF STONE FRUIT VIRUSES AT SEVERAL  
PRUNUS GENOTYPES

Poenaru Silvia<sup>1\*</sup>, Preda Silvia<sup>2</sup>

**KEY WORDS:** assay, genotype, natural infection, symptomless

**ABSTRACT**

*Plum pox virus infection was serologically established at the plum genotypes Dobrovica, Minerva CL27, Otesani 8 and at the japanese bush cherry, thus no infection was registered in peach, apricot, japanese plum, nanking cherry, blackthorn and canadian plum. Apple Chlorotic Leaf Spot Virus wasn't serologically determined in any of the plum genotypes evaluated. Regarding the two Ilarviruses only the peach cultivars were determined positive to the viral infection in natural field conditions: all for the infection with Prune Dwarf Virus and one, respectively Cresthaven for Prunus Necrotic Ring Spot Virus. Thus, was registered a mixed viral infection in case of one peach genotype.*

**INTRODUCTION**

*Prunus* genotypes are predisposed to varying degrees to infections by a wide range of pathogens (Laimer, 2003). Viruses can remain latent, spreading through an orchard and inflicting damage, sometimes without the growers' knowledge, these latent infestations often can produce small to moderate losses in fruit production (Agrios, 1997; Cembalia et al., 2003). Annually, plant viruses cause severe economic losses in crop production (Nemeth, 1994; Capote et al., 2008). The International Committee on the Taxonomy of Viruses published a list that included 1550 described species in 56 virus families (Van Regenmortel et al., 2000; Hillman, 2001).

Most cultivated *Prunus* genotypes are hosts for a large number of economically important plant viruses belonging to different genera including members of the genus Ilarvirus: Apple mosaic virus (ApMV), Prunus necrotic ring spot virus (PNRSV), Prune dwarf virus (PDV), American plum line pattern virus (APLPV); the Trichovirus Apple chlorotic leaf spot virus (ACLSV) and the virus responsible for the Sharka disease, Plum pox virus (PPV) from the genus Potyvirus (Sanchez-Navarro et. al., 2005)

In mixed viral infections, for example in case of peach cultivars, an attack of both PNRSV and PPV, diminished the yield capacity with 57%, conducted to a shortening of tree life and also to a poor quality of the fruits (Pusey and Yadava, 1991).

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The economic importance of ACLSV is largely due to its worldwide distribution and its capacity to induce severe graft incompatibilities in some *Prunus* combinations, causing major problems in nurseries (Ertunç and Ulubaş, 2005).

The aim of the present research is to evaluate the susceptibility of different *Prunus* genotypes to the most important viral diseases in natural field conditions.

### MATERIAL AND METHODS

The study was performed at Fruit Growing Research & Extension Station Vâlcea between 2009 and 2010. The biological material was represented by eight *Prunus* ssp. and the total number of genotypes was 29: plum (Tuleu gras CL 14, Minerva CL 27, Otesani 8, Empress, Blue free, Record, Ialomita, Dobrovica), peach (Romamer, Harbinger, Splendid, Cresthaven, Miorita, NJ265, Redhaven, Superba de toamna, Springold), apricot (Harleyne, Litoral, Dacia, Excelsior, Olimp, Mamaia, Sulina, Comandor, Goldrich, Harcot), japanese plum (Black Amber), nanking cherry (Orient), blackthorn, canadian plum and japanese bush cherry.

Plum cultivars were evaluated serologically regarding their behaviour in field conditions to four viruses: PPV, PNRSV, PDV and ACLSV. For peach, apricot, japanese plum, nanking cherry, blackthorn, canadian plum and japanese bush cherry cultivars the serological method was performed for three viruses: PPV, PNRSV and PDV.

Symptoless leaves and leaves with characteristic symptoms were collected in june and july 2009 and june 2010.

The serological method applied was DAS-ELISA (Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay) (Clark and Adams, 1977) (Figures 1, 2, 3). The readings of the assay were made with the microplate reader Stat Fax®3200 at the optical density of 405 nm.



Fig. 1 The distribution of the anticorp substrate



Fig. 2 The distribution of the positive and control, negative control and plants extracts



Fig. 3 The distribution of the conjugate substrate

## RESULTS AND DISCUSSIONS

In Figure 4 is showed the fourth stage of the serological assay, respectively the reaction substrate with pNPP at one hour after his distribution on the microplates for the four viruses studied: PPV (a), PDV (b), PNRSV (c) and ACLSV (d). Colorimetric reactions of the microplates wells indicates samples extracts with viral proteins.

Followig the microplates readings at 405 nm for each virus was determined the optical density of the samples extracts and was established which sample is infected.

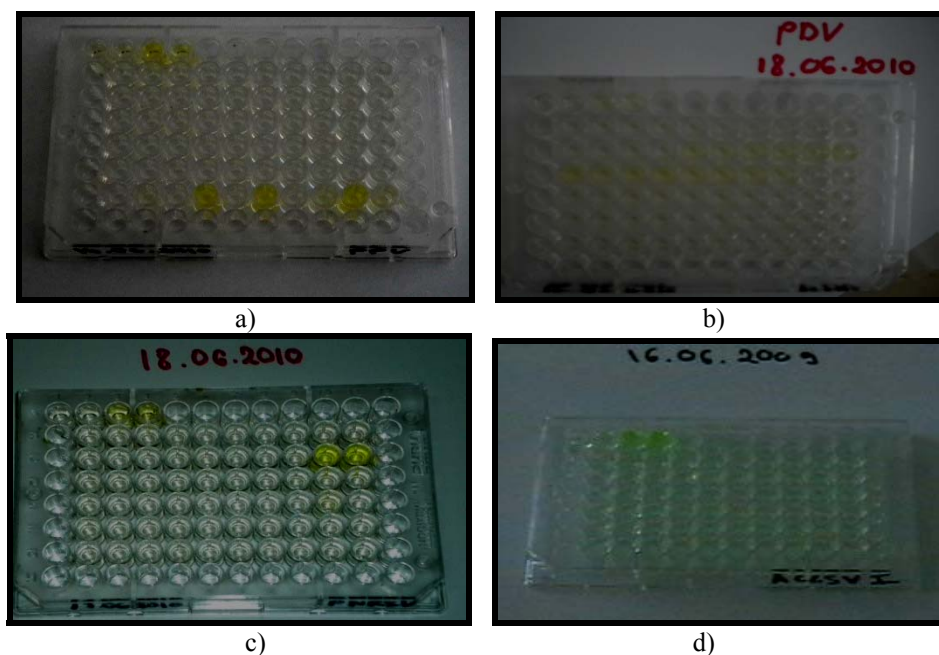


Fig. 4 Colorimetric reaction on the mycroplates indicates samples extracts with viral proteins: a) PPV test-strong evidency of viral protein in the wells G5,G7 and G11; b) PDV test-medium evidency of viral proteins in the wells C7 to C11 and from D2 to D9; c) PNRSV test-very strong evidency of viral proteins in the wells C10 and C11; d) ACLSV test-only the Positive Control is visually evident in the wells A3 and A4

Thus, no plum cultivar was found to be positive for ACLSV, PDV and PNRSV, the *Prunus* ssp.: apricot, peach, japanese plum, nanking cherry, blackthorn, canadian plum were not found positive for PPV, PDV and PNRSV (Tabel 1).

The japanese bush cherry was determined positive for PPV (values at 405 nm=3,163).

The plum cv. Dobrovica (values at 405 nm=2,766), Minerva CL27 (values at 405 nm=2, 975), Otesani 8 (values at 405 nm=3,222) and japanese bush cherry (values at 405 nm=3,163) were found to be infected with the Plum pox virus (PPV).

All the peach cultivars: Romamer (values at 405 nm=1,066), Harbinger(values at 405 nm=1,039), Splendid (values at 405 nm=1,071), Cresthaven (values at 405 nm=1,092), Miorita (values at 405 nm=1,239), NJ265 (values at 405 nm=1,077), Redhaven (values at 405 nm=1,098), Superba de toamna (values at 405 nm=1,046), Springold (values at 405 nm=1,007) were found to be infected with the Prune dwarf virus (PDV).

From eight *Prunus* ssp. evaluated through DAS-ELISA, only in *Prunus persica* (L.) Batsch was determined the presence of the Ilarvirus Prunus necrotic ring spot virus (PNRSV). Only one peach cultivar was positive for this virus, respectively cv. Cresthaven (values at 405 nm=3,237).

Thus, a mixed infection with two Ilarviruses: PDV and PNRSV was determined in one of the peach cultivars evaluated, the cv. Cresthaven. Visually, it is very difficult to distinguish the two viruses according to their symptomatology in the stone fruits (Milusheva and Borisova, 2005).

### CONCLUSIONS

Regarding PPV infection, in natural field conditions at eight *Prunus* ssp, through DAS-ELISA was established his presence only in *P. domestica* (cv. Minerva CL 27, Otesani 8 and Dobrovica) and in *P. japonica*.

ACLSV wasn't determined at any of the plum cultivars tested.

*P. armeniaca*, *P. tomentosa*, *P. salicina* and *P. nigra* had a negative response through the serological assay at PPV, PDV and PNRSV.

*P. persica* (nine cultivars tested) proved to be infected with the Ilarvirus PDV. Also one cultivar was found to be positive regarding the infection with another Ilarvirus: PNRSV. Thus, in the peach cultivar Cresthaven was determined a mixed viral infection

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Tab. 1

The serological evaluation of several *Prunus* ssp. through DAS-ELISA  
 Note: " + " = positive response; " - " = negative response; " \* " = not tested.

<i>Prunus</i> ssp.	Cultivars	DAS-ELISA positive and negative response			
		PPV	PDV	PNRSV	ACLSV
<i>P. domestica</i>	Tuleu gras CL 14	-	-	-	-
	Minerva CL 27	+	-	-	-
	Otesani 8	+	-	-	-
	Empress	-	-	-	-
	Blue free	-	-	-	-
	Record	-	-	-	-
	Ialomita	-	-	-	-
	Dobrovica	+	-	-	-
<i>P. persica</i>	Romamer	-	+	-	*
	Harbinger	-	+	-	*
	Splendid	-	+	-	*
	Cresthaven	-	+	+	*
	Miorita	-	+	-	*
	NJ 265	-	+	-	*
	Redhaven	-	+	-	*
	Superba de toamna	-	+	-	*
<i>P. armeniaca</i>	Springgold	-	+	-	*
	Harleyne	-	-	-	*
	Litoral	-	-	-	*
	Dacia	-	-	-	*
	Excelsior	-	-	-	*
	Olimp	-	-	-	*
	Mamaia	-	-	-	*
	Sulina	-	-	-	*
	Comandor	-	-	-	*
	Goldrich	-	-	-	*
	Harcot	-	-	-	*
<i>P. tomentosa</i>	Orient	-	-	-	*
<i>P. salicina</i>	Black Amber	-	-	-	*
<i>P. japonica</i>		+	-	-	*
<i>P. nigra</i>		-	-	-	*
<i>P. spinosa</i>		-	-	-	*

ISOLATION AND IDENTIFICATION OF YEAST SPECIES PRESENTED IN  
BANU MARACINE VINEYARD SOILS

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**KEY WORDS:** yeast strains, types, species, morphological tests, physiological tests

ABSTRACT

*The vineyard center Banu Maracine is one of the main centers of Oltenia where quality wines are obtained. This is largely due to the spontaneous microflora with high biochemical and physiological attributes. The yeast conveyer in this center is very rich, 15 species of yeasts being isolated of which the *Saccharomyces* species predominated. Yeast strains of this type have been tested to highlight the morphological features but also the biochemical and physiological ones.*

INTRODUCTION

Numerous studies have shown that a first step in selecting a yeast, specific for vineyards areas is the knowledge of the yeast flora of the area (Schütz and Gafner, 1994; Sabate et al., 1998; Beltran et al., 2002). These studies concluded that many strains of *Saccharomyces* lead spontaneous alcoholic fermentation and a large share at the end of fermentation. In Lodder's work since 1970 is given a key role in systematic yeast fermentation or physiological characteristics or assimilation, leaving a secondary role to morphological characters and extremely low to the organic ones. Under this new classification, which brought many changes of name, sporogenous yeasts have 25 types with 190 species and 12 types with 170 species of asporogenous yeasts.

To achieve the proposed objective, the aim was to isolate and identify species of yeasts from the plantation soil taken in the research (Banu Maracine).

MATERIALS AND METHODS

The researches were made in the vineyard center Banu Maracine of Oltenia, during 2003-2008. From this vineyard center soil samples were collected for isolation and identification of yeasts. Soil samples were collected from a depth of 10 cm to 35 cm. the preparation of soil samples for analysis: the soil sample is placed in a sterile flask over which is added sterile distilled water. Shake for 5-10 min. on the magnetic stirrer, centrifuge clear liquid, is seeded from the soil extract on solid culture medium.

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Microbiological analysis: the identification of isolated yeasts colonies was done by subjecting them to standard identification tests (Barnett and colab., 1970), based on examination of morphological and physiological features of isolated yeasts in pure culture. This method is applied worldwide in laboratories for the selection of yeasts for wine. Besides classical methods, other modern methods were used, namely: to achieve morphological tests an optical microscope equipped with a video camera was used. The analysis of the images obtained was performed with a computer program (MACE 100), a system of counting, characterization and analysis of microorganisms colonies, a system developed at Yale University, USA.

Culture media used for isolation and identification of yeast strains: YPG enriched medium, culture medium grape, grape growing medium agarised, YM culture medium (yeast extract-malt extract), Gorodkova classic culture medium for testing the ability of sporulation, culture medium for fermentation of sugars, culture medium for sugar assimilation, culture medium for the use of alcohol as the sole source of carbon, culture medium for the use of nitrogen by the yeasts, the presence of cicloxamide.

The shape and dimensions of cells were examined in cultures on standard mediums: solid medium with malt extract MYPG agarised and liquid medium MYPG enriched. To examine the cultural characteristics of liquid medium was used enriched liquid YPG medium and YM. To complete the taxonomic profile the following classic tests were performed: the carbohydrate fermentation and assimilation test, alcohol use as the sole carbon source, nitrate assimilation, arbutine splitting capacity, growth in the absence of vitamins (Lodder, 1934). Studying a strain to identify her implies, first, the obtaining of a pure culture, from a single cell. Solid and liquid culture media are used and a special microbiological technique is applied (Tanner, 1944 cited by Popa A. et al., 1990).

The identification of yeasts (Popa A. et al., 1990) is made having regarded to the following aspects: the shape and the size of cells, the examination of cultural characters, the form and the spore germination, the fermentation and the assimilation of various sugars, the selective fermentation of glucose and fructose, the respiratory and fermentative intensity, the exogenous vitamin requirement, the temperature reaction, the resistance to sulfuric acid, etc.

The identification of yeasts can be achieved through the use of commercial systems (multitest), which are: API 20 C, which gives 95% concordance with conventional methods, system Microdrop -84% concordance and Uni-Yeast-Tek system-99% (Anghel I. et al., 1991).

## RESULTS AND DISCUSSIONS

The study conducted at the vineyard center Banu Maracine during 2003-2008 aimed to isolate and identify strains of yeast. After passing the yeast strains isolated from vineyards soil microflora studied through morphological and physiological tests these were placed in the following types: *Saccharomyces*, *Saccharomycodes*, *Zygosaccharomyces*, *Pichia*, *Hansenula*, *Hanseniaspora*, *Schizosaccharomyces*, *Metschnikowia* from sporogenous yeasts and *Brettanomyces*, *Kloeckera*, *Candida*, *Rhodotorula* from the non-sporogenous yeasts.

The yeast strains belonging to the *Saccharomyces ellipsoideus* species, on microscopic examination, the cells present elliptical or oval shape, isolated or often grouped two by two (Figure 1). Present polar or bipolar budding type. The cell sizes range from (3,8-8,3) x (4,0-9,3)  $\mu\text{m}$ , (4,5-8,7) x (3,9-8,1)  $\mu\text{m}$ , (4,5-8,0) x (4,8-9,6)  $\mu\text{m}$



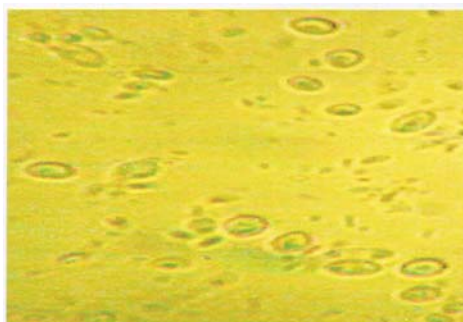


Figure1. *Saccharomyces ellipsoideus*- cells in liquid medium

In liquid medium, in the first days after sowing it becomes cloudy, starting to clear up after 6-7 days, becoming perfectly clear in about a month. Strains belonging to *Saccharomyces ellipsoideus* species, don't form a pellicle or a ring on the medium surface and the deposit can be made very thin, adherent to the sides, tamped or granular deposit, the liquid being very clear.



Figure 2. *Saccharomyces ellipsoideus*- colonies grown on solid medium

On solid culture medium, colonies have developed creamy white, smooth surface, withered in the middle, entire or finely toothed edges (Figure 2), creamy-yellow colour, smooth opaque surface, edges finely festooned or whole, cream and brown, wrinkled central surface, entire edges, slightly wavy. The colonies showed growth protruding, opaque surface, creamy consistency.

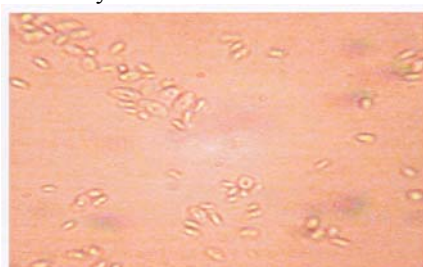


Figure 3. *Saccharomyces ellipsoideus*- sporulation phase cells

Grown on culture medium Goorodkova, the strains formed ascus with 1-4 round spores (Figure 3), and on PDA culture medium don't form mycelium and pseudomycelium.

The yeast strains belonging to the *Saccharomyces oviformis* species, on microscopic examination, the cells have round, oval or weak round oval form, being

isolated, often grouped two by two or short chains and groups, which are polar budding (Figure 4). The cell sizes range from  $(5,5-6,5) \times (7,5-9,5) \mu\text{m}$ ,  $(4,0-5,6) \times (6,2-10,8) \mu\text{m}$ .

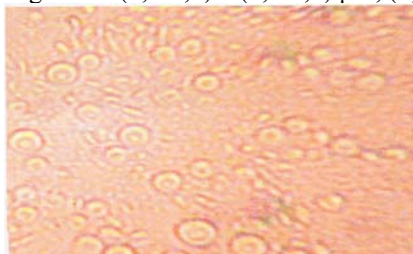


Figure 4. *Saccharomyces oviformis*- cells in liquid medium

In liquid medium, in the first days after sowing it starts to be cloudy, becoming clear after about 60 days. None of the strains belonging to the *Saccharomyces oviformis* species, don't form a pellicle or a ring on the medium surface, and the store formed can be sandy, slightly sandy, showing the tendency to join the sides.

On solid culture medium (Figure 5), the colonies developed a yellow-brown colour, striated surface fully or marginally or cream-white, smooth surface, slightly lobed margins, rizoide here and there.

Grown on culture medium Goorodkova, the strains formed asce with 1-4 round spores, and on PDA culture medium don't form mycelium and pseudomycelium.



Figure 5. *Saccharomyces oviformis*- culture on solid medium

Regarding the physiological peculiarities of yeast strains discussed, it appears that the strains that belonged to the *Saccharomyces ellipsoideus* species ferment glucose, galactose, sucrose, maltose, raffinose (third), the only unfermented sugar is lactose. To test the use of sugars as carbon the isolated strains assimilated the same sugars as for fermentation, the lactose remaining "unassimilated". The yeast strains do not "assimilate" nitrates as nitrogen source, don't use ethylic alcohol as carbon source, don't splits arbutine, don't grow on culture medium with added cycloamide results, the tests being negative.

The strains that belonged to the *Saccharomyces oviformis* species at the sugar fermentation test, they have fermented glucose, sucrose, maltose, raffinose being partially fermented. Unfermented sugars were galactose and lactose. Upon the completion of the test to use sugars as carbon source is noted that the isolated strains assimilated the same sugars as in the case of fermentation, galactose and lactose were not assimilated. In particular cultural medium using nitrate as nitrogen source is not growing, does not assimilate nitrates, the test being negative. The yeast strains do not use ethylic alcohol as carbon source, don't splits arbutine, and will not grow on culture medium with added cicloxamide results, the tests being negative.

## CONCLUSIONS

The isolated yeast strains from vineyard soil center Banu Maracine were taxonomic classified into the following types of which: *Saccharomyces*, *Saccharomycodes*, *Zygosaccharomyces*, *Pichia*, *Hansenula*, *Hanseniaspora*, *Schizosaccharomyces*, *Metschnikowia*, from sporogenous yeasts and *Brettanomyces*, *Kloeckera*, *Candida*, *Rhodotorula* from the non-sporogenous yeasts.

Within these types there were identified 15 species of yeasts of which 10 species: (*Saccharomyces ellipsoideus*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomycodes ludwigii*, *Pichia membranefaciens*, *Hansenula anomala*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Schizosaccharomyces pombe*, *Zygosaccharomyces baillii*), within the sporogenous yeasts and five species *Candida vini*, *Brettanomyces intermedius*, *Kloeckera apiculata*, *Rhodotorula glutinis*, *Rhodotorula rubra*) belonging to the non-sporogenous yeasts.

The best represented vine yeasts from the soil center Banu Maracine were found to be those of the *Saccharomyces* species. The results obtained show that we are found in vineyards areas where yeasts with high technological and physiological traits are well represented.

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ISOLATION AND IDENTIFICATION OF YEAST SPECIES PRESENTED IN  
SEGARCEA VINEYARDS

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**KEY WORDS:** morphological characters, physiological characters, yeasts

ABSTRACT

*Segarcea is a vineyard center with a great potential on cultivating wine grapes, one of the major wine centers of Oltenia. For the finished product, the wine, we need the intervention of spontaneous microflora. Therefore, has to be known the spontaneous microflora and to be studied the characteristics of the isolated yeast strains. From the vineyard soil center of Segarcea nine species of isolated yeast were assigned in seven types. In this paper I shall take the following species of yeasts found in soil samples: Candida vini, Pichia membranefaciens.*

INTRODUCTION

To make a yeast study, the scientists have established that it is necessary to examine the specific spontaneous fermentation of the area studied. The spontaneous fermentation should be compared with a conducted one by inoculation with selected strains to highlight and ensure that the yeast strains involved in spontaneous fermentation are local and specific to the studied area (Constanti M. et al., 1997; Croitoru C., 2008b personal communication ; Van Rensburg P. et al , 2007; Dunoiu A. et al., 2008).

In Europe the most numerous and lengthy studies on the influence of climate were carried out in Italy. From the researches conducted in Perugia and continued by Castelli, and others split the general conclusion: the yeast species presented are very numerous, but the most common (about 80%) are *Kloeckera apiculata* and *Saccharomyces ellipsoideus*, responsables for alcoholic fermentation. After these the most important in terms of frequency are *Saccharomyces rossei*, *Saccharomyces bayanus*, *Saccharomyces oviformis*, *Torulopsis baccularis*, *Saccharomyces mangini*, *Saccharomyces italicus*, etc.

To achieve the proposed objective, the aim was: to isolate and identify species of yeasts from the plantation soil taken in the research (Segarcea).

The yeast strains isolated from the vineyard soil center Segarcea were classified into the types: *Saccharomyces*, *Pichia*, *Hanseniaspora*, *Metschnikowia* from sporogenous yeasts and *Kloeckera*, *Candida* și *Rhodotorula* from the non-sporogenous yeasts.

Within these types there were identified nine species of yeasts: six species (*Saccharomyces ellipsoideus*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Pichia membranefaciens*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima* within the

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sporogenous yeasts and three species (*Candida vini*, *Kloeckera apiculata*, *Rhodotorula glutinis*) belonging to non-sporogenous yeasts.

The overall conclusion of the process of isolation, identification and quantification of the yeasts is that this process is complex and depends on many factors (Fleet and Heard, 1993).

## MATERIALS AND METHODS

The researches were made in the vineyard center Segarcea of Oltenia, during 2003-2008. From this vineyard center soil samples were collected for isolation and identification of yeasts. Microbiological Analysis: identification of isolated yeasts strains was done by subjecting them to standard identification tests (Barnett et al., 1990; Popa A. et al., 2007), based on examination of morphological and physiological features of isolated yeasts in pure culture. This method is applied worldwide in laboratories for selection of yeasts for wine. Besides classical methods, other modern methods were used, namely: to achieve morphological tests an optical microscope equipped with a video camera was used. The analysis of the images obtained was performed with a computer program (MACE 100), a system of counting, characterization and analysis of microorganisms colonies, a system developed at Yale University, USA.

Culture media used for isolation and identification of yeast strains: YPG enriched medium, culture medium grape, grape growing medium agarised, YM culture medium (yeast extract-malt extract), Gorodkova classic culture medium for testing the ability of sporulation, culture medium for fermentation of sugars, culture medium for sugar assimilation, culture medium for the use of alcohol as the sole source of carbon, culture medium for the use of nitrogen by the yeasts, the presence of cicloxamide.

The shape and dimensions of cells were examined in cultures on standard mediums: solid medium with malt extract MYPG agarised and liquid medium MYPG enriched. To examine the cultural characteristics of liquid medium was used enriched liquid YPG medium and YM. To complete the taxonomic profile the following classic tests were performed: the carbohydrate fermentation and assimilation test, alcohol use as the sole carbon source, nitrate assimilation, arbutine splitting capacity, growth in the absence of vitamins (Lodder J., 1970). Studying a strain to identify her implies, first, the obtaining of a pure culture, from a single cell. Solid and liquid culture media are used and a special microbiological technique is applied (Tanner F.W., 1944, Popa A. et al., 1990).

The identification of yeasts (Popa A. et al., 1990) is made having regarded to the following aspects: the shape and the size of cells, the examination of cultural characters, the form and the spore germination, the fermentation and the assimilation of various sugars, the selective fermentation of glucose and fructose, the respiratory and fermentative intensity, the exogenous vitamin requirement, the temperature reaction, the resistance to sulfuric acid.

## RESULTS AND DISCUSSIONS

The study conducted at the vineyard center Segarcea during 2003-2008 aimed to isolate and identify strains of yeasts by crossing isolates strains through morphological and physiological tests. Here were identified the following types: *Saccharomyces*, *Pichia*, *Hansenula*, *Hanseniaspora*, *Metschnikowia* from sporogenous yeasts and *Kloeckera*, *Candida*, *Rhodotorula* from the non-sporogenous yeasts.

The yeast strains belonging to the *Candida vini* species, on microscopic examination, the cells present elliptical shape, oval, sometimes cylindrical, being grouped

or in short chains, which present multilateral budding. The cell sizes range from (3,5 - 4,9) x (3,2 - 8,3)  $\mu\text{m}$ .

In liquid medium, it remains cloudy, and on the surface it forms a pleated pellicle, having white or yellowish colour in stemming fragments, that falling to the bottom of the vessel, leading to the formation of a slightly sticky deposit that goes through a simple motion to the liquid disorder (Figure 1).

On solid culture medium, the developed colonies presented a white-gray or gray-brown colour smooth surface, slightly pleated (Figure 2).

Grown on Goorodkowa culture medium, the strains did not form spores, and on PDA culture medium, 15 strains form the pseudomycelium.



Figure 1. *Candida vini*-Cells in liquid medium.

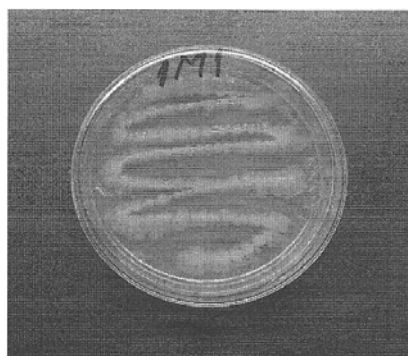


Figure 2. *Candida vini*-Colonies grown on solid medium

The yeast strains belonging to the *Pichia membranefaciens* species, grown in liquid medium for 3 days at 25 ° C, on microscopic examination, the cells have oval shape, elongated even cylindrical. The cells are found in simple or branched chains of cells. The cell sizes range from (3,1-4,8) x (5,7-10,3)  $\mu\text{m}$ .

In liquid medium, it is noted that it remains cloudy at the end of fermentation. The strains form on the liquid's surface a ring and a pleated white pellicle, the smell of ethyl acetate and formed store is adhering to the bowl's sides (Figure 3).

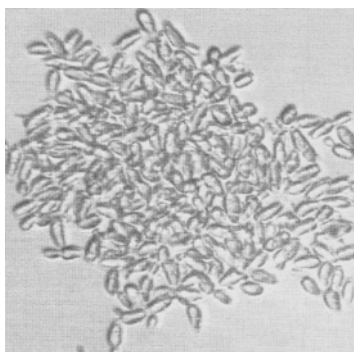


Figure 3. *Pichia membranefaciens* – cells in liquid medium

On solid culture medium, the developed colonies presented a white or creamy colour, the surface is matt, raised, partially or completely folded, with rizoidal edge (Figure 4)

In sporulation environment, the isolated strains have ascus formed with 1-4 ascospores and round shape. The Isolated strains formed the characteristic pseudomycelium on PDA medium.

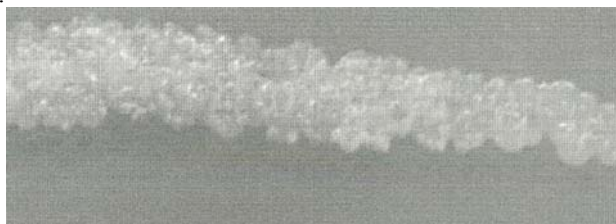


Figure 4. *Pichia membranefaciens* – culture on solid medium

The strains that belonged to the *Candida vini* species at the sugars fermentation test, it is noticed that these haven't fermented any of the tested sugars, the tests being negative. After the completion of the test using sugars as carbon source, it was noticed that isolated strains assimilated only glucose; the remaining sugars (galactose, sucrose, maltose, lactose and raffinose) were not assimilated, the test being negative.

In a particular medium, the use of nitrates as nitrogen source, they do not grow, do not assimilate nitrates, the test being negative. The yeast strains use ethylic alcohol as carbon source (when in the medium is found only ethylic alcohol), the test being positive. They don't splits the arbutine, and will not grow on culture medium with added cicloxamide, the tests being negative.

At the test of the fermentation of sugars, it was noticed that some strains which belonged to *Pichia membranefaciens* species (not all), it ferments only glucose. Galactose, sucrose, maltose, lactose and rafmose were not fermented. To test the use of sugars as carbon source, only isolated strains assimilated glucose, the rest of the sugars remained unassimilated.

The yeast strains do not assimilate nitrates as nitrogen source, the test being negative. This test is very important because it distinguishes the *Pichia* species of the *Hansenula* species, which uses the nitrates as a nitrogen source, in this case the test being positive. They do not use ethylic alcohol as carbon source, don't splits arbutine, and will not grow on culture medium with added cicloxamide, the tests being negative.

## CONCLUSIONS

The isolated yeast strains from vineyard soil center Segarcea in 2003-2008 period, were taxonomic classified into the following types of which: *Saccharomyces*, *Pichia*, *Hanseniaspora*, *Metschnikowia* from sporogenous yeasts and *Kloeckera*, *Candida* și *Rhodotorula* from the non-sporogenous yeasts.

Within these types there were identified 9 species of yeasts of which 6 species (*Saccharomyces ellipsoideus*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Pichia membranefaciens*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima* ), within the sporogenous yeasts and three species (*Candida vini*, *Kloeckera apiculata*, *Rhodotorula glutinis*) belonging to the non-sporogenous yeasts.

In this paper I chose to treat two significant yeast species from Segarcea area: *Candida vini* and *Pichia membranefaciens*. Microscopic examination was followed, where the species *Candida vini* strains showed several forms and were grouped in chains. A deposit was formed on liquid medium, forming colonies on solid medium with polished surface and in the sporulation environment did not form spores.

Strains of *Pichia membranefaciens* species have a characteristic shape; the cells are placed in chains. In liquid medium are forming a ring and white film and odor, formed colonies on solid medium, white or cream, and on sporulation medium formes ascospores.

Tested physiologically, the strains of *Candida vini* assimilates only glucose, the test of using nitrate as nitrogen source is negative, don't splits the arbutine, will not develop in culture medium with added cicloxamide. Some strains of *Pichia membranefaciens* to test the fermentation of sugars, ferment only glucose, and to test the use of sugars as carbon source. The strains not assimilate nitrates as nitrogen source, do not use alcohol as carbon source, it cleaves arbutine, do not grow on medium with added cicloxamide.

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THE YEAST'S CONVEYER STRUCTURE FROM THE VINEYARD SOIL  
CENTER BANU MARACINE

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**KEY WORDS:** soil, distribution, weight, yeasts

**ABSTRACT**

*The researches were conducted in the vineyard center Banu Maracine, during 2004 and 2007. In these years were taken soil samples that identified the presented yeast strains (14 strains in 2004 and 9 strains in 2007). Then was made the distribution by years of isolated strains from the soil, the distribution of isolated strains in 2004, the proportion of strains in May and September (which coincides with the harvest soil samples) and distribution of isolated strains from the soil in 2007.*

**INTRODUCTION**

Over the years, the scientists have shown that a soil vineyard, which is loaded with yeast spores collected from the fallen grains, can cause alcoholic fermentation of unfermented wine. At the end of grape harvest, much of the yeasts die, others are soil resistant as spores, others are taken by winter insects (bees, wasps, drosophiles) and will invade again the environment next year (Vasserot et al, 2001; Baoshan, 2005; Popa, 2007, Dunoïu et al., 2008).

To achieve the proposed objective, the aim was: the distribution of yeasts according to the environment in which it was collected and the climate year.

**MATERIALS AND METHODS**

The researches were conducted in vineyard center Banu Maracine, in Oltenia, during 2004 and 2007. From this vineyard center soil samples were collected for the isolation and identification of yeasts.

Microbiological analysis: the identification of isolated yeasts strains was done by subjecting them to standard identification tests (Barnett et al., 1990), based on examination of morphological and physiological features of isolated yeasts in pure culture (Popa et al., 2005). This method is applied worldwide in laboratories for the selection of yeasts for wine. Besides classical methods, other modern methods (Dunoïu et al., 2008) were used, namely: to achieve morphological tests an optical microscope equipped with a video camera was used. The analysis of the images obtained was performed with a computer program (MACE

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100), a system of counting, characterization and analysis of microorganisms colonies, a system developed at Yale University, USA.

The identification of yeasts can be achieved through the use of commercial systems (multitest), which are: API 20 C, which gives 95% concordance with conventional methods, system Microdrop -84% concordance and Uni-Yeast-Tek system-99% (Anghel et colab., 1991).

## RESULTS AND DISCUSSIONS

To highlight the differences of the influences of climate factors, we chose the years 2004 and 2007, different years in terms of climate.

The soil and climatic conditions in the vineyard center Banu Maracine, during 2004 and 2007, from the soil samples were isolated 23 strains of yeasts, of which 14 strains, which represent 61%, that were found in 2004 and 9 strains representing 39% in 2007 (Table 1).

From the soil samples collected in 2004 were isolated a number of 14 strains of yeasts, that represent 61% of the total yeasts isolated in 2004 and 2007 (Figure 1).

Table 1

The Distribution by years of isolated yeast strains from vineyard soil samples in the Banu Maracine center

The yeast species	Soil 2004	<i>Soil 2007</i>
<i>Saccharomyces ellipsoideus</i>	2	<i>1</i>
<i>Saccharomyces oviformis</i>	1	<i>1</i>
<i>Saccharomyces rosei</i>	2	<i>1</i>
<i>Saccharomycodes ludwigii</i>	-	-
<i>Candida vini</i>	1	<i>1</i>
<i>Pichia membranefaciens</i>	2	<i>1</i>
<i>Hansenula anomala</i>	1	<i>1</i>
<i>Brettanomyces intermedium</i>	-	-
<i>Kloeckera apiculata</i>	2	<i>1</i>
<i>Hanseniaspora uvarum</i>	1	<i>1</i>
<i>Metschnikowia pulcherrima</i>	1	-
<i>Schizosaccharomyces pombe</i>	-	-
<i>Zygosaccharomyces baillii</i>	-	-
<i>Rhodotorula glutinis</i>	1	-
<i>Rhodotorula rubra</i>	-	<i>1</i>
<i>Total</i>	<i>14</i>	<i>9</i>

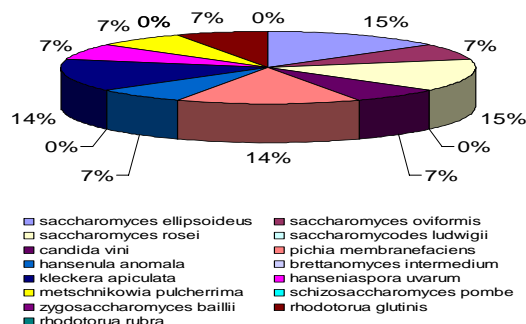


Figure 1. The Distribution of isolated yeast strains from Banu Maracine's soil (2004).

The soil samples collected in May 2004 (when the monthly average temperature recorded the value of 15.6 ° C and the rainfall amount was 28.8 mm/m<sup>2</sup>) from 10 to 15 cm depth, were isolated:

- one *Saccharomyces ellipsoideus* strain
- one *Saccharomyces rosei* strain
- one *Hansenula anomala* strain
- one *Kloeckera apiculata* strain
- one *Metschnikowia pulcherrima* strain
- one *Rhodotorula glutinis* strain; and in September, when monthly average temperature reached 17.2 ° C, and the amount of the rainfall was 80.9 mm/m<sup>2</sup>, were isolated:
- one *Saccharomyces ellipsoideus* strain
- one *Saccharomyces oviformis* strain
- one *Saccharomyces rosei* strain
- one *Candida vini* strain
- two *Pichia membranaefaciens* strains
- one *Kloeckera apiculata* strain
- one *Hanseniaspora uvarum* strain

From the things mentioned above and the graphs represented below (Figures 2 and 3) is noticed that in May compared with September, the yeast strains belonging to the *Candida* and *Pichia* type are missing, instead the yeast of strains *Rhodotorula glutinis* and *Metschnikowia pulcherrima* species appear. Of all the yeasts isolated in September, *Pichia membranaefaciens* was found in 25%, and *Candida vini* in 12,5%. Overall, in 2004, from the soil samples, it is noted that the share is held by the sporogenous yeasts (71%), the non-sporogenous being isolated in 29%.

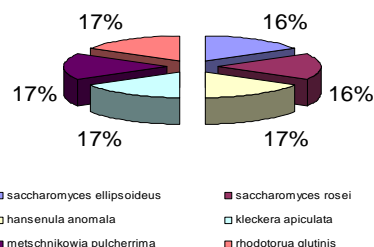


Figure 2. The share of isolated yeast strains from the soil samples in May, in the vineyard center Banu Maracine

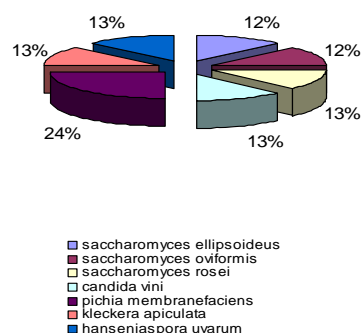


Figure 3. The share of isolated yeast strains from the soil samples in September, in the vineyard center Banu Maracine

From the soil samples collected in 2007, a number of nine strains of yeasts were isolated, representing 39% of the total isolated yeasts in 2004 and 2007. The below figure notes the distribution on the isolated yeast strains species (Figure 4).

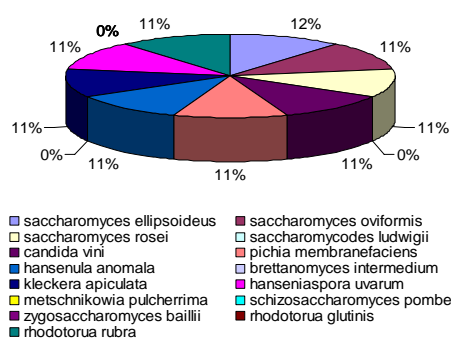


Figure 4. The distribution of isolated yeast strains from the Banu Maracine soil (2007)

Although 2007 was characterized by lower rainfall than 2004, were isolated from the soil samples only 39% of the total isolated yeasts from the soil samples during the years of study, it is noticed however, that the share was held by the sporogenous yeasts that had a percentage of 67%, while non-sporogenous yeasts had a percentage of 33%.

The large share of sporogenous yeast species can be explained by the fact that the soil samples were collected in September, were characterized by monthly average temperature of 15.8 C and rainfalls of 65.6 mm/m<sup>2</sup>, values of climatic elements that favored the emergence sporogenous yeast species.

Due to the different climatic conditions in those two years (2004 and 2007) studied, it varied also the number of isolated strains from the soil; in 2007 being less isolated yeast strains compared with 2004.

Although during 2004 were isolated more yeast strains compared with 2007, it can be seen significant presence of *Candida vini* (50%) and *Pichia membranefaciens* (67%) strains, their development being driven by heavy rainfall. In 2007, which was characterized by lower rainfall compared with 2004, it appears that yeast strains belonging to *Candida*

and *Pichia* species were isolated in 50%, respectively 33% and occur strains of yeast *Rhodotorula rubra* species that were missing in 2004.

## CONCLUSIONS

The influence of pedoclimatic factors of the viticulture area is given by the weight of these micro-organisms, ranging from one year to another and from one vineyard center to another.

Overall we can see that in the vineyard center Banu Maracine sporogeneous species predominate, the non-sporogeneous being isolated in smaller numbers.

So far the conclusion of the researches is that the most selected yeasts would be the ones in the vineyard center that uses it, resulting the need for major vineyards regions in Romania, to study the yeast conveyor and to select the strains with the most important characteristics .

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THE YEAST'S CONVEYER STRUCTURE FROM THE VINEYARD SOIL  
CENTER SEGARCEA

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**KEY WORDS:** *distribution, weight, yeasts, vineyard center*

**ABSTRACT**

*Vineyard Center Segarcea is a traditional center for its wines with special qualities. To their fermentation it seems that special contributions have the yeasts of spontaneous microflora of the area. Therefore, the yeasts must be studied, especially their distribution, which is known to be done according to the living environment and climatic conditions. This paper summarizes data on the distribution by years of isolated yeast strains from the soil, weight of stems in May and September (which coincides with the soil samples), the distribution of isolated strains in the two years and the weight of the yeasts species compared, in 2004 and 2007.*

**INTRODUCTION**

The results of all researches conducted on the entire globe shows that in the microfloral vineyard the same ecological balance between different species of yeasts (Ribereau-Gayon and Peynaud, 1960; Van Rensburg et al, 2007; Cotea, 2006).

The need to produce wines that have flavor qualities, food value, robustness and higher preservation, determined me to explore the structure of the yeast conveyer with oenological interest from the vineyard soil center Segarcea, a specific spreading area of a specimen with a great vocation for quality. This vocation seems to be determined also by the microbial structure of oenological interest.

In Oltenia first such work was undertaken by Felicia Dragomir in 2001. To achieve the proposed objective, the aim was: the distribution of yeasts according to the environment in which they were collected and the climate year in the vineyard center Segarcea.

**MATERIALS AND METHODS**

The researches were made in the vineyard center Segarcea in Oltenia, during 2004 and 2007. From this vineyard center soil samples were collected for the isolation and the identification of yeasts for their distribution. The soil samples were collected from a depth of 10 cm to 35 cm, were taken from the base, middle and top of slope.

The preparation of the soil samples for analysis: the soil sample is placed in a sterile flask and then is added sterile distilled water. Shake for 5-10 min. On the magnetic

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stirrer centrifuge clear liquid, is seeded from the soil extract on solid culture medium. Microbiological analysis: the identification of isolated yeasts strains was done by subjecting them to standard identification tests (Barnett et al., 1990), based on the examination of morphological and physiological features of isolated yeasts in pure culture. This method is applied worldwide in laboratories for the selection of yeasts for wine. Besides classical methods (Lodder, 1970; Barnett et al., 1990), other modern methods (Cretu, 2006) were used, namely: to achieve morphological tests an optical microscope equipped with a video camera was used. The analysis of the images obtained was performed with a computer program (MACE 100), a system of counting, characterization and analysis of microorganisms colonies, including mamaliene cells, bacteria, yeasts and molds, a system developed at Yale University, USA (Popa and Giurgiulescu, 2007; Dunoiu et al., 2008).

For identification various culture media have been used (Anghel et al., 1991).

## RESULTS AND DISCUSSIONS

From the soil samples collected from vineyard center Segarcea in the studied years (2004 and 2007), were isolated a total of 18 strains of yeasts, including seven in 2004 (39%) and 11 in 2007 (61%) (Table1).

Table 1.

