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ON AN OPILIONID (ARACHNIDA, OPILIONES) ASSEMBLAGE ON THE WALLS OF A MOUNTAIN CHALET

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ABSTRACT

This paper presents some characteristics of an opilionid assemblage observed to populate the walls of an inhabited chalet in Parâng Mountains – Romania: the species diversity and some aspects of Platybunus pinetorum (C. L. Koch, 1839) biology. The observations indicate territoriality and the chemical marking of the territory in Platybunus pinetorum.

INTRODUCTION

Most opilionids are arachnids living in humid places; they are found in various natural or anthropic environments: forests, caves, cultivated lands or buildings.

Habitat occupancy depends of numerous factors among which the biology of species plays an important role. One aspect of species biology is communication between individuals. In Opilionids, a major role in communication is attributed to the scent glands (=defence = repugnatorial glands). The scent glands are exocrine structures situated in the cephalothorax; they communicate with the exterior by two lateral openings, the ozopores.

The Opilionid scent glands are well studied in respect with their **structure/function/role** (Clawson 1988; Gutjahr et al. 2005 and included reference; Juberthie 1961; Schaider & Raspotnig 2009 a, b; Schaider et al., 2010); **chemistry and role of their secretions** (Acosta et al. 1993; Duffield et al. 1981; Eisner et al. 2004; Föttinger et al. 2010; Hara et al. 2005; Jones et al. 1976; Jones et al. 2009, Machado et. al 2000; Machado et. al 2002; Machado et al. 2005; Meinwald et al. 1971; Raspotnig et al. 2005; Raspotnig et al. 2010; Raspotnig et al. 2011; Raspotnig et al. 2014 a, b; Rocha et al. 2013 a, b; Segovia et al. 2015; Shear et al. 2010 a, b; Souza & Willemart 2011; Wiemer et al. 1978).

MATERIAL AND METHODS

The results of this paper arise from the daytime visual inspection and collectings of Opilionids from the walls of the chalet "Cabana Botanică Rânca Lac" (N 45° 19' 872" E 23° 40' 496", altitude 1529 m) – Parâng Mountains during 10 - 14 July 2015, 17 June 2016 and 30 June - 12 July 2016.

In the first four days the Opilionids were observed (obs.)/collected (coll.) from the western wall of the building, thereafter from all the walls, as follows: *10*

July 2015 – collecting W – 16^{00}; *11 July 2015* – observation W – 7^{45}, 14^{30}; *12 July 2015*– collecting W – 19^{30}; *13 July 2015*– collecting W – 7^{45}; *14 July 2015* – collecting – 9^{30}; *17 June 2016* – collecting – 11^{30}; 30 *June 2016* – observation – 21^{20}; *1 July 2016* – observation – 7^{30}; *2 July 2016* – observation –18^{00}; *3 July 2016* – observations – 6^{45}, 18^{45}; *4 July 2016* – observation – 8^{00}; *5 July 2016* – observation – 8^{40}; *6 July 2016* – observation – 8^{30}; *10 July 2016* – observation – 15^{00}; *11 July 2016* – collection – 20^{15}; *12 July 2016* – observation – 8^{00}.

The Opilionids were collected by hand; they were grabbed by their legs or by the body. For every collecting, all the specimens observed on the walls were gathered. The material is preserved in 75° ethylic alcohol and partly deposited in the collection of the Faculty of Horticulture, the Department of Biology and Environmental Engineering, University of Craiova.

In order to observe the scent gland secretions under binocular, the bodies of few *Platybunus pinetorum* males were gently pressed, after which the males were released.

The species identification was done according to Martens, 1978 and Avram, 1968.

RESULTS AND DISCUSSIONS

1. The Opilionid assemblage

The specific and numerical structure of the Opilionid assemblage found on the walls of the building is included in Table 1.

Table 1

The specific and numerical structure of the Opilionid assemblage

Species Date, nr. of ♂♂ and ♀♀	*Platybunus pinetorum*	*Platybunus bucephalus*	*Leiobunum tisciae*	*Mitopus morio*	*unidentified Oligolophinae*	*Parane-mastoma silli*
W, 10th July 2015,16^{00}, coll.	36 ♂♂ 12 ♀♀		1 ♂ 3♀♀	6 juveniles	2 juveniles (subadults)	0
W, 11th July 2015, 7^{45}, obs.	15 ♂♂ 5 ♀♀		0	0	0	
W, 11th July 2015, 14^{30}, obs.	58 ♂♂ 5 ♀♀		1	4 juveniles	0	0
W, 12th July 2015, 19^{30}, coll.	45 ♂♂ 5 ♀♀		0	8 juveniles	0	0
W, 13th July 2015, 7^{45}, coll.	50 ♂♂ 5 ♀♀	1 ♂	2	3 juveniles	0	0

W, 14th July 2015, 9^{30}, coll.	50 ♂♂ 3 ♀♀		1	4		
14 July 2015, 9^{30}, (all the walls) coll.	74 ♂♂ 3 ♀♀					1
17th June 2016, 11^{30}, coll.	1 ♂ 1 ♀					
30th June 2016, 21^{20}, obs.	10 ♂♂ 4 ♀♀		-	1 juvenile	1	-
1st July 2016, 7^{30}, obs.	9 ♂♂ 3 ♀♀		1	3 juveniles	-	-
2nd July 2016, 18^{00}, obs.	11 ♂♂ 7 ♀♀		-	1 juvenile	-	-
3rd July 2016, 6^{45}, obs.	16 ♂♂ 3 ♀♀		-	1 juvenile	-	-
3rd July 2016, 18^{45}, obs.	14 ♂♂ 5 ♀♀		-	-	-	-
4th July 2016, 8^{00}, obs./coll.	24/1 ♂♂ 8/2 ♀♀		-	3 juveniles	1	-
5th July 2016, 8^{40}, obs.	20 ♂♂ 2 ♀♀		-	2 juveniles	-	-
6th July 2016, 8^{30}, obs.	18 ♂♂ 4 ♀♀		-	2 juveniles	-	-
10th July 2016, 15^{00}, obs.	24 ♂♂ 4 ♀♀		-	1 juvenile	?	1
11th July 2016 20^{15}, obs./coll.	23/19 ♂♂ 4/3 ♀♀		-	3 juveniles	-	1
12th July 2016, 8^{00}, obs.	44 ♂♂ 2 ♀♀		-	2 juveniles	-	-

As only one male specimen of *Platybunus bucephalus* was identified in the collected evidence, (by supposition) we considered that individuals of the genus Platybunus counted during the observations belong to the species *Platybunus pinetorum*. Between 10 and 14 July 2015 the population of this species observed on the walls of Cabana Botanica Rânca comprised a relatively constant number of individuals distributed in a characteristic pattern, represented by groups of 3 to 7 specimens which maintained an approximate distance of 10-50 cm between them.

The distance between the groups was of 1 to 5-7 metres. The Opilionids preferred the constantly shaded areas of the walls where the groups maintained a relatively constant position.

A new population of Opilionids appeared on the walls after each collecting, with a similar number of individuals and distribution as the groups existent before the collecting. The repetition of the "experiment" (observation and collecting) in the time interval 30 June- 12 July 2016 led to the same findings: the population resumes after each collecting and approximately maintained the same number of individuals and similar distribution patterns on the walls. In 2016 the groups were smaller at the beginning of the study (30 June, as this was not the peak growth period for the adults). The groups did not maintain the position on the walls observed in the precedent year.

2. Some aspects of *Platybunus pinetorum* biology

Mobility

The females always stayed motionless in the shadowed areas. Most males were motionless, their only movements were to avoid the sun or bobbing or dropping from the walls when disturbed. Few males made some chaotic movements, to all appearances without any reason. No hunting or mating activity was noticed.

Observations on diet

A very small number of *Platybunus pinetorum* males showed interest in fruit (apple) in the absence of drought – Fig. 1a, while the chance landing of a dipter on the pair I of legs of a male triggered no hunting reaction – Fig. 1b. No specimen of Platybunus was observed hunting or eating live or dead prey in the daytime.

Chemical secretions

Platybunus pinetorum appeared to release two liquids under stress conditions: when grabbed and pressed with fingers on the dorsum and ventral at the same time, both males and females released a yellow-brown liquid on the finger touching the venter of the animal. The liquid has a soft iodine-Juglans green nut smell. When pressed under binocular, a quite large leak of a clear, transparent and colourless liquid has been observed in the proximity of the ozopore (in the dorsal area, between the cephalothorax and chelicerae, pedipalps, the coxae of the first and second pairs of legs) – Fig. 2a. No drops or jet secretions were noticed on the ozopores. Instead, the ozopore of a female collected in alcohol showed a brown solid formation (sediment) – Fig. 2b.

3. Discussions

The reappearance of the population after each collecting with an approximate equal number of individuals as the previous one, as well as the redistribution of the individuals of the new population in a pattern similar to that observed prior to the collecting (in groups that maintained approximately the same position on the walls and had a relatively constant number of individuals and a relatively constant distance between the group individuals) suggests a phenomenon of territoriality and of space demarcation for the species *Platybunus pinetorum*. This species may well be characterised by a phenomenon of chemical territorial marking, a phenomenon also found in other Opilionids, e.g.: *Goniosoma*

spelaeum (Gnaspini 1996 cited in Willemart et al. 2007), *Prionostemma* sp. (Donaldson & Grether 2007).

The chemical marking can be done by the secretion of some glands that may vary in location and openings: **A)** cephalothoracic scent glands, **B)** integumentary glands located throughout the body or on the legs. The chemicals released by Opilionids are referred/suggested to be used for territorial marking/chemical marking by other species as well (e.g. Grether et al. 2014; Holmberg 1986; Schaider & Raspotnig 2009b; Teng et al. 2012; Willemart & Hebets 2012).

If we assume that the colourless liquid leaked in the vicinity of the ozopores represents the secretion of the scent glands then we may consider some possible explanations for the presence of the yellow-brown liquid left on the hand: a) *Platybunus pinetorum* releases only one yellow-brown liquid secretion which seems colourless on the dark-brown background of the cephalothorax; b) *Platybunus pinetorum* releases only one colourless liquid which turns yellow-brown as a result of an oxidation process; c) *Platybunus pinetorum* releases 2 liquids: a yellow-brown enteric liquid and a colourless scent gland liquid, thus, a mix of enteric fluid and glandular secretions, unusual for Phalangiidae. Such a mechanism of producing a mix of enteric fluid and glandular secretion (with a defensive role) has been described so far in some Laniatores – Gonyleptidae: *Pachyloidellus goliath* (Acosta et al. 1993), *Acanthopachylus aculeatus* (Eisner et. al 2004), *Goniosoma spelaeum* (Gnaspini & Cavalheiro 1998), *Discocyrtus pectinifemur* (Segovia et al. 2015), *Goniosoma albiscriptum* (Willemart & Gnaspini 2004) and Stygnopsidae: *Haplobunus mexicanus* (Pomini et al. 2010). Giving the fact that the ozopore of a female revealed a brown sediment (a solid formation) we also may assume that the transparent liquid in the vicinity of the ozopore might represent the haemolimph leaked from an open wound, like a broken leg – Fig. 2a.

In respect with the iodine smell of the *Platybunus pinetorum* secretions, a similar odour is reported for the scent gland secretions of *Larifuga capensis*, a South African Opilionid belonging to Laniatores (Lawrence, 1937). Also Simon (1879) likened the odour of the scent gland fluid of an Opilionid to the walnut scent (Hillyard & Sankey 1989).

A different way to chemically mark of the territory is by releasing secretions from the integumentary glands of the entire body or of the legs, by dragging or rubbing the legs against the substrate (Willemart et al. 2007). No specimen of *Platybunus pinetorum* has been observed to drag the legs against the substrate. The administration of repellent liquids by leg dabbing in Opiliones has been reported by Eisner et al. in 1971 (Machado et al. 2002).

The chemical secretions of *Platybunus pinetorum* also might have a repellent role – ants and Platybunus avoided each other; no ants attack against Platybunus has been noticed.

The Opilionid diet is still a subject of research and debate; information on the Opilionid diet and feeding biology is still scarce. Most harvestmen are considered generalist feeders. Phillipson reported in the carnivorous species *Mitopus morio* that “a food capture was made only after contact with a leg had occured” (Hillyard & Sankey 1989). In this study the contact of a dipteran with a leg of *Platybunus pinetorum* did not trigger a hunt response. Harvestmen are also reported to “visit fallen or over-ripe fruits to suck the juices” (Hillyard & Sankey 1989), which is also found in *Platybunus pinetorum*.

The presence of *Platybunus pinetorum* on the walls in the daytime cannot be explained. The walls *Platybunus pinetorum* populate must represent the ecological neighbourhood = ambit (Wiens, 1989) of an unknown daytime activity (resting) for this species.

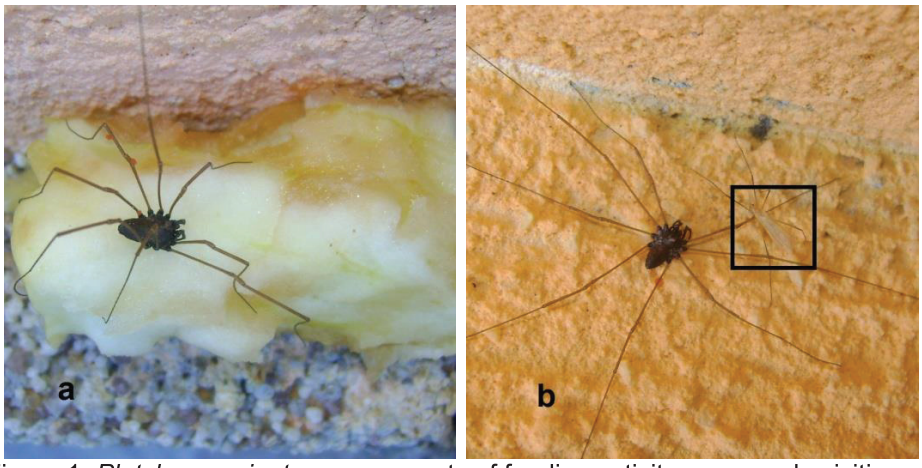

Figure 1. *Platybunus pinetorum* – aspects of feeding activity: a – a male visiting an apple, b – a dipteran sitting on first leg of a male.

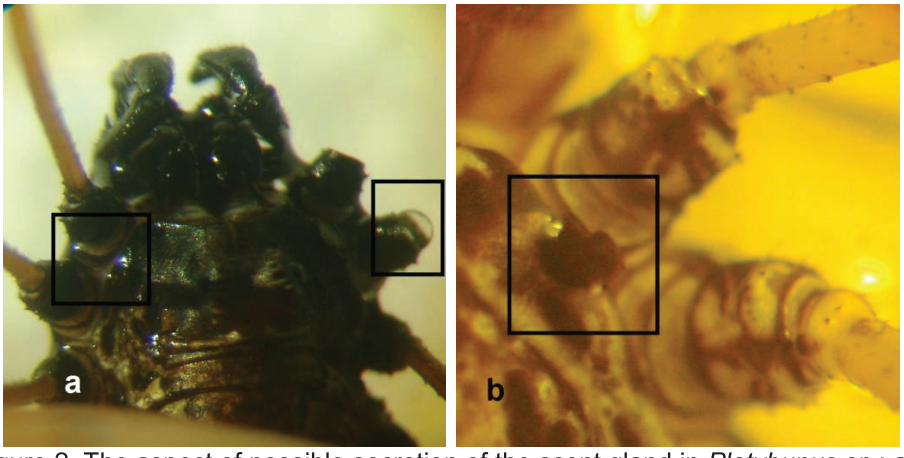

Figure 2. The aspect of possible secretion of the scent gland in *Platybunus sp.*: a – a colourless liquid in the vicinity of a male ozopore (left square), also on an open wound of a broken leg (right square), b – a brown sediment on the ozopore (in square) of a female collected in ethilic alcohol.

CONCLUSIONS

1. *Platybunus pinetorum* is the best represented species in the collected opilionid assemblage on the walls of the building “Cabana Botanică Rânca Lac”.
2. The pattern of distribution on walls indicates territoriality and the chemical marking of the territory in *Platybunus pinetorum*.
3. *Platybunus pinetorum* produces chemical secretions having a iodine, green walnut smell.

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SPECIES DIVERSITY OF THE GENUS PLATYBUNUS (ARACHNIDA, OPILIONES) IN RÂNCA – MOHORUL AREA (PARÂNG MTS., ROMANIA) – A MORPHOLOGICAL APPROACH

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ABSTRACT

This paper presents the intraspecific variability in 5 Platybunus species. Four main morphological features have been investigated and compared with the available literature: the habitus, the pedipalp, the penis and the seminal receptacles. The most variable characters are: the habitus in Platybunus pinetorum female and the apophysis of the pedipalpal patella in all species. In the case of some characters, important differences have been noticed in comparison with the references. Such differences either extend the range of intraspecific variability of the already described species or determine the description of new species. The paper suggests the existence of hybrid populations. Gynandromorphy is suggested in Platybunus banarescui.

INTRODUCTION

In Europe, the genus Platybunus is represented by many species: *Platybunus bucephalus* (C.L. Koch, 1835); *Platybunus pinetorum* (C.L. Koch, 1839); *Platybunus pallidus* Šilhavý, 1938; *Platybunus juvarae* Avram, 1968; *Platybunus decui* Avram, 1968; *Platybunus jeporum* Avram, 1968; *Platybunus alpinorelictus* Martens, 1978; *Platybunus banarescui* Babalean, 2004.

In the Parâng Mt. the following species have been recorded: *Platybunus bucephalus* – Rânca (Babalean 2011), *Platybunus pinetorum* - Cabana Rusu (Dumitrescu 1972), Rânca (Babalean 2001a, 2011), *Platybunus pallidus* – Mohorul (Babalean 2001a, 2011), *Platybunus decui* – Rânca (Babalean 2001b, 2011), *Platybunus banarescui* – Rânca (Babalean 2004, 2011).

