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FAUNISTIC DATA ON THE OPILIONES (ARACHNIDA) IN CRAIOVA AND PERI-URBAN AREAS

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Key words*: Opiliones, biodiversity, urban location*

ABSTRACT

This paper presents the results of the faunistic investigation on Opiliones in six parks of Craiova and peri-urban areas. The systematical list includes five species: Carinostoma elegans (Soerensen, 1894); Trogulus tricarinatus (Linnaeus, 1767); Phalangium opilio Linnaeus, 1761; Zacheus crista (Brullé, 1832) and Egaenus convexus (C.L. Koch, 1835).

INTRODUCTION

The Opilionids represent an important part of the biological diversity. They are to be found in various undisturbed and disturbed habitats and biocoenosis.

Craiova is an urban location in the S-W part of Romania, on the left bank of the Jiu River. It is situated on the border between the steppe and forrest-steppe regions.

In the urban and peri-urban region of Craiova there are more than 14 parks and gardens of different areas and different types of habitats, e.g. "Nicolae Romanescu" Park, "Tineretului" Park ("Lunca Jiului" Park), "Cornițoiu" Park, "Alexandru Buia" Botanical Garden, "Craiovița-Nouă" Park, "Hanul Doctorului" Park, "Lacul Tanchiștilor" area, "Sf. Dumitru" Park, "Mihai Bravu" Garden, the Central Garden, "A.I Cuza" Garden, the National Theatre Park, "Frații Buzești" Garden, "1 Mai" Garden (Ciobotea et al. 1999).

"Nicolae Romanescu" Park, with a surface of about 96 ha, is situated at the southern limit of Craiova. Its existence as a park - Bibescu Park, dates back to 1844-1845. During the history of the city, the park had different destinations and underwent numerous modifications and arrangements. It has numerous alleys that cross a forest and a water surface of about 4 ha. The forest is part of the nemoral zone, with spontaneous vegetation represented by *Quercus robur*, *Q. cerris*, *Q. frainetto*. Alongside, many other species of trees and ornamental plants have been planted during time – *Celtis australis*, *Fraxinus excelsior*, *Acer negundo*, *A. platanoides*, *A. pseudoplatanus*, *Tilia tomentosa*, *T. platyphyllos*, *Sophora japonica*, *Taxodium distichum*. The water surface consists of a chain of small lakes supplied by underground waters. Nowadays, the park is a main point of recreation, being subjected to numerous planning works.

"Tineretului" Park ("Youth" Park or "Lunca Jiului" Park), with a surface of nearly 50 ha, is located at the western limit of the city, on the left bank of the Jiu River. It is less modernized than "Nicolae Romanescu" Park. The spontaneous vegetation is represented mainly by *Quercus robur*, *Fraxinus angustifolia*, *Acer tataricum*, *A.*

campestre and *Ulmus glabra*. Few specimens of *Quercus rubra* and *Acer palmatum* have been relatively recently planted. The park is crossed by the Șerca Rivulet.

"Cornițoiu" Park is a small, abandoned recreation park situated in the north-west part of the city, between two residential districts. Its vegetation consists only of cultivated trees, mainly *Acer negundo* and *Fraxinus excelsior*.

"Alexandru Buia" Botanical Garden, with a surface of around 17 ha, is located in the south-west part of Craiova, being surrounded by residential districts. The garden is divided in many sectors comprising a diversity of rather cultivated species.

"Craiovița-Nouă" Park is a relatively large area, of about 50 ha, situated in the north-west part of the city. It was created during the communist period, on the course of the Cornițoiu Rivulet as a recreation zone, with a lake, numerous alleys and many cultivated species of trees and bushes. Nowadays the park is abandoned and highly deteriorated by the anthropogenic factor – grazing, industrial and domestic waste.

"Hanul Doctorului" Park is located on the upper terraces of the Jiu from the N and E area of Craiova, in the vicinity of the airport and Banu-Maracine research station, along the European road DE 70, which continues Calea Bucureşti Street. It was arranged as a recreational park during the communist period along the Valea Hanul Doctorului Creek. The park includes a rich natural and ornamental (planted) arboreal and bush vegetation, alleys and a three small water bodies. The park has now lost its recreational destination, being abandoned and damaged.

"Lacul Tanchiștilor" is an abandoned land located in the northern part of Craiova, at the exit towards Melinești. It has two small lakes supplied with water from CET (the thermal electric power plant). The main lake is surrounded by grassy vegetation characteristic to the steppe, ruderalized, plus isolated specimens of Salix and Populus, as well as a planted forest of *Pinus nigra*.

The purpose of this paper is to inform the public about the opilionid species identified in some of the parks of Craiova and its surroundings.

MATERIAL AND METHODS

The opilionids were sampled between 2008 and 2016, occasionally or on purpose in 6 parks of Craiova: "Nicolae Romanescu" Park, "Tineretului Park" (The Youth Park), the Botanical Garden (the Barber pitfall traps were set in an orchard of apple trees), "Cornițoiu" Park, "Hanul Doctorului" Park and "Lacul Tanchistului" area.

Most of the opilionids were collected by author directly by hand or by the use of the Barber pitfalls and Kraatz-Reiter-Weise strain and preserved in 75 % ethilic alcohol. The species identification was done according to Šilhavý (1956) and Martens (1978), using the habitus, the chelicera, the ocular tubercle, the armature of the pedipalps, the second leg for Trogulus and the genitals (penis, ovipositor and seminal receptacles). The photos were taken with a Sony cyber-shot camera.

RESULTS AND DISCUSSIONS

Results

The list of species systematically arranged according to Martens (1978):

Subord. Palpatores

Suprafam. Troguloidea

Fam. Nemastomatidae

1. *Carinostoma elegans*

"Nicolae Romanescu" Park: 21.04.2015 (6 ind.), 21.05.2015 (5 ind.), 13.06.2015 (3. ind.), 4.07.2015 (6 ind.)

Fam. Trogulidae

2. *Trogulus ticarinatus* – Fig. 1

"Nicolae Romanescu" Park: 21.11.2008 – 11.03.2009 (1 ind. - leg. Lavinia Bârlogeanu), 21.04.2015 (1 ind.), 21.05.2015 (1 larvae.), 13.06.2015 (3 ind.)

Suprafam. Phalangioidea

Fam. Phalangiidae

3. *Phalangium opilio*

"Nicolae Romanescu" Park: 2.07.2014 (2 ind.), 4.07.2015 (3 ind.); "Tineretului"Park: 28.05.2016 (3 larva), 2.06.2016 (4 immature), 2.06.-15.06.2016 (14 imm. – males and females), 15.06.-19.07.2016 (12 females); "Hanul Doctorului" Park: 2.06.-15.06.2016 (3 imm.), 15.06.-19.07.2016 (2 females)

4. *Zacheus crista* – Fig. 2

"Nicolae Romanescu" Park: 21.04.2015 (1 imm.), 21.05.2015 (2 imm.), 27.05.2015 (3 ind.), 13.06-4.07.2015 – no specimen found; "Tineretului" Park: 28.05.2016 (1 male), 2.06.-15.06.2016 (43 ind.), 10.06.-19.07.2016 (40 ind.); "Lacul Tanchistului" area: 15.06-19.07.2015 (5 ind.); "Hanul Doctorului" Park: 2.06.-15.06.2016 (23 ind.), 15.06.-19.07.2016 (7 ind.)

5. *Egaenus convexus* – Fig. 3

"Tineretului" Park: 2.06.-15.06.2016 (1 female)

No opilionid was found in the Botanical Garden (the experimental apple orchard), neither in "Cornițoiu" Park.

Discussions

In the six investigated parks five opilionid species belonging to five genera were recorded. The five species were present only in the large, outlying parks. In the small parks, situated between residential districts – the Botanical Garden and "Cornițoiu" Park, the opilionids were absent.

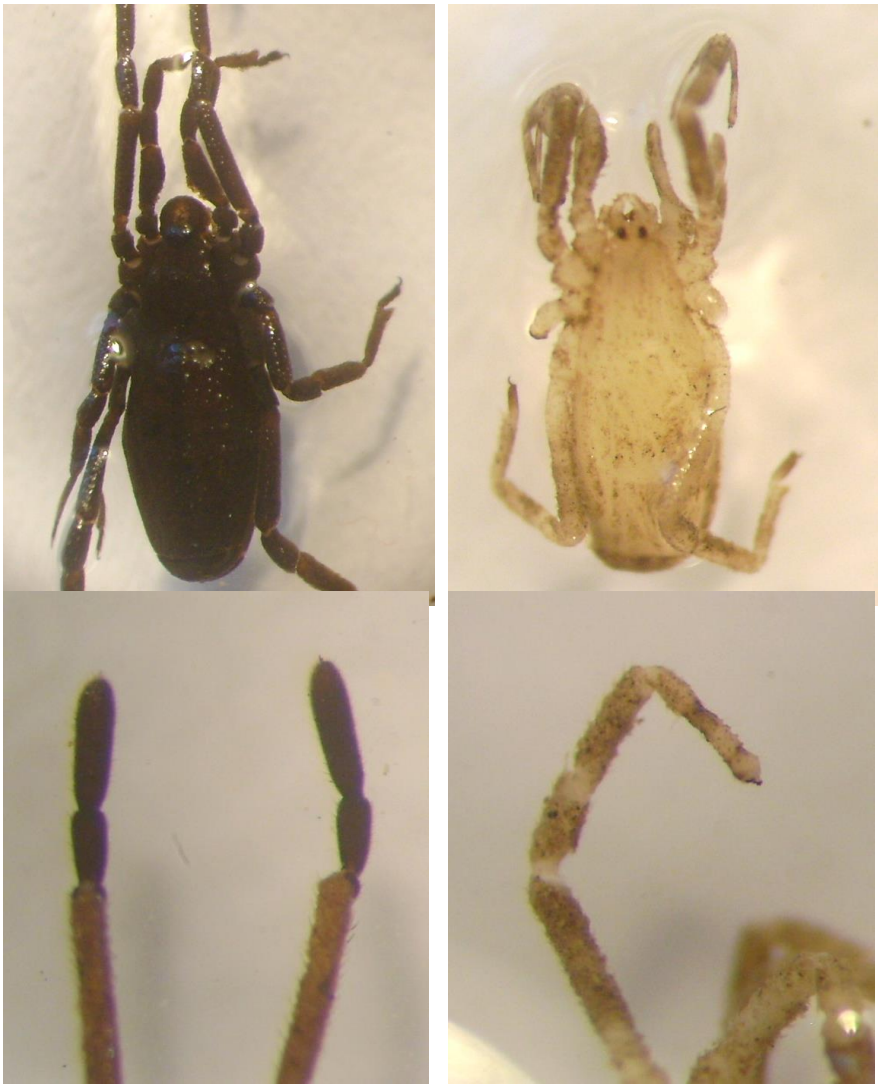
Out of the five collected species, *Zacheus crista* was present in all of the four outlying parks. In the *Pinus nigra* patch forest of "Lacul Tanchistului" area *Zacheus crista* was the only opilionid species, suggesting that it might be the only opilionid species able to occupy this type of habitat.

The species *Egaenus convexus* was found only in "Tineretului" Park, namely, only a female in the period June 6 – June 15, completely lacking in the next period June 15 – July 19.

The species *Egaenus convexus* (S-E European element) and *Zacheus crista* (Ponto-Mediterranean, eremial element) (Martens, 1978) are frequently found together in the leaf litter especially of the Quercus forests in different localities in the S-W part of Romania (Babalean, 2011). It was therefore expected to find the same species association *Egaenus convexus* – *Zacheus crista* also in the analyzed parks. The absence of *Egaenus convexus* in the large parks of Craiova can be correlated with some factors:

a) The setting of the Barber pitfall traps out of the species maximum level of activity. In other localities near the Jiu River (Bucovăț-Dolj) *Egaenus convexus* showed a maximum level of activity (the highest number of adults) between May 15 and June 15, thereafter the population decreases. The maximum level of activity of *Egaenus convexus* precedes the maximum level of *Zacheus crista* (Babalean et al., 2015; Babalean – unpublished data).

b) The species biological requirements: optimum temperature; humidity – the



females hygrotaxis during ovipositing (Weiss, 1985); luminosity, etc. are not naturally met in the investigated parks or are not met as a result of the human factor.

Figure 1 *Trogulus tricarinatus*: up, left – adult habitus, L=5 mm., dorsal view; up, right – larvae habitus, L=3 mm., dorsal view; down, left – adult Ta II; down, right – larval Ta II (samples: adult – 13.06.2015, "Nicolae Romanescu" Park; larvae – 21.05.2015, "Nicolae Romanescu" Park)

Figure 2 *Zacheus* crista: left – male habitus dorsal; middle – male habitus ventral, the genital operculum removed in order to see the penis; right – the penis in lateral view with the dorsal curvature of the glans (sample: “Nicolae Romanescu” Park, 27.05.2015)

Figure 3 *Egaenus convexus*: female habitus, dorsal view (sample: “Tineretului” Park, 2-15.06. 2016)

CONCLUSIONS

The results of this study reveal a low diversity of Opiliones in the parks of Craiova and surroundings.

In 2015 and 2016 the opilionids were absent in the small parks, located between the residential districts.

The present study should be continued as part of a larger project on the fauna of Opiliones and its biogeographical and ecological characteristics in different habitats of an anthropized zone.

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THE INFLUENCE OF CERTAIN PHYTOSANITARY TREATMENTS ON THE QUALITY INDICES IN THE CASE OF SOME VARIETIES OF VINES FROM VÂNJU MARE-OREVIŢA VITICULTURAL CENTER

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Key words*: phytosanitary treatment, quality indices, grapevine*

ABSTRACT

By applying certain phytosanitary treatments in conventional and unconventional systems on the two grapevine varieties, i.e. Fetească neagră and Cabernet Sauvignon, which are cultivated within Vânju Mare-Oreviţa viticultural center, it was possible to reveal their influence on some production quality indices, knowing the fact that the average weight of the grapes (of the clusters) represents both a productivity element, as well as a quality feature of the grapevine. Moreover, the sugar content and the acidity of the must were determined for each variety in correlation with the treatment type, as the two components are essential in ascertaining the grapevine quality.

INTRODUCTION

The protection of vineyards by using treatments meant to prevent and to combat diseases and pests represents an objective necessity within the framework of a modern viticulture that is competitive at national and international levels (Tomoiagă et al., 2006). The pesticides are used to stop the spreading of harmful organisms and to combat them, but, besides their capacity to fight the pests, they also led to some severe dysfunctions within the dynamic natural equilibrium and generate irreversible changes in the genotype of the useful organisms, including that of the human beings and of the cultivated plants (Voloşciuc, 2007).

Bucur, 2007 mentions that ecological agriculture might be considered a viable solution to the negative impact that traditional agriculture has on the environment and on the quality of production. In this framework, replacing the pesticides with natural mineral and organic substances represents an alternative to traditional agriculture.

Furthermore, as mentioned by Ţârdea and Dejeu, 1995, within the modern viticulture, the optimization of the viticultural ecosystem must follow the achievement of maximal production, quality and profit, with minimum cost and labour farce involvement, as well as the rational use of ecologic and economic resources and the protection of the habitat against pollution.

MATERIAL AND WORK METHOD

The research was conducted within Vânju Mare-Oreviţa viticultural centre, on two grapevine varieties: *Feteasca neagră*, cultivated on a surface of 7 ha and *Cabernet Sauvignon*, which covers a surface of 12 ha.

In order to keep under control the pathogens specific to grapevine, two treatment schemes were applied during 2016, one of them being conventional and the other unconventional, as showed in tables 1 and 2.

Tabel nr. 1

Conventional treatment scheme used in Vânju Mare-Oreviţa vineyard during the year 2016

No. of treat.	Phenological phase	Product used	Active substance (%)	Dose/ha	Pest	Observations
1	Appearance of clusters – first decade of May	Shavit F 72 WDG Nissorum10W P	Folpet 70%; Triadimenol 2% Hexithiazox 10%	2 kg 0.5 kg	Downy mildew Powdery mildew Mites	
2	Before flowering – third decade of May	Armetil M Falcon 460 EC Fastac 10 EC	Mancozeb 8%, Metalaxyl 64% Spiroxamine 250 g/l + Tebuconazole 167 g/l + Triadimenol 43 g/l Alpha-Cypermethrin 100g/l	2.5 kg 0.3 l 0.075 l	Downy mildew Powdery mildew Mites	
3	After flowering – second decade of June	Alleato 80 WG Folicur Solo 250EW	Aluminium Fosetyl 800 g/kg Tebuconazole 250 g/l	2 kg 0.4 l	Downy mildew Powdery mildew	
4	Growth of berries - third decade of June	Funguran OH 50 WP Cosavet 80 DF	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80%	2 kg 3 kg	Downy mildew Powdery mildew	On the 24th of June, 2016 the hail appears
5	Growth of berries – first decade of July	Armetil M Bumper 250 EC Novadim Progress	Mancozeb 8%, Metalaxyl 64% Propiconazole 250 g/l Dimethoate	2.5 kg 0.2 l 0.8 l	Downy mildew Powdery mildew Mites	
6	Compaction of grape clusters – second decade of July	Funguran OH 50 WP Kumulus 80 DF	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80%	2 kg 0.3 kg	Downy mildew Powdery mildew	
7	Start of fruit ripening – first decade of August	Funguran OH 50 WP Cosavet 80 DF Teldor 500 SC	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80% Fenhexamid 500g/l	2 kg 3 kg 1 l	Downy mildew Powdery mildew Botrytis Bunch Rot	

Tabel nr. 2

Unconventional treatment scheme used in Vânju Mare-Oreviţa vineyard during the year 2016

No. of treat.	Phenological phase	Product used	Active substance (%)	Dose/ha	Pest	Observations
1	Appearance of clusters – first decade of May	Funguran OH 50 WP Cosavet 80 DF Laser 240 SC	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80% Spinosad 240 g/l	2 kg 3 kg 0.15 l/ha	Downy mildew Powdery mildew Mites	
2	Before flowering – third decade of May	Funguran OH 50 WP Cosavet 80 DF Laser 240 SC	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80% Spinosad 240 g/l	2 kg 3 kg 0.15 l/ha	Downy mildew Powdery mildew Mites	
3	After flowering – second decade of June	Funguran OH 50 WP Cosavet 80 DF	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80%	2 kg 3 kg	Downy mildew Powdery mildew	
4	Growth of berries - third decade of June	Funguran OH 50 WP Cosavet 80 DF	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80%	2 kg 3 kg	Downy mildew Powdery mildew	On the 24th of June, 2016 the hail appears
5	Growth of berries - first decade of July	Funguran OH 50 WP Cosavet 80 DF Laser 240 SC	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80% Spinosad 240 g/l	2 kg 3 kg 0.15 l/ha	Downy mildew Powdery mildew Mites	
6	Compaction of grape clusters - second decade of July	Funguran OH 50 WP Cosavet 80 DF	77% Copper Hydroxide equivalent with 50% metallic copper Sulphur 80%	2 kg 3 kg	Downy mildew Powdery mildew	
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In order to establish certain quality indices, there were used physical and chemical methods for analysis and control. Assessments regarding the average weight of the grapes, the average number of clusters per rootstock, the grape production collected per hectare, the sugar content (g/l) and acidity (g/l tartaric acid) were conducted both on the field, as well as in the laboratory of *S.C. Vie Vin Vânju Mare*

S.R.L. society. The average weight of the grapes was assessed by weighting every 50 grapes per each type of treatment and it was expressed in grams. The average number of clusters per rootstock was determined at the same time with the evaluation concerning the average weight of the grapes.

After obtaining the data regarding the average number of clusters per rootstock and the average weight of the grapes, it was possible to determine the biological production per rootstock, which was expressed in kilograms. Furthermore, knowing the number of rootstocks/hectare, which is 4,500 in the case of *Fetească neagră* variety and 4,400 in the case of *Cabernet Sauvignon* variety, it was possible to finally compute the grape production on one hectare, for each type of treatment, by using the above formula:

Production/ha (t/ha) = production/rootstock (kg) x number of rootstocks

The sugar content was determined through density measurements (at the level of the must), as well as through the refractometric method, by using the ZEISS hand refractometer. The total acidity was chemically established, through the titrimetric method.

The frequency (F%) and the intensity (I%) of the pathogens *Plasmopara viticola*, *Uncinula necator* and *Botryotinia fukeliana* were determined three days after the administration of each phytosanitary treatment, both in the conventional and in the unconventional systems. The average attack degree for each phytoparasite was subsequently computed by using the above-mentioned indicators and following the formula:

$$AD\% = \frac{F\% \times I\%}{100}$$

RESULTS AND DISCUSSIONS

The results regarding the influence of the type of phytosanitary treatments on certain quality indices in the case of *Fetească neagră* and *Cabernet Sauvignon* varieties that are cultivated within Vânju Mare - Oreviţa viticultural area and in the climatic conditions of 2016 are presented in table 3.

With respect to the average number of clusters/rootstock, great differences between the varieties are to be noticed, irrespective of the treatment option. In the case of *Fetească neagră* variety, the average number of clusters/rootstock was 12 in the conventional system and 9 in the unconventional system, while in the case of *Cabernet Sauvignon* variety, the average number of clusters/rootstock was 21 in the conventional system and 17 in the unconventional system, respectively.

The average weight of the clusters also oscillated depending on the variety and the type of treatment; thus, in the case of *Fetească neagră* variety, the average weight of the clusters ranged from 255 g – in the conventional system to 248 g – in the unconventional one. A similar slightly decreasing tendency of the average weight of the clusters was also noticed in the case of *Cabernet Sauvignon* variety, cultivated in the unconventional system.

As expected, the grapevine production expressed in t/ha registered the highest value in the case of *Cabernet Sauvignon* variety in the conventional system; these are the conditions in which the highest number of clusters/rootstock was also registered, although the average weight of the clusters is less significant as compared to *Fetească neagră* variety.

Tabel nr. 3

The influence of the type of phytosanitary treatment on some quality indices

Variety \ Analysed character		Average number clusters/rootstock	Average weight cluster (g)	Grape production (t/ha)	Total acidity (g/l H_2SO_4)	Sugar content (g/l sugar)
Fetească neagră	Conventional	12	255	4	5.4	227.8
Cabernet Sauvignon		21	165	4.3	5.0	235.7
Fetească neagră	Unconventional	9	248	3.8	4.8	221.2
Cabernet Sauvignon		17	151	4.1	4.5	230.4

Higher values of the must acidity, irrespective of the type of treatment, were registered in the case of *Fetească neagră* variety; at the same time, irrespective of the grapevine variety, a decrease of the total acidity was registered after the treatment that used unconventional products.

With respect to sugar accumulation, the *Cabernet Sauvignon* variety is more adequate both for the conventional, as well as for the unconventional culture systems within Vânju Mare - Oreviţa viticultural area.

Regarding the attack of the monitored pathogens (table 4), it is to be noticed that it registered different values depending on the cultivar, the pathogen and the type of treatment.

Under the climatic conditions of 2016, the disease that generated problems within Vânju Mare-Oreviţa viticultural area was the grapevine powdery mildew. The attack degree values registered by this disease in the case of *Fetească neagră* variety reached 12.40% on the leaves and 10.20% on the clusters - in the conventional system, increasing to 15.42% on the leaves and 12.52% on the clusters – in the unconventional system. Moreover, the data comprised in the same table reveal the fact that the attack of the *Plasmopara viticola* and *Botryotinia fukeliana* micromycetes was weak, but it registered a slightly increasing tendency in the case of the treatment that used unconventional products.

Tabel nr. 4

The influence of attack degree, in conventional and unconventional systems, on *Fetească neagră* and *Cabernet Sauvignon* varieties within Vânju Mare-Oreviţa vineyard, in 2016

Variety	Analysed character	% of AD on the leaves		% of AD on the clusters		
		Plasmopara viticola	*Uncinula necator*	*Plasmopara viticola*	*Uncinula necator*	*Botryotinia fukeliana*
Fetească neagră	Conventional	2.20	12.40	2.32	10.20	2.12
Cabernet Sauvignon		1.78	2.12	1.60	1.20	0.60

Fetească neagră	Unconventional	3.82	15.42	2.93	12.52	3.02
Cabernet Sauvignon		2.24	3.58	2.01	1.97	1.04

CONCLUSONS

The research regarding the two grapevine varieties, i.e. ***Fetească neagră*** and *Cabernet Sauvignon*, cultivated within Vânju Mare-Oreviţa viticultural area, in 2016 revealed that their production amounted to higher quantities in the case of the traditional culture (4 t/ha ***Fetească neagră***; 4.3 t/ha *Cabernet Sauvignon*), as compared to the unconventional alternative of culture (3.8 t/ha ***Fetească neagră***; 4.1 t/ha *Cabernet Sauvignon*).

The highest number of clusters/rootstock was reached in the case of *Cabernet Sauvignon* variety, with an average value of 21 in conventional system and 17 clusters/rootstock in unconventional system, respectively.

The sugar content of the must also registered the best values in the case of *Cabernet Sauvignon* variety, both in the conventional (235.7 g/l) and in the unconventional (230.4 g/l) systems, whereas in the case of ***Fetească neagră*** variety, this indicator reached values of 227.8 g/l and 221.2 g/l, respectively.

In the conventional system, the total acidity of the must reached values of 5.4 g/l H_2SO_4 for ***Fetească neagră*** variety and 5.0 g/l H_2SO_4 for *Cabernet Sauvignon* variety, whereas in the unconventional system, the same indicator displayed values of 4.8 g/l H_2SO_4 for ***Fetească neagră*** and 4.5 g/l H_2SO_4 for *Cabernet Sauvignon*.

The attack of *Plasmopara viticola* and *Botryotinia fukeliana* micromycetes was weak, irrespective of the grapevine variety and of the applied treatment, this being a consequence of the response of the two varieties and of the climatic dynamics during the research year.

Under the climatic conditions of the analysed period, within Vânju Mare-Oreviţa viticultural area there was noticed that the ***Fetească neagră*** variety, displayed an average resistance to the attack of the *Uncinula necator* fungus, both of the leaves as well as on the clusters and irrespective of the type of treatment.

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RESULTS REGARDING THE INFLUENCE OF THE CLIMATIC CONDITIONS OF 2016 ON THE EVOLUTION OF PATHOGENS IN THE VINEYARD FROM S.C.VIE VIN VÂNJU MARE S.R.L., MEHEDINŢI COUNTY

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Key words*: grapevine, pathogens, climatic conditions*

ABSTRACT

S.C. Vie Vin Vânju Mare S.R.L. owns 240 ha of vineyards with quality wines such as: Cabernet Sauvignon, Fetească neagră, Riesling Italian, Merlot, Tămâioasă românească, Fetească regală, Pinoit noir, etc. at Vânju Mare Viticulture Center.

The data obtained from the controls carried out in the two agroecosystems (Vânju Mare-Oreviţa, Vânju Mare-Bucura) revealed the presence of certain harmful organisms, specific to vines. Under the climatic conditions of 2016, Drobeta Turnu Severin Forecasting and Warning Station issued a number of 8 warnings based on the phenological, biological and ecological criteria. For the main phytoparasites (Plasmopara viticola, Uncinula necator, Botryotinia fuckeliana), the dynamics of the evolution of the attack was correlated with the climatic conditions registered in the research year and there was also rendered the reaction of three grapevine varieties to their attack.

INTRODUCTION

Grapevine culture occupies an important place in Romania and would not be possible without a high quality propagation material (Vizitiu et al., 2015). Comes, 1995, shows that the large number of diseases and pests does not complicate the possibilities of parasite control too much, as only a few species are more frequent and truly damaging. For example, the grapevine downy mildew and powdery mildew can alone cause greater damage than all the others together. At the same time, the European grapevine moth is more dangerous than other pest species that occur less frequently or only under certain environmental conditions, requiring chemical treatments only to a certain degree of density.

Plasmopara viticola (Berk et Curt) Berl et de Toni, the pathogen that produces the grapevine downy mildew, is considered one of the most important pathogens attacking the grapevine.

According to Ulea, 2003, the attack of the downy mildew is manifested on all the aerial organs of the grapevine: leaves, shoots, tendrils, flowers, clusters and berries. The spots provoked by the downy mildew on leaves have a varied appearances; thus, in spring, there are yellow oilspots, with a diffuse contour - the oilspot stage, and, in time, their center gets brown, the leaf displaying a burned appearance - the leaf burning stage (Galet, 1991).

The attack on young clusters can be dangerous in years with abundant rainfalls; they become yellow and get covered by conidiophores and conidia when

weather is wet "rot-gris" or get brown on dry weather (Ulea, 2003). On young clusters, the downy mildew is frequent and damaging and is also called rot-gris.

Uncinula necator – the powdery mildew occurs throughout the vegetation period and affects all organs: leaves, shoots, tendrils and berries. On the leaves, there appears a grayish-white patch consisting of mycelium, conidiophores and conidia. In these areas, the tissues do not grow anymore and there appear corrugating on the limb and twists, and the mycelia and fructifications extend on the leaf petiole (Radulescu, Rafailă, 1972). When the attack affects the berries, the skin loses its elasticity and does not grow anymore; there appear cracks that deepen, revealing the seeds (Severin, Dejeu, 1994).

The attack of the Botryotinia fuckeliana fungus manifests with great intensity in rainy autumns, when the damage can be very high. However, the most popular attack known by viticulturists occurs on grapes after a sufficient sugar amount accumulates in the cells.

Ulea, 2003, also notes that the presence of hail damage, the cracks of the small berries during ripening are phenomena that occur frequently during the autumn rains.

Grape rot is the most serious form of attack caused by botrytis; thus, in the beginning, on the skin of the grape berries, there are observed yellowish-gray or red-violet spots that expand rapidly; the berries soften, crack and get covered with abundant mold (Tomoiaga Liliana, 2002).

Nowadays, viticulture cannot be conceived without chemical treatments against diseases and pests (Ilişescu Isabela, 2004).

MATERIAL AND WORK METHOD

The studies were carried out at Vânju Mare-Oreviţa, respectively Vânju Mare-Bucura Vineyards in 2016, the experiments taking place at Oreviţa Mare and Bucura, component villages of Vânju Mare town.

The biological material taken into study was represented by 3 varieties of vines, two varieties of red grapes (Fetească neagră and Cabernet Sauvignon) and a variety of white grapes (Riesling italian), varieties with the highest share in the two vineyards.

The climatic conditions of 2016, when the research was conducted, are rendered in Table 1.

Table 1

Climatic elements registered during the vine vegetation period that influenced the attack of pathogens at Vânju Mare - Oreviţa and Vânju Mare – Bucura vineyards in 2016 (Source: Drobeta Turnu Severin station)

Month	Year					
	2016			Average 2007-2016		
	T^0C	P l/mp	U%	T^0C	P l/mp	U%
April	15	54.2	65	13.48	50.28	62.6
May	16.6	81	71	18.21	82.98	65.7
June	22.7	90.6	70	22.37	70.22	62.8
July	24.5	127	60	24.97	53.99	56.6
August	23.3	39.2	66	21.95	31.51	58.1
September	20	15.2	65	19.17	64.31	63.9
October	10.9	73.2	79	11.2	83.18	77.4

According to the Methodologies used in the Forecasting and Warning Stations of the National Phytosanitary Network (M.A.I.A.-Metodici de Prognoză şi Avertizare /

Forecasting and Warning Methods, 1980), it was achieved the estimation of the attack for the three studied micromycetes by value (F%), intensity (I %) and attack rate (A.R.%).

Data collection on the behavior of the three studied varieties to the pathogen attack was carried out by field surveys, observations, counting and analyzing the plants or plant organs.

The evaluation of the attack on the analyzed organs was made using the assessment scale proposed by OIV1983 (code N05 OIV 452,453,455,456,458) presented in Table 2.

Table 2

The scale for assessing the resistance of vine to the main pathogens

Evaluation of leaf resistance	Character expression	Attack rate (A.R. %)
Very weak	1 2	>75% 50 - 75%
Weak (large necrotic spots on the leaves)	3 4	40 - 50% 25 - 40%
Medium (isolated, necrotic spots of about 1 cm in diameter)	5 6	15 - 25% 10 - 15%
Good (very small necrotic spots)	7 8	5 - 10% 1 - 5%
Very good (without symptoms of attack)	9	<1%
Evaluation of grape resistance	1 2	>75% 50 - 75%
Very weak	3 4	40 - 50% 25 - 40%
Weak (all grapes are attacked and they fall)	5 6	15 - 25% 10 - 15%
Medium (up to 20% of the berries are wilted or rotten)	7 8	5 - 10% 1 - 5%
Good (only some berries are wilted or rotten)	9	<1%
Very good (without symptoms of attack)	1 2	>75% 50 - 75%

RESULTS AND DISCUSSIONS

The obtained data emphasized that the action of the environmental factors (temperature, precipitation and relative humidity) determine a direct action on the dynamics of the evolution of the main pathogen attack.

In the climatic conditions of 2016, within the two studied vineyards, six warnings were issued by Drobeta Turnu Severin Forecasting and Warning Station for the prevention of the attack of *Plasmopara viticola* pathogen, namely on April 13, May 10, May20, June 28, July 21 and August 4 (Table 3).

The germination of the oospores of *Plasmopara viticola* fungus occurred gradually in May, when there were registered 81.0 l / m^2 of rainfall, 1.98 l / m^2 less than the average of this month in the last 10 years; there occurred the primary infection.

The incubation period of the pathogen was influenced by high temperatures of 15-16^{0}C, which led to the observation of the first oilspot at the Riesling italian variety on June 10, 2016. In case of the variety Feteasc Negr, the first oilspot was observed on June 20, 2016, and at Cabernet Sauvignon variety, the oilspot, which is a proof of the incubation period, was observed on June 30, 2016.

Table 3

Warnings issued by Drobeta-Turnu Severin Forecasting and Warning Station to combat pathogens in 2016

No. of warnings	**Warning date**	**The optimal period for treatment**	**Pest**	**Observations**
1	February 3	When temperature exceeds 10^0C and there are no precipitation conditions	Aphids Mites (*Tetranycus urticae, Panonycus ulmi, Colomerus vitis, Calipitrimerus vitis)* European fruit lecanium (*Parthenolecanium comi)* Woolly vine (*Pulvinaria vitis*) Willow beauty (*Peribatodes rhomboidaria*) Common Eastern Firefly (*Pyralis vitana*) *Uncinula necator*	The period of vegetative rest
2	March 28	March 29-31	Grape gall mite (*Eriophyes vitis*) *Uncinula necator*	Gemmate – fully open bud with 2 cm leaves –shoots with leaves of 2 cm
3	April 13	April 14-20	*Uncinula necator* *Elsinöe ampelina* *Plasmopara viticola* *Tetranycus urticae,* *Panonycus ulmi,* *Colomerus vitis,* *Calipitrimerus vitis*	In the period when shoots grow (Shoots of 5-15 cm)
4	May 10	May 11-18	*Plasmopara viticola* *Uncinula necator* *Tetranycus urticae* *Pseuodopeziza tracheiphilla*	Shoots=20-25 cm Appearance of clusters
5	May 20	Immediately	*Plasmopara viticola* *Uncinula necator* *Tetranycus urticae* *Lobesia botrana*	Appearance of clusters Treatment before flowering
6	June 28	June 30-July 8	*Plasmopara viticola* *Uncinula necator* *Botryotinia fuckeliana* *Tetranycus urticae* *Lobesia botrana*	Treatment after petals fall
7	July 21	July 22-27	*Plasmopara viticola* *Uncinula necator* *Botryotinia fuckeliana* *Tetranycus urticae*	Growth of berries and compaction of grape clusters
8	August 4	August 5-10	*Plasmopara viticola* *Uncinula necator* *Botryotinia fuckeliana*	Start of fruit ripening

The abundant rainfalls (127 l / m^2) and the recorded temperatures (average 24.5°C) made July favorable for the development of the pathogen and the occurrence of repeated secondary infections.