The Distribution by years of isolated yeast strains from vineyard soil samples in Segarcea center

The yeast species	Soil 2004	Soil 2007
<i>Saccharomyces ellipsoideus</i>	2	3
<i>Saccharomyces oviformis</i>	1	2
<i>Saccharomyces rosei</i>	-	1
<i>Saccharomycodes ludwigii</i>	-	-
<i>Candida vini</i>	-	-
<i>Pichia membranefaciens</i>	-	-
<i>Hansenula anomala</i>	1	1
<i>Brettanomyces intermedium</i>	-	-
<i>Kloeckera apiculata</i>	1	1
<i>Hanseniaspora uvarum</i>	1	1
<i>Metschnikowia pulcherrima</i>	-	1
<i>Schizosaccharomyces pombe</i>	-	-
<i>Zygosaccharomyces baillii</i>	-	-
<i>Rhodotorula glutinis</i>	1	1
<i>Rhodotorula rubra</i>	-	-
<i>Total</i>	7	11

The soil samples collected in May 2004 (when the monthly average temperature recorded the value of 15.9 ° C and the rainfall amount was 54 mm/m<sup>2</sup>) from 10 to 15 cm depth, were isolated:

- one *Saccharomyces ellipsoideus* strain
- one *Saccharomyces oviformis* strain
- one *Hansenula anomala* strain
- one *Hanseniaspora uvarum* strain



- one *Rhodotorula glutinis* strain; and in September, when monthly average temperature reached 17.5° C, and the amount of the rainfall was 12 mm/m<sup>2</sup>, were isolated:
- one *Saccharomyces ellipsoideus* strain
- one *Kloeckera apiculata* strain

The soil samples collected in May 2007 (when the monthly average temperature recorded the value of 18.6° C and the rainfall amount was 47 mm/m<sup>2</sup>) from 10 to 15 cm depth, were isolated:

- one *Saccharomyces ellipsoideus* strain
- one *Saccharomyces oviformis* strain
- one *Kloeckera apiculata* strain
- one *Metschnikowia pulcherrima* strain
- one *Rhodotorula glutinis* strain; and in September, when monthly average temperature reached 16° C, and the amount of the rainfall was 19 mm/m<sup>2</sup>, were isolated:
- two *Saccharomyces ellipsoideus* strains
- one *Saccharomyces oviformis* strain
- one *Saccharomyces rosei* strain
- one *Hansenula anomala* strain
- one *Hanseniaspora uvarum* strain

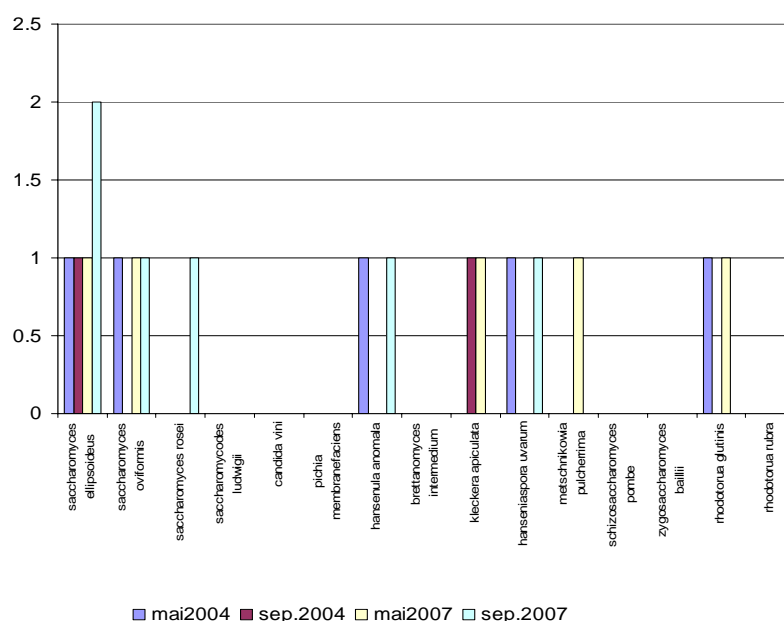


Figure1. The distribution of isolated yeast strains from the soil samples in May and September in 2004 and 2007, in the vineyard center Segarcea

From the things mentioned above, and the graphic representation of fig.1. , where the distribution of isolated yeast species were presented from the soil samples in May and September in 2004 and 2007, it can be seen that from the total of isolated yeasts in 2004, 71% (5 strains) were found in May , predominantly sporogenous yeast species and 29% (2 strains) in September when nonsporogenous yeasts prevailed. In 2007, in May were found

45% (5 strains) of all isolated yeast (11 strains), predominantly sporogenous yeasts and in September were isolated 55% (6 strains), predominantly sporogenous species.

Table 2.  
The share of isolated yeast species in 2004 and 2007 from the soil samples in the vineyard center Segarcea

The yeast species	Soil 2004	Soil 2007
<i>Saccharomyces ellipsoideus</i>	40	60
<i>Saccharomyces oviformis</i>	33	67
<i>Saccharomyces rosei</i>	-	100
<i>Saccharomycodes ludwigii</i>	-	-
<i>Candida vini</i>	-	-
<i>Pichia membranefaciens</i>	-	-
<i>Hansenula anomala</i>	50	50
<i>Brettanomyces intermedium</i>	-	-
<i>Kloeckera apiculata</i>	50	50
<i>Hanseniaspora uvarum</i>	50	50
<i>Metschnikowia pulcherrima</i>	-	100
<i>Schizosaccharomyces pombe</i>	-	-
<i>Zygosaccharomyces baillii</i>	-	-
<i>Rhodotorula glutinis</i>	50	50
<i>Rhodotorula rubra</i>	-	-

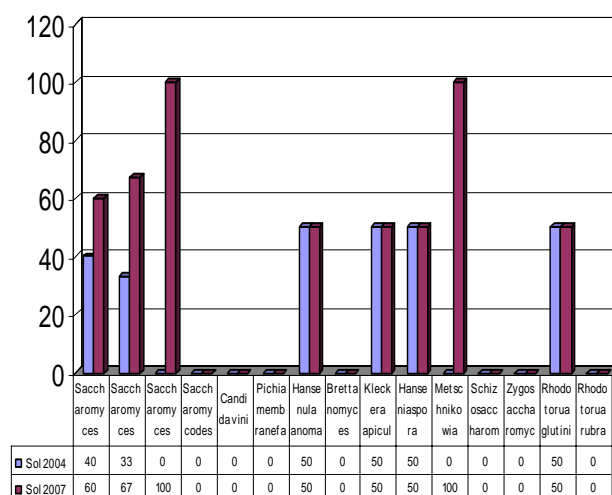


Figure 2. The share of isolated yeast species in 2004 and 2007 from the soil samples in the vineyard center Segarcea

The distribution of yeast strains species found in the soil samples in 2004 and 2007 is as it follows:

- *Saccharomyces ellipsoideus* 5 strains (40% in 2004 and 60% in 2007)
- *Saccharomyces oviformis* 3 strains (33% in 2004 and 67% in 2007)
- *Saccharomyces rosei* 1 strain (isolated in 2007)

- Hansenula anomala* 2 strains (50% in 2004 and 50% in 2007)
- Kloeckera apiculata* 2 strains (50% in 2004 and 50% in 2007)
- Hanseniaspora uvarum* 2 strains (50% in 2004 and 50% in 2007)
- Metschnikowia pulcherrima* 1 strain (isolated only in 2007)
- Rhodotorula glutinis* 2 strains (50% in 2004 and 50% in 2007) (Figure 2, Table 2).

## CONCLUSIONS

The influence of pedoclimatic factors of the viticulture area is given by the weight of these micro-organisms, ranging from one year to another and from one vineyard center to another.

The results of all researches of this kind conducted in Europe are similar to those obtained in Japan (Yokotsuka) Argentina (Mendoza-Vega Area), California (Mrak, Phaff, McCloung) which shows that on the entire globe is reflected in the microfloral vineyard the same ecological balance between different species of yeasts.

Important is that from the total yeast that pass in unfermented wine, 1-10% belong to the fermentation genus *Saccharomyces*, while a large share belong to *Kloeckera*, *Hanseniaspora*, *Torulopsis*, *Hansenula*, *Pichia*, *Candida* (Benda J., 1983).

It is necessary to deepen these researches and their extension throughout the entire vineyard area of Oltenia.

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**RESEARCH CONCERNING GRAPE YIELD IN THE HILLY AREA FROM THE  
EASTERN ROMANIA UNDER THE CLIMATE CHANGE DURING  
THE PERIOD 2007-2009**

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**KEY WORDS:** *Yield, grapes, Eastern Romania, climate change*

**ABSTRACT**

*The research is focused on 22 major climate factors and grape yield for the Vine types: Feteasca Alba, Feteasca Regala, Aligote, Sarba, Cabernet Sauvignon, Merlot, Babeasca Neagra, Feteasca Neagra, Chasselas Dore, Hamburg Muscat and Black selected Coarna in the hilly area from the Eastern Romanian, at Bujory Vineyard, Galatzi County in the period 2007-2009. The Index, Gain, Share and Comparison Methods have allowed the identification of the most important climate factors, the estimation of the production differences from a year to another and the type hierarchization. As a conclusion, the year 2008 was a dry one but 2009 was an excessive dry year compared to 2007. Grape yield was higher in 2008 and 2009 compared to 2007 but in 2009 its level was lower compared to 2008 for the most of Vine types.*

**INTRODUCTION**

Climate change affects agricultural ecosystems more and more (Anderson, 2008; Chiriac, 2007). Year by year, in viticulture, climate factors have an important impact upon Vine growing, grape production and quality, economic efficiency of the plantations (Enache, 2008, 2009). This aspect has determined the intensification of research work upon climate factors monitoring, Vine growing areas, creation of new types resistant to drought and pests, measures for fighting against drought (Hayes, 2007; Jones, 2007; Popescu, 2008; Schultz, 2008; Simion, 2008).

In this context, the purpose of the paper is to analyse the evolution of climate factors and grape yield in the vine plantations at Bujoru Vineyard, Galatzi County, situated in the hilly area of the Eastern Romania, within the partnership research project entitled „Research concerning the Impact of Global Climate Change upon viticultural ecosystems from the hilly area”, running during the period 2007-2010 (www.cnmp.ro 2007).

**MATERIAL AND METHODS**

The research work is carried out at Bujoru Research and Development Station for Viticulture and Vinification, Galati County, in the period 2007-2009. The experiments are running on 148 ha of Vine plantations belonging to 11 types for producing red and white wines as follows: Feteasca Alba, Feteasca Regala, Aligote, Sarba, Cabernet

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Sauvignon, Merlot, Babeasca Neagra, Feteasca Neagra, Chasselas Dore, Hamburg Muscat, Coarna Neagra Selectionata.

Grape Yield has been registered and analyzed year by year in close relationship with the evolution of climate factors.

The 22 climate factors are: global, active and useful thermic balance, annual rainfalls and during the vegetation period, annual average temperature, temperatures from the months of July, August and September, air minimum temperature, average temperature of August, average temperature of the 1st and 2nd decades of June, wind speed, air humidity, length of bioactive period, nebulosity, number of days with maximum temperatures, real heliothermic index, hydrothermic index, bioclimatic index of Vine and oenoclimatic index.

The level of climate factors has been studied from a year to another, identifying the differences, gains and establishing the weight of climate factors with a deep impact upon grape yield. The data are provided by the Meteo Station belonging to Bujoru Vineyard.

The year 2007 is considered the Control variant (M) and the years 2008 and 2009 are experimental variants (E1 and E2).

In order to process the data, the following mathematical methods are used:

-Index Method for calculating the individual indices of the 22 climate factors, according to the formula:  $R_i = X_{Ci}/X_{Bi}$ , where:  $R$  = individual index of  $i$  climatic factor,  $i=1, \dots, 22$ ; if  $R_i > 1$ , the factor is increasing, if  $R_i < 1$ , the factor is decreasing and if  $R_i = 1$ , the analysed factor is constant.

-Gain Method, based on the formula:  $S_i = R_i - 1$ , where  $S_i$  = gain of the  $i$  climate factor; if  $S_i > 1$ , the factor is increasing; if  $S_i < 1$ , the factor is decreasing and if  $S_i = 1$ , then the factor is constant.

-Share Method, based on the formula:  $P_i = |S_i| * 100 / \sum |S_i|$ , where  $P_i$  = the share of the climate factor.

-Point Method for establishing the Vine types hierarchy.

-Comparison Method for analysing the evolution of climate factors and grape yield from a year to another.

## RESULTS AND DISCUSSIONS

**Climate factors.** In the year 2008, lower rainfalls and higher temperatures than in 2007 have been noticed. Thermic balance, average annual temperatures, average temperature of the month of August, wind speed, air moisture and bioclimate index registered higher values in the year 2008 compared to 2007. The decreasing order of the climate factors based on their importance is: annual rainfalls, wind speed, number of days with temperatures higher than 30 degrees, average temperature of July and in the first decades of June, hydrothermic index, rainfalls during the vegetation period and heliothermic index.

In 2009, global and active thermic balance, average temperature of July and August, air minimum temperature and at the soil surface, average temperature in the first and second decades of June, number of days with higher temperatures than 30 degrees, real helio thermal index, Vine bio climate index registered lower values compared to the year 2007. Higher values than in 2007 have been noticed for: sunstroke during the vegetation period, average temperature in September, length of bioactive period, index of bio climate amplitude. The hierarchy of the climate factors based on their importance in 2009 is: annual rainfalls, average annual temperature, number of days with maximum temperatures over 30 degrees, air minimum temperature, average temperature in August, rainfalls during the vegetation period, hydro thermal index, minimum temperature at soil surface, global thermal balance, average temperature in September and bio climate index,

active thermal balance, maximum temperature in August, average temperature in the 1st and 2nd decades of June, index of Vine climate amplitude .

Table 1

Comparison of Climate Factors in the years 2007-2008 in the Bujoru Hilly Area

	M 2007	E 2008	E –M	Individual Index	Gain S <sub>i</sub>	Share P <sub>i</sub>
Global thermic balance (Σt°g)	3,671.5	3,694.9	+23.4	1.006	0.64	0.303
Active thermic balance (Σt°a)	3,618.3	3,645.3	+27	1.007	0.75	0.355
Useful thermic balance (Σt°u)	1,858.3	1,875.3	+17	1.009	0.91	0.431
Σ Annual rainfalls (mm)	554.4	285.7	-268.7	0.515	-48.47	22.948
Σ Rainfalls during the vegetation period (mm)	254.5	224.8	-29.7	0.883	11.47	5.525
Σ Sunstroke during the vegetation period	1,477.4	1,332.7	-144.7	0.902	9.79	4.635
Average annual temperature, °C	13.5	13.4	-0.1	1.037	3.70	1.752
Average Temperature of July, °C	28.1	24.0	- 4.1	0.854	-14.59	6.907
Average Temperature of August, °C	25.5	25.9	+0.4	1.015	1.57	0.743
Average Temperature of September ,°C	17.4	17.1	-0.3	0.982	-1.72	0.814
Air minimum temperature , °C	-13.0	-14.5	+1.5	1.110	7.41	3.508
Maximum average temperature of August ,°C	30.6	32.0	+1.4	1.045	4.58	2.168
Average temperature in the 1st and 2nd decades of June	25.2	21.9	-3.3	0.869	-13.10	6.020
Wind Speed (km/hour)	2.3	2.99	0.69	1.304	30.43	14.407
Air Moisture (%)	57	62.26	5.26	1.052	5.26	2.490
Nebulousness	5.6	5.99	0.39	1.071	7.14	3.380
Number of days with maximum temperatures > 30 °C	66	52	-14	0.787	-21.21	10.042
Length of bioactive period , days	178	177	-1	0.994	-0.56	0.265
Real heliothermic index	2.75	2.50	-0.25	0.909	-9.09	4.304
Hydrothermic Index	0.7	0.6	-0.1	0.857	-14.29	67.65
Bioclimatic index	11.5	11.8	+0.3	1.026	2.61	1.236
Oenoclimatic index	5,091.2	5,003.2	-88	0.982	-1.73	0.819
					Σ  S <sub>i</sub>   = 211.22	100.00

**Grape Yield** was higher in the year 2008 compared to 2007 for all the types, reflecting a favorable influence of climate factors. It has varied between 13,188 kg/ha for Feteasca Regala and 7,547 kg/ha for Babeasca Neagra . The decreasing order of the types based on grape yield in 2008 is : Feteasca Regala, Chasselas Dore, Hamburg Muscat, Coarna Neagra, Merlot, Fetesaca Alaba, Aligote, Feteasca Neagra , Cabernet Sauvignon and Babeasca Neagra.

In 2009, grape yield was higher than in 2007 for all the types, except for Cabernet Sauvignon, which registered a lower production. The highest average production was recorded by Chasselas Dore 21,510 kg/ha and the lowest grape yield, 9.000 kg/ha, was registered for Cabernet Sauvignon. In 2009, grape yield was lower than its level registered in the year 2008.

Table 2

Comparison of Climate Factors in the period 2007-2009 in the Bujoru Hilly Area

Climate Factor	M 2007	E 2009	E - M	Individual Index	Gain S <sub>i</sub>	Share P <sub>i</sub>
Global thermic balance ( $\Sigma t^{\circ}g$ )	3,671.5	3,664.2	-7.3	0.998	-0.002	0.030
Active thermic balance ( $\Sigma t^{\circ}a$ )	3,618.3	3,510.9	-107.4	0.907	-0.093	1.418
Useful thermic balance ( $\Sigma t^{\circ}u$ )	1,858.3	1,660.9	-197.4	0.893	-0.107	1.632
$\Sigma$ Annual rainfalls (mm)	554.4	0	-554.4	0	-1	15.253
$\Sigma$ Rainfalls during the vegetation period (mm)	254.5	172.4	-82.4	0.677	-0.323	4.926
$\Sigma$ Sunstroke during the vegetation period	1,477.4	1,560.6	-83.2	1.056	+0.056	0.854
Average annual temperature, °C	13.5	-	-13.5	0	-1	15.253
Average Temperature of July, °C	28.1	23.8	-4.3	0.846	-0.154	2.348
Average Temperature of August, °C	25.5	22.1	-3.4	0.866	-0.134	2.043
Average Temperature of September, °C	17.4	17.2	-0.2	0.988	-0.012	0.183
Air minimum temperature, °C	-13.0	-15.2	-2.2	1.169	+0.169	2.577
Maximum average temperature of August, °C	30.6	30.8	+0.2	1.006	+0.006	0.091
Average temperature in the 1st and 2nd decades of June	25.2	20.5	-4.7	0.813	-0.817	12.461
Wind Speed (km/hour)	2.3	2.5	+2.2	1.086	+0.086	1.311
Air Moisture (%)	57	67	+10	0	-1	15.253
Nebulousness	5.6	3.3	-2.3	0.589	-0.411	6.269
Number of days with maximum temperatures > 30 °C	66	44	-22	0.666	-0.334	5.094
Length of bioactive period, days	178	186	+8	1.004	+0.004	0.061
Real heliothermic index	2.75	2.59	-0.16	0.941	-0.059	0.899
Hydrothermic Index	0.7	0.49	-0.21	0.700	-0.300	4.575
Bioclimatic index	11.5	17	+5.5	1.478	+0.478	7.291
Oenoclimatic index	5,091.2	5,149.1	+57.9	1.011	0.011	0.167
					$\Sigma  S_i $ =6.556	Total 100

Table 3

The Hierarchy of types based on Grape Yield in the period 2007-2009

Vine Type	2007-M Grape Yield/ Position	2008-E1 Grape Yield/Position	2009-E2 Grape Yield / Position	E1/M	E2/M
Feteasca Alba	5,307/3	8,300/7	7,648/4	156.39	144.11
Feteasca Regala	7,119/1	13,188/1	5,376/9	185.25	75.51
Cabernet Sauvignon	2,720/9	7,650/10	5,620/8	281.25	206.61
Hamburg Muscat	1,163/11	11,438/4	9,332/1	983.49	802.40
Coarna Neagra	1,350/10	9,010/5	8,108/3	667.40	600.59
Merlot	2,888/8	8,800/6	6,664/6	304.70	230.74
Babeasca Neagra	3,677/5	7,547/11	4,524/11	205.24	123.03
Feteasca Neagra	3,515/6	8,000/9	5,340/10	227.59	151.92
Chasselas Dore	4,000/4	13,000/2	8,444/2	325.00	211.10
Aligote	6,290/2	8,200/8	6,948/5	130.36	110.46
Sarba	3,500/7	11,700/3	6,088/7	334.28	173.94

### CONCLUSIONS

1. The year 2008 was, in general, a dry one, with lower rainfalls than in 2007 and higher temperatures than in 2007.

2. The year 2009 could be considered an excessive dry one, characterized by insufficient rainfalls and higher temperatures than in 2007.

3. The climate factors with a major impact in 2008 have been: annual rainfalls, wind speed, number of days with higher temperatures than 30 degrees, average temperature in July and in the 1st and 2nd decades of June, hydrothermic index, rainfalls during vegetation period and heliothermic index.

4. In 2009, the order of climate factors based on their importance is: annual rainfalls, annual average temperature, number of days with maximum temperatures over 30 degrees, air minimum temperature, average temperature in August, rainfalls during the vegetation period, hydro thermal index, minimum temperature at soil surface, global thermal balance, average temperature in September and bioclimatic index, active thermal balance, maximum temperature in August, average temperature in the 1st and 2nd decades in June, vine amplitude index.

5. In 2008, grape yield was higher than in 2007, varying between 7,547 kg/ha for Babeasca Neagra and 13,188 kg for Feteasca Regala.

6. According to production level, the decreasing order of Vine types in 2008 has been: Feteasca Regala, Chasselas Dore, Hamburg Muscat, Coarna Neagra, Merlot, Feteasca Alba, Aligote, Feteasca Neagra, Cabernet Sauvignon and Babeasca Neagra.

7. In 2008, the Vine types recording high production performance in close relationship with climate conditions have been Feteasca Alba, Cabernet Sauvignon, Babeasca Neagra, Feteasca Regala, Feteasca Alba, Aligote, while Hamburg Muscat, Coarna, Merlot, Chasselas Dore and Sarba registered lower performances, due to the rainfalls and temperatures of that year.

8. In 2009, grape yield was higher than in 2007 for almost all the types, except Cabernet Sauvignon. Chasselas Dore registered the highest production 21,510 kg/ha, while Cabernet Sauvignon recorded the lowest yield, 9,000 kg/ha.

9. In 2009, grape yield was higher than in 2007 but lower than in 2008 for almost all the Vine types.



10. The aspects mentioned above show that climate factors should be analysed and monitored in close relationship with vine types and other technological items which are responsible for production performance.

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RESEARCH CONCERNING GRAPE QUALITY IN THE HILLY AREA OF  
EASTERN ROMANIA UNDER CLIMATE CHANGE DURING  
THE PERIOD 2007-2009

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**KEY WORDS:** *quality, grapes, Eastern Romania, climate change*

**ABSTRACT**

*The paper presents the analysis of grape quality in the hilly area of Eastern Romania, at Bujoru Vineyard, Galatzi County, during the climate years 2007-2009. The year 2008 was a dry one and 2009 was an excessive dry one compared to the year 2007. In the years 2008 and 2009 in comparison with 2007, acidity, weight of 100 berries and average weight of grape increased for almost all the studied vine varieties. The decreasing order of the vine varieties in experiment, depending of production quality and established by Point Method, is : Hamburg Muscat, Coarna Neagra, Babeasca Neagra, Chasselas Dore, Sarba, Feteasca Alba, Feteasca Regala, Merlot, Aligote, Cabernet Sauvignon. The evolution of climate factors should be continuously monitorized in close relationship with the vine varieties and viticultural area.*

**INTRODUCTION**

Climate Change affects not only the quantitative performance in viticulture but also grape and wine quality, market price and consumption (Anderson, 2008; www.cnmp.ro 2007).

For this reason, researches concerning the evolution and monitoring of climate factors, as well as the analysis of production trend but also of grape quality have a major importance for setting up the future strategies for the development of viticultural ecosystems (Enache, 2009; Hayes, 2007; Popescu, 2008; Simion, 2008).

In this context, the paper aimed to analyse the quality of grapes for red and white wines, obtained during the period 2007-2009 in the Vine plantations of the hilly area from the Eastern Romania, region where the effects of global climate change are more and more visible and require measures for monitoring climate factors and diminishing or eliminating their negative influences upon the economic performances in viticulture.

The experiments were achieved at Bujoru Vineyard, Galati County, within a partnership project entitled „ Research concerning the Impact of Global Climate Change upon viticultural ecosystems in the hilly area ”, which is running during the period 2007 - 2010 (www.cnmp.ro 2007).

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## MATERIAL AND METHODS

The research work were carried out at Bujoru Research and Development Station for Viticulture and Vinification , Galatzi County, during the perioad 2007-2009, using grapes obtained from 11 vine varieties for producing red and white wines as follows: Feteasca Alba, Feteasca Regala, Aligote, Sarba, Cabernet Sauvignon, Merlot, Babeasca Neagra, Black Feteasca, Chasselas Dore, Hamburg Muscat, Coarna Neagra Selectionata.

A number of 22 climate factors: global, active and useful thermic balance, annual rainfalls and during the vegetation period, annual average temperature, temperatures from the months of July, August and September, air minimum temperature, average temperature of August, avergae temperature of the 1st and 2nd decades of June, wind speed, air humidity, length of bioactive period, nebulosity, number of days with maximum temperatures, real heliothermic index, hydrothermic index , bioclimatic index of Vine and oenoclimatic index have been analysed during the period 2007-2009, based on the data registered by Meteo Station of Bujoru Vineyard .

In order to carry out the research work and obtain results the following modern methods have been used: (a) For climate factors assessment : Index Method ( $R_i = XC_i/XB_i$ ), Gain Method ( $S_i = R_i - 1$ ), Share Method ( $P_i = |S_i| * 100 / \sum |S_i|$ ) and (b) For grape quality assessment, laboratory determinations have been made concerning sugar content , acidity, weight of 100 berries, volume of 100 berries and average weight of grape. The level of these indicators has been comparatively analysed from a year to another based on the calculus of differences and vine types hierarchy with Point Method.

The year 2007 was conidered Control variant (M) and the years 2008 si 2009 were considered experimental years (E1 and E2).

## RESULTS AND DISCUSSIONS

### *Aspects concerning the evolution of climate factors .*

From a climate point of view, the year 2008 was a dry one while 2009 was an excessive dry year in comparison with the year 2007.

The hierarchy of climate factors in the year 2008 according to their imprtance is: annual rainfalls, wind speed , number of days with temperatures over 30 degrees, average temperature in July and the 1st and 2nd decades of June, hydrothermic index, rainfalls during the period of vegetation and heliothermic index.

The hierarchy of climate factors in 2009, based on their importance is: annual rainfalls, annual average temperature, number of days with temperatures over 30 degrees, air minimum temperature, average temperature in August, rainfalls during the period of vegetation, hydro thermal coefficient, minimum temperature at soil surface, global thermal balance, average temperature in September and bioclimatic index, active thermal balance , maximum temperature in August, average temperature in the 1st and 2nd decades of June , oenoclimatic amplitude index (Table 1) .

### *Aspects concerning grape quality.*

**Sugar Content**, in the year 2008, increased by 34 g for Babeasca Neagra, by 10 g for Feteasca Regala, but it has remained stationary for Feteasca Alba and smaller for the other vine varieties in comparison with the level recorded in the year 2007. In 2009 compared to 2007, sugar content decreased for almost all the vine varieties, except Babeasca Neagra and Feteasca Neagra. The differences varied between 12-13 up to 32 g/ liter must depending on vine variety (Table 2).

Table 1

## Climate Factors during the period 2007-2009 at Bujoru Vineyard

Climate Factor	M 2007	E1 2008	E2 2009
Global thermic balance ( $\Sigma t^{\circ}g$ )	3,671.5	3,694.9	3,664.2
Active thermic balance ( $\Sigma t^{\circ}a$ )	3,618.3	3,645.3	3,510.9
Useful thermic balance ( $\Sigma t^{\circ}u$ )	1,858.3	1,875.3	1,660.9
$\Sigma$ Annual rainfalls (mm)	554.4	285.7	0
$\Sigma$ Rainfalls during the vegetation period (mm)	254.5	224.8	172.4
$\Sigma$ Sunstroke during the vegetation period	1,477.4	1,332.7	1,560.6
Average annual temperature, $^{\circ}C$	13.5	13.4	-
Average Temperature of July, $^{\circ}C$	28.1	24.0	23.8
Average Temperature of August, $^{\circ}C$	25.5	25.9	22.1
Average Temperature of September, $^{\circ}C$	17.4	17.1	17.2
Air minimum temperature, $^{\circ}C$	-13.0	-14.5	-15.2
Maximum average temperature of August, $^{\circ}C$	30.6	32.0	30.8
Average temperature in the 1st and 2nd decades of June	25.2	21.9	20.5
Wind Speed (km/hour)	2.3	2.99	2.5
Air Moisture (%)	57	62.26	67
Nebulousness	5.6	5.99	3.3
Number of days with maximum temperatures $> 30^{\circ}C$	66	52	44
Length of bioactive period, days	178	177	186
Real heliothermic index	2.75	2.50	2.59
Hydrothermic Index	0.7	0.6	0.49
Bioclimatic index	11.5	11.8	17
Oenoclimatic index	5,091.2	5,003.2	5,149.1

Table 2

## Evolution of Grape Sugar Content by Variety at Bujoru Vineyard, 2007-2009 (g/l must)

	Feteasca Alba	Feteasca Regala	Cabernet Sauvignon	Hamburg Muscat	Coarna Neagra	Merlot	Babeasca Neagra	Feteasca Neagra	Chasselas Dore	Aligote	Sarba
2007	220	200	223	229	215	231	190	218	206	212	243
2008	220	210	168	195	154	214	224	213	173	175	203
2009	207	188	205	209	183	219	243	263	192	209	219

**Acidity** registered an increase for almost all the varieties, except Feteasca Alba and Feteasca Regala which have recorded a decrease in 2008 compared to 2007. Acidity increased in 2009 for Cabernet Sauvignon, Babeasca Neagra, Hamburg Muscat, Coarna Neagra, Feteasca Neagra, Chasselas Dore, Aligote and Sarba, but it registered a reduction for Feteasca Regala and Merlot and remained stationary for Feteasca Alba, in comparison with the level registered in the year 2007( Table 3).

Table 3

Evolution of Grape Acidity at Bujoru Vineyard, 2007-2009 (g/l must  $H_2SO_4$ )

	Feteasca Alba	Feteasca Regala	Cabernet Sauvignon	Hamburg Muscat	Coarna Neagra	Merlot	Babeasca Neagra	Feteasca Neagra	Chasselas Dore	Aligote	Sarba
2007	3.3	4.3	2.4	2.3	2.7	3.6	3.6	2.6	2.1	2.0	2.8
2008	3.3	3.6	6.4	3.5	3.5	3.3	4.0	2.8	3.1	4.0	4.2
2009	2.9	4.0	3.8	3.1	3.1	4.5	4.6	2.8	3.0	3.5	3.0

**Weight of 100 berries** increased in 2008 for Feteasca Alba (+59 g), Feteasca Regala (+64 g), Hamburg Muscat (+91 g), Coarna Neagra (+ 74 g), Babeasca Neagra (+37 g), Chasselas Dore (+37 g) and Sarba (+ 39 g), while in case of Cabernet Sauvignon, Feteasca Neagra, Merlot and Aligote decreased, compared with the records of 2007.

In 2009, the weight of 100 berries increased for almost all the vine varieties compared to 2007 but decreased for Cabernet Sauvignon, Feteasca Neagra, Merlot and Aligote (Table 4).

Table 4

Evolution of Weight of 100 Grape berries at Bujoru Vineyard, 2007-2009 (g)

	Feteasca Alba	Feteasca Regala	Cabernet Sauvignon	Hamburg Muscat	Coarna Neagra	Merlot	Babeasca Neagra	Feteasca Neagra	Chasselas Dore	Aligote	Sarba
2007	79	89	127	220	200	107	169	153	120	140	110
2008	120	153	67	312	275	104	206	97	158	96	149
2009	134	119	100	284	256	100	213	104	162	125	159

**Volume of 100 berries** increased in 2008 for Feteasca Alba (+ 15 c.m.), Feteasca Regala (+ 34 c.m.), Hamburg Muscat (+ 100 c.m.), Feteasca Neagra (+ 132 c.m.), Merlot (+ 6 c.m.), Chasselas Dore (+ 43 c.m.) and Sarba (+ 43 c.m.), while for Cabernet Sauvignon, Feteasca Neagra, Babeasca Neagra and Aligote decreased compared to the level recorded in 2007. In 2009, the volume of 100 berries increased for almost all the varieties compared to 2007 but it decreased in case of Cabernet Sauvignon, Feteasca Neagra and Aligote (Table 5).

Table 5

Evolution of volume of 100 Grape Berries at Bujoru Vineyard, 2007-2009

	Feteasca Alba	Feteasca Regala	Cabernet Sauvignon	Hamburg Muscat	Coarna Neagra	Merlot	Babeasca Neagra	Feteasca Neagra	Chasselas Dore	Aligote	Sarba
2007	84	94	95	200	180	82	180	137	100	122	90
2008	99	120	60	300	312	88	124	85	143	92	133
2009	117	100	90	257	243	87	193	95	135	107	137

**Grape Average Weight** increased in 2008 compared to 2007 for all the vine varieties. The decreasing order of the vine varieties depending of the gains in grape weight has been the following one: Coarna Neagra (+ 202 g), Hamburg Muscat (+ 105 g), Feteasca Neagra (+ 93 g), Sarba (+77 g), Cabernet Sauvignon (+ 52 g), Babeasca Neagra (+ 49 g), Chasselas Dore (+49 g), Merlot (+ 35 g), Aligote (+ 11 g), Feteasca Neagra (+ 3 g). The only variety where the average weight of grape has remained stationary was Feteasca Alba (Table 6).

Table 6

Evolution of Grape Average Weight at Bujoru Vineyard , 2007-2009 (g)

	Feteasca Alba	Feteasca Regala	Cabernet Sauvignon	Hamburg Muscat	Coarna Neagra	Merlot	Babeasca Neagra	Feteasca Neagra	Chasselas Dore	Aligote	Sarba
2007	90	89	45	80	102	50	90	59	54	84	78
2008	90	92	97	185	300	85	139	152	103	95	155
2009	85	79	67	243	239	78	196	85	113	98	123

***The Order of Vine Varieties based on the number of points achieved for grape quality.*** Taking into consideration that the smallest number of point reflects the highest grape quality, the hierarchy of the vine varieties, in 2008, in the decreasing order is: Hamburg Muscat, Coarna Neagra, Babeasca Neagra, Chasselas Dore, Sarba, Feteasca Neagra, Feteasca Alba, Feteasca Regala, Merlot, Aligote, Cabernet Sauvignon. In the year 2009, the decreasing order of the vine varieties depending on grape quality is : Hamburg Muscat, Sarba, Coarna Neagra , Babeasca Neagra, Chasselas Dore, Feteasca Neagra, Feteasca Alba, Aligote, Feteasca Regala , Merlot, Cabernet Sauvignon (Table 7).

Table 7

Hierarchy of Vine varieties depending on Grape Quality by Point Method

Vine Type	2008 Total points/ Position	2009 Total points/ Position	Vine Type	2008 Total points/ Position	2009 Total points/ Position
Feteasca Alba	29/7	27/5	Babeasca Neagra	21/3	20/3
Feteasca Regala	30/8	40/7	Feteasca Neagra	28/6	27/5
Cabernet Sauvignon	48/11	43/9	Chasselas Dore	24/4	25/4
Hamburg Muscat	16/1	12/1	Aligote	40/10	30/6
Coarna Neagra	19/2	20/3	Sarba	27/5	19/2
Merlot	34/9	42/8	-	-	-

## CONCLUSIONS

1. The reduced rainfalls and higher temperatures in the year 2008 have determined as this year to be considered a dry year compared to 2007 . The year 2009 was an excessive dry year due to the reduced rainfalls and increased temperatures compared to 2007 .

2. In the years 2008 and 2009 compared to the year 2007, acidity, weight of 100 berries and grape average weight increased for almost all the vine varieties. The decreasing order of the studied varieties depending on grape quality is: Hamburg Muscat, Coarna Neagra, Babeasca Neagra, Chasselas Dore, Sarba, Feteasca Neagra, Feteasca Alba, Feteasca Regala, Merlot, Aligote, Cabernet Sauvignon.

3. The evolution of climate factors during the last years have had an important impact upon grape quality in the hilly area of Eastern Romania. This aspect show how important is the continuous analysis and monitoring of climate factors in close relationship with vine varieties and growing area .

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**RELATION BETWEEN VEHICLE TRAFFIC AND HEAVY METALS CONTENT  
IN SOIL**

Popescu Camelia<sup>1\*</sup>

**KEY WORDS:** *atmospheric pollution, lead, cadmium, vehicle traffic*

**ABSTRACT**

*The aim of this paper is to test the relationships between vehicle traffic and the content of the two heavy metals, lead and cadmium, in soils. The field study was conducted for a year in a street canyon in Pitesti, a town in the southern Romania, which can be considered hot spot, by comparison with a blank area in Calinesti, a suburban area near Pitesti. Heavy metal content of soils sampled at progressively greater distance from the road was determined using atomic absorption spectroscopy. The horizontal, vertical and time variations of heavy metal content in soil from the two studied areas demonstrate that the vehicle traffic is a major emission source for lead and cadmium pollution. Lead concentrations in the urban soils were particularly high compared with any of the rural sampled sites.*

**INTRODUCTION**

Millions of tones of pollutants are emitted every year into the atmosphere, both from natural sources and especially from anthropogenic sources. There are four categories of emission sources: stationary (industrial processes, industrial and domestic combustions); mobile (road and stationary traffic); natural (volcanic eruptions, forest fires) and accidental pollutions (discharges, fires) (United States Environmental Protection Agency, 2007)

The systemic pollutants such as heavy metals are very dangerous because of their long time retention in soil and their accumulation by plants and animals. These pollutants can combine with minerals and oligo-minerals becoming blockers for these and the living organisms of these essential elements. The heavy metals do not decay by food preparation; they accumulate in the body and block the intracellular biochemical processes. The heavy metals represent a class of omnipresent pollutants, with toxic potential, in some cases even at low exposure level. They concentrate in each trophic level, because of their weak mobility, so the concentration in plants is higher than in soil, higher in herbivore animals than in plants, higher in carnivores' tissues than in herbivore, the highest concentration being reached at the end of the trophic chain, i.e. in large predators and in humans.

In most cases, heavy metal pollution is a problem associated with intensely industrialized areas. However, high vehicle traffic was proven to be one of the important heavy metal emission sources. Zinc, copper and lead are three of the most common heavy metals emitted by vehicle traffic, totaling at least 90% from the total emitted quantity. (United States Environmental Protection Agency, 2000) Also, vehicle traffic is responsible

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for the emission of some small quantities of other metals, like nickel and cadmium. In populous areas, where vehicle traffic is relatively high, the exposure of people to traffic – related pollution, especially particulate pollution is significant. In the recent years, vehicle traffic has increased especially in urban areas. In cities with densely packed with buildings, placed on both sides of the street, the pollution levels often do not comply with quality standards (European Environment Agency, 2006)

Heavy metals total deposition is the sum of dry (aerosol), wet (via rain, snow or hail) and fog and cloud water deposition. Most heavy metals get into the dust on the road surface. These metals become soluble during rainfall or are cleaned from the road in the same time with the dust. In both cases, the metals get into the soil and/or deposit on the vegetation and are transported by the chemical processes. Most metals have positive charge (are cations), while the organic matter from soil has positive or negative charge.

## MATERIAL AND METHODS

The field study was conducted for a year in a street canyon, which can be considered a pollution ‘hotspot’, by comparison with a suburban area, a blank area: 1. *Nicolae Balcescu Street, Pitesti* – an urban canyon street, with high buildings located both sides along the street the area and high vehicle traffic, that can achieve most times 60.000 transits in 24 hours, especially in the working days. 2. *Calinesti village, Arges County* – a rural area, about 20 Km east from Pitesti, characterized by very low vehicle traffic and away from any industrial influence.

The soils sampling were performed by three campaigns per year (spring, summer and autumn), from the two pedological levels (0-20cm, 20-40cm), at 10m, 20m, 50m away from the street axis. The soil samples were dried and milled till homogenization, digested by pressure disintegration in acid medium and then analyzed using atomic absorption spectroscopy. 4mg of each soil sample were digested with 6ml HNO<sub>3</sub> 65% and 2ml HCl 37% in a Berghof microwave oven using the temperature program from Table 1 (Berghof, Application Report)

Table 1

**The temperature program used for digestion of soil samples**

<i>Step</i>	<i>Temperature (°C)</i>	<i>Ramp time (min)</i>	<i>Time (min)</i>
1	160	6	0
2	180	5	0
3	180	0	14
4	Cooling	-	-

The solutions were analyzed according to the reference method SR ISO 11047/1999 “Soil quality. Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc from soil extracts in regal water. Method by flame atomic absorption spectrometry and electrochemical atomization” (Standard SR ISO 11047/1999) using an AA700 Perkin-Elmer spectrometer with hallow cathode lamp (User’s Guide, Atomic Absorption Spectrometer Perkin-Elmer)

## RESULTS AND DISCUSSIONS

### Heavy metals: theoretical air emissions

The annual quantities of **lead** and **cadmium** emitted by the vehicle traffic were calculated by using the EMEP/CORINAIR Methodology (Group 7-Road Transport), with the input data: the daily average vehicle traffic DAT [number of motor vehicles/24 hours], the average running speed ARS [Km/h] and the annual carburant consumption (ACC) estimated for each motor vehicle class [tonnes/year]. The DAT was determined from the

vehicle traffic manual counts made in three-hour intervals in different days along the year. (European Environment Agency, 2007) The results are presented in Tables 2 and 3.

Table 2

<b>Heavy metals calculated emissions – Nicolae Balcescu Street</b>						
<i>Motor vehicle class</i>	<i>Carburant</i>	<i>DAT [nr/24 h]</i>	<i>ARS [Km/h]</i>	<i>ACC [tonnes/year]</i>	<i>Emission(g/year/km)</i>	
					<b>Pb</b>	<b>Cd</b>
Cars	Gasoline	45500	40	1245	149.5	12.45
	Diesel oil	10800	40	265	0	2.65
Vans (<3.5 t)	Gasoline	4200	40	172	20.6	1.72
	Diesel oil	8300	40	305	0	3.05
Tracks	Gasoline	350	30	43	0	0.43
Buses	Diesel oil	1400	30	215	0	2.15
<b>TOTAL</b>					<b>170.1</b>	<b>22.45</b>

Table 3

<b>Heavy metals calculated emissions – Calinesti village</b>						
<i>Motor vehicle class</i>	<i>Carburant</i>	<i>DAT [nr/24 h]</i>	<i>ARS [Km/h]</i>	<i>ACC [tonnes/year]</i>	<i>Emission(g/year/km)</i>	
					<b>Pb</b>	<b>Cd</b>
Cars	Gasoline	80	50	1.64	0.197	0.0164
	Diesel oil	25	50	0.50	0	0.0050
Vans (<3.5 t)	Gasoline	5	50	0.12	0.014	0.0012
	Diesel oil	10	50	0.27	0	0.0027
Tracks	Gasoline	2	40	0.18	0	0.0018
Buses	Diesel oil	2	40	0.18	0	0.0018
<b>TOTAL</b>					<b>0.211</b>	<b>0.0289</b>

The vehicle traffic is shown to be an important emission source for heavy metals; in the urban area, the calculated air emissions are particularly higher than in the rural area.

### Lead Content

Lead content determination was performed at 283.3 nm wavelength using atomization technique in air-acetylene flame. (ASRO, 1999) Values obtained are presented in Table 4.

Table 4

Lead contents in soil (mg/kg dry matter)												
Pb (mg/Kg d.m.)	Nicolae Balcescu Street, Pitesti						Calinesti					
	10 m		20 m		50 m		10 m		20 m		50 m	
	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40
	March	37.15	40.23	48.23	38.05	32.15	19.06	12.34	10.22	10.06	11.11	10.12
June	35.31	36.15	44.86	34.45	28.25	16.26	11.46	9.98	9.85	10.62	10.06	11.58
September	46.23	48.02	55.79	43.62	38.86	22.85	11.78	11.15	10.84	11.01	10.84	12.06
Annual average	40.52		44.17		26.24		11.16		10.58		11.08	

Analyzing these results, it can be seen that in the urban area the lead concentrations are significantly higher than in rural area, exceeding the normal value of 20 mg/Kg dry matter, but being under the alert threshold for sensible usage of 50 mg/Kg dry

matter, according to the Order 756/1997 for approval of the Environmental Pollution Regulation (WFEP Ministry, 1997)

The horizontal variation is plotted in Figure 1 by representation of lead annual averages based on the distance from the street axis.

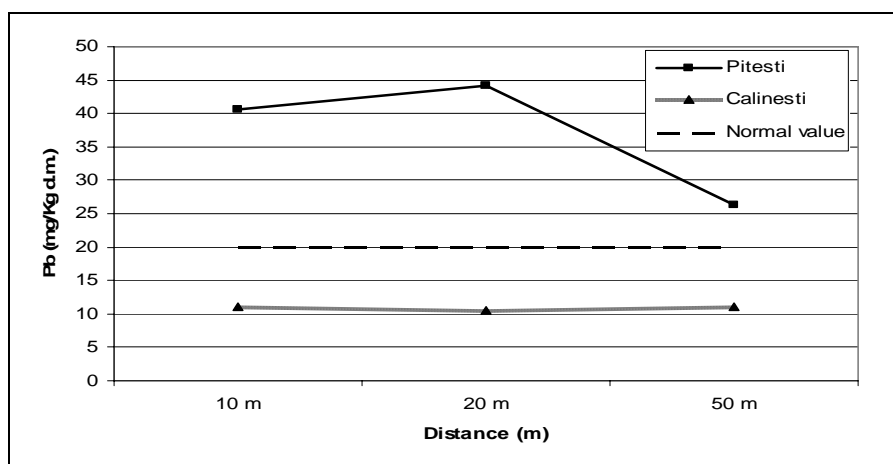


Figure 1. Horizontal variation of lead concentrations – 2008 annual averages

In the rural area, without vehicle traffic influence, average concentrations obtained are relatively constant at any distance from street axis. In the urban area, the highest lead concentration in soil was determined in the sample at 20m from the road axis. At greater distances from the road, the Pb values decrease, at 50 m being near the 'normal' value. Referring to soil samples at 20m, the time variation is plotted in Figure 2.

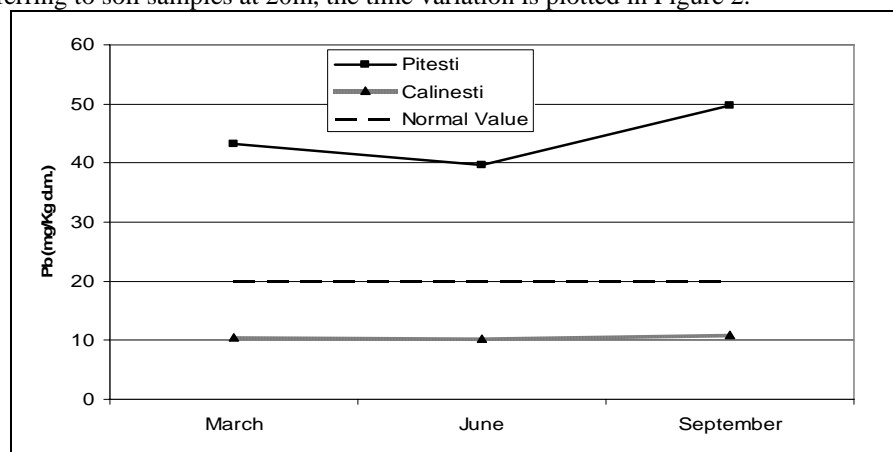


Figure 2. Time variation of lead concentrations at 20m from street axis – 2008

Again in the rural area, the values are relatively constant throughout 2008, whereas in the urban area, there is a slight decrease in June, while vehicle traffic is higher in this period of year. This variation may reflect the fact that in June low precipitation was recorded (Figure 3), so the wet deposition is less.

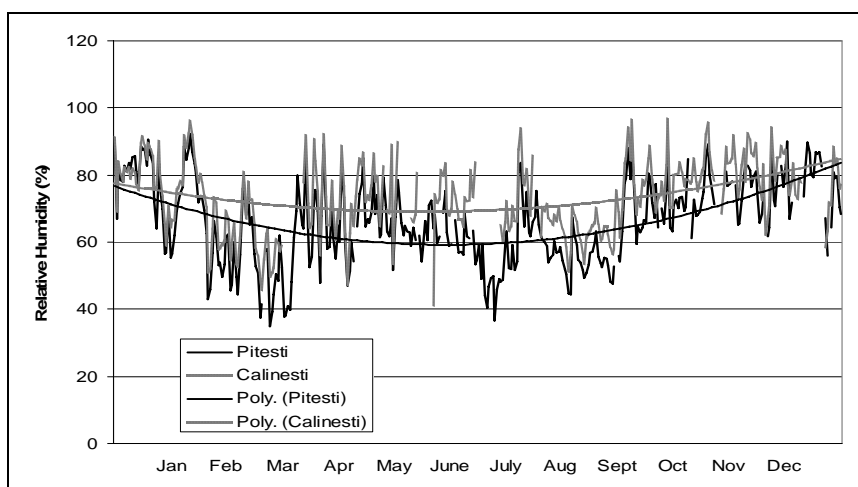


Figure 3. Relative humidity – 2008

The vertical variation is plotted in Figure 4 by representation of lead annual averages based on the sampling depth and distance from the street axis.