The presence of 5 Platybunus species in a small area – Rânca-Mohorul – represents a great diversity which should be investigated. In this context, the aim of the paper is the analysis of the main morphological features used in the species identification: the habitus (the dorsum), the armature of the pedipalp, the penis and the seminal receptacles.

MATERIAL AND METHODS

The material has been collected during June 1997 – August 2016 from sites of the Rânca Resort and Mohorul Peak – Parâng Mt. – Table 1. The habitus, the armature of the pedipalp, the penis and the seminal receptacles have been compared with the data of the available literature. Spinnentiere, Arachnida –

Weberknechte, Opiliones (Martens, 1978) was considered the most comprehensive reference; the old literature (Koch 1835, Roewer 1923) and the OmniPaper Project (Kury 2003) were also consulted. For the armature of the pedipalps we use the terminology provided by Hillyard & Sankey (1989) – adapted as follows: setae (hairs); spines; denticles, hair-tipped denticles, spine-tipped denticles for short structures; tubercles, hair-tipped tubercles, spine-tipped tubercles for the tall structures. The following abbreviations have been used both for singular and plural: Tr – trochanter, Fe – femur, Pt – patella, Ti – tibia, Ta – tarsus, Ap – apophysis. The photos have been taken with a Zeiss AxioCam ERc5s.

RESULTS AND DISCUSSIONS

1. Species diversity

Species diversity, the collecting date and place are included in Table 1.

Table 1

Platybunus species diversity in Rânca-Mohorul area

Species	*Platybunus pinetorum*		*Platybunus bucephalus*		*Platybunus banarescui*		*Platybunus pallidus*		*Platybunus decui*	
Collecting date and place	Nr. ♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
12-14 july 2015 Cabana Botanică, on walls	205	25	1							
11 july 2015 Pârâul Galbenul, surroundings	6	2								
17 July 2009 Pârâul Galbenul, surroundings	17	2	4	2						
23 July 1997 Pârâul Galbenul, surroundings	7			1						
23 July 1997 Cabana Botanică, surroundings	9		10	3					2	
16 August 1997 Cabana Botanică, surroundings	1		8							
14-17 June 1997 Cabana Botanică surroundings		1			3	3				
17 June – 12 July 2016 Cabana Botanica	28	7	1							
16 June 1997 Mohorul Peak							12 immatures			

24 July 1997 Mohorul Peak				9	18	
16 August 1997 Mohorul Peak				3	4	
27 July 2001 Mohorul Peak				2	6	
27 July 2001 Câlcescu Lake				3	5	
10 July 2009 Mohorul Peak				2		

2. The variability of the morphological features

Platybunus bucephalus – male

The male habitus is a constant character, consistent with the literature: most males presented a dark brown spotted dorsum; brown-spotted visible saddle; dark brown coxae of the legs, pedipalps and chelicerae (Fig. 1a).

The pedipalp (Fig. 4a, 5a) showed some constant features (present in all specimens) and consistent with the literature (Martens 1978, Iorio & Delfosse 2016): Fe-Pp with a ventral row of brown conspicuous spine-tipped tubercles and without tubercles in the apical medial (internal) position (near the Fe-Pt junction); Ap-Ti-Pp – cylindrical; Ti-Pp with 2 ventral unequal conspicuous spine-tipped tubercles; Ta-Pp with a basal protuberance and with a ventral row of brown inclined spine-tipped tubercles of moderate size.

The armature of Ap-Pt-Pp (Fig. 7a, b) is inconsistent with the literature (one medial hair-tipped denticle - Martens 1978 pg. 259, fig. 454) and shows variability, even the asymmetry of the pair pedipalps. Most males presented 2 medial short, unequal hair-tipped denticles on the Ap-Pt-Pp; few males presented 1-2 very short denticles on each patella or asymmetric pedipalps. Only 2 males presented the typical Ap-Pt-Pp with only one medial hair-tipped denticle, according to the literature (Martens, 1978).

The penis (Fig. 8a): the body is slender (thin) in the apical part, near the junction with the gland. Colour – yellow-brown with dark brown longitudinal edges.

Platybunus bucephalus – female

The habitus is a relatively constant character: yellow-brown spotted dorsum, visible saddle; few females presented a lighter dorsum (Fig. 2a, b, c).

The pedipalp (Fig. 6a) showed some constantly present features and in accordance with the literature (Martens 1978 - pg. 259, fig. 455; Iorio & Delfosse 2016 - pg. 46, fig. 143): Fe-Pp with a black-spine-tipped white tubercle in the apical medial position; Fe-Pp with a ventral row of black-spine-tipped white tubercles; Ti-Pp with 2-3 conspicuous spine-tipped white tubercles; Ta-Pp presents one ventral row of few conspicuous black-spine-tipped white tubercles.

The armature of Ap-Pt-Pp (Fig. 7c, d) shows a little inconsistency with the literature (Martens 1978, Iorio & Delfosse 2016) and rather a high degree of variability, up to asymmetry of the pair pedipalps. The armature ranges from 1 spine-tipped tubercle to 2 spine-tipped tubercles + 1 spine-tipped denticle on the same patella.

The seminal receptacles invariably consist of two pairs of unequal vesicles, of a more or less constant shape (Fig. 9a, b).

Platybunus pinetorum – male

The habitus is a constant character – spotted dark brown dorsum; visible saddle; dark brown coxae of the legs, pedipalps and chelicerae (Fig. 1b).

The pedipalp (Fig. 4b, 5b, e) showed some constantly present features (in all individuals) and in accordance with the literature (Martens 1978): Fe-Pp with a ventral row of brown spine-tipped tubercles and without apical medial spiniform tubercles; Ap-Ti-Pp – broad, triangular shape; Ti-Pp with 2 ventral unequal spine-tipped tubercles. Ta-Pp presents spines and in most males few ventral spine tipped-denticles.

Ap-Pt-Pp (Fig. 7e, f) differs from that of the literature (Martens 1978, pg. 259, fig. 457) by the presence of medial and external denticles. The presence of denticles is a constant character – almost all specimens have denticles. The number of denticles varies up to showing asymmetry of the pair pedipalps.

The penis (Fig. 8d): the body is slender (thin) in the apical part, near the junction with the gland. Colour – uniform dark brown

Platybunus pinetorum – female

The female habitus showed a high degree of variability (Fig. 2d, e, f, g).

The pedipalp (Fig. 6b) presented some constant features, in accordance with the literature (Martens 1978, Iorio & Delfosse 2016): Fe-Pp with an apical medial protuberance with spines but no tubercle; Fe-Pp with a ventral row of black-spine-tipped white tubercles. Other characters: Ti-Pp with 2 unequal spine-tipped white tubercles; Ta-Pp with spines and few ventral hair-tipped white denticles.

Ap-Pt-Pp (Fig. 7g, h) differs from that of the literature (Martens 1978, pg. 259, fig. 458) by the presence of denticles. Most females present 2 black-spine-tipped white denticles in the medial position. Few females present asymmetric Pt, even with an external denticle.

The seminal receptacles (Fig. 9c, d) are constantly represented by one pair of more or less elongated vesicles, in one case with a sinuous contour; in the upper region the vesicles show a chitinous brown “S” shape loop. A second upper and much smaller vesicle as described in Martens (Martens 1978, pg. 260, fig. 465) has not been observed.

Platybunus decui – male

Habitus: spotted yellow-brown dorsum with spots following the tergites; visible light saddle; brown coxae, pedipalps and chelicerae (Fig. 1c).

The pedipalp (Fig. 4c, 5c) is distinct by the particular broad, 2-branched Ap-Ti-Pp.; Fe-Pp – with a ventral row of dark tubercles and without medial apical tubercles (next to Pt-Pp); Ap-Pt-Pp – elongate-triangle shape; Ti-Pp with few ventral denticles and tubercles; Ta-Pp with a basal protuberance (similar to *Platybunus bucephalus*) and with few ventral tubercles of moderate size.

The penis (Fig. 8e): the body is thick near the junction with the gland and is ventrally curved like a gutter.

All characters are in accordance with the literature (Avram, 1968).

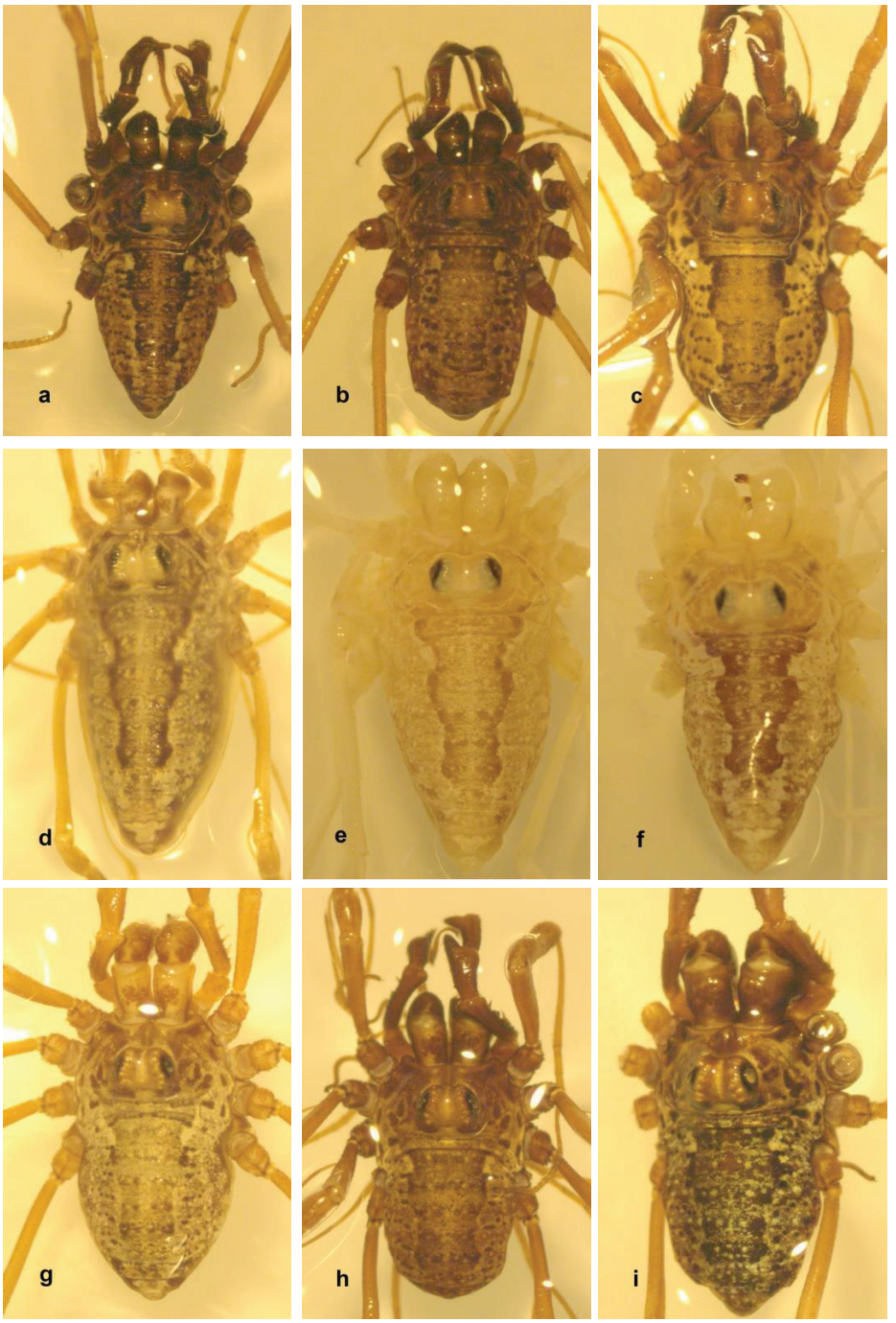

Figure 1. *Platybunus sp.*, male habitus: a – *Platybunus bucephalus*; b – *Platybunus pinetorum*; c – *Platybunus decui*; d – *Platybunus banarescui*; e, f – *Platybunus banarescui* albino; g, h, i – *Platybunus pallidus*.

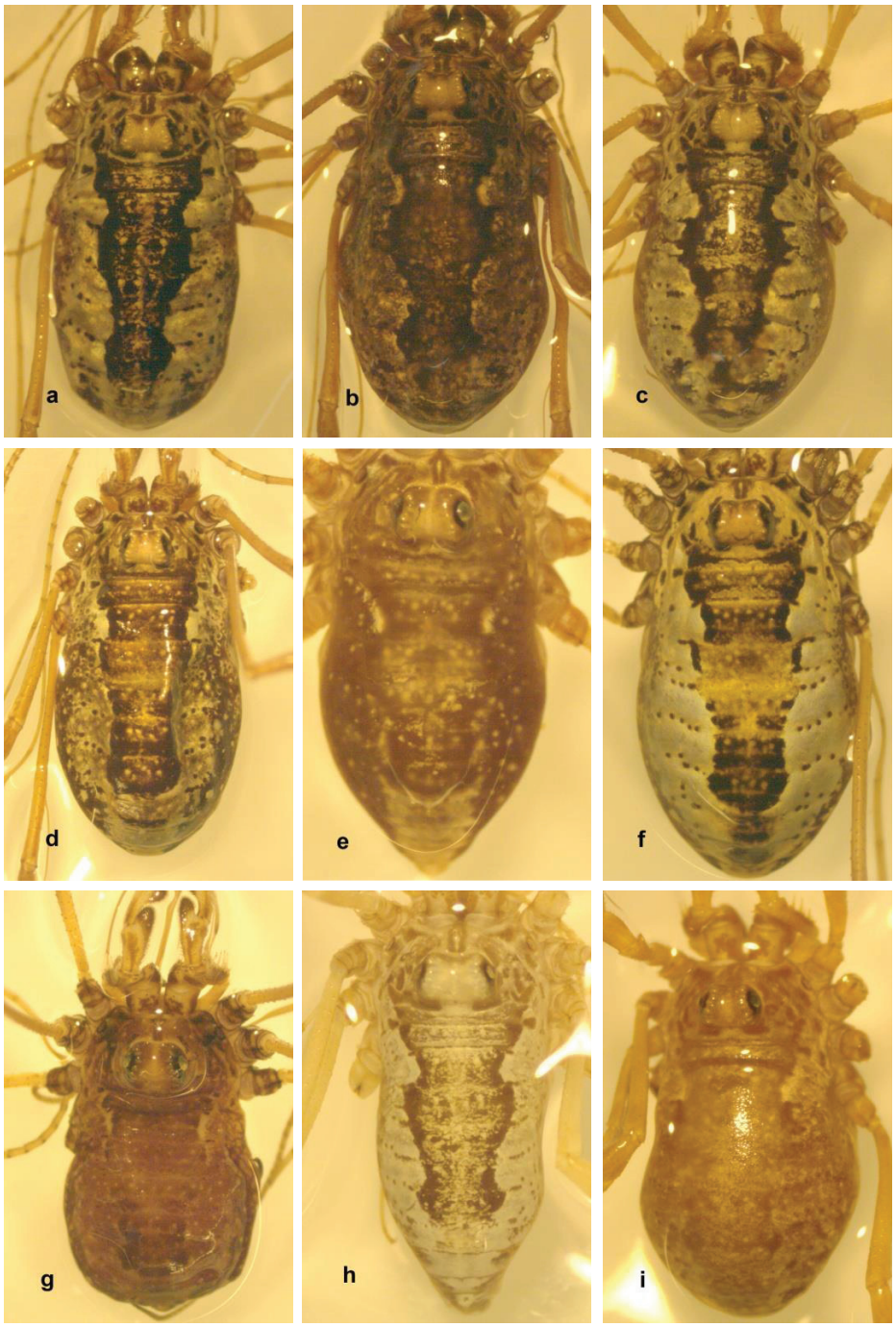

Figure 2. *Platybunus sp.*, female habitus: a, b, c – *Platybunus bucephalus*; d, e, f, g – *Platybunus pinetorum*; h – *Platybunus banarescui*; i – *Platybunus pallidus*.

Figure 3. *Platybunus pallidus* immature specimens: a – male like habitus, b – female like habitus.

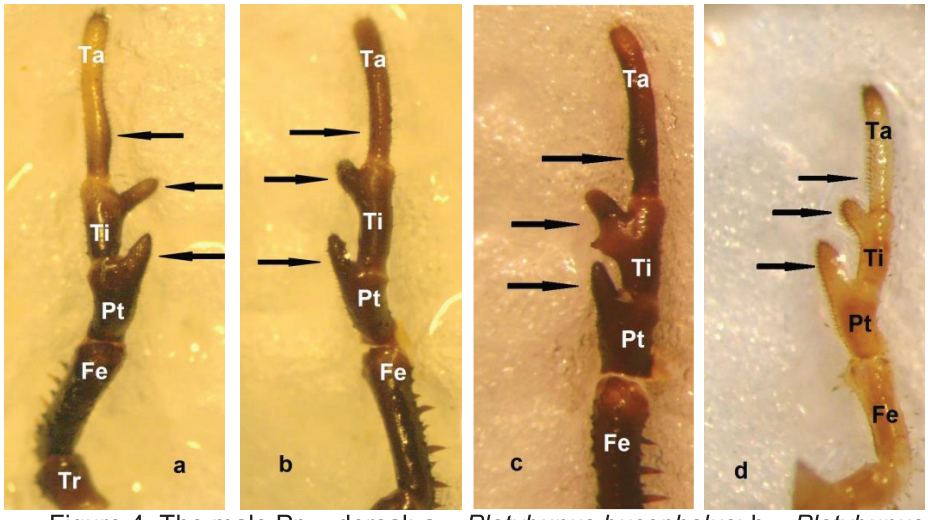

Figure 4. The male Pp – dorsal: a – *Platybunus bucephalus*; b – *Platybunus pinetorum*; c – *Platybunus decui*; d – *Platybunus banarescui*.