August and especially September, which were dry months, did not allow the stage of the *mosaic-spot stage* to occur, the biological stock of the pathogen for 2017 being thus reduced.

For the varieties analyzed in the year of research, the leaf attack rate varied between 1.78% for Cabernet Sauvignon variety at Vânju Mare-Oreviţa vineyard and 12.20% for Riesling italian variety at Vânju Mare-Bucura viticultural center (Table 4).

The varieties Cabernet Sauvignon and Fetească Neagră behaved like resistant varieties displaying a character expression 8, while Riesling italian variety behaved as a medium resistant with the character expression 6.

As it regards the cluster attack, it oscillated between 1.60% for Cabernet Sauvignon variety at Vânju Mare-Oreviţa viticultural center and 13.20% for Riesling italian at Vânju Mare-Bucura viticultural center, being a weak to medium attack.

For the control of *Uncinula necator* pathogen, Drobeta Turnu Severin Forecasting and Warning Station issued 8 warnings (Table 3) on February 3, March 28, April 13, May 10, May 20, June 28, July 21 and August 4.

The climate conditions registered in June and July favored the attack of *Uncinula necator* fungus. The first sign of attack was on June 22 on Riesling italian variety; then, in the first and second decades of July, it was found on Fetească neagră and Cabernet Sauvignon varieties.

The leaf and cluster attack recorded different values depending on variety and microclimate conditions (Table 5).

As for the leaf attack, it recorded values between 2.10% for Cabernet Sauvignon and 13.32% for Fetească neagră at the Vânju Mare-Bucura vineyard and 2.12%, respectively 12.40%, for the same varieties at the Vânju Mare-Oreviţa viticultural center.

Depending on the character expression, the varieties Riesling italian and Feteasca neagră behaved as medium resistant to the leaf attack of *Uncinula necator*, while Cabernet Sauvignon reacted as a resistant variety.

The cluster attack recorded values between 1.20% and 1.60% for Cabernet Sauvignon variety and between 10.20% and 10.44% for Fetească neagră variety in the two viticultural ecosystems, while for Riesling italian variety, the value of the attack rate reached 11.10%.

The analyzed varieties were classified into two classes of resistance, namely medium resistant (Riesling italian, Fetească neagră) and resistant (Cabernet Sauvignon).

Based on the obtained data, it can be stated that the critical period for the vine cultivated at Vânju Mare-Oreviţa and Vânju Mare-Bucura vineyards regarding the attack of *Uncinula necator* fungus is registered in the interval June-August.

To combat the pathogen *Botryotinia fukeliana*, Drobeta Turnu Severin Forecasting and Warning Station issued 3 warnings on June 28, July 21 and August 4 (Table 3).

The potential infection status for this facultative saprophytic pathogen was registered on June 22, when favorable weather conditions were also registered, namely an average temperature of 22.7°C and relative humidity of 70%.

The next potential states of infection occurred on July 14, when the average temperature was 24°C and the relative humidity 60%, and on August 6, when the average temperature was 23°C and the relative humidity 66%.In the phase of grape ripening, the phase of maximum vulnerability, in September, there were registered insignificant rainfalls (15.2 l / m^2) and a prolonged drought period began, which led to the cessation of the attack; thus, the production was not influenced.

Table 4

The dynamics of the attack caused by *Plasmopara viticola* fungus on the vine leaves and clusters at the vineyard S.C. Vie Vin Vânju Mare S.R.L in 2016

Viticultural ecosystem	Variety	Leaf attack			Cluster attack		
		A.R.%	Character expression	Resistance class	A.R.%	Character expression	Resistance class
Vânju Mare - Bucura	Riesling italian	12.20	6	MR	13.20	6	MR
	Cabernet Sauvignon	1.92	8	R	1.90	8	R
	Fetească Neagră	5.10	7	R	4.14	7	R
Vânju Mare – Oreviţa	Cabernet Sauvignon	1.78	8	R	1.60	8	R
	Fetească Neagră	2.20	8	R	2.32	8	R

Table 5

The dynamics of the attack caused by *Uncinula necator* fungus on the vine leaves and clusters at the vineyard S.C. Vie Vin Vânju Mare S.R.L in 2016

Viticultural ecosystem	Variety	Leaf attack			Cluster attack		
		A.R.%	Character expression	Resistance class	A.R.%	Character expression	Resistance class
Vânju Mare - Bucura	Riesling italian	12.20	6	MR	11.10	6	MR
	Cabernet Sauvignon	2.10	8	R	1.60	8	R
	Fetească Neagră	13.32	6	MR	10.44	6	MR
Vânju Mare – Oreviţa	Cabernet Sauvignon	2.12	8	R	1.20	8	R
	Fetească Neagră	12.40	6	MR	10.20	6	MR

This is also illustrated in Table 6, where the values of the attack rate on the grape clusters of the analyzed varieties are presented. As it can be noticed from this table, the maximum attack rate was 2.44% for Riesling italian variety, close values being registered in case of Fetească neagră variety in both viticultural centers. Cabernet Sauvignon behaved as a very resistant variety, the character expression being 9 in both viticultural ecosystems.

From the overall analysis of the obtained data, it was noticed that, in 2016, the climatic conditions registered during the vine vegetation period allowed possible infections starting with June, infections that were stopped in time.

Table 6

The dynamics of the attack caused by *Botryotinia fukeliana* fungus on the grape clusters in the vineyard
S.C. Vie Vin Vânju Mare S.R.L in 2016

Viticultural ecosystem	Variety	Cluster attack		
		A.R.%	Character expression	Resistance class
Vânju Mare - Bucura	Riesling italian	2.44	8	R
	Cabernet Sauvignon	0.80	9	FR
	Fetească Neagră	2.10	8	R
Vânju Mare – Oreviţa	Cabernet Sauvignon	0.60	9	FR
	Fetească Neagră	2.12	8	R

CONCLUSIONS

In order to keep the main phytoparasites under control at Vânju Mare-Oreviţa and Vânju Mare-Bucura vineyards in 2016, a number of 8 treatments were applied according to the phenological, biological and ecological criterion.

For the pathogen *Plasmopara viticola* 6 warnings were issued; 8 warnings were issued for the control of the *Uncinula necator* pathogen and 3 warnings for *Botryotinia fukeliana* by Drobeta Turnu Severin Forecasting and Warning Station.

The analyzed varieties behaved as medium resistant and resistant to the attack of *Plasmopara viticola* fungus on leaves and clusters; thus, Riesling italian variety behaved as a medium resistant in case of both vineyards and Cabernet Sauvignon and Fetească neagră behaved as resistant varieties.

The attack caused by *Uncinula necator* fungus on leaves and clusters ranks between medium for Riesling italian and Fetească neagră varieties in both the vineyards and weak for Cabernet Sauvignon variety.

Regarding the attack of *Botryotinia fuckeliana* fungus, Riesling italian and Fetească neagră varieties behaved as resistant, while Cabernet Sauvignon variety reacted as very resistant.

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THE PASSERIFORMES OF CRAIOVA CITY (DOLJ COUNTY, ROMANIA)

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Key words: *Order Passeriformes, phenology, seasonal dynamics, breeding*

ABSTRACT

This paper focuses on the main species of Passeriformes observed in the parks and gardens of Craiova city (Dolj County) during 2012-1016. The 61 species highlighted are distributed in 20 families. Most of them are migratory (17 summer visitors, 12 winter visitors and 17 passage migrants). The breeding habitats (trees, bushes, hollows, soil, buildings, wall cracks, etc.) have provided optimal conditions for 30 passerine birds considered certainly breeding species. The seasonal dynamics and numerical variations of birds are influenced by climatic conditions, food, anthropogenic activities.

INTRODUCTION

The studies carried out in the parks and gardens of Craiova the Romanescu Park (about 96 ha), Botanical Garden (about 17 ha), Craiovița Lake and Park, Tanchiştilor Lake (about 3 ha), Youth Park (about 55 ha), Şerca and Mofleni marshes or in different neighbourhoods: Mofleni, Rovine, Railway Station area, etc. have highlighted a great diversity and a characteristic dynamics of bird species, whose frequency has succeeded over the years.

The city of Craiova, the county-seat of Dolj County, is located in the south of Romania, on the left bank of the Jiu river. It is situated in the Oltenia plain, at an altitude ranging between 75 and 116 m and has an area of 81.41 km^2 with the coordinates 44°20'N 23°49'E (https://ro.wikipedia.org/wiki/Craiova). It has a temperate continental climate, specific for plains, with submediterranean influences.

The habitats in the city: parks, gardens, orchards (with appropriate vegetation), paludous vegetation, squares, buildings, etc. include a variety of bird species, of which the passerine birds are predominant in number. This is also due to the fact that among all orders of the Aves class, the order Passeriformes include most species: over 5,000 species (https://en.wikipedia.org/wiki/Passerine).

The data from this paper refer to the seasonal dynamics of passerine birds in the 2012-2016 period. The paper aims at establishing (as far as possible) the avifaunistic categories (sedentary and migratory species, nesting and non-nesting species), and some aspects of the behaviour of some passerines species (especially those related to the reproduction).

MATERIALS AND METHODS

The birds monitoring was based on classical methods: the method of transects, visual and optical observations (with the Buchnell 12x40 binoculars), photography (with Canon Sx40HS digital camera) and film shooting (with Panasonic SDR-H20 camera). The trips were performed on a monthly basis mostly in the morning. Thus all the seasons of the year were covered: prevernal (March-April), vernal (May-15 June), aestival (15 June-15 August), serotinal (15 August-15 September), autumnal (15 September-31 October) and hiemal (November, December, January, February) (Dajoz, 1970). Determinators were used in species identification (Bruun et al., 1999; Peterson et al., 1989).

The analysis of the obtained data was done taking into consideration also the literature (Croitoru, 2009; Munteanu, 2012; Radu, 1984; Tălpeanu, 1969, etc.). The birds observation was done in the urban area and, to a lesser extent in the periurban area.

RESULTS AND DISCUSSIONS

The information about the birds of Craiova since 2000 has revealed the presence of a rather large number of birds, about 119 species, of which 63 species are Passeriformes (Bălescu, 2008). In the 2012-2016 period, 61 species of Passeriformes were reviewed, most of them being migratory, which are taxonomically distributed in 20 families (Table 1). Muscicapidae with 9 species predominate, followed by Fringillidae and Acrocephalidae with 7 species each, Turdidae and Corvidae with 5 species each.

The diversity of passerine birds in the city is primarily conditioned by the favourable habitats that enable birds to carry out their main biological cycles. The anthropogenic pressure is low. The changes made in the city's gardens and parks for their modernization did not have any major effects on the birds in those areas. Birds have become accustomed, over time, to disturbing factors. Some species were absent only during the projects undertaken by the city hall. After the works were completed, the species returned.

Most of the passerine birds observed in the city have the same phenological status as the ones of the country.

Most identified species are migratory: summer visitors - 17 species. The winter visitors count 12 species. 17 species are in passage, observed during spring and/or autumn migration. For them, the city remains a passage area for resting in order to prepare for reproduction and/or wintering in some neighbourhoods (*Ficedula hypoleuca, Sylvia borin, Phylloscopus sibilatrix, Saxicola torquatus, Hippolais pallida* etc). If conditions allow, passage birds can become breeding birds.

The fewest species are included in the sedentary and partially migratory species group (15 species). Some birds have been identified as having a double phenology: summer visitors and passage migrants (*Sylvia curruca, Locustella luscinoides* etc) or winter visitors and passage migrants (*Carduelis spinus, Pyrrhula pyrrhula* etc). Some species predominate in some years, others predominate in other years. For example: *Locustella luscinoides*, *Acrocephalus palustris* have become rare in recent years, while *Lanius collurio* and *Muscicapa striata* are constantly observed and breeding in their characteristic habitats.

The fewest passerine birds were observed in the hiemal season. Along with sedentary species, 16 migratory species are observed. Of these, the most common species observed during the years in the city parks are: *Motacilla cinerea, Troglodytes troglodytes, Erithacus rubecula, Turdus pilaris, Fringilla coelebs, Coccothraustes coccothraustes*. From the numerical point of view, the number of species of corvids, fieldfare (over one hundred), hawfinch (tens of specimens), common chaffinch (small groups of specimens, but numerous), etc. increases, which can be observed in the

most diverse habitats. In heavy winters they are seen both in parks and in neighborhoods, often in trash cans, looking for food. The redwing was frequently seen in parks and gardens in flocks of 10-50 specimens in January-February. There are species that have been observed in small numbers but constantly, such as: Eurasian treecreeper (1-2 specimens), Eurasian nuthatch (2-5 specimens). As rare species, we mention the Eurasian siskin (in the Romanescu Park, the Botanical Garden), the yellowhammer (the Romanescu Park hippodrome in 2013, Banu Mărăcine in 2015 and 2016). In the last two years, the bullfinch (*Pyrrhula pyrrhula*) and brambling (*Fringilla montifringilla*) have become passage migrant species this season, being signaled during migration, at the beginning and end of the season.

In the prevernal season, most passerine birds were monitored: 50 species. 45 species and 42 species were observed in the serotinal and autumnal seasons. The increased number of species and individuals in these seasons is the consequence of the spring and autumn migration, respectively. This reinforce the conclusion that Craiova city is on the migration route of many species that find trophic resources and stopping places in the habitats of the city. The lower number of species inventoried on the field in the vernal season (35 species) and aestival season (32 species) is the consequence of the important reproduction function at birds, making them harder to find (being in the nests). The nesting passeriformes predominate in these seasons.

The presence or absence of passerines in the area during certain seasons of a year and numerical variations within the same species are influenced by climatic conditions and food (to a large extent) and anthropogenic activities (to a small extent). Birds return after the restoration of the biotope, some are nesting, others are just migrant. For example: *Oriolus oriolus* (golden oriole) nested in Lunca Jiului Park in 2006 (Bălescu, 2007). After the modernizations in the 2008-2010 period and the renaming of the park in the Youth Park, the golden oriole was seen only as a migrant. From 2015 the species breeds Tanchiştilor Lake area, located in the northern part of the city. In the vicinity of the lake there is a mosaic of habitats: paludous vegetation, pine plantation, deciduous trees plantation, open field with grassy vegetation and clusters of trees and shrubs, a favourable habitat for this species as well as for others (*Corvidae, Paridae*, reed warblers).

In the 2012-2016 period, we did not see the species highlighted in the previous years *Anthus pratensis, Regulus ignicapillus, Parus ater, Aegithalos caudatus, Carduelis cannabina* etc (Bălescu, 2005, 2006, 2007, 2008).

In this paper we have not mentioned other passerine birds such as *Alauda arvensis*, *Anthus campestris*, *Riparia riparia, Bombycilla garrulus, Prunella modularis, Parus lugubris, Lanius minor* etc., included in the paper on the distribution of birds in the Craiova Airport area, at a distance of 0-13 km (Ridiche & Munteanu, 2015). It is therefore necessary to continue monitoring the birds in the peripheral of the city as well as increasing the days of observation, especially during migration. We mention that we have strictly addressed our own observations in the above mentioned period. The comparisons will be included in a future study.

Regarding the breeding passerines, in the researched period, we signaled 30 certainly breeding species and 2 probably breeding species. Among the passerine birds that have not been constantly nested in the years of this study, there are several species such as: *Phoenicurus ochruros* breeding from 2014 - in the area of abandoned buildings, both in the urbane area of the city and the periphery, but also in the cemetery area (Sineasca and Ungureni). Own observations about the breeding of the *Sylvia atricapilla* species in the Botanical Garden are until 2014, etc. *Acrocephalus schoenobaenus* and *A. palustris* also sporadically nested in Șerca and Mofleni marshs,

in the Craiovița Lake (Bălescu, (2016). Possiblly breeding species are *Locustella luscinoides* and *Sylvia curruca*.

Taking into account the biotope where they nest, there are several groups of passerine birds.

-Species that nest in arborescent vegetation: in trees, shrubs, bushes in parks, gardens, orchards, trees along streets in different neighbourhoods, etc. Some passerines build nests in the trees and shrubs crown (*Corvus frugilegus, C. cornix, Oriolus oriolus, Pica pica, Garrulus glandarius, Passer montanus, Carduelis chloris, Sylvia communis, S. atricapilla, Lanius. collurio*); others in hollows (*Corvus monedula, Sturnus vulgaris, Parus major, Parus caeruleus*) or under the tree bark (*Sitta europaea*):

-Species that lay the eggs on the ground, among herbage, well camouflaged, hard to find (*Motacilla alba, Motacilla flava, Luscinia megarhynchos etc.*), on meadows or fallow land (*Galerida cristata* etc)

-Birds that breed in reed beds (warblers)

-Species that build their nests on/in buildings (in cracks/wall cavities, under the tiles, eaves, corners, on flat walls), etc.: *Hirundo rustica, Delichon urbicum, Passer domestica, Passer montanus, Phoenicurus ochruros* etc. We have some observations on the breeding of the common house martin (*Delichon urbicum*). Until 2015, there was a colony at the County Hall Building in the city centre. In 2015, the nests were destroyed due to the misery of the birds. Instead, a colony nests regularly at the platform 2 of the Railway Station (at the ceiling of the roof) in the last 6 years. The nests of mud, sand and saliva were placed above the neon lights (vertically). They are rounded with a small opening on top. In 2016, for each neon light we counted between 1 and 6 nests. Not all of them were occupied. Of the 36 nests (on the left) and 64 (on the right / the side of the Railway Station building) there were occupied 20 (on the left) and 35 (on the right). Annually, the pairs either consolidate the destroyed nests or build new ones. Most pairs laid eggs once/per year between May and June. Few pairs laid eggs twice/per year. The second time at the end of July/the first week of August. In 2016 we noticed in the last decade of August 11 nests of chickens, usually of 3-4 chickens. Beginning with September, they left gradually the nests, the last pair on September 14th. The breeding of this species will be further monitored both in the Railway Station area and in other areas where such colonies exist.

There are many species of Passeriformes in general that use more nesting habitats. For example, The great tit (*Parus major*), a common species, it nests both in the hollows of the various trees (very frequently) and in wall cracks, at the base of the pipes of some houses, in well-hidden cigarette boxes in the hedge (buxus), etc. The common starling (*Sturnus vulgaris*) nested both in hollows and at the roof of the houses, under the roof tiles, in the block cracks - on the top floor, in the free spaces of the telegraph poles. The presence of the common starling in the city is more dominant than that of the common blackbird (*Turdus merula*). The competition for food taken from the ground has led to the removal of the common blackbird from some parks: the Romanescu Park, Tineretului Park where it has not been seen nesting. In these areas it is sporadically seen, more in the cold season. Over the past 15 years, we have witnessed the steady breeding of the common starling in all the neighbourhoods of the city, usually they layed eggs twice/per year. In the summer, hundreds and thousands/tens of thousands of common starlings can be seen in reed (in the Şerca and Mofleni Marshs, the Craioviţa Lake etc.). In the future there will be tracked the evolution of this species and the relationships with other passerine birds.

In the last decades, we have reported the increase of the corvid population in the city's perimeter, especially western jackdaws (*Corvus monedula*) and common

magpies (*Pica pica*). Major rook colonies are met in the area of the Jiu Meadow and the Craiova Airport. In the last years, we report the reductions number of breeding couples, at other species, but also the reduction of breeding areas. We exemplify the crested lark (*Galerida cristata*), a sedentary species present constantly in about 3-10 specimens during the cold season, but in low flocks during the summer. The breeding area of the species was the peripheral area of the city: the fallow ground in the Șerca marshs, the land in the Banu Mărăcine area. *Sitta europea* nest in few pairs in the Romanescu and Youth Park.

The scientific information on the city's passerine birds should not stop here, they must be carried out and there must be observed other areas of the city in order to more accurately determine the populations of the birds (nesting, non-nesting species), to follow both the dynamics of the birds and their behaviour, always surprising. This makes it easier to identify the factors that act negatively on species, and there can be taken the most advantageous measures to maintain the biodiversity in the parks and gardens of the city.

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CONCLUSIONS

The 61 species of birds observed during the 2012-2016 period are grouped in 20 families: Alaudidae -1 species, Hirundinidae - 2 species, Motacillidae - 4 sp., Troglodytidae – 1 sp., Muscicapidae – 9 sp., Turdidae – 5 sp., Acrocephalidae – 7 sp., Sylviidae – 4 sp., Phylloscopidae – 3 sp.; Regulidae – 1 sp.; Paridae – 2 sp., Sittidae – 1 sp., Certhiidae – 1 sp., Oriolidae – 1 sp., Laniidae – 1 sp., Corvidae – 5 sp., Sturnidae – 1 sp., Passeridae – 2 sp., Fringillidae – 7 sp., Emberizidae – 3 sp.

Hirundo rustica, *Delichon urbicum, Motacilla alba, Motacilla flava*, *Luscinia megarhynchos, Muscicapa striata, Acrocephalus arundinaceus, Acrocephalus scirpaceus, Sylvia communis, Lanius collurio* are among the summer visitor species, which are constantly breeding in this period.

As species with irregular appearances there are: *Hippolais pallida, H.icterina Regulus regulus, Fringilla montifringilla, Carduelis spinus, Pyrrhula pyrrhula*. The months of April and September record a large number of species. Low number of species were recorded in November.

The diversity of city habitats (parks, gardens, orchards, buildings, etc.) influences the diversity of passerine birds, which have adapted to anthropogenic pressure over time, the city of Craiova being on the migration route of many species.

Table 1

The systematic list of the species of Passeriformes in Craiova city in the 2012-2016 period

No.	Taxonomy Family and Species	Months of the year												Observations
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
1.	**Alaudidae**													
	1. Galerida cristata	+	+	+	+	+	+	+	+	+	+	+	+	S, B
2.	**Hirundinidae**													
	2. Hirundo rustica				+	+	+	+	+	+				SV, B
	3. Delichon urbicum				+	+	+	+	+	+				SV, B
3.	**Motacillidae**													
	4. Anthus trivialis								+	+				P
	5. Motacilla flava				+	+	+	+	+	+	+			SV, B
	6. Motacilla cinerea	+	+	+							+	+	+	WV
	7. Motacilla alba			+	+	+	+	+	+	+	+			SV, B
4.	**Troglodytidae**													
	8.Troglodytes troglodytes	+	+	+							+	+	+	WV
5.	**Muscicapidae**													
	9. Erithacus rubecula	+	+	+							+	+	+	WV
	10. Luscinia megarhynchos				+	+	+	+	+					SV, B
	11. Phoenicurus phoenicurus									+	+			P
	12. Phoenicurus ochruros		+	+	+	+	+	+	+	+	+	+		P, SV, B
	13. Saxicola rubetra									+	+			P
	14. Saxicola torquatus									+	+			P
	15. Muscicapa striata				+	+	+	+	+	+	+			SV, B
	16. Ficedula albicollis				+	+			+	+				P
	17. Ficedula hypoleuca				+	+			+	+				P
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Observations

6.	**Turdidae**													
	18. Turdus merula	+	+	+	+	+	+	+	+	+	+	+	+	PM, B
	19. Turdus iliacus	+	+	+									+	WV, P
	20. Turdus philomelos			+	+				+	+	+			P
	21. Turdus viscivorus	+	+	+							+	+		WV, P
	22. Turdus pilaris	+	+										+	WV
7.	**Acrocephalidae**													
	23. Hippolais pallida			+	+				+	+				P
	24. Hippolais icterina								+	+				P
	25. Locustella luscinoides				+	+	+	+	+	+				SV, P, PB
	26. Acrocephalus schoenobaenus				+	+	+	+	+	+	+			SV, B
	27. Acrocephalus palustris				+	+	+	+	+	+				SV, B
	28. Acrocephalus scirpaceus				+	+	+	+	+	+				SV, B
	29. Acrocephalus arundinaceus				+	+	+	+	+	+	+			SV, B
8.	**Phylloscopidae**													
	30. Phylloscopus sibilatrix				+				+	+				P
	31. Phylloscopus collybita			+	+			+	+	+	+			P, SV
	32. Phylloscopus trochilus				+				+	+	+			P
9.	**Sylviidae**													
	33. Sylvia curruca			+	+	+	+	+	+	+				P, SV, PC
	34. Sylvia borin				+				+	+				P
	35. Sylvia atricapilla			+	+	+	+	+	+					P, SV, C
	36. Sylvia communis			+	+	+	+	+	+	+	+			SV, B
10.	**Regulidae**													
	37. Regulus regulus		+	+										P
11.	**Paridae**													
	38. Parus caeruleus	+	+	+	+	+	+	+	+	+	+	+	+	S, B
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Observations

	39. Parus major	+	+	+	+	+	+	+	+	+	+	+	+	S, B
12.	**Sittidae**													
	40. Sitta europaea	+	+	+	+	+	+	+	+	+	+	+	+	S, B
13	**Certhiidae**													
	41. Certhia familiaris	+	+										+	WV, P
14	**Oriolidae**													
	42. Oriouls oriolus					+	+	+	+	+				P, SV, B
15.	**Laniidae**													
	43. Lanius collurio					+	+	+	+	+	+			SV, B
16.	**Corvidae**													
	44. Pica pica	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	45. Garrulus glandarius	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	46. Corvus monedula	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	47. Corvus frugilegus	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	48. Corvus corone cornix	+	+	+	+	+	+	+	+	+	+	+	+	S, B
17.	**Sturnidae**													
	49. Sturnus vulgaris	+	+	+	+	+	+	+	+	+	+	+	+	PM, B
18.	**Passeridae**													
	50. Passer domesticus	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	51. Passer montanus	+	+	+	+	+	+	+	+	+	+	+	+	S, B
19.	**Fringillidae**													
	52. Fringilla coelebs	+	+	+	+						+	+	+	WV
	53. Fringilla montifringilla	+	+										+	WV
	54. Coccothraustes coccothraustes	+	+	+								+	+	WV
	55. Carduelis chloris	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	56. Carduelis carduelis	+	+	+	+	+	+	+	+	+	+	+	+	S, B
	57. Carduelis spinus		+	+										P
	58. Pyrrhula pyrrhula	+	+	+									+	WV

		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Observations
20.	**Emberizidae**													
	59. Emberiza citrinella		+	+										P
	60. Emberiza schoeniclus	+	+										+	WV
	61. Emberiza calandra		+	+	+	+		+	+	+	+			P, OV
	No. Total/months	27	32	35	40	35	32	34	43	44	35	22	26	
	Season	Hiemal		Prevernal		Vernal – Estival–Serotinal–Autum.						Hiemal		
	No total Passeriformes/season	32		50		35		33		45	42			

Legend. Phenology: S – sedentary species; PM – partially migratory species, P – passage visitors; SV – summer visitors, WV – winter visitors. B – breeding; PB – possiblly breeding; Months of the year: I – January, II –February, III – March, IV – April, V – May, VI – June, VII – July, VIII – August, IX – September, X – October, XI – November, XII – December

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HISTO-ANATOMICAL AND CHROMATOGRAPHIC RESEARCHES ON *ARTEMISIA ANNUA* L. (*ASTERACEAE*) SPECIES

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Key words*: Artemisia annua L., histo-anatomy, artemisinin, polyphenols, thin-layer chromatography.*

ABSTRACT

The paper presents the histo-anatomical researches on the root, stem and leaf of Artemisia annua L. species, as well as the chromatographic analysis of artemisinin and polyphenols content from the leaves and aerial parts, respectively. The samples contain both polyphenolic and isoprenoid derivatives. Chlorogenic acid was identified through the 11 specific bands for the polyphenolic components highlighted in the thin layer chromatogram.

INTRODUCTION

Artemisia annua L., Sweet wormwood, Annual wormwood (*Asteraceae*) is an herbaceous, annual, ruderal, aromatic and medicinal plant, native in temperate regions of Asia and used over 2000 years in traditional Chinese medicine under the name of *Qinghao*. It is naturalized and cultivated in many countries, from Europe to North America, as industrial raw material for artemisinin extraction (Ciocârlan 2000, Liu et al. 2013).

A. annua species is widely used in traditional Chinese medicine, mainly as a febrifuge, antimalarial, anti-icteric and hemostatic remedy (Liu et al. 2013). For the extracts obtained from leaves and aerial parts, the modern researches have highlighted some useful pharmacological actions: anti-inflammatory – triterpenoids, sterols, C-flavonoids (Zhang et al. 2017), antioxidant – polyphenols, essential oil (Iqbal et al. 2012, Pandey & Singh 2017, Zhang et al. 2017), antimicrobial – essential oil (Bilia et al. 2014, Zhang et al. 2017), antitumoral, antiviral, antiparasitic, insecticidal – artemisinin and derivatives (Li et al. 2016, Pandey & Singh 2017, Zhang et al. 2017).

The leaves of *A. annua* are the only industrial source for the extraction of artemisinin, a sesquiterpene lactone endoperoxide extensively used in the treatment of malaria (mainly for resistant forms) and as antitumoral (Guo 2016, Muangphrom et al. 2016).

In the specialty papers, there are some data concerning *A. annua* histo-anatomy (Noorbakhsh et al. 2008). The aim of our paper was the histo-anatomical researches on the root, stem and leaf of the above-mentioned species, as well as the

chromatographic analysis of artemisinin and polyphenols content from the leaves (*Artemisiae annuae folium*) and aerial parts (*Artemisiae annuae herba*), respectively.

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested from *A. annua* plants in blossom, in July 2017, from the surroundings of Scăeşti village, Dolj County (southwestern Romania).

Fixation and preservation of the roots, aboveground stems and leaves were made in 70% ethanol. The cross-sections and longitudinal-radial sections were obtained using botanical razor.

After washing with distilled water, the sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture (Genevese reagent) was used for the sections' staining. The reactive induced various colors, depending on the chemical composition of cell membranes: pink to red for cellulose and mucilage, pale red for cytoplasm, yellow for suberin and brown for lignin (Andrei & Paraschivoiu 2003).

Stained and mounted sections were analyzed on a Krüss binocular photon microscope (objectives ×4, ×10, ×40). The photos were taken with a Nikon Eclipse 55i binocular microscope coupled with a Nikon DS–Fi1 high definition video camera. The image acquisition and processing was done using the Image-Pro Plus ver. 6.0 software package.

The description of microscopic sections was achieved according to some classical authors (Toma & Ruginǎ 1998).

Thin-layer chromatography (TLC) analysis

Chromatographic analysis of artemisinin was performed using a CAMAG system (Muttenz, Switzerland). Extracts from leaf (*Artemisiae annuae folium*) and fruits (*Artemisiae annuae fructus* – negative control) were obtained in toluene, cca. 2 g of vegetal powder in 25 mL of solvent, by sonication, followed by centrifugation and supernatant filtration. The standard of artemisinin was also prepared in toluene (5.7 mg/25 mL). Chromatographic separation was performed on a stationary phase consisting of preformed glass plates (20×10 cm) coated with silica gel G 60 F_{254} high-performance thin-layer chromatography (HPTLC, Merck, Darmstadt, Germany). The mobile phase consisted of a mixture of cyclohexane–ethyl acetate–acetic acid (20:10:1, in volumes). Detection, UV examination (365 nm) and photodensitometric specific assessment, in fluorescence (VIS 510 nm, with "cut-off" filter at 540 nm), were performed after derivatization with anisaldehyde reagent (100^0C, 12 min.) (Gaudin & Simonnet 2002, Misra et al. 2014, Widmer et al. 2007).

The preliminary analysis of polyphenols was performed on the aerial parts of *A. annua* and *A. vulgaris* (as control) species, using a CAMAG system, in the following experimental conditions: stationary phase: TLC silica gel 60 F_{254} (Merck) 20×10 glass plates pre-washed with chloroform–methanol (1:1, *v/v*); mobile phase: chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes); samples – 20% methanolic extracts of *Artemisiae annuae herba* and *Artemisiae vulgaris herba*; standards (Merck) – 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin; migration distance 80 mm; samples (2, 4, 6, 8 and 10 µL) and standards (2 µL) application – CAMAG Linomat 5 semiautomatic system (spray gas nitrogen, dosage speed 150 nL/s, band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, data resolution 100 µm/step, measurement mode – absorption; winCATS software package (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014).

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Root

In cross-section, the root in the lower third has circular shape and secondary structure due to the presence of the two meristematic secondary areas: subero-phellodermic cambium (phellogen) and libero-ligneous cambium.

The following histological sequence was observed from the outside towards the inside of the root. Periderm is made of suber, phellogen and phelloderm. Suber is made of 3–4 layers of big, flattened cells, impregnated with suberin. From point to point, the suber is exfoliated. A single layer of anterior-posterior flattened cells, with thin walls, the radial slightly undulated, represents subero-phellodermic cambium. Phelloderm is made of 2–3 cellular layers with cellulosic thin walls. The ergastic substances are deposited into the cortical parenchyma of the primary structure. At this level, sclerenchyma fibers packs with periphloemic disposition are shown. The conducting tissues are arranged on two concentric rings. The phloem tissue forms a thin, external ring, made of sieve tubes, phloem parenchyma and annex cells. Libero-ligneous cambium is located between xylem and phloem tissues. The xylem tissue forms the internal ring, made of numerous metaxylem vessels of different calibers, disorderly placed into the libriform tissue mass, pushing to the center the protoxylem vessels with small diameter. Xylem vessels show reticulate thickenings, highlighted in longitudinal-radial sections. The protoxylem is poorly represented and accompanied by xylem parenchyma. The medullary rays are multicellular, uni- or bi-seriate, cellulosic at the level of phloem tissue ring and lignified at the level of xylem tissue ring. The medullary parenchyma is absent (Figures 1–3).

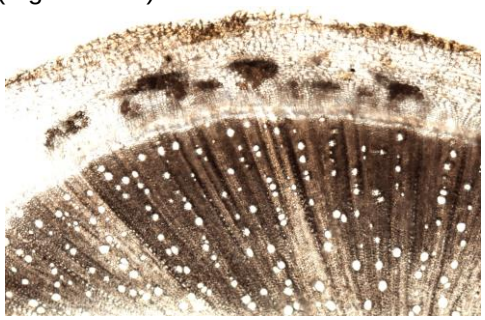

Figure 1. Cross-section through *A. annua* root: overview (Congo red–chrysoidine staining, ×40).

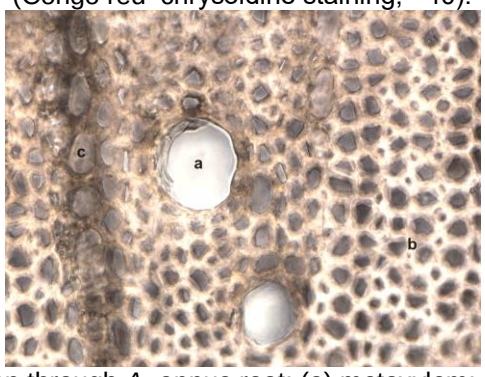

Figure 2. Cross-section through *A. annua* root: (a) metaxylem; (b) libriform tissue; (c) medullary ray (Congo red–chrysoidine staining, ×400).