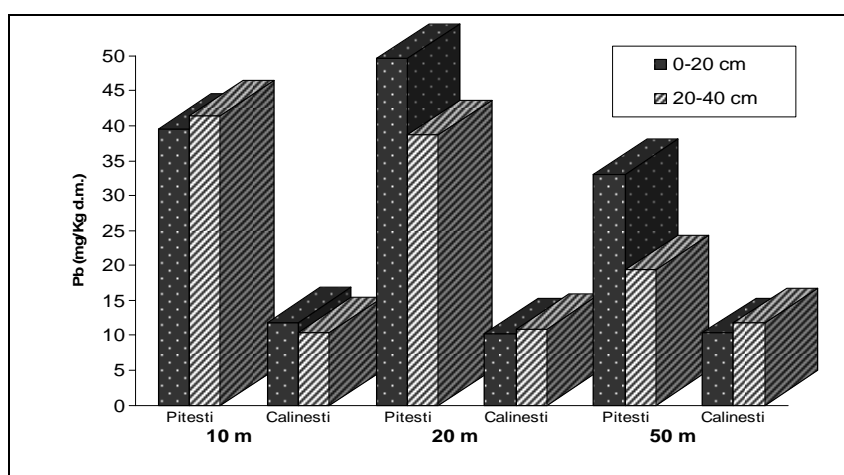


Figure 4. Vertical variation of lead concentrations based on the distance from street axis

In the rural area, the average concentrations obtained are relatively constant at any depth or distance from the street axis. In the urban area, at 10m from the street axis, the soil contamination increases with depth, while at 20m and 50m it decreases with depth. At the side of the street, the heavy metal deposition was higher through time, deeper into soil. As the distance from the street increases, the soil contamination becomes more superficial.

#### Cadmium content

Cadmium content determination was performed at 228.8 nm wavelength using atomization technique in air-acetylene flame. (ASRO, 1999) Values obtained are presented in Table 5.

Table 5

Cadmium contents in soil (mg/Kg dry matter)												
Cd (mg/Kg d.m.)	Nicolae Balcescu Street, Pitesti						Calinesti					
	10 m		20 m		50 m		10 m		20 m		50 m	
	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40
March	0.80	0.68	0.70	0.64	0.54	0.50	0.11	0.12	0.11	0.10	0.06	0.03
June	0.75	0.54	0.59	0.48	0.46	0.44	0.09	0.09	0.09	0.07	0.05	0.02
September	0.91	0.65	0.68	0.54	0.58	0.59	0.10	0.09	0.09	0.08	0.06	0.03
<b>Annual average</b>	<b>0.72</b>		<b>0.61</b>		<b>0.52</b>		<b>0.10</b>		<b>0.09</b>		<b>0.04</b>	

Analyzing these results it can observe that in urban area the cadmium concentrations are higher than in rural area, but do not exceed the normal value of 1 mg/ Kg dry matter according to the *Waters, Forests and Environmental Protection Ministry Order no. 756/1997 for approval of the Environmental Pollution Regulation* (WFEP Ministry, 1997)

The horizontal variation is plotted in Figure 5 by representation of cadmium annual averages based on the distance from the street axis.

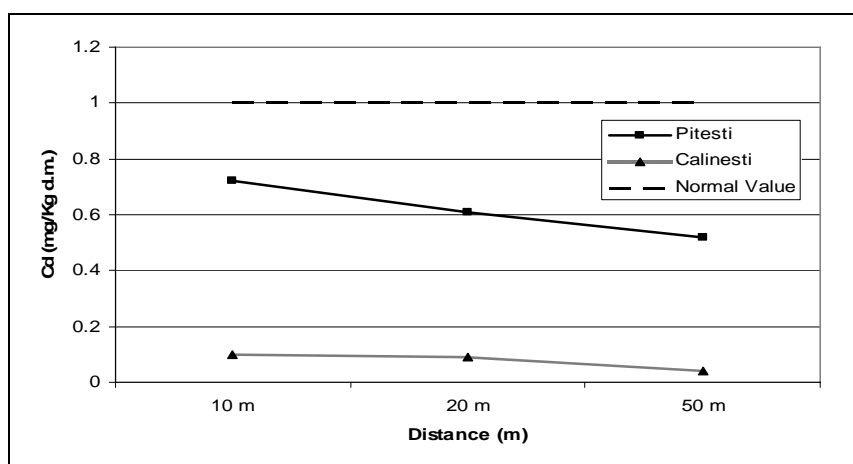


Figure 5. Horizontal variation of cadmium concentrations – 2008 annual averages

In the rural area, average concentrations are relatively constant, with a slight decrease at 50m. In the urban area, the cadmium concentration in soil decreases proportional with the distance from the street axis.

Referring to soil samples at 20m, the time variation is plotted in Figure 6

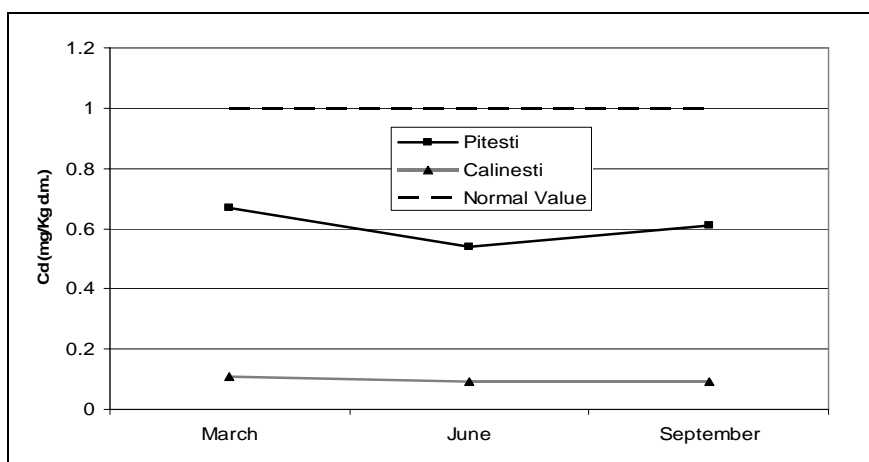


Figure 6. Time variation of cadmium concentrations at 20m from street axis – 2008

Again in the rural area the values are relatively constant along 2008, whereas in the urban area, there is a slight decrease in June, when vehicle traffic is higher. Like lead, this variation may be due to the low precipitation recorded in June, so the wet deposition is less.

The vertical variation is plotted in Figure 7 by representation of cadmium annual averages based on the sampling depth and distance from the street axis.

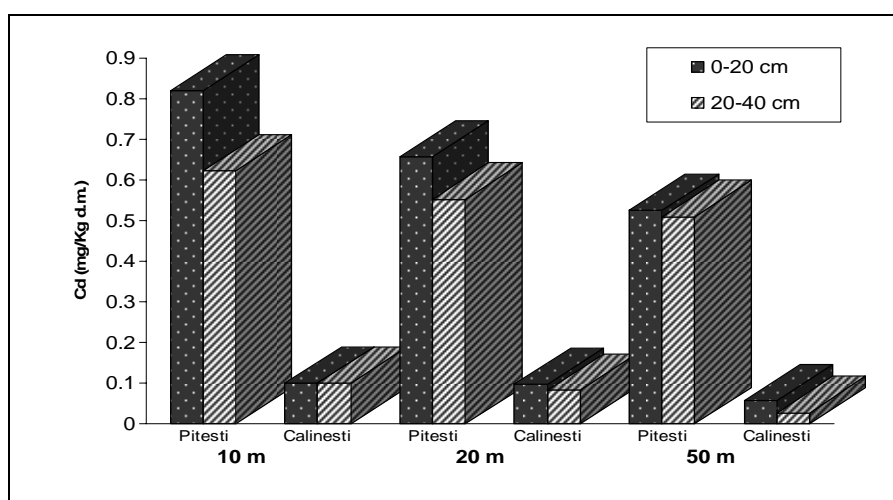


Figure 7. Vertical variation of cadmium concentrations based on the distance from street axis

In the rural area the average concentrations are relatively constant with a slight decrease at 50m and at 20-40cm depth. In urban area, the soil contamination decreases with depth and distance from street axis. At the side of the street, the soil contamination is more superficial.

## CONCLUSION

Lead and cadmium concentrations in soils from rural area do not exceed 'normal' values of 20 mg/ Kg dry matter, and 1 mg/ Kg dry matter, respectively, according to Romanian legislation. They remain relatively constant by vertical, horizontal and temporal distribution.

Lead concentrations in soils from the urban area exceed the 'normal' value, but do not exceed the 'alert' threshold of 50 mg/ Kg dry matter, according to the same Romanian legislation. Analyzing the horizontal distribution, the highest lead concentration was detected at 20 m distance from the street axis; the traffic influence weakens with distance from the road, so at 50 m concentrations decrease significantly, approaching the 'normal' value. In terms of vertical distribution, near the street the deposition of particulate heavy metal has been more intense through time, with higher lead values penetrating deeper into the soils, and as the distance from the street increases, the soil contamination becomes more superficial.

Analyzing the temporal variation, a slight decrease in June is established, although vehicle traffic is more intense during this period of year; this decrease is thought to reflect reduced rainfall and wet deposition of particulate pollutants at this time.

Cadmium concentrations in soils from the urban area are under the 'normal' value of 1 mg/ kg dry matter, recording a decreasing directly proportional to the distance from the street axis and to the sampling depth. Along the year the same decreasing in June was recorded, because of the low precipitation.

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THE EFFECTS OF TEMPERATURE ON SOME PHYSIOLOGICAL PROCESSES  
OF THE GRAPEVINE

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**KEY WORDS:** *grapevine, climate changes, temperature, photosynthesis, transpiration.*

**ABSTRACT**

*The effects of temperature on the physiological processes of the grapevine, was studied on 3 vines cv. Merlot grown in 5-l plastic containers in an environmental chamber having a controlled environment with temperatures of 20, 25, 30, 35, 40 °C and 50, 60 and 80% humidity. The determined parameters have been the photosynthesis, transpiration and stomatal conductance, monitored with the help of the ADC LC-PRO analyzer. The lowest values for the net rate of the photosynthesis have been obtained at the temperature of 40 °C, this being the main physiological effect influenced by the high temperatures. The obtained results highlight how whilst the temperature increases the values of photosynthesis drop and thus for temperatures over 35 °C it stops, but in exchange, the transpiration increases in value. Therefore this study points out the way high temperature influences not only photosynthesis but also the transpiration and in a minor way the stomatal conductance.*

**INTRODUCTION**

In the last years and up to the present day there have been observed major climate changes, explained by the increase of the carbon dioxide in the atmosphere, and that is the reason for which many research grants are meant to monitor the climate changes and their implications upon vine culture. Plants all over the globe, not only grape vine, have developed new mechanisms meant to face nowadays' climate changes. (Gordo and Sanz, 2009).

The temperature is the most important factor which influences the phenology of the grapevine and thus wields a major influence upon the development of grapes. ( Webb *et al.*, 2007), and that's why the influence of temperature upon the vital functions, photosynthesis being our main physiological process affected/ influenced by its changes, is of great importance and has been intensely researched. Future climate changes will produce, most likely, changes in the normal evolution of the phenologic phases, the rancidness of the composition and flavor of grapes and in the end of the wine, but also will produce changes in the confinement of the cultivation and growing of grape vine (Jones 2007). The next 30 years, it is envisioned that the average temperature in Europe will increase with 2,04 to 4,5 degrees in the summer and with 3,5 to 6 degrees in winter, having

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a major impact upon the traditional boundaries of growing grape vine, upon the phenological stages, the production and the quality of grapes and also upon the economical costs (Popescu Agatha *et al.*, 2009). For the most of the areas which grow/yield grapes, the forecast upon the evolution of the climate will determine a lower quality of the grape production and an endeavor to improve the resources management. (Schultz and Stoll, 2010). A study regarding the negative effects of global warming upon the grape vine through the implementation of a technological management meant to diminish the impact of thermal and water related stress, has been accomplished by Cichi Daniela Doloris *et al.*, 2007.

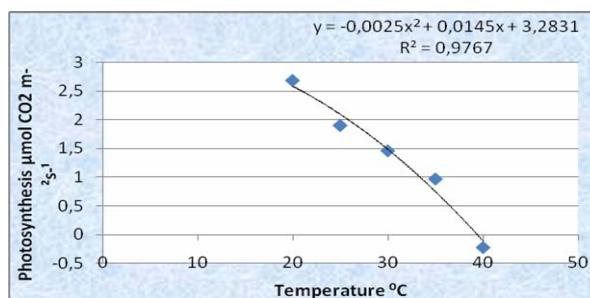
## MATERIALS AND METHOD

The research targeted some of the physiological changes undergone by the canopy of the grapevine, as a result of the high temperature within the environmental chamber. Thus, 3 plants of Merlot vine have been studied in an environmental chamber with controlled environment, VERSATILE ENVIRONMENTAL TEST CHAMBER MLR-351H at the temperatures of 20, 25, 30, 35 and 40 degrees C, with a humidity of 50%, 60% and 80%, for as long as 4 days for each value of humidity. The grape vine has been planted in 5 l plastic containers, the soil being brown-reddish and the plants watered properly. The physiological parameters that were determined were as follows: the photosynthesis, the transpiration and the stomatal conductance and they were determined with the help of the ADC LC-PRO analyzer which was kept for 4 days for each vine stock, these researches being conducted also by Cloete *et al.*, 2008.

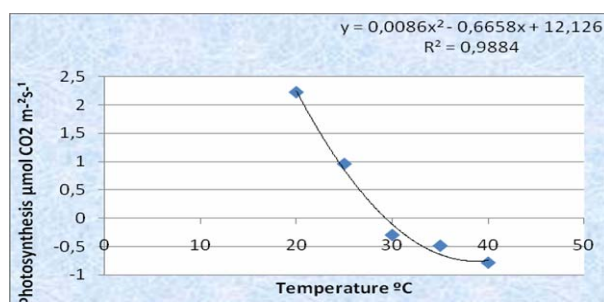
## RESULTS AND DISCUSSIONS

Temperature is one of the environmental factors that have major effects upon the growth and development of the grapevine. Researches regarding the thermal stress related effects and the response reactions of the grapevine were conducted by Gornik *et al.*, 2007. The obtained results have pointed out the significant differences from a certain temperature to the other regarding photosynthesis and transpiration. Regarding the stomatal conductance, this does not suffer great changes, the noted values being similar for the 3 different humidities studied.

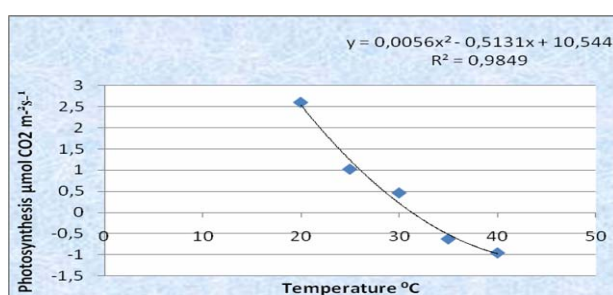
Photosynthesis represents the transformation of the solar energy in different forms of chemical energy through plants. Thus, the obtained results can be graphically expressed as follows:



Graph 1. The relationship between photosynthesis and temperature for 50% RH.



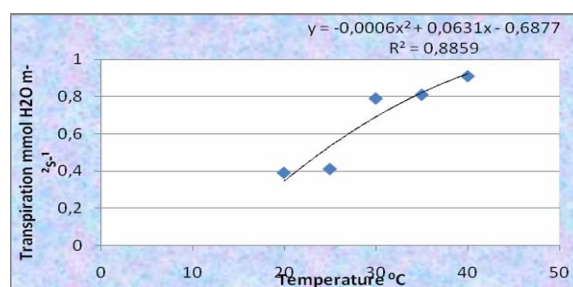
Graph 2. The relationship between photosynthesis and temperature for 60% RH.



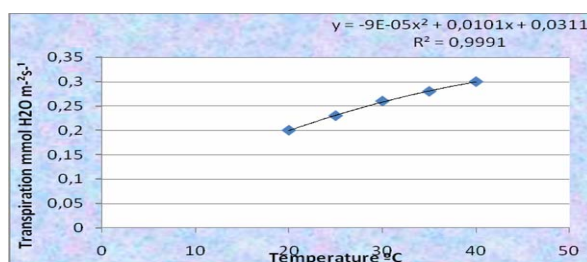
Graph 3. The relationship between photosynthesis and temperature for 80% RH.

From the 3 graphs presented above we can observe how the net value of photosynthesis drops whilst the temperature rises so that for temperatures that exceed 30°C this has negative values. The optimum values have been obtained for 20°C, for all 3 humidities (Graphs 1, 2 and 3). This represents a maximum 2,68 µmol CO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup> for a 50% humidity (Graph 1) and the minimum of -0,96 µmol CO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup> was obtained for 80% humidity at a 40°C temperature (Graph 3).

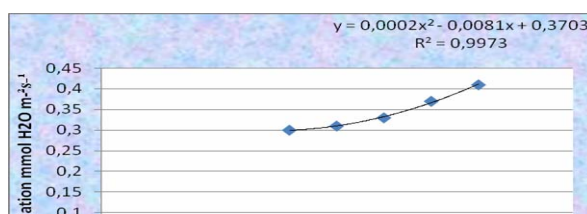
Transpiration represents the process of losing water as vapors through the aerial parts of the plant, and as the temperature of the air rises the more the transpiration grows, this fact being shown through the following graphs 4, 5 and 6.



Graph 4. The correlation between the rate of transpiration and the temperature for 50% RH.



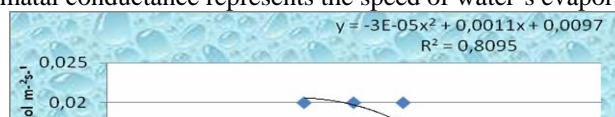
Graph 5. The correlation between the rate of transpiration and the temperature for 60% RH



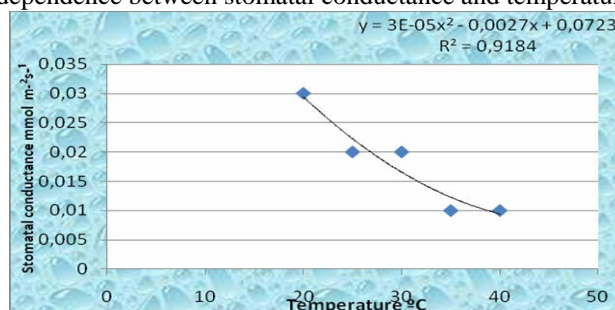
Graph 6. The correlation between the rate of transpiration and the temperature for 80% RH.

Unlike photosynthesis, transpiration rises along with the rise of temperature, the highest values of 0,91  $\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$  being obtained for a humidity of 40 °C and the lowest values of 0,20  $\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$  being obtained for a 20°C and a 60% humidity (graph 5).

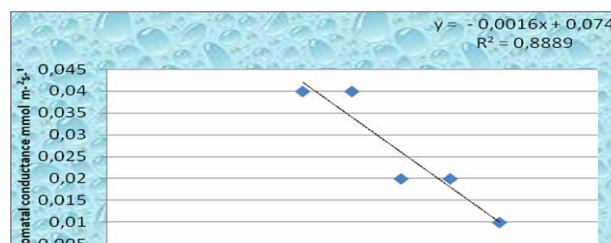
The stomatal conductance represents the speed of water's evaporation through the



Graph 7. The dependence between stomatal conductance and temperature, for 50% RH.



Graph 8. The dependence between stomatal conductance and temperature, for 60% RH.



Graph 9. The dependence between stomatal conductance and temperature, for 80% RH.

plants' vapors and is directly connected to the relative size of the stomata opening (graphs 7, 8 and 9).

According to the study, the stomatal conductance drops whilst the temperature rises, having a faster descent regarding the 80 % humidity at the temperature of 20 °C and registering the value of 0,04 mmol m<sup>-2</sup>s<sup>-1</sup>, then dropping at the value of 0,02 mmol m<sup>-2</sup>s<sup>-1</sup> at a 30 and 35°C temperature but the highlight being the value of 0,01 mmol m<sup>-2</sup>s<sup>-1</sup> at 40°C.

## CONCLUSIONS

The climate changes have become more obvious within the past recent years and are owed mostly to the rising concentration of the greenhouse gases in the atmosphere. The effects of the climate changes have also been observed in Romania, mostly in past recent years, the transition from the cold season to the hot one is no longer a step-by-step process but a sudden one with high variations of temperature. The results of the research show that high temperatures influence photosynthesis and transpiration and in lower degree it also affects stomatal conductance. Thus, as the temperatures grows, the photosynthesis decreases and so for temperatures over 30 °C it has a negative value, but in exchange, transpiration increases as the temperature rises. The grape vine is in fact a sensitive plant to high temperatures through its vegetation period, and that's why it is recommended that to be cultivated in favorable environments with a proper irrigation.

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RESEARCHES REGARDING THE EFFECTIVENES OF THE CERTIFICATION  
METHODS OF PLUM BIOLOGICAL CATEGORY

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**KEY WORDS:** Rootstock, serological assay, biological indexing, viruses.

ABSTRACT

*From the candidate vegetative rootstocks were obtained 35 Miroval plants, 34 Rival plants and 16 Oteşani 11 plants which were transferred in an isolated greenhouse, in individual containers on a free substrate of Xiphinema spp. and Longidorus spp. Subsequently, the 85 candidate plants were retested biological and serological, resulting 0 viral infections. In august 2009, each of the 3 candidate plum cultivars virus-tested were propagated in an isolated greenhouse using the 3 rootstocks virus tested mentioned above resulting 66 plum cultivars: 22 cv. Alina, 22 cv. Andreea and 22 cv. Tita (9 on Rival, 9 on Miroval and 4 on Oteşani 11). From each rootstock were preserved 4 plants in an isolated greenhouse in addition to the plum cultivars obtained. Also, at the beginning of July 2010, the plants obtained, were submitted to a serological test. Although the results were negative through DAS-ELISA, also a molecular test will be performed in order to obtain a better certainty of the results.*

INTRODUCTION

The main cause for the deterioration of crop health was the uncontrolled distribution of already infected planting materials (both scions and rootstocks) followed by subsequent vector-mediated spread, specifically Sharka disease produced by the Potyvirus Plum pox and thus was initiated national and international projects for the production of virus-free stocks through certification programmes (Di Terlizzi et al., 1998).

There are many examples, indicating that virus-free planting material even in comparison with plants only free of the main viruses in growth, feathering (compatibility), rooting; yeild and fruit production; tolerance towards different stress factors including replanting problems, sustaining production and duration of orchard (Lenz and Lankes, 2006).

Some viral infections effects of plant viruses cannot always be seen, which makes it difficult for the propagator to appreciate that stock may be deteriorating in quality and so plant material free from all known viruses has a major contribution to ensuring true-to-name healthy plants (Macdonald, 1986).

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Some viral infections cause incompatibility between rootstock and cultivar affecting yield in nurseries. A low percentage of successfully grafted trees in nurseries may be attributed to the viral infection (Nemeth, 1986; Cembali, 2003).

Severe controls regarding the importation and movement of propagating materials, indexing of imported germplasm in quarantine, production of virus-free propagating material in the frame of a certification program seem to be of main importance for the virus control (Myrta and Boscia, 1998).

The purpose of our research is to obtain the plum base biological category, thus the problems caused by the material of minor health status could be eliminated in time.

## MATERIAL AND METHODS

The identification and the selection of the plum candidate material was made in trial comparative crops and in the mother cuttings of Fruit Growing Research & Extension Station Vâlcea between 2008-2009.

The candidate biological material was represented by the plum cultivars Alina, Tita and Andreea and the vegetative rootstocks Miroval, Rival and Oteşani 11.

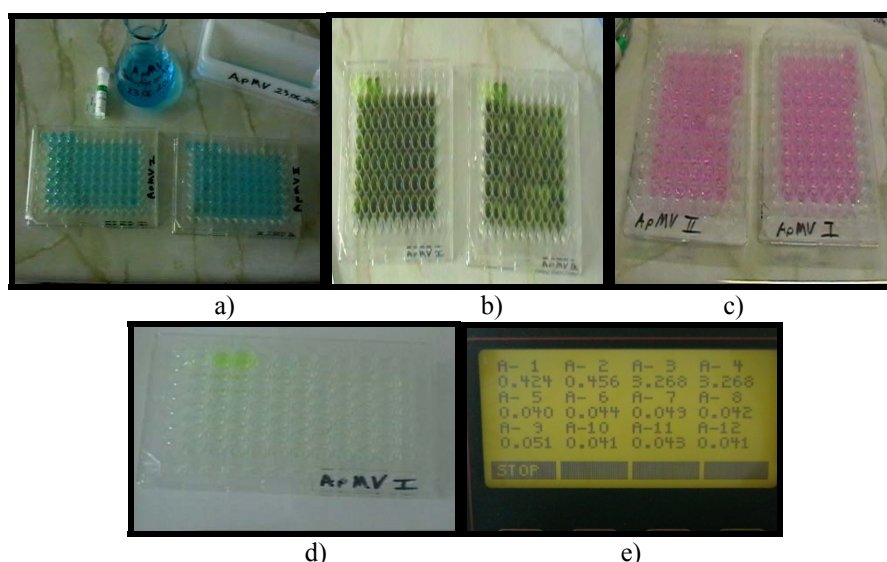


Figure 1. Steps of DAS ELISA serological assay of ApMV: a) anticorp substrate; b) plant extracts and positive and negative controls; c) conjugate substrate ; d) visual colorimetric reaction of two wells of the microtier plate; e) chromatically readings at 405 nm

Each selected plant was subjected to the biological and serological DAS-ELISA regarding the infections with the viruses Plum pox virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ring spot virus (PNRSV), Apple chlorotic leaf spot virus (ACLSV) and Apple mosaic virus (ApMV). For the serological assay was used the work protocol recommended by Bioreba (Switzerland), wich includes four major steps: repartition of the anticorp substrate, repartition of the plant extracts and positive and negative controls, repartition of the conjugate substrate and of the reaction substrate with pNPP (for each step were applied 200µl/well) (Figure 1). The incubation temperature for the anticorp and



conjugate substrates was 30°C. The microtiter plates were read chromatically at 405 nm with the instrument Stat Fax 3200. Biological indexing was made on the polivalent seedlings *Prunus persica* GF 305 virus-free.

In august 2009, each of the 3 candidate plum cultivars virus-tested were propagated in an isolated greenhouse using the 3 rootstocks virus tested: Miroval, Rival and Oteşani 11.

Another serological tests were made in june 2010 to see the phytosanitary state of the candidate material.

## RESULTS AND DISCUSSIONS

Through both serological and biological tests regarding the five viruses PPV, PDV, PNRSV, ACLSV and ApMV in case of the plum cultivars Andreea, Tita and Alina and also of the plum rootstocks Rival, Miroval and Otesani 11 were obtained negative results, thus no natural viral infection was found.

The initial biological material was found free of the viruses mentioned in Anexa no.1 from the G.O. no.136/2000 and was considered to be candidate biological material.

From the candidate rootstocks were obtained 35 Miroval plants, 34 Rival plants and 16 Oteşani 11 rooted cuttings wich were planted in containers on a free substrate of *Xiphinema* spp. and *Longidorus* spp. and were preserved in isolated conditions (Figure 2).

Each of them was again tested serologically for the five viruses mentionaded above and were found 0 infections. Thus, this material obtained directly from the candidate mother plants represents the prebase material.



Figure 2. Candidate plum rootstocks preserved in isolated conditions

In august 2009, each of the 3 candidate plum cultivars were propagated in an isolated greenhouse using the 3 prebase rootstocks ggmentioned above resulting 66 plum cultivars: 22 cv. Alina, 22 cv. Andreea and 22 cv. Tita (9 on Rival, 9 on Miroval and 4 on Oteşani 11).



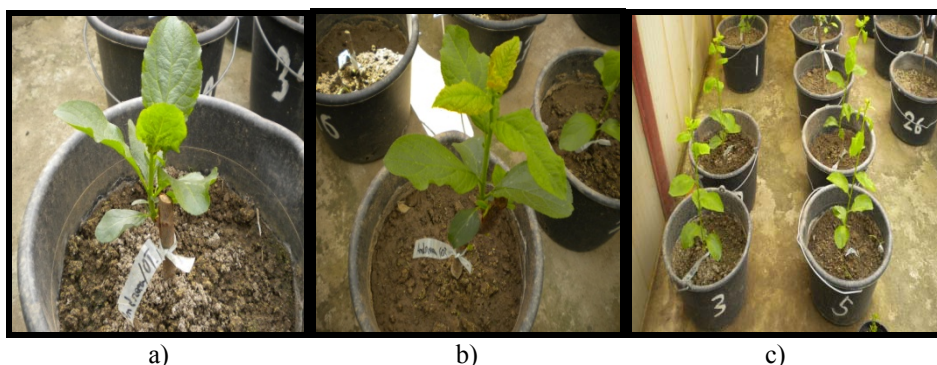


Figure 3. Prebase biological category: a) Andreea/Otesani 11; b) Alina/Otesani 11; c) Tita/Otesani 11

From each rootstock were preserved 4 plants in an isolated greenhouse in addition to the plum cultivars obtained. Also, at the beginning of July 2010, the plants obtained, were submitted to a serological test. Although the results were negative through DAS-ELISA, also a molecular test will be performed in order to obtain a better certainty of the results. This biological material represents the prebase biological category and it will be subjected to the evaluation process and phytosanitary testing in order to obtain the plum base biological category.

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ASPECTS REGARDING THE DYNAMICS OF THE PHYLLAPHIS BEECH  
POPULATION IN THE BEECHWOODS FROM THE UPPER POND OF THE  
DOAMNEI RIVER

Retevoi Gh. R.<sup>1</sup>

**KEY WORDS:** Beechwood, *Phyllaphis fagi*, density, numerical evolution, forecasting

ABSTRACT

*Phyllaphis fagi* is an aphid frequently found in the beechwoods in our country. During the year it presents an alternation of generations asexual and sexual. Their attack is strong in spring when, whitish colonies form leaves on the back of the beech. The collected data on the field have allowed the identification of the distribution type, the determination throughout counting the number of the sample units, the density, the numerical evolution of the population on the sample surfaces, the breeding rate and an association between the climate factors and the dynamic of the population.

INTRODUCTION

The research of the vermin for the beechwoods was realized in the hill area of the Pietroșani locality (county Argeș) during 2008-2009. On the studied surface along with *Phyllaphis fagi*, we have also identified the species *Mikiola fagi*, *Orchestes fagi* that, together with other biotic and abiotic factors determine the schorch of the beech tree (Chira et al., 2003; Gaëlle et al., 2006). *Phyllaphis fagi* is a species that, during one year presents an alternance of asexual generations (fundatrix, fundatrigenae) and sexual generations (Marcu, 1995). The specialized literature describes the morphology and the ecology of the species (Stroyan, 1977; Heie, 1980; Blackman and Eastop, 1994), but also the structure of the reproductive system (Wieczorek and Switek, 2008) and the influence of the temperature over the development of the population (Iversen and Harding, 2007). The attack is strong in spring, when whitish colonies are formed on the back of the leaves (fig. 2). Because they have an oral system adapted to stick and suck, they feed themselves with the sap from the leaves. During their strong attack we can notice the leaves becoming darker because of the mould that determines a productivity decrease. Because we have noticed a decline of the beech tree (Chira et al., 2003), a supervision of the vermin population is necessary, in order to forecast the numerical growth. The purpose of our study has been the identification of the population density, the breeding rate and the association between the climate factors and the dynamic of the population.

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## MATERIAL AND METHODS

In the hill area of Pietroșani there are different types of woods: beechwoods, quercines, acacia, spruces (fig. 1). The dominant species are the beechtrees; the oak, the acacia tree and the spruce tree form either island clusters of different dimensions or form transversal parcels along the hills in the beechwoods. This arrangement is due to some reafforestation which took place 57 years ago. The research of the insect took place in a beech wood along a transect which comprised the margin of the Stufosa Wood (locality Retevoiești, Argeș) but also trees from the massif. On the transect we have established three points for sampling the assay. Each sample surface has been established at 100 m<sup>2</sup>. The sample surface limited in the margin of the wood has a southern positioning and it is occupied by young beech trees. The sample surface limited in the middle of the transect has a southern positioning and it is occupied by beech trees of an intermediate dimension in comparison to those in the margin and in the massif. The sample surface limited in the massif has a western positioning and it is occupied by a mature and vigorous beechwood. The sampling was made weekly starting with May, 15th until September, 17th. From the limited surfaces we have sampled, randomly, branches with leaves (5) from the inferior third part. We have counted the insects on the first 20 leaves from each branch, and based on the data obtained we have identified the statistical values. In order to determine the way in which the density varies every month and year, we have applied the ANOVA test (Zamfirescu and Zamfirescu, 2007) bifactorial without replication.



Fig. 1. Image taken from a satellite of the researched area. S1, S2, S3-points of taking datas along the way. M=spruce groves; ST=oak forest; SL=locust trees.



Fig. 2. Colony of *Phyllaphys fagi*

## RESULTS AND DISCUSSIONS

The sampling of the data took place weekly in the three points of the transect. In the three sample areas which are different in what regards the age of the tree and positioning, we have registered different numerical values of the *Phyllaphys fagi* population. In calculating the statistical data we have used the data obtained in June 15th, 2008 in the middle of the transect (tab. 1).

Table 1

Sampling date ( <i>Phyllaphis fagi</i> )																			
X	0	1	2	3	4	5	6	7	9	11	12	14	16	18	20	30	40	42	58
f	27	30	8	3	5	5	5	2	4	3	3	1	1	3	2	1	1	1	1

x = the number of individuals/ sample unit (one leaf);

f = the number of units that had 0, 1, 2, 3, .. individuals;

Starting from these data we identified: the average of the individuals on the leaf, the deviation of the average, the variation, the standard deviation, the number of the sample units, the distribution type (tab. 2).

Table 2

Statistic data obtained for *Phyllaphis fagi*

The average of individuals per leaf	Standard error of the average mean	Variance	Standard deviation	Determination of number of sample units	Distribution type
5.24	0.53	29	5.38	26	Grouped

Considering that the surface unit is a leaf, we identified monthly the density in the three points of the transect, in 2008 and 2009 (tab. 3) .

Table 3

The density of the *Phyllaphis fagi* population

Months	Density					
	Skirt		Middle of transect		Massive	
	2008	2009	2008	2009	2008	2009
May	3.6	3.78	5	0.35	2.29	0.04
June	5.49	2.71	5.09	0.87	2.1	0.09
July	4.02	2.58	3.39	0.89	0.74	0.11
August	2.32	1.6	0.5	0.34	0	0.02

From the analysis of the table it results the fact that, the maximum density was realized in June at the margin of the wood. Starting with June the population starts to decrease numerically in all the points of the transect. The lower densities were registered in the massif. These differences are due to the different positioning of the trees but also due to the different age of the trees from the three sample areas. Starting with the logarithmic values of the densities in the margin, we applied the ANOVA test, bifactorial without replication and we obtained the following results (tab. 4).

Table 4

ANOVA statistical table

Source of variation	SP	gl	$\bar{SP}$	F
External, between columns	0.26	1	0.26	5.2
External, between rows	0.63	3	0.21	4.2
Internal	0.16	3	0.05	
Total	1.05	7		

Because  $F_c$  and  $F_r$  have lower values than  $F(\alpha, c-1, gl_{int})$  respectively  $F(\alpha, r-1, gl_{int})$ , it results that, in the margin the densities do not differ significantly from one month to another and from one year to another (Zamfirescu and Zamfirescu, 2007). If we repeat the test for the densities registered in the massif (2009) and margin (2008), we may notice that there is a difference of the population density from one year to another. If we totalize the number of the existing individuals at a certain moment in all the points of the transect, we obtain the curves of the population evolution in 2008 and 2009. From the analysis of this chart (fig. 3), which mentions the number of the individuals during every decade of May, June, July and August, it results that, the population has, during the year, two high seasons. The first numerical growth of the population takes place at the end of May and the beginning of June and the second growth takes place at the end of July and the beginning of August. The second growth coincides with the emergence of the sexual generation which spawns eggs.

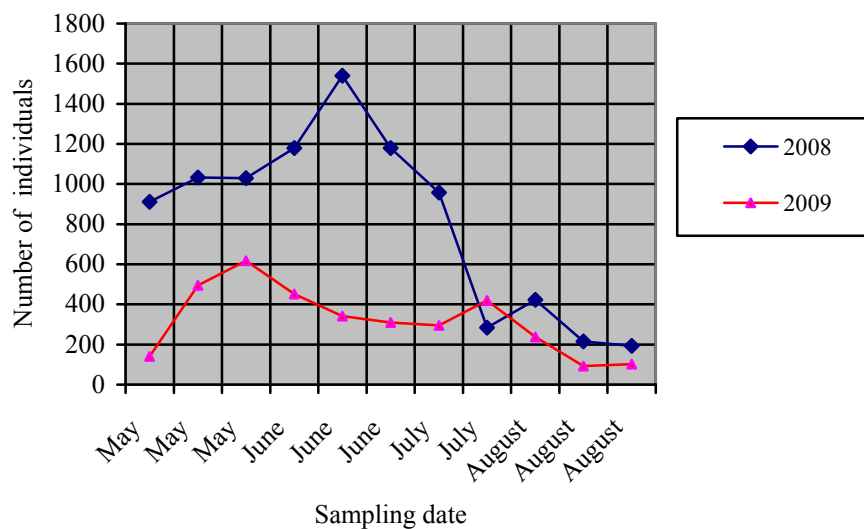


Fig. 3 Numerical evolution of *Phyllaphis fagi* population during 2008 and 2009

In comparison with 2008, in 2009 there were not any sudden growths or decreases of the population. The maximum number of the individuals was at the level of the margin in May, 30th. The population registered lower values in comparison with the previous years. The dynamic of the *Phyllaphis fagi* population was supervised under the influence of the climate factors, too: temperature and rainfall. In order to describe the climate conditions in the research area we have realised a climate-diagram of the type Walter and Lieth which mentions the periods that are very humid, the humid periods, the dry periods and the drought periods. In contrast with the original climate-diagram that it is established starting with the monthly temperature average and the monthly sum of the rainfall, this climate-diagram has at its basis the average temperature and the rainfall sum for each decade of May, June, July and August. If we compare the curve of the population evolution with the climate factors, we may notice that the growth seasons of the population coincide with the

humid periods, and the numerical decrease with the dry and drought periods (fig. 4). The thermal optimum for the development of the asexual generation is between 16.4 -18.4 C°.

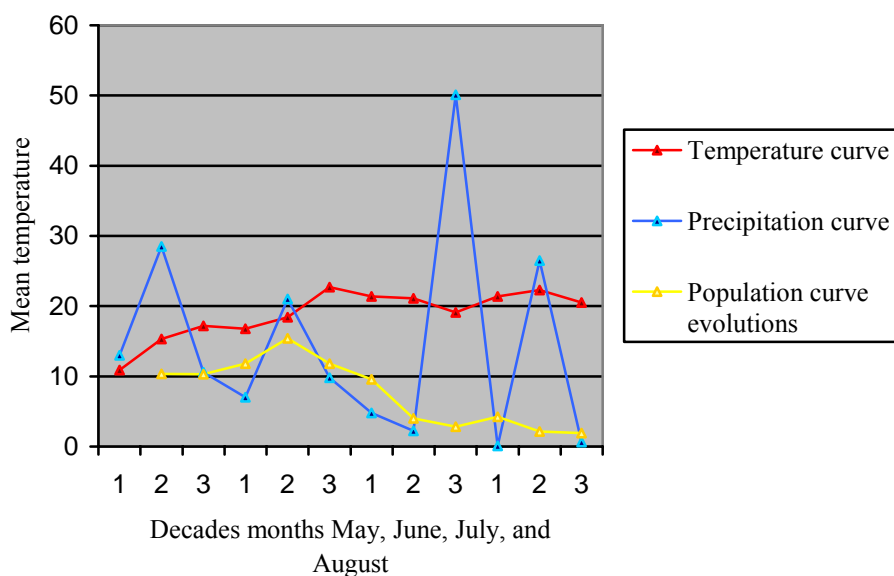


Fig. 4: Climatediagram of Walter and Lieth type for Retevoiești in 2008

These data confirm the results obtained by Iversen and Harding (2007) who have studied the development of the species at different temperatures, in laboratories. The numerical growths coincide or are preceded by humid periods and the numerical decreases are associated with the dry and drought periods. We may notice a numerical decrease of the population at an average temperature of 20 C°. In 2009 the intensity of the attack has reached lower values than in 2008, even though the climate conditions have been similar. This difference is given by the low temperatures from the last decade of April when there were recorded also temperatures below 0 degrees C.

The growth rate of the population was of 0.74. Sequeira and Dixon (1997) have issued a thesis according to which there might be a correlation between the density of the sexual population (the autumn generation) and the density of the population from the following spring.

According to this thesis, in the spring of 2010, the density of the population should have similar values with the density of the spring generation from the previous year because the densities of the autumn generations have similar values. At the beginning of May (2010) in the three points of the transect we have identified an average density of 1.58 individuals on a leaf which attests this hypothesis. Based on this comparison we can make useful forecasts in silviculture. In conclusion, the density of the population during one year is influenced by the climate and the density along the year is influenced by the autumn densities of the population.

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THE DYNAMICS OF THE POPULATION OF PARECTOPA ROBINIELLA AND  
PHYLONORYCTER ROBINIELLA IN THE ACACIA WOODS

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**KEY WORDS:** *Parectopa robiniella*, *Phylonorycter robiniella*, acacia, the dynamic of the populations, forecasting.

**ABSTRACT**

The research of the two vermin for the acacia tree has been made in the hill area of the locality Pietroșani (county Argeș) in 2008, 2009 and 2010. They mine the acacia leaves reducing the productivity and determining a premature fall of the leaves. The species have been researched in the margin of the forest and in the massif. For the two species we have identified the distribution type, the density, the intensity of the attack, the frequency of the leaflets attacked according to the number of mines on the leaves, the breeding rate, the mortality rate, the numerical evolution of the population and a forecast of the numerical evolution. We have noticed that there is a connection between the intensity of the attack, the blossom period and the number of flowers.

**INTRODUCTION**

The two invasive species are original from North America (*Parectopa robiniella* Clemens – 1863, *Phylonorycter robiniella* Clemens – 1859) and have reached most of the countries in Europe, too (Šefrová, 2002). *Parectopa robiniella* was mentioned in Italy in 1970 (Vidano, 1970) and *Phylonorycter robiniella* was mentioned in 1983. In Romania, *Parectopa robiniella* was discovered in Drobeta Tr. Severin (Nețoiu, 1990) and *Phylonorycter robiniella* in Vâlcea county (Nețoiu, 2003). The researchers have studied the morphological description of the species (Martinez and Chambon 1987; Whintebread, 1990), the life cycle (Csoka, 1999; Nețoiu, 2003), the description of the ecological effects (Nețoiu and Tomescu, 2006), the influence of the parasitoid over these species (Csoka and Zsolt, 2009; Hansson and Shevtsova, 2010). On the present paper, we have described the dynamic of the two populations in 2008-2010 by taking into consideration some characteristics: the distribution, the density, the intensity of the attack, the breeding rate, the mortality rate, the numerical evolution of the population and a forecast of the numerical evolution. In the specialized literature, these aspects are not mentioned, that is why I consider them very useful for silviculture in order to predict the divisions, but also to find some ecologically and economically efficient control methods. The convention regarding the biological diversity (2008) emphasizes the process of researching the invasive species in order to control the population and prevent the numerical explosions.

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## MATERIAL AND METHODS

The research of the vermin took place in the acacia woods in the hill area from Pietroșani (county Argeș) in 2008-2010. The two species have been researched in the margin of the forest and in the massif where there are vigorous trees. In these two areas, we have set out a surface of 400 m<sup>2</sup>. In the margin, the trees are very heterogeneous being of different classes and ages. We have taken the data randomly, along the diagonal of the square of the sample area.

The branches have been collected from the inferior third part of the canopy and we have counted the individuals from the first 10 leaves starting with the top of the branches. The samples were taken weekly and we made comparisons between the two studied areas. The sampling method was similar to the ones used in other regions from Europe (Povilas and Jolanta, 2008; Csoka and Zsolt, 2009).

## RESULTS AND DISCUSSIONS

In the studied region we have noticed that the moth had two generations. In other parts of the country, this species can develop even three generations per year (Nețoiu and Tomescu, 2006). The first generation emerged and developed between June 10th and July 20th, the second generation emerged and developed in the first decade of August until the first decade of September.

The eggs are spawned on the inferior epidermis of the young leaves. The larvae come out of the eggs forming triangular mines visible only on the inferior part of the leaf. These mines are placed at the insertion place of a secondary string with the main string (Martinez and Chambon, 1987). The larva produces then, a pinnate mine which extends itself in the mesophile of the leaf. When the larva reaches its maturity it leaves the mine getting down to the surface of the litter with the help of a silk thread. Once it reached the litter it forms a white cocoon where it stays during the winter. In summer the adults come out of the cocoons.

The breeding flight takes place during the evening, and afterwards the eggs are spawned on the leaves. The breeding flight takes place in the last decade of May and in the first decade of August. As we know the temperature average for these decades, we may say that the thermal optimum for this insect varies between 16.5-21.4 C° (Mărăcineni weather base). The distribution type:

In determining the type of distribution we used the data obtained in July 6th, 2008 (tab.1).

Table 1

Sampling date ( <i>Parectopa robiniella</i> )										
x	0	1	2	3	4	5	6	9	11	12
f	53	15	14	7	3	3	1	2	1	1

x = the number of the individuals/ sample unit (a leaf);

f = the number of unities that had 0, 1, 2, 3,...individuals;  $S^2 / x = 22.7 / 1.38 = 16.35$ ;  
Because  $16.5 > 1$ , it results that the distribution is grouped.

While observing the tree we noticed that out of the 30 researched trees, a percent of 40% present signs of attack during the first generation, while in the second generation all the trees are attacked. In the massif, in the summer of 2008 we didn't identify the species.

Analyzing the frequency of the attacked leaflets by the mine number on the leaf (fig. 1) we can notice that during the first generation (2008) only half of the leaves are attacked and most of these have 1-2 individuals.