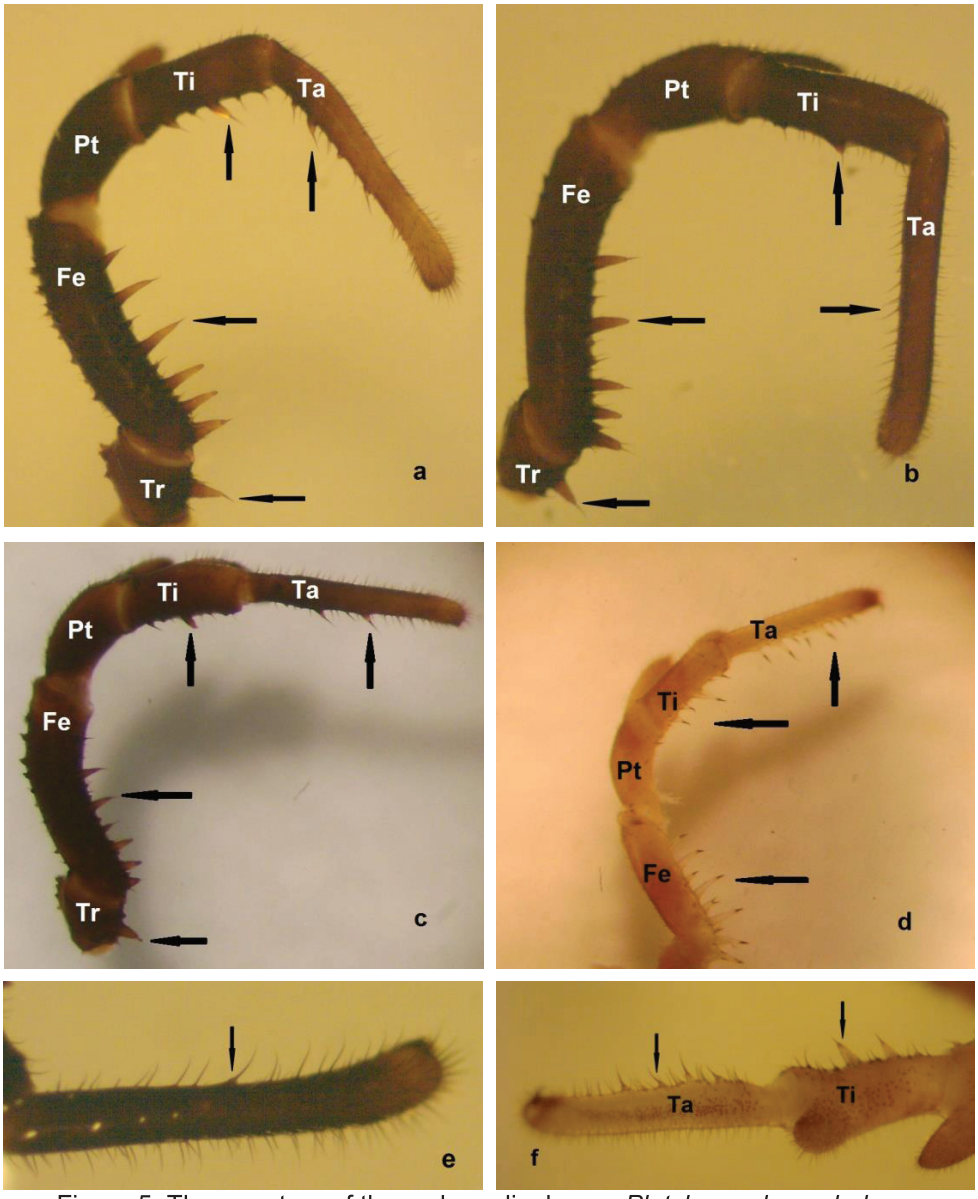

Figure 5. The armature of the male pedipalp: a – *Platybunus bucephalus* – pedipalp lateral-extern; b – *Platybunus pinetorum* – pedipalp lateral-extern; c – *Platybunus decui* – pedipalp lateral-extern; d – *Platybunus banarescui* – pedipalp lateral-exten; e – *Platybunus pinetorum* – Ta-Pp, f – *Platybunus pallidus* – distal part of the pedipalp – lateral-intern.

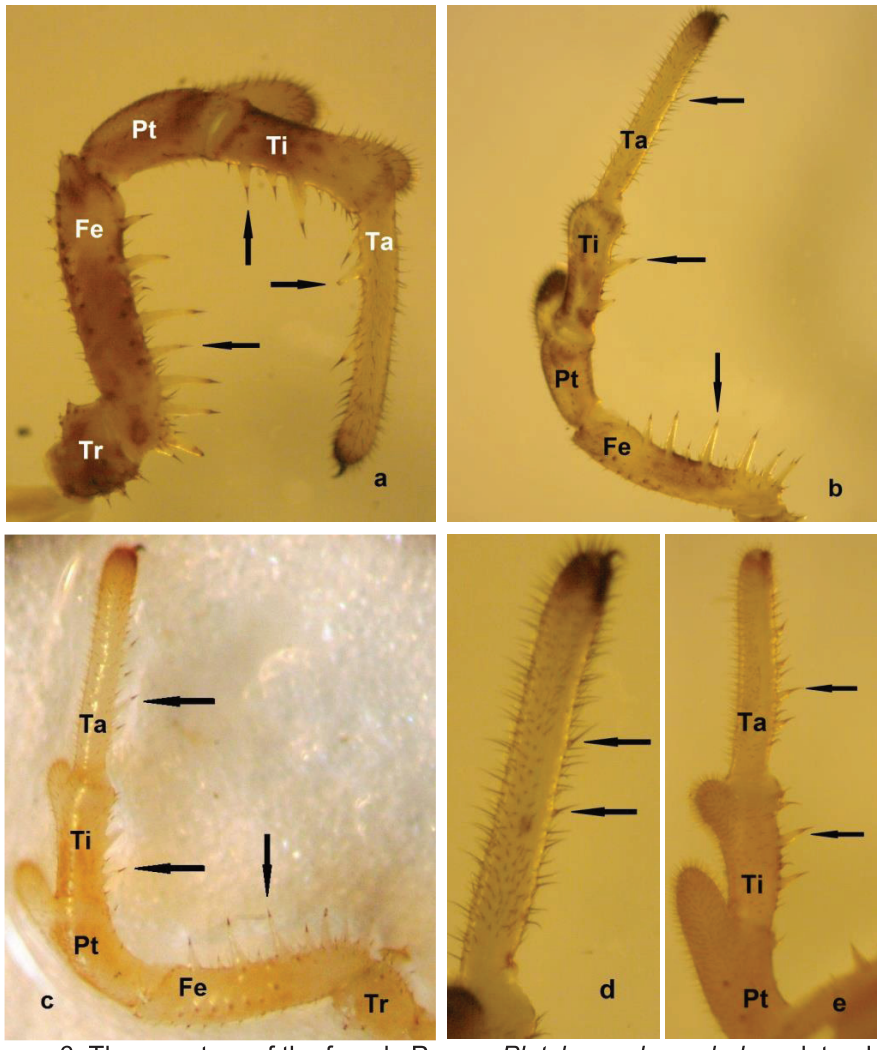

Figure 6. The armature of the female Pp: a – *Platybunus bucephalus* – lateral-extern; b – *Platybunus pinetorum* – lateral-extern; c – *Platybunus banarescui* – lateral-extern; d – *Platybunus pinetorum* – Ta-Pp; e – *Platybunus pallidus* – the distal part of the Pp – lateral-intern.

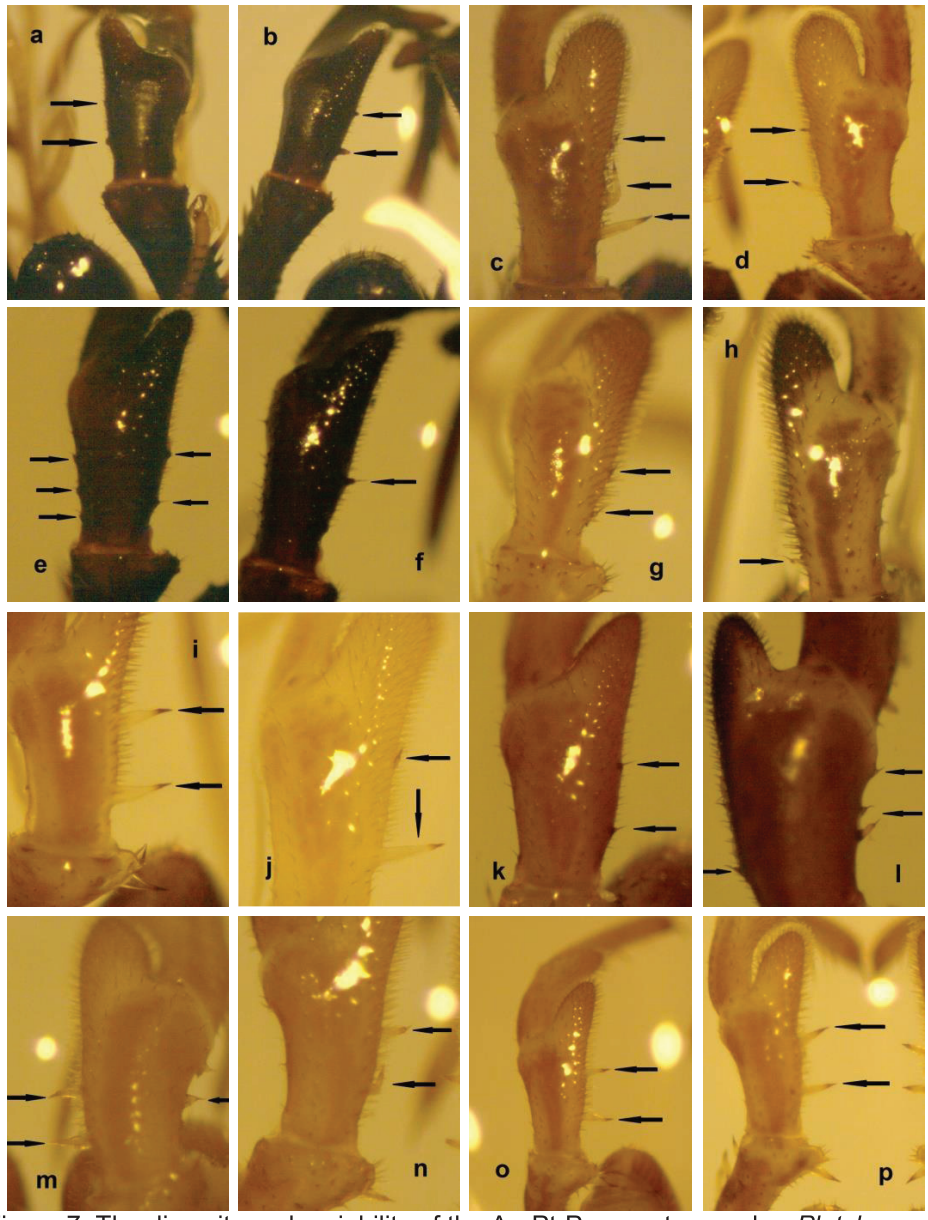

Figure 7. The diversity and variability of the Ap-Pt-Pp armature: a, b – *Platybunus bucephalus* male; c, d – *Platybunus bucephalus* female; e, f – *Platybunus pinetorum* male; g, h – *Platybunus pinetorum* female; i – *Platybunus banarescui* male; j – *Platybunus banarescui* female; k, l – *Platybunus pallidus* male; m, n – *Platybunus pallidus* female; o – *Platybunus pallidus* male habitus, immature; p – *Platybunus pallidus* female habitus, immature.

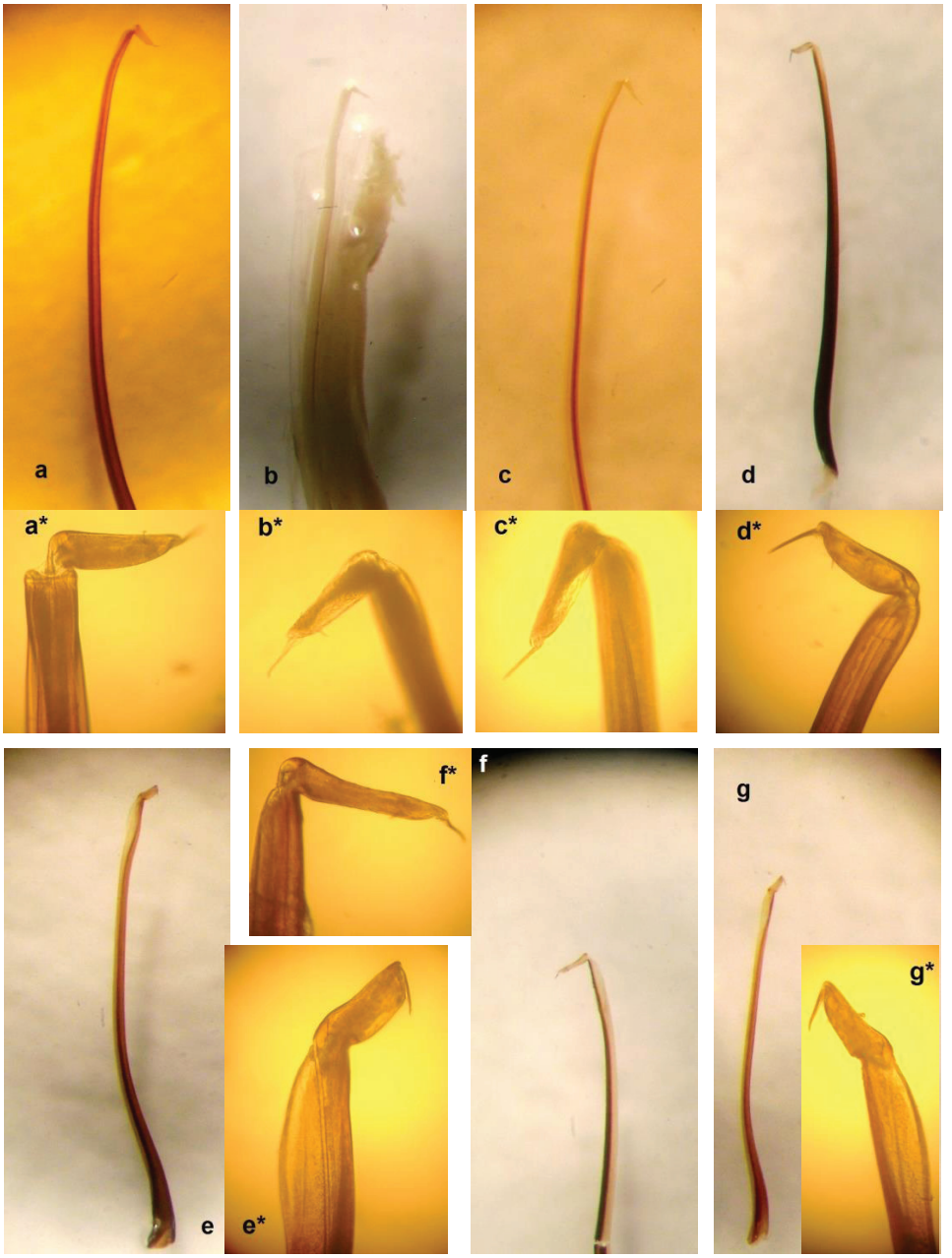

Figure 8. The penis: a, a* - *Platybunus bucephalus*; b, b* - *Platybunus banarescui*; c, c* - *Platybunus banarescui* albino; d, d* - *Platybunus pinetorum*; e, e* - *Platybunus decui*; f, f*, g, g* - *Platybunus pallidus* (* - the gland).

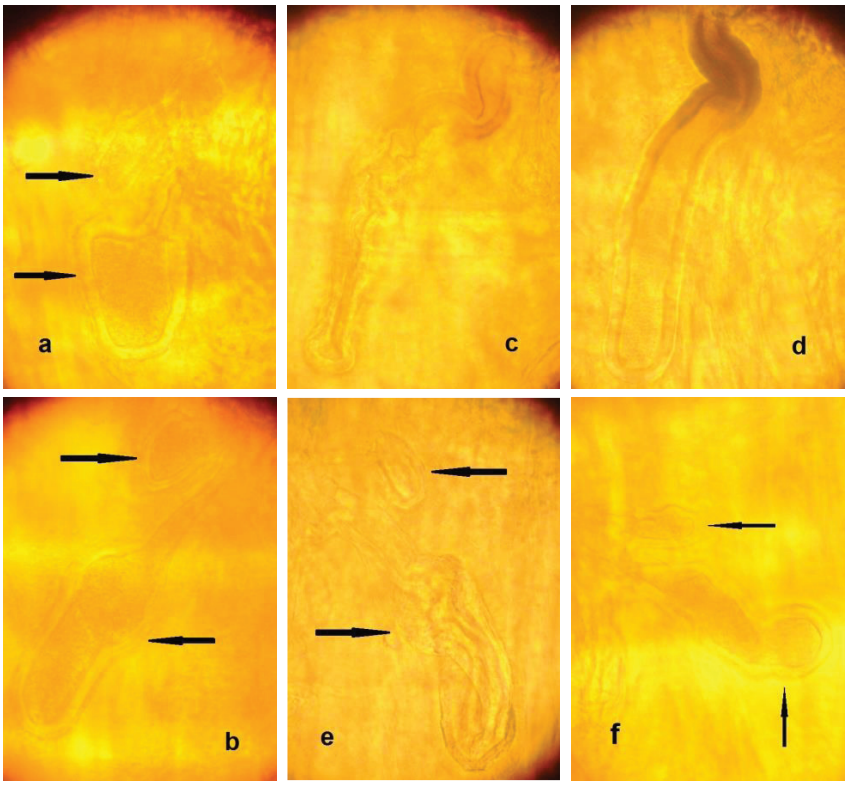

Figure 9. The seminal receptacles: a, b – *Platybunus bucephalus*; c, d – *Platybunus pinetorum*; e – *Platybunus banarescui*, f – *Platybunus pallidus.*

Platybunus banarescui – male

Habitus: light dorsum; visible saddle; light coxae, pedipalps and chelicerae (Fig. 1d).