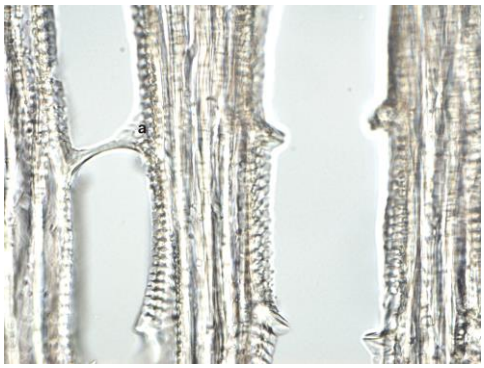

Figure 3. Longitudinal-radial section through *A. annua* root: (a) metaxylem with reticulate thickenings (Congo red–chrysoidine staining, ×400).

In cross-section, the rootlet in the upper third has circular shape and secondary structure due to the meristematic secondary zones: phellogen and libero-ligneous cambium. From the outside towards the inside of the rootlet, the same histological sequence as the main root is observed, with the difference that there are only two packages of sclerenchyma fibers in the cortical parenchyma (Figure 4).

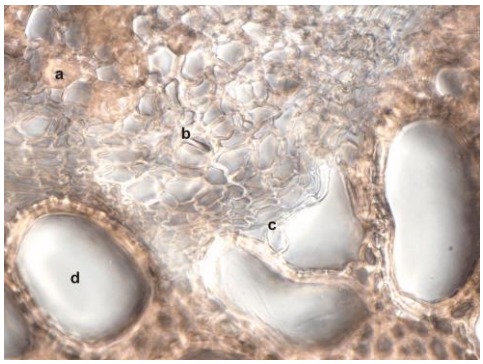

Figure 4. Cross-section through *A. annua* rootlet: (a) sclerenchyma fibers pack; (b) phloem tissue; (c) libero-ligneous cambium; (d) metaxylem (Congo red–chrysoidine staining, ×400).

Aboveground stem

In cross-section, the aboveground stem in the upper third has circular shape strongly modified by some ribs and secondary structure generated by the libero-ligneous cambium. The epidermis shows heterodiametric cells, with thickened outer wall covered by a thick cuticle. The epidermal cells are tangential elongated, with thin radial walls and thick tangential external and internal walls. From place to place, there are multicellular, uniseriate, long tector hairs, glandular hairs and stomata. The cortex is made up of 5–6 layers of angular collenchyma, at the ribs' level, less developed cortical parenchyma and sclerenchyma caps into the periphloemic area of the conducting fascicles. The conducting tissues are organized in numerous collateral-open libero-ligneous fascicles, with large sizes in front of the ribs and smaller between them, generated by the libero-ligneous cambium. The phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. The xylem tissue is made up of metaxylem vessels with different calibers and radial arrangement, accompanied by xylem parenchyma. The xylem vessels have spiral and reticulate thickenings. Primary xylem is poorly represented, with some protoxylem vessels and xylem parenchyma.

Large medullary rays are multicellular, pluriseriate, cellulosic. The medullary parenchyma is well developed, of meatus type (Figures 5 and 6).

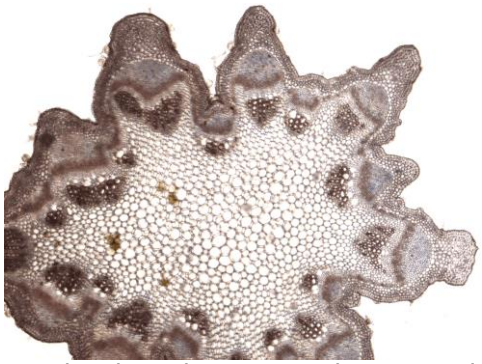

Figure 5. Cross-section through *A. annua* aboveground stem: overview (Congo red–chrysoidine staining, ×40).

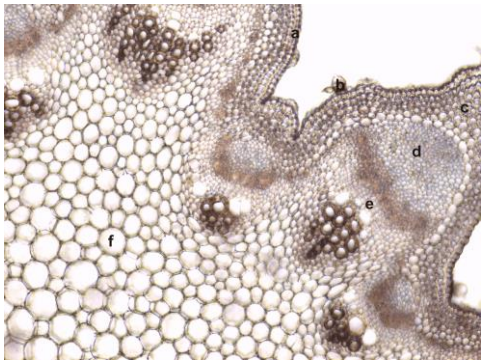

Figure 6. Cross-section through *A. annua* aboveground stem: (a) epidermis; (b) glandular hair; (c) angular collenchyma; (d) sclerenchyma fibers pack; (e) libero-ligneous conducting fascicle; (f) medullary parenchyma (Congo red–chrysoidine staining, ×100).

Leaf's limb

In cross-section, from the outside towards the inside of the leaf's limb, the following histological sequence is evidenced. Upper epidermis is made of a single layer of flattened large cells, with thickened tangential external and internal walls and thin radial walls. The external walls are bulged and covered by a thick cuticle with dentate relief. The mesophyll is made up of two layers of palisade parenchyma with large cells, slightly elongated and rich in chloroplasts, as well as of 3–4 layers of lacunose parenchyma composed of disorderly arranged small cells with aeriferous spaces. Into the mesophyll are found numerous small libero-ligneous conducting fascicles, each surrounded by an assimilatory sheath. The mesophyll is of bifacial type with dorsiventral structure. Lower epidermis is made of a single layer of tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. At this level, we found stomata, multicellular, uniseriate, long tector hairs and glandular hairs. The median rib is prominent on both the adaxial and the abaxial sides. On the outside, it shows the epidermis made up of small cells, slightly anterior-posterior flattened, having external wall covered by a thin cuticle with dentate relief. In the central area is located a single libero-ligneous conducting fascicle surrounded by assimilatory sheath and disposed in a mass of leaf's parenchyma. Into the libero-

ligneous fascicle, the xylem vessels are seriate disposition and the medullary rays are multicellular, uniseriate, cellulosic. The leaf's limb has bifacial, dorsiventral, hypostomatic structure (Figure 7).

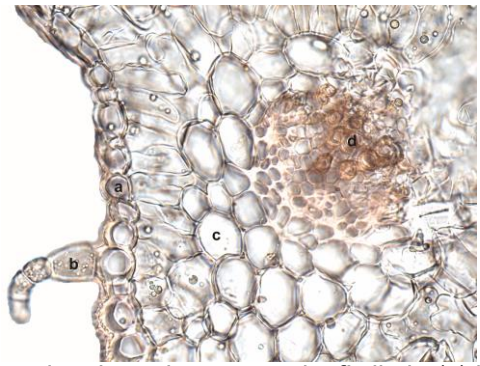

Figure 7. Cross-section through *A. annua* leaf's limb: (a) lower epidermis; (b) tector hair; (c) leaf's parenchyma; (d) libero-ligneous conducting fascicle (Congo red–chrysoidine staining, ×400).

TLC analysis

Figures 8–11 highlighted the experimental data on the HPTLC analysis of artemisinin from *Artemisiae annuae herba*. The analyzed samples include isoprenoid derivatives but also some polyphenolic compounds. *A. annua* fruits do not contain artemisinin. Artemisinin was appropriately separated in the form of bands, examined and delineated at R_f 0.44±0.01. The amount of artemisinin (cca. 0.1% – 102.45 mg per 100 g of dried vegetal product) was determined based on the calibration curve, which showed good linearity (Figure 11).

Analysis of artemisinin using HPTLC-photodensitometry method proved to be rapid, accurate, relatively inexpensive and can be applied in routine tests for the quality control of standardized herbal powders or extracts.

The results on the preliminary TLC analysis of polyphenols from *Artemisiae annuae herba* and *Artemisiae vulgaris herba* are presented in the Figures 12–15. Chlorogenic acid (R_f 0.09±0.01) was identified in different amounts – 313.25 mg and 106.95 mg per 100 g of dried vegetal products (*Artemisiae annuae herba* and *Artemisiae vulgaris herba*, respectively).

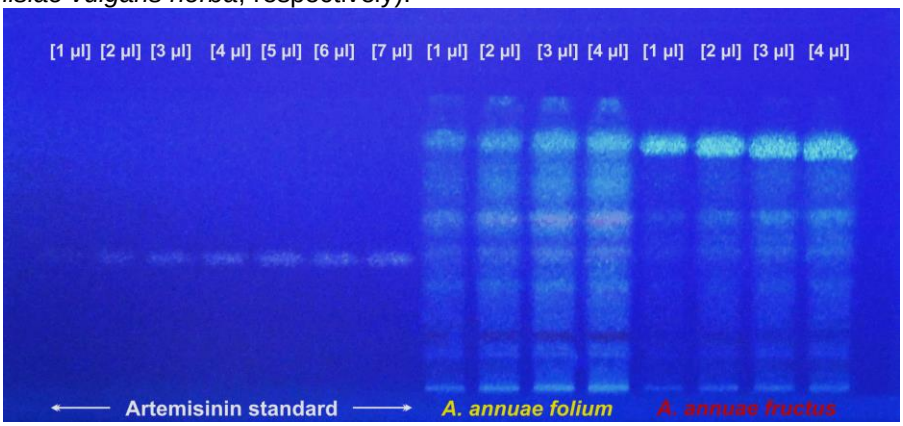

Figure 8. HPTLC chromatogram of artemisinin, isoprenoids and polyphenols from *Artemisiae annuae folium* and *Artemisiae annuae fructus* toluene extracts (UV 365 nm, derivatization with anisaldehyde reagent).

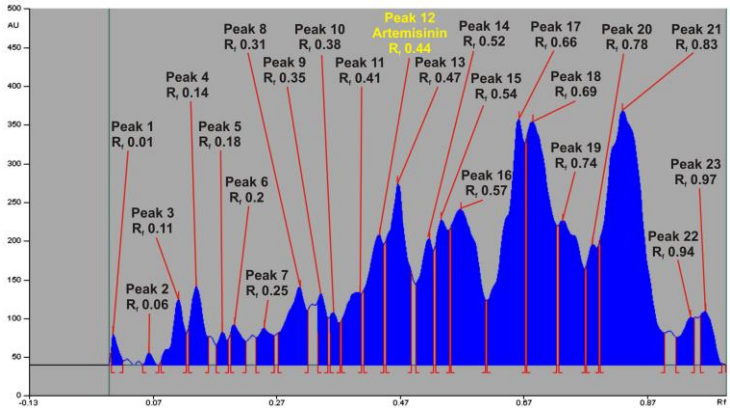

Figure 9. Photodensitogram of the compounds separated from *Artemisiae annuae folium* toluene extract (VIS 510 nm, derivatization with anisaldehyde reagent).

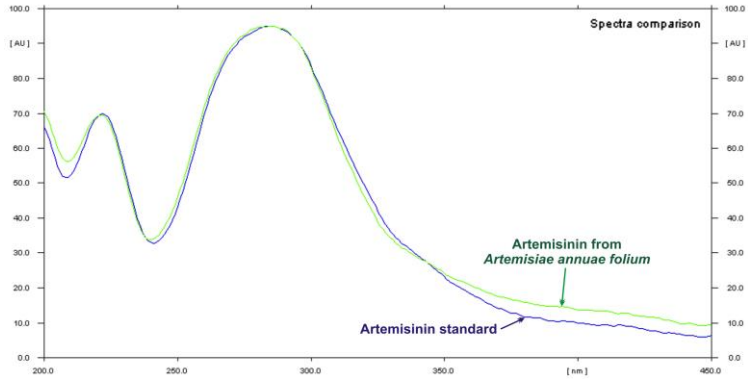

Figure 10. *In situ* UV spectrum of artemisinin standard and compound separated from the analyzed sample (*Artemisiae annuae folium* toluene extract).

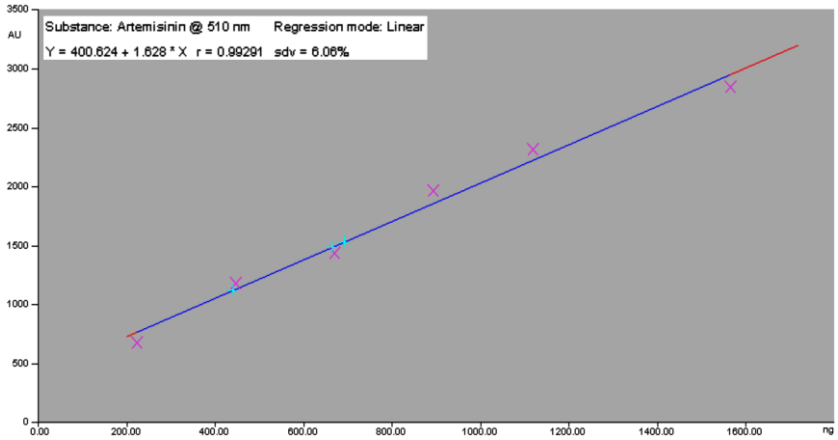

Figure 11. Calibration curve for artemisinin standard (510 nm).

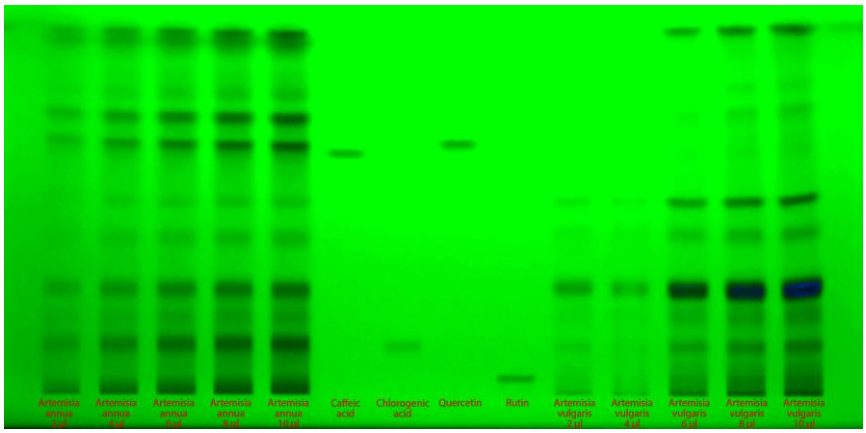

Figure 12. TLC chromatogram of polyphenols from *Artemisiae annuae herba* and *Artemisiae vulgaris herba* methanolic extracts (UV 254 nm, without derivatization). From left to right: first five applications – *Artemisiae annuae herba* sample (2, 4, 6, 8 and 10 µL); subsequent four applications – standards (2 µL); last five applications – *Artemisiae vulgaris herba* sample (2, 4, 6, 8 and 10 µL).

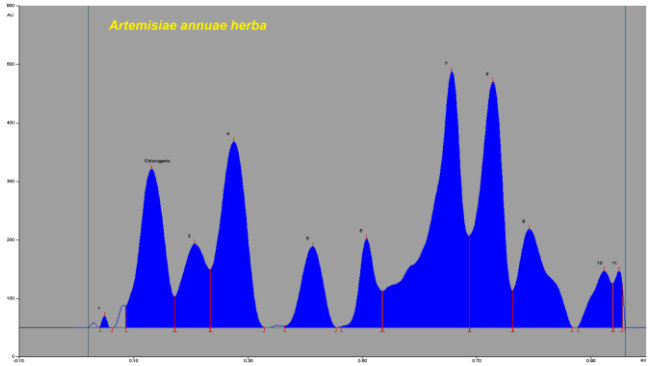

Figure 13. Densitogram of polyphenols (UV 254 nm) separated from *Artemisiae annuae herba* methanolic extract. From left to right, No. of peak/R_f: 1/0.04, 2/0.09 – chlorogenic acid, 3/0.18, 4/0.24, 5/0.37, 6/0.47, 7/0.54, 8/0.69, 9/0.77, 10/0.88, 11/0.94.

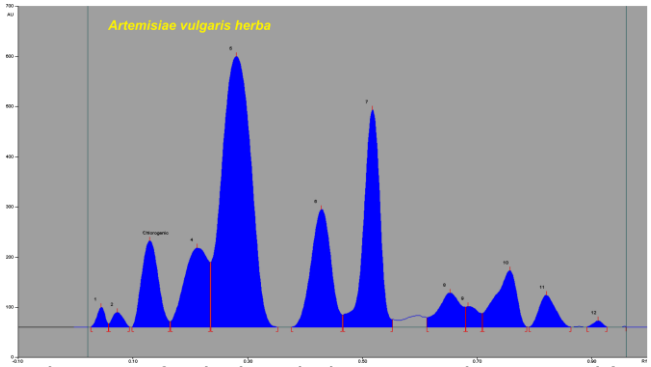

Figure 14. Densitogram of polyphenols (UV 254 nm) separated from *Artemisiae vulgaris herba* methanolic extract. From left to right, No. of peak/R_f: 1/0.03,

2/0.06, 3/0.10 – chlorogenic acid, 4/0.17, 4/0.24, 5/0.24, 6/0.38, 7/0.47, 8/0.62, 9/0.68, 10/0.71, 11/0.79, 12/0.90.

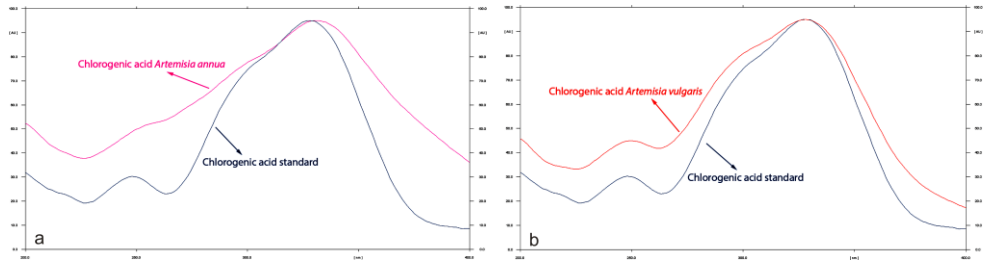

Figure 15. *In situ* UV spectra comparison of chlorogenic acid standard and compound separated from *Artemisiae annuae herba* (a) and *Artemisiae vulgaris herba* (b) methanolic extracts.

CONCLUSIONS

The histo-anatomical investigation of the root, stem and leaf of *Artemisia annua* species and the chromatographic analysis of artemisinin and polyphenols content from the leaves and aerial parts were accomplished. The root in the lower third has circular shape and secondary structure due to the presence of phellogen and libero-ligneous cambium. The aboveground stem in the upper third has circular shape strongly modified by some ribs and secondary structure generated by the libero-ligneous cambium. The leaf's limb has bifacial, dorsiventral, hypostomatic structure. The amount of artemisinin (cca. 0.1%) was established starting from the calibration curve, which showed good linearity. Chlorogenic acid was identified in different amounts – 313.25 mg and 106.95 mg per 100 g of dried vegetal products (*Artemisiae annuae herba* and *Artemisiae vulgaris herba*, respectively).

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ANATOMY OF *BEGONIA FISCHERI* SCHRANK (BEGONIACEAE) LEAF

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Keywords*: anatomy, leaf, mesophyll, photosensitive papillae, Begonia fischeri*

ABSTRACT

The paper presents anatomical aspects concerning the leaf structure of Begonia fischeri *Schrank. belonging to Begoniaceae family. Anatomically, the petiole has a single-layered epidermis and a differentiated mesophyll. The vascular system is of fascicular type, with a large number of collateral bundles placed into a basic tissue. The lamina is composed of an upper epidermis (with photosensitive papillae) and a lower one, a hypodermis, as well and the mesophyll. The mesophyll is differentiated into palisade and spongy tissue with the same vascular bundle structure such as that of the petiole, but with foliar arrangement of the conductive tissues. Cluster stomata are present on the lower epidermis.*

INTRODUCTION

The family Begoniaceae consists of two genera: *Begonia* Linnaeus, with approximately 1,500 species and pantropical distribution (Central and South America, Asia, Africa, the Pacific Islands) and the monospecific *Hillebrandia* Oliver, from Hawaiian (Jacques & Mamede 2005). The family comprises perennial herbaceous plants, suffrutescent or frutescent, with alternate leaves, sometimes asymmetrical, stipellated, with entire edge or a lobed side, lobed or divided, with variate colours. Fruits are three-winged capsules (Cruceru 2011).

Most Begoniaceae are monoecious perennials with very few dioecious exceptions. Begonias are widely spread in rainforests in the humid mountain areas, inside the woods, on the edge of water courses, on rock walls. Over 10,000 begonia hybrids and cultivars have been introduced by commercial growers. Many begonias are popular ornamentals (Awal et al. 2008).

Begonia genus is divided into 66 sections. *Begonia* is now considered to be one of the five largest genera of vascular plants (Hoover et al. 2004). These plants display a high variety of shapes, colours, patterns and textures in their leaves, rarely seen in other groups of plants. Sheue et al. (2012) concluded that the variegation is structural, due to intracellular spaces, where the light areas were created by internal reflection between the intercellular spaces. The intracellular space may occur below the superior epidermis or below the water storage tissue. Some investigations evaluate the antimicrobial and in vitro antioxidant potential of extracts of *Begonia* (Indrakumar et al. 2014), histoanatomical and physiological aspects (Lee 1974, Stratu et al. 2011), chromosome cytology (Zeilinga 1962, Peng et al. 2014), phylogenetic relationships

(Tebbitt et al. 2006), somatic embryogenesis and plant regeneration (Rosilah et al. 2014) etc.

Figure 1. Natural view of *Begonia fischeri S*chrank.

Many species were observed to have a hypoderm and abnormal stomatal patterning ("stomatal cluster"; Dehnel 1961, Tang et al. 2002). Medullary and cortical vascular bundles in the petiole and stem show an anatomical pattern more similar to that in monocotyledons than dicots. The stem has surficial cork-cambium. Physiologically, a distinctive feature of *Begonia* is the presence of oxalic acid in cystoliths, another characteristic rarely found in angiosperms (Pireyre 1961). *Begonia* species have paedomorphic secondary xylem containing thin-walled, wide, libriform fibers (Dulin 2008).

*Begonia fischeri S*chrank is a ruderal species, originating from South America: Brazil, Bolivia. It is synonymous with *Begonia fischeri* var. *elata* (Klotzsch) Irmsch. (Irmscher 1953). This species is decorative through its asymmetrical leaves. The tip is sharp, and the margins of the blade have short teeth, with numerous whitish hairs. The upper face of the blade is dark green, velvety and soft. The lower face is lighter green and glossy. The petiole is short, cylindrical, yellowish-green, all over the surface with short, very fine and whitish hairs. There are numerous brownish hairs at the junction of the petiole with the blade (Fig. 1). The fruit is a capsule with many very small seeds. It is an apartment plant, rather hardy, which grows on all types of soil (Cruceru 2011). The flowers are mostly white but also pink, grouped in 4-5 in dichasiums but may also be solitary.

The aim of this work is to analyze the anatomy of the petiole and lamina of *Begonia fischeri*. In this way we believe that the present paper will bring additional knowledge about this group of plants (*Begonia* genus), in general and, in particular, about *Begonia fischeri*.

MATERIAL AND METHODS

Plant mature leaves were collected from S.C. Iris International S.R.L in August, 2016. Small pieces of petiole and lamina were fixed in FAA (formalin: glacial acetic acid: alcohol 5:5:90). Cross sections of the leaf were performed by freehand technique (Bercu & Jianu 2003). The samples were stained with alum-carmine and iodine green. Anatomical observations and micrographs were performed with a BIOROM–T bright field microscope, equipped with a TOPICA 6001A video camera.

RESULTS AND DISCUSSIONS

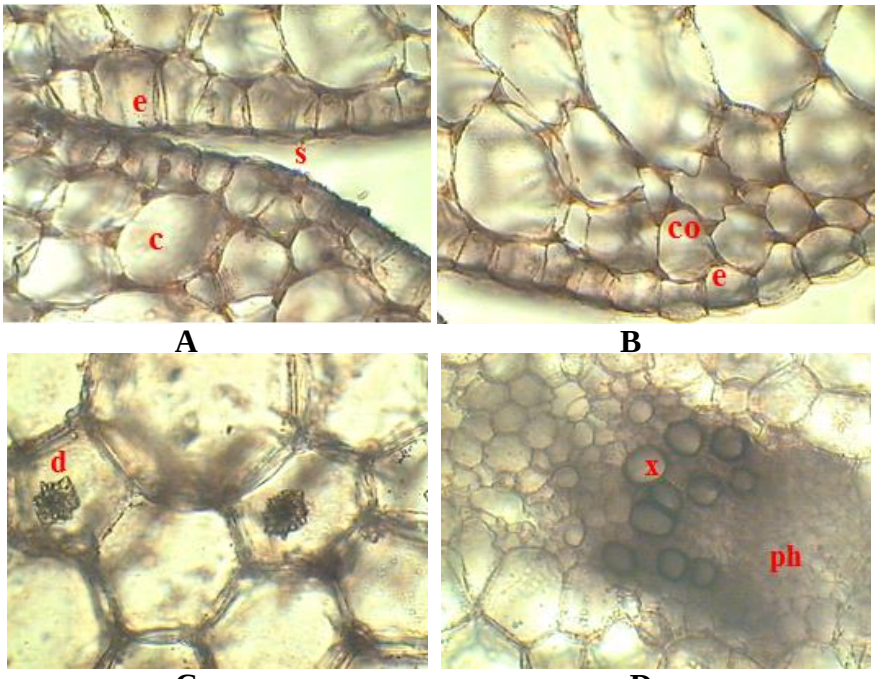

Figure 2. Cross sections of the petiole. Portion with epidermis and cortex (A, B). Portion of the basic tissue with druses (C). A petiole vascular bundle (D) (A-D, x 160): c- cortex, co- collenchyma, d- druse,e- epidermis, ph- phloem, s- slit, x- xylem.

The petiole, in cross section, is circular in shape, interrupted in the abaxial zone by a deep slit. The outer epidermis has a single layer of cells covered by a thick cuticle. It is followed by the cortex, differentiated into an external and an internal region. The external cortex is represented by one or two layers of angular collenchymatous cells, and the inner one, more developed, consisting of a number of parenchymatous cell layers (Fig. 2, A, B).

Such as in other *Begonia* species leaves (Bercu 2005), the vascular system, embadded into a basic tissue with druses (8-9 vascular bundles; Fig. 2, C), is of fascicular type, composed of a number of collateral vascular bundles, arranged on one circle. The vascular bundles have variable sizes, due to different developmental phases. Each vascular bundle has the phloem tissue towards the epidermis and the xylem tissue towards the central zone. The xylem tissue is composed of xylem vessels (4-5) with strongly lignified walls and xylem parenchyma. Phloem tissue is composed of few phloem vessels, companion cells and phloem parenchyma (Fig. 2, D).

The vascular bundles are surrounded by a bundle sheath (Fig. 2, D). The centraly located zone is composed of large, thin-walled cells with intercellular spaces.

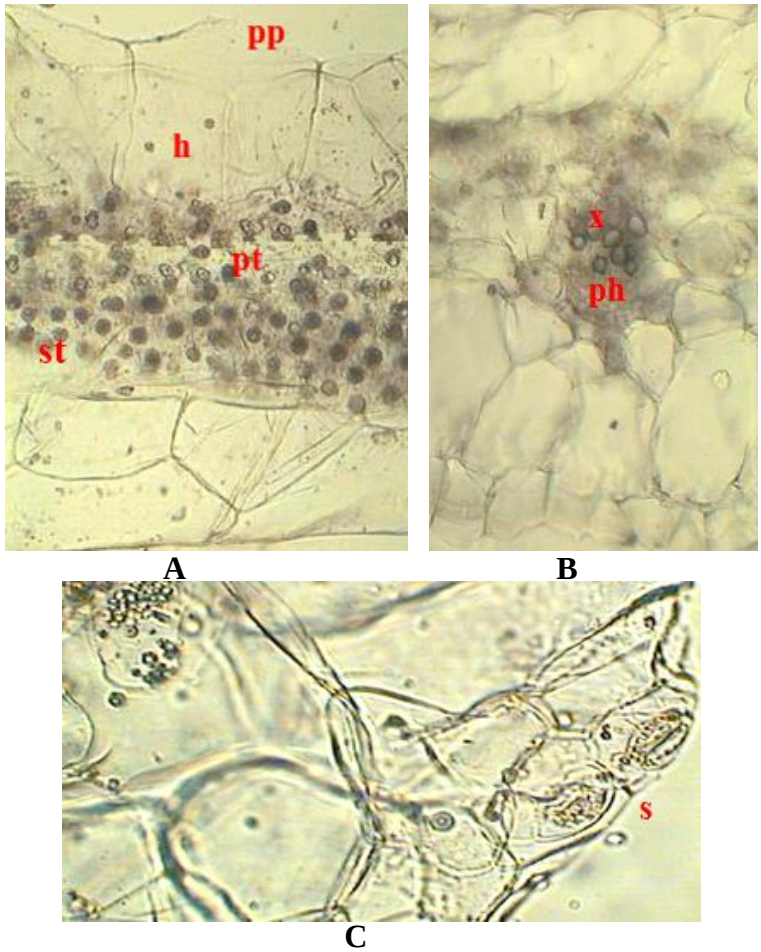

Figure 3. Cross sections of the leaf. Portion with epidermises and mesophyll (A). the midrib vascular bundle (B) (A, B, x 235). Portion of lower epidermis with stomata (C, x 320): h- hypodermis, st- spongy tissue, ph – phloem, pp- photosensitive papillae, s- stoma, st- spongy tissue, x- xylem.

Concerning the blade, the upper epidermis is made up of a layer of slightly tangentially-elongated cells, without intercellular spaces, covered by a thin cuticle. The upper epidermis shows photosensitive papillae with thick walls only in the median portion (Fig. 3; A). These papillae are specific to Begonias adapted toshadow or semi-darkness, such as Brodersen and Vogelmann (2007) reported for *B. erythrophylla* and *B. bowerae*. It seems that the plants with this type of cell usually have a highly hydrophobic surface, and the convex shape prevents water accumulation (Bhushan & Jung 2006). It is followed by a single-layered hipodermis, protodermal in nature, composed of large, radially-elongated cells. The mesophyll is differentiated into palisade and spongy tissue (heterogenous mesophyll; Ianovici 2010). The palisade tissue is more developed, composed of several cell layers (7-8 layers). Some taniniferous cells are present in the mesophyll. The spongy tissue is relatively poorly developed (3-4 cell layers; Fig. 3, A).

The midrib is slightly prominent towards the lower epidermis and less towards the upper one. The lower epidermis is also single-layered and its cells are smaller than

in the upper one, with the outer walls covered by a fine cuticle and a hypodermis below (Fig. 3, A). The vascular system of the midrib is composed of five vascular bundles. The vascular bundles have the same structure to those of the petiole but with typical foliar arrangement of conductive tissues (Fig. 3, B). Stomata, grouped (2-4) in clusters are present to the lower epidermis (hipostomatic lamina; Fig. 3, C). Neubauer & Beissler (1971) and Scott (1986) reported, for numerous *Begonia* species (122 species), that stomata clusters is a characteristic of this genus. Secretory or protective hairs are absent.

CONCLUSIONS

The petiole has a single layered epidermis and a differentiated cortex (collenchyma and parenchyma or inner cortex). The vascular system is of fascicular type, composed of a large number of collateral vascular bundles. The lamina has single-layered upper and lower epidermises and a hypodermis.

Remarkable is the presence of photosensitive papillae on the upper epidermis.The mesophyll is heterogenous and hipostomatic. The vascular system is represented by a number of vascular bundles with the common foliar arrangement of the conductive tissues. Collenchymatous tissue, placed bellow the petiole epidermis strengthens the foliar structure.

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DIVERSITY OF Lolium multiflorum Lam. CHARACTERS FROM WINTER WHEAT CROP

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Key words: *L. multiflorum, characters, diversity, fruits, winter wheat crop*

ABSTRACT

Weed control has entered a new stage. The herbicide is restricted for protect the environment and existing species, along with new ones, evolving. Adapted to the new conditions weeds might demonstrate by morphological variability. It was found that a weed with more variability of characters (including reproductive) have had adapted better and control measures will be necessary complex and diversified. Between monocots weeds that have adapted in wheat crop is ryegrass- Lolium multiflorum Lam. Weed-crop interaction produce significant damage (McKell et al. 1969; Stone et al. 1998) and to certain measures necessary to establish its integrated management (Beam et al. 2005; Hoskins et al. 2005),, were needed some studies of diversity. The way the plant grows here, reflects the specific existing ecotype at a time (Beddows 1973; Whitson et al. 2000). Does the research showed that ear was 29.7 cm long and 25.6 spikelets formed. Spikelet was 18.9 mm long, 11.2 kernels formed, had glume of 9.4 mm, 6.3 mm lower palea, and awn 5.03 mm. Kernels from the ear weight was 0.39 g, and the mass of thousand kernels weight (TKW) 1.36 g. Among some of the fruit characters were established correlations. This have been positive and very significant. The present study demonstrated the wide possibilities (Hides et al. 1993) that adapted weed in winter wheat crop.

INTRODUCTION

Species *Lolium multiflorum* Lam. [pro syn. *L.italicum* A.Braun; *L.perenne* L. ssp. *multiflorum* (Lam)Husnot; *L.aristatum* (Wild)Leg, LOLMU code Bayer] (Italian ryegrass, ryegrass, damel) has been an important increasing in winter wheat crop (Scursoni et al. 2001). Generally there are 2 types of Italian ryegrass: the *westerwoldicum*, westerwold type that is annual, and *multiflorum* or *italicum* that is short living perennial, 2-3 years. Here, the plant is autumn annual with a reduced potential for 2-3 years (Wilken 1993; Cole & Johnson, 2006). Ecotype existing in the area is robust (Bolland et al. 2001), with the strain that can reach 100 cm, are hairy, glabrous or caespitous. By improvement is a fodder plant quality (Moseley et al. 1988; Rutledge & McLendon, 1996). The inflorescence is an ear that contains a variable number of spikelets, between 10 and 25, fastened one on each side of rachis. Each spikelet has one single glume, except spikelet of the peak that may have 2 glumes.

The mass of 1000 caryopsis (thousand caryopsis mass/weight- TCW) is an average of 2.0-2.5 g and namely *L.multiflorum* diploid (2n = 14). For *L.multiflorum* diploid varieties the caryopsis grown TCW to 2.5-3.0 g. Tetraploid cultivars (2n = 28) have kernels with TCW up to 5.1 g, the big ones. Between different ecotypes of *L.multiflorum* and *L.perenne*, *Festuca pratensis* and *F.arundinacea* hybridization can occur with formation of new bodies with better adaptability to drought and persistence (Bulinska-Radomska & Lester 1988). Weed adaptability in a habitat (Thompson & Grime 1979; Rutledge & McLendon 1996) can observed by carrying out studies of diversity. In general, inflorescence of ear type is 20 cm long, terminal, wide, with spikelets 10-20 mm long, and 8-14 flowers each. Sessile flowers are gathered in spikelets placed with side on the rachis, to alternative nodes. The glume is acute, 7-12 mm long, paleas are strong and 2-veined. Each spikelet has more kernels. The caryopsis are wrapped by paleas, glumella. Research conducted to establish the diversity of plant included the main characters of ear length, number of spikelets, length of spikelet, number of kernels in a spikelet, length of glume, the length of palea, length of awns, weight of kernels per ear, and thousand of caryopisis weight (TCW).