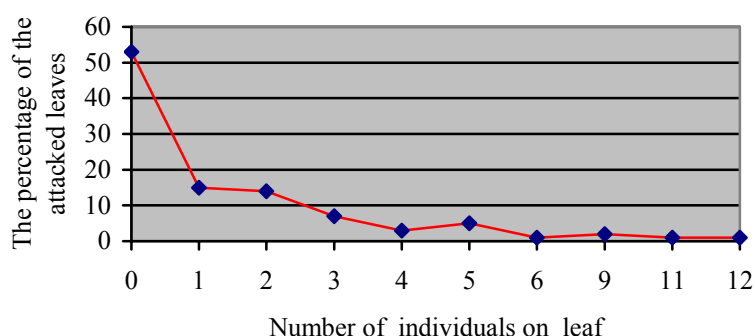


Fig. 1. The frequency of the leaflets attacked according to the number of mines on the leaves produced by the first generation larvae (2008)

If we analyze the frequency of the attacked leaflets by the number of mines on the leaf for the second generation (fig. 2) we shall notice that, more than 80% of the leaves are attacked and most of these have 2-4 individuals.

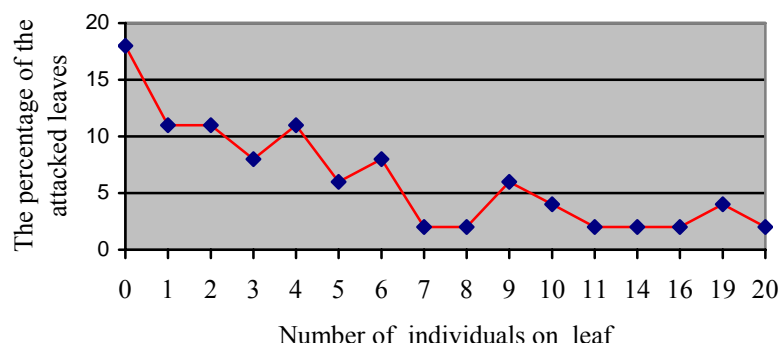


Fig. 2. The frequency of the leaflets attacked according to the number of mines on the leaves produced by the second generation larvae (2008)

The results were compared with those obtained by Nețoiu and Tomescu (2006) and we have discovered that, for the first generation they are similar, but for the second generation the results are different. The frequency curve of the attacked leaflets by the number of mines on the leaf in the second generation presents an increasing and a decreasing line with a maximum percent of leaves that have 6 individuals (Nețoiu and Tomescu, 2006). The density:

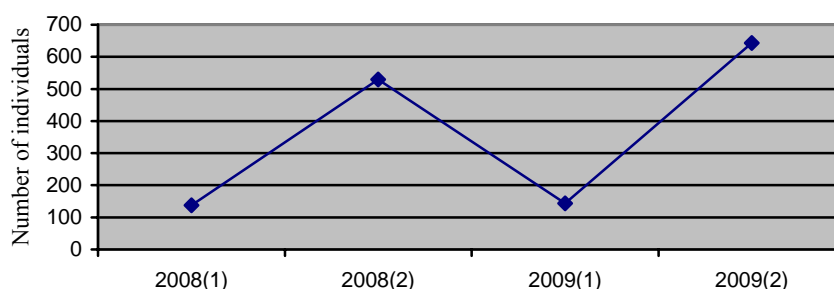
In determining the density we took a leaf as a sample unit. In table no. 2, there are presented the data obtained in 2009 in comparison with those in 2008.

Table 2

The density of the *Parectopa robiniella* population

	Skirt		Massive	
	First generation individuals density	Second generation individuals density	First generation individuals density	Second generation individuals density
2008	1.38	5.29	0	0
2009	1.44	6.43	0	0.17

If we analyze the data in the table we shall notice a light numerical increase of the population in 2009 which, in contrast with 2008, is more obvious during the second generation. In winter, the mortality was of about 73%. The breeding percent in 2009 for *Parectopa robiniella* is of 4.4. In the first decade of August we identified the presence of the species in the massif, too. Analyzing the numerical evolution of the population (fig. 3), we observe that the species present numerical variations from one generation to another.

Fig. 3 Numerical evolution of *Parectopa robiniella* population

If we take into consideration the ratio between the number of individuals from the second generation from 2008 and the number of individuals from the first generation from 2009, we may forecast a number of 175 individuals/100 leaves for the first generation in 2010. If we consider an average breeding rate of about 4 for the second generation, we estimate an average number of 700 individuals/ 100 leaves.

#### *Phylonorycter robiniella*

The researches have shown that it is a bivoltine species, but some researchers suggest that in the years with a long fall they develop a third generation (Nețoiu, 2003). The adult fly in July and August and after breeding the female spawns an egg on the inferior part of the leaf. The larva comes out of the egg and it feeds itself with the lacunar tissue and then it gets further to the palisadic tissue.

As opposed to the *Parectopa robiniella*, the dejection of this larva remains in the central area of the mine. When the larva reaches to its maturity, at the level of the mine remain the two epidermis and the libero-wooden vessels. The inferior epidermis from the

mine level has the aspect of a whitish felt while the superior one has a tessellated aspect (green-yellow). The larval-pupal transformation takes place in the mine in a white cocoon. The identification of the insect took place in the first decade of August (2008), the attack coincided with the second generation of the species *Parectopa robiniella*, but as opposed to this moth, the attack is much weaker.

Analyzing the frequency of the attacked leaflets by the number of the mines on the leaf, we have noticed that about 80% of the leaves are not attacked, and out of those attacked, the majority presents one individual. The average of the individuals, from the first generation, on a leaf was of about 0.25 and in the second generation of about 0.42.

In 2009 the first generation coincided with the second generation of the *Parectopa robiniella* species. In the first decade of September we noticed an average of 0.86 individuals on each leaf. In October, 10th, 2009 we have sampled 100 leaves from the studied area and by counting the mines we reached an average of about 1.61. If we analyze the numerical evolution of this species (fig. 5), we notice a duplication of the individuals' number from each generation. This characteristic is important in realizing some forecasts of the species, but we can establish also the year in which the species entered the ecosystem.

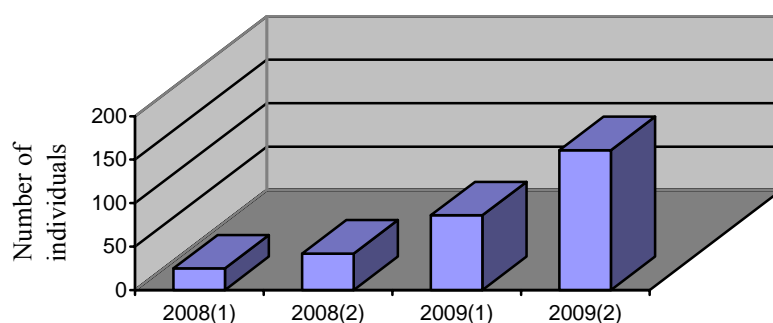


Fig. 5 Numerical evolution of *Phylonorycter robiniella* population

The mortality rate during the cold season is insignificant. In 2010 the species will present an average number of about 3 individuals per leaf and 6 in the second generation. Knowing that a leaf has in average 13-15 leaflets, and on a leaflet 2-3 mining larvae can be formed, we may say that in 2012 the insect enters in age-class. In the spring of 2010 a comparison was made regarding the blossom capacity for the acacia trees strongly attacked and those that haven't been attacked. In the case of those attacked, the blossoming took place later and it was 45% reduced. This association between the intensity of the attack, the blossoming period and the number of flowers was analyzed by Maceljski and Mešić (2001).

## CONCLUSIONS

The species *Parectopa robiniella* and *Phylonorycter robiniella* present two generations per year. If we compare the two attacks, the second is much more powerful and it has more important effects. Between the two species there is a competition for the same ecological niche. If we compare the two attacks produced by the two species we realize that *Parectopa robiniella* has a larger spread and the intensity of the attack is much more

pronounced. If we compare the life cycles, we shall notice that, *Parectopa robiniella* starts the attack in the first decade of June and *Phyllonorycter robiniella* in the first decade of August. The first generation of *Phyllonorycter robiniella* coincides with the second generation of the insect *Parectopa robiniella*. The breeding rate for *Parectopa robiniella* is of about 4 between the generations of the same year. The mortality is quite high (73%), it decelerates the numerical explosions. Because *Phyllonorycter robiniella* has the first generation in the first decade of August, it can not have but two generations per year because the temperature is getting lower. The duplication of the number of individuals at every generation and the low mortality rate will determine numerical explosions. *Phyllonorycter robiniella* will not be able to eliminate the competitor species because of the gap between the attacks. The attack is stronger in the margin of the forest, in the sunny places, while in the massif there are vigorous trees and we didn't identify the presence of the species in 2008. In the fall of 2009, in the massif we noticed the presence of *Parectopa robiniella*.

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CONSIDERATIONS CONCERNING PNEUMATIC SHOCK WAVES  
UTILIZED FOR BULK GRAIN DISINFECTION STOCKED IN SILOS

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**KEY WORDS:** *pneumatic shock waves, grain disinfection*

**ABSTRACT**

*The paper presents theoretical and experimental considerations concerning the principle due to the pneumatic shock waves can be utilized for bulk grain disinfection stocked in silos: working principle for pneumatic shock waves device; pneumatic shock waves velocity/energy; static and dynamic pressure into the bulk grain stockage silos; FEM simulation for silos walls' deformation.*

**INTRODUCTION**

The pneumatic shock wave systems (Big Blaster, Air Cannon, Air Chock) are extensively used to prevent the bulk material blockage in the bunker, and to determine the bulk materials flow from storage bunker/hopper/silos/bin.

There are known several technical applications for solid or powder bulk materials for large electro-thermal plants, metal molding, cement plants, raw materials for metallurgy, dust filtering system for belt conveyors (Roşca *et al.*, 2006; Roşca *et al.*, 2008), or for viscous materials (Roşca, 2004) in food industry (Figure 1).

An unconventional experimental equipment was designed to replace the effect of the windblasts, with orientated air blaster shock waves (with adjustable velocity / intensity), which replace the velocity and orientation of strong winds. In figure 2 is presented the main component of the experimental equipment for *Modular Equipment for Nuts Harvesting by Pneumatic Impulses - MEHPI* (Roşca, 2005; Roşca *et al.*, 2005; Roşca *et al.*, 2006).

This new equipment is proposed to replace the traditional harvesting machines which produce strong vibrations to the tree. During harvesting with the traditional vibration machines, the vibrations cause severe damage to the roots of the tree, and the scratching of the tree trunk causes the premature drying of the tree. During harvesting with the traditional vibration machines, the vibrations cause severe damage to the roots of the tree, and the scratching of the tree trunk causes the premature drying of the tree.

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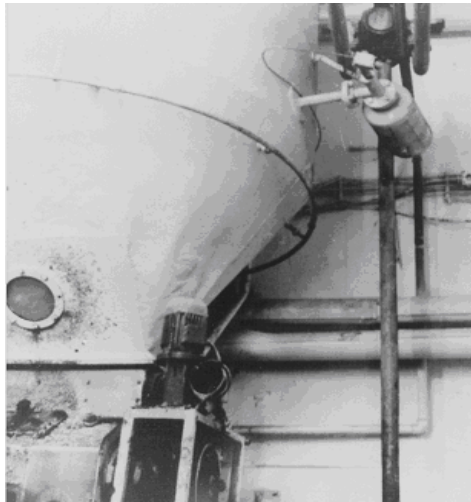


Figure 1. Pneumatic shock wave system on metallic bunker for viscous milling in beer plant

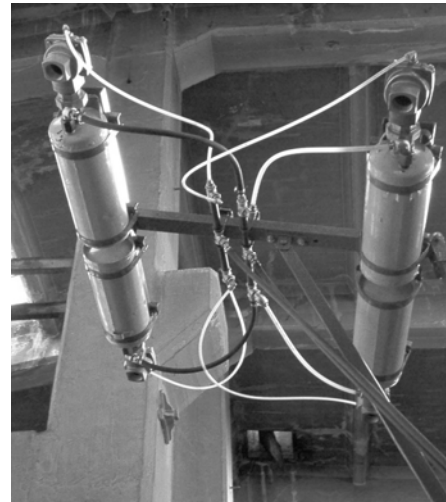


Figure 2. Modular Equipment for Nuts Harvesting by Pneumatic Impulses

### EXPERIMENTAL EQUIPMENT

Pest/insects control for bulk agricol product (stocked bulk grains in silos) is based on preventive and curative complex methods (Mitrea, 2004).

An important preventive method consists in the monitoring of the environmental conditions (temperature, humidity, chemical and biological conditions) inlet silos bulk grains, to combate or interrupt the biologic cycle of pest/insects.

For medium or longer period of grains' stockage in silos, an usual method recommends more often energetically manual or mechanical spooner.

Due to the intensive impact and friction phenomena between grain particles during mechanical spooner, the sensible life stage of insects (eggs, larva) are almost inactivated or killed. In the same time, during the mechanical spooner the bulk grain a short natural ventilation is realized. The traditional bulk grain stockage in large silos permits a longer artificial ventilation, but no mechanical spooner is possible.

The main operational element of MEHPI or bunkers discharge systems consists in fast discharge device (FDD). In principle, FDD is composed in a small capacity vessel with a special fast discharge pneumatic valve (figure 3).

Due to the special fast discharge pneumatic valve, the compressed air stocked in the vessel, is discharged in supersonic velocity regime (Roşca, 2005; Roşca *et al.*, 2005; Roşca *et al.*, 2006).

The pressured gas (air, CO<sub>2</sub>, nitrogen) is supplied at nominal work pressure 3...10bar, by an air compressor or by an independent pressurized gas recipient.

In order to use pneumatic shock waves for bulk grain disinfection stocked in silos, a FDD with 8dm<sup>3</sup> vessel capacity is proposed.

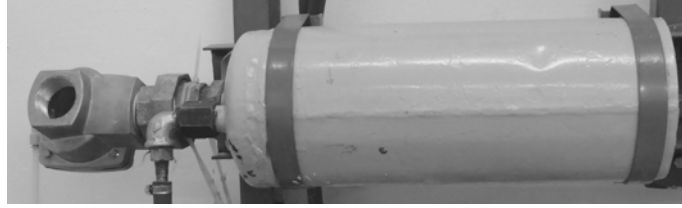


Figure 3. Fast discharge device

#### CONSIDERATION CONCERNING PNEUMATIC SHOCK WAVES VELOCITY

The FDD operation is based on the effect of the compressed gas discharge with high velocity from a storage vessel. During this fast process, the gas flow is characterized by high rate pressure variation; therefore there is no heat exchange with the outside environment, and the flow process can be considered adiabatic.

For compressible fluids, the Bernoulli equation for adiabatic process is:

$$\frac{v^2}{2} + \frac{k}{k-1} \cdot \frac{p}{\rho} = \frac{v_o^2}{2} + \frac{k}{k-1} \cdot \frac{p_o}{\rho_o}, \quad (1)$$

where  $p_o$  and  $\rho_o$  are the initial parameter of the gas;  $p$  and  $\rho$  are the final parameter of the gas;  $k$  is the adiabatic coefficient;  $v_o$  is the initial gas velocity (in the storage vessel  $v_o=0$ ) (Roşca, 2001; Roşca *et al.*, 2008; Roşca *et al.*, 2009).

The dynamic adiabatic transformations are irreversible (the entropy increase due to the internal heat stored in gas due to viscosity forces). Neglecting the viscosity force, this motion can be considered isentropic (admissible hypothesis for gas blaster discharging phenomena).

When the compressed gas is discharged from a storing vessel (initial parameter  $p_o, \rho_o, T_o$ ) through a nozzle in the atmosphere (characterized by parameter  $p_{at}, \rho_{at}, T_{at}$ ), the gas velocity is determined with relation:

$$v_{max} = \left\{ \frac{2k}{k-1} \cdot \frac{p_o}{\rho_o} \cdot \left[ 1 - \left( p_{at} / p_o \right)^{\frac{k-1}{k}} \right] \right\}^{1/2} \quad (2)$$

Because the ratio  $(p_{at} / p_o) < 0,5283$ , in the minimum cross section of the convergent nozzle/pipe the critical regime is realized, and the maximum flow that is obtained (passing through this cross section)  $Q_{max}$  can be determined with relation:

$$Q_{max} = 0,04042 \cdot S_p \cdot p_o / T_o^{1/2} \quad (3)$$

where cross section area of the convergent nozzle/pipe (the convergent nozzle  $D_p=44\text{mm}$ ).

Considering the initial and the final parameters of the gas ( $p_o=3\text{-}7$  bar;  $p_{at}=1$  bar;  $T_o = T_{at} = 293^\circ\text{K}$ ;  $k = 1,4$ ), the maximum velocity  $v_{max}$  of the pressured  $\text{CO}_2$  discharged from the storing vessel, and the maximum flow  $Q_{max}$  passing through the cross section  $S_p$  are presented in table 1.



Maximum velocity and maximum flow  
for pressured CO<sub>2</sub> fast discharging

Table 1

$p_o$ [bar]	$\rho_o$ [kg/m <sup>3</sup> ]	$v_{max}$ [m/s]	$Q_{max}$ [kg/s]
3	3,72	402,3	1,098
4	4,87	443,8	1,467
5	6,09	469,7	1,817
6	7,31	493,4	2,172
7	8,56	506,2	2,568

Theoretical and experimental medium  
velocity for pressured CO<sub>2</sub> fast discharging

Table 2

$p_o$ [bar]	$v_{med}$ [m/s]	
	Theoretic	Experim
3	80,5	75,3
4	86,8	81,2
5	93,9	86,2
6	98,7	91,5
7	101,2	95,3

The medium velocity of jet  $v_{med}$  is determined knowing that the medium flowing velocity in a flow section is determined with relation  $v_{med} = 0,2 v_{max}$ . The medium velocity determined using theoretical method and the experimental method are presented in table 2.

The experimental method to determine the shock wave velocity proposes high speed camera Fastec Imaging type (Roşca *et al.*, 2009).

To determine the shock wave velocity for pressured CO<sub>2</sub> fast discharging (initial pressure  $p_o = 3 \dots 7$  bar), a contrast colored fine powder was introduced into FDD convergent nozzle. A white panel with 0,5m horizontal and vertical grids was used (figure 4).



Figure 4. Shock wave velocity using high speed camera.

According the shock wave velocity value', the image capturing sequence was set for 500 frames per second, and 320x240 sensor resolution. The high speed camera MiDAS 4.0 Express Control Software start was simultaneous triggered with the FDD's electro-pneumatical fast discharge valve. The values for shock wave velocity determined with this innovative experimental method are 2...7% smaller then those obtained using theoretical method (in the front and at the border of the shock wave, due to viscosity force, the turbulent flow determines smaller values then the theoretical shock wave velocity).

#### CONSIDERATION CONCERNING PNEUMATIC SHOCK WAVES ENERGY

The properties of the stocked/processed materials, the wear and permanent deformations of the bunker walls, determine the flow blocking of the evacuation zone of the bunker, which is the main cause in quality and productivity of the technological process.

The method concerning in compressed gas (air/N/CO<sub>2</sub>) fast discharge is preferred because the gas action is directly distributed in the material inlet the bunker.

During materials stocking or in slow velocity homogenization, the bunker walls are stressed by the weight of the material which is distributed on the walls surfaces as static pressure characterized by various distribution laws.

The walls of the bunker are stressed by vertical and horizontal static pressures which are calculated with simplified method described by the relations (Banu, 2000; Roşca, 2004).

$$P_{SV} = \frac{\rho_m \cdot g \cdot R_{H,b}}{f_{m,b} \cdot m_m} \cdot \left[ 1 - \exp\left(-h_m \frac{f_{m,b} \cdot m_m}{R_{H,b}}\right) \right], \quad [\text{N/m}^2] \quad (4)$$

$$\text{respectively } P_{SH} = P_{SV} \cdot m_m, \quad (5)$$

in which  $\rho_m$  is the density of the stocked/processed material,  $[\text{kg/m}^3]$ ;  $f_{m,b}$  is the friction coefficient between the material and the bunker wall;  $m_m$  is the mobility coefficient of the material, calculated with the relation:

$$m_m = (1 - \sin \varphi_m) / (1 + \sin \varphi_m), \quad (6)$$

in which  $\varphi_m$  is the internal friction angle between the material particles;  $g$  is the gravitational acceleration,  $[\text{m/s}^2]$ ;  $h_m$  is the material depth in the bunker,  $[\text{m}]$ .

The hydraulically radius  $R_{H,b}$  is determined with relation

$$R_{H,b} = A_b / P_b \quad (7)$$

where  $A_b$  is the transversal section of the bunker;  $P_b$  is the perimeter of the bunker section.

For dried bulk materials is recommend  $m_m = 0,18 f_{m,b}$ , therefore after replacements, the relation (4) become

$$P_{SV, \max} \cong 55 \rho_m \cdot R_{H,b} \quad [\text{N/m}^2] \quad (8)$$

In the pyramidal or conical walls bunker, the vertical static pressure is depending on inclination angle  $\alpha_p$ , therefore is calculated with relation

$$P_{SV, \alpha} \cong P_{SV} (\cos^2 \alpha_p + m_m \cdot \sin^2 \alpha_p) \quad [\text{N/m}^2] \quad (9)$$

The total static pressure is calculated taking into account the horizontal pressure and the vertical pressure that action on bunker walls  $P_{st} = \sqrt{P_{SH, \alpha}^2 + P_{SV, \alpha}^2}$  (Banu, 2000; Roşca, 2004).

In main, the pneumatic shock waves unblocking method is based on the fast discharge of pressured gas from a stocking vessel.

In figure 5 is presented the putting into operation principle schema, for a FDD (2) which has a discharge pipe (3) and a convergent - divergent nozzle (4) mounted on the bunker wall (1).

The shock wave generates a dynamic pressure  $P_{din}$  which has an impulsive effect toward the adherent material on bunker walls.

To realize the unblocking it is necessary to respect the condition  $P_{din} > P_{st}$ , where  $P_{st}$  is the total static pressure of the bulk material on bunker walls.

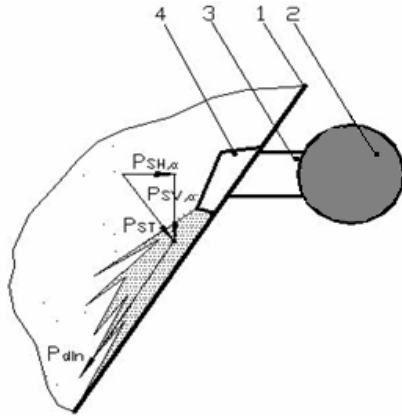


Figure 5. FDD mounting schema on bunker wall.

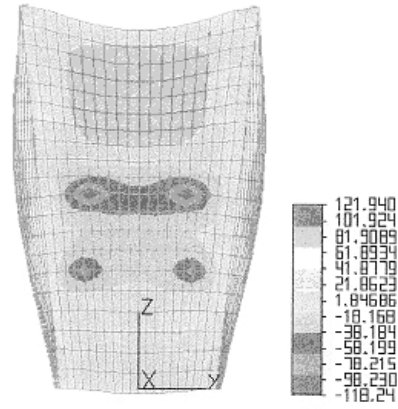


Figure 6. FEM simulation for four FDD on bunker wall.

The kinetically energy realized by the direct shock wave action produced by the gas fast discharging effect, can be calculated with relation (Roșca, 2001):

$$\Delta W_{cin} = \frac{1}{\rho_o \cdot c_o} \int_0^{t_m} p^2(t) \cdot dt = \frac{1}{\rho_o \cdot c_o} \int_0^{t_m} (p_{max} \cdot e^{-t/\theta})^2 \cdot dt, \quad (10)$$

where  $t_m$  is the time moment when is achieved the maximum wave pressure ( $p_{max}$ ).

Replacing the initial pressure values  $p_o = 3 \dots 7 \text{ bar}$  in relation (10), there were obtained direct shock wave energy in  $12 \div 110 \text{ J}$  range values.

In order to determine the bunker wall deformation during the dynamic kinetically energy realized by the direct shock wave action produced by the gas fast discharge, FEM simulation for bunker wall dynamic stability are requested.

In figure 6 is presented the FEM simulation for four FDD on bunker wall: conical shape, 3m high, 1mm wall thickness, grains inlet (Năstăsescu, 2005; Roșca *et al.*, 2008).

The figure presents the von Misses equivalent strength in bunker wall, which permit to determine if static and dynamic stability reglementations are respected during the bunker dynamic discharging process.

## CONCLUSIONS

The pneumatic shock wave energy make possible mechanical spooner of the bulk grains inlet the bunker. The pneumatic shock wave energy is able to realize a fast and intensive moving of important grain particles quantities, that will inactivate or kill the sensible stage life of insects.

In the same time artificial ventilation is realized, when the pneumatic shock waves contains gaseous fumigant or powder / liquid disinfectant substances, fumigant disinfection, or liquid disinfection, respectively.

The maximum values of the dynamic pressure of the pneumatic shock waves determine dynamic loads both to the bulk material inside the bunker, and to the bunker's walls, too. To prevent any damages on the bunker's structure are necessary theoretical simulations and experimental research to determine the bunker stability depending on the bunker's walls ware degree.

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EXPERIMENTAL EQUIPMENT TO STUDY  
THE INFLUENCE OF LOW VACUUM PROCESSING  
ON NON – THERMIC PRESERVATION OF ORANGE FRUIT

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**KEY WORDS:** *equipment, low vacuum, non-thermic preservation*

**ABSTRACT**

*The paper presents experimental equipment special made for interdisciplinary research studies concerning the influence of low vacuum processing on non-thermic preservation technology methods of fruits. The research studies concern in infusion speed and quality of the orange fruit utilizing a non-thermic preservation method based on vacuum process up to 0,01bar (-0,99bar). The paper presents experimental results proving the possibility of increasing the infusion speed and the fruit infusion quality depending on the vacuum values, and the processing duration and succession.*

**INTRODUCTION**

The increased consumers demand for improved nutritional and sensorial of food characteristics, with no "fresh taste" loss, in the last years, non-thermal preservation method such as high pressure process, or medium and low vacuum processing, made necessary extensively studies in food industry research (Larousse, 1993).

The European "Novel Foods" Directive reviewed in 2004, recommend few limits for high pressure process, or low vacuum processing products research development.

During high pressure processing (Larousse, 1997) and low vacuum processing (Rosca *et al.*, 2008) isostatic process is realized: the food product is immersed in an incompressible liquid which transmits uniformly the pressure into the food product, with non - thermic preservation process.

In order to begin the interdisciplinary studies concerning high pressure research, in the Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Horticulture in Craiova (research laboratory financed by CNCSIS grant), experimental equipment was made. This experimental equipment consists in stainless steel vessel (0,5 liter capacity; designed and made according ISCIR norms; maximum pressure up to 800bar obtained using a test strength machine, and usual pressure up to 500bar obtained using a special screw mechanism) (Rosca *et al.*, 2004; Nour *et al.*, 2004).

In order to determine the influence of high pressure process on fruit preservation, Jonathan apple variety with 12,3 % dried soluble mater content, were infused in sugar syrup with 70 % dry soluble mater content (Nour *et al.*, 2004). It was determined that after fruits

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infused at continuum pressure of 600 bar for 10 min, the dried soluble mater content in apples increase up to 26,8%, and the dried soluble mater content in syrup decrease up to 61,8%.

After fruits infusion using increasing pressure up to 600 bar (three consecutive stages consisting in 200 bar/2min, 400 bar/2 min and 600 bar/ 6 min), it was observed the dried soluble mater content in apples increase up to 28,2%, and the dried soluble mater content in syrup decrease up to 59,4%. There are several experimental studies concerning the influence of vacuum process in non-thermic fruit preservation (Rosca *et al.*, 2008; Rosca *et al.*, 2009).

In this variant were used strawberry, orange and pear. To observe the influence of low vacuum processing in fruits infusion, sugar syrup with 71% dried soluble mater content, and several fruits variety (orange, pear, strawberry) was used. The vacuum process consists in 5 processing stages (22 min total vacuum process time; depressurization up to 0,97% absolute pressure, maintaining up to 0,85% absolute pressure, pressurization to 0bar absolute pressure). For pear (14,2<sup>0</sup> R initial dried soluble mater content), after vacuum infusion it was observed the dried soluble mater content in fruit increasing up to 28,8<sup>0</sup> R, and the dry soluble mater in fruit' syrup decreasing up to 63,2<sup>0</sup> R (Rosca *et al.*, 2009).

## EXPERIMENTAL EQUIPMENT

In order to put in evidence the quantitative comparisons between the high pressure process method, and the influence of low vacuum processing on non-thermic preservation in fruits preservation, in the Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Horticulture in Craiova, experimental equipment was made.

In main, the experimental equipment is composed in *vacuum pump*, *vacuum processing vessel*, and *condensed gases dryer module* (figure 1). The main characteristics of the *vacuum pump* (HYVAC type): maximum flow rate up to 40 l/min; absolute pressure up to 0,5 militorr.

The main characteristics of *vacuum processing vessel* (designed and made according Romanian ISCIR norms): absolute pressure up to 0,5 militorr; stainless steel W1.4571; welding coefficient 1; 100% ultrasonic control for welding assemblies. To observe the inlet vessel during vacuum process, one of the flanges is made in transparent visor/plate (high resistant polycarbonate). The *condensed gases dryer module*, in main, consists in stainless steel vessel (designed and made according to Romanian ISCIR norms). The vessel contains 13X and 5A type molecular sieve.

The experimental equipment fitting accessories (ISO/NW/NPT type) are made in AISI 316L stainless steel. In order to observe the pressure/vacuum losses, the condensed gases dryer module and the processing vessel are provided with special manovacuumeter gauges (stainless steel W1.4571; 1,6 precision class).

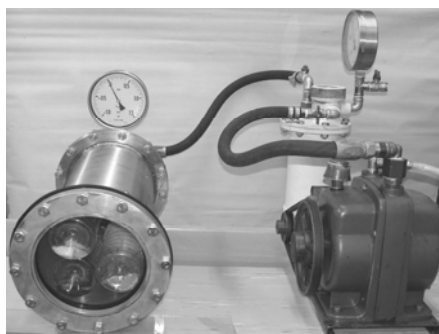


Figure 1. Experimental equipment for low vacuum processing of fruit non – thermic preservation.

## MATERIAL AND METHOD

In order to determine the influence of low vacuum processing on non – thermic preservation of orange fruit, experimental research using commercial orange were performed. The selected fruits were cutted in pieces, then weighted to be introduced in sugar syrup. The same quantity of fruit and sugar syrup were put in transparent food grade plastic bags to be introduced into vacuum processing vessel of the experimental equipment.

To observe the influence of low vacuum on non-thermic preservation of orange fruit, honey syrup with 85% dried soluble mater content, and oranges with 10,6% dried soluble mater content were used.

Before and after the low vacuum processing, the dried soluble mater content in orange, and the dried soluble mater content in syrup after the non-thermic infusion process was determined with ABBE portable refractometer.

The proposed low vacuum processing is a *non-thermic preservation method*; therefore, the temperature of low vacuum processing is necessary to be permanently monitored. Due to the low vacuum process value, it was used a non contact method (FLIR Infrared Thermovision Camera, provided by Thermomechanical Parameter Monitoring Laboratory, within Faculty of Horticulture). During the non-thermic infusion, 24...27°C range temperature into the vacuum processing vessel was measured.

## RESULTS AND DISCUCTIONS

The paper presents two types of experimental research concerning the influence of low vacuum processing on non-thermic preservation in oranges preservation: low vacuum continuum processing, and low vacuum cyclic processing, respectively.

Low vacuum continuum processing represents 22min total time vacuum process consisting in 5 processing steps (depressurization, maintaining, pressurization) presented in table 1.

Table 1

Processing steps for low vacuum continuum preservation method

Processing step		Variant 1	Variant 2	Variant 3
1. Depressurization	Vacuum rate, [bar]	0 ↓ - 0,97	0 ↓ - 0,97	0 ↓ - 0,97
	Time rate, [min]	1	1	1
2. Maintaining	Vacuum rate, [bar]	- 0,97 → - 0,95	- 0,97 → - 0,95	- 0,97 → - 0,95
	Time rate, [min]	5	5	5

3. Pressurization	Vacuum rate, [bar]	- 0,95 ↑ - 0,7	- 0,95 ↑ - 0,85	- 0,95 ↑ - 0,9
	Time rate, [min]	1	10	10
4. Maintaining	Vacuum rate, [bar]	- 0,7	- 0,85 → - 0,8	- 0,9 → - 0,85
	Time rate, [min]	14	5	5
5. Pressurization	Vacuum rate, [bar]	- 0,7 ↑ 0	- 0,8 ↑ 0	- 0,85 ↑ 0
	Time rate, [min]	1	1	1

The process diagram for Variant 1 and Variant 2 are presented in figure 2, and in figure 3, respectively.

Low vacuum cyclic processing presented in this paper consists in 2 variants with up to 6 process steps (depressurization, maintaining, and pressurization), with total time process 12min, respectively 9min (table 2).

The process diagram for Variant 4 and Variant 5 are presented in figure 4, and figure 5, respectively.

Detail image of low vacuum preservation/infusion due to osmosis process during the vacuum evaporation in Variant 5 is presented in figure 6.

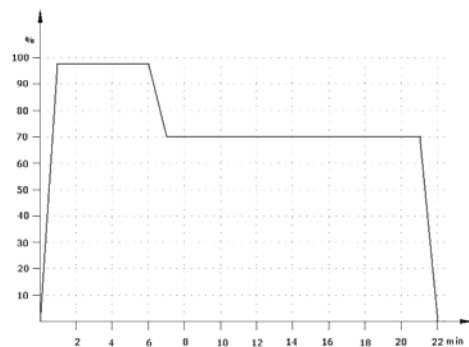


Figure 2. Process diagram for Variant 1.

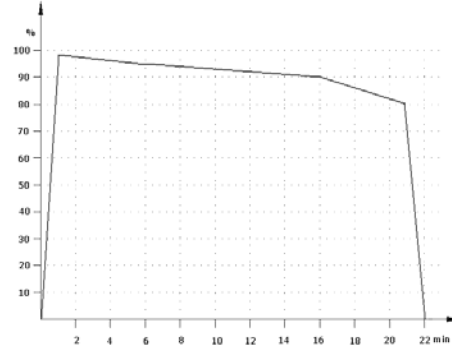


Figure 3. Process diagram for Variant 2.

Table 2

Processing steps for low vacuum cyclic preservation method			
Processing step		Variant 4 (12min total time)	Variant 5 (9min total time)
1. Depressurization	Vacuum rate, [bar]	0 ↓ - 0,97	0 ↓ - 0,97
	Time rate, [min]	1	1
2. Maintaining	Vacuum rate, [bar]	- 0,97 → - 0,95	- 0,97 → - 0,95
	Time rate, [min]	3	2
3. Pressurization	Vacuum rate, [bar]	- 0,95 ↑ - 0,75	- 0,95 ↑ - 0,5
	Time rate, [min]	2	1
4. Maintaining	Vacuum rate, [bar]	-	- 0,5
	Time rate, [min]	-	1
5. Depressurization	Vacuum rate, [bar]	- 0,75 ↓ - 0,97	- 0,5 ↓ - 0,97
	Time rate, [min]	1	1
6. Maintaining	Vacuum rate, [bar]	- 0,97 → -0,95	- 0,97 → -0,95
	Time rate, [min]	2	2
7. Pressurization	Vacuum rate, [bar]	- 0,95 ↑ - 0,75	- 0,95 ↑ - 0
	Time rate, [min]	2	1
	Vacuum rate, [bar]	- 0,75 ↑ 0	-
	Time rate, [min]	1	-



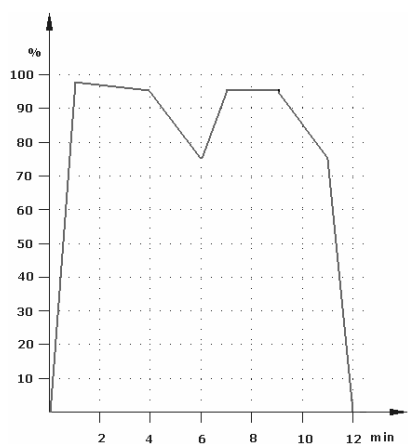


Figure 4. Process diagram for Variant 4.

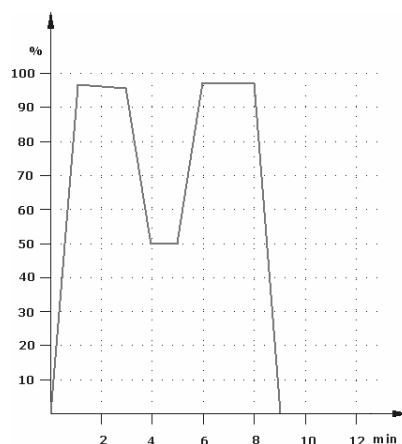


Figure 5. Process diagram for Variant 5.



Figure 6. The vacuum infusion due to osmosis process during the vacuum evaporation in Variant 5.

The dried soluble mater content in orange and the dried soluble mater content in honey' syrup before and after the preservation/infusion, using *low vacuum continuum processing*, are presented in table 3.

In table 3 is observed that after the low vacuum continuum infusion process, the dried soluble mater content in orange increase more then 150%, and the dried soluble mater content in honey' syrup decrease up to 20%.

Table 3

Dried soluble mater in orange and the dried soluble mater in honey' syrup, before and after the low vacuum continuum processing

Variant	Dried soluble mater in orange, [ $^{\circ}$ R]		Increase of dried soluble mater in orange, after the infusion process, [ $^{\circ}$ R]	Dried soluble mater in honey' syrup, after infusion process, [ $^{\circ}$ R]
	before infusion	after infusion		
1	10,6	20,5	9,9	75,1
2		24,2	13,6	72,4
3		25,9	15,3	70,7

The dried soluble mater content in orange and the dried soluble mater in honey' syrup before and after the preservation/infusion, using *low vacuum cyclic processing*, are presented in table 4.

In table 4 is observed that after the low vacuum cyclic infusion process, the dried soluble mater content in orange increase up to 170%, and the dried soluble mater content in honey' syrup decrease up to 23%.

Table 4

Dried soluble mater in orange and the dried soluble mater in honey' syrup,  
before and after the low vacuum cyclic processing

Variant	Dried soluble mater in orange, [ <sup>0</sup> R]		Increase of dried soluble mater in orange, after the infusion process, [ <sup>0</sup> R]	Dried soluble mater in honey' syrup, after infusion process , [ <sup>0</sup> R]
	before infusion	after infusion		
4	10,6	25,5	14,9	70,1
5		28,2	17,6	67,4

## CONCLUSIONS

The non-thermic infusion is based on the osmosis process between fruits and syrup. During this process the sugar content in fruits gradually is increasing, and in the same time the juice of the fruits dilutes the syrup, until the equilibrium stage is realized.

The oranges pieces infused using low vacuum cyclic process were translucent, with no browning, good shape and with typical organoleptical standard properties (figure 7).



Figure 7. Organoleptical probe for Variant 5.

The quality of non-thermic infusion process is increased by low vacuum cyclic process (more economic then the continuum process), with comparable results obtained using high pressure process non-thermic preservation method.

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## DIFFERENT APPROACHES TO DEVELOPMENT AN INFORMATION SYSTEM

Roșca Doina<sup>1,\*</sup>, Bănică Logica<sup>2</sup>, Sîrbu Mirela<sup>3</sup>

**KEY WORDS:** *database, system design, system building, , system life cycle, organization*

### ABSTRACT

*The core activities in systems development are system analysis, system design, programming, testing, conversion, and production and maintenance. Systems analysis is the study and analysis of problems of existing systems and the identification of requirements for their solution. Requirements must consider economic, technical, and temporal constraints, as well as the goals, procedures, and decision processes of the organization. There are various systems-building alternatives: the traditional systems life cycle, prototyping, application software packages, end-user development, and outsourcing.*

### 1. Introduction

Wherever their origin, new information systems are an outgrowth of a process of organizational problem solving. A new information system is built as a solution to some type of problem or set of problems that organization perceives it is facing.

The resulting information systems is the product of a series of activities called **systems development**. Systems development is a structured kind of problem solving with distinct activities: *system analysis; system design; programming; testing; conversion; production and maintenance* [3]. Each activity involves strong interactions with the organization.

### 2. Database Trends

Recent database trends include the growth of distributed databases and the emergence of object-oriented and hypermedia databases. Beginning in the early 1970s, information processing became more distributed with the growth of powerful telecommunications networks and the decline in computer hardware costs.

The dispersion and use of computers among multiple geographically or functionally separate locations so that local computers handle local processing needs is called *distributed processing*. Although early distributed systems worked with a single centralized, over time the smaller local systems began to store local databases as well. A *distributed database* is one that is stored in more than one physical location.

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There are several ways of distributing a database:

- a. the central database can be partitioned so that each remote processor has the necessary data on customers to serve its own local needs; changes in local files can be justified with the central database on a batch basis, often at night;
- b. the central database can be duplicated at all remote locations; also require updating of the central database after hours;
- c. another possibility is to maintain only a central index distributed database and to store complete records locally; a query to the central name index identifies where the full record can be found; there are no central database and no updating costs.
- d. an "ask-the-network" distributed database, the network polls its remote processors to locate a record and transfers the complete record to whatever processor requests it.

Distributed systems have *benefits*: reduce the vulnerability of a single, massive central site; permit increases in systems power by purchasing smaller, less expensive minicomputers; they increase service and responsiveness to local users; and *drawbacks*: are dependent on high-quality telecommunications lines, which themselves are vulnerable; local databases can sometimes depart from central data standards and definitions and pose security problems by widely distributing access to sensitive data.

For large national organizations working in several regions, the question is no longer whether to distribute but how to distribute in such way to minimize costs and improve responsiveness without sacrificing data and system integrity.

*Conventional database* management systems (DBMS) were designed for homogeneous data that can be easily structured into predefined data fields and records. Conventional DBMS are not well suited to handling graphics-based or multimedia applications. An *object-oriented database*, on the other hand, stores both data and procedures as objects that can be automatically retrieved and shared. The *hypermedia database* approach to information management transcends some of the limitations of traditional database methods by storing chunks of information in the form of nodes connected by links established by the user. The nodes can contain text, graphics, sound, full-motion video, or executable computer programs. Searching for information does not have to follow a predetermined scheme; the user can choose his own path to move from node to node. For each node displayed on the screen is shown the links between the node depicted and other nodes in the database.

### 3. Management Requirements for Database Systems

Perhaps the most difficult task of the systems analyst is to define the specific information requirements that must be met by system solution selected. Requirements analysis carefully defines the objectives of the new or modified systems and develops a detailed description of the functions that the new system must perform. Requirements must consider economic, technical, and temporal constraints, as well as the goals, procedures, and decision processes of the organization. Much more is required for the development database systems than simply selecting a logical database model: the database is an organizational discipline, a method, rather than a tool or technology. It requires organizational and conceptual change.

The critical elements [3] in management requirements for a database environment are:

- a. *data administration*: data planning, information policy, maintenance of data dictionaries, data quality standards;

b. *data planning and modeling methodology*: enterprise analysis, which addresses the information requirements of the entire organization is needed to develop databases; the purpose is to identify the key entities, attributes, and relationships that constitute the organization's data;

c. *database technology and management* refers to the more technical and operational aspects of managing data, including physical database design and maintenance; these functions are performed by **database administration**;

d. *users*: a database serves a wider community of users than traditional systems; more resources must be allocated to training end users.

Techniques used in requirements determination have involved over time to become more structured and increasingly rely on computer support. The more traditional requirements determination methods include interviewing, observing users in their work environment, and collecting procedures and other written documents. One of the current method is Joint Application Design (JAD); CASE (Computer-aided software engineering) tools are useful in requirements determination; prototyping has become a key tool for some requirements determination efforts.

#### **4. Different Approaches to Improving Development**

In the continuing effort to improve the systems analysis and design process, several different approaches have been developed. Attempts to make systems development less of an art and more of a science are usually referred to as system engineering or software engineering. As names indicate, rigorous engineering techniques have been applied to systems development.

The *system life cycle* is the oldest method for building information systems and is still used today for medium or large complex system s projects. This methodology assumes that an information system has a life cycle similar to that of any living organism, with a beginning, middle, and end. The life cycle methodology has a very formal division of labor between end users and information systems specialists. Technical specialists such as systems analysts and programmers are responsible for much of systems analysis, design and implementation work; end users are limited to providing information requirements and reviewing the work of technical staff.

The systems life cycle is still used for building large transaction processing systems and management information systems where requirements are highly structured and well defined. However, it is costly, time-consuming and inflexible. Volumes of new documents must be generated and steps repeated if requirements and specifications have to be revised.

**CASE Tools** is software that provide automated support for a wide variety Systems Development Life Cycle (SDLC) activities. An integrated and standard database called a *repository* is the common method for providing product and tool integration, and has been a key factor in enabling CASE to more easily manage larger, more complex projects and to integrate data across various tools and products.

The term CASE is now extended to includes all types of computer tools from business modeling and requirements capture to implementation tools.

The general types of CASE tools are:

- diagramming tools enable system process, data and control structures to be represented graphically;
- form (computer display) and report generators that make it easier for the systems analyst to identify data requirements and relationships;

- automatically check for incomplete, inconsistent, or incorrect specifications in diagrams, forms, and reports;
- a central repository enables the integrated storage of specifications, diagrams, reports, and projects management information;
- documentation generators produce technical and user documentation in standard formats.

**Joint Application Design** is a process for collecting information system requirements and reviewing system designs. The basic idea is to bring structure to the requirements-determination phase of analysis to the reviews that occur as part of design. Users, managers, and system developers are brought together for structured meeting run by a JAD session leader. By gathering all the people directly affected by an information system to agree on system requirements and design details, time and organizational resources are better managed. In many organizations, end users are developing a growing percentage of information systems with little or no formal assistance from technical specialists. This phenomenon, called **end-user development**, has been made possible by fourth-generation software tools: query languages, report generators, graphics languages, application generators, application software packages, very-high-level programming languages, and microcomputers tools (general-purpose application packages that have been developed for microcomputers).

**Prototyping** is less formal than the life cycle method. Instead of generating volumes of detailed specifications and documents, prototyping rapidly generates an experimental working model of a system for end users to evaluate. This method is most useful when some uncertainty exists about requirements or design solutions; it is especially valuable for the design of the **end-user interface** of an information system. Prototyping is best suited for smaller applications - large systems would have to be subdivided so that prototypes can be built one part at a time.

Prototyping consists in designing and building a scale-down but functional version of a desired system. A prototype can be built with any computer language or development tool, with query screen and report design tools of a database management, and CASE tools.