The pedipalp (Fig. 4d, 5d): Fe-Pp with a medial apical tubercle and with a ventral row of spine-tipped white tubercles; Ap-Pt-Pp (Fig. 7i) with two medial conspicuous spine-tipped tubercles; Ti-Pp with 2-3 conspicuous unequal tubercles; Ta-Pp with a ventral row of conspicuous spine-tipped tubercles.

The penis (Fig. 8b): the body is getting thin near the gland; the gland is long and very flat.

In 14-17 June 1997 two unusual albino males, combining characters of both *Platybunus bucephalus* and *Platybunus banarescui* were collected.

Habitus: depigmented to white dorsum; visible saddle; depigmented to white coxae, pedipalps and chelicerae (Fig. 1e, f).

The armature of the pedipalps is *Platybunus bucephalus* specific: Ap-Pt-Pp with 2 medial denticles.

The penis (Fig. 8c) is *Platybunus banarescui* specific, with a very flat gland.

Platybunus banarescui – female

Habitus: androgin, male like; a light yellow to whitish dorsum, visible saddle with brown borders, light spotted chelicerae, pedipalps and coxae (Fig. 2h).

The armature of the pedipalp (Fig. 6c): Fe-Pp – with a medial apical tubercle (absent on one pedipalp of a female) and with a ventral row of conspicuous spine-tipped tubercles; Ap-Pt-Pp (Fig. 7j) with one spine-tipped tubercle and one spine-tipped denticle; Ti-Pp with 2-3 unequal ventral tubercles; Ta-Pp with a ventral row of conspicuous spine-tipped tubercles.

The seminal receptacles (Fig. 9e) consist of 2 pairs of unequal vesicles – the upper one is smaller, of a triangular shape; the lower one is elongated, with two prominences on their contour.

Platybunus pallidus – male

The male habitus is a character that showed variability in time. Thus, the males collected in June 1997 had a spotted yellow-brown dorsum and yellow-brown coxae, pedipalps and chelicerae (Fig. 1g). The males collected in August 1997, July 2009 and July 2009 had a dark brown dorsum, coxae, pedipalps and chelicerae (Fig. 1h, i).

The armature of the pedipalp (Fig. 5f) showed little variation and generally fit to the original description of Šilhavý (1938). All individuals had: Fe-Pp without apical medial tubercle and with a ventral row of spine-tipped yellow to brown tubercles; Ap-Ti-Pp – broad, of a triangular shape, with a small groove (hollow) near the Ti-Ta junction; Ti with 2 ventral unequal spine-tipped tubercles; Ta – without a basal protuberance and with a ventral row of low spine-tipped tubercles.

Ap-Pt-Pp (Fig. 7k, l) showed little variability and inconsistency with the literature (2 unequal denticles, Martens, 1978 fig. 460, pg. 259). On the medial side of the Ap-Pt-Pp, most males have 2 short spine-tipped denticles or simple spines originating from a darker chitinous area. Few males have external denticles on the Ap-Pt Pp.

The penis (Fig. 8f, g) has a thick body near the junction with the gland and is curved ventrally in a gutter shape, similar to *Platybunus decui*. The gland showed variability, from a moderate thickness to very flat.

Platybunus pallidus – female

Similar to males, the female habitus showed variability in time: the females collected in July 1997 (Fig. 2i) have a lighter dorsum, coxae, pedipalps and chelicerae than those collected in August 1997, July 2001 and 2009.

The pedipalp (Fig. 6e) presented some constant features (in all individuals) consistent with the literature: Fe-Pp with a spine-tipped tubercle in the medial apical position – just two females lacked the tubercle on one or both pedipalps and one female had two tubercles on a pedipalp and one tubercle on the other pedipalp. Other constant features: Fe – with a ventral row of spine-tipped yellow to brown tubercles; Ti-Pp with 2 ventral unequal spine-tipped tubercles, more developed than of the males; Ta-Pp with a ventral row of spine-tipped tubercles, longer than in the males.

Ap-Pt-Pp (Fig. 7m, n) showed a high degree of variability and sometimes inconsistency with the literature (2 equal tubercles on each Ap – Martens 1978, pg. 259, fig. 462). Most collected females show the asymmetry of the pair pedipalps

with 1 – 4 medial and ventral-external denticles and tubercles, which are sometimes ”L” shaped.

The seminal receptacles (Fig. 9f) are constantly represented by 2 pairs of vesicles: an upper smaller one and a lower, tubular vesicle with a small median strangle.

All the immature specimens collected in June 1997 present a female-like armature of the pedipalp, with 2 spine-tipped tubercles on the Ap-Pt-Pp (Fig. 7o,p), although 3 – 4 individuals have a male-like aspect (Fig. 3a) and 8-9 individuals present a female-like aspect (Fig. 3b). The dissection of a female-like specimen revealed an immature ovipositor, while the dissection of male-like specimen did not reveal a penis.

3. Discussions

Some morphological characters, especially the female habitus and the armature of the pedipalp showed a certain degree of variability and inconsistency with the literature.

The habitus

Proved to be a highly variable character, difficult to be used in species delimitation:

a) *Platybunus pinetorum* females with different dorsum (Fig. 2d, e, f, g);

b) The great resemblance between some *Platybunus pinetorum* and *Platybunus bucephalus* females (*P. pinetorum* female – 17 June 2016 Cabana Botanică, on walls – Fig. 2f; *P. bucephalus* female – 23 July 1997 vicinity of Galbenul River – Fig. 2c.

The male appearance of *Platybunus banarescui* females suggests gynandromorpy, namely, “individuals in which both sexes are discretely combined” (Cockendolpher & Sissom, 1988). *Platybunus banarescui* females might be transverse gynandromorphs, although it is less probable for 3 gynandromorph females to appear at the same time in nature. Gynandromorphy has been reported in some Opiliones species, e.g: *Mitopus morio* (Cokendolpher & Sissom, 1988), *Melanopa grandis* (Tsurusaki, 1982), *Leiobunum globosum* (Suzuki 1980 in Tsurusaki 1982).

The pedipalp

The male pedipalp

On the whole, the armature of the male Pp showed a certain degree of variability and inconsistency with the literature in *Platybunus pinetorum*, *Platybunus bucephalus* and *Platybunus pallidus*. This variability was obvious at the level of the armature (denticulation) of Ap-Pt-Pp, which is constantly present in all three species (in all or in most of the individuals). The number of the denticles, however, varies showing even the asymmetry of the pedipalps of the same individual.

The presence of two denticles in medial position on Ap-Pt-Pp is *Platybunus pallidus* specific, as shown in Martens (Martens, 1978, pg. 259, fig. 460). A similar characteristic – 1 to 4, most frequently two medial punctiform denticles on the Ap-Pt-Pp is also present in *Platybunus pinetorum* and *Platybunus bucephalus* as a constantly present feature (related to number of individuals and time interval), which however is not specific to these species (inconsistent with the literature).

Ta-Pp is given as - ”aucun tubercule spiniforme bien distinct” in the ventral part in both sexes in *Platybunus pinetorum* (Iorio & Delfosse 2016) and figured by Martens (1978, pg. 259, fig. 456) with few spiniform denticles in *Platybunus*

pinetorum males. In our population both males and females presented few spiniform denticles in the ventral part of Ta-Pp (Fig. 5e – male, Fig. 6d – female).

The ventral part of Ta-Pp in *Platybunus bucephalus* is reported with ”plusieurs tubercules spiniformes de longueur modérée” (Iorio & Delfosse 2016) in both sexes and figured by Martens (1978, pg. 259, fig. 453) with the same moderate long spiniform tubercles in males. This aspect of the Ta-Pp is available in our studied population.

The female pedipalp

Similarly to the male’s pedipalp, the armature of the female’s pedipalp presented a certain degree of variability and inconsistency with the literature in *Platybunus pinetorum*, *Platybunus bucephalus* and *Platybunus pallidus*. This variability is present at the level of the Ap-Pt-Pp denticulation. The Ap-Pt-Pp denticulation is constantly present in the three species (in all or in most of the individuals), but the number of denticles varies, even showing asymmetry of the pedipalps of the same individual.

The denticulation of the Ap-Pt-Pp (2 medial denticles) in *Platybunus pinetorum* is a constantly present character, but it is not species specific for *Platybunus pinetorum*, as described in literature.

The armature of the Ap-Pt-Pp (2-3 unequal tubercles) in *Platybunus bucephalus* is a constantly present feature which showed variability in respect with the number of the medial tubercles, up to the asymmetry of the pair pedipalps. This aspect of the Ap-Pt-Pp is one between Martens – one medial tubercle – (Martens 1978) and Iorio & Delfosse – 2 equal spiniform tubercles (Iorio & Delfosse 2016).

Ap-Pt-Pp in *Platybunus pallidus* constantly has 2 medial tubercles more or less equal. Some females have ventral-external denticles, a feature which is not consistent with the literature (Martens, 1978).

The seminal receptacles

The appearance of the seminal receptacles differs from that recorded in the literature in *Platybunus pinetorum* (Martens 1978-p. 260, fig. 465) and *Platybunus pallidus* (Martens 1978-p.260, fig. 464; Šilhavý 1956, pg. 235, fig. 417). If in *Platybunus pallidus* the seminal receptacles are at least represented by 2 pairs of unequal vesicles, in none of the *Platybunus pinetorum* females the upper smaller vesicle could be visualised.

The penis

It is rather a character difficult to be used because of its great resemblance between species and because of the gland variability in some species: the penis revealed variability in *Platybunus pallidus*, ranging from a moderate thick gland (Fig. 8g) to a very flat gland (Fig. 8f), thus resembling the gland of *Platybunus banarescui* (Babalean 2011, pg. 102, fig. 39h). The body of the penis near the junction with the gland – thick and curved ventrally is a common feature in *Platybunus decui* and *Platybunus pallidus*.

On the basis of the Pp and seminal receptacles one can assign at least the collected *Platybunus pinetorum* females from Rânca to a different species, thus unreasonably increasing the species diversity.

Because of their flattened shape of the penis gland and of the fact that they were recorded in June, the albino male individuals with a particular habitus should be included in *Platybunus banarescui*, even if the armature of their pedipalps is *Platybunus bucephalus* specific. The two individuals might be considered as individual variations within *Platybunus banarescui*.

At present it is impossible to provide an explanation for the morphological aspects presented in this paper (variability and inconsistency with the literature). Three independent or correlated factors might be considered:

1) The clinal variation of the morphological characteristics, which may also influence the seminal receptacles and the penis;

2) The appearance of hybrid populations.

The observation of the species succession during the entire summer season shows the coexistence in the same habitat of at least two sexually mature species in each summer month - Table 2.

Table 2

Platybunus species succession during the entire summer season

June	July	August
Platybunus banarescui	*Platybunus pinetorum*	*Platybunus pinetorum* – very few
Platybunus pinetorum	*Platybunus bucephalus*	*Platybunus bucephalus*
	Platybunus decui	

The succession of the sexually mature adults along the summer months and the previously analysed morphological characters suggest the development of hybrid populations. Proving the existence of such hybrid populations would have a major bearing on the structure and systematic of the genus Platybunus.

3) Parthenogenesis, considering the fact that the variability of the morphological characteristics is also encountered in some invertebrates with a partenogenetic reproduction (rotifers, inferior crustaceans...).

Parthenogenesis is facultative in Opilionids and has been reported for various species, including *Platybunus pinetorum* (Tsurusaki 1986 and included references). Tsurusaki (1986) assumes in the light of the Red Queen hypotheses that parthenogenesis might act as a selective factor in species competition ("the thelytokous species with little genetic variance can survive only in a less competitive community" = cannot survive in competitive communities). We may also assume that the species with facultative parthenogenesis can "escape" the competitive environment (community) by leaving it and thus, changing the areal. In parthenogenetic species, sex-ratio might be a good indicator of areal changing – a female biased sex-ratio indicates the occurrence of parthenogenesis which is presumed to act in the expansion of territory in *Platybunus pinetorum* in Sweden (Fritzén et al. 2015 and included references).

A male biased sex-ratio might indicate a stable (settled) or an in regression areal. The sex-ratio for the best represented Platybunus species in Rânca – Mohorul area is male biased in *Platybunus pinetorum* and *Platybunus bucephalus* and female biased in *Platybunus pallidus*.

Morphologically speaking, the 5 Platybunus species encountered in Rânca-Mohorul area can be separated in 2 groups:

1) *Platybunus pinetorum* group – with one species: *Platybunus pinetorum*;

2) *Platybunus bucephalus* group – including: *Platybunus bucephalus*, *Platybunus banarescui*, *Platybunus decui* and *Platybunus pallidus*.

In the latter group *Platybunus decui* and *Platybunus pallidus* seem to be closely related by the aspect of the male habitus, penis and armature of the pedipalp (Fe, Ap-Pt, Ti, Ta), except the Ap-Ti-Pp. *Platybunus decui* and *Platybunus bucephalus* share the same basal protuberance on the Ta-Pp. Until a full systematic revision is made, including one on a molecular basis, we shall consider *Platybunus bucephalus* and *Platybunus banarescui* to be two similar but yet distinctive species, separated in terms of morphology and time. As far as the males are concerned, the morphological differences include: *Platybunus banarescui* – Ap-Pt-Pp with 2 conspicuous spiniforme tubercles, Ta-Pp with a ventral row of conspicuous tubercles, a much thinner gland of the penis; *Platybunus bucephalus* – Ap-Pt-Pp without tubercles (maximum with short denticles), Ta-Pp with a ventral row of tubercles of moderate size, a thicker gland of the penis. The females of the 2 species show a great resemblance; the single distinctive character is the habitus. The seminal receptacles vary a little in terms of shape.

CONCLUSIONS

In the region Rânca-Mohorul the genus Platybunus is represented by 5 species: *Platybunus bucephalus*, *Platybunus pinetorum*, *Platybunus decui*, *Platybunus banarescui* and *Platybunus pallidus*.

The morphological characteristics presented increase the variation limits of these species.

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BREEDING AQUATIC BIRDS ON THE TERRITORY OF CRAIOVA CITY (DOLJ COUNTY, ROMÂNIA)

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KEYWORDS: *aquatic birds, aquatic habitats, breeding pairs, nests, chicks*

ABSTRACT

The present work brings additional information on the breeding of aquatic birds within the municipality Craiova (plain city located in the South-West of Romania). We monitored the number of breeding species, their breeding area, location of nests, estimated the breeding pairs, the presence of chicks and juveniles. Of the 46 aquatic species inventoried since 2000 within the aquatic habitats of the city, there were registered 20 species that nested between 2013 and 2016, while 5 species are considered to be possible breeding species.

INTRODUCTION

Craiova Municipality, Dolj County seat, is an important urban centre, located in the plain area in the South-West of Romania, on the left bank of the Jiu River. Bird monitoring occurred in the main parks of the city (Nicolae Romanescu Park, Craioviţa Lake and Park, the Botanical Garden, Youth Park), along the Jiu River and near the marshes located on the outskirts of Mofleni district (Figure 1).

Nicolae Romanescu Park is situated in the southern part of Craiova. It has a surface of about 96 ha, being one of the largest and most picturesque parks in the country. The aquatic habitat is represented by some lakes with a surface of about 4 ha (water surface). There is an island with willows within the central lake.

Craiovița Lake is located in the northwestern part of the city. It is separated by an isthmus in two lakes of unequal sizes. They are surrounded by characteristic aquatic vegetation (reed, rush, clumps of willows /or in certain sectors willows etc.); within their perimeter, there can be also found some small islands. In many sectors of the lakes, there appear compact reed beds. By 1990, the lake together with the nearby park had 85 ha, 32 ha being covered by water (Ciobotea et al., 1999). After 1990, the land became the private property of a company. Consequently, there were many modifications with major changes in the original habitat. This led to the reduction of the habitat for different constructions (hotels - Flormang, Articus; hypermarkets - Auschan, Bila; gas stations, etc.).

The Botanical Garden "Alexandru Buia" is situated in the southwestern part of Craiova Municipality. It has a surface of about 17 ha. Along the creek that crosses the garden from East to West, there were built three lakes totalizing a surface of 0.3 ha. The lakes contain typical aquatic plants with numerous water

lilies. Starting with August 2015, the Botanical Garden is subject to a modernization process that has greatly affected the aquatic avifauna.

The Youth Park, with a surface of about 55 ha, is situated East of the Jiu River and is limited by Șerca creek in the North. Along the banks of this creek there grow rush and reed on a width of 0.5/1 m – 2 m; from place to place, there also grow trees, such as willows, alders, etc.

Within the territory of Mofleni district, there are two marshes, namely Mofleni and Șerca. Mofleni Marsh (near the former ceramics factory) is permanent, with a surface of about 3-4 ha. It is surrounded by reed and rush on a width of 0.5-2 m. In the eastern part of the marsh, the reed beds are compact. Șerca Marshes appeared in the pits (of different sizes and depths) used to deposit the residues from the ceramics factory. After the failed privatization and destruction of this factory, the pits filled with rain water were invaded by characteristic paludous vegetation (*Phragmites australis, Typha latifolia, Scirpus lacustris, Oenanthe aquatic*, etc.). In certain parts, the reed beds are compact. The southernmost marshs gets dry during hot summers. The marshes extend over about 1 km, being separated by paths. Their names come from Șerca creek that flows North of them. Besides Șerca marshes, there is found some uncultivated land, where cattle, goats and sheep graze.