MATERIALS AND METHODS

Measurements were made in the second half of June, in the past 3 years, on the *L.multiflorum* plants spread across many areas under winter wheat crops, from the middle of Argeș county. They were chosen randomly among weed precincts, with 100 strains of *L.multiflorum*. To each of the stems were cut out the ears, after which they were brought into the laboratory. The measurements and determinations of ear included: length, the total number of spikelets, length of spikelet, number of kernels in a spikelet, the length of the glume, length of lemma, length of awn, and weight of kernels of an ear and the weight of a thousand kernels, caryopsis (TCW).

To express the diversity of characters analyzed using an appropriate statistical method of *frequency specific polygon* or histogram. Evolution ranges of values established either by class or by absolute values as such. The specifics of each ecotype character analyzed revealed the modal value (higher frequency) and variation limits concerned.

Among the main characters were established some correlations. These are important developments of these characters by observing the trend in the newly studied ecotype. Charts were developed using Excel software.

On the other hand, measurements were processed statistically by means of analysis of variance on the ranks of variation. The indices were calculated: average ($\bar{a} = \frac{\Sigma x}{n}$), variance ($s^2 = \frac{1}{n-1}\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]$), standard error ($s = \sqrt{S^2}$) and the coefficient of variation ($s\ \% = \frac{S}{\bar{a}}$. 100).

RESULTS AND DISCUSSIONS

Diversity of ear characters**.** From what is known, *L.multiflorum* ear have the lengths of 10-25 cm (Beddows 1973). Measurements have shown that the new ecotype species had greater length, between 16 and 36 cm. The frequency distribution of these was different lengths, specific- figure 1. This highest frequency had a 27-30 cm ears long (37%), followed by those with 30-33 cm (28%), while the ears of 33-36 cm have the frequency was 17%. Shorter ears, under 21 cm accounted 1%. The graph shows a high specific variability due to competition with winter wheat (McKell et al. 1969)- figure 2.

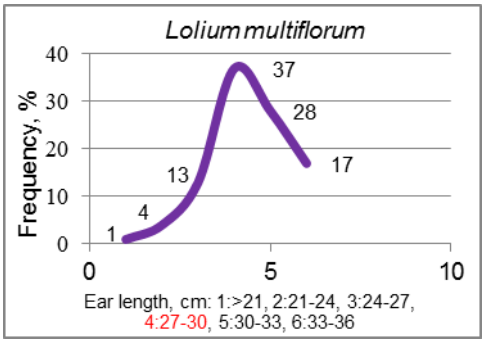
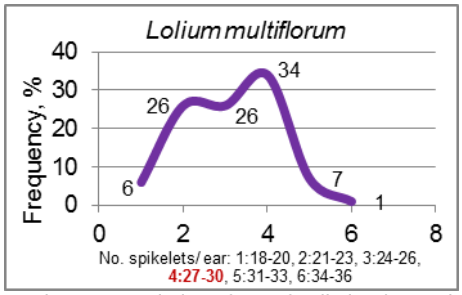


Figure 1. Evolution of ear length of *L.multiflorum*. Figure 2. The ear of weed (original)

Number of spikelets was between 18 and 36. Their frequency distribution was different. The ear with their most frequent were those with 27-30 spikelets (34%). However, 86% of ears were between 21 and 36 nember of spikelets. Spikelet length was variable- figure 3. They dominated those with 18-19 mm (43%). Spikelet length limit was between 14 and 26. High frequency was 43% at 18-19 mm spikelet length- figure 4.



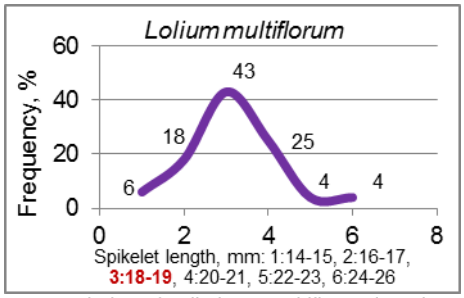
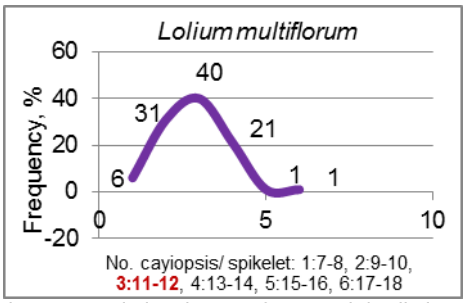


Figure 3. Evolution of no. of spikelets/ ear. Figure 4. Evolution of spikelet *L.multiflorum* length

As a number, spikelet formed kernels between 7 and 18. The higher frequency has a spikelet with 11 and 12 kernels (40%). Near they were spikelets with 9-10 kernels (31%) and with 13-14 kernels (21%). Spikelets with fewer kernels accounted for 6% of the total, and spikelets with 15-16 and 17-18 kernels were by 1% each- fiure. 5. Glume length of weed had specific variability. Thus, it was between 6 and 13 mm. The highest frequency have had the 8-9 mm (49%), followed by those with 10 to 11 mm (40%)- figure 6. Shorter glume, 6-7 mm, accounted for 6%, while those with 12 to 13 mm, 5%.



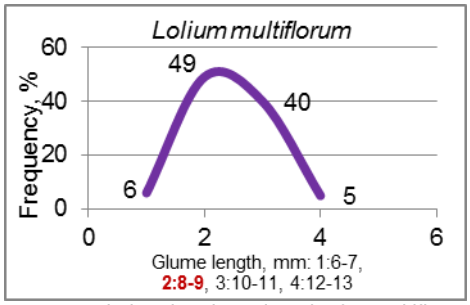
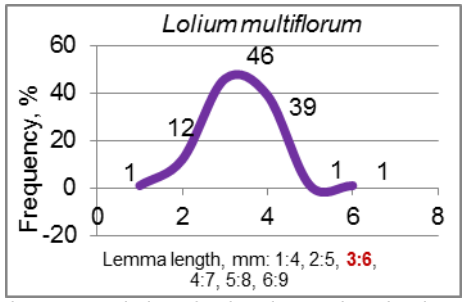


Figure 5. Evolution the no. of caryopsis/ spikelet. Figure 6. Evolution the glume length of *L.multiflorum*

The length of the down palea- lemma had a characteristic variability. Thus, it had lengths between 4 and 9 mm. The highest frequency had a glume than 6 mm long (46%)- figure 7. Awns length known limits between 1 and 10 mm- figure 8. Modal value stood at 42% for 5-6 mm lengths. It was near lengths of 3-4 mm (36%), and then the 7-8 mm (14%). Awns of 1 to 2 mm were with 5% frequency, while those with 9-10 mm, 3%.



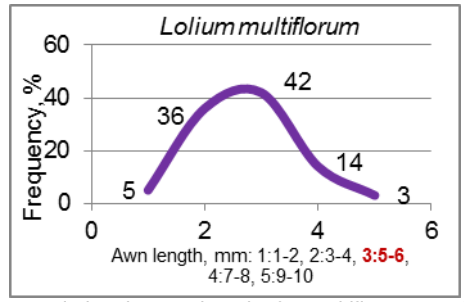


Figure 7. Evolution of palea- lemma length. Figure 8. Evolution the awn length of *L.multiflorum* weed

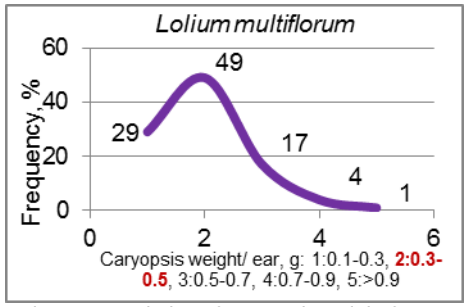


Figure 9. Evolution of caryopsis weight from a ear. Figure 10. Caryopsis of *L.multiflorum* weed

Kernels/ caryopsis from the ear weight was 0.10- 0.99 g as limits. The highest frequency had a 0.30-0.50 g (49%). Spikelets with lighter kernels, of 0.10-0.30 g represented 29%, and 0.50-0.70 g of 17%. Ears with kernels heavier than 0.71-0.90 g and over 0.90 g were below 5%- figure 9. The weed produce a lot of caryopsis- figure 10.The mass of 1,000 kernels (thousand caryopsis weight-TCW) was the limit between 0.50 and 2.3 g. The modal value was at 1.1-1.5 g (45%). Kernels of 0.7-1.1 g were 24% of the total, while those with 1.5-1.9 g, 20%. Kernels heavier, 1.9-2.3 g accounted for 10%, and those below 0.7 g, 1%- figure 11. The caryopsis details are characteristic- figure 12.

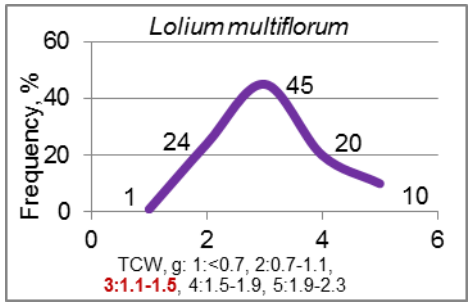


Figure 11. Evolution of TCW values. Figure 12. Caryopsis with and without palea

Correlations between different morphological characters**.** In general, the characters determined have demonstrated both negative aspects, as well as positive- table 1. Negative correlations were obtained between the number of flowers/ spikelet with awn length (r = -0.323^{000}) and between the spikelets/ spike with down palea length (r = -0.163). Positive correlations have been established in many situations. Stood out most of thousand caryopsis wieght (TCW) with ear length (r = 0.282*) and between TCW with spikelet length (r = 0.361***). Some present them in detail.

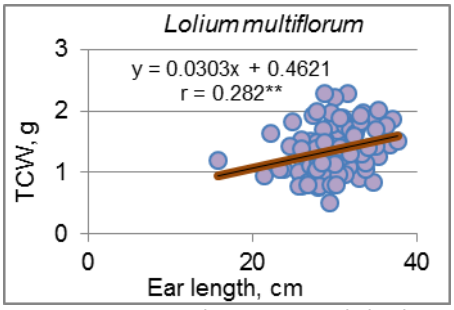
Table 1

Correlations between differing characters of *L.multiflorum* weed fruits

Character	Ear length	Total no. spikelets	Spikelet length	No.flowers spikelet	Glume length	Down palea length	Awn length	Caryosis weight	TCW
Ear length	1	0.417***	0.426***	0.355***	0.186	0.026	-0.063	0.517***	**0.282***
Total no. spikelets		1	0.173	0.127	0.020	**-0.163**	**-0.012**	0.443***	-0.014
Spikelet length			1	0.482***	0.455***	0.136	-0.024	0.566***	**0.361*****
No. flowers/ spikelet				1	-0.003	-0.032	**-0.323^{000}**	0.561***	0.058
Glume length					1	0.229*	**0.230***	0.039	0.036
Down palea length						1	**0.222***	0.061	0.197*
Awn length							1	-0.120	0.076
Caryopsis weight								1	0.738***
TCW									1
LSD 5 % = 0.19 LSD 1 % = 0.25 LSD 0.1 % = 0.32									

From the study of the correlation between ear length and weight of 1,000 kernels (TCW) resulted in a favorable situation. The correlation coefficient, positive, was r = 0.282**- figure 13. The relationship between the number of spikelets from ear with the number of caryopsis formed in a spikelet was weaker and characteristic, r = 0.127- figure 14. Both correlations show that the weed had a good adaptability under crop conditions analized.

Between spikelet length and weight of the kernels of an ear, was obtained also a highly positive and significant correlation (r = 0.566***)- figure 15. In another correlation between the length of the glume and palea length, a positive correlation was obtained, providing significant, r = 0.229*- figure 14. The explanation could be that the plant forms different lengths of glume that have a less dependent with palea length.



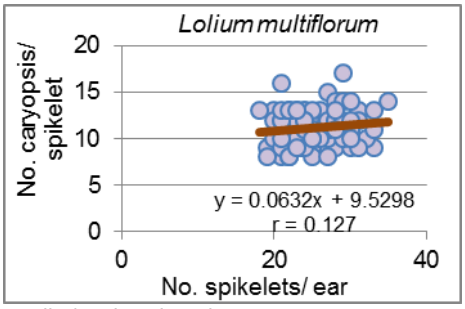
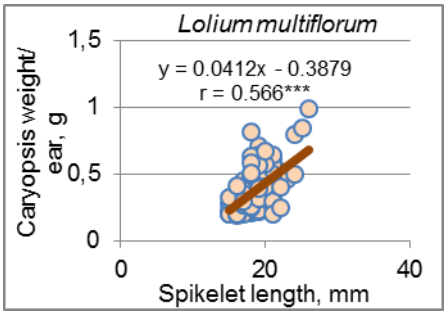


Figure 13. Correlation between spike/ear length and TCW.

Figure 14. Correlation between no. spikelets/ spike and no. of caryopsis from of *L.multiflorum* spikelet



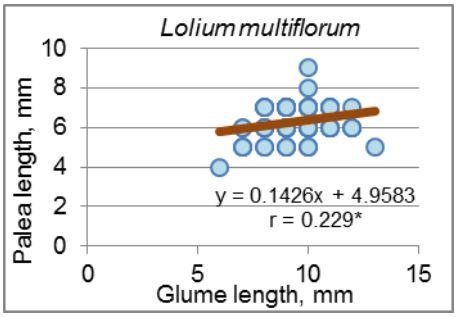


Figure 15. Correlations between spikelet length with caryopsis weight/ ear (spike).
Figure 16. Correlation between glumes length with palea length of *L.multiflorum* weed

Statistical indices of characters diversity. From the statistical values that have emerged highlighted specific new *L.multiflorum* ecotype, that was spread to southern of territory- table 2 and table 3. The ear length was 29.7 cm, and were formed in the each ear 25.6 spikelets. Spikelet length was 18.9 mm, number of kernels/ spikelet 11.2, glume length 9.4 mm, length of down palea 6.3 mm, and awn length was 5.03 mm. Kernels from the ear weighted 0.39 g, and the weight of 1,000 kernels was 1.36 g. The coefficients of variation were decreased for the length of ear (11.90%), spikelet length (11.39%) a,d length of lower palea (11.99%). Middle averages were number of spikelets of ear (15.35%), number of kernels in a spikelet (16.34%), and length of glumes (13.17%). High variability had awn lwngth (32.76%), weight of kernels in a ear (34.46%) and especially the weight of 1,000 kernels (TCW)(87.05%).

Table 2

Statistic indices of *L. multiflorum* spikes (ears)

Indices	Ear length, cm	No. spikelets/ ear	Spikelet length, mm	No. caryopsis/ spikelet
Media, ā	**29.74**	**25.62**	**18.9**	**11.15**
Variance, s^2	12.52	15.47	0.046	3.321
Standard error, s	3.54	3.93	0.215	1.822
Coef. of var. %	**11.90**	**15.35**	**11.39**	**16.34**

Table 3

Statistic indices of *L. multiflorum* caryopsis

Indices	Glume length, mm	Lemma length, mm	Awn length, mm	Weight caryopsis/ ear, g	TCW, g
Media, ā	**9.42**	**6.28**	**5.03**	**0.391**	**1.362**
Variance, s^2	0.015	0.006	0.027	0.018	0.116
Standard error, s	0.124	0.075	0.165	0.135	0.340
Coef. of var. %	**13.17**	**11.99**	**32.76**	**34.46**	**87.05**

CONCLUSIONS

Ecotype *L.multiflorum* weed that has spread recently in the area analyzed, showed good adaptability in winter wheat crop. This is demonstrated by the diversity of its characters. Such study could help to promote more complete it's specific management. The presence of this monocots in wheat crop has some new characters, specific. Thus, the ear was much longer than normal and formed more spikelets. Spikelet length was greater, with relatively normal glume. Down palea was 0.4-0.9 cm

and awn of 0.1-0.9 mm. Kernels formed in an ear was 8-17 with MMB's something less, between 0.5-2.3 g- table 4.

Table 4

Characters variability of *Lolium multiflorum* Lam. Fruits

No.	Fruit characters	Literature	Determinations
1.	Ear length, cm	20	15.8 – 37.6
2.	No. spikelets/ ear	10 - 25	18 - 33
3.	Spikelet length, cm	1.0 2.0	1.5 – 2.6
4.	Glume length, cm	0.7 – 1.2	0.6 – 1.2
5.	Down palea length, cm	-	0.4 – 0.9
6.	Awn length, mm	-	0.1 – 0.9
7.	No. caryopsis/ ear	-	8 - 17
8.	Caryopsis weight/ ear, g	-	0.20 – 0.81
9.	Thousand caryopsis weight, TCW, g	2.0 – 2.5	0.496 – 2.276

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CLIMATIC CONSIDERATIONS ON THE DROUGHTY SUMMER OF 2016 IN OLTENIA

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Key words*: drought, scorching heat, climate risks.*

ABSTRACT

The year 2015 has been the warmest year on record coinciding with the intensity peak of the El Nino which prolonged until May 2016 (as noted by NOAA and WMO). Owing to the intense El Nino, from a temperature standpoint,in 2016 the global mean has exceeded the pre-industrial average by 1.0°C for the first time. In Oltenia, the winter of 2015-2016 has been the warmest on record, and was followed by the earliest spring arrival ever registered. From June 15th until September 15th the weather was warm. The drought started mid-June in the Oltenia Plain and the Southern hill area, July brought about a gradual extension, and by August the entire region was affected. August was very droughty, its first day being the warmest when the maximum thermal value of the summer 2016, 38.0°C, was recorded in Bechet. All crops were seriously affected leading to significant negative impacts on the national economy.

INTRODUCTION

Year 2015 has been, according to global average, the warmest year registered since the beginning of weather records and coincided with the intensity peak of El Nino climate phenomenon. Global mean air temperature has exceeded the global mean from preindustrial era for the first time with 1.0°C. In Oltenia, the winter of 2015-2016 has been the warmest since the beginning of weather records, although January 2016, was a normal thermal month during which a cold wave in the interval 19-26 January. During this cold wave the minimum air temperature dropped below -20.0°C in some localities (in the morning of 24 January 2016 minimum temperature values were comprised between -23.3°C in Caracal and -11.1°C in Drăgășani) and there were human casualties because of frost. The spring arrival in 2016 has been the earliest since measurements are performed in Romania, and indexes of spring arrival have become absolute climate records excepting the values registered in 2002 in Calafat, Apa Neagră and Voineasa. February has been for the entire Romania the warmest month ever registered in the measurements history. We will further analyse climate aspect of the droughty summer of 2016, in Oltenia. The paper is part of series of extended studies on the variability of the climate in Oltenia and climate changes and is useful for all people interested in climate changes and their consequences (Marinică, Andreea, Floriana Marinică 2014, 2016).

RESULTS AND DISCUSSIONS

3.1. **Climatic characteristics of June 2016.** ***Thermal regime of June 2016.*** *Monthly air temperature means* were comprised between 17.8°C in Voineasa Intracarpathian Depression and 23.1°C in the extreme South-West in Calafat, and their *deviations* from the multiannual means were comprised between 1.3°C in the central part of Oltenia in Craiova and 3.6°C in Subcarpathian Depressions in Polovragi, leading to classifications of thermal time type on relief levels of Oltenia from warmish (WS) in most part of Oltenia Plain and on restricted areas at the Southern limit of hills and in Subcarpathians to warm (W) in most part of the region (56.3%). *Air temperature mean for the entire region* was 21.0°C, and its deviation from the multiannual mean was 2.6°C leading to the classification of *warm month on average for the entire region*.

Monthly maximum values of air temperature were registered in the interval 18-24 June and were comprised between 31.9°C in Subcarpathian Depressions in Polovragi and 36.8 in South-West in Calafat and Olt Couloir in Caracal, and their mean for the entire region was 34.5°C. Most of them exceeded the scorching heat threshold, and the first heat wave lasting 7 days was registered during this interval (18-24 June). *Monthly minimum air temperature values* were registered in the first decade of the month when the weather was cool (CO) and were comprised between 6.8°C in Voineasa and 12.2°C in the extreme West in Dr. Tr. Severin and in Olt Couloir in Drăgășani, and their mean for the entire region was 10.0°C.

Parameters variation characterizing air temperature has been increasing, and the fast increase was recorded by the maximum temperature values.

Most of the *monthly maximum temperatures on ground surface* were registered on 22 and 23 June (during the heat wave) and were comprised between 39.7°C in Caracal and 63.6°C in Dr. Tr. Severin, and their mean for the entire region was 51.5°C. Most of the *monthly minimum temperature values on ground surface* were registered on 9 June and were comprised between 7.4°C in Polovragi and 14.7°C in Caracal, and their mean for the entire region was 11.1 °C.

Pluviometric regime of June 2016. *Monthly quantities of precipitation* were comprised between 30.2 mm in Băilești, 199.0 mm in Polovragi, being the highest monthly quantity of summer and 103.1 mm in Parâng, and *their deviations from the multiannual means* were comprised between -54.6% in Oltenia Plain in Băilești and 101.4% in the hilly area in Tg . Logrești, which leads to classifications of pluviometric time type from exceedingly droughty (ED) in Oltenia Plain in Băilești and exceedingly rainy (ER) in hilly area, Subcarpathian Depressions, in Olt Couloir and at the mountain (Tg. Logrești, Apa Neagră, Polovragi, Rm. Vâlcea and Parâng) (Table no. 1).

Mean of monthly quantities of precipitation for the entire region was 104.4 mm, and its percentage deviation from the normal was 24.0%, which designates a rainy month (R) overall for all Oltenia region. In June only two days with significant rains were registered on extended areas on 1st June and 13th June. The maximum quantity of precipitation registered in 24 hours was 105.5 mm in Costeşti, in Vâlcea County on 13th June.

On the same date significant values of precipitation were registered in 24 hours in: Craiova in Dolj County (38.6 mm), Bâcleş in Mehedinți County (41.7 mm), Balta in Mehedinți County (62.9 mm), Tg. Logreşti in Gorj County (65.4 mm), Podari in Dolj County (66.3 mm), Baia de Fier in Gorj County (72.3 mm). On 13th June the highest quantities of precipitation were registered of all summer 2016, and the mean calculated for the entire region was 26.5 mm, namely 25.4% of the mean for the whole region. In the end of June, on 28 June, in wheat crop, on the depth of 0-100 cm, ***water reserve*** was almost optimum (AlO) and optimum (O) in the Northern third of the region, and in most part of the region almost satisfactory (AS). The phenomenon of

moderate pedological drought (MD) occurred on restricted area in the South-West (Băileşti Plain-Calafat), in South-East (Corabia) and in Olt Couloir in Rm. Vâlcea. In corn crop, on the depth of 0-100 cm, water reserve was almost optimum (AlO) in most part of the region and almost satisfactory (AS) in Băileşti and Romanaţi Plain.

Table no. 1.

Quantities of precipitation (mm) registered in the summer of 2016 (Σ), compared to the normal values (N) for the period 1901-1990, deviation (%) and pluviometric time type according to Hellmann criterion (CH) (ED = excessively droughty, VD = very droughty, D = droughty, LD = little droughty, N = normal, LR = little rainy, R = rainy, VR = very rainy, ER = excessively rainy).

Meteorological Station	Hm	June 2016				July 2016			
		ΣVI	N	Δ%	**CH**	**ΣVII**	N	Δ%	**CH**
Dr. Tr. Severin	77	90.6	72.5	25.0	**R**	127	49.3	**157.6**	**ER**
Calafat	66	49.8	65.6	-24.1	**D**	88.6	45.6	94.3	**ER**
Bechet	65	67.4	62.3	8.2	**N**	***8.2***	46.6	***-82.4***	**ED**
Băileşti	56	***30.2***	66.5	***-54.6***	**ED**	21.5	45.0	-52.2	**ED**
Caracal	112	72.4	73.7	-1.8	**N**	41.0	53.8	-23.8	**D**
Craiova	190	73.4	71.2	3.1	**N**	39.8	51.4	-22.6	**D**
Slatina	165	48.6	80.6	-39.7	**VD**	35.0	57.5	-39.1	**VD**
Bâcleş	309	97.8	72.0	35.8	**VR**	61.9	47.1	31.4	**VR**
Tg. Logreşti	262	145.6	72.3	**101.4**	**ER**	54.8	49.5	10.7	**LR**
Drăgăşani	280	46.6	87.6	-46.8	**VD**	28.8	51.6	-44.2	**VD**
Apa Neagră	250	149.1	99.2	50.3	**ER**	**129.4**	72.7	78.0	**ER**
Tg. Jiu	210	71.2	93.0	-23.4	**D**	125.4	61.9	102.6	**ER**
Polovragi	546	**199.0**	112.3	77.2	**ER**	114.4	88.9	28.7	**R**
Rm. Vâlcea	243	143.4	86.9	65.0	**ER**	59.0	98.0	-39.8	**VD**
Voineasa	587	132.2	106.7	23.9	**R**	116.2	88.6	31.2	**VR**
Parâng	1585	253.0	124.1	**103.1**	**ER**	**154.0**	132.1	16.6	**LR**
Mean Oltenia	**-**	104.4	84.2	24.0	**R**	75.3	65.0	15.9	**LR**
Ob. Lotrului	1404	266.0				151.9			
Meteorological Station	Hm	August 2016				Summer 2016			
		ΣVIII	N	Δ%	**CH**	**ΣS**	N	Δ%	**CH**
Dr. Tr. Severin	77	39.2	38.2	2.6	**N**	256.8	160.0	**60.5**	**ER**
Calafat	66	39.8	35.6	**11.8**	**LR**	178.2	146.8	21.4	**R**
Bechet	65	12.0	37.9	-68.3	**ED**	87.6	146.8	-40.3	**VD**
Băileşti	56	26.4	39.0	-32.3	**VD**	***78.1***	150.5	-48.1	**ED**
Caracal	112	***4.4***	39.9	***-89.0***	**ED**	117.8	167.4	-29.6	**VD**
Craiova	190	34.2	42.1	-18.8	**LD**	147.4	164.7	-10.5	**LD**
Slatina	165	36.2	46.8	-22.6	**D**	119.8	184.9	-35.2	**VD**
Bâcleş	309	33.1	33.4	-0.9	**N**	192.8	152.5	26.4	**R**
Tg. Logreşti	262	28.4	43.6	-34.9	**VD**	228.8	165.4	38.3	**VR**
Drăgăşani	280	18.6	46.4	-59.9	**ED**	94.0	185.6	***-49.4***	**ED**
Apa Neagră	250	44.1	60.1	-26.6	**D**	322.6	232.0	39.1	**VR**
Tg. Jiu	210	29.2	64.3	-54.6	**ED**	225.8	219.2	3.0	**N**
Polovragi	546	35.8	76.5	-53.2	**ED**	**349.2**	277.7	25.7	**R**
Rm. Vâlcea	243	**44.4**	69.4	-36.0	**VD**	246.8	254.3	-2.9	**N**
Voineasa	587	36.0	72.8	-50.5	**ED**	284.4	268.1	6.1	**N**
Parâng	1585	37.4	90.6	-58.7	**ED**	**444.4**	346.8	28.1	**VR**
Mean Oltenia	**-**	31.2	52.3	-40.3	**VD**	210.9	201.5	4.7	**N**
Ob. Lotrului	1404	**116.9**				534.8	0.0		

(Source: Data processed from NMA archive)

3.2. Climatic characteristics of July 2016.

Thermal regime of July 2016. *Monthly average temperatures* were comprised between 18.1°C in Voineasa and 24.4°C in Caracal in Romanați Plain, and the deviations from the multiannual means were comprised between 0.6°C in Apa Neagră in the area of Subcarpathian Depressions and 1.9°C in Slatina and Drăgășani in Olt Couloir, leading to classifications of thermal time type of warmish month (WS) in most part of the region excepting some restricted areas where it was normal (Băilești, Tg. Logrești and Apa Neagră). *Monthly temperature mean for the entire region* was 22.2°C, only 1.2°C higher than the mean of June, and the deviation from the normal was 1.3°C, confirming the classification of warmish month (WS), overall for the entire region.

Monthly minimum temperatures were registered on 8 and 9 July and were comprised between 7.7°C in Voineasa and 14.4°C in Caracal, and their mean for the region was 10.8°C, only 0.8°C higher than the mean of June. *Monthly maximum air temperatures* were registered on 14 July, excepting the mountainous area and were comprised between 30.3°C in Polovragi and 37.5°C in Calafat, and their mean for the entire region was 33.5°C, with 1.0°C lower than June. In July there wasn't any heat wave, only ***one warming on 14 July***, the preceding day of the most significant rains of July.

Parameters charts characterizing air temperatures were slightly increasing, and the fastest increase was recorded by the maximum value.

On ground surface, most of the monthly minimum temperature values were registered on 8 and 9 July and were comprised between 8.3°C in Polovragi and 17.2°C in Caracal, and their mean for the entire region was 12.3°C. Monthly maximum thermal values on ground surface were comprised between 43.1°C in Calafat and 67.0°C in Dr. Tr. Severin, and their mean for the entire region was 55.1°C, with 3.6°C higher than the mean of June.

Pluviometric regime of July 2016. *Monthly quantities of precipitation in July* were comprised between 8.2 mm in Bechet in the South of Oltenia, which is the lowest value of summer and 129.4 mm in Apa Neagră, and in the mountains 154.0 mm in Parâng, and *their percentage deviations* from the normal value were comprised between -82.4% in Bechet and 157.6% in Dr. Tr. Severin, leading to classifications of pluviometric time type from exceedingly droughty (ED) in Băilești Plain (Băilești-Bechet area) and very droughty (VD) on the entire Olt Couloir in the South of Carpathians to exceedingly rainy (ER) in the West and extreme South-West in Dr. Tr. Severin and Calafat and in the Subcarpathian Depression in Apa Neagră and Tg. Jiu. Droughty weather (D) was registered in the central part of Oltenia (Craiova area) and in Romanați Plain (in Caracal) (Table no. 1). ***In Olt Couloir in Drăgășani and Slatina the very droughty weather was a continuation of the same weather registered in June.*** Monthly mean of precipitation for the entire region was 75.3 mm, and the deviation from the multiannual mean was 15.9% leading to the classification of little rainy month (LR) overall for the entire region, but with high pluviometric contrasts between East and West. One significant rainy interval was registered on 15th and 16th July during which daily means for the entire region were 31.1 mm and 22.0 mm, but even in these two days the quantities of precipitation were not uniform (comprised between 8.2 mm in Bechet and 118.2 mm in Dr. Tr. Severin). In Bechet area in the extreme South from 19th June to 15th July (26 days) there was no rain, and then during the interval 17th July-31st July (14 days) it was the same situation, and in August only 12 mm were registered, distributed on 5 days with significant precipitation.

3.3. Climatic characteristics of August 2016

Thermal regime of August 2016. *Monthly average temperatures* were comprised between 17.4°C in Voineasa and 24.0°C in Caracal, and their deviations from the multiannual means were comprised between 0.1°C in the hilly area and Subcarpathian Depressions in Tg. Logrești and Apa Neagră and 1.8°C in Olt Couloir in Rm. Vâlcea, leading to classifications of pluviometric time type on Oltenia relief levels from normal (N) in most part of the region (62.5%) to warmish (WS) in the extreme West in Dr. Tr. Severin, in Romanați Plain in Caracal, in Olt Couloir in Drăgășani and Slatina and in the mountainous area in Voineasa and Parâng. *Monthly mean* for the entire region was 21.5°C, lower with 0.7°C than the mean of July, and its deviation from the normal was of 1.0°C at the lower limit of classification of warmish month (WS) overall for the entire region.

Most of the *monthly maximum air temperature values* were registered on 1 August and were comprised between 31.3°C in Voineasa and 38.0°C in Bechet, the latest being ***the maximum thermal value of the summer of 2016 in Oltenia.***

For the entire country the maximum thermal value of the summer of 2016 was 38.2°C registered in Turnu Măgurele and Giurgiu in the same day on 1st August 2016. *Mean of monthly maximum thermal values in the air* for the entire region was 33.8°C, with only 0.3°C higher than the mean of July. Most of *minimum air temperatures* were registered on 14th August and were comprised between 5.9°C in Voineasa and 12.3°C in Caracal, and their mean for the entire region was 9.2°C, lower with 1.6°C than the mean of July. ***The warmest day of the summer of 2016 was 1st August,*** and then there was a very slow and long term cooling process, and air temperature has decreased until December.

On ground surface, most of the *monthly maximum temperatures* were registered and were comprised between 41.5°C in Apa Neagră and 63.6°C in Dr. Tr. Severin, and their mean for the entire region was 54.3°C, with 0.8°C lower than the mean of July. Most of the monthly minimum temperatures on ground surface were registered on 14th August and were comprised between 6.6°C in Polovragi and 12.8°C in Calafat, and their mean for the entire region was 10.6°C, with 1.7°C lower than the mean of July. Therefore, ***excepting the monthly mean for the entire region of maximum air temperatures, the other monthly means for the entire region have dropped compared to July.***

Parameters charts *characterizing air temperature* had slightly decreasing values, and the fastest decrease was registered by daily minimum temperatures.

Pluviometric regime of August 2016. *Monthly quantities of precipitation* were comprised between 4.4 mm in Caracal and 44.4 mm in Rm. Vâlcea, and *their percentage deviations* were comprised between -89.0% in Caracal and 11.8% in Calafat (only two positive deviations, in Calafat and Dr. Tr. Severin), leading to classifications of pluviometric time type from exceedingly droughty (ED) and very droughty (VD) in the whole region to little rainy (LR) in Calafat (Table no. 1). *Mean of monthly quantities of precipitation for the entire region* was 31.2 mm, and its percentage deviation from the normal was -40.3% leading to the classification of very droughty month (VD) overall for the entire region. Only on 1st and 24th August significant precipitation were registered for agriculture locally, while in the other days there were no precipitation or there were insignificant. In Olt Couloir and Băilești Plain July drought continued in this month, as well as the droughty regime (D) in the central part.

On 31 August, water reserve in the ground layer of 0-20 cm field was at the level of extreme (ED) and severe drought (SD) in most part of the region and only in

the North region was almost satisfactory (AS). The same situation was for the ground layer on the depth of 0-100 cm in corn crop.

CONCLUSIONS

The summer of 2016 was warm and droughty, summer drought has gradually expanded after 14th June, and on 13th June there were the most significant rains of this summer. In the middle of June on 15th and 16th July there were significant rains, but without any notable effect on crops, because drought and scorching heat have soon come back.

Daily maximum temperatures reached and exceeded 35.0°C starting from 17th June, and the monthly temperature means have increased, the warmest month from this point of view was July, although all the maximum thermal values of summer were registered on 1 July, then the decreasing temperature has been maintained on long term until the end of December.

Two moderate heat waves were registered in the interval 18-24th June and 27th July-1st August and one single weather warming on 14th July.

Drought became more severe due to temperatures exceeding 30.0°C, registered for long periods of time with minimum values comprised between 15 and 22.2°C. Atmospheric and pedological drought was more intense in the East of the region, especially in Băilești Plain, Romanați Plain and Olt Couloir, where the water reserve maintained low during the entire summer.

All crops, especially spring crops have been damaged.

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HISTO-ANATOMICAL AND PRELIMINARY CHROMATOGRAPHIC RESEARCHES ON *GLECHOMA HEDERACEA* L. (*LAMIACEAE*) SPECIES

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ABSTRACT

The paper presents the histo-anatomy of the root, stem and leaf of Glechoma hederacea L. species and the preliminary chromatographic researches of the polyphenols content from the aerial parts (Glechomae hederaceae herba). In the thin-layer chromatogram, rutin was identified from 11 specific bands of the polyphenolic compounds.