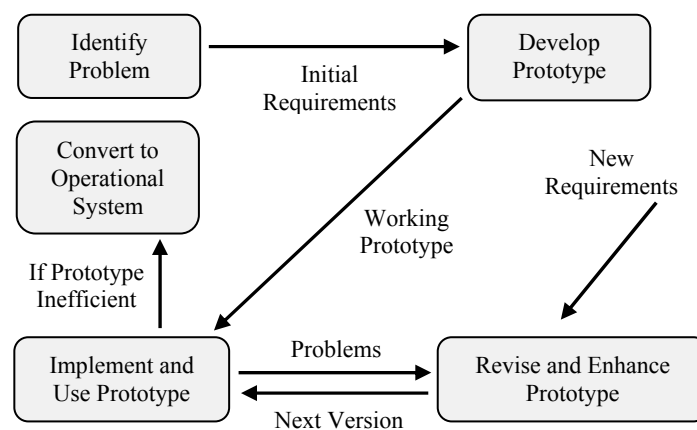


Figure 1. The prototyping methodology [6]

Using prototyping as a development technique, the analyst works with users to determine the initial or basic requirements for the system; once the prototype is completed,

the users work with it and produce feedback to improve the prototype; this iterative process continues until the users are satisfied with it. The advantage of this technique is that involves the user in analysis and design and its ability to capture requirements in concrete, rather than verbal or abstract form.

If one organization develops or runs a computer application for another organization, that practice is called **outsourcing**: a firm develop and run your application on its computers, so all you do is supply input and take output.

### 5. Sources of Software

The specialists group the sources of software into six major categories: information technology services firms, packaged software producers, enterprise-wide solutions, application service providers, open source software, and in-house developers.

Information technology (IT) services firms help companies to develop custom information systems for internal use, or they develop, host, and run applications for customers.

On the top of global software companies is IBM who has been moving away from a reliance on hardware development towards services and consulting: middleware software and application server, Web server, system integration services, IT consulting. Some of the largest computer companies in the world produce software exclusively, like Microsoft, the top in operating systems and its personal productivity software (Microsoft Office Suite).

Software companies develop what are sometimes called prepackaged software or off-the-shelf systems: Microsoft's Project is popular example of such software. The packaged software development industry serves many market segments.

Some off-the-shelf software systems cannot be modified to meet the specific, individuals needs of a particular organization. Such application systems are sometimes called turnkey systems. The producer of a turnkey system will only make changes to the software when a substantial number of users ask for a specific change. Even though many organizations perform similar functions, no two organization do the same thing in quite the same way. A turnkey system may be good enough for a certain level of performance, but it will never perfectly match the way a given organization does business.

More and more organizations are choosing complete software solutions, called enterprise solutions or enterprise resource planning (ERP) systems, to support their operations and business processes. Each module supports an individual, traditional business function, such as accounting, distribution, manufacturing, human resources. The difference between the modules and traditional approaches is that the modules are integrated to focus on business processes rather than on business functional areas. Using enterprise software solutions, a firm can integrate all parts of a business process in a unified information system. The benefits include a single repository of data for all aspects of a business process and the flexibility of the modules. a single repository ensures more consistent and accurate data, as well as less maintenance; the modules are flexible because additional modules can be added as needed once the basic system is in place.

Another method for organization to obtain applications is to rent them or license them from third-party providers who run the applications at remote sites. Users have access to the applications through the Internet or through virtual private networks. The companies that host the applications are called application service providers (ASP). As a business grows or changes, using an application through an ASP becomes limiting. Responding to the need for growth and flexibility is a new generation of providers called managed service providers (MSP). An MSP can offer customized applications and also include business processes, engineering, security and maintenance.



Although exist several different types of external sources of software, in-house development remains an option, when resources and staff are available and system must be built from scratch.

## **6. Paradoxes of Information Systems Development Methods**

Despite the efforts in method development and research, the question "*are methods actually used in practice*" is more often raised. It is a contradiction between the great efforts made to promote text-books methods and their surprisingly low use in practice. The companies still use empirical researches, or develop their own variants. Thus, there is a paradox between the claimed advantages of methods, which should indicate high use, and the empirical observations revealing low acceptance of methods.

It was identified some major *benefits and drawbacks* of method use [4].

Major benefits include enhanced documentation, systematized information systems development process, meeting requirements better, and increased user involvement.

Organization which do not use methods consider that these are labor-intensive, difficult to use and learn, and as having poorly defined and ambiguous concepts. The methods are also seen as limiting and slowing down development, generating more bureaucracy and being unsuitable. Most methods are proposed as universal, therefore the methods is not to apply them as given. The local methods are more popular than their commercial counterparts; this explains the low acceptance of CASE tools which normally necessitate the use of fixed methods.

To sum up, many of the organizations which apply methods do not use totally the methods propose by other: commercial methods are modified, simplified or combined them with other methods, or organizations develop their own methods.

The development of the local method is relatively high costs and requires significant expenditure of resources, but they consider more applicable for the organization.

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THE INFLUENCE OF ANTHROPIC CONDITIONS IN PITESTI,  
MIOVENI AND MARACINENI ON THE NON-PHOTOCHEMICAL QUENCHING  
(NPQ) PARAMETERS OF CHLOROPHYLL FLUORESCENCE AT SOME  
SYNANTHROPIC PLANT SPECIES

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**KEY WORDS:** *thermic dissipation, photoprotection, photoinhibition, photodamage*

**ABSTRACT**

*The way PS II functions is the most sensitive indicator of environmental stress in plants. NPQ test was applied to highlight the photoprotection processes as a result of plant exposure to light excess. Thirteen indicators were calculated and interrelations were stated among. The experiments were carried out in 2009 for six dominant species on the wastelands around the cities Pitesti, Mioveni and Maracineni. They are: Cichorium intybus L., Conyza canadensis (L.) Cronq., Erigeron annuus L. (Pers.), Lactuca serriola Torn., Polygonum aviculare L. and Echinochloa crus-galli (L.) Beauv., by the use of FluorPen FP 100 fluorometer. Non-photochemical thermal dissipation, as a photoprotective mechanism, was less productive at Conyza canadensis, Polygonum aviculare and Echinochloa crus-galli, which means that these species are less affected by excess light exposure and, respectively, less affected under the conditions of soil vegetation*

**INTRODUCTION**

Maxwell and Johnson (2000) show that PS II is the most vulnerable component of the photosynthetic apparatus regarding the photodamage process induced by light excess exposure. Damages at PS II level often represent the incipient manifestation of stress to which the leaf/plant is subject. Thus, chlorophyll fluorescence may offer information about the ability of plants to cope with the environmental stress and about the way this stress can damage the photosynthetic system. NPQ varies linearly depending on the heat dissipation on a scale from 0 to infinite. In plants, the values of NPQ are generally between 0.5 and 3.5, in saturation light. The changes of NPQ reflect the efficiency of thermal dissipation. A rise can occur as a result of the processes which protect the leaf against the damages induced by light excess. (Maxwell and Johnson, 2000).

The parametres of quenching allow the cuantification of the photochemical state PS II regarding the fraction of PS II centers which remain open or oxidized at every moment, as well as the photosyntetic non-photochemical mechanisms involved in photoprotection; quenching phenomena related to the transitions of the state 1 and state 2; photoinhibition and photodamage.

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NPQ test includes five measurements in actinic light and three measurements while dark adaptation.. As the Manual of Opti-Sciences assesses the leaf is prior dark adapted. The test starts at  $F_0$  or minimum fluorescence, measured in the absence of actinic light. Then a pulse of saturation light occurs, which causes a total closure of the receivers in PS II by completely reducing the PS II, the result being maximum fluorescence  $F_M$ . The pulse of saturation is followed by the activation of an actinic light and, once the fixation of  $CO_2$  begins, the fluorescent signal decreases to a steady-state. Photochemical quenching is a measure of the open centres of PS II, of the non-photochemical quenching photoprotection and of other mechanisms of thermal dissipation which may occur.

The pulse of saturation occurring during the steady-state produces  $F_M'$  – maximum fluorescence, in this situation NPQ being balanced with photochemistry.  $F_s$  stands for the fluorescence related to the present level of the photochemical balance. From this point where the actinic light is cut off, a source of far-red light is turned on to facilitate the rapid transfer of electrons to reduce PS I and subsequently PS II re-oxidization.  $F_0'$  stands for this value of the non-relaxed non-photochemical quenching.

The increase of the rates of the saturation pulses after the actinic light has just been cut off represents the relaxation of NPQ in time

NPQ has more components (parameters), (according Manual of Opti-Sciences) and it is a measure of heat dissipation, a combination for photoprotective mechanisms, for the quenching phenomena of the transition in stage 1 and stage 2, photoinhibition and photodamage

## MATERIAL AND METHODS

The index of NPQ was studied and registered for the following species: *Conyza canadensis* (L.) Cronq., *Erigeron annuus* L. (Pers.), *Lactuca serriola* Torn., *Polygonum aviculare* L., *Echinochloa crus-galli* (L.) Beauv., *Sonchus oleraceus* L., *Taraxacum officinale* Weber ex Wiggers, *Amaranthus retroflexus* L. and *Ambrosia artemisiifolia* L. Under the specific conditions offered by the towns of: Pitești, Mioveni și Mărcăneni, between June and August 2009. The parameters of chlorophyll fluorescence for OJIP test (Chlorophyll Fluorescence Induction Kinetics) and NPQ (Non-photochemical Quenching) test – were measured by the use of FluorPen FP 100 fluorometer. The following parameters regarding the NPQ rates were analysed:

- $F_0$  (the dark adapted initial minimum fluorescence when  $Q_A$  is oxidized ( $q_p=1$ ) and the non-photochemical quenching is relaxed (NPQ=0);
- $F_M$  (maximal fluorescence after dark adaptation when  $Q_A$  is reduced ( $q_p=0$ ) and the non-photochemical quenching is relaxed (NPQ=0);
- $F_P$  (the peak of fluorescence during the incipient phase of Kautsky effect (Kautsky effect is related to complex dynamics of the chlorophyll fluorescence emission as well as to photochemical productions during the transition from dark to light (Kautsky and Hirsch, 1931), when a local maximum of  $F$  occurs by rapid reduction of the amount of plastoquinone (PQ) and the slight stimulation of redox mechanisms and non-photochemical quenching
- $F_{M\_Lss}$  (steady-state maximum fluorescence in light, when  $Q_A$  is reduced and the non-photochemical quenching is at maximum (NPQ=1);
- NPQ\_Lss (steady-state non-photochemical quenching in light)
- $Q_p\_Lss$  (steady-state photochemical quenching parameter estimated as fraction of open PS II reaction centers:  $PSII_{open}/PSII_{open}+PSII_{closed}$ )

- $R_{fd}$  (steady-state fluorescence decline ratio, parameter used to assess plant vitality  $R_{fd}=(F_p-F_t/F_p)$ );
- $NPQ\_D_3$  (non-photochemical quenching during dark relaxation)  $NPQ\_D_3=(F_M-F_{M\_D_3})/F_{M\_D_3}$ ;
- $Q_p\_D_3$  (parameter of photochemical quenching during dark relaxation when the reaction centers of PSII are open, estimate of the fraction of open PS II reaction centers)  $PSII_{open}/(PSII_{open}+PSII_{closed})$
- $QY\_max$  (PS II maximum yield during dark adaptation =  $F_v/F_M$ )
- $QY\_Lss$  (photochemical production of FS II during the steady-state, in light  $QY\_Lss=(F_{M\_Lss}-F_{t\_Lss})/F_{M\_Lss}$ )
- $QY\_D_3$  (FS II production during dark relaxation  $QY\_D_3=F_{M\_D_3}-F_{t\_D_3}/F_{M\_D_3}$ ).

The statistic method used to process the experimental data was polifactorial experiments variance analysis, and the test used to establish the statistic significance was DUNCAN multiple range test for a confidence level of  $\alpha=0,05$ . Statistical analyses were computed using SPSS 14 for Windows.

## RESULTS AND DISCUSSION

The 30 values of the test specimen represent the total number of determinations for the following parameters:  $F_0$ ,  $F_M$ ,  $F_p$ ,  $F_{M\_Lss}$ ,  $NPQ\_Lss$ ,  $Q_p\_Lss$ ,  $R_{fd}$ ,  $NPQ\_D_3$ ,  $Q_p\_D_3$ ,  $QY\_max$ ,  $QY\_Lss$  și  $QY\_D_3$ . Significant correlations and discrete significant positive and negative ones were proved to be established among the analysed parameters.  $F_0$  correlates with  $F_M$ ,  $F_p$ ,  $F_{M\_Lss}$  and  $Q_p\_D_3$ , and  $F_M$  positively correlates and all the parameters except for  $NPQ\_Lss$  and  $Q_p\_D_3$ .

The variation of  $F_0$ ,  $F_M$ ,  $F_p$  și  $F_{M\_Lss}$ . parameters, for different species, is illustrated in Figure1.

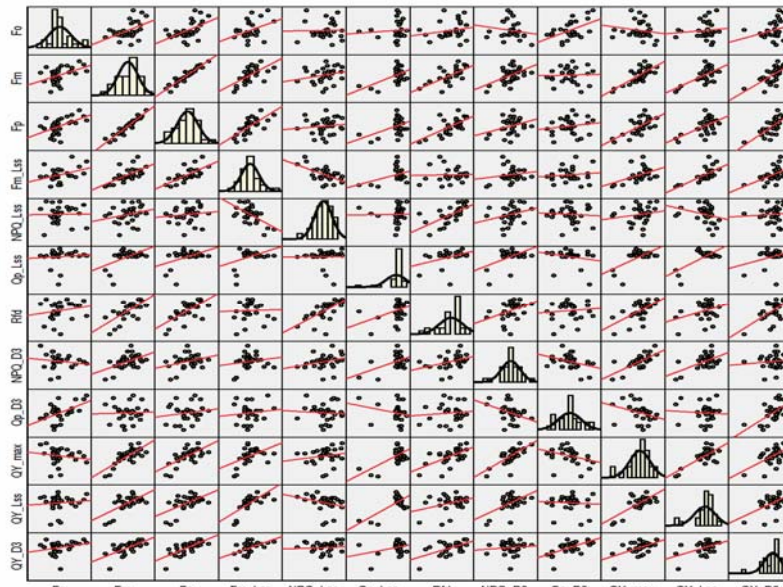


Figure 1. Correlations matrix

The research shows that under the environmental conditions in Pitești, inside the soil, the highest value of  $F_M$  and  $F_P$  were registered in the case of *Erigeron annuus*, which also registered the steepest variation of momentary fluorescence. In the mean time relaxation is more rapid for this species, which registers the highest value of  $F_{M\_D3}$ . This means that the reaction centres become able to capture photons soon after being blocked by the light excess. The values of NPQ\_Lss,  $R_{fd}$  and QY\_Lss in the case of this species were 2.04, 0.77 și respectively 0.37. The lowest values of these parameters (NPQ\_Lss=1.36,  $R_{fd}$ =0.68, QY\_Lss=0.28) were registered in the case of *Echinochloa crus-galli*. For this species the photochemical reactions are reduced as well as NPQ whose value shows that this species is hardly stressed by light (Figure 2).

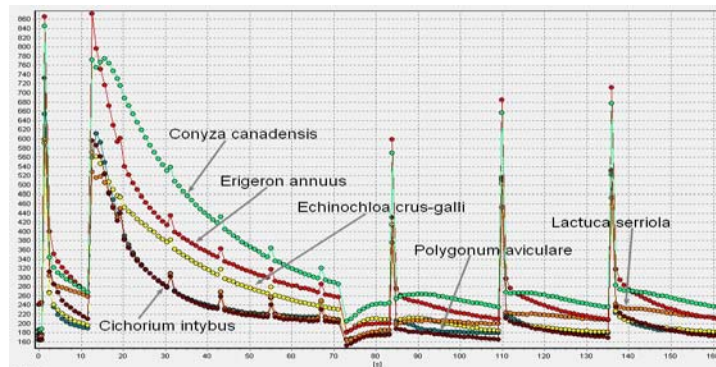


Figure 2. NPQ test – Pitești, soil

Under the conditions provided by the environment of Pitești, in asphalt, the species which displays the highest values of  $F_M$  and  $F_P$  is *Echinochloa crus-galli*, which also displays an elevated graph-curve compared to the other species. The values for NPQ\_Lss,  $R_{fd}$  and QY\_Lss in the case of the above mentioned species were: 0.76, 0.65 respectively 0.37. In the case of the plants stressed by light, the graph-curve registered a steep decrease, and NPQ\_Lss had high values (1.82 for *Erigeron annuus*, 1.74 for *Cichorium intybus*). These plants responded to light excess by closing the reaction centers and subsequently displayed the lowest values of QY\_Lss (0.30 for *Erigeron annuus*, 0.31 for *Cichorium intybus*) (Figure 3).

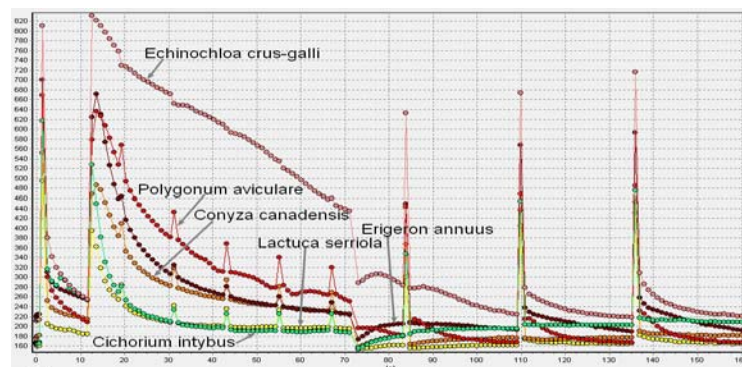


Figure 3. NPQ test – Pitești, asphalt

Under the conditions provided by the environment of Mioveni, in soil, the lowest value of NPQ\_Lss was calculated for *Conyza canadensis* (0.82), which also displayed the highest value of QY-LSS (0.43) and a graph-curve situated above those calculated for the rest of the species, this means this species was able to get the better of the light conditions provided while the measurements were conducted. The lowest values of  $F_M$ ,  $F_P$  and  $F_{M\_D_3}$  (rapid relaxation after the stress caused by intense light exposure) were displayed by *Lactuca serriola*, in which case the graph-curve registered a steep decrease and NPQ\_Lss registered a higher value (1.59), which means that this species is stressed by the photons excess (Figure 4).

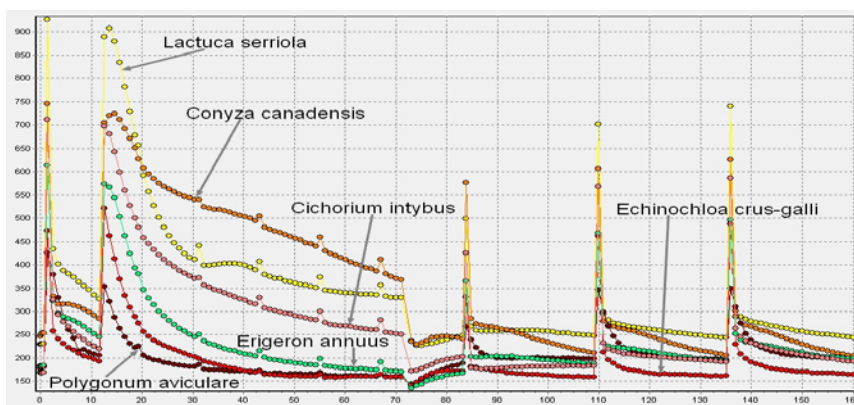


Figure 4. NPQ test – Mioveni, soil

Under the conditions provided by the environment of Mioveni, in asphalt, based on the calculated values of NPQ\_LSS, it was found that *Erigeron annuus* (1.87) displays the most intense non-photosynthetic thermal dissipation, when the light intensity is at a high level, which was also registered for this species in Pitești, în asphalt. This species also relaxes rapidly having the highest value of  $F_{M\_D_3}$ . In spite of displaying the lowest value of NPQ-Lss (1.47), *Echinochloa crus-galli* has the smallest photochemical effect and the lowest value of QY\_Lss (0.11). In general, for all the analysed species  $Q_P$ , as a measure of photochemical quenching, has small values, which situation was also found in Mioveni, in asphalt (Figure 5).

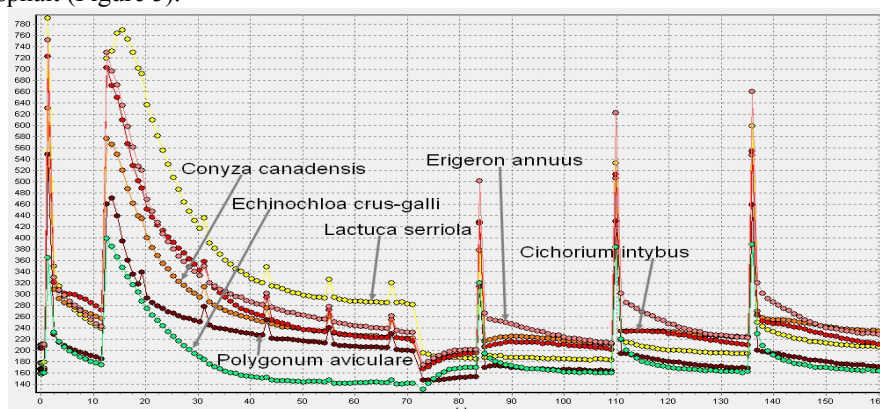


Figure 5. NPQ test - Mioveni, asphalt



Under the conditions provided by the environment of Mărăcineni, in soil, the highest values of  $F_M$ ,  $F_P$  and  $F_{M\_D3}$  were calculated at *Lactuca serriola*, which, however, displayed a steep graph-curve for the momentary fluorescence and a relatively high NPQ\_Lss (1.3) compared to other species (1.24 in the case of *Polygonum aviculare* and 0.46 at *Echinochloa crus-galli*). *Echinochloa crus-galli* also had the lowest ratio of the decrease of fluorescence, as a parameter which stands for the plant vitality ( $R_{fd}=0.6$ ) as well as a high value of QY\_Lss (0.40) comparable to the one calculated for *Polygonum aviculare* (0.45), which displayed the highest photochemical production of PS II during the steady-state, in light.(Figure 6).

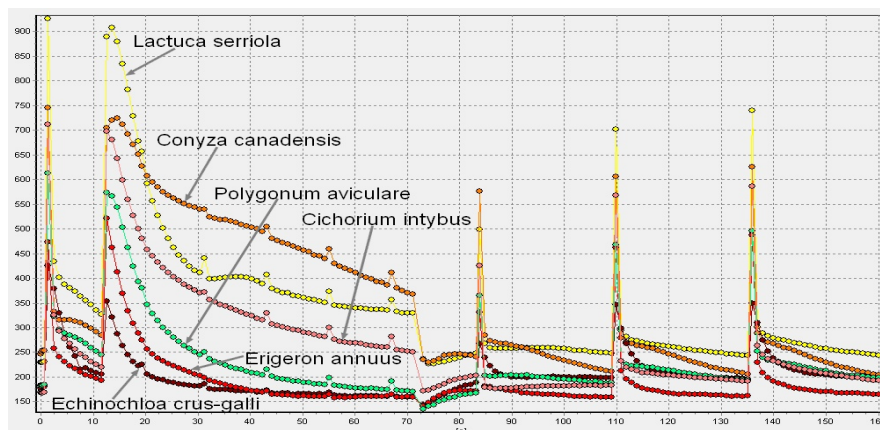


Figure 6. NPQ test – Mărăcineni, soil

## CONCLUSIONS

NPQ test was applied to study the synanthropic plants in order to mark out the photoprotection processes as a result of plant exposure to light excess. Thirteen indicators were calculated and interrelations were stated among. Non-photochemical thermal dissipation as a photoprotective mechanism was less emphasised at *Conyza canadensis*, *Polygonum aviculare* and *Echinochloa crus-galli*, which means that these species are less affected by light excess exposure. NPQ registered lower values under the conditions of plants soil vegetation.

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CONTRIBUTIONS TO THE INTRODUCTION OF THE SPECIES *PAULOWNIA*  
*TOMENTOSA* (THUNB.) STEUD. BEHIND ROMANIAN GREEN SPACES

Simion F.O.<sup>1</sup>

**KEY WORDS:** *Paulownia tomentosa*, green spaces, introduction

**ABSTRACT**

*Paulownia tomentosa* (Thunb.) Steud. has a high ornamental value, is resistant to pollution and valuable as a possible tree forest for wood quality and increased yield of timber. Limited frequency of *Paulownia* as ornamental plant and forest species can be explained by high sensitivity to frost, difficulties in planting material production, and insufficient knowledge of its behavior in nursery culture.

**KNOWLEDGE ABOUT THE CURRENT STATE OF SPREAD OF SPECIES IN  
ROMANIA**

In the Faculty of Horticulture, University of Craiova, species *Paulownia tomentosa* has been studied for a long time, over 25 years, recent research is how to obtain industrial plant for the garden seedlings and species behavior in different areas of the country and different types of green spaces (Simion, 2004, 2005, 2006, 2009).

In the last 30-50 years, the species existed only in botanical gardens in the country and in some cities in very limited number of copies, which were identified by ICAS Bucharest. From our past and present observations, we can indicate that *Paulownia* species, grows much better in urban green spaces, with specific microclimate, than forest crops. The main cities where *Paulownia* species can be meet, planted singly or in small groups, aged 30-50 years are located on and between the annual isotherms of 11:10 ° C.

Places where the *Paulownia* trees aged 30-50 years exist are: Arad, Baia Mare, Baile Herculane Bucharest, Buzau, Horezu-Monastery, Govora-Spa, Caracal, Craiova, Cluj, Minis, Slatina, Targu Jiu, Timisoara, Vaslui. *Paulownia* is also found in other localities in the country: dendrological collections, botanical gardens and private gardens - have been listed only places with trees which observations were made, mostly located in favorable ecoclimate.

Species presence in the mentioned cities, characterized by different climate conditions, is highlighting good regeneration capacity and adaptation to climatic conditions, the urban microclimate being friendly to this (Cristescu et al., 1989; Simion 2009).

There are prerequisites expansion in culture and other areas of Romania with similar conditions or associated with other species of trees, to protect them.

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## PROPOSED OBJECTIVES

- Ecological Zoning in the species they use urban green spaces;
- Establishment of planting green technology;
- Diversify the composition of existing and new green spaces;
- Implementation of protection curtains with rapid achievement of the environmental protection function.

## METHOD AND CONDITIONS OF PLANTING

In order to zone *Paulownia tomentosa* (Thunb.) Steud ecological species and knowledge of how to behave in culture, because the species is extended, its have been inventorying the trees species in favorable areas based on thermophilic character and microclimate provided by urban green spaces, association with other trees to protect them, also planting in the vicinity buildings, etc..

New plantations were made in several different systems of green areas of the country, characterized by different climatic conditions, but no absolute minimum lethal temperatures for the *Paulownia* species.

Places where observations were made are: Arad, Minis, Baile Herculane Bucharest, Buzau, Caracal, FARC, Stoenesti, Vădastra, Olt County, Cluj, Craiova, Govora - Spa Resort, Pitesti, Slatina - Plantation Line, Targu Jiu-ARTECO SA Tâmburești and Bechet – Dolj (Figure 2,3,4,5).

Planting points lie at great distances between them and the number of individuals existing or planted at each point show very little research orientation imposed at the preliminary observations. In this study mature trees were taken into consideration, but were also made new plantings with seedlings of different sizes and ages.

In order to achieve the concerns and goals, the first step was the planting seedlings of seedlings at different ages, the option of planting small seedlings aged 2-3 months or a year is indicated, because it eliminates the difficulties of transport juveniles large (Barnhill et al., 1982). Production of seedlings in these areas and in larger quantities, planting age could be a valuable opportunity for planting *Paulownia* species.

Seedlings were planted in green spaces in different climatic conditions.

Ground vegetation as a factor in urban green areas needs improvement in terms of physical and chemical traits. The plot is often uncovered in site activity where normal sequence of horizons disappear and the fertile horizon can often be uncovered and removed.

For this reason all new plantings were made in pits with dimensions of 70 x 70 X70, these dimensions are normal for the bundle of land planted with seedlings in the green space. Planting seedlings in the planting hole was added to peat and manure soil (natural fertilizer): 10% peat [10% manure soil and the difference soil. When the soil is clayey, sandy texture improvement of 10-20% is justified].

Planting seedlings with root systems in unincorporated bowl was good to catch, only for the one year old seedlings (Beckjord, 1982; Burger et al., 1985). When seedlings have 3-4 years of age, grip is very weak, losses caused by leaf-rich device early, intense sweating and weak between absorption of water by reduced root system to malfunction physiological due to injuries.

In order to prevent consequences of physiologically active roots are destroyed when possible when removing seedlings from nursery seedlings from nursery to experienced out annually or once in two years, followed by shorter operation root system

and crown design. Repeated shortening of the roots and planting seedlings in the nursery has improved in the pits with soil favored branching root system. Planting of seedlings was done in 12 localities in Romania, with conditions favorable species.

## RESULTS

The climatic conditions for the specific area and planting seedlings, were made in two ways: with the root bale of land and land without the root bundle, the results were different.

Good behavior of seedlings planted and mature specimens of *Paulownia tomentosa* (Thunb.) Steud., studied in green places indicate a favorable ecological plasticity and expansion of the species used as ornamental plants.

In terms of planting used at SEMTEST - Craiova species was studied as a decorative tree with ecological functions, indicated for protection curtains. Green behavior was studied in different parts of the country and gave results that are favorable conclusions that can lead to a expansion in culture.

In the research and production base of SEMTEST Craiova, where the produced seed for research was made, a curtain was realized made of *Paulownia* 100% protection.

- Location: SEMTEST Craiova, Craiova on 7 km distance between localities Facai and Malu Mare
- Land-surface: 2500 sqm land rectangular plan form;
- Soil: sandy-clay, poor nutritional components, but improved by addition of nutrient mixture consisting of peat and manure soil;
- Planting distances, very small, 1.5 x 2.0 m
- The average height of trees: 15.0 m, after eight years of its establishment.
- Average trunk diameter 10 cm, measured at 1m height from the ground;
- Consistency plantation: solid state forms with full consistency;
- General Appearance: culture acts as a protective curtain compact;
- Plant Condition: good tree trunk comply with natural cleaning;
- The environmental protection: very good, after 5 year after planting, note the noise and dust from wind, ground shading is strong;
- Resistance to cold: quite good, 3-5% loss in early years increases terminal freezes, but the plant is recovering quickly;
- Soil: nisipo-clay, poor nutritional components, but improved with manure soil 10 cubic decimetres / plant, added at planting;
- Planting distances, very small, 1.5 x 2.0 m
- High trees average, after eight years of its inception: 15.0 m
- Trunk diameter average - 10 cm;
- Consistency plantation: solid state forms with full consistency;
- General Appearance: culture acts as a protective curtain compact;
- Plant Condition: good tree trunk comply with natural cleaning;

By doing stem at ground level, were reached by autumn a plant height of 2-3 m at the Romanian Television Park plantings in 2000.

Bale out saplings with the earth at the root was only possible to produce seedlings in the nursery on clay or clay soil, - sandy soil texture makes it impossible to support the root system of land mass.

Seedlings planted in residential neighborhoods have flourished in all cases after the third and fourth year later after planting, planting seedlings age being two years. Tree

height achieved in the fourth year after planting is 5-7 m and average diameter of 10 cm trunk.

Craiova plantings in neighborhoods and private gardens, Bucharest, TVR Park, Govora-Spa, Herculane-Spa, Targu Jiu.

Observations on the crown tree: crown is young, transparent, well-storey, pyramid. In all cases we wanted to mitigate damages that can be caused by cold currents, by planting trees in areas away from streams, protect adjacent buildings or groups planted woody vegetation perpendicular to the direction of air currents that could affect the loss of trees annual increases and therefore the flowers. Regarding flowering - in winter season with very low minimum temperatures in areas with cold air currents, floral dial buttons frozen. Very dry summers affect the flowering trees and therefore the growth for the following year. In spring 2008, *Paulownia* had a very poor flowering in all cities where observations were made. Annual increases and accumulation were small and large losses made by cold.

Observations on tree growth and development in green areas show a good adaptation of species to specific conditions of the urban environment. Dust, sunlight and toxic gases do not produce in life species evident disturbance. Cold air currents and strong frosts, with values lower minimum temperatures of -20 ... -25 0C, causing flowers freezing and low annual increases the lemniscate upper third. Under these conditions the tree may not blossom a year, but quickly recover crown.

For observation, *Paulownia* was planted isolated in small groups, and street alignment plantations. Street alignment plantations made Slatina and Pitesti city highlight decorative proving the utilitarian value of species for this purpose unquestionable, but also its negative peculiarity – the phenomena of degarnish after 25-30 years of age, dried branches break. Among specific defects of *Paulownia* in culture, in addition to high sensitivity to frosts may be mentioned: the relatively low mechanical strength and high sensitivity to older branches of pest *Hyphantria cunea* excessive hunger.

Characterization of specimens studied for this purpose existing or planted. Seedlings planted in Bucharest at the Romanian Television, abundant flowering, frost-resistant in recent years and also confirmed specimens adult aged over 50 years, good ability to adapt to climatic conditions of the environment. Sheltered microclimate offered by Romanian Television park and small distances from buildings - 10 to 15 m of ice, prevent adverse consequences. One year old seedlings were frozen and have even rebuilt the following year, growth has exceeded 3m (Figure 1).

Planting seedlings in the resorts: Baile Herculane and Baile Govora enjoy the features of a mild climate, favorable species. Trees planted in Baia Mare Borhutului Valley, repeatedly freeze because of cold air currents, but the tree recovers very fast. Pitesti and Slatina: observations led to good results on the behavior of trees in urban green spaces. In both cases the species was used in plantations and street alignment. Trees after reaching 20-25 years after planting, tend to garnish the crown and dried branches can break easily in the wind.

In I.C.P.D. Pitesti Mărcineni: Mărcineni road planted specimens, at the point of junction with the Budeasa Valley, the trees froze repeatedly, their flowering is casual, floral buds even in mild winters freeze because cold air currents. Tree trunk is characterized by obvious necrotic phenomena caused by cold currents. In most cases, observations have

confirmed the possibility of extending the species *Paulownia tomentosa* and outside areas of the highest favorability - with mild climate, microclimate provided maximum recovery.

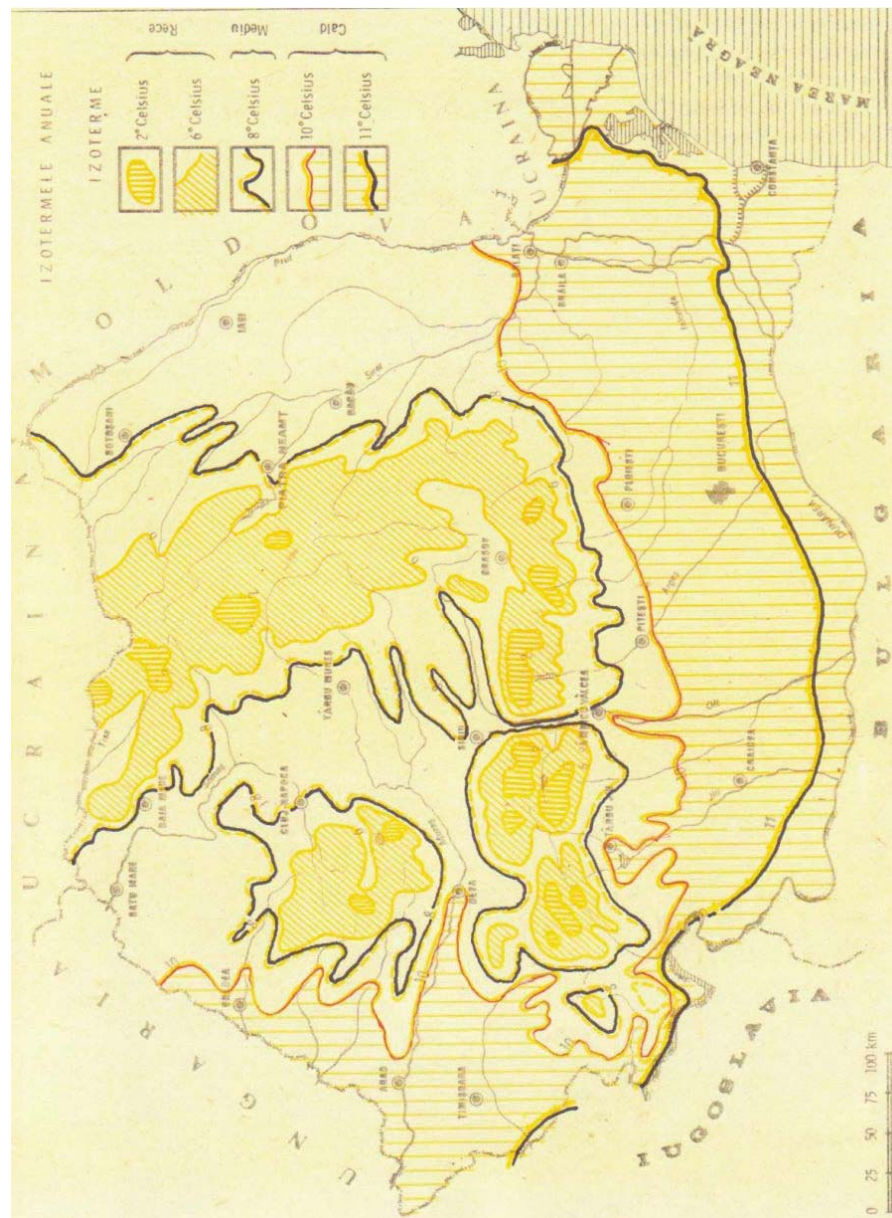


Figure 1. Isotherms in Romania, ecological zone for *Paulownia tomentosa* (Thunb.) Steud  
 10 – 11°C warm zone – favorable; 8°C medium favorable; 6 – 2°C cold zone, less favorable



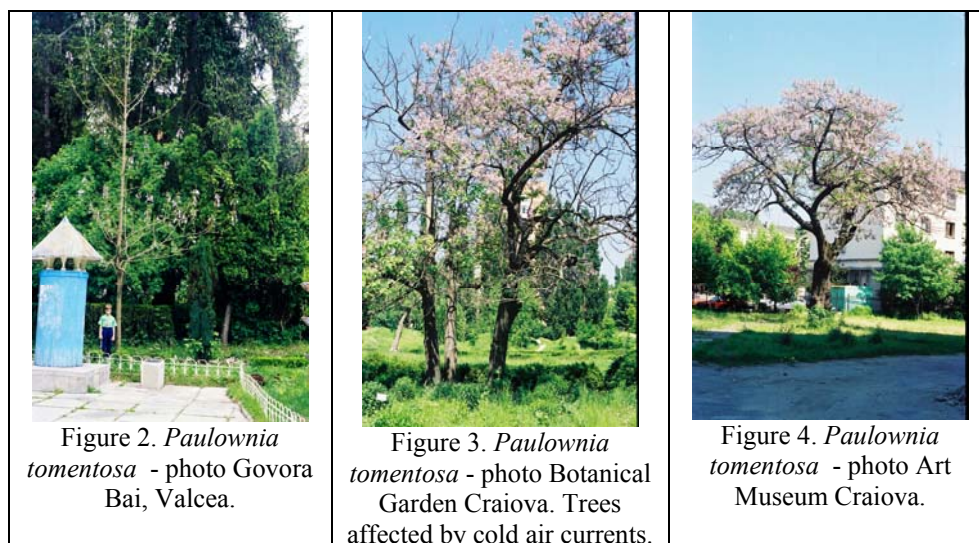


Figure 5. *Paulownia tomentosa* - environmental protection curtain, Craiova, Malu Mare Dolj.

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✓ Horticultură  
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          produselor agricole  
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**OBSERVATIONS AND RECOMMENDATIONS CONCERNING THE FREE  
PROPAGATION AT *PAULOWNIA TOMENTOSA* (THUNB.) SIEB. ET ZUCC. IN  
THE CONDITIONS OF ROMANIA**

Simion F. O.<sup>1</sup>

**KEY WORDS:** *Paulownia tomentosa*, propagation, free

**ABSTRACTS**

*Paulownia tomentosa* is a tree valued by its ornamental qualities, ecological and useful. *Paulownia tomentosa* is multiplying spontaneously (generative and vegetative) in the conditions of Romania. The spontaneous propagation takes place in humid places, protected from the direct solar radiation and supposes the existence of a sample near by. The observations were made a long time to University of Craiova.

**IMPORTANCE OF THE SPECIES**

Lately the *Paulownia tomentosa* plantations have known a great spreading both in China, the country of provenience, and also other countries from Asia and even other continents. This is due both to the valuable wooden mass with numerous uses as well as to the therapeutic action of some chemical components of this tree.

The *Paulownia tomentosa* wood, known and used starting from the antiquity is yellow- whitish towards fading red, granulated, right, shining after processing, light, smell-less. In order to keep its initial qualities long time after processing it is recommended to be cut during autumn or winter in the repose time. It is a type of wood easily to process and the products made of it do not break, do not crack and do not deform. The permeability to water is slower than to other wooden species. As far as the resistance to rotting is concerned on behalf of the observations from ancient writings are concerned, it has been stated to be very big- at the Chinese the coffins made of this wood rotted harder (Cristescu, 1989).

This wood has good acoustical features, so that the traditional musical Chinese instruments are made only of *Paulownia* wood, because of the better sound resonance.

Having a small density, it is not resistant, not being recommended for elements which cannot hold great tensions like in constructions. It is used for the construction of doors, windows, separating walls, for the beams of the roof since they maintain its shape by not curbing. It is also used for the furniture fabrication.

Lately it is more and more used in the construction of airplanes, ships, cars because it is resistant to rotting, light. It is used to the fabrication of toys, in obtaining the plating and the paper.

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The population of China, Japan and the countries from the South- East of Asia has a long tradition in the confection of vases, Buddha, wooden fish, fabrication of paint, matches.

Beside the importance offered by the wooden mass, *Paulownia tomentosa* is outlined also through the medicinal properties given by the chemical substances it contains. The plants which contain ursolic acid have been used in the traditional medicine before knowing the components responsible for the therapeutic effects are. The contemporary studies which led to the identification of the ursolic acid contained by the Paulownia leaves confirmed its therapeutic effects such as: anti- microbial, anti-viral, anti- hepatic, anti-inflammatory, for the treatment of tumors, of ulcer. Also it is a good stimulant for the hair regeneration, the head's skin irritation and has anti-dandruff effect. It is also used in cosmetics.

The pharmaceutical experiments have shown that the extracts from the Paulownia fruits can ease the cough, the asthma and reduce the blood pressure. The leaves and fruits have a high percentage of nutritive substances so that they can constitute a good fodder.

The big and pubescent Paulownia leaves play an important role in air purification of smoke and dust so that *Paulownia tomentosa* have become the main species for afforesting in numerous areas where the pollution is a problem.

In order to obtain seedlings from seeds it was used the material research base in the laboratory of Ornamental Arboriculture and Landscape Architecture science in Horticulture Faculty of the University of Craiova, training and research fields in the Botanical Garden of the University of Craiova, the Laboratory of Horticulture Faculty in SEMTEST Craiova, tree nursery of Craiova Public Land Company, and other towns that received seedlings 1-year aged or transplanted in pots and nutritive cubes, in order to fortify them in transplantation areas within own nurseries (Simion, 2004, 2005, 2006, 2009).

By taking into account the small extent in green areas of *Paulownia tomentosa* wood species, and also the fact that it is characterized by special ornamental and useful features, it is justified to produce propagating material by generative and vegetative ways.

## **MATERIAL AND METHOD**

The main working methods were the observation. Were made observations on the possibility of generative and vegetative free propagation and development condition. Observations were made in different areas from Craiova (Agronomy Complex, Carol I High School, Botanical Garden, etc).

## **RESULTS AND DISCUSSIONS**

*Paulownia tomentosa* is existing in Romania in several areas, the number of trees is very small, depending on ecologic conditions which the species could benefit.

*Paulownia tomentosa* barely multiplies spontaneously (freely) in the conditions of Romania. The spontaneous propagation takes place in humid places, protected from the direct solar radiation and supposes the existence of a sample near by. The plant easily regenerates from the roots remained in the soil, after the removal of the saplings from the seedbed or after the cutting of the old plants (Figure 1-9).

### **I. The propagation in natural conditions (free) from seeds (figure 1-4).**





Figure 1. Plants appeared from seeds in the rotten and humid stump of *Paulownia tomentosa* Craiova – Agronomy Complex – 2007



Figure 2. Plants appeared from seeds on shadowed roof – Craiova 2007



Figure 3. Plants appeared from seeds in the spaces between the concrete paving blocks. Craiova 2007

Conditions:

Shadowed pavement, with various spaces where there have been formed soil particles. N-E exposition. High humidity, favourable to the germination of seeds and the growth of plantlets.



Figure 4. Plants appeared in clefts in asphalt. Craiova 2007

Conditions:

Carol I High School in Craiova. Asphalted platform with several clefts, situated at 2 metres below the pavement. The Northern exposure, protected by the direct action of the solar radiation. The inferior level of the field favoured the humidity of the raising and the natural establishment of the *Paulownia* species.

**II. The propagation in natural conditions (free) from root suckers (Figure 5-9).**

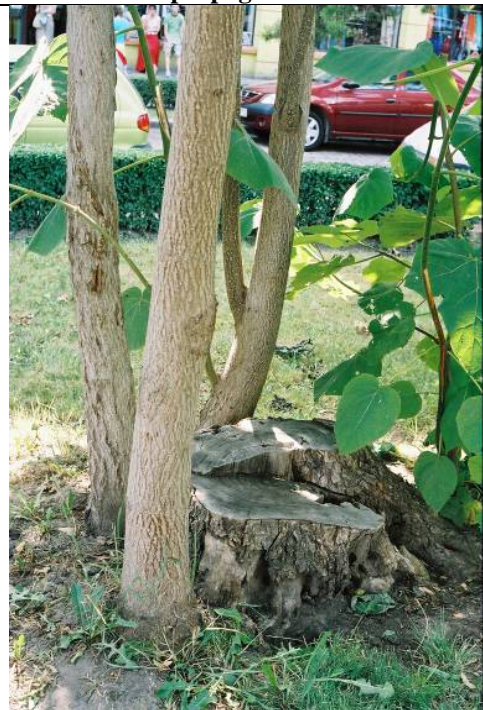


Figure 5. Young plants appeared from root suckers. Craiova 2007. The National Theatre in Craiova.

After the cutting of the tree, from the superficial roots appeared various root suckers.

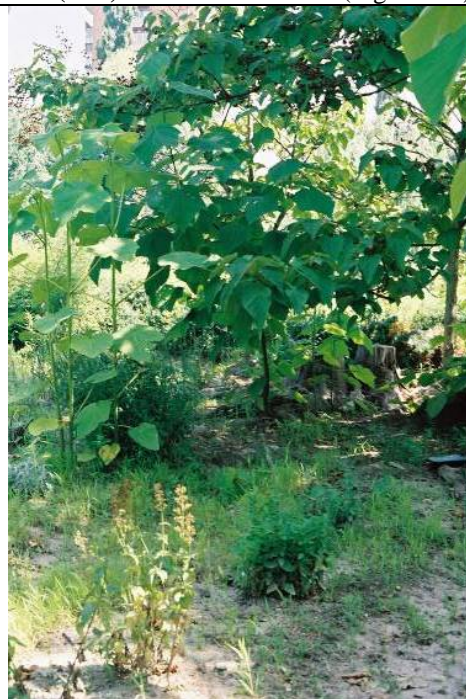


Figure 6. Plants appeared from root suckers, actinoid toward the bole. Craiova 2000.