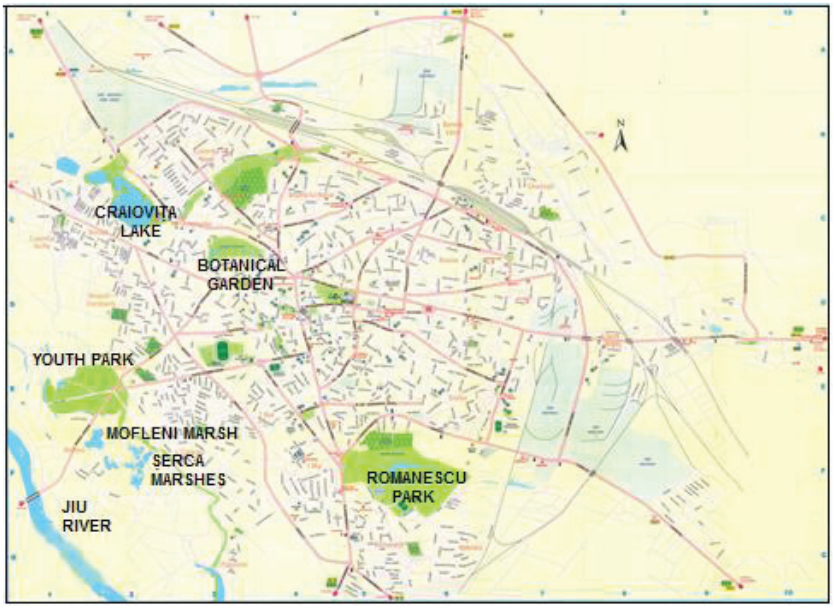

Figure 1. Map of Craiova Municipality rendering the studied habitats (achieved by SC InDesign SRL Craiova).

This paper continues the ornithological observations started in 2000 bringing further information on the reeding aquatic species from the last 4 years. Our attention turned to: the phenology of aquatic avifauna of Craiova, the places where they build their nests, the estimated number of breeding couples, the number of chicks and juveniles.

MATERIALS AND METHODS

The monitoring of the species of breeding aquatic birds was based on the classical methodology. The field trips in the parks and gardens from Craiova were performed monthly, most of them in the morning (8 a.m.-2 p.m.), but also in the afternoon (5 p.m. to 8 p.m.). The observations along transects were made with optical instruments: binoculars (Buschnell), Canon SX40 HS camera, Panasonic videocamera. The classification of the species from the systematic viewpoint was based on the atlas of birds of community interest (2015). In determining the species, there were also used different guides (Bruun et al. 1999; Peterson et al. 1989). The processing of the information obtained in the field from the biological, ecological and ethologically point of view was performed according to specialized literature (Munteanu 2005, 2009, 2012, Tălpeanu 1969). The results of the observations rendered in the present paper are from the last four years: from 2013 to 2016.

RESULTS AND DISCUSSIONS

In spite of all the changes made over the years in the infrastructure of the city, birds were attracted to water basins that provided food source, shelter and breeding ground. In time, birds have adapted to anthropogenic conditions so that their presence in these areas has become habitual for local people.

Of the 46 aquatic and semiaquatic species (including the warbler and wagtail species of Passeriformes, as well as the marsh harrier) identified within the territory of Craiova (Bălescu, 2013), in the period 2013-2016, we observed 20 certainly breeding species (3 sedentary and 17 migratory) and 5 possibly breeding species (Table 1).

During the monitoring we found that the number of species and nesting pairs varied depending on both climatic (rainfalls, storms, droughts) and anthropogenic factors. Rainy years had negative effects on certain limicolous and tern (Sternidae) species and prolonged drought made some bird species seek more favourable territories. Generally, aquatic species tolerated each other and there was not a serious competition among them. The first species that came in the area occupied the adequate places. The ones that arrived later moved to other water bodies of the city or remained in the area in small numbers. They usually breed in mixed or monospecific colonies (small) or in solitary pairs. Most species (18 species) built their nests near Șerca marshes and Craioviţa Lake (15 species). At Mofleni marsh, there nested 12 species (Table 1). While some species are frequently breeding, others breed just sporadically.

We shall further render some aspects referring to the breeding of the aquatic species from the last four years.

- *Cygnus olor* (The mute swan), which is a winter or a passage guest species has nested in the reed beds at Șerca marshes in the last two years. In 2015, they had nine cygnets and, in 2016, eight cygnets. The voluminous nest was placed in the reed bed, at the edge of the water body in the same place in both years).
- *Anas platyrhynchos* (The mallard) is a common sedentary species, constantly breeding in the reed beds located near Craiovița Lake and the marshes from Mofleni district. The number of chicks accompanying the female duck varied between 8 and 14. Starting with 2014, it has also nested within Romanescu Park: two-three pairs in 2014 and 2015; four pairs in 2016 with seven-nine chicks. Here, they laid their eggs on the ground, at the base of hollow willows.

- *Aythya ferina* (The common pochard) is a common passage species. It sporadically breeds in a small number of specimens, one-two pairs in 2013 and 2015, at Craiovița Lake, respectively 2013 and 2014 at Șerca marshes and, in 2014, at Mofleni marsh.
- *Aythya nyroca* (The ferruginous duck) is a constant reed breeding species, but in small numbers, two-four pairs. We remarked it at Craiovița Lake together with four-six chicks on the water in each of the last four years. It sporadically breeding at Șerca marshes (in 2014 and 2015) and Mofleni marsh (2016).
- *Tachybaptus ruficollis* (The little grebe), a very discrete species, regularly bred at Craiovița Lake - three to eight pairs. The floating nests were placed on small water surfaces within the reed beds. The breeding period extended from May till July. In 2016, only three pairs bred on the small lake and five on the larger lake. On the 15th of August, we identified between three and five juveniles accompanying the pairs. As for Șerca and Mofleni marsh, breeding was irregular (sporadic) and there were between one and four pairs.
- Four species of Ardeidae are breeding in the area. *Ixobrychus minutus* (The little bittern) is a constant breeding species in the aquatic habitats. It usually arrives at the beginning of May. The lowest number of pairs was registered at Mofleni in 2015 – two pairs, and the largest in 2013, +9 pairs at Craiovița Lake. Juveniles that could not fly were observed at the edge of the reed bed, sitting on rush or reed stems.
- *Nycticorax nycticorax* (The black-crowned night heron), a frequent species, breeds within the aquatic habitats of Craiova in mixed but small colonies, together with other heron species. They first arrive in the area by the end of March, respectively the beginning of April. Starting with 2013, three – nine pairs have bred at Craiovița Lake. The nests were built in the willows located in the middle of the island within the larger lake. On the 28th of April 2016, nine nests were occupied by adults. In the first half of July, there were noticed between two and three non-flying juveniles in the nest. Starting with 2014, breeds within the undergrowth on the bank of the Jiu. In 2016, at Șerca marshes, two pairs (with three chicks each) also nested in the compact reed bed together with other herons (little bittern, squacco heron and little egret). The increase of the number of the breeding pairs of the black-crowned night heron from one year to another is due to the adaptation of the species to the existent conditions.
- *Egretta garzetta* (The little egret) is a species frequently noticed within the aquatic habitats of Craiova. Starting with 2014, the species is breeding in mixed colonies together with the black-crowned night heron in the undergrowth near the Jiu (four-seven pairs); in 2016, two pairs both at Craiovița Lake in association with the black-crowned night heron and in the compact reed beds from Șerca marshes.
- We mention the breeding of a pair of squacco heron (*Ardeola ralloides)* for the first time since monitoring birds in the area in the compact reed beds from Șerca marshes, in the period May-June. On the 27th of July, at the edge of the paludous vegetation, in the area of the highly eutrophized pool, we noticed the presence of four juveniles besides the adults. One month later, on the 24th of August 2016, the four juveniles were present in association with other herons in the dry area of the marsh, looking for food. We no longer noticed the adults. The four specimens of heron left the territory in the second half of September (on the 18th of September they were no longer observed).

- *Ciconia ciconia* (The white stork), constantly breeding (from 2010) in Mofleni district, there being identified only one pair. The number of chicks/year varied: four chicks in 2013, two chicks in 2014, three chicks in 2015. This year, of the three chicks, there survived only two. One of them fell down from the nest when trying to fly and could not be recovered. The behaviour to attract a partner for mating was a nice surprise.
- The two Rallidae: *Gallinula chloropus* (common moorhen) and *Fulica atra* (Eurasian coot) are common, sedentary species that breed within the reed beds, at the edge of the water bodies, at Craiovița Lake, Mofleni and Șerca marshes. Generally, the common moorhen has a lower number of breeding pairs compared to the Eurasian coot. We remarked pairs that laid eggs twice a year. *Fulica atra* laid eggs two/three times per year, the earliest by the end of February and the latest by mid-August. Between March and September, on the lakes and marshes from Craiova, there were noticed specimens in different stages of development. There were certain situations when, because of bad weather conditions, pairs laid eggs only once a year. The black chicks with red feather on the head and neck are very cute, standing beside their parents and trying to copy their behaviour. In Romanescu Park, till 2015, we noticed the presence of two-three pairs of Eurasian coot. Starting with 2015, the Eurasian coot has been replaced by two pairs of common moorhen. In the Botanical Garden, the Eurasian coot nested until 2015 (one-two pairs). However, the common moorhen remained and nested here even in 2016 (a pair with seven chicks), although the modernization works continued after the 5th of April. The adults of the Eurasian coot appeared on the lake together with four-seven chicks and the common moorhen with three-six chicks.
- *Chlidonias hybrida* (The whiskered tern) is one of the common species of the aquatic habitats of the city. It comes in the interval April-May (the last decade of April till the first half of May). On Craiovița Lake, we remarked a smaller number of pairs – two-five pairs. In 2016, at Șerca marsh, the first specimens of whiskered tern arrived on the 23rd of April. The extremely noisy pairs built nests in the southern part marsh. About one month later, on the 25th of May 2016, they had already laid eggs in their nests. We counted 15 nests placed in shallow water. The aspect of the nest was erratic. The nests were built from reed and rush stems. They were grouped in two or three nests at a distance of 0.5-3 m from each other. Other nests were at 7-8 m from the first groups. Between the first and the last nest, there was a distance of about 30 m. In the nests, we noticed one-two and even three eggs. Some nests were in an incipient phase, unfinished, and partners were carrying materials in their beak in order to finish it. One month later, on the 26th of June, we observed the extremely cute and noisy chicks - two nests with one chick each, four nests with two chicks each, and the other nests with three chicks each. In two nests, we could not observe the chicks as they were protected by adults. Generally, our presence within the habitat led to more aggressive manifestations of the adults, namely flying and stridently screaming above us. On the 27th of July, the juveniles were able to fly. On the 23rd of August, they were no longer observed. During our field research, we were informed that some hunters had come in the area to shoot ducks, in order to reach the hunting quota. The consequence was that a part of the species (whiskered tern, little egret) or specimens of big ducks, common moorhen, Eurasian coot, etc. left the breeding area.

- *Vanellus vanellus* (The northern lapwing) constantly breeding (between five and -+nine pairs) in the field near Șerca marshes in the last years. The nests were built in the unevennesses of the ground, in abundant herbaceous vegetation.
- In case of the two species of gull, *Larus ridibundus* (black-headed gull) and *Larus cachinnans* (Caspian gull), we did not remark any nests within the administrative territory of Craiova or nadification activities. Instead, we observed juveniles together with adults in almost all months of the year, both in the aquatic habitats of the city and the sands near the Jiu. This makes us think that they have nests near the city. It remains to be found the breeding area.
- *Himantopus himantopus* (The Common stilt) it is possible to have nested in 2014 and 2015 at Șerca marshes. In these years, in June, we noticed a noisy pair above the reed bed, which made us conclude that they defended their chicks present in the nest. In the first week of August, we saw both juveniles and adults in the area without water in search for food. Among all stilt species, this one is the most sensitive to human activity. It reacts to any noise in the area (the presence of humans and mammals that graze in the area, dogs, etc.).
- *Circus aeruginosus* (The western marsh harrier) was noticed within the territory of Craiova in the interval March/April - September, hovering above the reed beds. We do not have clear observations with regard to the nests of the species in the studied area. However, both in 2013 at Craioviţa Lake and in 2014 at Șerca marshes, we noticed one-two specimens overflying the area in the breeding season. We witnessed how the western marsh harrier raised above the paludous vegetation, hovered in circles to appreciable heights, then plunged into the reed bed. After a few minutes, it raised up again. These activities – raising, hovering, descending made by one or both partners, led us to the idea that they remained here both for food and for breeding.
- *Motacilla flava* (The western yellow wagtail) breeds in the unevennesses of the ground, in the herbaceous vegetation near Șerca marshes. In the area, there predominate specimens of *Motacilla flava feldegg, Motacilla flava flava* and *Motacilla flava thunbergi*. In the evening, many adult and juvenile specimens (+25 specimens) shelter in the reed beds.
- *Motacilla alba* (The white wagtail) did not breed in the area of Șerca marshes and that of the Botanical Garden. In the other analysed areas, we noticed the species breeding in the hydrophilous vegetation near the water bodies, in reed piles but also in other habitats. It is possible that the breeding population exceeds 10 pairs in the area of Craiovița Lake.
- In the reed beds, we also noticed warbler nests, which were fixed on the stems of the paludous vegetation (most of them reed stems). In the area, are nesting *Acrocephalus arundinaceus* (the most widespread warbler) and *Acrocephalus scirpaceus*. *Acrocephalus schoenobaenus* and *A. palustris* are nesting sporadically. None of the warbler species nested in Romanescu Park, while at the Botanical Garden, there bred only *Acrocephalus arundinaceus* that remain the most dominant warbler in this area. Human impact (manifested by the reduction of the space for parks in favour of the construction of hotels and supermarkets; the noise of daily activities; sport fishing; grazing on the meadows near the marshes that leads to the destruction of the nests located at the ground level; cutting and burning reed at inappropriate times – exactly during the breeding period, occasional hunting at Șerca marshes, etc.).

Table 1

Phenological category and estimative appreciation of the breeding pairs from the aquatic habitats of Craiova (2013-2016) and their conservation and protection status

No	Species	Phenology Craiova	Craiovița Lake	Șerca Marshes	Mofleni Marsh	Youth Park	Jiu	Botanical Garden	Park Romanesu	Red book of Vert. in Rom	Bird Directive
			Breeeding Pairs								
1.	***Cygnus olor***	WV,SV,B		1							
2.	*Anas crecca*	WV,P									
3.	***Anas platyrhynchos***	S, B	9 – 15	4 – 12	2 – 4	2			2 – 4		
4.	*Spatula (Anas) querquedula*	P, SV									
5.	*Spatula (Anas) clypeata*	P, SV									
6.	***Aythya ferina***	SV, P, B	1 – 2	1 – 2	1 2014						
7.	***Aythya nyroca***	SV, B	2 – 4	2 – 4	2 2016					VU	Annex 1
8.	*Mergus albellus*	WV									Annex 1
9.	***Tachybaptus ruficollis***	SV, WR, B	3 – 8	3 – 4	1 – 3						
10.	*Podiceps cristatus*	P, SV									
11.	*Podiceps nigricollis*	WV									
12.	*Phalacrocorax carbo*	P, WR									
13.	*Microcarbo pygmaeus*	P, SV, WR								VU	Annex 1
14.	***Ixobrychus minutus***	SV,B	6 – 9	5 – 7	2 – 4						Annex 1
15.	***Nycticorax nycticorax***	SV,B	3 – 9	2 2016			5 – 8			VU	Annex 1
16.	***Ardeola ralloides***	P, SV, B		1 2016						VU	Annex 1
17.	***Egretta garzetta***	SV, B	2 2016	2 2016			4 – 7			EN	Annex 1
18.	*Ardea alba*	P, WV								EN	Annex 1
19.	*Ardea cinerea*	SV, WV									
20.	*Ardea purpurea*	P								EN	Annex 1
21.	***Ciconia ciconia***	SV, B		Mofleni Neighborhood (one pair)						VU	Annex 1
22.	***Circus aeruginosus***	SV, PB	1	1							Annex 1

			2013	2014							
23.	***Gallinula chloropus***	S, B	8 – 14	4 – 6	2 – 4	1 – 3		1 – 3	2		
24.	***Fulica atra***	S, B	10 – 16	8 – 10	3 – 5	1 – 2		1 – 2	2 – 3		
25.	***Himantopus himantopus***	SV, PB		1						EN	Annex 1
26.	*Recurvirostra avosetta*	P									Annex 1
27.	*Charadrius dubius*	P									
28.	***Vanellus vanellus***	SV, B		5 – 9							
29.	*Calidris minuta*	P									
30.	*Tringa ochropus*	P,WV									
31.	*Larus fuscus*	P, RWV									
32.	***Larus (Chroicocephalus) ridibundus***	SV,WV, PB					PB				
33.	***Larus cachinnans***	SV,WV,PB					PB				
34.	***Chlidonias hybrida***	SV,B	2 – 5	5 – 15	2 – 3						Annex 1
35.	*Chlidonias niger*	P									Annex 1
36.	*Chlidonias leucopterus*	P									
37.	*Alcedo atthis*	WV									
38.	***Motacilla alba***	SV, B	4 – 10		5 – 8	+6			+5		
39.	***Motacilla flava***	SV, B		+9							
40.	*Motacilla cinerea*	WV									
41.	***Locustella luscinoides***	SV, P, PB	PB	PB							
42.	***Acrocephalus schoenobaenus***	SV, B	+3	+4	2 – 3						
43.	***Acrocephalus palustris***	SV, B	2-3	+3							
44.	***Acrocephalus scirpaceus***	SV, B	+4	+6	+3						
45.	***Acrocephalus arundinaceus***	SV, B	+5	+8	3 – 4			1 – 2			
46.	*Emberiza schoeniclus*	WV									

Legend: Phenological status: SV-summer visitor, WV – winter visitor, P – passaje species, S – sedentary species, B – breeding, PB – possiblly breeding; Red book of Vert.in Rom.= Red book of vertebrates in Romania: VU – vulnerable, EN – endangered

bad weather conditions (high winds, prolonged drought, etc.) has not obviously prevented the presence and nesting of the species within the aquatic ecosystems, as birds adapted to these perturbation factors in due time. The very rich trophic offer, compact and thick reed beds (in certain parts) at Craioviţa Lake, Mofleni and Șerca marshes represented an attraction to stay in the area and certainly breeding for a diverse number (relatively large) of aquatic and semi-aquatic species (Podicipedidae -1 species, Anatidae - 4 species, Ardeidae - 4 species, Ciconiidae - 1 species, Rallidae - 2 species, Charadriidae - 1 species, Sternidae – 1 species, Motacillidae – 2 species, Sylviidae - 4 species), although the number of breeding couples is relatively low. The behaviour of birds (breeding, feeding, etc.) was always attractive and surprises in a pleasant way. Therefore bird monitoring will continue.