INTRODUCTION

Glechoma hederacea L., Ground-ivy, Gill-over-the-ground (*Lamiaceae*), is an Eurasian species, herbaceous, perennial, which blooms in April–June, in forest edges, hedges, wet and shady places (Ciocârlan 2000).

From the phytochemical point of view, the medicinal product *Glechomae hederaceae herba* contains a wide range of active principles, as follows: flavonoids (Belščak-Cvitanović et al. 2014, Kikuchi et al. 2008, Yamauchi et al. 2007), essential oil (Judzentiene et al. 2015), sterols (Kikuchi et al. 2008), pentacyclic triterpenoids (Kikuchi et al. 2008), lignans and neolignans (Kikuchi et al. 2008, Yamauchi et al. 2007), heteropolysaccharides / polyuronides – mucilages (Belščak-Cvitanović et al. 2014), polyphenolcarboxylic acids (Belščak-Cvitanović et al. 2014), lectins (Wang et al. 2003).

Glechomae hederaceae herba extracts highlighted some pharmacological actions, such as: antibacterial, antioxidant, immunomodulatory, antitumoral, diuretic, spasmolytic, antitussive, anti-inflammatory, capillaroprotective, cicatrizing, emollient (Judzentiene et al. 2015, Kikuchi et al. 2008, Yamauchi et al. 2007).

In the specialty papers, there are some data concerning *G. hederacea* histo-anatomy (Akçin et al. 2011, Ninova et al. 1984, Siebke & Weis 1995). The aim of our paper was the histo-anatomical investigation of the root, stem and leaf of the above-mentioned species and the preliminary chromatographic analysis of the polyphenols content from the aerial parts (*Glechomae hederaceae herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested from *G. hederacea* plants in blossom, in May 2016, from the surroundings of Radovan village, Valea Rea zone, Dolj County (southwestern Romania).

The fixation and preservation of roots, aboveground stems and leaves were made in 70% ethanol. The cross-sections and longitudinal-radial sections were obtained using botanical razor.

After washing with distilled water, the sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture (Genevese reagent) was used for the staining of sections. Depending on the chemical composition of cell membranes, the reactive induced various colors: pink to red for cellulose and mucilage, pale red for cytoplasm, yellow for suberin and brown for lignin (Andrei & Paraschivoiu 2003).

Stained and mounted sections were analyzed on a Krüss binocular photon microscope (objectives ×4, ×10, ×20). The photos were taken with a Nikon Eclipse 55i binocular microscope coupled with a Nikon DS–Fi1 high definition video camera. The image acquisition and processing was done using the Image-Pro Plus ver. 6.0 software package.

The description of microscopic sections was made according with some classical authors (Toma & Ruginǎ 1998).

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was performed on the aerial parts of *G. hederacea* species (*Glechomae hederaceae herba*), using a CAMAG (Muttenz, Switzerland) system, in the following experimental conditions: stationary phase: TLC silica gel 60 F_{254} (Merck, Darmstadt, Germany) 20×10 glass plates pre-washed with chloroform–methanol (1:1, *v/v*); mobile phase: chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes); sample: 20% methanolic extract of *Glechomae hederaceae herba*; standards (Merck): 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin; migration distance: 80 mm; sample (1–10 µL) and standards (2 µL) application – CAMAG Linomat 5 semiautomatic system (spray gas nitrogen, dosage speed 150 nL/s, band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, data resolution 100 µm/step, measurement mode – absorption; winCATS software package (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014).

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Root

In cross-section, the root in the lower third has circular shape and primary structure. From the outside towards the inside of root, the following histological sequence was evidenced. Rhizodermis is exfoliated but in some areas, it consists of small cells with thin, cellulosic walls and with long absorbent hairs. Exodermis is made up of a single layer of large heterodiametric cells, with suberin-impregnated walls and from point to point with passage cells. The cortical parenchyma consists of five layers of large oval cells with thin, cellulosic walls and with variable size intercellular spaces. Endodermis is made up of a single layer of large cells, antero-posterior flattened and provided with Casparian strips. A single layer of cellulosic pericycle, having cells

disposed alternately with endodermic cells, delimits the central cylinder. Xylem fascicles are placed alternately with the phloem ones, and separated by multicellular, uniseriate, cellulosic medullary rays. Within the fascicles, protoxylem and protophloem are disposed near the pericycle, and metaxylem and metaphloem in the central part of the fascicles. Metaxylem has reticulate thickenings and occupies the central part of the root, replacing the medullary parenchyma (Figure 1).

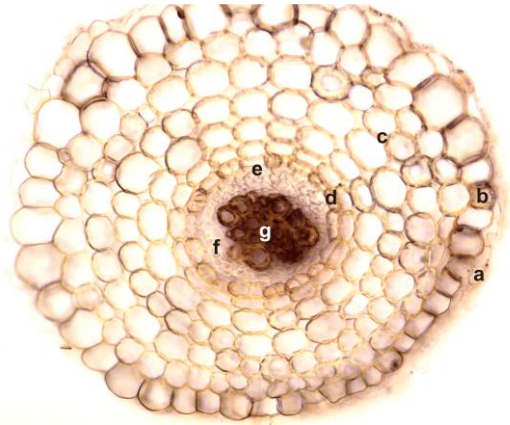

Figure 1. Cross-section through *G. hederacea* root: (a) rhizodermis; (b) exodermis; (c) cortical parenchyma; (d) endodermis; (e) pericycle; (f) phloem fascicle; (g) xylem fascicle (Congo red–chrysoidine staining, ×200).

Aboveground stem

In cross-section, the aboveground stem in the lower third has four-rib shape and secondary structure generated by the libero-ligneous cambium. The epidermis is made up of heterodiametric cells with thickened outer wall covered by a thick cuticle with dentate relief. The epidermal cells are antero-posterior flattened, with thin radial walls and thick tangential external and internal walls. Tector hairs, glandular hairs and stomata are observed from place to place. The cortex is made up of two areas: the outer area consisting of 4–5 layers of angular collenchyma disposed at the level of the four edges and 2–3 layers of tabular collenchyma between the edges. The inner zone of the cortex is composed of 4–5 layers of cortical parenchyma bounded inward by a single layer of primary-type endodermis made up of large, suberin-impregnated and flattened cells. The conducting tissues are organized into four large libero-ligneous fascicles of collateral-opened type, placed at the edges. Between these appeared new, small-sized libero-ligneous fascicles emerged from the libero-ligneous cambium activity. Within large fascicles, the phloem is made up of sieve tubes, phloem parenchyma and annex cells. At this level, the medullary rays are multicellular, pluriseriate, cellulosic. The secondary xylem is made up of metaxylem vessels with different calibers scattered into the well-represented libriform mass. The primary xylem is poorly represented, made up of some protoxylem vessels and xylem parenchyma. At this level, the medullary rays are large, multicellular, pluriseriate, strongly lignified. The medullary parenchyma is well developed, of meatus type, and contains ursines (Figures 2 and 3).

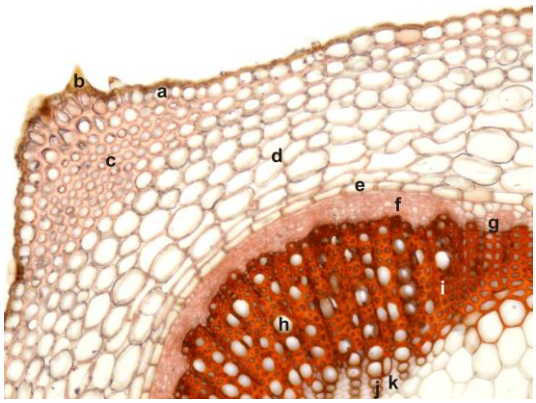

Figure 2. Cross-section through *G. hederacea* aboveground stem: (a) epidermis; (b) tector hair; (c) angular collenchyma; (d) cortical parenchyma; (e) endodermis; (f) phloem tissue; (g) libero-ligneous cambium; (h) metaxylem; (i) libriform tissue; (j) protoxylem; (k) xylem tissue (Congo red–chrysoidine staining, ×100).

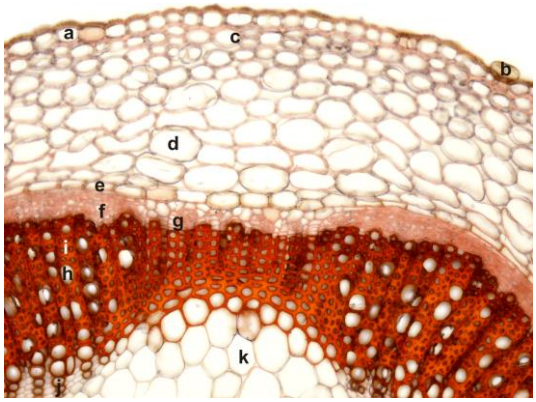

Figure 3. Cross-section through *G. hederacea* aboveground stem: (a) epidermis; (b) glandular hair; (c) tabular collenchyma; (d) cortical parenchyma; (e) endodermis; (f) phloem tissue; (g) libero-ligneous cambium; (h) metaxylem; (i) libriform tissue; (j) protoxylem; (k) medullary parenchyma (Congo red–chrysoidine staining, ×100).

Leaf

Leaf's limb

In cross-section, the following histological sequence is observed from the outside towards the inside of leaf's limb. Upper epidermis is made up of one layer of flattened large cells with thickened tangential external and internal walls and thin radial walls. The external walls are bulged and covered by a thick cuticle with dentate relief. The mesophyll is organized in one layer of palisade parenchyma made up of large, elongated cells, rich in chloroplasts, as well as of 4–5 layers of lacunose parenchyma composed of disorderly arranged small cells with aeriferous spaces. Multiple small libero-ligneous conducting fascicles are found into the mesophyll. The mesophyll is of bifacial type, with dorsiventral structure. Lower epidermis is made up of one layer of tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. At this level, we found stomata, unicellular tector hairs, sharpened at the tip, and glandular hairs. The median rib is protruding on the abaxial side, as well as in the adjacent secondary ribs. On the outside, the epidermis consists of small cells,

slightly anterior-posterior flattened, the external wall being covered by a thin cuticle with dentate relief. At the adaxial pole, rare unicellular tector hairs, sharpened at the tip, are evidenced. Under the epidermis, at both adaxial and abaxial poles, we evidenced 2–3 layers of angular collenchyma. In the central zone is found one libero-ligneous conducting fascicle, disposed in the leaf's parenchyma mass. Into the libero-ligneous fascicle, xylem vessels have seriate disposition and the medullary rays are uniseriate, cellulosic. The leaf's limb has hypostomatic, bifacial dorsiventral structure (Figures 4 and 5).

Figure 4. Cross-section through *G. hederacea* leaf's limb: overview (Congo red–chrysoidine staining, ×40).

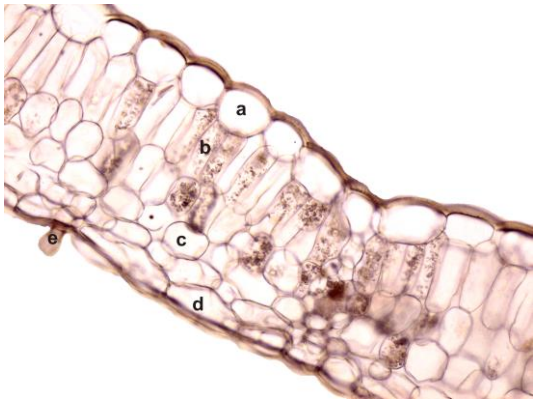

Figure 5. Cross-section through *G. hederacea* leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) lower epidermis; (e) glandular hair (Congo red–chrysoidine staining, ×200).

Petiole

The petiole has semicircular shape, with flat adaxial side modified by two adaxial extensions. The following histological sequence was evidenced in cross-section, from the outside towards the inside of petiole. A single epidermal layer consists of heterodiametric small cells, with thin radial walls and thickened tangential external and internal walls. A thin cuticle with dentate relief covers the external wall. From point to point, we found stomata. The angular collenchyma is organized in 2–3 layers under the epidermis. In the leaf's parenchyma, there are three libero-ligneous

conducting fascicles, one centrally arranged and the other two placed one in each extension (Figure 6).

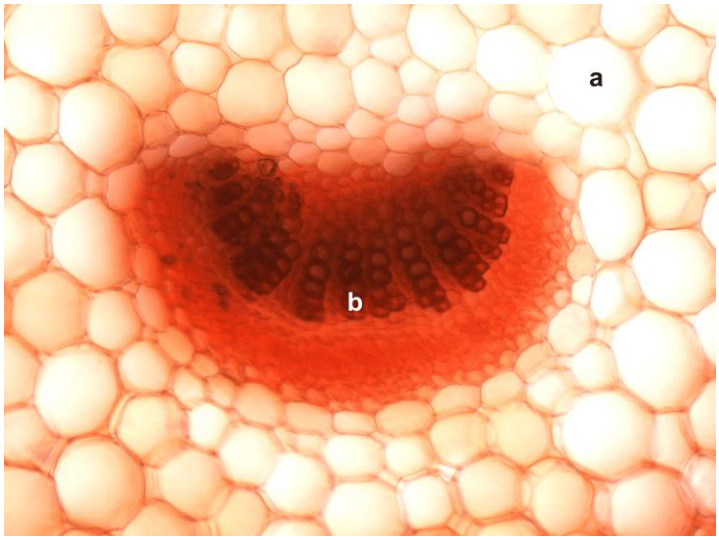

Figure 6. Cross-section through *G. hederacea* petiole: (a) leaf's parenchyma; (b) libero-ligneous conducting fascicle (Congo red–chrysoidine staining, ×200).

TLC analysis

The experimental results about the preliminary chromatographic analysis of polyphenols from *Glechomae hederaceae herba* are exhibited in Figures 7–9. Rutin (R_f 0.05) was identified in a concentration of 164.5 mg/100 g of dried vegetal product.

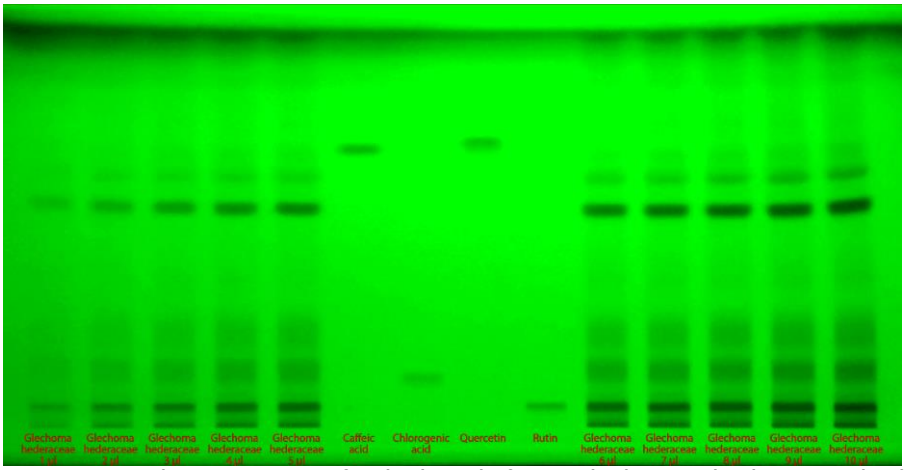

Figure 7. TLC chromatogram of polyphenols from *Glechomae hederaceae herba* methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

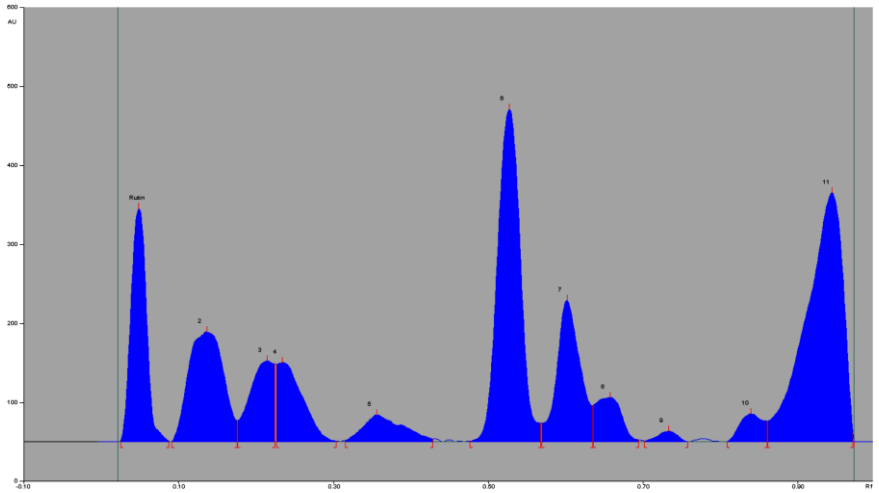

Figure 8. Photodensitogram of polyphenols (UV 254 nm, without derivatization) separated from *Glechomae hederaceae herba* methanolic extract. From left to right, No. of peak/R_f: 1/0.05 – rutin, 2/0.14, 3/0.22, 4/0.24, 5/0.36, 6/0.53, 7/0.60, 8/0.66, 9/0.74, 10/0.84, 11/0.95.

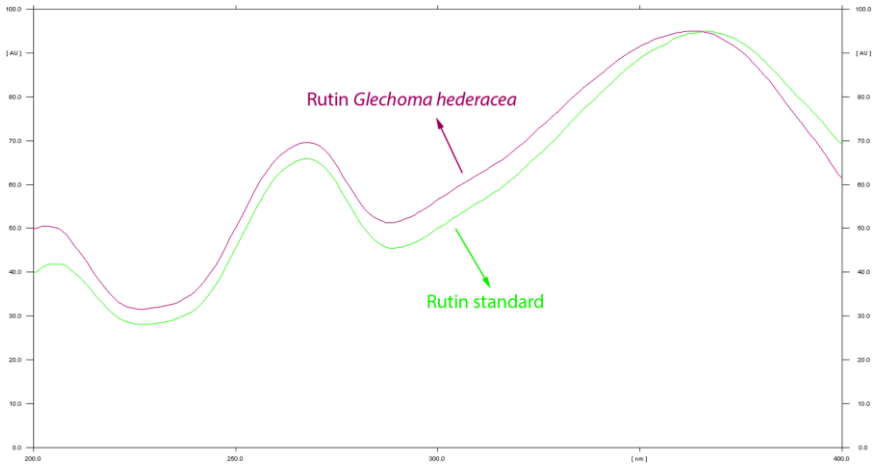

Figure 9. *In situ* UV spectra of rutin standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical research of the root, aboveground stem and leaf of *Glechoma hederacea* species and the preliminary chromatographic assay of the polyphenols from *Glechomae hederaceae herba* were achieved. In the lower third, the root has circular shape and primary structure. The aboveground stem, in the lower third, has four-rib shape and secondary structure generated by the libero-ligneous cambium. The leaf's limb has hypostomatic, bifacial dorsiventral structure. The petiole has semicircular shape, with flat adaxial side modified by two adaxial extensions. Of the 11 specific bands for the polyphenolic compounds evidenced in the thin-layer chromatogram, only rutin was identified and quantified.

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HIGH-SENSITIVITY C-REACTIVE PROTEIN - PREDICTOR MARKER FOR CARDIOVASCULAR DISEASE

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Key words*: inflammation, CRP, hs-CRP, inflammatory marker*

ABSTRACT

It has been known for ages that inflammation plays a major role in the development of heart disease. High-sensitivity C-reactive protein (hs-CRP) is produced by the body when blood-vessel walls are inflamed. hs-CRP is a marker of inflammation that predicts incident myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death in healthy individuals without history of cardiovascular disease and relapsing events and death in patients with acute ailments or stable coronary syndromes. In recent years, hs-CRP has been endorsed by several public health organizations as a biomarker of cardiovascular disease risk.

INTRODUCTION

The “inflammation hypothesis” sidelined for decades in favor of the so-called cholesterol hypothesis has resurfaced and many scientists believe that inflammation is a primary causative factor in many chronic diseases of today (https://www.docsopinion.com/health-and-nutrition/hscrp/).

Advances in vascular biology have shown that inflammation plays an integral role in the pathogenesis of cardiovascular disease and some inflammatory markers such as CRP and hs-CRP actually constitute cardiovascular risk factors (Ridker 2001, 2004, https://www.revistagalenus.ro/practica-medicala/evaluarea-riscului-cardiovascular-proteina-c-reactiva/, https://www.docsopinion.com/health-and-nutrition/hscrp/).

Studies on the etiology of cardiovascular disease (CVD) have highlighted numerous serum markers as candidates for "non-traditional" risk factors. The correlation of CRP serum concentration with the severity, extent and progression of many pathologies and the prognostic significance of these associations emphasizes that CRP is not only a marker of disease, but also contributes to pathogenesis (https://bioclinica.ro/analize/biochimie/proteina-c-reactiva).

CRP (C-reactive protein) is one of a group of proteins called acute phase reactants that go up in response to inflammation, most notably in response to pneumococcal (bacterial) infectious, histolytic disease and a variety of disease states. (http://www.drg-diagnostics.de/65-1-Equipment+DRGHYBRiD-XL.html, https://www.docsopinion.com/health-and-nutrition/hscrp/).

The levels of acute phase reactants increase in response to certain inflammatory proteins called cytokines. released by damaged tissue These proteins are produced by white blood cells during inflammation (Devaraj 2015, Starkebaum

2017). CRP is also, produced by adipose tissue and by cells in the vascular wall such as endothelial cells, smooth muscle cells (Devaraj 2015).

C-reactive protein is a non-glycosylated protein with the calcium dependent pentameric structure, which belongs to the pentraxin family.

(https://www.revistagalenus.ro/practica-medicala/evaluarea-riscului-cardiovascular-proteina-c-reactiva/)

The kinetics evolve rapidly with a half-life of 18 hours. The value increases at 6-7 hours after contact with the pathogen to reach a maximum of 72 hours.

The level returns to normal in about a week. In order to quantify the inflammatory response and once, in the inflammatory profile CRP dosing is often associated with other inflammation proteins with slow kinetics (haptoglobin) (https://bioclinica.ro/analize/biochimie/proteina-c-reactiva).

Originally discovered by Tillet et al. in 1930 in patient sera with acute infection, CRP has now come to be used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to therapy and surgery. Furthermore, regular measurements of CRP in infants can be a useful aid in the early diagnosis of infectious disease (http://www.drg-diagnostics.de/65-1-Equipment+DRGHYBRiD-XL.html, https://www.docsopinion.com/health-and-nutrition/hscrp/).

There are two different tests for CRP assessment. The standard test measures a much wider range of CRP values, but is less sensitive on the lower ranges. The hs-CRP test can more accurately detect low protein concentrations (more sensitive), making it more useful than the CRP test in estimating the risk of a healthy person doing cardiovascular disease.

(http://www.labtestsonline.ro/tests/hs_CRP.html?tab=5)

Both levels of CRP and hs-CRP become elevated in a wide range of acute and chronic inflammatory conditions who cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP by the liver.

(https://www.docsopinion.com/health-and-nutrition/hscrp/).

The C-reactive protein is measured to concentrations of 3 to 5 mg/l. In contrast, hs-CRP is measured to concentrations of about 0.3 mg/l, improved sensitivity that allows it to be used to detect low levels of chronic inflammation (https://www.pritikin.com/high-sensitivity-c-reactive-protein).

Blood measurements of hs-CRP are often performed to assess the risk of future cardiovascular disease (Ridker 2001, https://www.docsopinion.com/health-and-nutrition/hscrp/).

Atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process. The researchers have hypothesized that inflammatory markers such as hs-CRP may provide an adjunctive method for global assessment of cardiovascular risk (Ridker 2001).

The high-sensitivity C-reactive protein is a marker of inflammation that predicts incident myocardial infarction, stroke (Bassuk et al. 2004, Ridker 2001) peripheral arterial disease (Bassuk et al. 2004, Pearson et al. 2003) and sudden cardiac death in healthy individuals without history of cardiovascular disease and relapsing events and death in patients with acute ailments or stable coronary syndromes (Bassuk et al. 2004).

Studies seem to indicate that CRP prediction power is independent of the presence of hypertension, diabetes, high cholesterol, age, despite the evidence that CRP levels are somewhat elevated in those with these risk factors. Because of this independence, CRP could be added as predictive value near the Framigham

score(https://www.revistagalenus.ro/practica-medicala/evaluarea-riscului-cardiovascular-proteina-c-reactiva/).

These data suggest that hs-CRP may have a role in risk stratification of patients with established CVD, although more data are needed that compare the prognostic value of elevated levels of hs-CRP with other prognostic measures currently in use. (Pearson et al. 2003, Aldica et al. 2006).

Because hr-CRP is a marker of inflammation, it can detect low levels of inflammation, but can manifest itself in the future in different ways. Most studies have focused on heart disease, but recent research shows that CRP values at the upper limit of the normal range may also be associated with other diseases such as increased risk of cancer death (Young-Jin Ko et al. 2012), colon cancer with complications diabetes and obesity

(https://labtestsonline.org/understanding/analytes/hscrp/tab/faq).

Obesity is associated directly with increased plasma levels of hs-CRP, an observation consistent with findings that adipocytes secrete interleukin-6, a primary hepatic stimulant for CRP production. Individuals with clinically apparent inflammatory conditions such as rheumatoid arthritis or lupus are likely to have elevations of hs-CRP well into the clinical range (Ridker 2001).

MATERIAL AND METHODS

Since hs-CRP is a predictor marker for coronary artery disease, I have studied its dynamics determined by the immunoturbidimetric method, a simple and sensitive method which allows the risk stratification in patients with CHD.

The DRG:HYBRID-XL CRP-HS is an immunoturbidimetric assay for the quantitative *in vitro diagnostic* measurement of CRP in serum and plasma (EDTA-, heparin- or citrate plasma). The measuring range of the assay is between 0.054 mg/L – 10 mg/L.

The hs-CRP levels was determined with the DRG:HYBRID-XL analyzer (http://www.drg-diagnostics.de/65-1-Equipment+DRGHYBRiD-XL.html).

The hs-CRP test is for apparently healthy people to determine the risk of cardiovascular disease (http://www.labtestsonline.ro/tests/hs_CRP.html?tab=5).

This paper is based on a retrospective statistical study that includes individuals who had completed medical check-up and had been screened for serum hs-CRP concentrations at the Medical Analysis Laboratory Priority Medical, Craiova, between February 2017 and June 2017.

hs-CRP determinations were also correlated with LDL cholesterol (LDL-C) determinations of the same patients.

It is important that any person having this test be in a healthy state for the results to be of value in predicting the risk of coronary disease or heart attack.

Any recent illness, tissue injury, infection, or other general inflammation will raise the amount of CRP and give a falsely elevated estimate of risk (https://labtestsonline.org/understanding/analytes/hscrp/tab/faq).

Because hs-CRP levels increase with acute infections and trauma, testing should be avoided within 2 to 3 weeks in patients who have had a respiratory tract infection or other acute disease (Ridker 2001).

Subjects with inflammatory/infectious associated illnesses whose serum hs-CRP concentrations >10 mg/L were excluded by monitoring other inflammatory markers.

In the final analysis were included a total of 140 participants. The study included subjects with or without coronary disease, men and women, aged between 21 and 80 years. Individuals in the study were divided according to clinical criteria, normal

or pathological results of hs-CRP and LDL-C determinations, epidemiological criteria, gender and age of the patients (reviewed in the analysis report of each patient) (Table 1, Table 2).

Table 1

hs-CRP dynamics based on risk group, age group and gender

Age group	hs-CRP low risk		hs-CRP average risk		hs-CRP high risk	
	male	female	male	female	male	female
21-40 years	4	12	16	16	16	8
41-60 years	4	10	1	4	15	2
61-80 years	8	4	4	4	8	4
Total	16	26	21	24	39	14

Table 2

The correlation between hs-CRP and LDL-C based on risk groups and gender

LDL-C low risk						LDL-C high risk					
hs-CRP low risk		hs-CRP average risk		hs-CRP high risk		hs-CRP low risk		hs-CRP average risk		hs-CRP high risk	
M	F	M	F	M	F	M	F	M	F	M	F
8	8	13	12	19	8	8	20	8	8	20	8

Through the statistical processing of the results from the analysis bulletins a data base was developed which was used for their graphical and tabular representation, interpretation and discussion of the results, as well as draw conclusions by reading them.

RESULTS AND DISCUSSIONS

Individuals in the study were divided by gender (76 male subjects and 64 female subjects) and by age groups (21-40 years, 41-60 years, 61-80 years).

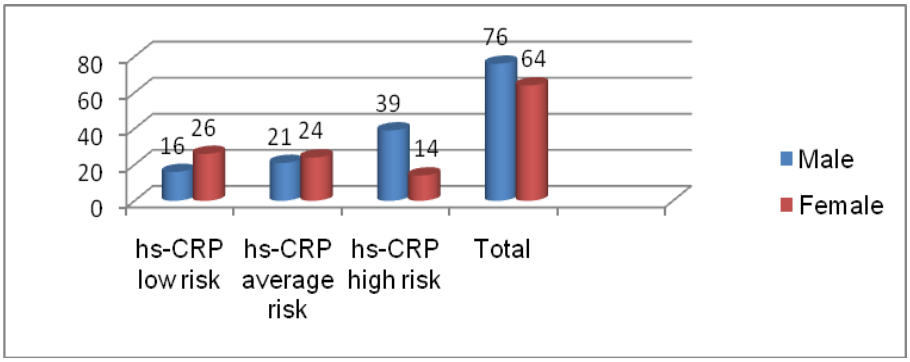
The American Heart Association and U.S.Centers for Disease Control and Prevention have defined hs-CRP risk groups as follows:

- hs-CRP less than 1.0 mg/L - low risk,
- hs-CRP - 1.0 to 3.0 mg/L - average risk,
- hs-CRP above 3.0 mg/L-high risk (Starkebaum 2017, https://labtestsonline.org/understanding/analytes/hscrp/tab/faq).

So, according to their serum levels of hs-CRP, the participants were categorized into 3 groups: low, average and high risk.

According to their serum levels of LDL cholesterol (LDL-C), the participants were categorized into 2 groups, as follows: LDL-C < 125 mg/dl - low risk, LDL-C > 125 mg/dl - high risk.

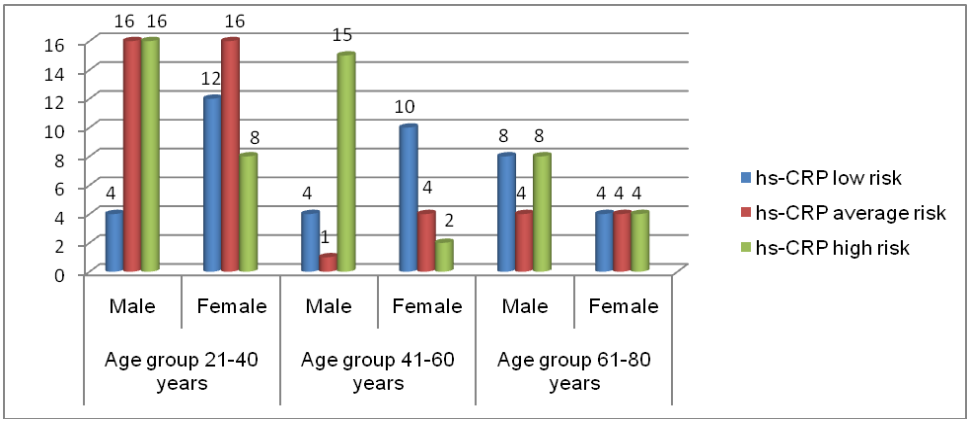
The analysis of the distribution according to the hs-CRP risk group determines that the number of patients belonging to the hs-CRP low risk group was the smallest, 42 (30%), followed at a small distance by the one belonging to the hs-CRP average risk group, 45 (32,15%). Most individuals, 53 (37,85%), have shown values that have fallen into the hs-CRP high risk group (Table 1, Graphic 1).



Graphic 1. hs-CRP dynamics based on risk group and gender

Regarding the gender distribution within the same groups of risk, it is noticeable that in the first two groups, female sex is predominant, unlike the third group. In this group, the hs-CRP high risk, the number of male subjects, 39 (73,58%), who recorded high values, was significantly higher compared to that of female subjects, 14 (26,42%). In the first group, the hs-CRP low risk, the number of male subjects, 16 (38,10%), was lower than the number of female subjects, 26 (61,90%). In the second group, the hs-CRP average risk, the two genders were close to value, 21 male subjects (46,66%) and 24 female subjects (53,33%).

Distribution by age group (Table 1, Graphic 2) releaved an unbalanced impairment, since more than half of all subjects, 72 (51,42%), belonged to the 21-40 age group. The rest were divided almost equally between the other two groups, 36 subjects (25,71%) belonging to the age group 41-60 years and 32 subjects (22,85%) to the age group 61-80 years.



Graphic 2. hs-CRP dynamics based on risk group, age group and gender

If we take into account only the first group, 21-40 years, we notice that the number of female subjects, 36 (50%), was equal to the number of male subjects, 36 (50%). Concerning the dynamics according to the hs-CRP risk group, it can be seen that both female and male subjects with low risk are fewer, 16 (22,22%), compared to those at average risk, 32 (44,44%) or high risk, 24 (33,33%) subjects. Of the hs-CRP high risk subjects, 16 are males and only 8 are females (Table 1, Graphic 2).

In the 41-60 age group, it was noted that of the 20 males (55,55%) and 16 females (44,44%), 14 (38,88%) were at hs-CRP low risk, 5 (13,88%), hs-CRP average

risk and 17 (47,22%), hs-CRP high risk. This time, the number of hs-CRP high risk males also significantly exceed the number of females.

In the 61-80 age group, the number of males, 20 (62,50%), was also superior to that of females, 12 (37,50%). The hs-CRP low risk and hs-CRP high risk groups recorded the same number of males, 8 and the hs-CRP average risk group had a half-value, only 4 males. Females had an equal distribution to all of the three hs-CRP risk groups.

This result is correlated with literature data.

The Physician's Health Study (PHS) performed in 1997 in initially healthy males showed that the initial levels of hs-CRP were significantly higher among those who subsequently had a heart attack or stroke compared to those who did not. The results showed that the predictive value of hs-CRP was independent of other risk factors such as blood cholesterol and smoking. The results also suggested that hs-CRP was a better predictor of cardiovascular events than several other inflammatory biomarkers (https://www.docsopinion.com/health-and-nutrition/hscrp/).

A guideline from the American College of Cardiology Foundations and the American Heart Association says that hs-CRP testing may be useful when men 50 years old or younger and women 60 years old or younger have intermediate risk.

It also may be useful for treatment decisions when men and women are older than these respective ages and have LDL-C less than 130 mg/dl and meet several other criteria, such as no existing heart disease, diabetes, kidney disease, or inflammatory conditions.