Conditions: The Botanical Garden in Craiova. The ground-water layer situated at 1.5 m. The soil is clay-argillaceous, humid. Area naturally shadowed.





Figure 7. Plants appeared from tracing roots, Craiova 2000

Place: The Botanical Garden in Craiova. The soil is humid, clayey. A two-year-old plant, that comes from actinoid buds, at different distances from the bole.



Figure 8. Plants grown from root suckers – detail. Craiova 2000.

The Botanical Garden in Craiova. Root suckers that are at different distances from the bole of a mature tree. The superficial roots with mechanical damages of different intensities favoured the appearance of root suckers.



Figure 9. Plants grown from root suckers – detail. Craiova 2000.

The Botanical Garden in Craiova. Conditions: The soil is humid, clayey. Plants that have appeared from root suckers, at different distances from the bole, after the cutting of the tree.

**Conclusions concerning the free gathering (in natural conditions) of the planting material at *Paulownia Tomentosa* SIEB. ET ZUCC. in the conditions of Romania.**

1. The seeds drawn by air currents, in humid and with sufficient shadow areas, give birth to saplings with sudden appearance, in unpredictable places; the roof and the humid walls of buildings, clefts in foundations, etc.
2. From fragments of the leaves shifted by the wind to humid shadowed places, favourable to rooting, come bines that form roots, then plants.
3. The old trees, which are in the phase of the biologic decline, produce several root suckers, which spring from the developed roots with obvious prominences at the surface of the soil.
4. From the superficial roots of the trees, remained in the soil after the cutting, there appear root suckers that can be separated and formed in the seedbed.
5. After the removal of the saplings from the seedbed, there remain roots in the soil, which give birth to bines and can be separated at autumn as plants that result from "cuttings" or "rooted marcotte", when the annual bines grow from roots and become distinct as well conformed samples.

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THE SPECTRUM OF THE BENEFICIAL ENTOMOFAUNA FROM THE  
VITICULTURAL ECOSYSTEM SEGARCEA

Stan C.<sup>1</sup>, Țucă O.<sup>1</sup>, Mitrea I.<sup>1</sup>, Genoiu C.<sup>2</sup>, Nitu Tantica<sup>3</sup>

**KEY WORDS:** *beneficial entomofauna, parasites, predators*

**ABSTRACT**

*The profound intervention of the human in the viticultural ecosystems has led to powerful disequilibrium, especially the pesticides applying it is one of the main factors which affect the biodiversity, the pesticides being synthesis chemical substances, toxic or very toxic, from outside of the agricultural ecosystems.*

*In order to counterbalance these negative effects, we have to know the beneficial entomofauna (parasites and predators), we have also to protect and eventually to help through our actions it presevation and development.*

*The beneficial entomofauna (parasites and predators) has been represented by 15 species systematically framed in four orders. The most numerous order has been **Coleoptera** with 7 species, followed by **Hymenoptera** with 4 species and **Neuroptera** with 3 species, and from the **Diptera** order has been identified a single species.*

**INTRODUCTION**

The main primary producer existent in the viticultural biocoenosys it is the vine, often represented by the bio-system grat/stock. Besides the vine, there are other primary producers represented by different herbal species (weeds), which usually make the object of some controlling measures (Stan et al., 2008).

The monocotyledonated weeds as well the bycotyledonated weeds, perenial or annual represent host plants for a series of damaging secondary species (*Anomala spp.*, *Melolontha melolontha*, *Polyphylla fullo*), speciesiwith damaging potential (*Ephippiger ephippiger*) and migratory species (*Dociostaurus maroccanus*, *Gryllus spp.*, *Eurydema spp.*, *Graphosoma lineatum*, *Opatrum sabulosum*, *Agriotes spp.* *Bothynoderes punctiventris*). Also, the weeds from the vineyard ensure shelter and conditions for laying eggs and the developemnt of new generations of predators (beneficial entomofauna: *Calosoma spp.*, *Carabus spp.*, *Adalia spp.*, *Coccinela spp.*, *Trichogramma spp.*) of the vine damaging species (Mitrea et al., 2008).

From the consumers presents in the viticultural biocoenosys, the ones from the first order are the best represented. They are represented by the phytopathogen agents and pests. The consumers from the second order within the viticultural biocoenosys has a

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reduce representation, more numerous being the micro-organisms species (the reducers) which ensure the decomposing of the organical matter from the vegetal and animal organisms (Rosca et al., 2008).

The profound intervention of the human in the viticultural ecosystems has led to powerful disequilibrium, especially the pesticides applying it is one of the main factors which affect the biodiversity, the pesticides being synthesis chemical substances, toxic or very toxic, from outside of the agricultural ecosystems (Simeria, 2003).

The targeted organisms (damaging organisms) receive a very small quantity of pesticide, which represent in fact the most important quantity for controlling, the rest go into the soil and on other elements of the agricultural ecosystem (the beneficial entomofauna).

Thus, the pesticides generate important dangers regarding the environment pollution (water, air, soil) and crop, but also the appearance of the resistant phenomenon of the pests toward some phytosanitary products.

In order to counterbalance these negative effects, we have to know the beneficial entomofauna (parasites and predators), we have also to protect and eventually to help through our actions it presevation and development (Simeria, 2007).

## MATERIAL AND METHODS

In order to establish the beneficial entomofauna from the Segarcea vineyard ecosystem, during the research period (2009-2010), there has been made collecting of the entomological material using different means and methods: collecting with the entomological net, tests in the soil or on the soil surface (on the interval between the rows), lightning traps, coloured traps, visual control, analyzing the sample with magnifying glass, microscope in the field or laboratory (Figure 1, 2, 3).

The collecting has been made in different vegetation phase of the vine.

The collected species has been analyzed and identifiicated according to the determinators published in the Romanian Fauna (Panin, 1951) and European Fauna (Chinery, 1998).



Figure 1. Barber Trap



Figure 2. Entomological net



Figure 3. Yellow trap

## RESULTS AND DISCUSSIONS

The beneficial entomofauna (parasites and predators) has been represented by 15 species systematically framed in 4 orders (Table 1).

The most numerous order has been **Coleoptera** with 7 species (*Adalia decimpunctata*, *Adalia bipunctata*, *Coccinella 7 punctata*, *Carabus ulrichi*, *Carabus violaceus*, *Carabus cancelatus*, *Calosoma sycophanta*), followed by **Hymenoptera** with 4 species (*Trichogrammaspp.* (*T. dendrolimi*, *T. evanescens*, *T. minutum*), *Scolia flavifrons*) and **Neuroptera** with 3 species (*Chrysopa perla*, *C. carnea*, *Hemerobius humulinus*.) From the **Diptera** order has been identified the species *Syrphus ribesii* (Figure 4, 5)

Table 1

The structure of the beneficial entomofauna		
Nr.	Order	Nr. of beneficial species
1	Hymenoptera	4
2	Neuroptera	3
3	Coleoptera	7
4	Diptera	1
TOTAL		15

The role and the evolution of the zoophagus from the vine plantation during the vegetation period are presented in the Table 2.

Table 2

The zoophagus from the vine plantation and their evolution during the vegetation period

The group of the pests	Zoophagus (ord.)	Month						
		III	IV	V	VI	VII	VIII	IX
Acarians, wooly scale, cycads	Diptera							
	Neuroptera							
	Hymenoptera							
	Coleoptera							
Moth eggs, first instar larva, moth	Diptera							
	Neuroptera							
	Hymenoptera							
	Coleoptera							
Beetle, cockchafer, locusts	Diptera							
	Neuroptera							
	Hymenoptera							
	Coleoptera							



Present low activity (nule)



Present- medium activity



Present- intense activity



Figure 4. *Adalia bipunctata*,

*Coccinella 7 punctata*,  
(<http://images.google.ro>)

*Trichogramma spp.*



Figure 5. *Scolia flavifrons*

*Chrysopa spp.*  
(<http://images.google.ro>)

*Syrphus ribesii*

## CONCLUSIONS

The beneficial entomofauna (parasites and predators) has been represented by 14 species systematically framed in four orders.

The most numerous order has been **Coleoptera** with 7 species, followed by **Hymenoptera** with 4 species and **Neuroptera** with 3 species, and from the **Diptera** order has been identified a single species.

The fungicides used for control the phytopathogen agents had a different efficient action on the pathogens, but the great majority are not selective for the beneficial entomofauna. The acaricides, insecticides used for controlling the pests are not selective for the beneficial entomofauna (parasites and predators), thus we recommend their using only a advertising.

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THE STRUCTURE OF THE DAMAGING ENTOMOFAUNA FROM THE  
VINEYARD S.D. BANU MARACINE

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**KEY WORDS:** harmful entomofauna, key pests, potential pests, migratory species

**ABSTRACT**

*For the quantitative determination of the pests population dynamic, in order to establish the necessity of control treatment, during 2006-2008, there has been made investigation, in the vineyard from the winegrowing center Banu Mărăcine.*

*Within the viticultural ecosystem from the S.D. Banu Mărăcine there has been identified a number of 36 harmful species of Arthropods, sistematically framed in 8 order.*

*The most numerous order has been **Coleoptera** with 15 species, followed by the **Orthoptera** order with 7 species **Lepidoptera** order with 4 species, **Homoptera** and **Hymenoptera** order with 3 species each, **Acari** order with 2 species, **Dermaptera** and **Thysanoptera** order with one species.*

**INTRODUCTION**

The classification of the animal pests comprise besides the key species, the group of the secondary pests, which can produce damages in certain area and conditions being known as occasional pests. Although they do not produce damages year after year, they must be monitorized, for they will not turn on key pests as a consequence of some human activities. Usually these species are under the economically treshold (P.E.D.) due to their natural control. The following groups are represented by the one of the potential pests, which do not produce significant damages from the economically point of view but sometimes they can produce surprise turning into the group of secondary or key pests, and the one of the migratory species, which proceed from other crops and can became dangerous for the vine in certain conditions (Smith and Van der Bosch, 1967).

Function the area and pedological and climatic conditions, the key pests can vary as density and damaging treshold, from one year to another, and that is why there can not be apply standard integrate management (Rosca et al., 2008).

**MATERIAL AND METHODS**

For the quantitative determination of the pests population dynamic, in order to establish the necessity of control treatment, during 2006-2008, there has been made investigation, in the vineyard from the winegrowing center Banu Mărăcine, estabilishing the structure of the biocoenosis and the critical damaging treshold.

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In order to establish the harmful entomofauna from the vineyard of the S.D. Banu Maracine, during the research period (2006-2008), there has been made collecting of the entomological material using different means and methods: collecting with the entomological net, tests in the soil or on the soil surface (on the interval between the rows), lightning traps, coloured traps, pheromonal traps, visual control, analyzing the sample with magnifying glass, microscope in the field or laboratory (Mitrea et al., 2008).

The collecting has been made in different vegetation phase of the vine (Figure 1, 2, 3).

The collected species has been analyzed and identificated according to the determinators published in the Romanian Fauna (Panin, 1951) and European Fauna (Chinery, 1998).



Figure 1. Yellow trap



Figure 2. Barber Trap



Figure 3. Pheromonal traps (AtraBOT, AtraAMBIG)

## RESULTS AND DISCUSSION

Within the viticultural ecosystem from the S.D. Banu Mărăcine (2006-2008) there has been identified a number of : 36 species of harmful Arthropods (Table 1), sistematically framed in 8 order.

Table 1

The vine harmful entomofauna identified in the viticultural ecosystem from S.D. Banu Mărăcine

Nr. crt	Order	Scientific denomination
1	<b>ACARI</b>	<i>Eriophyes vitis</i> Page.
2		<i>Tetranychus urticae</i> Koch.
1	<b>ORTHOPTERA</b>	<i>Gryllotalpa gryllotalpa</i> L.
2		<i>Gryllus campestris</i> L.
3		<i>Gryllus desertus</i> L.
4		<i>Ephippiger ephippiger</i> Fieb.
5		<i>Caliptamus italicus</i> L.
6		<i>Locusta migratoria</i> L.

Nr. crt	Order	Scientific denomination
7		<i>Dociostaurus maroccanus</i> Thunb.
1	<b>DERMAPTERA</b>	<i>Forficula auricularia</i> L.
1	<b>THYSANOPTERA</b>	<i>Anaphothrips vitis</i> Priesner
1	<b>HOMOPTERA</b>	<i>Empoasca vitis</i> Goethe
2		<i>Phylloxera vastatrix</i> Planch.
3		<i>Pulvinaria vitis</i> Targ..
1	<b>HYMENOPTERA</b>	<i>Vespa vulgaris</i> L.
2		<i>Vespa germanica</i> L.
3		<i>Vespa crabro</i> L.
1	<b>COLEOPTERA</b>	<i>Melolontha melolontha</i> L.
2		<i>Amphimalon solstitialis</i> L.
3		<i>Rhizophagus aequinoctialis</i> Herb.
4		<i>Polyphylla fullo</i> F.
5		<i>Anoxia orientalis</i> L.
6		<i>Anomala solida</i> Er.
7		<i>Anomala vitis</i> F.
8		<i>Anomala dubia</i> Scop.
9		<i>Lethrus apterus</i> L.
10		<i>Phyllopertha horticola</i> L.
11		<i>Agriotes obscurus</i> L.
12		<i>Agriotes ustulatus</i> Schall.
13		<i>Agriotes lineatus</i> L.
14		<i>Byctiscus betulae</i> L.
15		<i>Otiorrhynchus ligustici</i> L.
1	<b>LEPIDOPTERA</b>	<i>Lobesia botrana</i> Den et Schif.
2		<i>Sparganotis pilleriana</i> Den et Schif.
3		<i>Clysia ambiguella</i> Hb.
4		<i>Hyphantria cunea</i> Drury.

The most numerous order has been **Coleoptera** with 15 species, followed by the **Orthoptera** order with 7 species **Lepidoptera** order with 4 species, **Homoptera** and **Hymenoptera** order with 3 species each, **Acari** order with 2 species, **Dermaptera** and **Thysanoptera** order with one species.

Regarding the gravity of the attack, the harmful entomofauna from the vineyard of the S.D. Banu Mărăcine can be structured as follow:

- **species of economical importance (key species)** *Eriophyes vitis* Page., *Tetranychus urticae* Koch. belonging to the **Acari** order and *Lobesia botrana* Den et Schif., *Sparganotis pilleriana* Den et Schif., *Clysia ambiguella* Hb. belonging to the **Lepidoptera** order.

- **secondary pests** belonging to the orders: **Coleoptera** (*Melolontha melolontha* L., *Anomala* spp., *Polyphylla fullo* F. ) and **Homoptera** (*Empoasca vitis* Goethe, *Pulvinaria vitis* Targ..).

- **potential pests** that don't produce significantly damages from the economical point of view, within the viticultural ecosystem S.D. Banu Mărăcine, has been collected: *Phylloxera vastatrix* Planch., *Vespa* spp. L., *Agriotes* spp. L., *Byctiscus betulae* L. *Otiorrhynchus ligustici* L.

- **migratory species**, originated from the crops within the viticultural ecosystem S.D. Banu Mărăcine, these species can not be neglected because they can become very dangerous for the vine.

Regarding strictly to the feeding way, from the 36 harmful species collected from the viticultural ecosystem S.D. Banu Mărăcine, only *Eriophyes vitis* Page. and *Anaphothrips vitis* Priesner attack only the vine or different species of the **Vitis** Genre. (*Phylloxera vastatrix* Planch.) the rest of the harmful species are polyphagous.

The polyphagous species identified in the viticultural ecosystem S.D. Banu Mărăcine attack plants from cultivated or spontaneous flora, but some of them feed preferable on vine (*Lobesia botrana* Den et Schiff., *Sparganotis pilleriana* Den et Schiff., *Clysia ambiguella* Hb, *Anomala* spp., *Polyphylla fullo* F, *Empoasca vitis* Goethe, *Pulvinaria vitis* Targ., etc.).

Analyzing under this aspect it come out that the number of the harmful species, with their evolution cycle tied to the presence of the vine it is limited, while the number of the polyphagous species it is relatively high explained through the richness of the cultivated and spontaneous species from the S.D. Banu Mărăcine.

## CONCLUSIONS

Following the recorded results we can conclude that the main group of vine pests from the viticultural ecosystem at S.D. Banu Mărăcine are:

**Key pests:** vine acarids (*Eriophyes vitis* Page. and *Tetranychus urticae* Koch. and the vine moth (*Lobesia botrana* Den et Schiff., *Eupoecilia ambiguella* Hb. and *Sparganotis pilleriana* Den et Schiff.).

**Secondary pests (ocasional):** *Anomala* spp. Sam., *Pulvinaria vitis* Targ., *Melolontha melolontha* L., *Polyphylla fullo* F.,

**Potential pests:** *Phylloxera vastatrix* Planch., *Vespa* spp. L., *Otiorrhynchus ligustici* L., *Agriotes* spp. L., *Byctiscus betulae* L.

Besides these ones, there are frequently encountered a series of other species considered to be **migratory**: *Gryllotalpa gryllotalpa* L., *Phyllopertha horticola* L. *Amphimalon solstitialis* L., *Rhyzotrogus aequinoctialis* Herb., *Hyphantria cunea* Drury. etc.

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**PRELIMINARY REZULTS REGARDING THE INFLUENCE OF BLACK  
CHARCOAL APPLICATION IN APPLE ORCHARDS ON CHEMICAL AND  
MICROBIOLOGICAL PROPERTIES OF THE SOIL**

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**KEY WORDS:** *apple, modern orchards, black charcoal*

**ABSTRACT**

*The work presents some preliminary results regarding the influence of black charcoal in a modern superintensive apple orchard, in supplying the soil with organic carbon (humus ingredient), with nitrogen (total, nitric or amonical formes), on soil reaction (pH) and on microbiological activity one year after the charcoal application. Afther this period the soil total carbon content increased between 1.08% and 2.30%. Thogether with organic matter mineralisation, the soil assimilable nitrogen content increased between 0.02% and 0.05%, the total nitrogen content, decreased between 0.31% and 0.08%, but a part of it can be found adsorbed on charcoal surface. At a low soil reaction (pH=5.8), the organic matter mineralisation is slow, ideal for fruit species, which are perennials. The isolation and growing of some fungi as Cladosporium, Paecylomyces, Sporobolomyces and Myrothecium genus, are indicators of the soil microbiological activity stimulation, especially the degradation process of cellulose and green manure, manure resulted by orchard floor management, with favorable impact on soil organic matter content and fertility. Moreover, grace to micropores structure of charcoal, the soil phisical and chemical properties were enhanced, by retention on its surface of additional quantities organic and mineral substances and water, and slowly release them for the plants benefits.*

**INTRODUCTION**

Nowaday to obtain high quality yields of fruits at the lower costs is a very actual objective of the growers, due the demografical increase and the necessity to increase the fruit consumption in the people daily diet. In perspective, the achievement of these goals must stand with sustainable use of the natural resources and modern ecological fruit growing technologies as well. For many plants, the main source of carbon and nitrogen is the soil humified organic mater.

Into the soil, under natural conditions and without any fertilisation, the mineral compounds of the nitrogen are generated as a result of the organic matter transformation by microorganismes (Papacostea, 1976). Because the plants nitrogen needs and consumption is higher than the soil capacity to mineralize the organic nitrogen and to release nitrates and

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ammonium, these ions are rapidly assimilated by the plants, and by consequence they can be hardly detected and revealed by analyses.

Among the factors affecting the organic nitrogen mineralisation, an important role is held by the soil reaction (pH). In the acidic soils, the nitrification process is inhibited but the ammonification process is stimulated. Many researches show the positive effect of the vegetal charcoal, vegetal ash and similar compounds incorporation, in the improvement of soil fertility and productivity (Steiner et al., 2007).

The soil microorganisms convert and recycle the organic matter and the nutrients, and they act both as reservoirs - during the immobilisation on the charcoal surface and as nutrients source - during the mineralisation process. (Błaszowski, 1994, 1994, 1995; Leath and Kendall, 1983; McKemy and Morgan-Jones, 1991; Bordjiba et al., 2001; Owens, 2005; Papacostea, 1976).

For the mentioned reasons, in a modern high density apple orchard was studied the influence of black charcoal on supplying the soil with organic carbon (humus ingredient), and nitrogen (total, nitric or ammoniacal forms), on soil reaction (pH) and on microbiological activity one year after the charcoal application. The charcoal content in organic carbon, total nitrogen and its pH were analysed as well.

## MATERIAL AND METHODS

The researches regarding the application of the black charcoal were conducted at the Research Institute for Fruit Growing Pitesti Romania, in a 5 years old high density apple orchard. The experiment type is a bifactorial one, 2 x 4: **A factor - the application depth** with the graduations **a1** - into the soil layer 0-0,2 m; **a2** - into the soil layer 0-0,4 m; **B factor the charcoal dose**: with the graduations **b1** - untreated control variant - without black charcoal use, **b2** - black charcoal 1Kg/m<sup>2</sup>; **b3** - black charcoal 5Kg/m<sup>2</sup> and **b4** - black charcoal 10Kg/m<sup>2</sup>.

From yielded samples, the soil pH was determined in soil-water solution, using the potentiometric method, the soil/water ratio being 1 : 2.5.

The soil total nitrogen content was determined by mineralisation in the presence of the catalysts (potassium sulphate and copper sulphate), followed by distillation and titration (Kjeldahl method). The nitrates and the ammonia forms of the nitrogen were assessed using the same method but other catalyst (Devarda alloy). The organic carbon was determined using the Walkley-Black oxidimetric method modified by Gogoasă.

The researches goal was also to evidence the influence of the black charcoal application on the soil fungi community of the high density apple orchard.

To accomplish these goals and to evidence as many taxons in the experimental variants, from the soil samples were realised water suspensions and then were initiated fungi cultures on artificial growing media. The associated fungi genus and species grown were observed both under the stereomicroscopes Carl-Zeiss DDR and Bell-Photonics and then examined under an IOR ML-4 microscope.

The pictures in the experimental variants and in the lab were taken using the digital camera SONY Cybershoot F828 8.0 MP, optic block Carl Zeiss Vario-Sonnar T\* 2-2.8/7.1-51, and HP Photosmart 525 6.0 MP, optic block HP 6.0-18.0. The images were stored under \*.jpg and \*.png format, and then processed and analysed using Irfan View and Photo Filter software.

The great amount of experimental data were stored, processed and represented in a graphical form using Instat+ version 3.036 and MS 2003 Excel 7.0. software.

## RESULTS AND DISCUSSIONS

### A. Results on the soil chemistry dynamic-total carbon, total nitrogen, reaction (pH).

The soil total organic content in the variants treated with black charcoal increased with 56.00% up to 88.52% compared to the untreated control variant (table 1 and figure 1). On short term this increase is not very important because this organic carbon from black charcoal is not fully mineralised. It will take a long time period till the organic carbon mineralization and its transformation in accesibles compounds to plants. It is a sort of carbon temporary imobilised, with a limited accesibility in the time, because of the long degradation period of the black charcoal between 5 and 100 years (depending on biomass carbonisation technology) [Murariu, 2008]. However, the black charcoal has a beneficial effect on the physical properties of the soil increasing the water and minerals holding capacity, and their availability for plants roots as well, being considered a nutrients reservoir due its great capacity of adsorbtion and desorbtion.

The total nitrogen contents obtained one year after the charcoal application decreased in all experimented variants. An important drop of the total nitrogen content was obtained in the variants treated with the higher rates of charcoal (5 kg/m<sup>2</sup> and 10 kg/m<sup>2</sup>), with 60.8% compared to untreated control variant. (Table 2 and figure 2). The reduction of the total nitrogen content in the variants treated with charcoal can be explained by the fact that in the soil, the organic nitrogen was mobilised on the charcoal and slowly mineralised and released to the plants, avoiding the loosess by levigation. On the other hand, it is very possible that the soil nitrogen and its formes (organic or anorganic ones) to be held (adsorbed) on the charcoal and will be released by time. This affirmation is sustained by the fact that the analyses of the charcoal yielded from the variants experimented, revealed a very high total nitrogen content (Nt = 0.60%). It is also true that the black charcoal has ascertain amount of total nitrogen content generated by its production technology (combustion up to 450°C), but at this temperature it is unlikely to remain into the charcoal such higher total nitrogen content (Nt = 0.70%).

The results of the chemical analyses of the soil mineral nitrogen (nitric and amoniacal forms) revealed some increases compared to the last year in all experimented variants including also untreated control variant (table 3). The increases of mineral nitrogen content of soil (accessible to the plants) can be explained by soil aeration improvement, which led to the mineralisation of the organic mater existent in the soil, previous to black charcoal application.

The mineral nitrogen adsorption on charcoal, prevented its levigation through the soil profile. Although the total organic content rised up when the charcoal was applied, the C/N ratio, calculated for all variants was much lower than previous year (Table 3). This thing is dued to the soil mineral nitrogen content (accesible) under this year conditions. The pH analysis showed no differences compared to the previous year. At that time, the soil was classified in the acid soils category, the classification being still the same, also one year after the black charcoal application (Table 3). The pH determinations carried on the charcoal sampled fom the experimented variants showed the modification of the values from the neutral domain (pH=6.8-7.0) to the acide one (pH=4.8).

Table 1

**Influence of black charcoal doses on the soil organic carbon content**

Nr.	Variant	Total organic carbon [%]	Carbon increase compared to control [%]
1	Soil Control 1	1.2200	-
2	Soil+1 Kg charcoal/m <sup>2</sup>	1.9050	56.00
3	Soil+5 Kg charcoal/m <sup>2</sup>	2.0700	69.67
4	Soil+10 Kg charcoal/m <sup>2</sup>	2.1900	79.50
5	Soil+10 Kg charcoal/m <sup>2</sup>	2.2900	87.70
6	Soil Control 2	1.2000	-
7	Soil+1 Kg charcoal/m <sup>2</sup>	1.9150	56.96
8	Soil+5 Kg charcoal/m <sup>2</sup>	2.0400	67.21
9	Soil+10 Kg charcoal/m <sup>2</sup>	2.1400	75.40
10	Soil+10 Kg charcoal/m <sup>2</sup>	2.3000	88.52

Table 2

**Influence of charcoal doses on soil total nitrogen**

No.	Variant	Total nitrogen [%]	Carbon decrease compared to control [%]
1	Soil Control 1	0.204	-
2	Soil+1 Kg charcoal/m <sup>2</sup>	0.184	9.80
3	Soil+5 Kg charcoal/m <sup>2</sup>	0.080	60.78
4	Soil+10 Kg charcoal/m <sup>2</sup>	0.080	60.78
5	Soil+10 Kg charcoal/m <sup>2</sup>	0.104	49.01
6	Soil Control 2	0.200	-
7	Soil+1 Kg charcoal/m <sup>2</sup>	0.172	15.68
8	Soil+5 Kg charcoal/m <sup>2</sup>	0.116	43.13
9	Soil+10 Kg charcoal/m <sup>2</sup>	0.096	52.94
10	Soil+10 Kg charcoal/m <sup>2</sup>	0.108	47.05

Table 3

**Evolution of soil agrochemical indicators in 2009, one year after charcoal incorporation in the orchard soil**

N o.	Variant	pH in water	Total organic C [%]	Mineral N (accessible) %	N-NH <sub>4</sub> ppm	N-NO <sub>3</sub> ppm	C/N
1	Soil Control 1	5.30	1.2200	0.028	302.26	288.31	20.60
2	Soil+1 Kg charcoal/m <sup>2</sup>	5.45	1.9050	0.025	288.31	251.88	35.27
3	Soil+5 Kg charcoal/m <sup>2</sup>	5.55	2.0700	0.023	274.36	237.16	40.58
4	Soil+10 Kg charcoal/m <sup>2</sup>	5.85	2.1900	0.031	279.01	316.21	37.11
5	Soil+10 Kg charcoal/m <sup>2</sup>	5.60	2.2900	0.028	289.20	280.91	40.17
6	Soil Control 2	5.80	1.2000	0.023	297.61	223.21	23.07
7	Soil+1 Kg charcoal/m <sup>2</sup>	5.65	1.9150	0.023	260.41	237.16	38.53
8	Soil +5 Kg charcoal/m <sup>2</sup>	5.35	2.0400	0.022	250.21	220.17	43.40
9	Soil+10 Kg charcoal/m <sup>2</sup>	5.80	2.1400	0.021	234.35	210.15	48.19
10	Soil+10 Kg charcoal/m <sup>2</sup>	5.45	2.3000	0.028	270.45	280.65	41.74



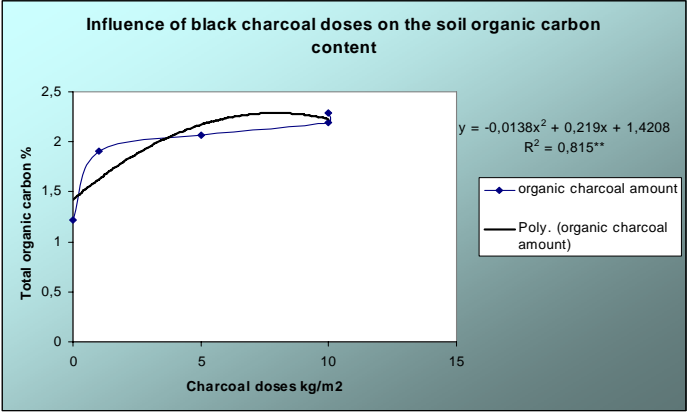


Figure 1

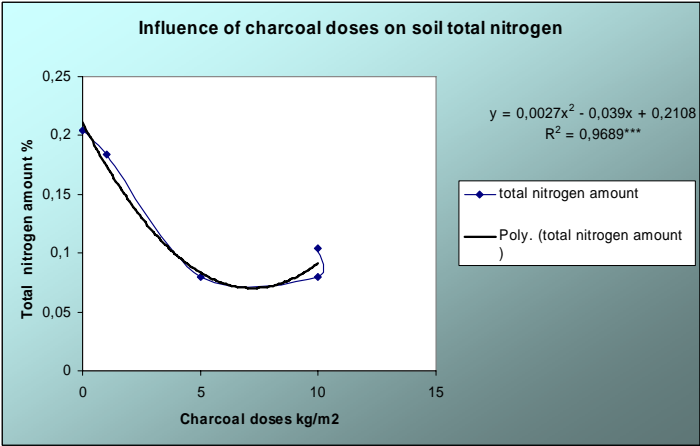


Figure 2

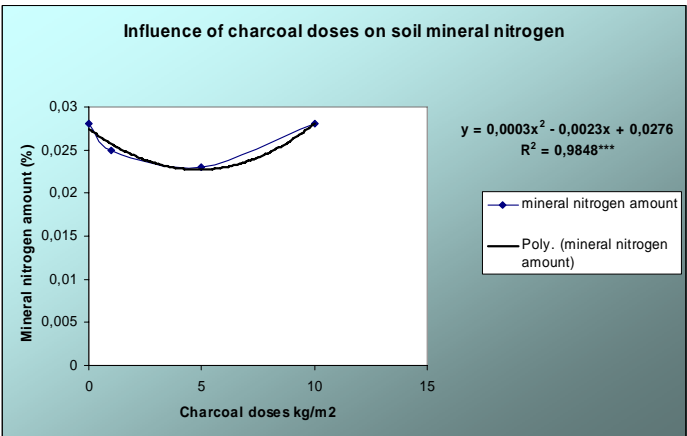


Figure 3

## B. Results regarding the soil microbiology

The researches carried out aimed to evidence the activity of the soil fungal community, one year after black charcoal application. To reveal the main fungi taxa living in the soil of the experimented variants, many artificial growing media were tested. On these media, but especially on PDA, was noticed the abundant development of some fungi genus well known as pathogens or potential pathogens as *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Verticillium*, *Fusarium*, *Mucor*, *Rhizopus*, *Monilia*, but also some other genera as *Dactylaria*, *Humicola* (sin. *Scoleobasidium*), *Myrothecium*, *Paecylomyces*, *Sporobolomyces* etc., involved in green manure degradation, with potential influence on soil fertility improvement. Among the saprophyte species *Trichoderma harzianum* and *Trichoderma viride* are now well known for their remarkable capacity to transform the cellulose from the green manure and ground cover waste, also for their role as antagonists of the soil born pathogens.

The obtained results are presented in the charts 4 to 8.

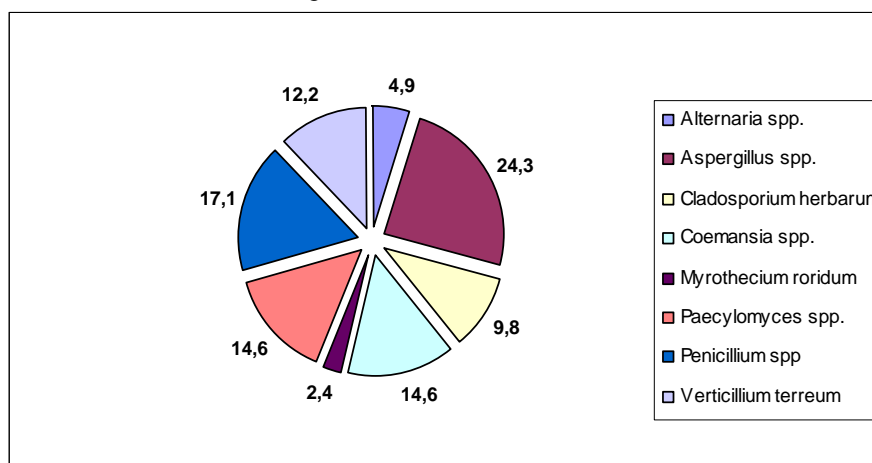


Figure 4. The ratio between the main fungi genera and species isolated from the experimental variants and grown on standard artificial media PDA.

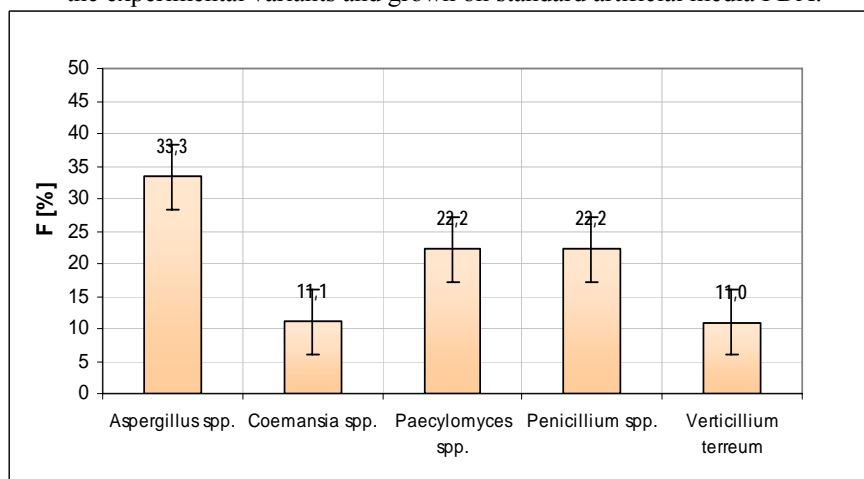


Figure 5. Frequency of the main genera in variants 1 and 5 - (untreated control)

By assessment of the figure 5 can be observed that in the variants 1 and 5 (untreated control) the main fungi genera and species that isolated and grown on standard PDA media were *Aspergillus* spp. (33.3%), *Coemansia* spp. (11.1%), *Paecylomyces* spp. (22.2%), *Penicillium* spp. (22.2%) and *Verticillium terreum* (11.0%), the ratio between the fungi genera was 3:1:2:2:1.

The analysis of the figure 6 can reveal that in the soil of the experimental variants 2 and 6 treated with black charcoal at a rate of 1 kg/m<sup>2</sup>, the main fungi genera and species that developed on standard PDA media were *Alternaria* spp. (14.3%), *Aspergillus glaucus* (14.3%), *Coemansia* spp. (14.3%), *Paecylomyces* spp. (28.6%), *Penicillium* spp. (14.3%), *Verticillium terreum* (14.3%), the ratio between the genera and species changing at 1:1:1:2:1:1.

The dominance of the *Paecylomyces* spp. in the soil of the variants 2 and 6, can be considered a reliable indicator of the active cellulose transformation by the microorganisms.

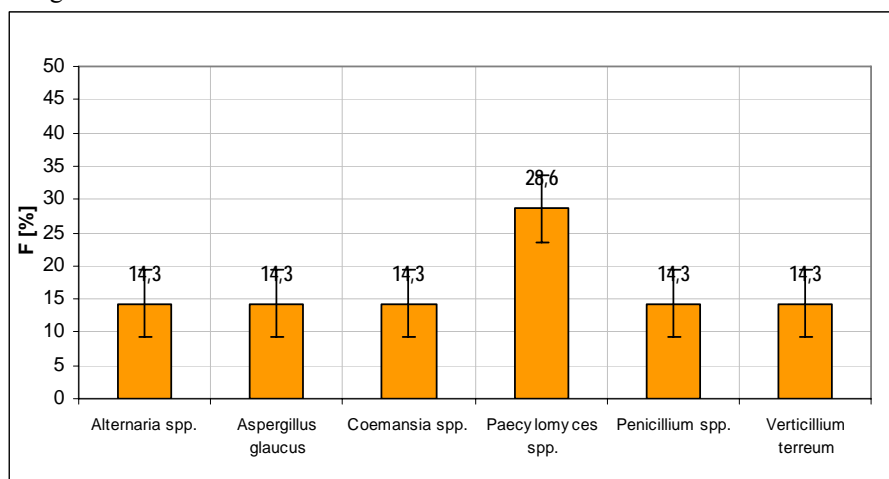


Figure 6. Frequency of the main genera in the variants 2 and 6

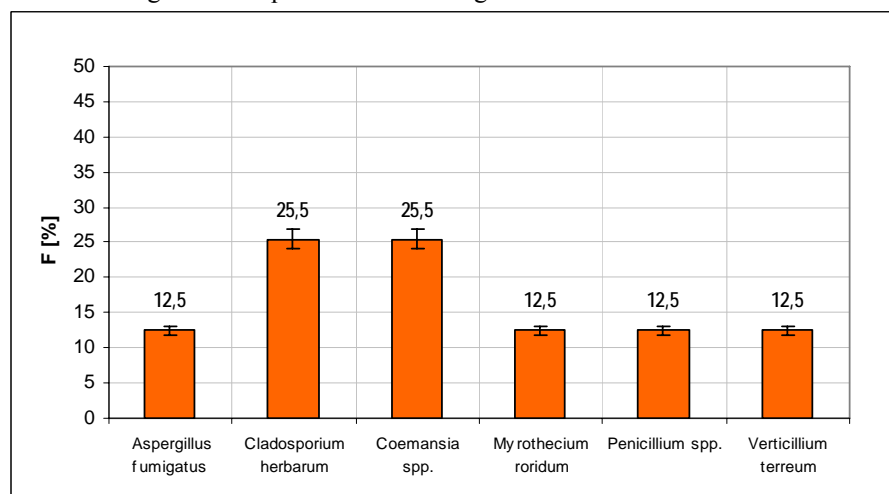


Figure 7. Frequency of the main genera in the variants 3 and 7

The examination of the figure 7 reveal that in the soil of the variants 3 and 7, treated with black charcoal at a rate of 5 kg/m<sup>2</sup>, the most important fungi genera and species, isolated and grown on standard PDA media, were *Aspergillus fumigatus* (12.5%), *Cladosporium herbarum* (25.5%), *Coemansia* spp. (25.5%), *Myrothecium roridum* (12.5%), *Penicillium* spp. (12.5%) and *Verticillium terreum* (12.5%), the ratio between the genera and species being 1:2:2:1:1:1.

The dominance of the *Cladosporium herbarum* in the soil of the variants 3 and 7, can be considered a reliable indicator of the organic matter transformation at the interface with the applied black charcoal. Also the presence of fungus *Myrothecium roridum* in the soil extractes obtained from the variants 3 and 7 and its development on the growing media was associated to the process of descomposiotion of the roots and green manure waste, derived from *Leguminosae* plants family (also prezent in the orchard floor of the experience, trimmed and incorporated with the occasion of the orchard soil management works).

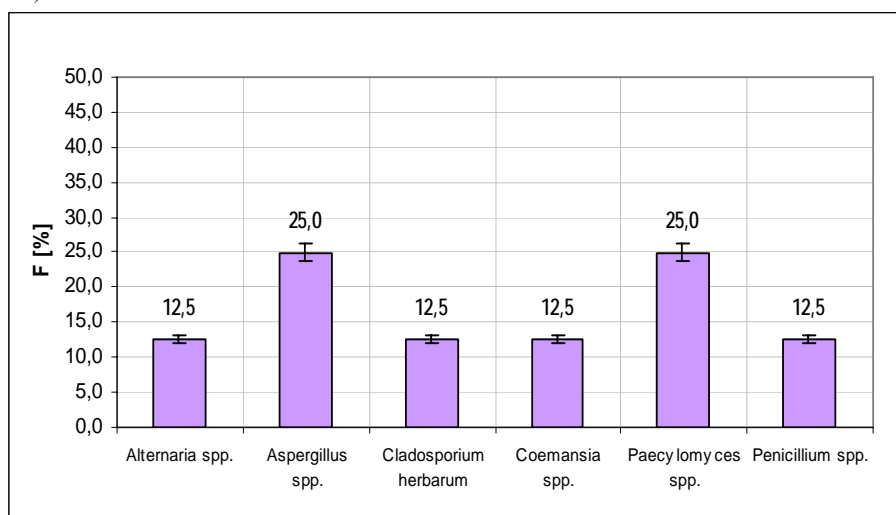


Figure 8. Frequency of the main genera in the variants 4 and 8

Analysis of the figure 8 shows that in the soil of the variants 4 and 8, treated with black charcoal at a rate of 10 kg/m<sup>2</sup>, the main genera and species of fungi isolated and grown on the standard growing media PDA were *Alternaria* spp. (12.5%), *Aspergillus* spp. (25.0%), *Cladosporium herbarum* (12.5%), *Coemansia* spp. (12.5%), *Paecylomyces* spp. (25.0%), and *Penicillium* spp. (12.5%), the ratio between genera and species being 1:2:1:1:2:1.

## CONCLUSIONS

- Using black charcoal, the total content of soil in the organic carbon risid up between 1.08% and 2.30%, but as regard the accessibility for plants, this is quite limited because the black charcoal will be degradated in time (up to 15 years). This aspect is an advantage, a modern orchard can being exploited for 15-20 years period.

- ✚ The black charcoal has also a very high total nitrogen content (Nt=0.67%), in time, its mineralization will bring into the soil an important amount of nitrogen compounds, accessible to the plants.
- ✚ Concomitant with the organic matter mineralization into the soil the content in assimilable nitrogen increased between 0.02% and 0.05%, the total nitrogen content decreased between 0.31% and 0.08%, but a part of it can be found adsorbed on the charcoal, but in time will be desorbed and put at the plants disposal.
- ✚ The soil reaction was not influenced by the charcoal incorporation at the experimented rates. Although the black charcoal application led to a slightly increase of the soil acidity, due to the adsorption of the nitrogen compounds and some others acid radicals on the charcoal surface, this fact is not negatively influencing the grown fruit species (the apple)
- ✚ If the soil reaction is acid (pH=5.8), the mineralization of the organic matter is slower, ideal for the fruit species, which are perennials (the modern orchards can be exploited over a 15-20 years period).
- ✚ The black charcoal incorporation into the orchards soil has the property to adsorb on its surface the organic and mineral substances and the water as well, increasing the soil retention capacity, due to the charcoal microstructure.
- ✚ The phytopathogen species belonging to the genera *Fusarium*, *Monilia*, *Pythium* and *Rhizoctonia*, identified with significant frequencies in the soil, had the status of constants or accessories of the soil fungal community, but the saprofit species as *Trichoderma harzianum* and *Trichoderma viride*, are well known both as cellulose transforming species and antagonists of the soil borne pathogens.
- ✚ Isolation and growing of some fungi species as *Cladosporium herbarum* represent a reliable indicator of the organic matter transformation at the interface with the fragments of black charcoal, but isolation of some fungi as *Paecilomyces* spp., *Sporobolomyces* and *Myrothecium roridum* represent reliable indicators of the stimulation of cellulose transformation and decomposition of the green manure waste and herbs roots belonging to the orchard floor, with favorable effects on soil fertility.
- ✚ The management of the orchards exploitation technologies must insure the soil microbiological re-equilibration, by stimulation of the beneficial and antagonists species and must stimulate the physical and chemical processes mediated by the soil microbiological community.

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Black charcoal application, November, 2008

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**OBSERVATIONS USEFUL AND HARMFUL SPECIES IN SWEET CHERRY AND  
SOUR CHERRY PLANTATION FROM THE NATIONAL COLLECTIONS OF  
THE SCDP IASI**

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**KEY WORDS:** *Barber traps, beating, cherry orchards*

**ABSTRACT**

*Observations were made on the farm Station for fruit growing Iasi, from May to September 2009. This regular observations were made directly on the orchard, and is also collected samples were then analyzed and determined in the laboratory.*

*Species observated in this period was: Dermestes lanarius Illig., Carabus violaceus L., Harpalus calceatus Duft., Otiorhynchus raucus F., Amara aenea DeGeer., Omias rotundus F.*

**INTRODUCTION**

Of many tree species which are cultivated in our country, cherry is one of the most valuable. It is a fruit growing species with great economic importance, given the nutritional characteristics, technological and commercial fruits.

Although the cherry crop is present throughout the country, due to weather conditions, there was a concentration of them in Iasi, Botosani, Bacau, Vrancea, Buzau, Prahova, Arges and Valcea, namely Eastern hilly country (Cociu, 1981). Like all cherry and sour cherry fruit species are affected by many diseases and pests which attack fruit, leaves, shoots, flowers, branches, stems and roots. Affected trees were growing poor condition, is wholly or partly calamitează fruit depreciates their quality and longevity of trees decreases (Cârdei, 1987).

Obtaining high yields and fruit quality is correlated with a good health of trees. With our country by neglecting to pests and diseases can cherry and cherry harvest loses about 45-100% mid and late maturing varieties .

**MATERIAL AND METHODS**

Experience was held for observation useful and harmful insect species in the National Cherry Collection of Station for fruit growing from Miroslava.

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The research method consisted of setting soil traps type Barber, six traps with formalin concentration 3-4% and bimonthly beating method was applied to 10 trees and that was the sudden shaking of two branches with a length of 30-50 cm (Talmaciu M. et al, 2003).

Throughout the period of observations from May and ending in August we made a number of 7 harvesting following:

- The first harvest on 18.05;
- The second collection on 29.05;
- The third harvest on 22.06;
- Fourth harvest on 06.07;
- Fifth harvest on 15.07;
- Sixth harvest on 28.07;
- Seventh harvest on 18.08.