CONCLUSIONS

In the period 2013-2016, in the aquatic habitats from Craiova city (Craioviţa Lake, Șerca and Mofleni Marshes, Șerca creek, the Jiu River, the lakes from the Botanical Garden and Romanescu Park, etc.), there were identified 20 certainly breeding species and 5 possibly breeding species.

The main species, constantly breeding in the area in these years are: *Aythya nyroca*, *Anas platyrhynchos*, *Ixobrychus minutus*, *Nycticorax nycticorax*, *Ciconia ciconia*, *Tachybaptus ruficollis*, *Gallinula chloropus, Fulica atra*, *Vanellus vanellus, Chlidonias hybrida*, *Acrocephalus scirpaceus, A. arundinaceus, Motacilla alba, Motacilla flava.* At this list, we can also add *Egretta garzetta* starting with 2014.

Species breed in solitary pairs or in mixed or monospecific colonies. Most species have bred at Șerca Marshes (18 species) followed by Craioviţa Lake (15 species) and Mofleni Marsh12 species).

Depending on the location of the nests, we distinguish: - birds breeding in the reed beds, most of them (Anatidae, Rallidae, Ardeidae, warblers, etc.); -birds that build floating nests (*Chlidonias hybrida, Tachybaptus ruficollis*); -birds that breed in trees (willows, undergrowth) (*Nycticorax nycticorax, Egretta garzetta)*; - birds that breed on telegraph poles (white stork); -birds that breed on the ground, between weeds (V*anellus vanellus,* Motacillidae).

From year to year, the number of breeding aquatic species and pairs varied depending on the vegetation of the water basins, trophic resources, water level, weather conditions (rainfalls or drought) or anthropogenic factors (felt differently by each species).

Of the breeding and possibly breeding species six species drew our attention as they are found both in the Red Book of Vertebrates of Romania as having a certain status of endangerment and in Annex 1 of the Directive, species requiring increased conservation measures (*Aythya nyroca*, *Nycticorax nycticorax, Ardeola ralloides, Egretta garzetta, Ciconia ciconia, Himantopus himantopus*).

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ASPECTS REGARDING THE INFLENCE OF SOME CLIMATIC FACTORS IN DINAMIC EVOLUTION OF SOME PATHOGENS SPECIFIC TO APPLE CULTURE IN MĂRĂCINENI AREA BETWEEN 2014-2015

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Keywords: *Idared, climate condition, pathogen, frequency, intensity*

ABSTRACT

The continuously analysis of weather conditions gives to us important clues in dynamic evolution and the way action of a pathogen. Besides, each step inside the lifecycle of a pathogen is conducted in an optimal range of temperature, precipitation, including the duration of leaves wetting which add the wind speed with role in conveying inoculum. Monitoring the conditions to realise infections for Venturia inaequalis Wint and Podosphaera leucotricha (Ell. Et Ev.) Salmon in climatic conditions in 2014 and 2015 has been made using WATCHDOG station, the data given by it being in accordance with the situation in the field. The study reveals that for keeping these pathogens under control warning is a necessary measure, skin completed with the biological reserve evaluation, all correlated with plant phenophase.

INTRODUCTION

Apple is one of the most sensitive species to the pest attack. The continuous monitoring by forecasting dynamics, preparation of warning bulletins and protective measures are essential steps within each cycle of vegetation.

Corellation of pests biology with climatic conditions in order to determine the moment of maximum vulnerability is mandatory, every day of delay in implementing protection measures could lead to loss of quality and quantity, as evidences by a series of researches in this sphere of activity.

Thus, extensive studies on the ecology of *Venturia inaequalis* fungus to establish minimum threshold temperature for infection were taken by Stensvand and colab., 1997. Their object was to quantify the effects of low temperature on release and realization the infection throw the ascospores and conidia. Thus, the average time required to a catch of 1% was 143 minutes at 2°C, 67 minutes at 4°C, 56 minutes at 6°C and 40 minutes at 8°C. The authors had as references in comparing data the table of Mills, considered standard. According to Mills, at least two days are required for wetting the foliage, at temperatures below 6°C for realization the infection throw the ascospores.

Researches on the climatic conditions necessary for infection with *Podosphaera leucotricha* were taken by Turechek et al. (2004) from Cornell Univerity. According to them, the infection take place when the relative humidity

exceeds 70% and the temperature oscillates between 10°C and 25°C, the optimum being of 19-22°C. Under optimum conditions, the symptoms are apparent within 48 hours after the infection and new infections produce ascospores in 5 days.

A complex study regarding *Erwinia amylovora* biology was made in 2000 by Joël L.Vanneste. 2000.

In the same year studies about *Monilinia fructigena* distribution and yield loss are found in the paper written by G.C.M. van Leeuwen and colab.

MATERIAL AND RESEARCH METHOD

The researches were made between 2014-2015 at ICDPP-Mărăcineni, which, under climatic aspect, belongs to the second climatic zone, moderately warm, semi-humid, which includes area characterized by annual average temperature between 8,0 and 10,5°C, solar radiation of 114-128 Kcal/cm^2, a sum of temperatures above 0 ^{0}C between 3400-4100 ^{0}C, of higher than 10 ^{0}C between 2800-3500 ^{0}C and those above 10 ^{0}C of 1100-1600 ^{0}C .

The average annual rainfall is between 450-700 mm, in April-October, 325-475 mm in drier years, in the same period, the hydric deficit being of 220-391 mm.

Inside this area, the territory covers the fourth subarea and subclass of weak hydroclimatic surplus balance of 91-105 and aridity index of 28-38.

The observations and measurements took place in an apple culture, Idared variety, trees planted in 1992, at a distance planting of 3,5 x 2,5 (1842 trees/ha).

Data regarding the frequency (F%) and intensity (I%) of the attack were extracted using WATCHDOG station, dedicate soft and IRFAN VIEW (Freeware).

RESULTS AND DISCUSSIONS

Analysis of the data presented in 1, 2 and 3 figures highlights that 2014, in terms of climatic, was a very favourable year for the attack of *Venturia inaequalis* and *Podosphaera leucotricha*.

In this year the average monthly temperature was 10.9 ºC in Aprili, 16.6 ºC in May and 17.6 in June (figure 1).

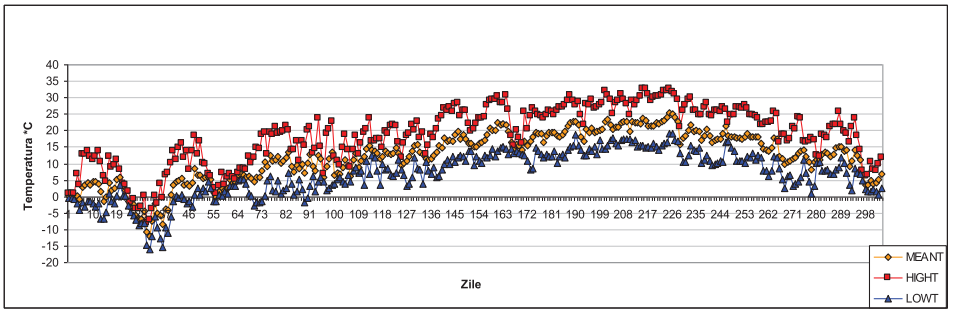

Figure 1. Daily temperature values at ICDP Piteşti-Mărăcineni in 2014.

Number of days with precipitation was 15 in Aprili, 19 in May and just 9 in June, while the sum of precipitation was 410 l/m^2 (figure 2)

In the same period the sum of foliage wetting increased from 44 hours in April to 154 hours in May and 129 hours in June (figure 3).

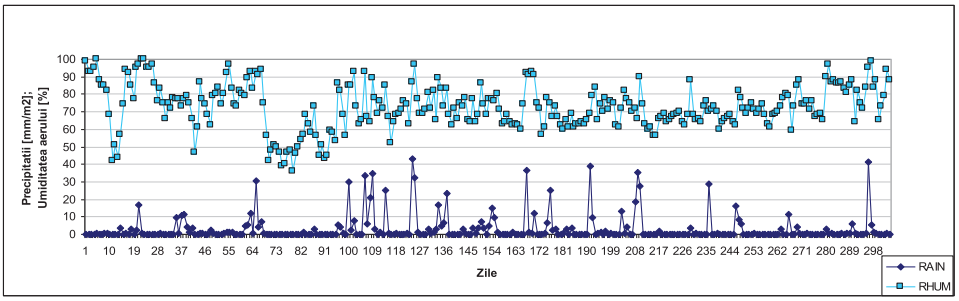

Figure 2. Daily precipitation and relative air humidity at ICDPP - Mărăcineni in 2014.

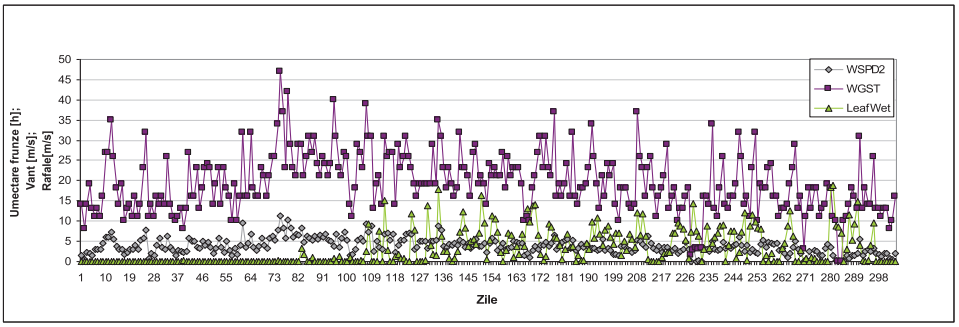

Figure 3. Dailiy wind speed, blasts and duration of leaves umectation at ICDPP-Mărăcineni in 2014.

As a result of the evolution of climatic conditions, in this year (2014) the primary infections with *Venturia inaequalis* occurred in the first part of March, when the maxim daily temperature were between 5,7-12,1ºC and the wetting canopy exceeded sometimes 12h/zi.

The secondary infection occurred during May and June, when the leaves wetting was between 4,4-15 and 9,7-21,2 h/day, and the spore maturation degree was between 85% and 99%. During the first decades of June the leaves wetting was between 10,8 h/day and 19,8 h/day, and the spore maturation degree was very high (99%), even to the middle of October (figure 4).

In the same year, we can observe that the risk of primary infection with *Podosphaera leucotricha* was maximum (3 in a scale 0-3) from the middle of April. It was constant during the third decade of April until the middle of May (figure 5).

In the second decade of May, the risk of secondary infection with *Podosphaera leucotricha* was maximum (3 in a scale 0-3), the index of spore maturation often exceeding 50-60%. Also in July and August were conditions for disease installation, the sum of precipitation until 1 August reaching 700 l/m^2, exceeding the average of annual rainfall from the area.

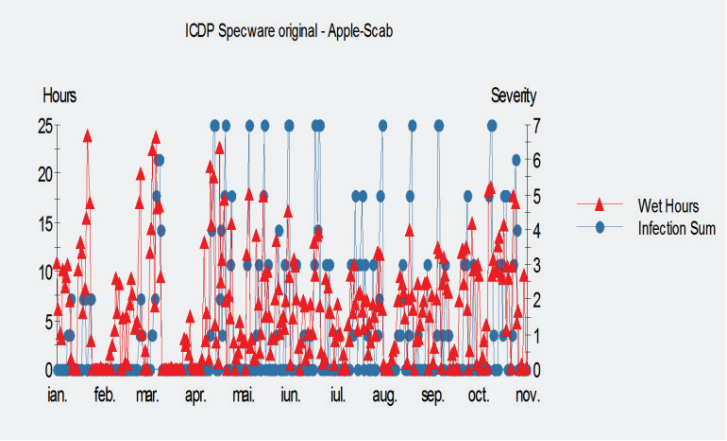

Figure 4. The risk of primary and secondary infections with *Venturia inaequalis* in 2014.

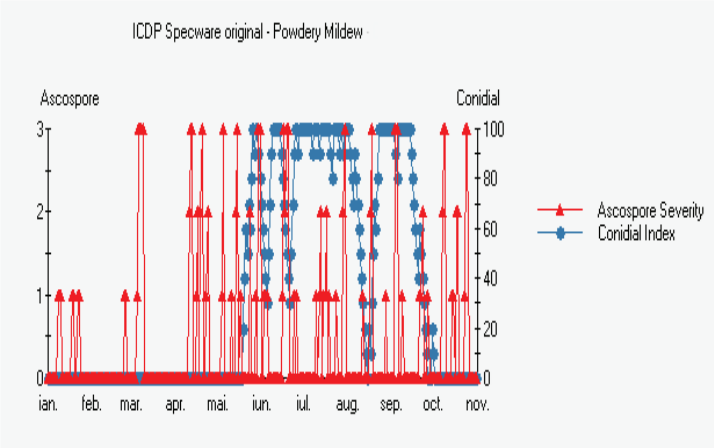

Figure 5. The risk of primary and secondary infections with *Podosphaera leucotricha* in 2014.

To illustrate what can happen in an aplle culture, in case of lack protection, in a very favourable year to the attack of *Venturia inaequalis* and *Podosphaera leucotricha*, in an untreated variant were made notes regarding frequency (F%) and intensity (I%) attack of two pathogens, for calculating the degree attack (GA%) on different organs.

As shown in table 1, in case of *Venturia inaequalis* the values of attack degree, whatever the analyzed organ, were above 50%, values done by the high frequency and intensity of the attack.

In case of *Podosphaera leucotricha*, the values of degree attack (GA%) were 58,7% on leaves and 67,5% twigs.

Table 1

The influence of climatic conditions in 2014 on the attack of *Venturia inaequalis* and *Podosphaera leucotricha*

Pathogen	Affected organ											
	leaves			rosette			twigs			fruits		
	F%	I%	GA%	F%	I%	GA%	F%	I%	GA%	F%	I%	GA%
V. inaequalis	86.7	58.33	50.6	80	73.33	58.6	-	-	-	80	75	60
P. leucotricha	78.3	75.0	58.7	-	-	-	90	75	67.5	-	-	-

In 2015 the climatic conditions were favourable to the attack of *Venturia inaequalis* and *Podosphaera leucotricha*, the monthly average temperature being 10°C in April, 16.7°C in May and 19.1°C in June (table 2).

Table 2

Dynamic of climatic conditions in 2015

	Temperature										
Date	High	Day	Low	Day	Mean	Degree Days	Chill Hours	Wet hours	Wet days	Rain Fall	Rain Days
ian	15.2	11	-20.9	01	0.4	1.74	37.8	0.0	25.0	61.0	14
feb	15.2	28	-13.3	10	1.0	1.73	48.3	0.3	27.0	37.3	14
mar	18.7	26	-5.6	20	5.5	11.55	42.5	1.5	22.0	74.2	12
apr	25.6	27	-2.2	09	10.0	75.63	46.0	32.8	17.0	44.8	10
mai	29.1	19	5.3	30	16.7	213.5	68.0	101.0	27.0	42.6	8
iun	32.8	14	8.2	22	19.1	272.4	11.3	112.8	25.0	82.0	12
iul	35.3	30	9.0	12	23.3	399.3	2.3	61.3	19.0	38.3	8
aug	35.3	16	10.9	26	21.5	346.4	0.0	86.8	17.0	55.3	7
sep	35.3	02	13.7	02	23.1	74.5	0.0	13.8	5.0	9.6	2
						1396.7	1756.0	410.0	184.0	445.0	87

In this year the number of precipitation days was 10 in April, 8 in May and just 12 in June while the sum of rainfall was 169.4 l/m^2. In the same period, the sum of leaves wetting hours increased from 32.8 hours in April to 101 hours in May and 112.8 hours in June. July was extremely dry with only 2.6 l/m^2 and 13 hours of leaves wetting, the average maxim temperature being 34.9 ºC with extreme above 38 ºC.

As a result of climatic conditions, in 2015, the primary infections with *Venturia inaequalis* occurred in the first part of March, when the maxim daily temperature often exceeded 18.0ºC and the period of leaves wetting was more 12hours/day (figure 6).

From the data presented in the same figure, we can observe that the secondary infections appeared during April, May and June, when the period of leaves wetting was 32.6, 101.6 respectively 110.6 hours/month. The infections risk was lower during July.