(https://labtestsonline.org/understanding/analytes/hscrp/tab/faq)

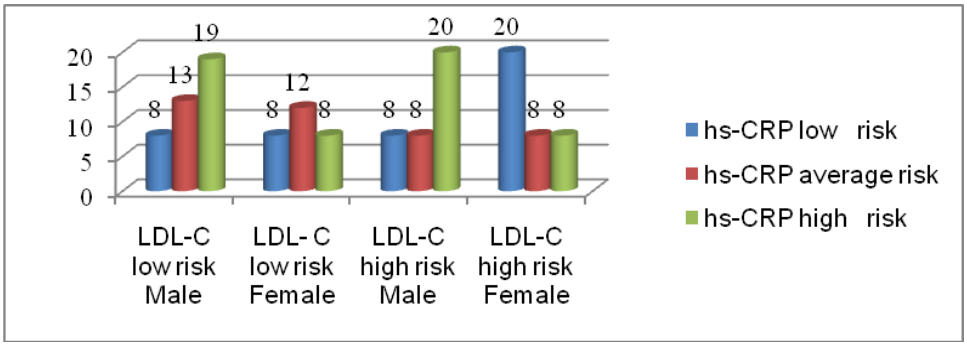
Several studies from both the United States and Europe indicate that elevated levels of hs-CRP among apparently healthy men and women are a strong predictor of future cardiovascular events (Ridker 2001).

People with higher hs-CRP values have the highest risk of cardiovascular disease and those with lower values have less risk. Specifically, individuals who have hs-CRP results at the high end of the normal range have 1.5 to 4 times the risk of having a heart attack as those with hs-CRP values at the low end of the normal range (https://labtestsonline.org/understanding/analytes/hscrp/tab/faq).

Regarding the correlation between hs-CRP and LDL-C values, a balance is observed between subjects with LDL-C low risk, 68 (48,58%), and those with LDL-C high risk, 72 (51,42%). If in the first group, the LDL-C low risk group, the number of males, 40 (58,82%) exceeded that of females, 28 (41,18%), at the second, LDL-C high risk group, the number of males and females was equal, 36 (50%).

In the LDL-C low risk group, 16 subjects (23,52%) had hs-CRP low risk values, 25 subjects (36,76%) recorded hs-CRP average risk values and 27 subjects (39,70%) had hs-CRP high risk values. 19 of these were males.

In the LDL-C high risk group, an equal number of subjects, 28 (38,88%) recorded hs-CRP low and high risk values. In terms of the number of subjects with hs-CRP values indicating an average risk, it was lower, 16 (22,22%), but the same for both genders, 8 (Table 2, Graphic 3). Thus, a large number of subjects with LDL-C high risk values have hs-CRP high risk values, so increased risk also this time.



Graphic 3. The correlation between hs-CRP and LDL-C based on risk groups and gender

These findings are consistent with previous studies showing an association between hs-CRP serum levels and LDL-C serum levels.

Patients with elevated hs-CRP levels in the absence of elevated cholesterol appear to derive preventive benefit from statin therapy.

The results of the JUPITER study may indicate that hs-CRP levels may be useful for selecting individuals who may benefit from statin therapy. Patients selected for the JUPITER study should have hs-CRP > 2 mg/L and LDL-C <130 mg/dl. At the end, there was significantly fewer cardiovascular events, Rosvastatin significantly reducing both hs-CRP and LDL-C (Ridker, 2004, https://www.docsopinion.com/health-and-nutrition/hscrp/).

Finding a high relative risk level of hs-CRP (>3.0 mg/L) may allow intensification of medical therapy to further reduce the risk and to motivate some patients to improve their lifestyle or comply with medications prescribed to reduce their risk. An interesting use for hs-CRP is to motivate persons with moderate to high risk levels to improve their lifestyles (smoking cessation, dietary modification, exercise, weight loss) or to comply with drug therapies (Pearson et al. 2003).

According to the WHS (Women's Health Study), hs-CRP has been shown to be a stronger predictor of cardiovascular events than LDL-C. Women in the low-level hs-CRP and LDL-C group presented an absolute higher risk than the subgroup with low levels of hs-CRP and high levels of LDL-C. However, screening for both biological markers provided better predictive value than any other test (https://www.docsopinion.com/health-and-nutrition/hscrp/).

CONCLUSIONS

hs-CRP is considered to be an useful test in determining the potential risk level for cardiovascular disease, heart attack and stroke and may play a role in the patient assessment process before confronting one of these health problems.

More clinical trials are being carried out to measure hs-CRP in an effort to better understand its role in cardiovascular events and could eventually lead to recommendations on the use of hs-CRP in screening and in making treatment decisions.

The high levels of CRP are correlate with the presence of traditional cardiac risk factors, including hypertension, high cholesterol, diabetes, obesity, aging, smoking and a family history of heart disease. Diet, exercise, smoking cessation, and an appropriate medical approach to these risk factors can improve cardiovascular disease prevention.

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***https://labtestsonline.org/understanding/analytes/hscrp/tab/faq

***https://www.docsopinion.com/health-and-nutrition/hscrp/

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***https://www.revistagalenus.ro/practica-medicala/evaluarea-riscului cardiovascular-proteina-c-reactiva/

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STUDY REGARDING THE FEMALE REPRODUCTIVE SYSTEM AND THE FREQUENCY OF THE CLINICAL AND CYTOLOGICAL EXAMINATIONS

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Key words*: genital tract, clinical examination, cytology*

ABSTRACT

The main of this study was to evaluate the frequency of the periodic examination of the genital tract and the pathological aspects which determinate the women to come into the medical office.

The study made on a lot of 146 women showed that 64,38% of them asked for the medical examination because of symptoms like vaginal smears associated with burning, abnormal odor, itching, bleeding, irregular menstrual cycle or pelvic pain. Only 35,61% of women come for a routine evaluation.

The majority of patients were between 20 and 29 years old, a period when the sexual activity is at the maximum level. 14.38% of women were never screened for the cervical cancer by Pap test and only 39.73% of them did the Pap test periodically every 2 or 3 years.

INTRODUCTION

Genital cancers are ones of the most frequent cancers in women.

The cervical cancer is the second cause of mortality in Romania. The detection and prevention of cervical cancer include screening tests, clinical and sonographic evaluation and diagnostic biopsies.

The microscopic examination of the cervico-vaginal cytology or Papanicolau test (Pap test) is the most important screening for precancerous lesions and cervical cancer. The Pap test is named after the Greek doctor George Nicolas Papanicolaou, who developed this method.

Cervical cytology (the Pap test) is the most widely used cancer screening test in most industrialized countries. It is the first step in the diagnosis of early cervical cancer stages and cervical cancer precursors. It can detect cell changes before develop into a tumor.

Papanicolaou smear sampling of the cervix involves scraping of the cervical surface and a portion of the nonvisualized cervical using various sampling devices (Apgar et al. 2008).

MATERIALS AND METHODS

The study was made between January 2016 and July 2017.

A number of 146 women with ages between 16 and 69 years old were enrolled in this study.

It was performed an anamnestic study regarding the next questions:

-the age of subjects;

-the reasons for asking a medical examination: routine evaluation (asymptomatic subjects) or gynecological symptoms (pathology);

-the types of the symptoms;

-the frequency of the Pap test (cervical cancer screening by performing the Pap test).

RESULTS AND DISCUSSIONS

1. The evaluation of the patients age.

The majority of the women were in the group of age 20-29, followed by the patients with ages between 40 and 49 (table 1, figure 1).

The first group of patients were in the reproductive period, when the sexual activity is at the maximum level. The second group of patients were women in premenopausal period, when appear hormonal disfunctions. There were only 7 patients over 60 years old and 5 cases under 19 years old.

Table 1

The repartition of the cases according to the age

The age group	<19	20-29	30-39	40-49	50-59	>60	Total
No. of cases	5	65	23	34	12	7	146
Percentage	3.42%	44.52%	15.75%	23.29%	8.22%	4.79%	100%

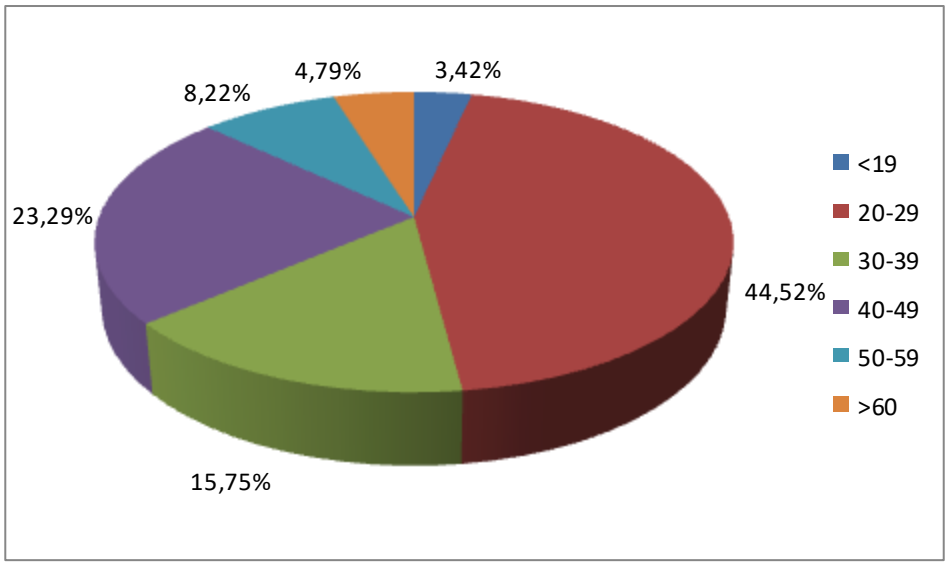

Figure 1. The repartition of the cases according to the age

2. The evaluation of reasons for asking a medical examination

The majority of patients came for a gynecological symptomatology and only in 35.62% of cases they came for a routine evaluation (table 2, figure 2).

Table 2

The repartition of the cases

The cause of the exam	Number of cases	Percentage
Routine examination	52	35.62%
Symptomatology	94	64.38%
Total	146	100%

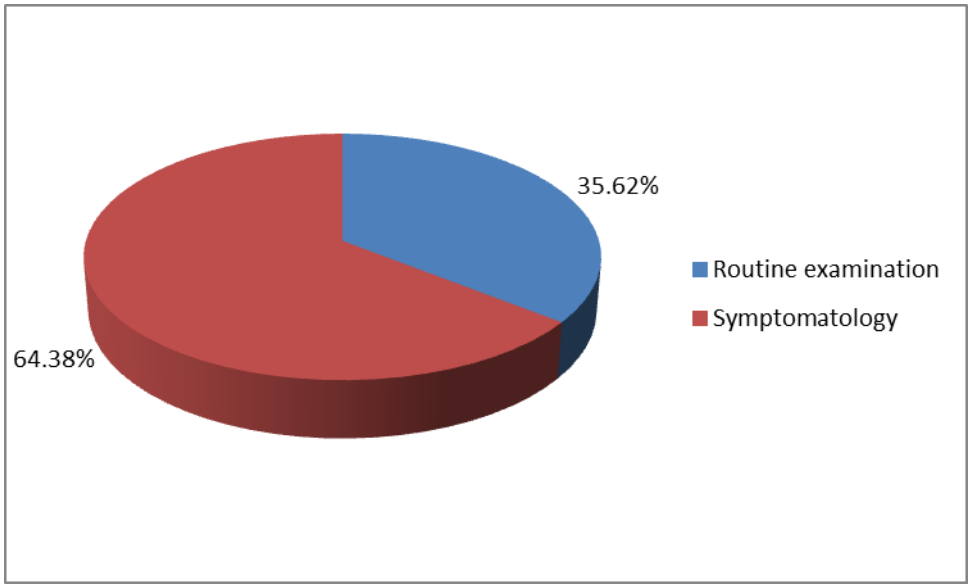

Figure 2. The repartition of the cases

3. The evaluation of the symptomatology

The gynecological symptoms were divided into the next categories:

-cervico-vaginal smears associated with vaginal itching, burning, irritation, abnormal odor;

-vaginal bleeding (hemorrhage);

-irregular menstrual cycle;

-pelvic or abdominal pain;

-pelvic tumors (table 3, figure 3).

Table 3

The repartition of the cases according to the symptoms

Symptoms	No. of cases	Percentage
Vaginal smears	33	35.11%
Irregular menstrual cycle	21	22.34%
Bleeding	17	18.09%
Pain	20	21.28%
Tumors	3	3.19%
Total	94	100%

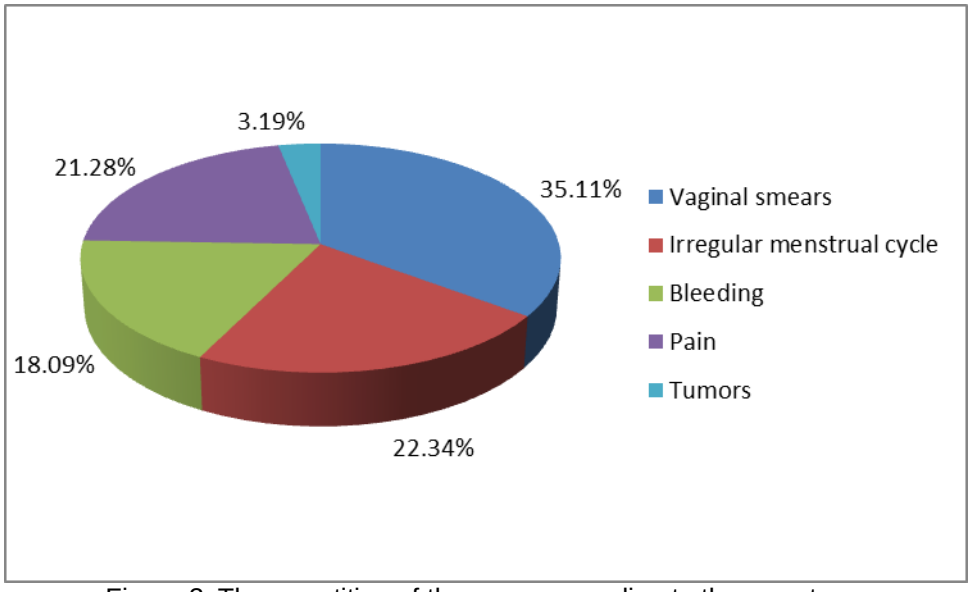

Figure 3. The repartition of the cases according to the symptoms

The majority of women (35,76%) came for vaginal symptomatology, followed by irregular menstrual cycle and pelvic pain.

4. The cervical cancer screening by Pap test

21 patients (14.38%) from 146 never have been followed by screening test (cervico-vaginal cytology) even they did clinical examination.

9 patients with ages between 34 and 69 years old have a period of 6 until 12 years from the last screening test, even they gave births or had abortions in their medical history.

Only 58 women (39.73%) did the Pap test periodically, every 2 or 3 years.

A percentage of 17.81% (26 patients) could not say if they were screened or not and mentioned they didn't because the family doctor or the gynecologist did not recommended (table 4, figure 4).

A 34 years old women has mentioned that she asked to her doctor to perform this test, but he has told her that she is too young for cervical cancer screening.

Table 4

The periodicity of the screening

Period of time	No. of cases	Percentage
No follow-up	21	14.38%
Every year	32	21.92%
Every 2-3 year	58	39.73%
Period longer than 4 years	9	6.16%
No mention	26	17.81%
Total	146	100%

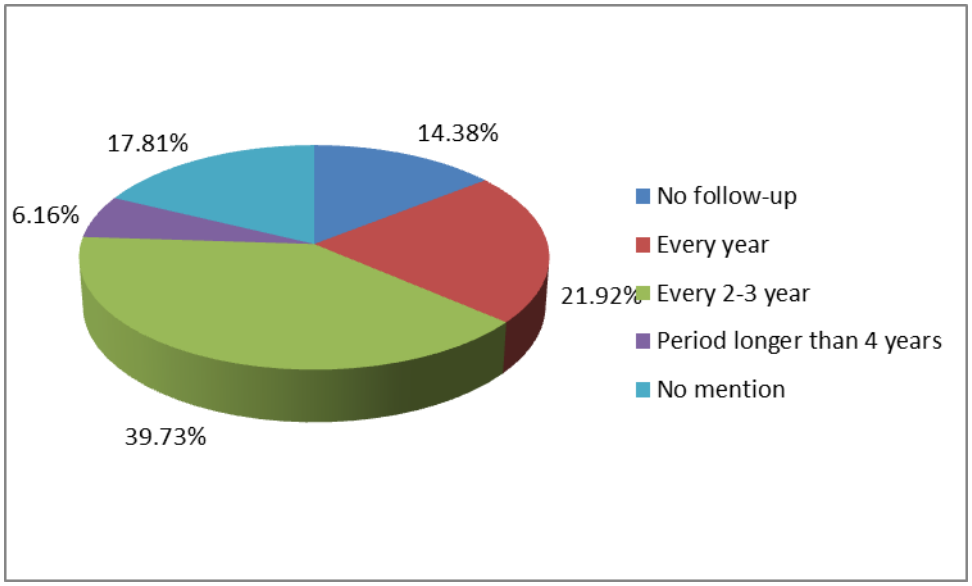

Figure 4. The periodicity of the screening

On a practical level, the patients should be managed according to the clinical evidence-based guidelines.

In Europe the guidelines recommend to start the screening at 25 years old and to repeat it every 3-5 years up to age 65 years old. A woman older than age 30 is screened with a Pap test and DNA-HPV (Human Papilloma Virus) detection. For women age 30 and older Pap and HPV contesting is less likely to miss an abnormality than Pap testing alone (Giorgi Rossi 2013).

It is necessary to test cytologically normal women initially positive by an HPV carcinogenic screening test to determinate whether there is infection by HPV 16, 18 or 45 (Clifford 2006).

More than 100 HPV viral types have been identified, 30 or 40 are known to infect the lower anogenital tract. Fifteen high-grade (HR-HPV) types have been identifies. (Munõz et al. 2003).

Approximately ten million DNA-HPV tests are conducted in the US annually. It appears that incorporation of HPV testing into routine clinical practice continues to increase (Apgar et. al. 2008).

The HR-HPV types 16, 18 and 45 are associated with 70% to 80% of invasive cervical cancer. These women may need more vigilant follow-up (Wright 2007).

Women infected by only low risk HPV need to repeat HPV and cytology test in one year (Castellsague et al. 2006).

The relative risk for cervical cancer in infected women is almost a hundredfold higher that in uninfected women. HPV is highly prevalent in young, sexually active populations with an incidence of up to 20%. HPV is extremely common in adolescents but the spontaneous clearance occurs in the majority.

These high rates of infections are due to sexual behavior and biological vulnerability. Initiating screening at appropriate times is essential. The risk of progression to cancer is minuscule (Bosch et al. 2002).

The vaccines have no effect on women who have already been infected.

It is recommended the routine vaccination of females 11 to 12 years of age with three doses. With the HPV vaccine, it will be essential to vaccinate children prior to onset of sexual activity. All adolescents vaccinated must be counseled about the important of continued cancer screening (Apgar et al. 2008).

Older guidelines in the United States recommended that all adolescents begin screening once sexually active (ACOG 1995).

Recent guidelines are based on risk behaviors. Screening should be initiated 3 years after the onset of sexual activity or by 21 years of age.

According to the updated guidelines, women ages 21 through 29 should be screened with a Pap test every 3 years. Women ages 30 through 65 can be screened every 3 years with a Pap test alone (ACOG 2009, Saslow et al. 2002).

The upper age limit for initiating screening is much higher in many other countries. In country with organized screening such as the United Kingdom, new recommendations are to begin screening at 25 years of age (NHCS 2015).

In Australia the screening begin at the age of 18 years, or 1 to 2 years after first sexual intercourse (NHMRR 2007).

In the European Community the screening programs show real results in decrease of mortality by cancer. In states where the screening involved over 60% of population, like Norway (78.9%), Denmark (66.3%) or United Kingdom (78.5%) the mortality was reduce with 50%-65% (WHO 2015).

Cervical cancer is a very important problem of public health care in our country. More than 4000 women are diagnosed every year in Romania. This type of cancer is the second most frequent in women between 15 and 44 year old, after the breast cancer. In Romania the cervical cancer is the 4th cause of mortality in women after the breast cancer, colorectal and lung cancer and the first cause of mortality in women between 15 and 44 years old, with more than 1900 cases every year (WHO 2014).

In our country, every year, occur between 300 and 350 new cases. Cervical cancer affects especially the young women, the majority of cases occur between 35 and 60 years old (www.insse.ro).

The report of the new cases is mandatory in Romania from 1981 because the cancer is the second cause of death after cardio-vascular diseases.

In 2012 in our country it was developed a national screening program for cervical cancer. The purpose of this public health program it was to reduce the mortality and the incidence of the invasive forms of cervical cancer. The screening program was available for all women with ages between 25 and 64, over a period of 5 years (www.insp.gov.ro).

In 2002 there were an estimated 493.000 worldwide cases of cervical cancer and 273.000 deaths (Sankaranarayanan et al. 2006).

Conform GLOBOCAN, in 2012, the worldwide incidence was 527.624 new cases (Globocan 2012).

The cervical cancer remains the second leading cause of cancer-related female deaths in resources-poor regions of the world. 85% of cases occur in developing countries without national screening programs (Monsonego 2004).

In 2012, in Europe were diagnosed 58.373 new cases of cervical cancer.

In Europe, in 2012, the cervical cancer was on the 6th place as incidence and on the second place as frequency and mortality in women with ages between 15 and 44 years old (Bruni et al. 2015)

In 2013 the highest rates of incidence were in Montenegro (32.46%), Bulgaria (28.36%) and Romania (28.18%) and the lower rate in Malta (4.24%). The lower rates

of mortality were in Finland (1.22%), Turkey (1.69%), Netherlands (1.94%) and Spain (1.97%) (MDB-WHO 2015).

In Germany, in 2013, became effective the National Cancer Plan. The Gynecology Oncology Working Group (AGO) defined the parameters for cervical cancer screening from 2018. The new organized screening program supposed that women aged between 20-60 years with healthcare insurance will receive a letter every five years inviting them to be screened for cervical cancer. The women above the age of 30 years can request an HPV test every 5 years. Cytology is then only done if the findings of the HPV detection are abnormal. Women can continue with the annual Pap test as an alternative to the new screening strategy (Hillemanns et al. 2016).

In the United States, in 2014, were 12.360 new cases. The cervical cancer is on the 8th place as incidence and the average age of diagnosis is 48 years (ACS 2015).

CONCLUSIONS

Cervical cancer screening is an essential part of a woman's routine health care. It is very important to educate the young women regarding the risk factors, symptoms and prevention

It is the role of the medical doctor to inform the patients about the importance of the long term follow-up.

If the patient can be assured that follow-up is in her interest, she is more likely to accept the recommendation.

An important role have the public health authorities and media channels.

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SEASONAL EVOLUTION OF FOLIAR CHLOROPHYLLS, CAROTENOIDS AND FLAVONOIDS IN *PLATYCLADUS ORIENTALIS* (L.) FRANCO

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Key words*: Platycladus orientalis, seasonal evolution, pigments*

ABSTRACT

This paper presents the seasonal variation of foliar pigments (chlorophylls a and b, xantophylls and carotins and flavonoids) in oriental arborvitae from in the October-May period, determined through pigment extraction and spectrophotometric analysis.

Results show major differences on a monthly basis. Chlorophyll a varied between 124.45-433.37 µg/g average values, with a minimum in February-March for all analyzed individuals. Chlorophyll b had similar concentrations, with minimum values in January-April.

Carotenoids (42.86-433.37 µg/g) had their minimum in March. Flavonoids (7.31-12.36 µg/g) had a different evolution, with the highest foliar concentrations in January-March.

Pigment concentrations were weakly correlated to ambient temperature and leaf water content.

INTRODUCTION

Pigments play an important role in plant life. They are the primary receptors of light energy in photosynthesis, they provide protection from damaging radiation and determine the specific color of each plant organ, with specific ecological roles.

These compounds can be classified into several groups. Porphyrins derive from porphin (four heterocyclic, nitrogen-containing rings bound together) and they include chlorophylls a (green-blue) and b (green-yellow), the main compounds involved in photoautotrophic nutrition in plants. Their synthesis needs ambient light and the presence of key minerals, such as magnesium. The ratio between the two pigments is highly variable (3:1-1:1); lower light exposure favors chlorophyll b (Młodzińska 2009).

Carotenoids are yellow, orange or red terpenes and derivatives (a group of hydrocarbons), coloring various organs. They are present, as acccesory pigments, in the photosynthetic apparatus. Their concentration in plant leaves is much more constant than that of chlorophylls, since they are harder to degrade and do not require light for synthesis. They include orange-colored carotenes (α,β,γ) and yellow xanthophylls (Alkema & Seager 1982). They absorbtion range is 400-500 nm, and their purpose in plant leaves is photoprotection and energy transfer to chlorophyll (Młodzińska 2009).

Flavonoids derive from a basic structure, with two aromatic rings. Flavones and flavonols are yellow, while anthocyanidins are red, purple or blue. Their absorption range is 280-315 nm (Młodzińska 2009).

The concentration and ratio of various leaf pigments varies throughout the year (especially in deciduous species) and with leaf age. Basically, it seems that, while incident light is directly connected to a high chlorophyll content, high sugar levels stimulate flavonoid synthesis (Alkema & Seager 1982).

The concentration of various leaf pigments is a good indicator of plant health and nutrition status (Popoviciu et al. 2016).

Platycladus orientalis (formerly *Thuja orientalis*) – Chinese arborvitae, tree-of-life, biota, or simply thuja – is a common ornamental, evergreen, coniferous shrub, a member of the Cupressaceae family. A native of Northeast China, Korea and Eastern Siberia, it is now grown worldwide, in various cultivars. A shrub or medium-sized tree (<20 m), it has spreading branches, scale-shaped leaves (yellow-green to light green), small (2-3 mm) male cones and 20-25 mm female cones, with a characteristic glaucous color. Besides the decorative features, it is used in folk medicine in East Asia and other regions, for a wide variety of purposes (Gilman & Watson 1994).

MATERIAL AND METHODS

Samples consisting of fresh foliar material were collected on a monthly basis from three *P. orientalis* individuals from Tăbăcăriei Park, Constanța. Pigments were determined throughout the October 2016 (November, for flavonoids) – May 2017.

For chlorophyll analysis, 0.1 g of each sample were ground in 10 mL 80% acetone and filtered (Popoviciu et al. 2016). Spectrophotometric absorbance was determined by using a WPA S106 device. Absorbances read at 470, 647 and 663 nm were introduced in specific trichromatic equations for determining the concentration of chlorophyll a, chlorophyll b and total carotenoids (xantophylls and carotins; Lichtehenthaler & Buschmann 2001).

For flavonoids, 1 g of leaf material were ground in 5 mL methanol and the filtered extract was diluted in 8:17 (v/v) aqueous methanol solution prior to reading spectrophotometric absorbance at 340 nm and calculating total flavonoid concentration according to Szabo et al. (2012).

Foliar water content was determined by drying fresh samples at 80ºC. Ambient temperature at sample collection time was also recorded. For determining possible correlations among the parameters, Pearson's correlation coefficients were determined (Paulson 2008).

RESULTS AND DISCUSSIONS

Fig. 1-4 show the evolution of chlorophyll a, b, total carotenoids and total flavonoids in *P. orientalis* leaf samples from the three analyzed individuals.

In all analyzed individuals, chlorophyll a concentrations showed a descending evolution from October (an average of 433.37 µg/g) with minimal values in February-March (124.45 µg/g being the lowest average). A fast recovery was observed in April and May (345.74-486.50 µg/g on average).

Chlorophyll b concentrations followed a different evolution. The peak was reached in November, with major differences among individuals (449.43 µg/g average). A drop followed, during winter months (131.52 µg/g lowest average in January). A new decreased was observed in April (190.28 µg/g), followed by a steep increase (328.96 µg/g average).

Carotenoid concentration had major variations even among the individuals. From 338.04 µg/g in October, a decrease to 173.15-311.07 µg/g followed during winter and a minimum in March (42.86 µg/g), followed by a steep increase.

Flavonoids increased from 7,31 7,31 µg/g in November to a maximum of 12.34-12.36 µg/g (January-February) and a decrease during spring months.

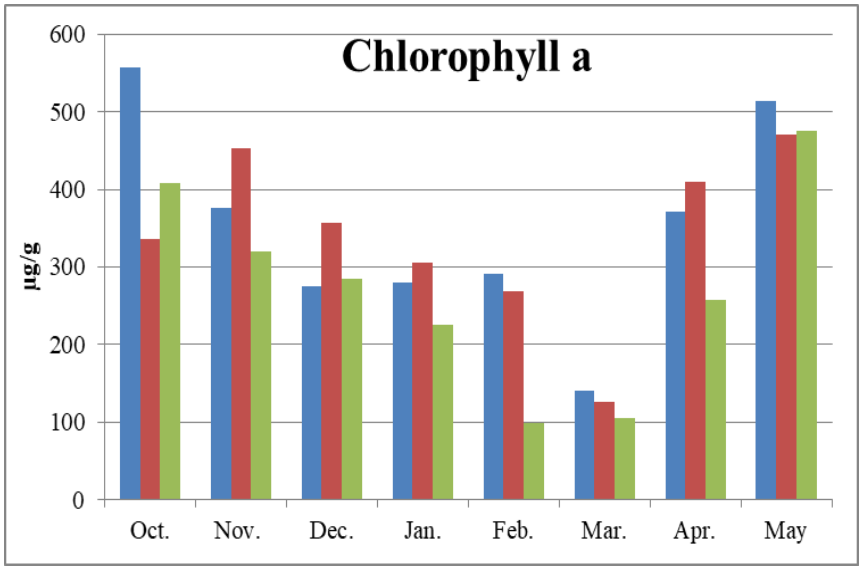

Figure 1. Monthly evolution of chlorophyll a concentration (µg/g) in selected three *Platycladus orientalis* individuals.

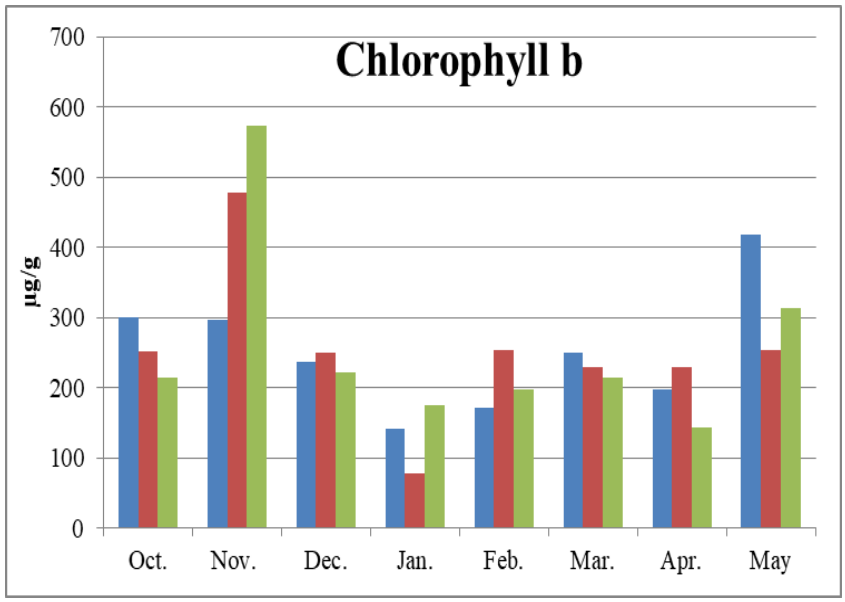

Figure 2. Monthly evolution of chlorophyll b concentration (µg/g) in selected three *Platycladus orientalis* individuals.

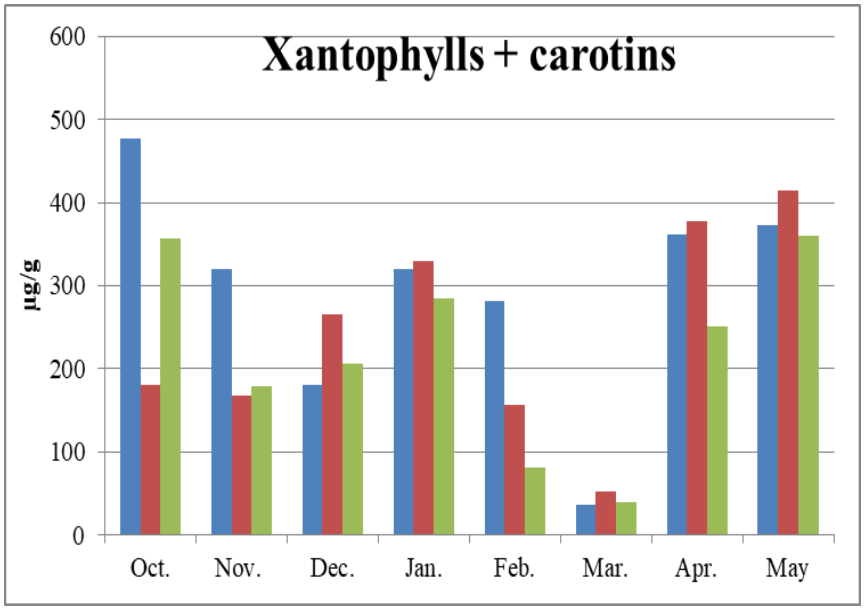

Figure 3. Monthly evolution of carotenoid concentration (µg/g) in selected three *Platycladus orientalis* individuals.

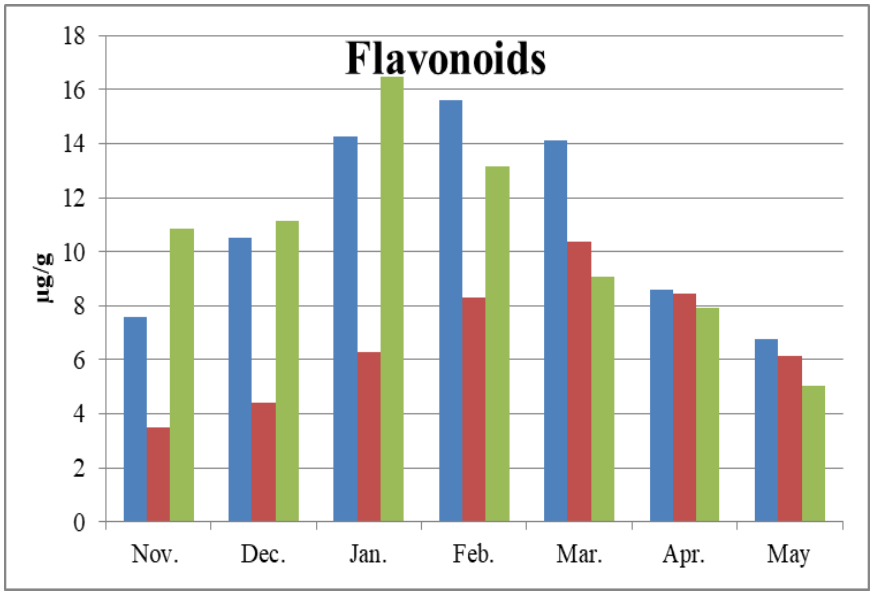

Figure 4. Monthly evolution of flavonoid concentration (µg/g) in selected three *Platycladus orientalis* individuals.

As for correlation coefficients, values above 0.5 (indicating some positive/negative correlation were found only for chlorophyll a and carotenoids (positive) and flavonoids (negative), with no significant correlation to temperature (Table 1).

Generally, 100-1,200 µg/g can be considered as the normal variation limits for total chlorophylls and 100-500 µg/g for carotenoids in most coniferous species (Jarvis 1977, Linder 1980, Gond et al. 1999).

Temperature is known to influence pigment amount, with higher chlorophyll concentrations in warmer periods of the year. A higher insolation, on the other hand favors carotenoid synthesis, as a protective mechanism (Lewandowska & Jarvis 1977, Linder 1980).