## RESULTS AND DISCUSSIONS

The obtained results consolidate entomofauna found useful and harmful in the national collection of cherry Miroslava, Iasi County over seven harvest using traps type Barber and beating method(Chatened du Gaetan, 1990; Panin I., 1951; Rogojanu V., Perju T., 1979).

Table 1.  
Data collection, number of samples collected using traps soil type Barber in 2009

Data collection	No.	Species name	No. of samples	Total
18.05.2009	1	<i>Dermestes lanarius</i> Illig.	6	<b>23</b>
	2	<i>Cantharis fusca</i> L.	3	
	3	<i>Panageus crux major</i> L.	1	
	4	<i>Harpalus tardus</i> Panz.	2	
	5	<i>Harpalus calceatus</i> Duft.	3	
	6	<i>Otiorhynchus ovatus</i> L.	2	
	7	<i>Adalia bipunctata</i> L.	1	
	8	<i>Cymindis humeralis</i> Fourc.	1	
	9	<i>Omius rotundus</i> F.	2	
	10	<i>Opatrum sabulosum</i> L.	1	
	11	<i>Phyllotreta nemorum</i> L.	1	
29.05.2009	1	<i>Harpalus tardus</i> Panz.	1	<b>20</b>
	2	<i>Amara fasciata</i> F.	1	
	3	<i>Coccinella 7punctata</i> L.	1	
	4	<i>Dermestes lanarius</i> Illig.	6	
	5	<i>Omius rotundatus</i> F.	3	
	6	<i>Otiorhynchus raucus</i> F.	3	
	7	<i>Amara aenea</i> DeGeer.	1	
	8	<i>Harpalus calceatus</i> Duft.	3	
	9	<i>Psylliodes chrysocephala</i> L.	1	
22.06.2009	1	<i>Carabus violaceus</i> L.	4	<b>51</b>
	2	<i>Dermestes lanarius</i> Illig.	21	
	3	<i>Otiorhynchus raucus</i> F	2	



	4	<i>Brachinus crepitans</i> L.	2	
	5	<i>Amara aenea</i> DeGeer	4	
	6	<i>Harpalus pubescens</i> Mull.	2	
	7	<i>Harpalus calceatus</i> Duft.	7	
	8	<i>Psylliodes chrysocephala</i> L.	2	
	9	<i>Psylliodes cuprea</i> L.	1	
	10	<i>Curculio nucum</i> L.	1	
	11	<i>Harpalus azureus</i> F.	3	
	12	<i>Carabus coriaceus</i> L.	1	
	13	<i>Harpalus distinguendus</i> Duft.	1	
	14	<i>Harpalus aeneus</i> F.	1	
06.07.2009	1	<i>Dermestes lardarius</i> L.	2	9
	2	<i>Carabus violaceus</i> L.	1	
	3	<i>Cymindis vaporariorum</i> L.	1	
	4	<i>Dermestes lanarius</i> Illig.	2	
	5	<i>Meligetes aeneus</i> F.	2	
	6	<i>Harpalus punctifolis</i> L.	1	
15.07.2009	1	<i>Carabus violaceus</i> L.	4	8
	2	<i>Harpalus tardus</i> Panz.	1	
	3	<i>Dermestes lanarius</i> Illig.	1	
	4	<i>Longitarsus tabidus</i> F.	2	
28.07.2009	1	<i>Harpalus griseus</i>	1	4
	2	<i>Carabus violaceus</i> L.	1	
	3	<i>Propilea quatuordecimpunctata</i> L.	2	
18.08.2009	1	<i>Carabus violaceus</i> L.	16	16

Using traps type Barber (Table 1) at first harvest of 18.05 since we recorded a total of 23 samples of beetles belonging to a total of 11 species: *Dermestes lanarius* Illig., *Cantharis fusca* L., *Panageus crux major* L., *Harpalus tardus* Panz., *Harpalus calceatus* Duft., *Otiorhynchus ovatus* L., *Adalia bipunctata* L., *Cymindis humeralis* Fourc., *Omas rotundus* F., *Opatrum sabulosum* L., *Phyllotreta nemorum* L..

The second harvesting the date 29.05 is represented by nine species of beetles with a total number of 20 samples. The nine species of beetles are: *Harpalus tardus* Panz., *Amara fasciata* F., *Coccinella 7punctata* L., *Dermestes lanarius* Illig., *Omas rotundatus* F., *Otiorhynchus raucus* F., *Amara aenea* DeGeer., *Harpalus calceatus* Duft., *Psylliodes chrysocephala* L..

Three harvesting we totaled the number of 51 samples of 14 species of beetles represented as follows: *Carabus violaceus* L., *Dermestes lanarius* Illig., *Otiorhynchus raucus* F., *Brachinus crepitans* L., *Amara aenea* DeGeer., *Harpalus pubescens* Mull., *Harpalus calceatus* Duft., *Psylliodes chrysocephala* L., *Psylliodes cuprea* L., *Curculio nucum* L., *Harpalus azureus* F., *Carabus coriaceus* L., *Harpalus distinguendus* Duft., *Harpalus aeneus* F.

Fourth harvest on 06.07 we saw a number of nine samples belonging to six species of beetles: *Dermestes lardarius* L., *Carabus violaceus* L., *Cymindis vaporariorum* L., *Dermestes lanarius* Illig., *Meligetes aeneus* F., *Harpalus punctifolis* L..

In the fifth collection of 28.07 I collected a 8 of samples belonging to four species of beetles: *Carabus violaceus* L., *Harpalus tardus* Panz., *Dermestes lanarius* Illig., *Longitarsus tabidus* F..

Harvest number six recorded only four samples collected, represented by three species: *Harpalus griseus*, *Carabus violaceus* L., *Propilea quatuordecimpunctata* L..

At the last harvest of 18.08 I caught wide for a single species, *Carabus violaceus* L. with a total of 16 samples.

Table 2

Structure, dynamics and abundance of species of beetles collected in the farm Miroslava cherry orchard in 2009

No.	Species name	No of samples							Total
		Collection							
		1	2	3	4	5	6	7	
1	Dermestes lanarius Illig.	6	6	21	2	1	-	-	36
2	Cantharis fusca L.	3	-	-	-	-	-	-	3
3	Panageus crux major L.	1	-	-	-	-	-	-	1
4	Harpalus tardus Panz.	2	1	-	-	1	-	-	4
5	Harpalus calceatus Duft.	3	3	-	7	-	-	-	13
6	Otiorhynchus ovatus L.	2	-	-	-	-	-	-	2
7	Adalia bipunctata L.	1	-	-	-	-	-	-	1
8	Cymindis humeralis Fourc.	1	-	-	-	-	-	-	1
9	Omius rotundus F.	2	3	-	-	-	-	-	5
10	Opatrum sabulosum L.	1	-	-	-	-	-	-	1
11	Phyllotreta nemorum L.	1	-	-	-	-	-	-	1
12	Amara fasciata F.	-	1	-	-	-	-	-	1
13	Coccinella 7punctata L.	-	1	-	-	-	-	-	1
14	Otiorhynchus raucus F.	-	3	2	-	-	-	-	5
15	Amara aenea DeGeer.	-	1	4	-	-	-	-	5
16	Psylliodes chrysocephala L.	-	1	2	-	-	-	-	3
17	Brachinus crepitans L.	-	-	2	-	-	-	-	2
18	Harpalus pubescens Mull.	-	-	2	-	-	-	-	2
19	Psylliodes cuprea	-	-	1	-	-	-	-	1
20	Curculio nucum L.	-	-	1	-	-	-	-	1
21	Harpalus azureus F.	-	-	3	-	-	-	-	3
22	Carabus coriaceus	-	-	1	-	-	-	-	1
23	Harpalus distinguendus Duft.	-	-	1	-	-	-	-	1
24	Harpalus aeneus F.	-	-	1	-	-	-	-	1
25	Dermestes lardarius L.	-	-	-	2	-	-	-	2
26	Carabus violaceus L	-	-	-	1	4	1	16	22
27	Cymindis vaporariorum L.	-	-	-	1	-	-	-	1
28	Melighetes aeneus F.	-	-	-	2	-	-	-	2
29	Harpalus punctifolis L.	-	-	-	1	-	-	-	1
30	Longitarsus tabidus F.	-	-	-	-	2	-	-	2
31	Harpalus griseus	-	-	-	-	-	1	-	1
32	Propilea quatuordecimpunctata L.	-	-	-	-	-	2	-	2
TOTAL		23	20	41	16	8	4	16	128

Analyzing the structure, dynamics and abundance of species of beetles from the farm Miroslava in cherry orchard I totaled a number of 128 samples belonging to 32 species of beetles, including those with the highest number of samples (Chatened du Gaetan, 1990;

Panin I., 1951; Rogojanu V., Perju T., 1979).: *Dermestes lanarius* Illig (36)., *Carabus violaceus* L. (22), *Harpalus calceatus* Duft. (13), *Otiorhynchus raucus* F. (5), *Amara aenea* DeGeer. (5), *Omius rotundus* F. (5), *Harpalus tardus* Panz. (4).

Tabel 3

Useful and harmful Entomofauna collected using the beating method in cherry orchard at the Miroslava farm in 2009

Harvest - Date	No.	Name	No samples collected	Total samples collected
<b>Harvest no.1-</b> 18.05.2009	1	Homoptera	15	36
	2	Hymenoptera	3	
	3	Coleoptera	11	
	4	Arachnida	1	
	5	Diptera	1	
	6	Larva de tip protopod	5	
<b>Harvest no.2-</b> 29.05. 2009	1	Coleoptera	8	62
	2	Hymenoptera	5	
	3	Homoptera	38	
	4	Arachnida	3	
	5	Diptera	2	
	6	Neuroptera	1	
	7	Larva de tip protopod	5	
<b>Harvest no.3-</b> 22.06.2009	1	Dermaptera	1	9
	2	Heteroptera	1	
	3	Neuroptera	1	
	4	Coleoptera	2	
	6	Homoptera	2	
	7	Hymenoptera	2	
<b>Harvest no.4-</b> 06.07.2009	1	Neuroptera	2	17
	2	Hymenoptera	2	
	3	Arachnida	3	
	4	Homoptera	5	
	5	Coleoptera	5	
<b>Harvest no.5-</b> 15.07.2009	1	Coleoptera	3	11
	2	Larava de tip protopod	1	
	3	Homoptera	2	
	4	Hymenoptera	5	
<b>Harvest no.6-</b> 28.07.2009	1	Neuroptera	2	21
	2	Diptera	1	
	3	Homoptera	5	
	4	Coleoptera	9	
	5	Hymenoptera	3	
	6	Lepidoptera	1	
<b>Harvest no. 7 -</b>	1	Diptera	1	15

	2	Hymenoptera	2	
	3	Coleoptera	8	
	4	Homoptera	4	
<b>TOTAL</b>			<b>171</b>	

After applying the beating method (Table 3) in cherry orchard, the SCDP Iași I have totaled seven harvesting a total of 171 samples of useful and harmful insects (Donald J. Borror et al ,1963; Stănoiu I et al., 1995 )as follows:

- Collecting the date of 18.05 has a total of 36 samples belonging to five orders: Homoptera, Hymenoptera, Coleoptera, Arachnida, Diptera.
- second collection dated 29.05, totaling a number of 9 samples belonging to seven orders: Coleoptera, Hymenoptera, Homoptera, Arachnida, Diptera, Neuroptera.
- Three harvesting dated 29.05, totaling a number of 62 samples belonging to six orders Dermaptera, Heteroptera, Neuroptera, Coleoptera, Homoptera, Hymenoptera.
- Fourth harvesting on a total of 06.07 totaling 17 samples belonging to five orders: Neuroptera, Hymenoptera, Arachnida, Homoptera, Coleoptera.
- Fifth harvesting on a 15.07 totaling 11 samles belonging to three orders: Coleoptera, Hymenoptera, Homoptera.
- Sixth collection totaling 28.07 dated a number of 21 samples belonging to six orders: Neuroptera, Diptera, Homoptera, Coleoptera, Hymenoptera, Lepidoptera.
- Seventh collection of numbers totaling 18.08 on 15 samples belonging to four orders: Diptera, Hymenoptera, Coleoptera, Homoptera.

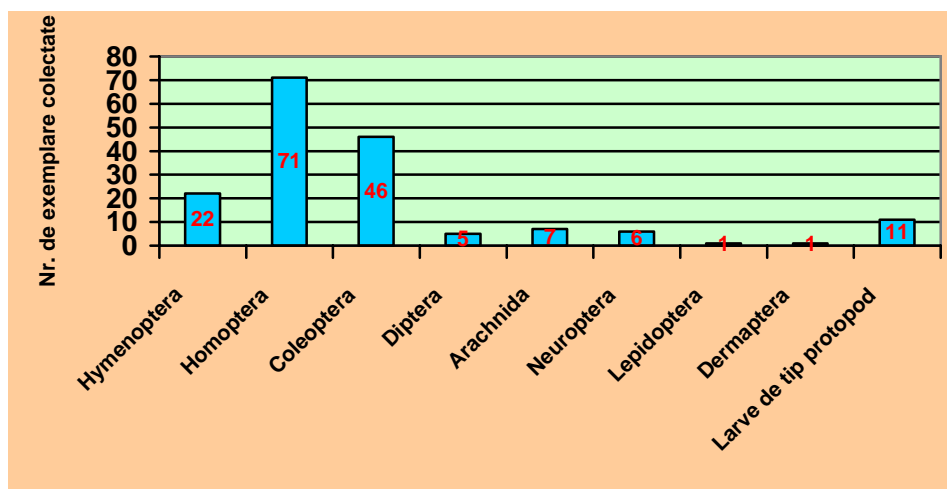


Fig.1 Centralization number of insects collected by the beating method according to the systematically order

From the graphical representation is noted that the order Homoptera has the highest number of insects collected (71) followed the order Coleoptera with a number of 46 samples , then follows the order Hymenoptera with 22 samples, 7 samples ordinal Arachnida, order Neuroptera 6 samples , order Diptera with five samples, and orders Dermaptera Lepidoptera and represented by a single copy.

## CONCLUSIONS

Period of the observations were contained in period May to August 2008, where we collected 128 samples of beetles, belonging to 32 species.

Species with the largest number of samples collected were: *Dermestes lanarius* Illig., *Carabus violaceus* L, *Harpalus calceatus* Duft., *Omius rotundus* F., *Coccinella 7punctata* L. , *Otiorhynchus raucus* F., *Harpalus tardus* Panz..

Harvesting no. 3 dated 22.06, using soil traps, it was noted by (totaling) a number of 51 samples belonging to a number of 14 species of beetles. The lowest number of specimens was collected on 28.07, meaning the collection with number five.

By using the beating method have collected a total of 171 samples of insects belonging to eight orders Homoptera, Coleoptera, Hymenoptera, Arachnida, Diptera, Lepidoptera, Dermaptera and Neuroptera.

The largest number of samples collected belong to the order Homoptera find 71 and the lowest number of samples are equal to one and Dermaptera Lepidoptera orders.

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## BIOACTIVITY OF OLIVE OIL MILL WASTEWATER AGAINST POST-HARVEST DISEASES

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**KEY WORDS:** Olive oil mill wastewater, antifungal activity, post-harvest diseases

### ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) was investigated. The effect of sterilized, filtered sterilized and non sterilized olive OMW was tested a) on mycelium growth of *Botrytis cinerea* in vitro and b) on strawberries fruits infected with the fungus *Botrytis cinerea* in vivo. The results show that the filtered sterilized olive OMW inhibits the growth of *Botrytis cinerea* mycelium in vivo confirming the antifungal activity of the phenolic compounds which contained on olive OMW in vitro.

### INTRODUCTION

During olive oil extraction a large amount of solid and aqueous residues known as olive oil mill wastewaters (olive OMWs) are produced annually worldwide where the majority of it is produced in the Mediterranean basin. The uncontrolled disposal of olive OMW is becoming a serious environmental problem due to its high content in phenolic compounds: tannins and flavonoids (Gonzales *et al.* 1999; Hamdi, 1992). Some of these phenols are responsible for several biological effects, including antibiosis (Rodríguez *et al.*, 1988) and phytotoxicity (Capasso *et al.*, 1992). They also appear to be involved in the defense of plants against invading pathogens, including bacteria, fungi and viruses (Marsilio *et al.* 2001). The use of olive OMW for plant and harvested fruits protection against microorganism could be a solution for residues management and nature protection. The main objective on this study was to examine the post-harvest biological control of grey mould (*Botrytis cinerea* Pers.: Fr.) on fresh-market strawberries with olive OMW.

### MATERIAL AND METHODS

*Effect of olive OMW on biological control of grey mould (Botrytis cinerea Pers.: Fr.) on fresh-market strawberries*

*Botrytis cinerea* isolated from market strawberries was used for this experiment. Spores suspension was prepared by isolating spores of above *Botrytis* species, from 7 days old cultures. Three agar plates per fungus culture were used to collect spores. Spores were collected in 1 l Erlenmeyer flask which contained distilled water by washing the agar

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surface with 3ml distilled water and filter the produced solution through sterilized muslin. In each flask spores suspension was adjusted at  $10^6$  spores/ml. A 50ml of olive OMW were added in each flask.

Fresh-market strawberries were surface sterilized and soaked for 3 min in 11 beakers contained 500 ml of the above spore and olive OMW solution. After that time fruits removed from the flasks, dried for 10 min in a laminar flow unit and incubated at 21°C for 12 days. Olive OMW was passed through Whatman filter paper No 2 before added to each beaker. After the incubation time, the spores number of each strawberry fruits was counted by scraping fruits surface into 200ml beaker which contained 50ml distill water.

The spore number per treatment and per beaker was counted by optical microscope using a hemacytometer.

Also, after the incubation period, the mycelium (molt) formation of each strawberry fruit was recorded and mold formation was sorted in six classes (0-5, as reported by Vagelas *et al.* 2009), where 0 is equal to healthy fruits, 1=slightly mold fruits and 5=heavy mold fruits. The experiment had fourteen replicates and four treatments; strawberry fruits infected with spores and olive OMW and strawberries infected only with spores, treated only in olive OMW and treated only with sterilized water were used as control.

#### *In vitro* assessment of antimicrobial activity of olive OMW on *Botrytis cinerea* mycelia

The antifungal effect of olive OMW against *Botrytis cinerea* mycelia was tested *in vitro*. Tests were made on PDA (Potato Dextrose Agar; DIFCO) in 9 cm Petri dishes. Treatments were PDA plates with a) olive OMW added into the medium and autoclaved and b) a drop of filter sterilized olive OMW (using a syringe filler 0.2  $\mu$ m) added onto the agar surface. In the first treatment a 25ml of olive OMW were added into 11 agar and further sterilized by autoclaving (121 °C for 20 min). In the second treatment a drop (50  $\mu$ l) of filter sterilized olive OMW was added onto the centre of each plate. Fifteen agar plates per treatment were inoculated with a mycelium plug (5 mm in diameter) of the above fungus which was taken from the periphery of 7 days old fungal colonies.

Mycelia plugs were placed onto the centre of each plate or next to the olive OMW drop. Equal plate numbers were used as control (without olive OMW). Plates were incubated at 21°C for six days and fungus mycelium growth was recorded.

#### **Statistical analysis**

Data were analyzed using the Minitab statistical package. Analysis of variance was used to assess treatments effect.

### **RESULTS**

#### *Effect of olive OMW on biological control of grey mould (*Botrytis cinerea* Pers.: Fr.) on fresh-market strawberries*

The olive OMW significantly reduced the number of *B. cinerea* ( $P<0.001$ ) spores. The average spore's number was  $3.4 \times 10^6$  for strawberry fruit infected only with *B. cinerea* and  $1.6 \times 10^2$  conidia/strawberry fruit infected with *B. cinerea* and treated with olive OMW. Further, a high mold formation was recorded only in treatments with strawberry fruits treated with fungus conidia suspension (Figure 1).

*In vitro* assessment of antimicrobial activity of olive OMW on *Botrytis cinerea* mycelia

There was a statistical significant difference between filtered sterilized olive OMW and control (untreated PDA and sterilized with olive OMW PDA), ( $P<0.001$ ). The total phenols content and antioxidants of filtered sterilized olive OMW could be an explanation of olive OMW antimicrobial activity *in vitro* confirming the biological control of grey mould by olive OMW as presented with the *in vivo* studies.

The filter sterilized olive OMW significantly inhibited the growth of *Botrytis cinerea* mycelia and showed only fungistatic activity against grey mould *in vitro* probably due to phenols content (Figure 2).

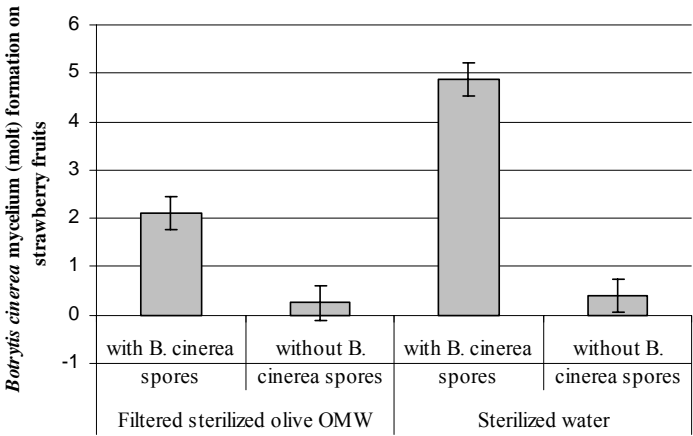


Figure 1. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on the mycelium mold formation of *Botrytis cinerea* on strawberry fruits.

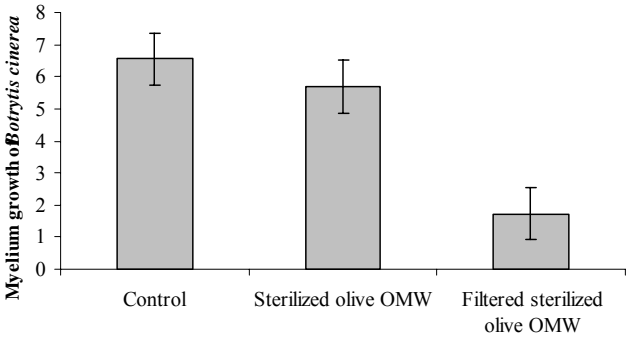


Figure 2. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on the mycelium growth of *Botrytis cinerea*.



## DISCUSSION

Olive oil mill wastewater (olive OMW) contains phytotoxic components capable of inhibiting the growth of microorganisms (Ramos-Cormenzana *et al.*, 1996) and plants (Martin *et al.*, 2002). Olive OMW contains phenolic compounds (Ramos-Cormenzana *et al.*, 1995) polysaccharides, lipids, proteins, and a number of monocyclic and polymeric aromatic molecules (Ethaliotis *et al.* 1999) which might exhibit inhibition effects towards some specific microorganism populations. In the current study filter sterilised olive OMW significantly reduced the growth of *Botrytis cinerea*. According to D'Annibale *et al.* (2004) phenolic compounds are the main determinants of the phytotoxic effect of olive residues.

Thus, the phenolics of olive OMW used in this experiment had negative effect on *Botrytis cinerea* mycelia *in vitro*. The used for olive OMW sterilization at 121 °C for 20 min probably removed or destroyed the phenolic compounds from olive OMW solution resulted a same or a better growth media for all tested fungi *in vitro*.

Furthermore, the production of *B. cinerea* spores on fruits inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses fungus reproduction and possible could offer a protection on strawberry fruits from post-harvest diseases. Overall we believe that the olive OMW due to phenolics have antifungal activity and could possible used against fruit fungal pathogens for preventing post harvest diseases.

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**SSR-TYPE AND SPOROPHYTIC INCOMPATIBILITY GENETIC ANALYSIS,  
DEFINING ELEMENTS FOR DETERMINING THE GENETIC KINSHIP FOR THE  
HAZELNUT**

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**KEY WORDS:** hazelnut, cultivar, genetic analyses, genetic incompatibility

**ABSTRACT**

*The hazel is a plant with high nutritional value of the fruits and with economic value. The modernization of the cultivation of this species is tied to the knowledge of the inheritances in the gene pools (species, cultivars, biotypes, mutants etc.) both regarding phenotypes and genotypes. The inheritances are used both for conservations in the gene banks for future generations and/or for programs of genetic improvement. The present study proves the link between genetic markings done with SSR-type (Simple Sequence Repeat) molecular markers and the allele composition for sporophytic-type incompatibility determined by using the fluorescence microscope. This was done in order to precisely establish the dendrographs and the grouping of the cultivars according to their degree of kinship, the duplicates etc. The UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) dendrographs have been used to create the phylogenetic tree for 81 cultivars of hazel, in 4 distinct groups using SSR markers. The analyses regarding the determination of sporophytic-type incompatibility alleles were done for a number of 8 Romanian cultivars. For the foreign cultivars, the allele formulas from specific literature were used. For the Romanian cultivars from SCDP Valcea, the following allele formulas were established: Arutela ( $S_2S_7$ ), Valverd ( $S_5S_{10}$ ), Roverd ( $S_5S_{11}$ ), Valcea 22 ( $S_2S_{10}$ ), Romavel ( $S_{10}S?$ ), Natval ( $S_{10}S?$ ), Uriase de Valcea ( $S_{20}S_{11}$ ) and Cozia ( $S?S?$ ).*

**INTRODUCTION**

The hazel is a diploid fruit-growing species which is important for the nutritional value of its fruits and for the economic value. Cultivation of the hazel is in full expansion along with the improvement of cultivation technique with the purpose of improving productivity and fruit quality.

The improvement of the value of the cultivars and of cultivation techniques is inevitably related to the fundamental knowledge of the genetics of the biologic material.

Recent research for the hazel have aimed to analyze the genetic diversity, to evaluate the germoplasm from gene-banks and to study genetic markings with the help of molecular markers etc. (Boccacci et al, 2005; Boccacci et al, 2006; Botta et al, 2004; Pop, 2009, etc.).

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The hazel is a plant with pollen incompatibility and is therefore dependant on the incompatibility gene S with its two alleles forms specific to each cultivar. The sporophytic-type incompatibility system is controlled by a single locus with multiple alleles (Botu, 1987;Thompson, 1979a) and is due to some chemicals in the walls of the pollen molecules which manifest at the moment of contact between the pollen tube and the cells of the stylus (Mehlenbacher and Thompson, 1988).

26 alleles S have been discovered to date and over 100 allele formulas have been determined for different cultivars (Thompson, 1979; Mehlenbacher 1997; Erdogan, 2005, etc.).

With the help of allele formulas for sporophytic-type incompatibility we can determine the pollinators for hazel cultivars in a plantation, the relationships between cultivars and their purity.

In this paper we aim to prove the link between genetic marking with the help of molecular SSR-type markers and the allele composition of the sporophytic-type incompatibility determined with the help of the fluorescence microscope. This would help to precisely establish the dendrographs and the grouping of the cultivars by the degree of kinship and also the resemblance between cultivars etc.

## MATERIALS AND METHODS

The genetic marking of Romanian and foreign cultivars has been done with the help of SSR-type markers. The analyses were done in the laboratories of Microsynth AG, Switzerland, with material from SCDP Valcea. The results were compared and completed with those published by Boccaci et al. (2006) in the paper 'DNA typing and genetic relations among European hazelnut (*C.avellana* L) cultivars using microsatellite markers' and Pop (2009).

Data interpretation and the design of the dendrographs were done using the UPGMA method (Un-weighted Pair Group Method with Arithmetic Mean). These were used to create the phylogenetic tree for 81 cultivars of hazel out of which 3 were Romanian cultivars (Valcea 22, Romavel and Uriase de Valcea) (Figure 1 and 2).

The allele formulas were established using the fluorescence microscope.

Each cultivar was pollinated with pollen from multiple tester cultivars for which the allele formula was known.

On the basis of the results obtained with the tester cultivars, with known allele formula, we succeeded in establishing the allele formula for the parent cultivars or just for the dominant allele in their allele formula.

The allele formulas for the Romanian cultivars were established at SCDP Valcea and for the foreign cultivars the formulas have been drawn from relevant literature (Mehlenbacher, 1996; Erdogan, 2005).

## RESULTS AND DISCUSSIONS

With the help of UPGMA dendrographs (Boccacci et al., 2006; Pop, 2009) (Figure 1 and 2) the phylogenetic tree has been drawn which includes 4 groups with the main lineages from the National Collection *Corylus* from SCDP Valcea.

The first group contains almost all the studied Italian and Spanish lineages. This is subdivided into 4 sub-groups:

The sub-group *Ia* contains cultivars from Spain (Alcover 236, Trenet, Negret, Pauetet etc.), one cultivar from Portugal (Da Viegá), 2 from Italy (TGDL and Nocciolino di San Grato) and 2 cultivars with unknown origin (Comen and Payrone).

The sub-group *Ib* contains 19 Italian cultivars (Ghirara, Nociara etc.), 7 Spanish cultivars (Queixal de Ruc, Barcelona, Gironell etc.) and 2 North-American selections (Royal and Willamette).

The sub-group *Ic* contains one Italian cultivar (Tonda Romana) and 2 Spanish cultivars (Morell and Queixal de Llop).

The sub-group *Id* contains one Italian cultivar (Tonda Bianca) and one German cultivar (Gunslebert).

The second group includes 2 sub-groups:

The sub-group *Ila* contains 2 cultivars from the Great Britain (Contorta and Du Chilly), 2 with unknown origin (Apolda and Fructo rubro), one from Turkey and one from Spain.

The sub-group *Ilb* contains 11 Turkish cultivars (Imperial de Trebizonda, Sivri etc.), one cultivar from Adzerbadjan (Ganja), one cultivar with unknown origin (Jann's) and two Romanian cultivars (Valcea 22 and Romavel).

The third group contains 3 English cultivars (Cosford, Daviana and Imparateasa Eugenia), 2 American cultivars (Ennis and Butler), one French cultivar (Fercoril – Corabel), 2 cultivars of unknown origin (Bearn and Codlinger) and one Romanian cultivar (Uriase de Valcea).

The fourth group contains an American cultivar (Gasaway), a German one (Heynick's Zellermuss), one of unknown origin (Pallagrossa) and Merweille de Bollwiller, same as Hall's Giant (France/Germany).

The research done between 2008 and 2010 on the sporophytic-type incompatibility system for the Romanian cultivars of hazel (Vicol 2010) has managed to show the complete or incomplete allele formulas of these cultivars (Table 1).

The allele formulas for sporophytic-type incompatibility obtained by Mehlenbacher (1996), Erdogan (2005), have been connected with the UPGMA dendrographs (Table 2) for comparison.

In accordance with the allele formulas for sporophytic-type incompatibility, the following groups have been obtained:

Group I contains 46 cultivars out of which: 25 cultivars have in common the allele S2; 20 cultivars have in common the allele S1; 3 cultivars have in common the allele S9; 2 cultivars have in common the allele S7; 2 cultivars have in common the allele S22; 4 cultivars have in common the allele S10.

A number of 18 cultivars from group 1 have the allele formula  $\underline{S_1}S_2$ , out of which only

12 have proved to be synonymous. This shows that some cultivars are not synonymous even if they have the same allele formula.

The cultivars Royal, Willamette, Nociara from group 1 and the cultivar Fercoril – Corabel from group IIIa have the same allele formula,  $S_1\underline{S_2}$  but have a high genetic distance in the dendrograph.

The only cultivar that does not have any sporophytic-type incompatibility allele in common with other cultivars is Gunslebert, which is also separate in the phylogenetic tree.

Group II contains 21 cultivars out of which: 6 cultivars have in common the allele S10; 6 cultivars have in common the allele S4; 2 cultivars have in common the allele S21; 5 cultivars have in common the allele S2;

The cultivars Extra Ghiaghli, Sivri Ghiaghli and Tombul have the same allele formula  $S_4S_{12}$  and in specialized literature (Erdogan, 2005) the cultivars Extra Ghiaghli and Tombul are presented as synonymous.

The Romanian cultivar Romavel has been placed in this group but we cannot yet say precisely if this is correct because his genitors are Urias de Halle x mix of pollen from 5 cultivars and therefore the unknown genitor might fall into Turkish cultivars group.

Group III contains 9 cultivars out of which: 5 cultivars have in common the allele S11; 5 cultivars have in common the allele S3; 2 cultivars have in common the allele S1; 3 cultivars have in common the allele S5;

In this group we can find the cultivars Ennis, Butler, Fercoril – Corabel, cultivars which are thought to be hybrids of the Barcelona cultivar. These cultivars have an allele formula which shows the kinship between them but in the phylogenetic tree they are at a large distance from the Barcelona cultivar.

The cultivars Bearn and Sodlinger are considered according to the dendrograph to be outside this group. However, the allele formula shows that Bearn cultivar has the allele S11 in common with three cultivars from his group. It seems like through the allele formula the bond of the 2 cultivars with the rest of the group is proven.

Group IV has 4 cultivars, 3 of which showing the allele S5 as common.

The Gasaway cultivar is the only one outside of the group and this is shown when using both methods.

From the analysis of the dendrograph and the allele formulas it has been observed that the grouping of the cultivars has been done in the same way in both types of analysis, establishing similar types of relationships based on the pedigree and the geographic origin, with certain remarks:

- Group I, sub-group Ia, with the Spanish and Italian cultivars can also include the Romanian cultivar Arutela, which has the same allele formula with TGD<sub>L</sub> ( $S_2S_7$ ) and the genetic origin is Urias de Halle x TGD<sub>L</sub>. The allele S2 is common to both genitors but  $S_7$  is the dominant allele and it comes from the TGD<sub>L</sub>.

- Group II, sub-group IIa – amongst other cultivars it also contains the cultivar Fructo rubro, synonymous with Red Lambert and Purple Filbert and it has unknown origin. The allele formula is  $S_5S_{10}$ . The Romanian cultivar Valverd is a genetic mutation with green-colored leafs and has the same allele formula as the parent genitor ( $S_5S_{10}$ ).

Another Romanian cultivar Roverd results from the mixing of cultivars Red Lambert x Daviana and has the allele formula  $S_5S_{11}$ . We propose the distribution of the cultivars Valverd and Roverd in the subgroup IIa next to Fructo rubro (Red Lambert). Roverd has inherited the dominant allele  $S_5$  from Red Lambert and the recessive allele  $S_{11}$  from Daviana.

The sub-group IIb contains the most cultivars of Turkish origin.

The Romanian cultivar Valcea 22 is a new cloning selection of Imperial de Trebizonda and they have the same allele formula ( $S_2S_{10}$ ). Its place in the dendrograph is next to the parent genitor and not next to the cultivar Extra Ghiaghli as in the dendrograph because the genetic distance between Imperial de Trebizonda and Extra Ghiaghli is too large.

Romavel comes from Urias de Halle x Imperial de Trebizonda which have the allele formulas  $S_5S_{15}/S_2S_5$ . The only highlighted allele for Romavel is  $S_{10}$ ? and it comes from Imperial de Trebizonda and this is the reason why we think it should be placed next to this

cultivar. The same applies to the cultivar Natval which comes from the cultivar Imperial de Trebizonda x mix of pollen and the highlighted allele formula was  $S_{10}S?$

Another observation can be made on the placement of the Turkish cultivars Tombul and Extra Ghiaghli considered to be the same cultivar (Erdogan, 2005), both having the same allele formula ( $S_4S_{12}$ ). They are however placed at a significant genetic distance.

- Group III contains the Romanian cultivar Uriase de Valcea with the genetic origin Ennis x Red Lambert and with the allele formula  $S_{11}S_{20}$ . In the dendrograph the genetic distance between Ennis and Uriase de Valcea is quite small. However, the place of this cultivar can be right next to the parent genitor. At present, the two are separated by the cultivar Butler.

- Group IV contributes few cultivars to the dendrograph. The Romanian cultivar Cozia comes from Urias de Halle x mix of pollen. From the measurements we could not establish an allele formula for sporophytic-type incompatibility and therefore its place in the dendrograph is uncertain, but closer to Urias de Halle.

## CONCLUSIONS

1. The modernization of plum cultivation is closely tied to the knowledge of the lineages (species, cultivars, mutants etc.) from the gene-banks, both regarding phenotypes and genotypes.
2. The genetic characterization of the biological material has been made by using SSR-type (Simple Sequence Repeat) molecular markers and the method of establishing allele formulas regarding sporophytic-type incompatibility.
3. The dendrographs UPGMA have been used to create the phylogenetic tree for 81 cultivars from 4 distinct groups using the SSR markers.
4. The analyses to determine the sporophytic-type incompatibility alleles have been carried out for a number of 8 Romanian cultivars: Arutela ( $S_2S_7$ ), Valverd ( $S_5S_{10}$ ), Roverd ( $S_5S_{11}$ ), Valcea 22 ( $S_2S_{10}$ ), Romavel ( $S_{10}S?$ ), Natval ( $S_{10}S?$ ), Uriase de Valcea ( $S_{20}S_{11}$ ) and Cozia ( $S?S?$ ).
5. Using both types of genetic analyses we have managed to more correctly place the Romanian cultivars in the phylogenetic tree as a result of the complementarity of the two methods (Arutela in group Ia; Valverd and Roverd in group IIa; Valcea 22, Romavel and Natval in group IIb; Uriase de Valcea in group III and Cozia in group IV).

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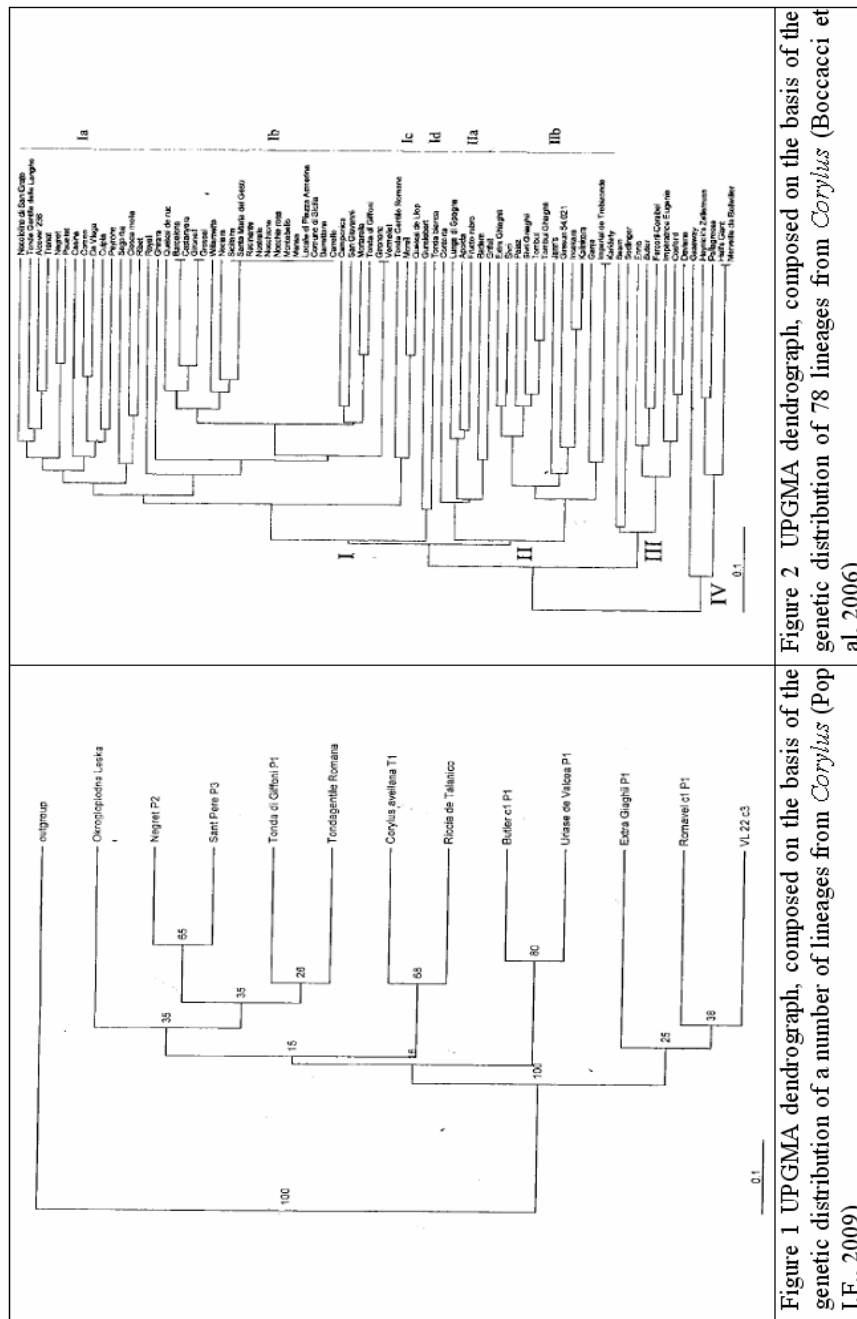




Table 1

The allele composition proposed for the Romanian cultivars regarding sporophytic-type compatibility

Number	Variety	Number of hybrid combinations	Formula proposed allele
1.	Vâlcea 22	11	$S_2\bar{S}_{10}$
2.	Romavel	13	$\bar{S}_{10} S_7$
3.	Uriaş de Vâlcea	17	$\bar{S}_{20}S_{11} / \bar{S}_{10}S_{11}$
4.	Arutela	15	$S_2\bar{S}_7$
5.	Cozia	14	$S_7S_7$
6.	Natval	6	$S_{10}S_7$
7.	Valverd	13	$S_5S_{10}$
8.	Roverd	13	$S_5S_{11}$

Table 2

Genetic distribution of a number of lineages from *Corylus* (according to Boccacci et al, 2006 and Pop I.F., 2009), complete with a number of cultivars and reported to the sporophytic-type incompatibility alleles.

Group	Sub-group	Variety	Allelic formula		Proposal of placement on the UPGMA dendrograph
			From literature	Personal measurements	
I	Ia	‘Nocciolino di San Grato’	$S_2\bar{S}_7$		
		‘TGDŁ’	$S_2\bar{S}_7$		
		*		$S_2\bar{S}_7$	‘Arutela’
		‘Alcover 236’	$\bar{S}_{15}S_{22}$		
		‘Trenet’	-		
		‘Negret’	$\bar{S}_{10}\bar{S}_{22}$		
		‘Pauetet’	$\bar{S}_{18}S_{22}$		
		‘Casina’	$\bar{S}_{10}S_{21}$		
		‘Comen’	$\bar{S}_2S_9$		
		‘Da Viega’	-		
		‘Culpla’	$S_9\bar{S}_{10}$		
		‘Payrone’	-		
		‘Segorbe’	$\bar{S}_9S_{23}$		
		‘Closca molla’	-		
		‘Ribet’	$S_2\bar{S}_{16}$		
	Ib	‘Royal’	$S_1\bar{S}_3$		
		‘Ghirara’	$S_2\bar{S}_{21}$		
		‘Queixal de ruc’	-		
		‘Barcelona’	$\bar{S}_1S_2$		
		‘Castanyera’	$\bar{S}_1S_2$		
		‘Gironell’	$\bar{S}_1S_2$		
		‘Grossal’	$\bar{S}_1S_2$		
		‘Willamette’	$S_1\bar{S}_3$		

		‘Nociara’	$S_1 S_3$		
		‘Siciliana’	$S_1 S_2$		
		‘Santa Maria del Gesu’	$S_1 S_2$		
		‘Racinante’	$S_1 S_2$		
		‘Nostrale’	$S_1 S_2$		
		‘Nocchione’	$S_1 S_2$		
		‘Nocchia rosa’	$S_1 S_2$		
		‘Montebello’	$S_1 S_2$		
		‘Mansa’	$S_1 S_2$		
		‘Locale di Piazza Amerina’	$S_1 S_2$		
		‘Comune di Sicilia’	$S_1 S_2$		
		‘Barrettona’	$S_1 S_2$		
		‘Carrello’	$S_1 S_2$		
		‘Camponica’	$S_1 S_2$		
		‘San Giovanni’	$S_2 S_8$		
		‘Mortarella’	$S_2 S_{17}$		
		‘Tonda di Giffoni’	$S_2 S_{23}$		
		‘Gironenc’	-		
		‘Vermellet’	-		
	Ic	‘Tonda Gentile Romana’	$S_{10} S_{20}$		
		‘Morell’	$S_1 S_2$		
		‘Queixal de Llop’	-		
	Id	‘Gunslebert’	$S_5 S_{23}$		
		‘Tonda bianca’	-		
II	IIa	‘Contorta’	-		
		‘Du Chilly’	$S_{10} S_{14}$		
		‘Apolda’	$S_{10} S_{11}$		
		‘Fructo rubro’	$S_5 S_{10}$		
		*		$S_5 S_{10}$	Valverd
		*		$S_5 S_{11}$	Roverd
		‘Badem’	$S_2 S_5, S_5 S_{15}$		
		‘Grifoll’	-		
		‘Extra Ghiaghli’	$S_4 S_{12}$		
	IIb	‘Valcea 22’	$S_2 S_{10}$		
		‘Romavel’	$S_{10} S?$		
		‘Sivri’	$S_8 S_4$		
		‘Palaz’	$S_2 S_4$		
		‘Sivri Ghiaghli’	$S_4 S_{12}$		
		‘Tombul’	$S_4 S_{12}$		
		‘Tombul Ghiaghli’	$S_4 S_8$		
		‘Jann’s’	$S_2 S_{10}$		
		‘Giresun 54.021’	-		
		‘Incekara’	$S_{21} S?$		
		‘Kalinkara’	$S_{21} S?$		
		‘Ganja’	-		
		‘Imperial de Trebizonde’	$S_2 S_{10}$		
		*		$(S_2 S_{10})$	‘Valcea 22’
		*		$(S_{10} S?)$	‘Romavel’

				( <u>S</u> <sub>10</sub> S?)	‘Natval’
		‘Karidaty’	S <sub>2</sub> S <sub>10</sub>		
III		‘Bearn’	<u>S</u> <sub>5</sub> S <sub>11</sub>		
		‘Sodlinger’	-		
		‘Ennis’	<u>S</u> <sub>1</sub> S <sub>11</sub>		
		*		<u>S</u> <sub>20</sub> S <sub>11</sub>	‘Uriase de Valcea’
		‘Butler’	S <sub>2</sub> <u>S</u> <sub>3</sub>		
		‘Uriase de Valcea’	<u>S</u> <sub>20</sub> S <sub>11</sub> / <u>S</u> <sub>10</sub> S <sub>11</sub>		
		‘Fercoril – Corabel’	S <sub>1</sub> <u>S</u> <sub>3</sub>		
		‘Imperatice Eugenie’	<u>S</u> <sub>3</sub> S <sub>14</sub>		
		‘Cosford’	<u>S</u> <sub>3</sub> S <sub>11</sub>		
		‘Daviana’	<u>S</u> <sub>3</sub> S <sub>11</sub>		
IV		‘Gasaway’	<u>S</u> <sub>3</sub> S <sub>26</sub>		
		‘Heynick’s Zellernuss’	-		
		‘Pallagrossa’	<u>S</u> <sub>5</sub> S <sub>25</sub>		
		‘Hall’s Giant’	<u>S</u> <sub>5</sub> <u>S</u> <sub>15</sub> / S <sub>7</sub> S <sub>5</sub>		
		‘Merveille de Bollwiller’	<u>S</u> <sub>5</sub> <u>S</u> <sub>15</sub> / S <sub>7</sub> <u>S</u> <sub>5</sub>		
		*		S?S?	Cozia

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CHEMICAL COMPOSITION OF BASIL ESSENTIAL OIL

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**KEY WORDS:** Basil, essential oil, Linalool, Estragol, Eugenol

ABSTRACT

The essential oils content and the chemical compositions of green and dry drogue, of two basil varieties, *Ocimum basilicum*, var. *genovese* (broad leaf basil) and *O. basilicum* var. *apalla* (narrow leaf basil) were assessed in this study. The essential oil was obtained via hydrodistillation and analyzed using Gas Chromatography. The essential oils yield was found to be higher in broad leaf basil variety compared to narrow leaf variety. Regarding the active substances, Linalool exhibits the highest concentration followed by Estragol and Eugenol for both basil varieties. Broad leaf basil variety showed slightly higher concentration in Eugenol compared with narrow leaf basil variety.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is member of the Lamiaceae family, which has about 3500 species, distributed among 210 genera. The genus *Ocimum*, includes around 30 plant species from tropical and subtropical areas. *Ocimum* is widely cultivated and extensively used for food, perfumery, cosmetics, pesticides, medicine and traditional rituals because of their natural aroma and flavor and other properties (Albuquerque, 1996; Darrah, 1974).