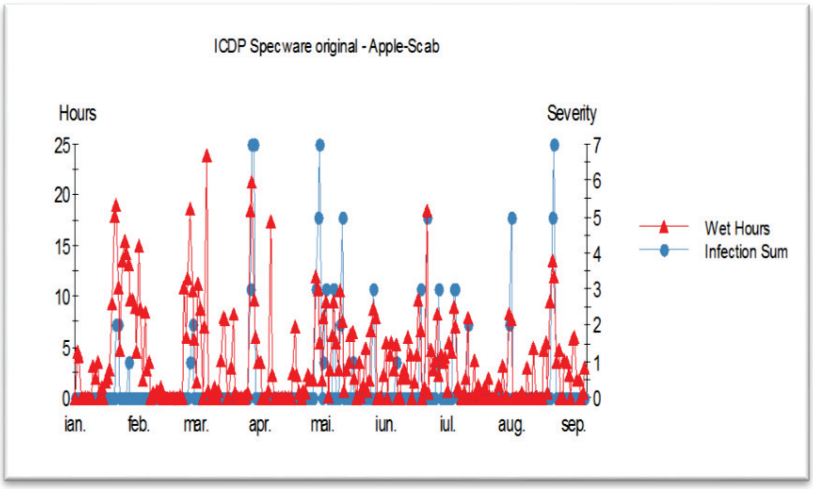

Figure 6. The risk of primary and secondary infections in case of *Venturia inaequalis* in 2015.

In case of *Podosphaera leucotricha*, in 2015, the primary infections began at the middle of April, the risk of secondary infections being relatively constant during summer, when conidia maturation index fluctuated between 50-60 and 100% (figure 7).

From the data presented in table 3 is visible that in 2015, favourable year to the attack of *Venturia inaequalis* and *Podosphaera leucotricha*, the values of attack degree (GA%) were lower than in 2014 (table 1), 2014 being a very favourable year.

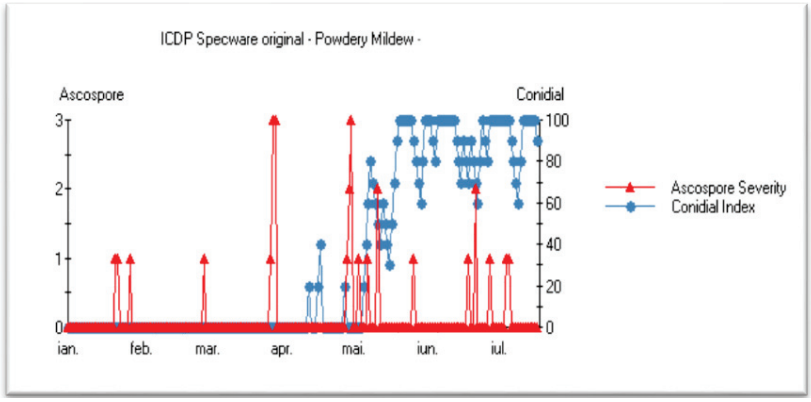

Figure 7. The risk of primary and secondary infection with *Podosphaera leucotricha* in 2015.

Table 3

The influence of climatic conditions in 2015 on the attack of *Venturia inaequalis* and *Podosphaera leucotricha*

Pathogen	Affected organ											
	leaves			rosettes			twigs			fruits		
	F%	I%	GA%	F%	I%	GA%	F%	I%	GA%	F%	I%	GA%
V. inaequalis	46.3	53.3	24.7	51.0	56.6	28.9	-	-	-	39.3	53.3	20.9
P. leucotricha	38.0	38.0	14.4	-	-	-	41	39.9	16.4	-	-	-

CONCLUSIONS

The analyse of frequency attack of *Venturia inaequalis* and *Podosphaera leucotricha* provides information about disease spatial distribution and the susceptibility of 'Idared' variety to be infected.

Primary infections with *Venturia inaequalis* occurred in the first decade of March in both two years of observation although the temperature difference was considerable (5.7-12.1ºC in 2014 respectively often exceeding 18ºC in 2015)

Regarding secondary infections, installation time was different, starting in May until June in 2014 and starting with April until June in 2015.

In 2014, because of constant precipitation, the degree of spore maturation was maintained until the middle of October (99%), different from 2015, when between July and September the precipitation volume was much lower.

In case of *Podosphaera leucotricha,* the installing of primary infections was similar in the two years, this being in the middle of April, with high infection risk, even there were differences of climatic factors.

The risk of secondary infections was high in 2014, when at the beginning of August, the sum of precipitation exceeded 700 l/m^2, with 7.7% more than multiannual average, different from 2015, when the precipitation volume was lower.

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THE EFFICIENCY OF SOME FUNGICIDES IN CONTROLLING THE MAIN PATHOGENS SPECIFIC TO APPLE CULTURE IN MĂRĂCINENI AREA BETWEEN 2014-2015

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Keywords: *disease, treatment, active substance, results*

ABSTRACT

The treatment order is looking to include products with new way of action, longer protection and larger spectrum of combat. For example, the active fungicide substance called fluopiram act on all development stages of the pathogens Venturia inaequalis and Podosphaera leucotricha, increasing in this way the range of product application. Fluopiram belongs to a newly discovered chemical called SDHI – inhibitors of succinat dehydrogenase, the enzyme responsible for electron transport in mitochondria, blocking pathogen respiration. It inhibts spore germination, the growth of germination tube and mycelium and also the sporulation.

INTRODUCTION

Apple, one of most wide-spread species in the world, with more than 10000 varieties, is attacked by a large number of parasities and pests.

From the large number of damaging agents, 4-6 pathogens and 12-15 pests can compromise the production of the sensitive varieties and also can debilitate the trees if defensive measures are not taken (Tomşa 2003).

Venturia inaequalis Cooke (Wint.), *Podosphaera leucotricha* (Ell. et. Ev.) Salmon, *Monilinia fructigena* Honey and *Erwinia amylovora* (Burrill) Winslow et.al. are pathogens whose attack can appear in all apple producing regions, causing the worst diseases (MacHardy şi colab., 2001, Ferree and Warrington, 2003, Mackie, 2005, Van der Zwet, 2002).

Over the time, many researches had concerns about the control of pathogens in apple culture by chemical an d non-chemical methods.

The papers where are is analyzed the sensitivity or the resistance of *Venturia inaequalis* to different chemical substances are many, for instance Köller. and colab., in 2004, studied its resistance to kresoximmethyl şi trifloxystrobin.

The behavior of the pathogen called *Podosphaera leucotricha* towards the sterol-inhibitors fungicides is analyzed in a study published in 1998 by Moshe Reuveni, Dov Oppenheim and Reuven Reuveni.

The fire blight world distribution was studied by Van der Zwet in 2002.

The research aim is to demonstrate that can be obtained good results of disease control in apple culture using chemical methods by applying products based on new active substances. These give protection as long as possible,

includind a lower number of treatments, a powerful way of action and a larger combat spectrum. It is also in compliance with environmental and food chain.

MATERIAL AND METHODS

The researches have been made between 2014-2015, at I.C.D.P.P.-Mărăcineni, in a 22 years old apple plantation, where the trees are planted at a distance of 3.5x2.5 (1842 trees/ha), sprinkler irrigated and fertilized with complex 16-16-16, in a dose of 90 kg active substance/ha and ammonium nitrate 30 kg active substance/ha.

The variety is Idared, especially choosen for its sensitivity to pathogens attack. The plant protection products used for protection against pathogens are presented in table 1.

Table 1

Plant protection products used for protection against pathogens

Fungicide	Product	Active substance	Target organism
	Antracol 70 WP	propineb 70%	*V. inaequalis*
	Aliette 80 WG	aluminum fosetil 80 %	*E. amylovora*
	Clarinet 200 SC	pirimetanil 150 g/l + fluquinconazol 50 g/l	*V. inaequalis* *P. leucotricha*
	Flint Plus 64 WG	trifloxistrobin 4% + captan 60 %	*V. inaequalis* *P. leucotricha*
	Luna Experience 400 SC	fluopiram 200 g/l + tebuconazol 200 g/l	*V. inaequalis* *P. leucotricha*
	Folicur Solo 250 EW	tebuconazol 250 g/l	*V. inaequalis* *P. leucotricha*

The treatments were applied using Osella 1000 pump spray, activated by a U-650 DTC tractor, the volume solution being 1000 l/ha.

In the research period, the products have been applied in some treatments scheme (tables 2 and 3), the number and data ranging in correlation with climatic factors evolution.

As is visible in table 2, in 2014, were necessary, for keeping the pathogens under control, a number of 17 treatment, in three cases being applied 2 products in one treatment, while in 2015, due to less favorable climatic conditions for diseases, were necessary only 14 treatments (table 3).

The treatment program was applied based on the correlation between target-organisms biology, environmental conditions and plant phenology, to determine the moment of maximum vulnerability to infection. For each target, were set the frequency (F%) and intensity (I%) of the attack, at different moments, the data collected in the field being picked using usual formulas. To highlight the experimental results, it was calculated the average of determinations which was compared with the untreated variant.

Table 2

The table of treatments made in 2014

Treatment number	Date	BBCH	Products and concentrations
1	28.03	55	Antracol 70 WP 4,5 kg/ha
2	9.04	57	Clarinet 200 SC 1,5 l/ha
3	15.04	61	Aliette 80 WG 4,5 kg/ha + Flint Plus 64 WG 1,875 kg/ha
4	23.04	65	Flint Plus 64 WG 1,875 kg/ha
5	29.04	69	Flint Plus 64 WG 0,75 l/ha
6	7.05	71	Luna Experience 400 SC 0,75 l/ha
7	19.05	71-72	Clarinet 200 SC 1,5 l/ha
8	28.05	72	Flint Plus 64 WG 1,875 kg/ha + Antracol 70 WP conc. 4,5 kg/ha
9	6.06	72-73	Clarinet 200 SC 1,5 l/ha
10	13.06	73	Antracol 70 WP conc. 4,5 kg/ha + Folicur Solo 250 EW 0,75 l/ha
11	23.06	73-74	Clarinet 200 SC 1,5 l/ha
12	4.07	75	Flint Plus 64 WG 1,875 kg/ha
13	16.07	77	Antracol 70 WP 4,5 kg/ha
14	28.07	78	Flint Plus 64 WG 1,875 kg/ha
15	8.08	79	Antracol 70 WP 4,5 kg/ha
16	21.08	81	Flint Plus 64 WG 1,875 kg/ha
17	5.09	85	Luna Experience 400 SC 0,75 l/ha

Table 3

The table of treatments made in 2015

Treatment number	Date	BBCH	Products and concentrations
1	16.04	55	Antracol 70 WP 4.5 kg/ha
2	22.04	57	Clarinet 200 SC 1.5 kg/ha
3	27.04	62	Aliette 80 WG 3 kg/ha
4	2.05	66	Flint Plus 64 WG 1.875 kg/ha
5	12.05	71	Luna Experience 400 SC 0.75 l/ha
6	19.05	72	Folicur Solo 250 EW 0.75 l/ha
7	26.05	74	Luna Experience 400 SC 0.75 l/ha
8	5.06	74-75	Flint Plus 64 WG 1.875 kg/ha
9	23.06	75-76	Antracol 70 WP 4.5 kg/ha
10	6.07	76-77	Folicur Solo 250 EW 0.75 l/ha
11	16.07	77-78	Flint Plus 64 WG 1.875 kg/ha
12	25.07	78-79	Clarinet 200 SC 1.5 kg/ha
13	10.08	79	Flint Plus 64 WG 1.875 kg/ha
14	28.08	81	Flint Plus 64 WG 1.875 kg/ha

RESULTS AND DISCUSSIONS

Regarding the treatment scheme efficiency in controlling the analyzed pathogens, in the climatic conditions of the year 2014, between 1 June and 14 August, there are visible differences in frequency and intensity attack between the experience and untreated variants (tables 4, 5, 6).

In case of *Venturia inaequalis* attack, in the untreated variant, was recorded an average frequency of 86,7% on the leaves and 80% on rosette and fruits while the average values of determination in the experience were 13,7% on leaves, 7% on rosettes and 29,3% on fruits (table 4).

Regarding the intensity of the attack, in the untreated variant, the values were 58,33% on leaves, 73,33% on rosettes and 75% on fruits while the average values of determination in the experience were 11,7% on leaves, 5% on rosettes and 13,3% on fruits.

For *Podosphaera leucotricha* the average values of frequency attack in the untreated variant were 78,3% on leaves and 90% on shoots while in the experience the values were 3,3% on leaves and 2,7% on shoots.

The intensity of the attack in the untreated variant has an average value of 75% both on leaves and shoots while in the experience was 10% on leaves and 50% on shoots (table 5).

The attack of *Monilia fructigena* in the untreated variant had an average frequency of 10,67% on rosettes, 8,33% on shoots and 6,67% on fruits, while in the experience the attack was not signaled, whatever the analyzed organ (table 6).

Table 4

The effect of products applied for disease control in 2014 for *Venturia inaequalis*

	Venturia inaequalis					
	leaves		Rosettes		fruits	
Date	F%	I%	F%	I%	F%	I%
12.06	2	5	3	5	17	10
23.06	19	15	9	5	32	15
14.07	20	15	9	5	39	15
14.08	stopped					
Lot average	13,7	11,7	7,0	5,00	29,3	13,3
Untreated	86,7	58,33	80	73,33	80,00	75,00

From the data presented in the figures 1,2 and 3,4 is visible the influence of the treatment scheme applied in 2015, for controlling the attack of *Venturia inaequalis* (Figure 1,2) and *Podosphaera leucotricha* (Figure 3,4) at 26 May 29 June and 30 July, compared with the untreated variant.

The preventive treatments, applied before 30.07.2015 have limited the attack of *Venturia inaequalis*, both in terms of frequency (F%) and intensity (I%) of the attack. Thus, in the experience, the frequency of the attack was 2.0-5.58% on leaves, 2.0-6.10% on rosettes and 2.0-5.33% on young fruits, compared with the untreated variant where the frequency of the attack was 37.67- 46.33% on leaves, 41.33-51.00% on rosettes and 27.00-39.33% on fruits.

Table 5

The effect of products applied for disease control in 2014 for *Podosphaera leucotricha*

Date	*Podosphaera leucotricha*					
	leaves		sprigs		fruits	
12.06	F%	I%	F%	I%	F%	I%
23.06	2	10	2	50	-	-
14.07	4	10	3	50	-	-
14.08	4	10	3	50	-	-
	stopped					
Lot average	3,3	10,0	2,7	50	-	-
Untreated	78,3	75,00	90,00	75,00	-	-

Table 6

The effect of products applied for disease control in 2014 for *Monilia fructigena*

Date	*Monilia fructigena*					
	rosettes		sprigs		fruits	
12.06	F%	I%	F%	I%	F%	I%
23.06	0	0	0	0	0	0
14.07	0	0	0	0	0	0
14.08	0	0	0	0	0	0
	-	-	-	-	-	-
Lot average	0	0	0	0	0	0
Untreated	10,67	11,66	8,33	13,33	6,67	15

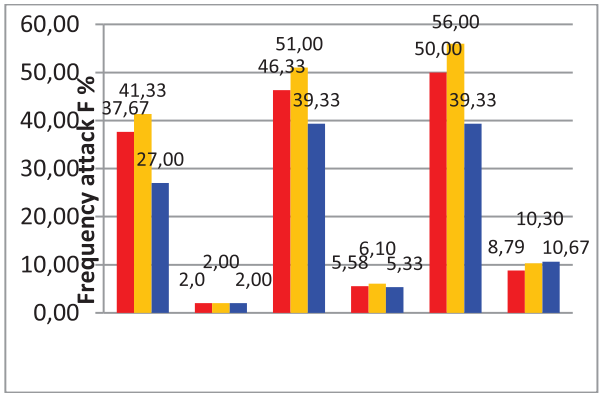

Figure 1. The influence of treatment scheme in controlling the fungal *Venturia inaequalis.*

The intensity of the attack with scab was 10.00-12.22% on leaves, 6.66-11.10% on rosettes and 0.25-0.33% fruits, compared with the untreated variant where the intensity was 50.00-53.33% on leaves, 50.00-56.66% on rosettes and 50.00-53.33% on fruits. Regarding the control of *Podosphaera leucotricha* in 2015,

the preventive treatments, applied before 30.07, have limited both the frequency (F%) and intensity (I%) attack.

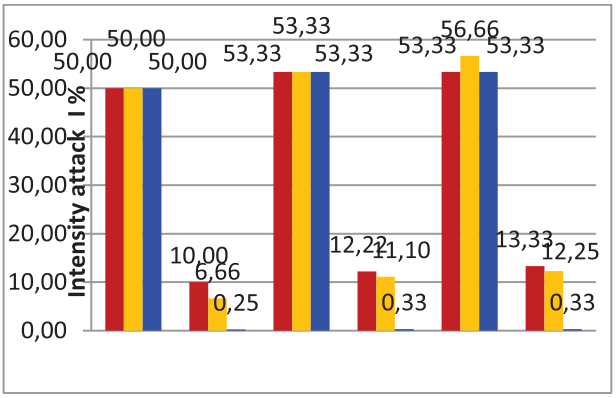

Figure 2. The influence of treatment scheme in controlling the fungal *Venturia inaequalis.*

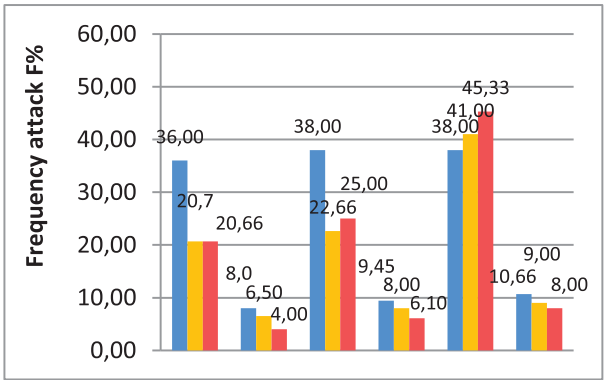

Figure 3. The influence of treatment scheme in controlling the fungal *Podosphaera leucotricha.*

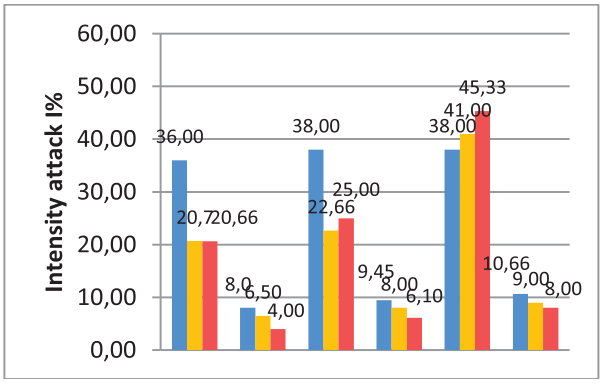

Figure 4. The influence of treatment scheme in controlling the fungal *Podosphaera leucotricha*.