Another imprtant factor is soil nitrogen availability, which is commonly seen as directly correlated to foliar chlorophyll concentration (Linder 1980).

Table 1

Pearson's correlation matrix for studied parameters

	Chl a	Chl b	Carotenoids	Flavonoids	% water	TºC
Chl a		0.41	**0.83**	**-0.63**	0.16	**0.49**
Chl b			-0.05	-0.33	0.12	0.22
Carotenoids				-0.30	0.18	0.35
Flavonoids					0.05	-0.42
% water						-0.20
TºC						

The main role of flavonoids is protection of foliar tissue against excessive UV-B radiation. Experiments on *Picea abies* have shown a tendency to increase foliar flavonoid concentration in summer and autumn (Fischbach et al. 1999). In this case, a higher flavonoid content during winter months is probably connected to other, internal factors.

CONCLUSIONS

In all *Platycladus orientalis* individuals, the foliar concentration of both chlorophylls decreased during winter months, with a minimum in Ferburary-March, for chlorophyll a, and January for chlorophyll b.

Total xantophyll and carotin concentration varied significantly among individuals, with a minimum in March and a srtong correlation to chlorophyll a.

Total flavonoids had a different evolution, with a peak in January-February.

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ABOUT THE MEADOWS EDIFIED BY *CHRYSOPOGON GRYLLUS* WITHIN OLTENIA REGION

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Key words*: Chrysopogon gryllus, Oltenia, pajiști, Romania.*

ABSTRACT

Starting from the scientific and practical importance of the meadows that were edified by Chrysopogon gryllus, in this paper, we try a comprehensive overview of the flora and vegetation of these areas of Oltenia and a corresponding framing to Natura 2000 habitats.

There have been identified three associations edified by Chrysopogon gryllus: Chrysopogonetum grylli Buia, Păun, Safta et Pop 1959; Thymio pannonici - Chrysopogonetum grylli Doniţă et al. 1992 şi Danthonio - Chrysopogonetum grylli Boşcaiu (1970) 1972.

The classification into three different associations is determined by the climatic conditions in which this phytocoenosis vegetates.

If we report the meadows edified by Chrysopogon gryllus to the Habitats from Romania (Doniţă & al. 2005), we can say that they fall in the following habitats: R3411 Daco-Balkan Meadows with Chrysopogon gryllus and Festuca rupicola and R3501 Balkan Meadows with Chrysopogon gryllus and Danthonia alpina.

All this information gives us an overview of the meadows with Chrysopogon gryllus, highlighting the scientific and economic importance of the fields with herbaceous vegetation.

In the plains of Oltenia, there are numerous rarities, some of which are Chrysopogon gryllus communities we studied: Ziziphora capitata, Dianthus leptopetalus, Trigonella monspeliaca – Radovan Rea Valley Reserve.

INTRODUCTION

Old province of Romania, Oltenia, a territory between the Danube, the Olt and the Southern Carpathians, is characterized by a varied relief. Its attractive landscape is a crossroads of three different climates: Central European, Pontic and SubMediterranean.

The climatic, edaphic, orographic and biotic factors that act within this wonderful part of the country have enabled the installation of a very rich spontaneous vascular flora (especially within meadows).

The surface covered by meadows is quite large in Oltenia. The largest ones are located within the hilly area and the sub-Carpathian depressions.

Data on the flora and herbaceous vegetation in Oltenia were provided by: Buia et al., 1959, Păun M. 1967, for Balş region; Roman N. 1962, 1974, for the South of

Mehedinţi Plateau; Zaharia C.I. 1972, for the Gilortului Basin; Păun M. & Popescu Gh. 1972, for the Jiu Alluvial Plain; Maloş C. 1977, for the Upper Basin of the Motru; Popescu Gh. 1974, for the Basin of the Bistriţa de Vâlcea; Popescu et al. (2001); Popescu et al. (2003) from Radovan Rea Valley, Costache I. (2005) for the Lower Basin of the Motru and Mariana Niculescu (2006) for the Basin of the Luncavăț River.

A synthesis of the flora and vegetation of Oltenia was carried out by a team coordinated by Prof. dr. Popescu Gh. (2001).

Chrysopogon gryllus is a subMediterranean taxon that has a wide vertical distribution in Oltenia (from the Oltenia Plain to the submountain region - Bărbătești, Piatra in Vâlcea County (Popescu et al., 2001)). It develops on poorly acidic or neutral soils, sometimes skeletal, as it is the case with those in the western part of Oltenia.

MATERIAL AND METHODS

The materials and working methods are the ones characteristic to such a study. These follow a few steps:

- bibliographic documentation,
- field trips to collect data and plant material,
- preservation and identification of uncertain material,
- processing of the obtained data.

In the study of vegetation we have monitored, as much as possible, its present stage, dynamics in time and space, the successive directions of the most important phytocoenoses within certain territories where the anthropogenic influence is more strongly felt.

The size of the sample areas for the meadow study was generally of 100 m^2 and the areas were square.

The relevés were chosen in the areas characteristic to the associations, taking into account the minimum areas corresponding to each type of phytocoenosis.

RESULTS

Following the processing of the material, a flora inventory of the species found in the meadows edified by *Chrysopogon gryllus* in Oltenia was carried out. It contains 88 taxa. The taxa belong to all associations edified by this species in Oltenia.

If we refer to the area where these meadows were found, we mention that those from the hilly area and the Subcarpathian depressions have a floral composition represented by 72 species, those from lower altitudes 57 species and those developed on skeletal soils 38 taxa.

In most cases, it can be noticed that the southern elements (sub-Mediterranean, Balkan, Balkan-Pontic or Pontic-Mediterranean) have a significant share.

The coenotaxonomic classification of the meadows edified by *Chrysopogon gryllus* in Oltenia:

FESTUCO – BROMETEA Br.-Bl. et Tx. ex Klika et Hadač 1944

FESTUCETALIA VALESIACAE Br.-Bl. et Tx. ex Br.-Bl. 1949

Festucion rupicolae Soó 1940

Chrysopogonetum grylli Buia, Păun, Safta et Pop 1959

Festucion valesiacae Klika 1931

Thymio pannonici - Chrysopogonetum grylli Doniţă et al. 1992

BRACHYPODIO – CHRYSOPOGONETALIA (Horvatic 1958) Boşcaiu 1972

Danthonio - Brachypodion Boşcaiu 1972

Danthonio - Chrysopogonetum grylli Boşcaiu (1970) 1972

Chrysopogonetum grylli Buia, Păun, Safta et Pop 1959 (*Agrosti capillaris – Chrysopogonetum grylli* Pușcaru-Soroceanu et al. 1963)

The meadows with *Chrysopogon gryllus* occupy large surfaces of tens of ha in Gorj County, on the terrace deposits within Câmpul-Mare inter-hilly depression and Vârtopu commune, but they also appear sporadically on small surfaces in the hilly area and the Subcarpathian depressions.

It is found in dry, sunny places, with deep groundwater, on slopes with small or high inclination angles.

About 60 species of vascular species have been identified in the floristic composition of these meadows.

The presence of the species *Festuca vallesiaca* in most of the phytosociological relevés shows that these meadows succeed to those edified by the tor grass.

They are different from the meadows located in the western part of the country because they have *Agrostis capillaris* and *Asperula cynanchica* as indicator species and from those in the northern part of Oltenia where we have *Trifolium patens*.

From the point of view of the bioforms, we can say that the hemicryptophytes are predominant (more than 60%), while if referring to geoelements, the Eurasian one have the highest rate (about 40%).

In Oltenia, the association has a wide distribution in the localities: Prunești, Ciuperceni, Desa, Nebuna, Seaca de Câmp, Râpa Roșie (Fig. 1), Verbița, Verbicioara, Segarcea, Șimnic, Coțofeni, Sadu, Vârtopu, Rugi, Horezu, Crasna, Aninișu din Deal, Schela, Pojogeni, Mogoșani, Mușetești, Purceleni, Curpen, etc., between 80 and 450 m alt.

Fig. 1. ***Chrysopogonetum grylli*** Buia, Păun, Safta et Pop 1959 from Râpa Roșie locality (orig.)

Thus, starting from Baia de Aramă to the neighbourhood of Rm. Vâlcea, these meadows develop on smaller or relatively larger surfaces.

Thymio pannonici - Chrysopogonetum grylli Doniţă et al. 1992 (Syn. *Chrysopogonetum grylli* Soó 1939)

The meadows edified by *Chrysopogon gryllus* from the area of Târgu Cărbuneşti, Mărăcinele (Fig. 2), Valea Rea-Radovan and Jitaru can be assigned to this association.

Fig. 2. The physiognomy of meadows from Mărăcinele locality (orig.)

The slightly xeromesophilic character of these places is reflected in the richer floristic composition of the phytocoenoses of this association.

They are greatly similar to those of *Dichanthium ischaemum* with which they are also associated.

Danthonio - ***Chrysopogonetum grylli*** Boşcaiu (1970) 1972

The coenoses edified by *Chrysopogon gryllus* in codominance relationship with *Danthonia alpina* cover large surfaces in Mehedinţi County (Gura Văii, Vârciorova). In spite of the xeric appearances, this group is predominantly mesophilic. It develops on sandy-clayish soils, usually loose, with sufficient moisture at the beginning of the vegetation season, covering both sunny slopes and plateaus.

HABITATS EDIFIED BY *Chrysopogon gryllus* FROM OLTENIA

The influence of man on the habitats edified by *Chrysopogon gryllus* is more and more increased.

If we report the grasslands edified by the scented grass to the Habitats in Romania (Donita et al., 2005), we can say that they fall into the following habitats:

- R3411 Daco-Balkan Meadows with *Chrysopogon gryllus* and *Festuca rupicola*
- R3501 Balkan Meadows with *Chrysopogon gryllus* and *Danthonia alpina*.

The first habitat has a low conservative value and the second one moderate.

CONCLUSIONS

The studies regarding the vegetation edified by *Chrysopogon gryllus* refers especially to the most representative and widespread phytocoenoses.

The surfaces with scented grass from the plain area of Oltenia are increasing compared to the one from the hilly area. Initially, there appear isolated bushes, which form a vegetation cover with a considerable coverage (75-85%) after about 5-8 years. The reason for their expansion is due to the water shortage in this part of the country.

The associations edified by *Chrysopogon gryllus* in Oltenia are deficient in terms of fodder value. If we refer to the quality of grass or hay, we can say that the plants in these places do not make good use of the land they cover.

If we refer to the counties of Oltenia, we can say that Gorj and Dolj have the best representation. These are followed by Vâlcea and Mehedinţi, and the last place is occupied by Olt.

In some areas of Oltenia (Gorj County), the dominant species is exploited by the locals for brush making (for domestic use only).

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HABITATS NATURA 2000 WITH HALOPHILOUS VEGETATION FROM OLTENIA (ROMANIA)

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Key words*: habitats, halophilles, Oltenia, Romania.*

ABSTRACT

The present paper aims at presenting all the halophilous habitats within the territory of Oltenia.

There were identified 10 habitats. Each of these contains a brief description, the edifying species and locations where they were found. Some of them have a high degree of anthropization - R1521.

The data rendered in this paper can contribute to the chorology of this type of vegetation in Romania.

INTRODUCTION

The halophilous vegetation from Oltenia covers a reduced surface, limited to several hundred hectares, located in a few localities in the plain and hilly regions: Gighera, Seaca de Câmp, Bratovoiești, Sadova, Tâmburești, Piscu Sadovei, Murta, Dobrești (Dolj County), Ocnele Mari – Ocnița (Vâlcea County), Osica de Sus, Gura Padinii (Olt County).

The studies referring to the halophilous flora and vegetation from Oltenia are sporadic and included in works that concern the flora or vegetation of certain areas in this part of the country (Buia et al. 1959, 1960, 1961; Doltu et al. 1977, Păun et Popescu, 1973, 1974; Țopa E., 1954, Dihoru, 1990, Popescu et al. 2000, Răduțoiu, 2013). Almost 40 years ago, Al. Buia et al. (1961) reported in the area, within meadows, on slightly saline soils, numerous species such as: *Trifolium ornithopodioides, T. subterraneum, T. echinatum, T. angulatum, Medicago arabica, M. hispida* and *Scorzonera cana* (*Podospermum canum*).

The only work that has as main objective the study of the halophilic flora of Oltenia is the one elaborated by the research team of the Department of Botany of the University of Craiova, led by Prof. Dr. Gheorghe Popescu (2000).

MATERIAL AND METHODS

For the identification of the habitats with halophilic vegetation within the territory of Oltenia, numerous trips were carried out in the aforementioned localities in order to monitor the vegetation at all stages of development. This is absolutely necessary if we take into

account that the identification and description of habitats is based on the characteristic vegetation (Doniță et al., 2005, Gafta et Mountford, 2008).

After identification, there is also made a characterization of the vegetation taking into account local particularities. Moreover, the areas where a negative influence of people and domestic animals was noticed are mentioned and we propose certain conservation measures for the corresponding surfaces. We also took original photos in the identified habitats, some of which being also included in the work.

RESULTS

Of the habitats with halophilous vegetation from Romania, in Oltenia, we find: R1509 Western Pontic communities with *Petrosimonia triandra* and *Artemisia santonicum*, R1511 Western Pontic communities with *Crypsis aculeata*, R1513 Western Pontic meadows of *Beckmannia eruciformis* and *Zingeria pisidica*, R1514 Western Pontic communities with *Trifolium fragiferum*, *Cynodon dactylon* and *Ranunculus sardous*, R1515 Western Pontic communities with *Heleochloa schoenoides*, R1516 Western Pontic communities with *Pholiurus pannonicus* and *Plantago tenuiflora*, R1518 Western Pontic communities with *Salicornia* (*europaea*) *prostrata* and *Suaeda maritima*, R1521 Pontic-Sarmatian communities with *Puccinellia limosa* and *Plantago maritima*, R1526 Pontic-Sarmatian communities with *Triglochin maritima*, *Aster tripolium* subsp. *pannonicum*, *Scorzonera parviflora* and *Peucedanum latifolium* and R1529 Pontic-Pannonian meadows of *Hordeum hystrix*.

R1509 Western Pontic communities with *Petrosimonia triandra* and *Artemisia santonicum* – it is a poorly represented habitat within Oltenia. It was identified on small surfaces only at Gura Padinii locality from Olt County. The physiognomy of these places is given by the two characteristic species: *Petrosimonia triandra* and *Artemisia santonica*, which cover the first layer of the herbaceous vegetation. Along with these, in the stationary where this habitat was identified, we can find isolated specimens of *Puccinellia limosa*, *Spergularia maritima*, *Suaeda maritima*, *Scorzonera cana*, *Juncus gerardii*, *Atriplex tatarica* or *Lepidium ruderale*.

R1511 Western Pontic communities with *Crypsis aculeata* – it covers small surfaces from Gighera and Goicea Mare localities. The vegetation of this habitat is smaller than that of the R1509. Besides *Crypsis aculeate*, there can be also found *Crypsis schoenoides*, *Camphorosma annua*, *Aster tripolium* subsp. *pannonicus*, *Taraxacum bessarabicum*, *Spergularia maritima*, *Cynodon dactylon*, *Trifolium fragiferum*, *Artemisia santonica,* etc.

R1513 Western Pontic meadows of *Beckmannia eruciformis* and *Zingeria pisidica* – it covers large surfaces within Bratovoiești locality and is less extended within Osica de Sus. It develops on flat or slightly inclined terrains. The dominant species (*Beckmannia eruciformis*) covers the first layer of this vegetation (Fig. 1). In a much smaller number, besides this species, we find *Oenanthe silaifolia*, *Carex melanostachya*, *Plantago tenuiflora*, *Puccinellia distans*, *Taraxacum bessarabicum*, *Alopecurus pratensis*, *Aster sedifolius,* etc.

Fig. 1. R1513 în localitatea Bratovoiești (orig.)

R1514 Western Pontic communities with *Trifolium fragiferum*, *Cynodon dactylon* and *Ranunculus sardous* is present in almost all the studied sites in areas characterized by slight salinization. In the floristic composition of this habitat, we remark *Cynodon dactylon*, *Ranunculus sardous*, *Gypsophila muralis*, *Rumex crispus*, *Rorippa austriaca*, *Agrostis stolonifera* and *Juncus bufonius*.

R1515 Western Pontic communities with *Heleochloa schoenoides* – it covers surfaces of a few square meters in Gighera locality. The floristic composition of this habitat is relatively poor and made up of *Crypsis schoenoides*, *Lotus tenuis*, *Artemisia santonica*, *Taraxacum bessarabicum*, *Bupleurum tenuissimum*, *Lepidium ruderale*, *Matricaria recutita*, *Atriplex tatarica* and *Polygonum aviculare*.

R1516 Western Pontic communities with *Pholiurus pannonicus* and *Plantago tenuiflora* – it was identified in the meadows from the Olteţ alluvial plain, within Osica de Sus locality (Olt County) and at Sadova and Tâmbureşti localities (Dolj County), in the areas where humidity is high in spring. Within the saline areas of these meadows, there can be found rare elements, such as *Limonium tomentellum*, *Dianthus guttatus*, *Cyperus pannonicus* and *Iris halophila*. At national level, the conservative value of this habitat is great, values supported by the presence of the species *Limonium tomentellum* in these places.

R1518 Western Pontic communities with *Salicornia* (*europaea*) *prostrata* and *Suaeda maritime* – it has the best area in Oltenia within Gighera locality. Small areas of several tens of square meters were also identified in Ocnele Mari - Ocnița area. The physiognomy of these places is given by *Salicornia europaea* (Fig. 2), besides which we can notice some halophilic taxa: *Puccinellia limosa*, *Aster tripolium* subsp. *pannonicus*, *Taraxacum bessarabicum,* etc.

R1521 Pontic-Sarmatian communities with *Puccinellia limosa* and *Plantago maritima* – the vegetation of this habitat develops on flat surfaces or surfaces with small unevenesses, moderately saline, in the localities from Dolj County where we meet this habitat: Ghighera, Seaca de Câmp, Sadova and Goicea Mare. The physiognomy of this habitat is given by *Puccinellia limosa* (Fig. 3). Besides this species, there also appear *Carex distans*, *Puccinellia convoluta* and isolated specimens of *Plantago maritima* or *Taraxacum bessarabicum*.

Fig. 2. R1518 în localitatea Gighera (orig.)

Fig. 3. R1521 în localitatea Goicea Mare (orig.)

On appreciable surfaces, it is practiced an irrational grazing as there are numerous sheep, cattle and horses. As a result, measures are needed to conserve the areas occupied by this habitat, knowing that it has a high conservative value at national level.

R1526 Pontic-Sarmatian communities with *Triglochin maritima*, *Aster tripolium* subsp. *pannonicum*, *Scorzonera parviflora* and *Peucedanum latifolium* – it covers small surfaces from Murta and Dobrești localities. In the floristic composition of these surfaces, we remark *Triglochin maritima*, *Aster tripolium* subsp. *pannonicus*, *Taraxacum bessarabicum*, *Puccinellia limosa*, *Juncus gerardi* or *Artemisia santonica*.

R1529 Pontic-Pannonian meadows of *Hordeum hystrix* – it is met in many localities from Oltenia (e.g. Seaca de Câmp, Osica de Sus, Piscu Sadovei, etc.), but on smaller surfaces compared to the other habitats with halophilous vegetation. The physiognomy of this habitat is induced by *Hordeum geniculatum* (*H. hystrix*), which is sometimes exclusivist. Besides this species, there can be also found *Puccinellia limosa*, *Pholiurus pannonicus*, *Plantago tenuiflora*, *Cerastium dubium*, *Lepidium ruderale* and *Artemisia santonica*.

CONCLUSIONS

As a conclusion, we can say that, following the research carried out in the sites with halophilous vegetation from Oltenia, the habitats that display a good representation are R1521 (Ghighera, Seaca de Câmp, Ocnele Mari Ocnița, Sadova and Goicea Mare), R1529 (Seaca de Câmp, Osica de Sus, Piscu Sadovei) and R1516 (Sadova, Tâmburești, Osica de Sus). The habitat 1513 covers large surfaces within Bratovoiești locality and smaller ones within Osica de Sus; R1514 is present in almost all the botanized sites in areas characterized by weak salinization. The habitats R1526 (Murta, Dobrești), R1518 (Gighera and Ocnele Mari - Ocnița), R1515, R1511 (Gighera), R1509 (Gura Padinii) cover small surfaces.

Of all the habitats with this type of vegetation within the territory of Oltenia, habitat 1521 is the most affected one by the zoo-anthropogenic factor.

Besides the aforementioned habitats, the surfaces edified by *Limonium tomentellum* play a special role. The analysis of their floristic composition does not allow their inclusion in any of the studied habitats. Here we find species that are characteristic to different habitats: *Puccinellia convoluta*, *Hordeum geniculatum* or *Pholiurus pannonicus*. The nucleus of species entitles us to say that these phytocoenoses can be included in *Puccinellion limosae*.

The necessity to protect the halophilous habitats from Oltenia is acute if we take into account that most of them have a high conservative value (R1509, R1513, R1516, R1518, R1521, R1526).

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PHENOLOGICAL DEVELOPMENT STAGES OF *COLCHICUM AUTUMNALE* L. (*LILIACEAE*) UNDER THE CONDITIONS IN THE FLORISTIC REGION OF THE RHODOPE MOUNTAINS OF BULGARIA

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Key words: *phenological stages, Colchicum autumnale, phenological spectrum*

ABSTRACT

The phenological stages of Colchicum autumnale L. in the Floristic Region of the Rhodope Mountains of Bulgaria were studied. The length of the vegetation period and the growth characteristics of the species were studied in three consecutive years (2014-2016). Observations on the growth and development of the species were carried out on three populations in the floristic region. The first studied population was established in the locality of Rozhen Meadows, located at 1423 meters above sea level, N 41040'331, E 24043'867. The next habitat of the species was located at the outskirts of the village of Chokmanovo at 1090 m a.s.l., N 41031'887, E 24043'793. The last population was found near the village of Smilyan, located at 799 m a.s.l., N 41030'650, E 24045'291. A detailed phenological spectrum was presented. The growth and development stages of the species are related to the content and supply of nutrients of the mother corm and its vitality. A direct relationship was established between the duration of the phenological stages and the climatic features of the respective year.

INTRODUCTION

Medicinal plants as a source of biologically active substances have been used and studied since ancient times. They are a major source material for the pharmaceutical industry as their therapeutic properties are well-studied in many fields of medicine (Newman and Cragg, 2016). Because of the increased interest in medicinal plants, some of them are seriously threatened with extinction. The anthropogenic impact, the irrational collection of herbs and the climate change are causing adverse effects on the populations of medicinal plants. Conservation of plant biodiversity and the sustainable use of the resources have been set as a priority in Bulgaria and on a global scale (Evstatieva et al., 2007).

C. autumnale (Meadow Saffron, Autumn Crocus) is a valuable medical plant containing more than 20 alkaloids such as colchicine, demecolcine, colchicoside, etc. Colchicine is one of the major alkaloids and it has an antitumor effect and high anti-inflammatory activity (Brossi, 1990; Kiraz et al., 1998; Ueda et al., 1987; Wetherley Mein et al., 1983). The extraction of colchicine from *C. autumnale* has a potential for the preparation of drugs for cancer treatment but with a narrow therapeutic index (Wallace et al., 1991). Some derivative alkaloids – demecolcine and deacetyl colchicine also possess antitumor activity, but are less toxic than colchicine (Cifuentes et al., 2006; Graening and Schmalz, 2004). Demecolcine is used for the treatment of

acute myeloid leukemia and malignant lymphoma, and, in the form of ointment – for the treatment of skin cancer (Gupta, 1985; Asenov et al., 1998; Rodríguez-Arnaiz et al., 2004). The use of drugs produced from the species in the veterinary medicine for treatment of arthritis and as a diuretic agent (Jaeger and Flesch, 1990) was also reported in the world literature. A tendency to increasing herb collection from the natural habitats has been observed in Bulgaria, including gathering of that species. That requires strict control and monitoring of populations (Hardalova et al., 1998; Evstatieva and Hardalova, 2004). Knowing their biology and the rhythm of growth and development are the basis, on which measures for the control and conservation of medicinal plants resources are planned.

The aim of the present study was to identify growth characteristics, development and phenological features of *C. autumnale* in the floristic region of the Rhodope Mountains in Bulgaria.

MATERIAL AND METHODS

The exact location, coordinates and altitude of the habitats were determined by GPS (Garmin Dakota 20). The rhythm of growth and development was determined according to the methods of Beideman (1954) and Schultz (1966). Observations were conducted in the period 2014-2016. In August-October, observations were conducted over five days, and in the rest of the period – every month. Plant development goes through the following phenological stages: Vegetation (**V**), Flowering (**F**), Fruit Set (**FS**) and Dormancy (**D**). According to the methods used, each stage is indicated by the first letter of the respective stage and by digits, when it is divided into sub-stages, namely: **V, 1.** – Beginning of vegetation; **V, 2.** - Formation of true leaves; **V, 3.** Formation of a daughter corm; **F, 1.** – Differentiation of the reproductive organs; **F, 2.** – Beginning of flowering; **F, 3.** – End of flowering; **FS, 1**. – Beginning of fruit setting (withering of the perianth); **FS, 2.** – Hidden fruiting; **FS, 3.** Fruit capsules appear above ground; **FS, 4.** – Fruits and seeds mature; **FS, 5.** – Full maturity of fruits and seeds; **D, 1.** – Summer dormancy (leaves turn yellow and drop); **D, 2.** – Winter dormancy; **Dy** –Mother corm dying.

RESULTS AND DISCUSSIONS

C. autumnale is a geophyte of the *Liliaceae* family. Plant growth and development processes of the species are unlike the other plants. Initially the plant is flowering in August-September, thereafter it passes a relative period of dormancy, and in the spring of the following year it forms the true leaves and fruits. The corm is a modified stem performing different functions during the growing season. It provides for the plant nutrition by accumulating reserve substances and water during periods of dormancy. It also provides for the vegetative propagation of the species. During observations, it was found that the corm of the species varies in size from 0.8 cm to 3-4 cm in diameter and 5-7 cm in length. It is pear-shaped, pointed at the base. The corm sheaths, red-brown in color, are formed at the base and in fact they are false leaves. They are arranged in 2-3 rows. One corm side is flattened, the opposite one convex . There are 3-4 buds at the upper side. The future daughter corm develops from the uppermost bud and feeds from the mother corm. The future leaves and flowers are set in the daughter corm, and they are developed in the next growing season. The daughter corm develops and grows in an elongated and concave groove, which is located at the flat side of the mother corm. Protuberances emerge at the bottom of the corm, from which the roots are initiated. The leaves of the species are linear lanceolate to oblong elliptic, glossy green. In the studied populations, their

number varied within 4-5-6. They are formed in the spring from April to June, depending on climatic conditions.

Flowers vary in number from 3-4-5 to 7 per plant. They are pink-purple with a simple perianth. The perianth leaves are 6 in number. They are located in two rosettes and are fused at the basis. The stamens are 6 with large yellow anthers.

The fruits are three-part splitting open capsules, which are initiated together with the leaves in the following year.

The growth and development cycle of the species is unusual. It is characterized by alternating stages of flowering and beginning of fruit setting, followed by a period of winter dormancy, after which the vegetative and generative stages (leaf and fruit formation) go in parallel. The calendar terms and the duration of the stages are closely related to the climate characteristics of the region.

The plant is wintering in the soil as a corm, marked with **D, 2.** on the phenological scheme (Fig. 1.). The depth of penetration in soil varies depending on the soil type and ranges within 15-45 cm. At the beginning of spring (in March), leaves appear from the new daughter corm, which has overwintered with the mother corm in the ground, (**V, 1.** and **V, 2**). Their development and growth is most vigorous in April – May (**V, 2**). Parallel to the appearance of the leaves above ground, fruit capsules also appear (**FS, 3**). They are egg-like boxes, splitting into three. Initially the fruit capsules are green and the seeds are at the stage of milk maturity (white in color), (**FS, 3**). They ripen in June-July (**FS, 4**), and the seeds pass from the milk, through wax to full maturity stage (**FS, 5**). In that period of plant growth, the mother corm changes its texture and appearance, marking the stage of its dying (**Dy**). This is the result of the reutilization and exhaustion of the nutritional reserves by the daughter corm. During spring months the new daughter corm forms protuberances and gradually reaches the size of the mother corm. Similar characteristics of the species development were established by Jung & al. (2014).

During the development and growth of the daughter corm, the reproductive organs are differentiated (the flowers, **F, 1**). It remains attached to the mother corm. After flowering of the meadow saffron, it performs the function of a reserve organ. Our observations showed that the new corm grows as a separate plant after exhausting the nutrients of the mother corm, which coincides with the end of flowering stage. It is buried deep down in the soil by contractile roots, reaching a great depth. After the leaves turn yellow, which is accompanied by ripening of the fruit capsules (stages **D, 1.** and **FS, 4.**), the plants enter the stage of summer dormancy (July till mid-August). At the end of August, the flowers emerge above ground. The beginning of flowering (**F, 2**.) of the studied populations was reported at the end of August till the first decade of September. The flower is in bloom for about 5 days. According to the requirements of the method used, the beginning of fruit setting is recorded with the withering of the perianth (**FS, 1.**). After the end of flowering, the species enters a relative period of winter dormancy (**D, 2.**). The dormancy is considered relative, because the processes of hidden fruit setting and the formation of vegetative propagation products continue below ground.

The active (visible) vegetation of the species begins from the end of May to July and from the end of August to the beginning of October. The growth and development stages of the species are directly related to the condition of the mother corm and the impact of the climatic factors. In the first two years of the study (2014-2015), the observations showed that species development begins at the beginning of March (**V, 1.**), (Fig. 1). At the end of the same month, the true leaves (**V, 2.**) appear above ground. Parallel to their emergence, fruit capsules also appear above the soil surface (**FS, 3.**). At the end of June, the leaves and capsules turn yellow, marking the

beginning of seed maturing (**FS, 4.**), (Fig. 1). The process of fruit and seed ripening in that year was delayed as a result of the amount of rainfalls reported. The amount was twice as high as the norm for that period. The flowering period in the studied populations in 2015 also lasted longer compared to the next year of the study. The reason for that prolonged flowering was the heavy rainfall reported for the period August-September.

In the following year (2016) it was found out that the phenological stages started ten days earlier. Probably the reason was the higher average monthly temperatures and the warmer winter. What is more, in the period of relative winter dormancy (**D, 2.**) the amount of precipitation was also well above the norm for the region, which also favoured the species development. During the period of fruit and seed ripening (July), the amount of rainfall was well below the norm and the average temperatures were optimal. Drought in combination with high temperatures was a prerequisite for the accelerated ripening of fruits and seeds and drying of the leaves. That was also the reason for the transition to relative summer dormancy (**D, 1.**).

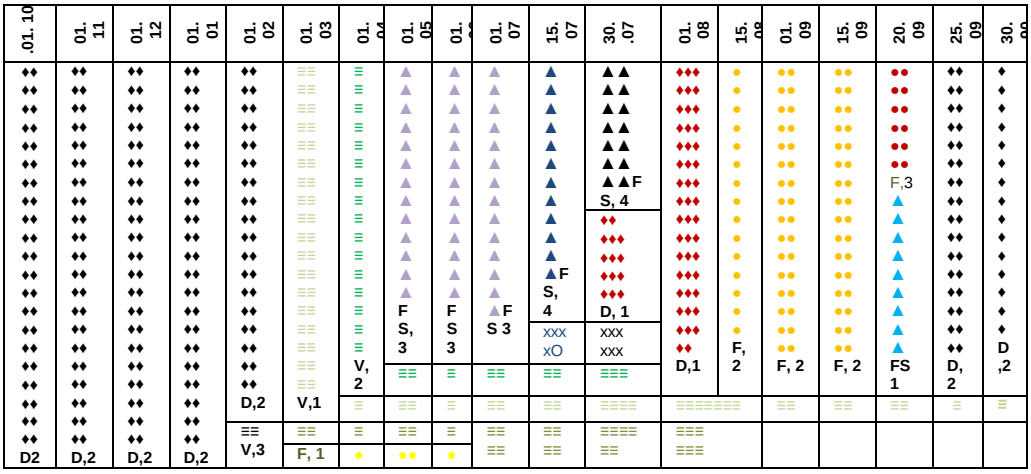
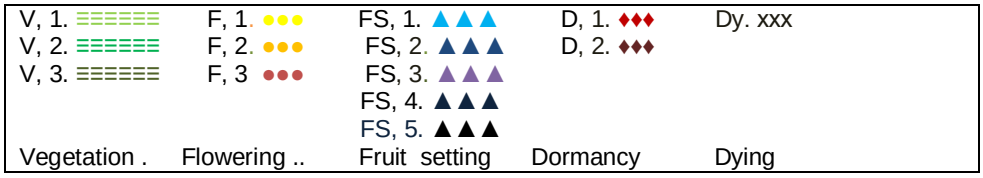


Fig. 1. Phenological spectrum of *C. autumnale*

Legend:

V, 1. – Beginning of vegetation
V, 2. – Formation of true leaves
V, 3. – Formation of a daughter corm
F, 1. – Differentiation of the reproductive organs
F, 2. – Beginning of flowering
F, 3. – End of flowering
FS, 1. – Beginning of fruit setting
FS, 2. – Hidden fruiting
FS, 3. – Fruit capsules appear above ground
FS, 4. – Fruits and seeds mature
FS, 5. – Full maturity of fruits and seeds
D, 1. – Summer dormancy
D, 2. – Winter dormancy
Dy – Mother corm dying



CONCLUSIONS

The growth and development stages of the species are related to the content and supply of nutrients of the mother corm and its vitality. A direct relationship was established between the duration of the phenological stages and the climatic features of the respective year.

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OBSERVATIONS REGARDING THE ENTOMOFAUNA FROM A NEWLY ESTABLISH VINE PLANTATION AT BANU MARACINE S.D.

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Key words*: vineyard ecosystem, beneficial entomofauna, harmful entomofauna*

ABSTRACT

During 2017, we have made some observation regarding the entomofauna from a newly establish vine planatation at S.D. Banu Maracine. According to our observation we have identified a number of 48 species of Arthropods, sistematically framed in 7 order.

From the total of 48 Arthropods species a number of 10 species represent species harmful for the vine, 28 species are indiferent for the vine and 10 species are beneficial.

Most beneficial insect species identified belonged to the Order Coleoptera (5 species) followed by the Diptera (2 species), Orthoptera (1 species), Hymenoptera (1 species) and Dermaptera (1 species).

INTRODUCTION

The viticultural biocenosys it is simpler than the natural biocoenosys (meadow, forest, etc.) and present a reduce stability and complexity. 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 (Stan C., et. all 2008)

In this paper we have propose to inventory the entomofauna present in Banu Maracine S.D. new vineyard ecosystem.

The observation has been made during 2017, in a vine planatation establish in 2015 on a location that was not cultivated anymore (in the past being cover with by spontaneous grassland vegetation and shrubs). Our purpose was to determine to what extent the vine key pest will develop in the vineyard, having in mind that the old vineyards has been clearance by the actual owners.

The research made in the old vine plantations has identified numerous pests, framed as key pests, secondary pests, potential or migratory pests (Stan C. et all 2010)

MATERIALS AND METHODS

To determine the structure of the beneficial populations were made collection of material using various means and methods: directly by hand from plants or soil, frame metric, soil surveys and soil surface collected with entomological net, visual inspection, collection with sticky traps for flying insects, analyzing samples with binocular magnifier glass directly in the field or laboratory.