The chemical composition of *O. basilicum* essential oils has been intensively investigated throughout the world indicating the presence of tanines, flavonoids, saponins and volatile terpenes like camphor, tymol, methylchavicol, linelool, eugenol, 1-8-cineol and pinenes. The aim of this work was to study the essential oils content and chemical composition of two species of basil.

MATERIAL AND METHOD

The experiment took place at the laboratory of aromatic and medical plants at the Technological Education Institute of Larissa. Two basil varieties *Ocimum basilicum*, var. *genovese* (broad leaf basil) and *O. basilicum* var. *apalla* (narrow leaf basil) were used for volatile oil isolation. A sample (10 g) of dried drogue was extracted by the hydrodistillation technique during 2 hours with essential oil determination apparatus. The oils obtained were then immediately analyzed using Hewlett-Packard 5890 type gas chromatograph (GC) equipped with Flame Ionization Detector (FID).

The components of oil samples were identified with the use of commercial standard Eugenol, Estragol and Linalool (99% pure).

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The experiment had two varieties, two treatments (green and dry drogue) and forty replications. Data were analyzed using the SPSS statistical package. Analysis of variance was used to assess treatments effect.

## RESULTS AND DISCUSSION

There were not significant differences on the green and dry drogue between the two basil varieties. The average fresh and dry weight of broad leaf basil was 887.5 g/m<sup>2</sup> and 126.5 g/m<sup>2</sup> respectively, while the narrow leaf basil had dry weight 689.6 g/m<sup>2</sup> and fresh weight 97.8 g/m<sup>2</sup>.

In this experiment, the essential oils yield differed between varieties ( $P < 0.001$ ). The narrow leaf basil variety had significantly lower content in oil (0.154 ml/10g dry weight) than broad leaf basil variety (0.225 ml/10g dry weight).

According to Hanus et al. (2006), the basil oil extraction yields were in the rage of 0.04 to 0.7%, while Dachler and Pelzman (1999) reported changes from 0.5-1.5%. The concentration of basil essential oil is depending from plant genotype and variety, year of culture, date of sowing and harvest time (Hanus et al. 2006; Dachler and Pelzman1999; Marquard and Kroth, 2002).

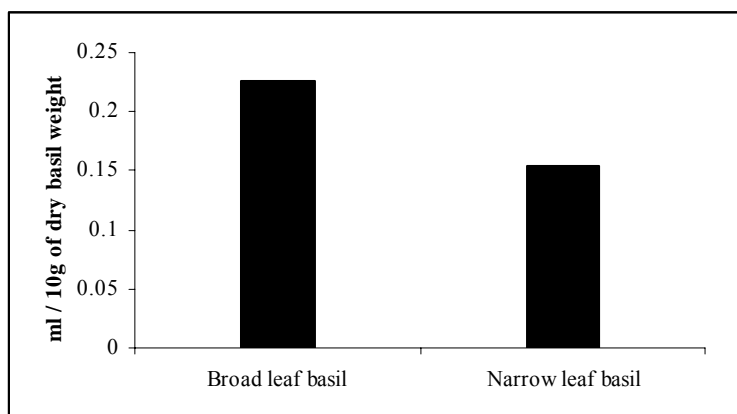


Figure 1. Essential oil yield in two basil varieties.

In addition, the main active substances show remarkable differences which depend from variety. According to Hanus et al. (2006), there are basil varieties with high concentration in Linalool (more that 75% of oil) and others with increased concentration in Eugenol.

In this study, the chemical composition of basil oil differed in both varieties ( $P < 0.001$ ). The Linalool was in greater content than Estragol and Eugenol. Moreover, the Eugenol concentration differed between varieties. Narrow leaf basil variety had lower content in this chemical substance that broad leaf basil variety.

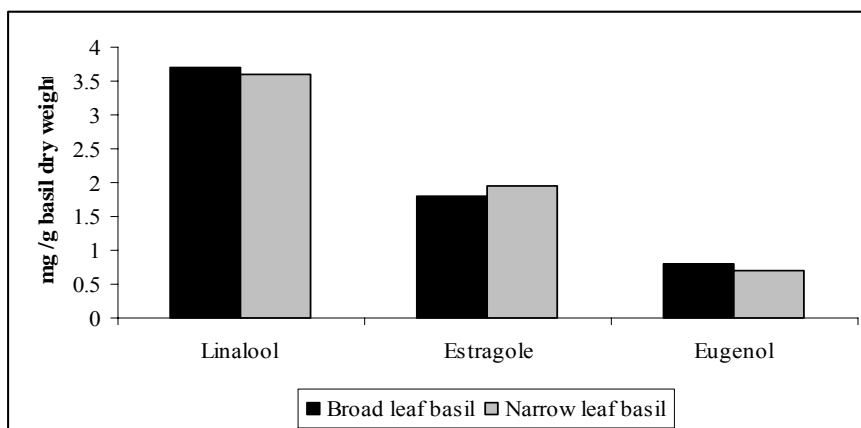


Figure 2. Concentration of main chemical substances in two basil varieties.

## CONCLUSION

The production of basil essential oil depends on the variety. Broad leaf basil variety was more productive than narrow leaf variety. However, the content of the main active substances Linalool and Estragole were formed independently of the variety in contrast with Eugenol. Linalool exhibits the highest concentration followed by Estragole and Eugenol in both basil varieties.

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**RESEARCH REGARDING THE EFFECTS OF WATER RETENTION  
MECHANISMS, RESISTANCE TO DROUGHT, HEATSTROKE AND HIGH  
TEMPERATURES OF THE *SEMPERVIVUM* ROSETTES**

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**KEY WORDS:** *Sempervivum* sp., rosettes, resistance to drought, heatstroke, high temperatures

**ABSTRACT**

*This paper presents the results of the research carried out within the experiences related to the behavior of the Sempervivum, rosettes and the effects of the water resistance mechanisms, resistance to drought, heatstroke and high temperatures. The experiment consisted in exposing the aerial parts of the Sempervivum plants to very high temperatures, sometimes exceeding 50°C, maximum sunlight and total lack of water. In order to develop the experiences one used small and large size rosettes of Sempervivum tectorum and Sempervivum montanum; the observations and determinations targeted the monitoring of changes, both in appearance and weight of plants' organs exposed to stressors. One could not say with certainty that the species would have influenced somehow the resistance to the excessive moisture, but concluded that the excess of humidity is the most worrying factor causing plants' death within a very short time*

**INTRODUCTION**

*Sempervivum* sp. is known as being resistant soil and atmospheric drought, low or very high temperatures, the maximum possible heatstroke (Mitchell 1973; Grey 1979; Zaharia 2007)

These affirmations are supported by various facts and circumstances when *Sempervivum* plants have survived to very critical conditions, taking into account the extreme values of environmental factors, conditions in which other plants died.

The studied scientific literature does not present any research results which can explain the mechanisms underlying these plants resistance to possible extreme values of the above mentioned factors.

**MATERIALS AND METHODS**

In order to understand the phenomenon one conducted an experiment which consisted in exposing the aerial parts of *Sempervivum* plants at very high temperatures, sometimes exceeding 50°C, maximum heatstroke (August 2008 - it was 90 000 lx) and complete lack of water.

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All observations and determinations aimed at monitoring changes in appearance and weight of plants'organs exposed to stressors. One considered that the changes are caused by the loss of water from the exposed plants'organs, and the exfoliation phenomenon had in view the specific mechanism of the evapo – transpiration.

Within the experience one used small and large rosettes of *Sempervivum tectorum* and *Sempervivum montanum*. After that, one removed the roots, the dried leaves and stem portion without leaves, the large rosettes were weighed and exposed to sunlight; the small rosettes were left with the heel portion on which they were formed.

Next, the rosettes were weighed with a precision electronic balance and the changes regarding their weight have been registered.

## RESULTS AND DISCUSSION

The results obtained within the research carried out on *Sempervivum* plants' behavior and the effects of water retention mechanisms, resistance to drought, high temperature and heatstroke are presented in tables and graphs.

Table 1  
Experimental results on the weight of *Sempervivum* rosettes exposed to water stress conditions, heat and sunlight (12 -24 august 2008)

No of days	Date VIII.2008	<i>Sempervivum tectorum</i>			<i>Sempervivum montanum</i>		
		Fresh weight (g)	Water losses (g)		Fresh weight (g)	Water losses (g)	
			Partial	Cumulated		Partial	Cumulated
1.	12	103,5	-	-	88,5	-	-
2.	13	82,0	21,5	21,5	44,8	10,7	10,7
3.	14	71,3	10,7	32,2	38,0	6,8	17,5
4.	15	65,5	5,8	38,0	32,1	5,9	23,4
5.	16	60,7	4,8	42,8	29,1	3,0	26,4
6.	17	58,2	2,5	45,3	27,3	1,8	28,2
7.	18	56,1	2,1	47,4	24,9	2,4	30,6
8.	19	54,3	1,8	49,2	24,0	0,9	31,5
9.	20	52,8	1,5	50,7	22,9	1,1	32,6
10.	21	49,9	2,9	53,6	21,9	1,0	33,6
11.	22	49,5	0,4	54,0	21,0	0,9	34,5
12.	23	49,1	0,4	54,4	20,4	0,6	35,1
13.	24	49,0	0,1	54,5	20,3	0,1	35,2

Table 1 presents the results obtained by weighing the *Sempervivum tectorum* and *Sempervivum montanum* rosettes at 7:00 am, within 24 hours.

Analyzing the data in the table one noticed that fresh weight (in grams) of rosettes permanently changed between two consecutive weightings. The changes regarding the fresh weight of the rosettes was due to water losses by evapo – transpiration and to specific consumption of other physiological processes that took place during this period in the rosettes of leaves.

One noticed that the changes that were registered took place in a single direction - from baseline toward lower values. After 13 days, period of time during which the rosettes



were subjected to more than 6 hours on temperatures above 50°C, light intensity above 85000 lx and a total lack of water, the rosettes weight decreased from 103,5 g to 49,0 g for *Sempervivum tectorum* and from 55,5g down to 20,3g for *Sempervivum montanum*.

The weight losses are 54,5g for *Sempervivum tectorum* and 35.2g for *Sempervivum montanum*.

Analyzing the same table, Table 1, it reveals that weight loss of the rosettes from a weighing to the next are not uniform. The highest losses are recorded in the first 3- 4 days, but not equally during this period of time - they are decreasing from the first day towards the last day.

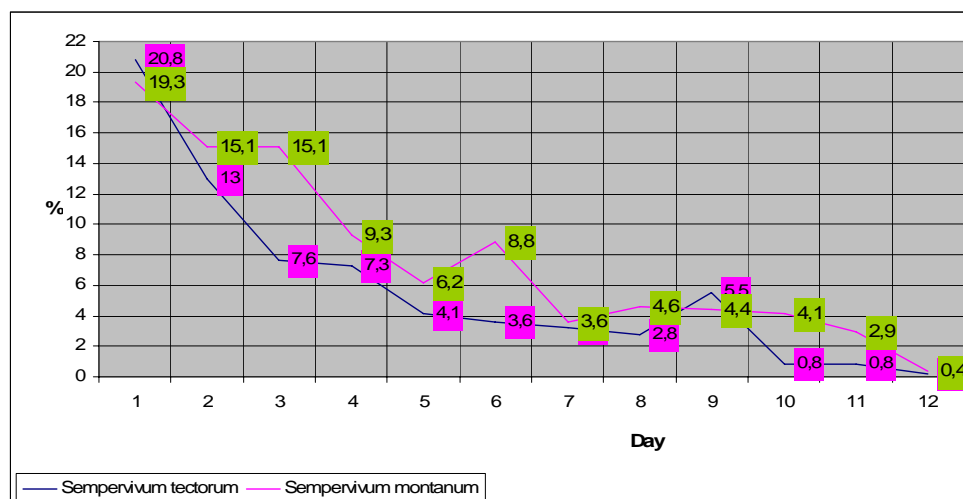
Furthermore, the data from the table highlight the fact that after 7 days for *Sempervivum montanum* rosettes and 10 days of the *Sempervivum tectorum* rosettes, weight loss stabilized at lower values and after another 3 - 4 days losses were very small.

For a more real perception of the changes on the weight of rosettes, weight loss from one weighing to another are presented are presented graphically and expressed as a percentage of fresh weight (graph 1.).

Analyzing the graphic diagrams of weight loss one observes that the weight loss of rosettes, the two species of *Sempervivum*, are very similar, have the same shape and mostly are pursuing each other, although the absolute values are very different.

The graphics are show very well how weight loss are reduced from one weighing to another, arriving after 7-8 days at very low values and after 10 - 11 days losses reach less than 0,2 - 0,4%.

After 12 days of rosettes' exposure in very difficult conditions, which remained weighed 49.0g and represented 47,3% of initial weight (103,5g) for *Sempervivum tectorum* and for *Sempervivum montanum* remaining fresh mass weighed 20,3g.k, equivalent of 36,5% of initial weight (55.5g).



Graph 1. Graphic presentation of weight loss (% of fresh weight) of *Sempervivum* rosettes exposed to water stress conditions, heat and sunlight (12 -24 august 2008)

The effect of the specific mechanisms of *Sempervivum* plants in terms of consumption and water management in critical situations made evident that water loss did

not occur in the same proportion in all rosettes' leaves. From the observations regarding the aspect of the rosettes one can say that the stress they were exposed to lead to different manifestations depending on age and position of leaves within the rosette.

This observation was supported by the fact that the first signs of distress on the water shortages were observed in the older leaf, positioned just to the bottom of the rosettes. The appearance of fading of leaf started at the edge and continued to its base. Step by step, each leaf followed and so on until phenomenon of wilting and drying leaves completely ceased (7-10 days after exposure to critical conditions).

Analyzing rosettes' leaves that have dried, these ones corresponded to those situated in horizontal position or with the tip bended to the ground.

In the absence of analysis that provide information about the existence in the leaves of various substances and the changes that occurred with these rosettes due to the exposure to critical conditions, explaining the phenomenon has been reported for some similar research and determinations, but for other plants of other researchers. Thus Burzo et al., 2005 affirms that water stress causes the disruption of physiological processes and biochemical repercussions of herbaceous plants at the cellular level and whose amplitude varies depending on the level of stress and species.

Among the consequences of water scarcity one enumerates the increasing fluid potential of plants and the reduction of water transport in growth areas (Stephenson 1994, Bradford and Msiao 1982).

The researches carried out by Escobar Gutierrez et al., 1998, quoted by Burzo et al., 2005, show that in severe water stress conditions, the relative water content of peach leaves decreased from 74% to 67%.

The same researchers mention that water stress induces and lead to the increase of the acid abscisic content, with inhibitory role of biochemical processes.

Given the above statements, one can assume that these phenomena also appeared in the *Sempervivum* experiences. Also the explanation for the reaction of the *Sempervivum* rosettes which lose less water, although stress conditions were maintained at the same critical level, is based on the fact that in such conditions, plants have a unanimous response, - the stoma close, process which helps to reduce the quantities of water lost through transpiration and photosynthesis.

In the situation where one would want to determine the hydric potential of leaf, the results of Lasko, 1985, who established that the stoma closure occurs when its value reaches -12 to -16 bars and in some conditions down to -25 bars.

The lack of smoothness analysis does not inhibit one to conclude that *Sempervivum* rosettes which reached the critical conditions of water shortage, accompanied by very high temperatures ( $> 40^{\circ}\text{C}$ ) and strong sunlight succeed in a short time (4 - 5 days) to reduce water loss at up to 0.2 - 0.3% of fresh weight, thereby retaining the rosettes' viable state.

Also loss of water in the early days and which are in large quantities which can reach up to 45-55% of the rosettes' weight, took place the basal leaves, which dry, while the leaves located in the apical area do not feel the effect of water stress, remain turgid and fully functional.

It is to be noted that rosettes exposed to profound drought conditions and high temperatures, at the end of the experiment have been trimmed (dried leaves were removed and the section was removed from the stem) and brought in rooting conditions where they behaved like those that were not subject to stress.

Drought situations, accompanied by hot temperatures and sun exposure may occur in different periods of summer and surprise the *Sempervivum* plants in various stages of development and vegetation phases.

One can say that in very difficult conditions, causing water stress, heat and strong sunlight, *Sempervivum* plants (rosettes), regardless of size (age) behaves similarly and have specific mechanisms to provide them protection against water loss which could cause dehydration (wilting) and plant death.

Water losses occur as soon as plants are exposed to stress, but for 3-4 days, water loss is blocked out of and plant viability is assured for a long time.

In this context the rosettes, both mature and daughters, exposed to this experiment were trimmed (removed dried parts) and were planted, providing them the conditions for rooting.

All rosettes, both mature and the daughters, had rooted and were further developed like those that were not subject to stress.

Water stress can be caused both by lack of water and it's excess.

If *Sempervivum* plants proved to be highly resistant to stress caused by lack of water, the same cannot be said about excess moisture resistance.

With regard to this matter one organized experiences which revealed that excess moisture causes the biodegradation of the whole plant in a very short time, slightly different depending on species and age of rosettes. The results of this test are shown in Table 2.

Table 2

Experimental results regarding the effects of the excessive humidity on  
*Sempervivum* rosettes

Variant	Large size – Ø> 5,0cm				Medium size 4.9 < Ø <3.0cm				Small size Ø <2.9cm			
	Excessive humidity (h)	Rosettes			Excessive humidity (h)	Rosettes			Excessive humidity (h)	Rosettes		
		Initial No.	Biodegradation			Initial No.	Biodegradation			Initial No.	Biodegradation	
			No.	%			No.	%			No.	%
Sempervivum tectorum	84	18	17	94,4	72	12	11	91,6	68	20	19	95,0
Sempervivum montanum	90	8	7	87,5	78	22	21	95,4	68	20	19	95,0

For organizing the experiences one used 50 rosettes of each species. As concerning to the size of the rosettes these ones were differentiated into large, medium and small size rosettes.

Pentru realizarea excesului de umiditate s-a folosit apă de ploaie, pentru a elimina probabilitatea și a altor factori care ar putea să se găsească în apa de robinet sau din alte surse.

The excess of humidity was actually flooding so that the rosettes and for the small ones even a part of the rosette were in the water.

Such conditions are specific to the lack of oxygen which adversely affects the roots' metabolism.

In such circumstances Burzo, 2005 claims that the synthesis of new proteins takes place, representing a class of enzymes involved in anaerobic biodegradation of carbohydrates by alcoholic means.

As a result – the appearance of acetic aldehyde and alcohol, which at one level become toxic to cells. Such a situation is possible in the case of *Sempervivum* plants.

What is certain and it was noticed after 60-70 hours from the beginning of the conditions of excessive humidity, were the specific aspects of these cases. Rosette leaves have changed their color into yellow or gray shades, and lost their stiffness becoming soft, without structure, and by pressing they were losing their shape. Biodegradation process started at the base of their leaves and fast forwarded toward the apical area and it affects all of rosettes' leaves.

Most sensitive proved to be the small rosettes, which bended due to the harmful effect of excess moisture, after 65-70 hours. Every 10 to 12 hours from the moment when it was established the loss of small rosettes, one observed the same phenomenon for the medium rosettes, followed by the larger ones.

One cannot affirm that the species would somehow influence the excess moisture resistance. In that regard, it should be noted that once harmfulness excess moisture symptoms appeared the affected rosettes cannot be saved. The phenomenon is irreversible and affects the whole rosette simultaneously. From this point of view, excessive humidity is the most worrying factor causing plant death within a very short time.

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**POPULATION DYNAMICS AND INCIDENCE IN PEST THRIPS SAMPLES  
(FRANKLINIELLA OCCIDENTALIS PERGANDE, HELIOTHIRIPS  
HAEMORRHODALIS BOUCHÉ) FROM THE TOMATOES GREENHOUSES,  
THE FIRST CYCLE OF CROP**

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**KEY WORDS:** *Frankliniella occidentalis* Pergande, *Heliothrips haemorrhoidalis* Bouché, population dynamics, samples incidence, protected spaces

**ABSTRACT**

From investigations made in the year 2008 on *Frankliniella occidentalis* Pergande adults it observed a significant and constant presence of californian thrips in samples. The average of 18.66 adults sample emphasized the pest importance thanks to its incidence during all the period of observation. In the tomatoes greenhouses cycle of crop, the experimental year 2008, it observed that the populations dynamics of adults *Frankliniella occidentalis* Pergande presented a maximum of examples in 13.06.2008 and a minimum of examples in 13.05.2008.

*Heliothrips haemorrhoidalis* Bouché number of thrips was relative low in comparison with *Frankliniella occidentalis* Pergande number of thrips. From the amount species of collected thrips in the year 2008 from tomatoes crop, the californian thrips was in 95.03% percentage, while thrips of greenhouse plants was in 4.96% percentage.

**INTRODUCTION**

International changes in floriculture domain, truck farming and agriculture constitute the cause of introduction a lot of pests in the crops. In ornamental vegetables and plants crop from the protected spaces, the pests are very dangerous because they easily spread, and could cover in a short time big surfaces from a greenhouse, producing great damages. The damages produced by thrips in protected spaces are frequently, the crop being or qualitative and commercial depreciated or could be totally compromised. *Frankliniella occidentalis* Pergande thrips was considered a relative pest recently identified in Romania greenhouses, being signaled for the first time at Isalnita, Dolj district, by Vasiliu Oromulu Liliana in the year 1993. Greenhouses plants thrips (*Heliothrips haemorrhoidalis* Bouché), observed the first time by Bouché (1883) in Europe, was introduced from tropical America (USA). The insect was broadcast 1951, Manoliu and colab., 1993). In Romania only in greenhouses on different plants leaves (Knechtel, 1951).

Investigations concerning in that domain were made in abroad by Higgins and Myers (1992) in S.U.A; in Netherlands by Vierbergen (1995); in Canada by Pearsall (1998), Pearsall and Myers (2000, 2001); Bielza et al. (2007) in Spain; Buitenhuis and Shipp (2008), Orosz et al. (2009) in Hungary; Raymond (2009), Brunner and Frey (2010) in SUA.

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In Romania, were made investigations in that domain by Knechtel (1951), Vasiliu-Oromulu (2001), Muntenașu (2006).

### MATERIALS AND METHODS

The entomological material which constituted that object of study was collected in the first cycle of the year 2008, at Agricultural Society Agro-Dor, from Dorobanti locality, Arad district. The samples were collected from a number of 3 repetitions, each one with a number of 10 plants. Every row (repetition) had a length of 3m, the distance between repetitions was 80 cm and between plant row was 30cm. The samples collecting from tomatoes crop was made during two months, beginning with 13<sup>ten</sup> May and ending with 27 Juin, with a collecting periodicity of 3-4 days. The insects collecting was made in the morning between 8-9 when the temperature was situated between 18-24 °C and the humidity 64-73%. The entomological material was collected in carrier bags through plant organs jarring which presented the samples to analyse. The insects were afterwards treated with acetic ethil, acetone, chloroform, etc., after that were transported in the Entomology Labor of Agricultural Faculty of Timisoara city for choosing, preserving and determination them. In the speciality literature from our country and abroad were presented a series of papers which represented the work methodology which could be applied in the investigations case concerning the dynamics populations and the samples incidence of thysanoptera on vegetables crop from the protected spaces: Vasiliu-Oromulu, 2002, 2004; Stanley (1957); Pearsal and Myers (2000, 2001).

### RESULTS AND DISCUSSIONS

From the effected investigations from the year 2008 on *Frankliniella occidentalis* Pergande adults it observed a constant presence of californian thrips in samples (table 1).

Table 1  
*Frankliniella occidentalis* Pergande adults collected from the tomatoes crop, the first cycle of crop, at Agricultural Society Agro-Dor., Dorobanti, 2008

Collecting date	<i>Frankliniella occidentalis</i> samples number - adults-			Total number of samples	Average
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>		
13.05.2008	10	8	10	28	9.33
16.05.2008	9	8	13	30	10.00
19.05.2008	11	9	9	29	9.66
22.05.2008	17	12	14	43	14.33
26.05.2008	17	16	18	51	17.00
30.05.2008	18	19	16	53	17.66
02.06.2008	21	24	20	65	21.66
06.06.2008	26	25	19	70	23.33
10.06.2008	33	33	21	87	29.00
13.06.2008	39	34	29	102	34.00
17.06.2008	34	32	27	93	31.00
20.06.2008	29	21	9	59	19.66
24.06.2008	17	19	9	45	15.00
27.06.2008	13	12	4	29	9.66
Average = 18.66667					

Thus, during the period 13.05.2008, after that it was registered an obviously increasing in 13.06.2008, when were collected in average 34.00 adults. Until the end of period it observed a gradual decreasing of samples adults incidents. The average of 18.66 adults/sample was emphasized the pest importance thanks to its incidence during all the observation period.

Table 2  
*Frankliniella occidentalis* Pergande larva collected from the tomatoes crop, the first cycle of crop at Agricultural Society Agro-Dor., Dorobanti, 2008

Collecting date	<i>Frankliniella occidentalis</i> samples number - larva-			Total number of samples	Average
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>		
13.05.2008	6	4	6	16	5.33
16.05.2008	8	9	5	22	7.33
19.05.2008	12	9	7	28	9.33
22.05.2008	11	10	8	29	9.66
26.05.2008	16	13	13	42	14.00
30.05.2008	18	12	16	46	15.33
02.06.2008	20	14	19	53	17.66
06. 06.2008	22	12	21	55	18.33
10. 06.2008	25	18	23	66	22.00
13. 06.2008	29	28	27	84	28.00
17. 06.2008	24	22	23	69	23.00
20. 06.2008	18	14	19	51	17.00
24. 06.2008	11	9	15	35	11.66
27. 06.2008	5	3	6	14	4.66
Average = 14,52381					

The first larva appearance was signaled for the first time in 13.05.2008 with a average of 5.33 species sample. From the date of 13<sup>rd</sup> May to 13 Juin it observed a larva number increasing from an average of 5.33 species sample to 28.00 species sample.

From table 2 we saw that in 13.06.2008 the collected larva number was maximum that means an average of 28.00 species/sample. From that date the collected larva number began to decrease, being in last day of collecting, 27 Juin, at an average of 4.66 species/sample. After examining table 3 it resulted that the number of *Heliothrips haemorrhoidalis* Bouché species was relative low.

Table 3  
*Heliothrips haemorrhoidalis* Bouché adults collected from the experimental field from Agricultural Society Agro-Dor., Dorobanti, 2008

May		Juin		
decade II	decade III	decade I	decade II	decade III
5	7	9	13	7

In the second decade of May month, its number was about 5 samples, after that its number will begin to increase, being in third decade of May month 7 species, in the first decade of Juin month about 9, continuing to increase being in the second decade of Juin month about 13 species, that increasing of its number being followed by a decreasing until the end of Juin about 7 species.

In the tomatoes greenhouses the first cycle of crop, the experimental year 2008, it observed that the populations dynamics of *Frankliniella occidentalis* Pergande adults

presented a maximum of samples in 13.06.2008 and a minimum of samples in 13.05.2008. (figure 1).

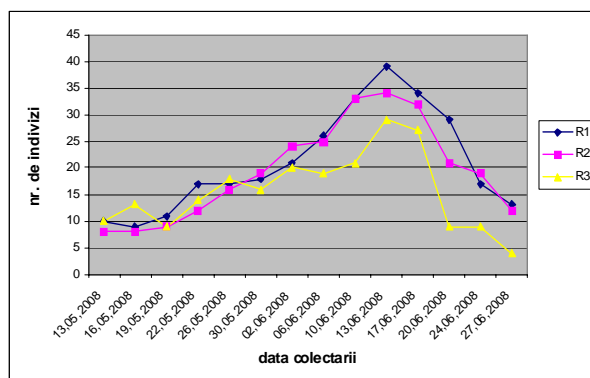


Figure 1. Populations dynamics of adults collected in the tomatoes greenhouse, the first cycle of crop, from Dorobanti locality, 2008

From 13.05.2008 it was emphasized a continu increasing of collected insects number to 13.06.2008 its number increasing 9.33 species/sample to 34.00 species/sample in average. From 13 Juin the thrips number decreased to 27 Juin from an average of 34.00 specimens/sample to 9.66 copies/sample. In function of populations dynamics the best moment to apply the treatments was situated during 10.06.2008-13.06.2008.

In case of the collected larva its number increasing from the second decade of May month to the second decade of Juin month, it was determinated a populations decreasing beginning with the third decade of Juin month, touching minimum of values at the end of the observations period (figure 2).

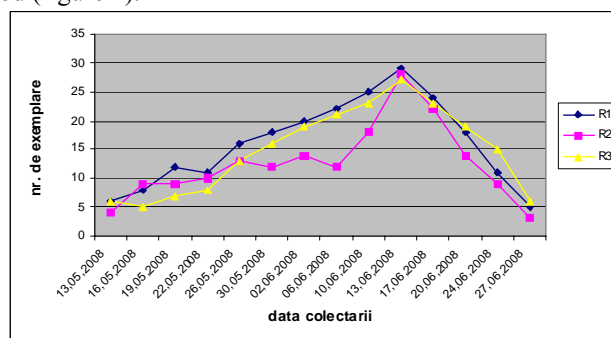


Figure 2. Populations dynamics of larva (*Frankliniella occidentalis* Pergande) collected in the tomatoes greenhouse, the first cycle of crop, from Dorobanti locality, 2008

In function of larva population dynamics the best moment to apply the treatments was situated like that in the adults case during the period 10.06.2008-13.06.2008.

*Heliothrips haemorrhoidalis* Bouché species was met in the tomatoes crop, in the year 2008 in a small number comparative with *Frankliniella occidentalis* Pergande species.

On May month of the year 2008 the number of thrips species of greenhouse plants was about 12 very reduced in comparison with the Californian thrips which had a number of 234 samples collected in two decades of May month.



On Juin month, in all of three decades the number of *Frankliniella occidentalis* Pergande species was very big in comparison with *Heliothrips haemorrhoidalis* Bouché species collected.

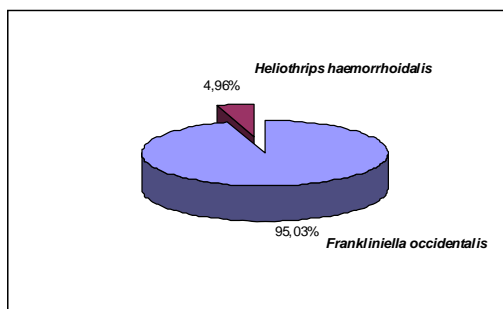


Figure 3. The report among the thrips species collected from the tomatoes greenhouses, the first cycle of crop in May-Juin months 2008 from Agricultural Society Agro-Dor., Dorobanti, represented in percentages

From the total of thrips species collected in the year 2008 from the tomatoes crop, the californian thrips was found in 95.03% percentage, while greenhouse plants thrips in 4.96% (figure 3) percentage.

### CONCLUSIONS

After investigations made in 2008 in the tomatoes crop, first cycle on *Frankliniella occidentalis* Pergande adults it determined a significant and constant presence of californian thrips in samples. In the period 13.05.2008-27.06.2008 the most reduced number of adults from the tomatoes crop was in average registered in 13.05.2008, and the biggest number of species was collected in 13.06.2008.

In tomatoes crop, the first cycle 2008, the larva number increased beginning with the second decade of May month to the first decade of Juin month, after that it decreased, and in the third decade of Juin month registered the smallest number of collected species.

In case of *Heliothrips haemorrhoidalis* Bouché species from the tomatoes crop, the first cycle 2008, the smallest number and the biggest number of samples registered in the second decade of Juin month.

In the tomatoes greenhouses, the first cycle of crop, experimental year 2008, it observed that the populations dynamics of *Frankliniella occidentalis* Pergande adults presented a maximum of samples in 13.06.2008 and a minimum of samples in 13.05.2008. In function of adults populations dynamics from the tomatoes crop, first cycle 2008, the best moment of treatment application was situated during the period 10.06.2008-13.06.2008.

In case of larva collected after an increasing of its number from the second decade of May month to the second decade of Juin month it observed an increasing of populations, beginning with the third decade of Juin month touching minimum of values to the end of observations period.

From the total of collected thrips in the year 2008 from the tomatoes crop, first cycle, the californian thrips was found in 95.03% percentage, while greenhouse plants thrips only in 4.96% percentage.

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THE CALIFORNIAN THRIPS CONTROLLING FROM CUCUMBER CROP  
USING BLUE STICKY TRAPS

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**KEY WORDS:** *Frankliniella occidentalis* Pergante, protected spaces, monitorization, physico-mechanical, coloured traps

**ABSTRACT**

In cucumbers crop, the collected species number varied not a little, both on reading and on trap; thus the biggest number of collected species/trap was on the second trap at the first reading, and the smallest number of collected species was at the third reading on the first trap.

The biggest average number of species/cm<sup>2</sup> registered at the third reading (63.05 species/cm<sup>2</sup>), and the smallest number of the collected species was (56.37 species/cm<sup>2</sup>) at the first reading. The total number of the collected species varied between 36 404 species/trap and 70 072 species/trap. Species number/cm<sup>2</sup> on the blue traps was 59.32 species/cm<sup>2</sup> in average.

**INTRODUCTION**

The physico-mechanical measures presented the advantage that didn't ask for cheap controlling measures. In the weather conditions of the greenhouses the preserving and accumulation in the soil of a lot of pests was inevitably.

A measure used in thysanoptera pests monitorization and controlling from the cucumbers crop from the protected spaces was the physico-mechanical measure through the adhesive traps usage of blue colour. In finding early the thrips were used those sticky plates of light blue, which could be the most attractive and specific for those insects.

The thrips identification species directly from the plates was very hard. Through the species which could be identified in that way was *Frankliniella occidentalis* Pergande. The other ones species identification was possible only through microscopical exam. The insects unsticking from the plates was realized with substances like the alcohol, diclometan, ethil acetate, chloroform, etc.

Investigations of that domain were effected abroad by: Chu Chang-Chi et al., 2000; Roditakis et al., 2001; Chen Tian-Ye et al., 2004; Natwick et al., 2007; Davidson et al., 2009; Raymond, 2009 in U.S.A., Al-Ayed and Al-Doghairi (2004) in Saudi Arabia, Andjus and Trdan (2005) in Serbia, De Villiers and Pringle, (2007) in South Africa, Milevoj et al., (2007) in Slovenia, Akadémiai, (2008) in Israel, Pizzol et al. (2010) in France, etc.

In Romania, investigations in that domain were made by: Maier and al., 1961; Raicu et al., 1979; Raicu and Mihailescu, 1982; Baicu and Săvescu, 1986, etc.

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## MATERIAL AND METHOD

The adhesive traps of blue colour were placed in the cucumbers greenhouses at Agricultural Society Agro-Dor., from Dorobanti locality, Arad district at the distance of 25 cm face to plant, 75 cm distance among plants, it were placed two traps at 100 ml. Investigations to control the pest thysanoptera controlling in the protected spaces with the help of blue traps, were realized during the period 10<sup>th</sup> Juin-01<sup>st</sup> July 2008. For pests monitorization were used the following materials: the adhesive traps of blue colour (figure 1) of Csalomon type, 10 db SZINb, MTA, Budapest etc.



Figure 1. Blue trap of type Csalomon, 10 db SZINb used for pest thysanoptera controlling in the protected spaces

The traps were replaced with the others new at every seven days. After traps replacement these were transported in a labor to number and select the insects but also to interpret the data.

The insect selection realized through dividing every trap in a rectangle with four cadres. Every trap was a rectangle with the length of 19 cm and latitude of 16 cm, the surface of that type of rectangle was 304 cm<sup>2</sup>. In every cadre delimited a surface of 1 cm<sup>2</sup>, surface unmethodical (random) on which it were numbered the thrips caught on the sticky surface. The insects numbering from the delimited surface realized by a help.

## RESULTS AND DISCUSSIONS

In the cucumbers crop, with the help of blue traps, at the 1<sup>st</sup> reading, the species number monitorized/cm<sup>2</sup> oscillated from an average of 26.25 species/ cm<sup>2</sup> to an average of 95.5 species/ cm<sup>2</sup> (table 1).

Table 1

*Frankliniella occidentalis* Pergande species monitorization from the cucumbers crop with the help of blue sticky traps, 1<sup>st</sup> reading, 17.06.2008

Trap no. 1		Trap no. 2		Trap no. 3		Trap no. 4	
no. species/cm <sup>2</sup>		no. species/cm <sup>2</sup>		no. species/cm <sup>2</sup>		no. species/cm <sup>2</sup>	
R <sub>1</sub>	37	R <sub>1</sub>	35	R <sub>1</sub>	73	R <sub>1</sub>	92
R <sub>2</sub>	29	R <sub>2</sub>	106	R <sub>2</sub>	67	R <sub>2</sub>	79
R <sub>3</sub>	17	R <sub>3</sub>	34	R <sub>3</sub>	21	R <sub>3</sub>	107
R <sub>4</sub>	22	R <sub>4</sub>	28	R <sub>4</sub>	51	R <sub>4</sub>	104
Total	105	Total	203	Total	212	Total	382
Average	26,25	Average	50,75	Average	53,0	Average	95,5

From figure 2 we could observe the thrips californian number of species/cm<sup>2</sup> captured with the help of blue sticky traps at the 1<sup>st</sup> reading from the cucumbers crop of Dorobanti vegetables greenhouses.



Figure 2. *Frankliniella occidentalis* Pergande species number/cm<sup>2</sup>, blue traps, cucumbers, 1<sup>st</sup> reading, Dorobanți, 2008

The second reading made in 24 Jun 2008 at the seven days face to the first reading, moment in which it were replaced both the sticky traps of blue colour.

Table 2  
*Frankliniella occidentalis* Pergande species monitorization from the cucumbers crop with the help of blue sticky traps, the 2<sup>nd</sup> reading, 24.06.2008

Trap no. 1		Trap no. 2		Trap no. 3		Trap no. 4	
no. species/cm <sup>2</sup>				no. species/cm <sup>2</sup>			
R <sub>1</sub>	61	R <sub>1</sub>	52	R <sub>1</sub>	23	R <sub>1</sub>	77
R <sub>2</sub>	73	R <sub>2</sub>	76	R <sub>2</sub>	45	R <sub>2</sub>	103
R <sub>3</sub>	56	R <sub>3</sub>	46	R <sub>3</sub>	40	R <sub>3</sub>	111
R <sub>4</sub>	32	R <sub>4</sub>	33	R <sub>4</sub>	46	R <sub>4</sub>	63
Total	222	Total	207	Total	154	Total	354
Average	55.5	Average	51.75	Average	38.5	Average	88.5

At the second reading on blue traps the number of californian thrips/ cm<sup>2</sup> had between 154 species/ cm<sup>2</sup> on trap number 3 and 354 species/ cm<sup>2</sup> on trap number 4 that means from an average of 38.5 species/ cm<sup>2</sup> to an average of 88.5 species/ cm<sup>2</sup> (table 2).



Figure 3. *Frankliniella occidentalis* Pergande number species/cm<sup>2</sup>, blue traps, cucumbers, the 2<sup>nd</sup> reading, Dorobanți, 2008

In the fig. 3 was presented the species number of californian thrips/  $\text{cm}^2$ , at the 2<sup>nd</sup> reading, captured through mechanical methods of controlling like blue sticky plates. The biggest number of captured species registered on the 4<sup>th</sup> and the smallest number of species/  $\text{cm}^2$  found on the 3<sup>rd</sup> trap.

The last reading made in 1<sup>st</sup> Juin 2008, also at an interval of a week face to the second reading.

Table 3

*Frankliniella occidentalis* Pergande species monitorization from the cucumbers crop with the help of blue sticky traps at 3<sup>rd</sup> reading, 01.07.2008

Trap no. 1		Trap no. 2		Trap no. 3		Trap no. 4	
no. species/cm <sup>2</sup>				no. species/cm <sup>2</sup>			
R <sub>1</sub>	137	R <sub>1</sub>	50	R <sub>1</sub>	42	R <sub>1</sub>	64
R <sub>2</sub>	93	R <sub>2</sub>	64	R <sub>2</sub>	36	R <sub>2</sub>	54
R <sub>3</sub>	54	R <sub>3</sub>	136	R <sub>3</sub>	15	R <sub>3</sub>	40
R <sub>4</sub>	61	R <sub>4</sub>	115	R <sub>4</sub>	20	R <sub>4</sub>	28
Total	345	Total	365	Total	113	Total	186
Average	86.25	Average	91.25	Average	28.25	Average	46.5

At the third reading on blue traps from the cucumbers crop, the average number of collected species on the first trap was 86.25 species/  $\text{cm}^2$ , on the second trap were collected 91.25 species/  $\text{cm}^2$ , on the third trap were registered a number of 28.25 species/  $\text{cm}^2$  and on the forth trap were collected a number of 465 species/  $\text{cm}^2$  (table 3).

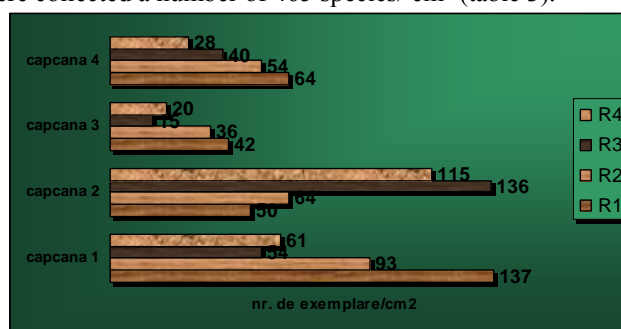


Figure 4. *Frankliniella occidentalis* Pergande species number/ $\text{cm}^2$ , blue traps, cucumbers, the 3<sup>rd</sup> reading, Dorobanți, 2008

From figure 4 it could be observed that the species number/  $\text{cm}^2$  in the cucumbers crop 2008 at the third reading varied between 15 species on traps no. 3 and 136 species registered on trap no.2.

Table 4

*Frankliniella occidentalis* Pergande species monitorization from the cucumbers crop with the help of blue sticky trap, 2008

Traps	no. species/ $\text{cm}^2$		
	1 <sup>st</sup> reading	2 <sup>nd</sup> reading	3 <sup>rd</sup> reading
Trap no. 1	26,25	55,5	86,25
Trap no. 2	50,75	51,75	91,25
Trap no. 3	53,0	38,5	28,25
Trap no. 4	95,5	88,5	46,5
Average	56,37	58,56	63,05

From table 4 it observed that the biggest average number of species/ cm<sup>2</sup> registered at the third reading (63.05 species/ cm<sup>2</sup>), and the smallest collected species was at the first reading (56.37 species/ cm<sup>2</sup>).

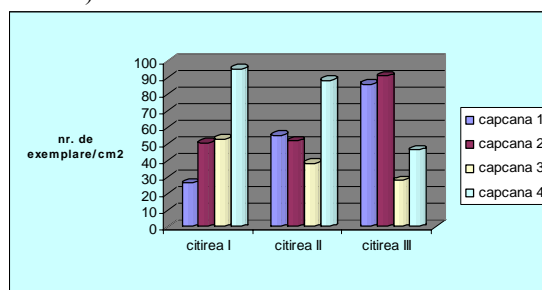


Figure 5. The variation of *Frankliniella occidentalis* Pergande species number at the all three readings, cucumbers, blue traps, 2008

From figure 5 it observed that the biggest number of the reading, and the smallest number of collected species was collected on trap no.1 at the first reading.

Table 5

*Frankliniella occidentalis* Pergande species number on a trap surface of blue colour to all the three readings, 2008

Traps	Readings			Total species./trap
	I	II	III	
Trap no. 1	7 980	16 872	26 220	51 072
Trap no. 2	15 428	15 732	27 740	58 900
Trap no. 3	16 112	11 704	8 588	36 404
Trap no. 4	29 032	26 904	14 136	70 072

From table 5 it was determinate that the total number of collected species varied between 36 404 species/trap and 70 072 species/trap.

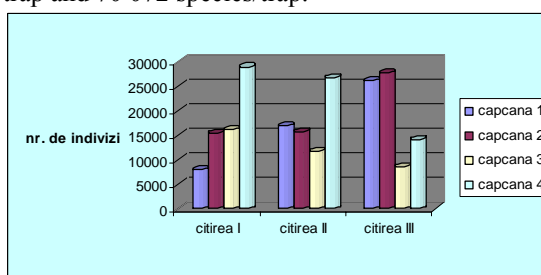


Figure 6. Total number of *Frankliniella occidentalis* Pergande species number collected on the surface of a blue trap, cucumbers, 2008

In the cucumbers crop of 2008, the collected number of species varied not a little, both on reading and on traps; so the biggest number of collected species/trap was at the third reading on trap no.2, and the smallest number of collected species at the first reading was on trap no. 1 (figure 6).

## CONCLUSIONS

In the cucumbers crop, with the help of blue traps, at the first reading, the number of monitored species/ cm<sup>2</sup> oscillated from an average of 26.25 species/ cm<sup>2</sup> to an average of 95.5 species/ cm<sup>2</sup>.

At the second reading on blue traps the number of Californian thrips species/ cm<sup>2</sup> was between 154 species/ cm<sup>2</sup> on trap no. 3 and 354 species/ cm<sup>2</sup> on trap no. 4.

At the third reading on blue traps from the cucumbers crop, the average number of collected species on the trap no. 1 was 86.25 species/ cm<sup>2</sup> and on the trap no. 3 registered a number of 28.25 species/ cm<sup>2</sup>.

The biggest average number of species/ cm<sup>2</sup> in the cucumbers crop, on blue traps, registered at the third reading (63.05 species/ cm<sup>2</sup>), and the smallest number of collected species at the first reading was 56.37 species/ cm<sup>2</sup>.

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