For as little impact on the ecosystem we have preferred to capture images with the camera than to capture live specimens were subsequently removed from their

natural environment.

After collecting, the biological material was analyzed and determined using the Identification Manual (Panin L, 1951, Chatened du Gaetan, 1990, Chinery M., 1998, Godeanu S.P., 2002, Szabó D. Zoltán et all 2010).

RESULTS AND DISCUSSIONS

During our observation there has been identified a number of : 48 species of Arthropods (table 1), sistematically framed in 7 order.

Table 1

The entomofauna identified during the research period

Nr. crt	Order	Scientific denomination
1	ORTHOPTERA	*Gryllus campestris* L.
2		*Gryllus desertus L.*
3		*Caliptamus italicus* L.
4		*Locusta migratoria* L.
5		*Dociostaurus maroccanus* Thunb.
6		*Tetigonia viridisima L.*
7		*Mantis religiosa L.*
1	DERMAPTERA	*Forficula auricularia* L.
1	HETEROPTERA	*Lygus pabulinus* L.
2		*Lygus pratensis* L.
3		*Dolycoris bacarum* L.
4		*Eurygaster maura* L.
5		*Aelia acuminata L.*
1	HYMENOPTERA	*Vespa vulgaris* L.
2		*Vespa germanica* L.
3		*Scolia flavifrons*
1	COLEOPTERA	*Melolontha melolontha* L
2		*Amphimalon solstitialis* L.
3		*Rhyzothrogus aequinoctialis* Herb.
4		*Polyphylla fullo* F.
5		*Anomala vitis* F.
6		*Phyllopertha horticola* L.
7		*Epicometis hirta* poda.
8		*Cetonia aurata* L.
9		*Oxythyrea funesta* Poda.
10		*Agriotes obscurus* L.
11		*Agriotes ustulatus* Schall.
12		*Agriotes lineatus* L.
13		*Opatrum sabulosum* L.
14		*Adalia bipunctata L.*
15		*Coccinella 7 punctata L*
16		*Carabus ulrichi L.*
17		*Carabus violaceus L.*
18		*Calosoma sycophanta L.*
1	LEPIDOPTERA	*Loxostege stiticalis* L.
2		*Scotia segetum* Schiff.
3		*Plusia gamma* L.
4		*Mamestra brassicae* L.
5		*Euxoa nigricans* L.
6		*Vanessa polychloros* L.
7		*Pieris brassicae* L.

Nr. crt	Order	Scientific denomination
8		*Pieris rapae* L.
9		*Pieris napi* L.
10		*Aporia crataegi* L.
11		*Hyphantria cunea* Drury.
1	**DIPTERA**	*Musca domestica*
2		*Syrphus ribesii L.*
3		*Syrphus torvus L.*

The most numerous order has been **Coleoptera** with 18 species, followed by the **Lepidoptera** order with 11 species and **Orthoptera** order with 7 species.

From the total of 48 Arthropods species a number of 10 species represent species harmful for the vine, 28 indiferent species for the vine, and 10 useful species (table 2).

Table nr. 2

The structure of the entomofauna identified

Order	Number of damaging species for the vine	Number of indiferent species for the vine	Number of useful species	TOTAL
Orthoptera	0	6	1	7
Dermaptera	0	0	1	1
Heteroptera	0	5	0	5
Hymenoptera	2	0	1	3
Coleoptera	8	5	5	18
Lepidoptera	0	11	0	11
Diptera	0	1	2	3
TOTAL	10	28	10	48

As it can be observed in table 2 most beneficial entomofauna identified belonged to the Order Coleoptera (5 species) followed by the Diptera (2 species), Orthoptera (1 species), Hymenoptera (1 species) and Dermaptera (1 species *Forficula auricularia* which we have considered as a beneficial species because the vine plantation is young, and the adults of this species manifest as predator for others small arthropods.

Figure 1 Aspects from the vine plantation and the spontaneous grassland vegetation and shrubs from nearby

CONCLUSIONS

Following the recorded results we can conclude that the main group of vine pests under the conditions of 2017 and of the development period of the vineyard, are represented by species considerered as secondary pests (ocasionaly): *Anomala* Sam.**,** *Melolontha melolontha* L., *Polyphylla fullo* F. and potential pests:*Vespa spp.* L., *Agriotes spp.* L. *Amphimalon solstitialis* L. *Rhyzothrogus aequinoctialis* Herb

Besides these ones, there are frequently encountered a series of other species

considered to be migratory: *Gryllus spp., Dociostaurus marocanus* L., Caliptamus italicus, *Dolycoris baccarum* L., *Lygus pratensis* L., *Hyphantria cunea* Drury. etc., which was to be expected due to the fact that on this area in the previous years the vegetation has been represented by spontaneous grassland vegetation and shrubs.

During our reserach we haven`t identified any main harmful species for the vine, key pests like vine acarians and the vine moth (which we have ifdentified on other area during previous years 2005 – 2008) probably due to the fact that vine plantation is two years old, as well the absence of other vineyard nearby.

Trophic activity of the predators takes place in good conditions and the population of predators provide a reasonable level of pest control under the conditions of the year 2017, as well the fact that no control treatments has been yet made in this young vineyard.

Also, there has been identified some birds species considered to be beneficial like: great tit, hoopoe, cuckoo, common blackbird, woodpecker, which also contributes to diminishing the pest populations (Szabó D. Zoltán, et. all 2010).

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THE INFLUENCE OF TEMPERATURE IN THE FLIGHT DYNAMIC OF THE *MACROSIPHUM ROSE L.* SPECIES, AT THE AL. BUIA BOTANICAL GARDEN, CRAIOVA

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Key words*: Macrosiphum rose L., temperature, flight dynamic.*

ABSTRACT

Roses for the longest time have enjoyed the honor of being the most popular flowers in the world. With its vast collections of great diversity, the Al.Buia Botanical Garden from Craiova, owns living biological material for study and basic and applied research. Rose is attacked by numerous pests; amongst them, aphids are considered as a major pest. The rose aphid (Macrosiphum rosae L.), Homoptera Order, Aphididae Familiy, present a large area of spreding, being one of the main rose pest.

During 2015 - 2016 at Al. Buia Botanical Garden there has been made research regarding the influence of the temperature on the flight dynamic of the alate form. The study has been conducted during May, there has been recorded the flight dynamic of the rose aphid alate form, during 24 h, in five consecutive days.

Following the observations made during the period 2015 - 2016 regarding the flight dynamics of the Macrosiphum rosae alate form, it came out that within 24 hours the activity of the aphids is decreased in the interval 5.00 – 7.00 h due to the lower air temperatures, below 18°C, and around noon when temperatures rise above 24°C. The flight activity of the winged aphids begun in the morning, reduce as intensity during the day, reaching a maximum to the evening.The flight dynamics of the alate forms is maximum in the range 9.00 – 11.00 and 18.00 – 20.00 due to the fact that during this interval air temperatures between 19 and 22°C are considered as optimal temperatures.

INTRODUCTION

Roses are one of the most popular garden plants. These beauties come in a range of colours, many with scented blooms, and they can be grown in borders, containers, over arches, pergolas and as groundcover. They are easy to grow and live for a long time, if looked after.

A rose is a woody perennial flowering plant of the genus *Rosa*, in the family *Rosaceae*. There are over a hundred species and thousands of cultivars. They form a group of plants that can be erect shrubs, climbing or trailing with stems that are often armed with sharp prickles.

Roses have acquired cultural significance in many societies (Krussma, 1981). Rose plants range in size from compact, miniature roses, to climbers that can reach seven meters in height. Different species hybridize easily, and this has been used in the development of the wide range of garden roses.

There are evidences that *Rosaceae* family is an ancient plant (Cox 1999). Some fossils of rose are found in America that are 30 million years old (Vetricka, 1997). Ornamental roses have been cultivated for millennia, with the earliest known cultivation known to date from at least 500 BC in Mediterranean countries, Persia, and China (Goody, 1993). The Rosa genus is endemic to temperate regions of the northern hemisphere, including North America, Europe, Asia and the Middle East (Phillips & Rix 1988).

The genus *Rosa* is comprised of hundreds of species of prickly shrubs which may also have a climbing or trailing habit. The roses are used for beauty and decoration of garden, extraction of attar for making fragrant mixtures. But main use of roses is in cut flower industry and land scaping where it is mainly use in production as trade (Datta 1997).

Roses are susceptible to a number of pests, diseases and disorders. A large number of the problems affecting roses are seasonal and climatic (Ross,1985). Certain varieties of roses are naturally more resistant or immune than others to certain pests and diseases. Cultivation requirements of individual rose species and cultivars, when observed, often assist in the prevention of certain pests, diseases and disorders.

Various species of aphids feed on roses, but the predominant species is the rose aphid (*Macrosiphum rosae* L.). Rose aphids are small (3 – 4 mm). They are soft-bodied, pear-shaped, pink or green insects that are found in clusters on new growth of buds, leaves and stems. Aphids feed on plant sap with their piercing-sucking mouthparts. A low population of aphids does little damage to a rose bush; however, aphids reproduce very rapidly and can quickly reach numbers that cause damage. Their feeding results in distorted growth. Heavy infestations can reduce the number and quality of blooms. As they feed, aphids excrete honeydew, a sugary substance that attracts ants and wasps.

The honeydew supports the growth of unsightly, dark-colored sooty mold fungi on the leaves. reported damage and tender unfolding eaves and buds by the clusters of aphid colonies. Aphid eggs live through the winter in protected nooks and crannies on the plant. In the spring, eggs hatch into females that are capable of reproducing without mating. They give birth to live female aphid young that have the same capability. This process of asexual reproduction is called parthenogenesis. In the fall, triggered by the change in day length, winged sexual forms (males and females) are produced (Alford 1991). They mate, and the females lay eggs for overwintering, (Dixon 1987), (Blackman & Eastop 2000).

Aphids feed on plant cell contents and sap by piercing the plant and sucking up the liquids (Minks & Harrewijn 1989). These colonies make for easy pickings by aphid predators and parasites. However, many of the natural enemies of aphids are more susceptible to chemical controls than are the aphids. Aphids have several natural enemies, including parasitic wasps, ladybird beetles (ladybugs) and larvae, and green lacewing adults and larvae. However, it is important to note that aphids are very difficult to control because they multiply so rapidly. Leaving even one aphid alive can result in a large population very quickly.

MATERIALS AND METHODS

The research on aphids flight dynamic was carried out at the rose collection from Al. Buia Botanical Garden. During 2015 - 2016 at Al. Buia Botanical Garden there has been made research regarding the influence of the temperature on the flight dynamic of the alate form. The study has been conducted during May, there has been recorded the flight dynamic of the rose aphid alate form, during 24 h, in five consecutive days.

The migration of the alate form has been recorded using yellow traps (10 x 20 cm), placed in the rose collection and in the spontaneous flora nearby.
The flight dynamic, within 24 hours, has been recorded at 7:00, 9:00, 11:00, 13:00, 15:00, 17:00, 19:00 and 20:00 hours, also there has been recorded at these hours the air temperasture.

RESULTS AND DISCUSSIONS

In order to study the *Macrosyphum rosae* alate form migration, we have made some studies regarding the biology and the life cycle of this species. During the spring when the average daily temperature increase over 5^0C, the species start he`s development. Thus, from the eggs that survived the winter, hatch the fundatrix, a female that can reproduce parthenogenetically, therefore this female is the founder of the aphid colony. They give birth to live female aphid young that have the same capability.

Our observations has shown that the optimal average temperature for reproduce and development of this species ranged between 19^0 – 22^0C. It take 10 days to complete a generation at 21^0C.

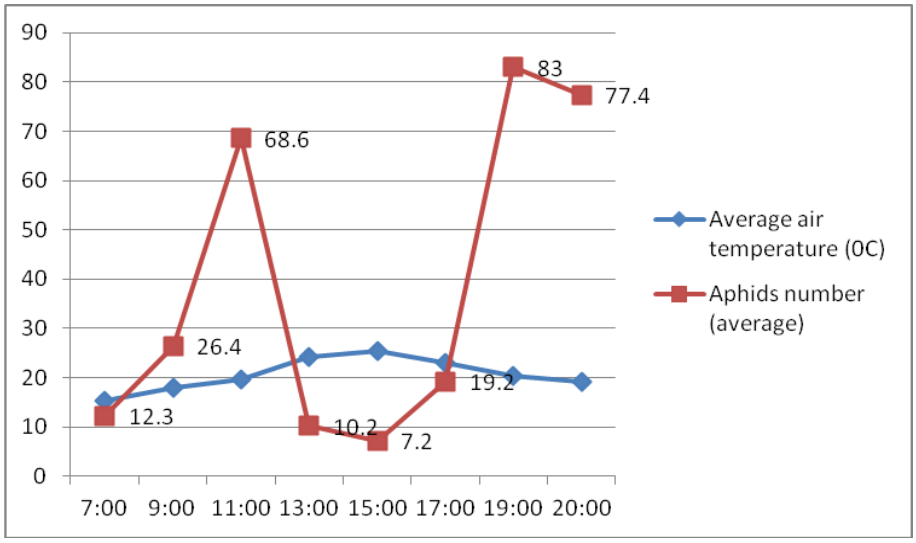
During 2015 we have noticed that the flight activity of the *Macrosipum rosae* L. alate form reached a maximum beyween 19^0 – 22^0C. Thus< the flight begin in the morning, during the day decrease as intensity< especially when the air temperature surpass 26^0C, reaching a maximum in the evening< around 19:00 – 20:00 hours (Table 1, Graphic 1) average of five consecutive days

Table 1

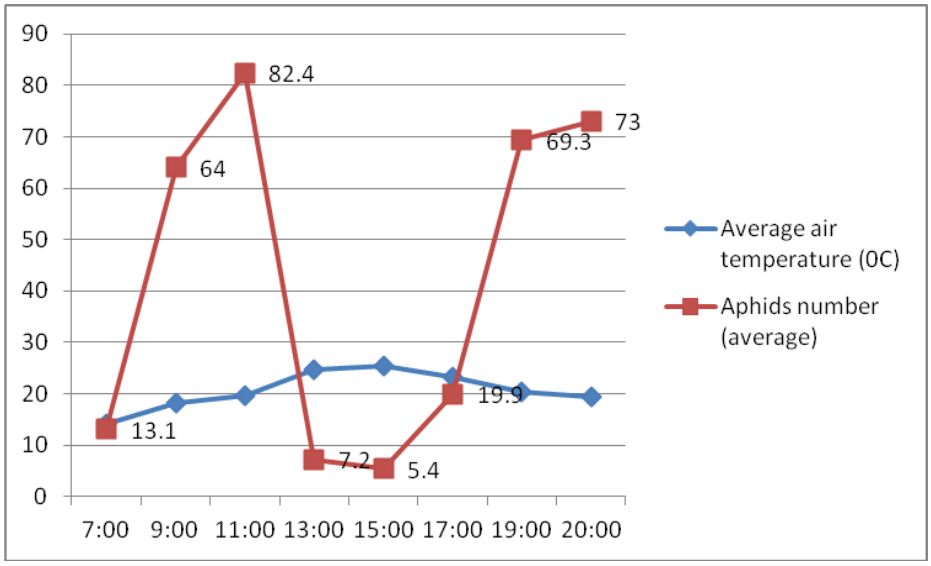
The flight dynamic of *Macrosiphum rosae* L., at rose collection from Al. Buia Botanical Garden, 2015

Hour	Average air temperature (^{0}C)	Aphids number (average)
7:00	15.3	12.3
9:00	18.00	26.4
11:00	19.7	68.6
13:00	24.1	10.2
15:00	25.4	7.2
17:00	23.1	19.2
19:00	20.3	83
20:00	19.2	77.4

During 2016, the flight dynamic of the *Macrosiphum rosae* L. alate form has presented the same looks. Thus, at 7:00 there has been recorded an average of 13.1 aphids, Their number has increase during 9:00 – 11:00 hours to 64.0 respectively 82.4 specimens. During 13:00 – 15:00 hours, the number of flying aphids has decrease to 7.2 respectively 5.4. After 17:00 hours when the air temperature begun to decrease, the flight dynamic has begun a significant increase reaching 69.3 and 73.0 aphids at 19:00 respectively 20:00 hours (Table 2).



Graphic 1. The flight dynamic of *Macrosiphum rosae* L., at rose collection from Al. Buia Botanical Garden, 2015



Graphic 2. The flight dynamic of *Macrosiphum rosae* L., at rose collection from Al. Buia Botanical Garden, 2016

Analyzing the flight dynamic of the *Macrosiphum rosae* L. alate form, during 2016, it came out that at 7:00 hours there has been recorded first aphids (13.1), their number increasing up to 64.0 respectively 82.4 between 9:00 – 11:00 hours (Graphic 2).

Table 2. The flight dynamic of *Macrosiphum rosae* L., at rose collection from Al. Buia Botanical Garden, 2016

Hour	Average air temperature (^{0}C)	Aphids number (average)
7:00	14.1	13.1
9:00	18.0	64,0
11:00	19.7	82.4
13:00	24.1	7.2
15:00	25.4	5.4
17:00	23.1	19.9
19:00	20.3	69.3
20:00	19.2	73.0

CONCLUSIONS

From our observations regarding the Macrosipum rosae L., species, it come out that during the spring when the average daily temperature increase over 5^{0}C, the species start he`s development. Our observations has shown that the optimal average temperature for reproduce and development of this species ranged between 19^0 – 22^{0}C. It take 10 days to complete a generation at 21^{0}C.

As a result from our observations during 2015 – 2016, regarding the flight dynamic of the Macrosiphum rosae L. Alate form, we can acertain that in an interval of 24 hours, there is no fly activity during the night until 7:00 hours. The aphids flight activity it`s low between 7:00 – 9:00 hours due to the average air low temperature, below 18^{0}C and at noon when the average air temperature surpass 23^{0}C.

The flight dynamic of the Macrosipum rosae L., alate form is maximum during 9:00 – 12:00 and 17:00 – 20:00 hours, due to the fact that within these intervals there has been recorded an average temperature of the air ranged between 19^{0}C and 22^{0}C, considered as optimal temperature.

According to our research, the air relative humidity had lower influence on the reproducing and developing within the aphids colonies. We consider that this factor had a higher influence on the flight activity of the alate form, taking care that the high value of the air temperature has been associated with an air relative humidity of 60%.

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PATHOLOGICAL ASPECTS OF INTERNAL ORGANS IN BOVINE LEUCOSE

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Key words*: leucosis, leukemia, malignancy, contagious diseases*

ABSTRACT

This paper presents the study of cases of leucosis encountered in domestic animals. Leukosis is a disease produced by a retrovirus - the bovine enzootic leukosis virus. Bovine animals can infect at any age, including embryonic stage. Most infections are subclinical and 30% of bovine animals over three years have persistent lymphocytosis and a lower percentage of lymphosarcomas (tumors) in different internal organs.

Clinically, bovine enzootic leukosis presents: exophthalmia, cataracts, lymph node hypertrophy, weight loss. Diagnosis is determined by serological tests and hematological examinations.

INTRODUCTION

Mammalian leukosis are infectious diseases of the reticulohistiocitar system, of the neoplastic, malignant type, and with usual chronic evolution. It occurs especially in adult animals under a leukemic, intravascular, and other extravascular, tumor form, being produced by viruses belonging to the Retroviridae family, Oncovirus group, type C (Pop M. and collab., 2012).

Naturally, leucosis can transmit both vertically and horizontally.

Enzootic bovine leukosis (LEB) is characterized by intense multiplication of hematopoietic tissue cells, which can enter the bloodstream and then infiltrate the organs. This increase is often caused by nucleated cells, especially lymphocytes, but also by other blood cells. Due to the rapid growth process of these cells, many immature, poorly differentiated cells with atypical pathological forms can be discovered (Miclea and collab., 1978).

The main hematological and cytotoxic changes encountered in bovine leucosis are represented by increased leukocytosis (is considered when the leukocyte number exceeds 9000 /mm^3), leukopenia (when the leukocyte number drops below 4000 /mm^3), lymphocytosis (when lymphocyte proportion is greater than 65%).

In the course of LEB evolution, blood changes are variable, translated by hematological remissions and ascents, which can lead to diagnostic escapes or confusion in some pseudo-leukemic cases (Pop and collab., 1980)

MATERIAL AND METHOD

The monitoring of the animals in the household is permanent and the preventive tests against the contagious diseases are done annually.

For detection of cases of enzootic bovine leukosis (LEB) blood is taken for analysis. Antibodies are detected by ELISA - enzyme linked immunosorbent assay and ID-agar gel immuno-diffusion. Bovine animals over 24 months of age are tested.

One of the cases found to be positive was subjected to both macroscopic and microscopic investigations. Obvious traces of leukosis have been observed particularly in the liver and spleen.

Within these organs, LEB-specific signs can be seen, of which more obvious: multiple hepatic lymphosarcomas and diffuse lymphoblastic tumor hyperplasia.

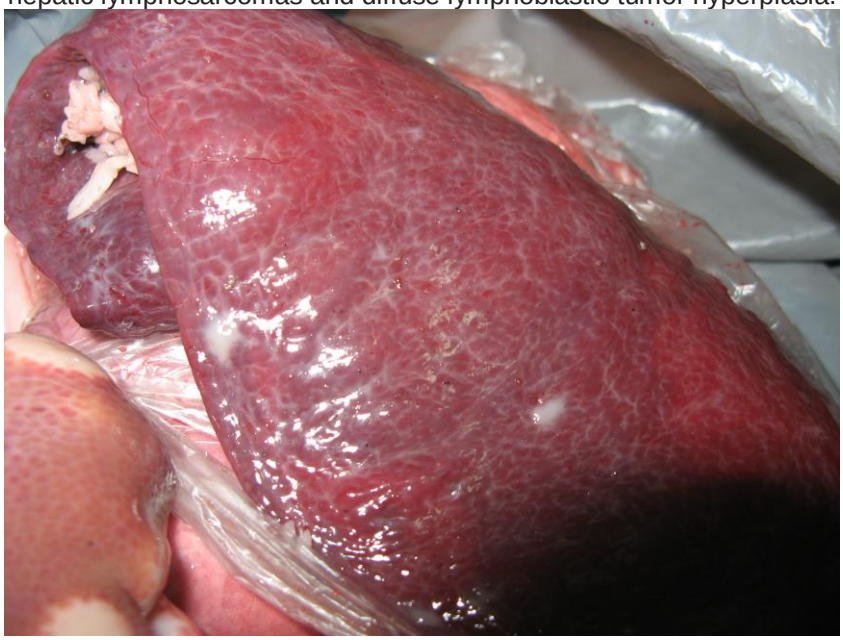

Figure 1. Macroscopic study of internal organs showing signs of leucosis – spleen.

Following the macroscopic study of the liver, multiple hepatic lymphosarcomas can be seen.

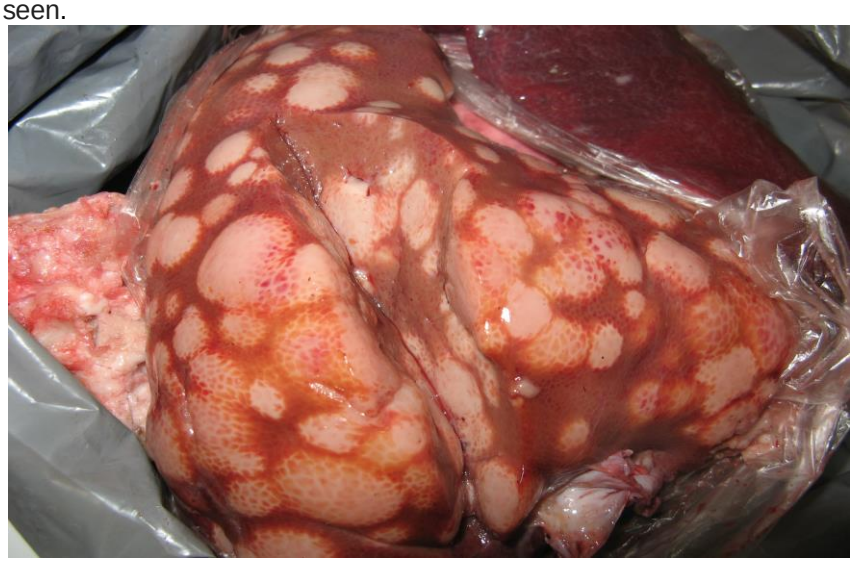

Figure 2. The macroscopic study of internal organs showing obvious signs of leukosis - the liver.

RESULTS AND DISCUSSIONS

Both the laboratory analysis and histopathological analysis confirmed the diagnosis of enzootic leukosis.

After performing the hematological analysis, it was noticeable that the number of white blood cells was extremely high compared to the number of red blood cells; white globules have phagocytes red blood cells - which confirms the diagnosis of LEB.

Following histopathological analysis, as shown in Figure 3, the tumor masses are characterized by the presence of well differentiated lymphocytes.

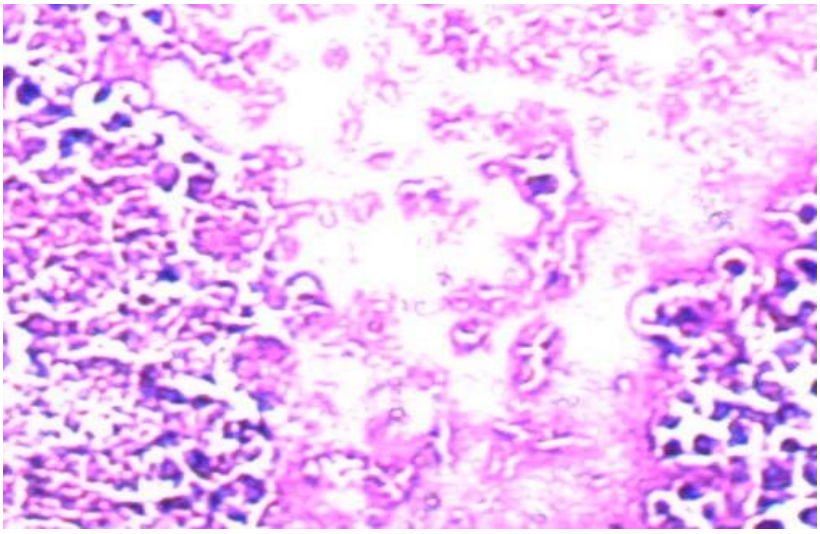

Figure 3. Microscopic appearance (liver) - Enzootic bovine leukosis (col. H&E, 200x objective).

CONCLUSIONS

Enzootic bovine leukosis is an infectious, incurable, cancer-causing disease that causes serious economic harm to livestock and presents a particular danger to human health. The disease is caused by carcinogenic retroviruses that have a close antigenic link with AIDS retroviruses and human T-cell lymphoma.

Many animals remain in the preclinical stage of disease evolution even years, sometimes throughout their lives.

Most infections are asymptomatic and are only detected by serological tests. Of the infected animals, 30% have persistent lymphocytosis without being associated with clinical signs.

No treatment has been developed so far.

Prevention of the disease is done through the strict application of nonspecific prophylactic measures: observance of technology, provision of quarantine period, periodic serological checks, control of hematopoietic vectors, application of selection programs for disease resistance, etc.

The National Reference Laboratory for Enzootic Bovine Leucosis operates within the Institute of Diagnosis and Animal Health.

No reference laboratory is established at European Union level. In the last 5 years, in our country the incidence of cases has dropped significantly. Six LEB cases have been reported in a community in the last 5 years.

During January, 2017, 11 cases of leucosis were reported from a herd of 21,000 cattle.

Currently, leukosis is considered a disease undergoing eradication.

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STUDY ON THE MACROSCOPIC AND MICROSCOPIC ASPECTS ON DOG MAMMARY TUMOR

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Key words*: mammary tumors, malignant tumors, hormones, histopathological examination*

ABSTRACT

This paper presents the study of a mammary tumor at a female dog. Mammary tumors, benign or malignant, are the most common form of canine cancer. As in humans, breast cancer can be lethal if necessary action is not taken in time.

This type of cancer affects unsterilized female dogs over the age of six years, the overall average is somewhere around the age of 10. In many cases, breast cancer occurs in male dogs also. Breast tumors are heavily influenced by sex hormones, so sterilization can be a successful alternative to avoid them.

INTRODUCTION

Tumor growth is a process of progressive tumor multiplication of tissue and organs, with the appearance of a new tissue, with or without differentiation, with a special metabolic activity and which influences the general condition of the body to varying degrees.

The tumors present a wide variety of structural aspects, but much of them resembles normal tissues or certain precursor cells. All neoplasias are characterized by excessive and abnormal hyperplasia of cells from preexisting normal tissues. They can develop from both tissue with less differentiated cells, and in the highly specialized cell (Coman M., 2006).

The mammary glands in dog are 10 (plus or minus, depending on the dog's breed and size) arranged on two rows parallel to the median line of the abdomen. The position of each mammary gland is authenticated by the presence of nipples. The 5th gland (numbered from the front to the back) is the largest and most frequently affected by neoplasms. Breast tissue is strongly influenced by sex hormones and therefore the incidence of tumors is so high in this region.

MATERIAL AND METHOD

At the veterinarian a 12-year-old female Metis breed was presented. Following a thorough clinical examination, a tumor was revealed, located at the last mammary gland on the right side, and the female has undergone surgery to remove the present formation.

The resection piece resulting from surgery was sent to the laboratory for histopathological analysis, in order to determine precisely its nature.

The resection piece, a tumor formation with dimensions of 20 cm long / 18 cm wide / 15 cm thick, shows increased consistency with polylobulised areas partially covered by the skin.

Figure 1. Macroscopic study of the tumor – tumor measurement.

On the section, the partially encapsulated tumor shows a non-homogeneous appearance, with white yellow areas, alternating with cystic areas (cystic formations with variable dimensions starting with 0.3 mm), with yellow-brown gelatinous content, alternating with firm grayish brown areas.

On the sections made at 0.5 cm, in the central portion of the tumor was found a cystic cavity (0.5 cm in diameter) with hematic content, as well as other cystic formations immediately below the tegument (2 cm in diameter) with brownish black content.

Figure 2. Macroscopic study of the tumor – the non-homogenous appearance of the tumor on different sections.

Figure 3. Macroscopic study of the tumor - the area of necrosis.

RESULTS AND DISCUSSIONS

The diagnosis of malignant breast tumor was established following the histopathological examination.

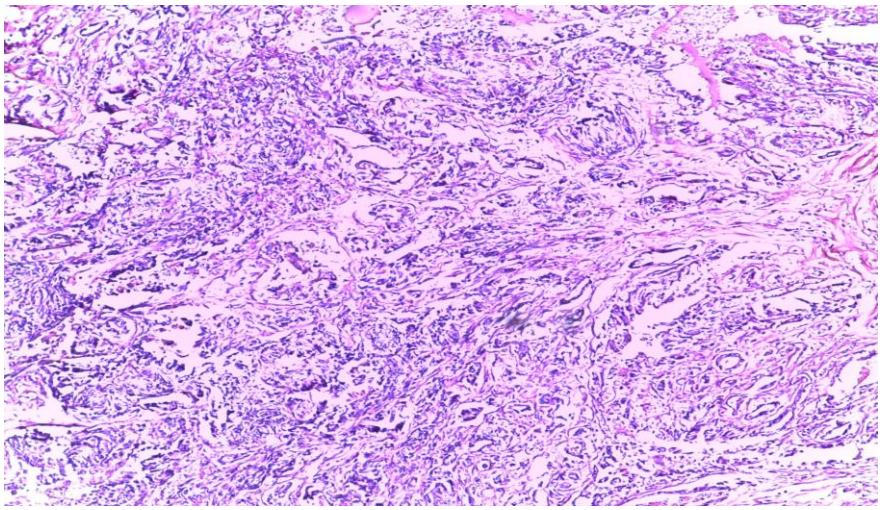

Figure 4. The microscopic appearance of the tumor.

Figure 4 highlights the nodal region of the tumor (adenocarcinoma): fibrocollagen tissue including a spread proliferation of uneven glands with infiltrative nature, lined with epithelium atypical (col. H&E, 100x objective).

Figure 5 - the nodal region of the tumor (adenocarcinoma): infiltration of connective tissue by the islets of adenocarcinoma and discrete infiltrate chronic peritumoral inflammation (col. H&E, 200x objective).

Following the intervention, the female dog was given antibiotic treatment for 5 days. The female dog returned to the medical office after 8 days to remove the suture of the wounded skin. The overall status was good to very good. Despite the histopathological examination diagnosis advocating an unfavorable evolution, the

female dog lived for 3 more years without relapse. One year after the intervention, the dog gave birth to 4 puppies.

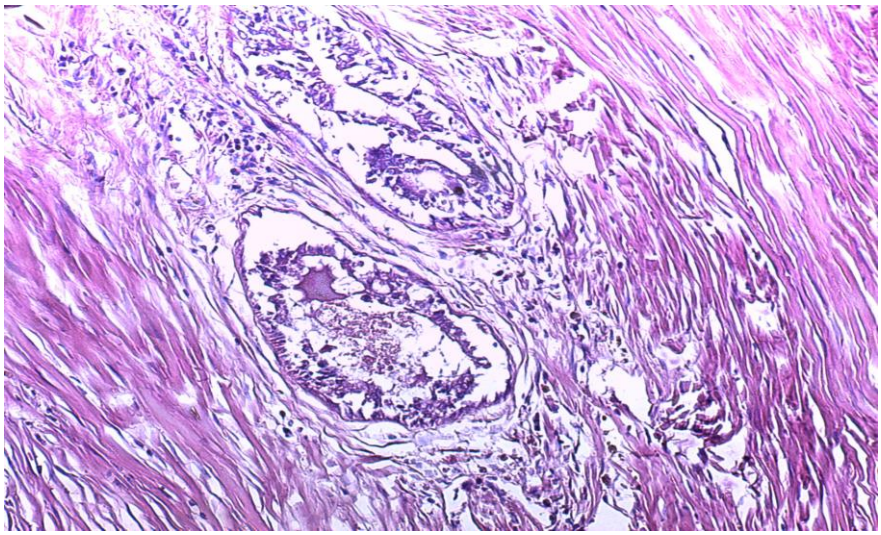

Figure 5. The microscopic appearance of the tumor – nodal region.

CONCLUSIONS

Tumor removal is the first option for mammary cancers in dogs. Unlike women, for female dogs, the breast tissue is easily removed, only with the skin above, without involving the muscles in the area. In the case of elderly females who are unable to undergo surgery or the neoplasm is advanced, the tumor excretion will be discontinued.

Depending on the clinical case, the veterinarian will decide which formations are removed. The tumor is excised with safety margins alone or together with neighboring mammary glands, surrounding tissue, lymphatic network etc. Total mastectomy (removal of all mammary glands) or partial (removal of mammary glands on one side) are often performed.

To prevent relapses, some doctors prefer to carry out sterilization of the dog in the same session. For dogs, recovery after such surgery is done quickly in about 2 weeks (Dutu R, 1985).

Breast tumors can be prevented by sterilization. The risk of breast cancer in sexually active female dogs is 26%. In those sterilized after the first heat cycle, risk falls to 8%, and sterilized before the first heat, the risk is almost non-existent, of only 0.5% (Hasegan N.).

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