In the experience area, the frequency of the attack was 8.00-10.66% on leaves and 6.50-9.00% on shoots, compared with the untreated variant where the frequency attack was 36.00-38.00% on leaves and 20.7-41.0% on shoots.

Referred to intensity of powdery attack, this was 8.00-10.66% on leaves and 6.50-9.00% on shoots. In the untreated variant, the powdery attack was 36.00-38.00% on leaves and 20.00-41.00% on shoots (fig.4).

Results regarding the control of *Monilia fructigena* and *Erwinia amylovora*

Because of the preventive treatments, the frequency and intensity of *Monilinia fructigena* and *Erwinia amylovora* were 0 (zero) until the fruits harvest.

CONCLUSIONS

Because of different weather conditions between the two years, the treatments data were also different. The time difference between T_1 fungicid in 2015 and T_3 fungicid in 2014 was only one day and in 2014 were applied 3 more fungicide than in 2015.

In 2014, in case of 3 treatments, because of high pressure of diseases, were used 2 products in the same time for increase the efficieny against pathogens.

In 2014, in case of *Venturia inaequalis* and *Podosphaera leucotricha*, the differences recorded in case of frequency and intensity ao the attack, between the experience and untreated variant, were notable differences at the level of leaves, rosettes, shoots and fruits.

Inside the experience, both the frequency and intensity attack of *Monilia fructigena* and *Erwinia amylovora* were 0 (zero) until the fruits harvest.

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HISTO-ANATOMICAL AND PRELIMINARY PHYTOCHEMICAL RESEARCHES ON *SIDERITIS MONTANA* L. (*LAMIACEAE*) SPECIES

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Keywords: *Sideritis montana L., histo-anatomy, polyphenols, thin layer chromatography.*

ABSTRACT

By microphotography technique, the cross-sections through root, stem and leaf of Sideritis montana L. species were obtained for the first time. Also, the polyphenols content has been researched and interpreted for the reason of pharmacognostic expertise on Sideritis montanae herba medicinal product. Using thin layer chromatography analysis, in the aerial parts, two main polyphenolic components were identified from the 13 characteristic bands: one rutin derivative and one chlorogenic acid derivative.

INTRODUCTION

Sideritis montana L., Mountain ironwort, *Lamiaceae* family, is a herbaceous annual species, up to 35 cm tall, spontaneous in arid zones on the rocky substrate, flowering in May–July, in Mediterranean zone, Balkan Peninsula, Central Europe, Southern and Central Asia (Ciocârlan 2000).

The aerial parts of *Sideritis* species contain different active principles, such as: essential oil rich in *α*-pinene, *β*-pinene, carvacrol, *α*-cadinol, *β*-caryophyllene, nerolidol, hexadecanoic acid, octadecenol (Meshkatalsadat et al. 2007, Miladinović et al. 2012, Todorova et al. 2000, Venditti et al. 2016), distinctive flavonoids with chemotaxonomic value – hypolaetin, isoscutellarein and their methoxy derivatives, 5,7-dihydroxy-flavones (apigenin, luteolin) (Menković et al. 1993, Todorova et al. 2000), diterpenoids – ferruginol acetate, siderol, sideridiol, sideroxol, epoxysiderol, 18-acetoxy-leucanthol (Todorova et al. 2000), sterols, triterpenoids (amyrin, ursolic acid, oleanolic acid), catechic tannin, phenylpropane derivatives, polyphenolic acids, heteroglycans (mucilage), coumarins, simple carbohydrates, fatty acids, proteins, organic acids, vitamins, mineral salts (Fraga 2012, González-Burgos et al. 2011, Petreska et al. 2011, Petreska Stanoeva et al. 2015, Pljevljakušić et al. 2011).

The active principles from *Sideritis* sp. exhibit some useful pharmacological actions: antifungal (Radojevic et al. 2011a), antibacterial properties of essential oil (Miladinović et al. 2012) and ethyl acetate extract (Radojevic et al. 2011b), excitatory

and smooth muscle-relaxing effect on Guinea-pig ileum (Tóth et al. 2015), enhancing memory and learning in Alzheimer's *β*-amyloidosis aged C57Bl/6 mice (Hofrichter et al. 2016), triple monoamine reuptake inhibition (Knörle 2012), cytotoxic effect on B16 melanoma and HL-60 leukemic cells (Tadić et al. 2008 & 2012), antiglioma properties against C6 rat glioma cells (Jeremić et al. 2013, Tadić et al. 2009), anti-oxidant, anti-inflammatory, analgesic, gastroprotective, emollient, cicatrizing, against cough (González-Burgos et al. 2011, Todorova et al. 2000, Zengin et al. 2014).

In the specialty papers, there are scarce and incomplete data concerning *S. montana* histo-anatomy (Dehshiri & Azadbakht 2012, Güvenç & Duman 2010) and chemical composition (González-Burgos et al. 2011, Petreska et al. 2011, Todorova et al. 2000).

The aim of our paper was the histo-anatomical investigation of the root, stem and leaf of the above-mentioned species and the preliminary analysis of the polyphenols content from the aerial parts (*Sideritis montanae herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested from *S. montana* 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 achieved 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 stains: 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) and then photographed using a Sony DSLR-A380 digital system adapted to the microscope.

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

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was performed on the aerial parts of *S. montana* species (*Sideritis montanae herba*), using a CAMAG (Muttenz, Switzerland) system in the following experimental conditions (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014):

▪ stationary phase: TLC silica gel 60 F_{254} 20×10 precoated glass plates (Merck, Darmstadt, Germany) 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) in a vapor-equilibrated chromatographic tank (20×10 cm twin trough chamber, CAMAG);

▪ sample: 20% methanolic extract of *Sideritis montanae 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 and 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;

▪ spectra acquisition, processing and quantification analysis: winCATS ver. 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Root

In cross-section, the root has circular shape and secondary structure due to the presence of the two meristematic secondary areas: phellogen and libero-ligneous cambium.

In cross-section, from the outside towards the inside of the root, the following histological sequence was found.

Periderm consists of suber, phellogen and phelloderm. Suber is composed of 4–5 layers of flattened cells impregnated with suberin. From point to point, suber is exfoliated. A single layer of anterior-posterior flattened cells, with thin walls, the radial slightly undulated, makes subero-phellodermic cambium. Phelloderm consists of 2–3 layers of flattened cells, with cellulosic thin walls (Figures 1–3).

Conductive tissues are arranged in two concentric rings. Phloem forms a thin, external ring, consisting of sieve tubes, phloem parenchyma and annex cells. Libero-ligneous cambium is placed between xylem and phloem tissues. Medullary rays are multicellular, uniseriate, cellulosic. Xylem forms the internal ring consisting of metaxylem vessels of different sizes, placed on radial rows in a mass of libriform tissue, pushing to the center protoxylem vessels with small diameter. Metaxylem shows reticulate thickenings. Protoxylem is accompanied by xylem parenchyma and occupies the central area of the root. Medullary rays from the xylem zone are multi-cellular, uniseriate, lignified. Medullary parenchyma is missing (Figures 1–3).

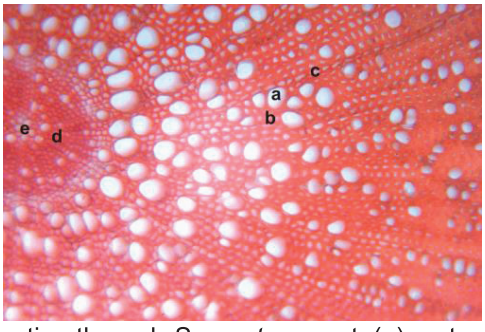

Figure 1. Cross-section through *S. montana* root: (a) metaxylem; (b) libriform tissue; (c) medullary ray; (d) xylem parenchyma; (e) protoxylem (Congo red–chrysoidine staining, ×100).

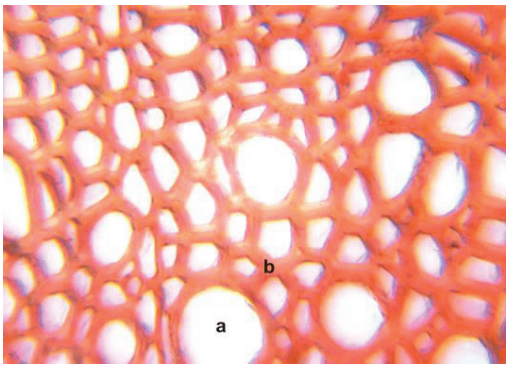

Figure 2. Cross-section through *S. montana* root: (a) metaxylem; (b) libriform tissue (Congo red–chrysoidine staining, ×400).

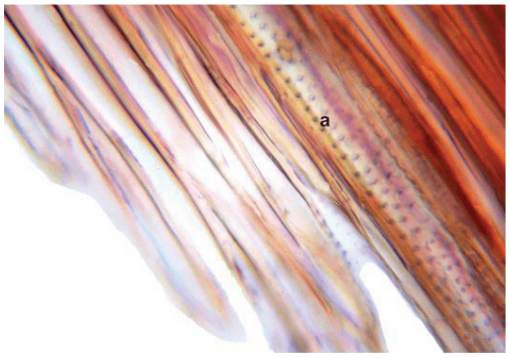

Figure 3. Longitudinal-radial section through *S. montana* root: (a) metaxylem with reticulate thickening (Congo red–chrysoidine staining, ×400).

Aboveground stem

In cross-section, the aboveground stem has four-rib quadratic shape and secondary structure due to the intrafascicular libero-ligneous cambium.

Epidermis presents heterodiametric cells with thickened outer wall covered by a thick cuticle with papiliform relief. Epidermal cells are tangential elongated with thin radial walls and thick tangential external and internal walls. Stomata and multicellular uniseriate tector hairs, sharp peak and slightly bent, are found from place to place. Tector hairs from the four ribs are fixed to an epidermal pedestal (Figures 4–6).

Cortex is made up of two areas: 5–6 layers of angular collenchyma to the ribs and intercostal chlorenchyma. The inner zone is cortical parenchyma. After cortical parenchyma, a single layer of primary endodermis is evidenced. From point to point, sclerenchyma fibers are found in periphloemic area, under endodermis. Conducting tissues are organized into four large collateral-open libero-ligneous fascicles disposed opposite ribs. Libero-ligneous cambium is intrafascicular. Phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. Secondary xylem is made up of metaxylem vessels with different calibers scattered into well-represented libriform tissue. In longitudinal-radial sections, metaxylem has spiral and reticulate thickenings. Primary xylem is poorly represented, consisting of some radial-arranged protoxylem vessels and xylem parenchyma. Large medullary rays

are multicellular, multiseriate and cellulosic to the phloem and lignified to the xylem tissue. Medullary parenchyma is well developed, of meatus type (Figures 4–6).

Figure 4. Cross-section through *S. montana* aboveground stem: overview (Congo red–chrysoidine staining, ×40).

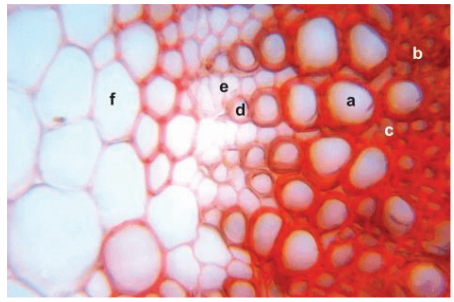

Figure 5. Cross-section through *S. montana* aboveground stem: (a) metaxylem; (b) libriform tissue; (c) medullary ray; (d) protoxylem; (e) xylem parenchyma; (f) medullary parenchyma (Congo red–chrysoidine staining, ×400).

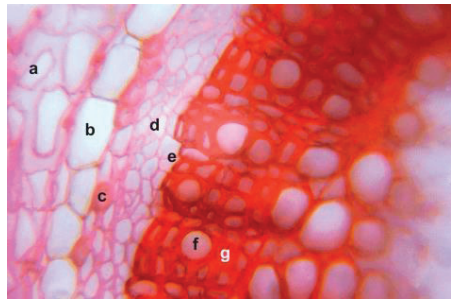

Figure 6. Cross-section through *S. montana* aboveground stem: (a) angular collenchyma; (b) endodermis; (c) sclerenchyma fibers; (d) secondary phloem tissue; (e) libero-ligneous cambium; (f) metaxylem; (g) libriform tissue (Congo red–chrysoidine staining, ×400).

Leaf's limb

In cross-section, from the outside to the inside of leaf's limb, the following histological sequence is observed. A single layer of upper epidermis is composed

of flattened large cells with thickened tangential external and internal walls and thin radial walls. External walls are bulged and covered by a thick cuticle. From point to point, unicellular tector hairs are fixed in an epidermal base. Mesophyll is organized in a single layer of palisade parenchyma consisting of small slightly elongated cells, rich in chloroplasts, as well as of 4–5 layers of lacunose parenchyma composed of disorderly arranged small cells with aeriferous spaces. Many small libero-ligneous fascicles, each surrounded by an assimilatory fascicular sheath, are found into the mesophyll. Mesophyll has bifacial 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, there are many stomata and unicellular tector hairs, sharp peak and drop in an epidermal base. At median rib's level, in the center, is located a single libero-ligneous fascicle surrounded by an assimilatory fascicular sheath and arranged into a leaf's parenchyma mass. Into the libero-ligneous fascicle, xylem vessels are seriate disposed and the medullary rays are uniseriate, cellulosic. Phloem tissue is underrepresented. Leaf's limb has hypostomatic bifacial dorsiventral structure (Figures 7 and 8).

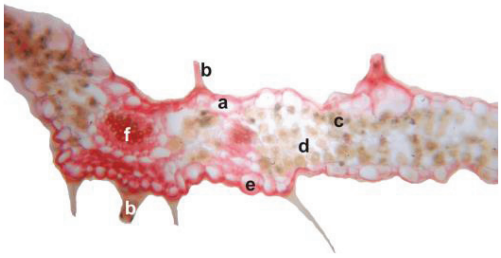

Figure 7. Cross-section through *S. montana* leaf's limb: (a) upper epidermis; (b) tector hair; (c) palisade parenchyma; (d) lacunose parenchyma; (e) lower epidermis; (f) conducting fascicle (Congo red–chrysoidine staining, ×100).

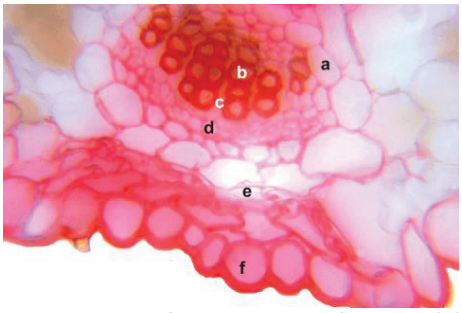

Figure 8. Cross-section through *S. montana* leaf's limb: (a) fascicular sheath; (b) xylem vessel; (c) medullary ray; (d) phloem tissue; (e) leaf's parenchyma; (f) lower epidermis (Congo red–chrysoidine staining, ×400).

TLC analysis

The experimental data on the preliminary TLC analysis of polyphenols from *Sideritis montanae herba* are highlighted in Figures 9–12. One rutin derivative (R_f 0.07) and one chlorogenic acid derivative (R_f 0.63) were identified in an amount of 312.2 mg% and 295.6 mg%, respectively (per 100 g of dried vegetal product).

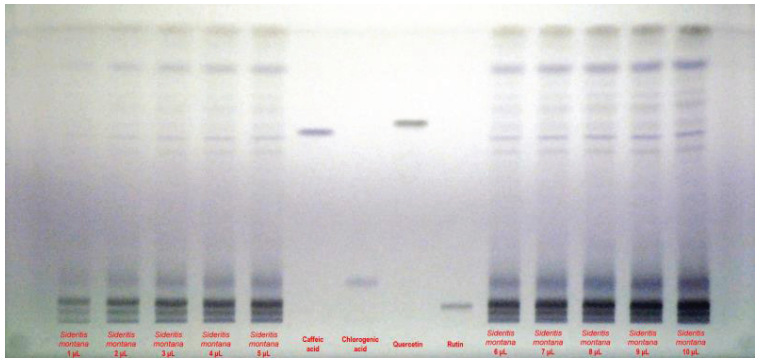

Figure 9. TLC chromatogram of polyphenols from *Sideritis montanae herba* methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

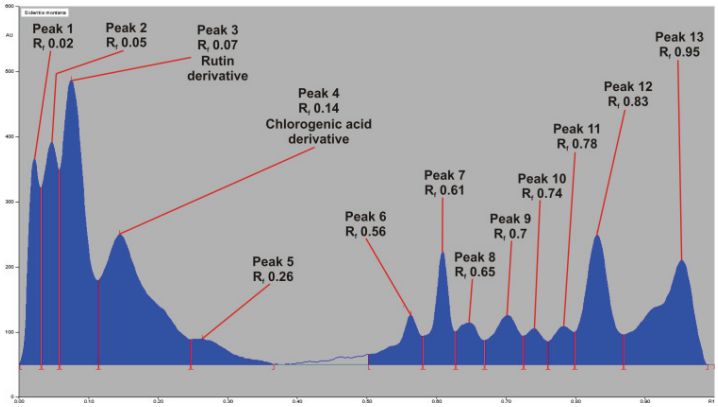

Figure 10. Densitogram of polyphenols (UV 254 nm) separated from *Sideritis montanae herba* methanolic extract.

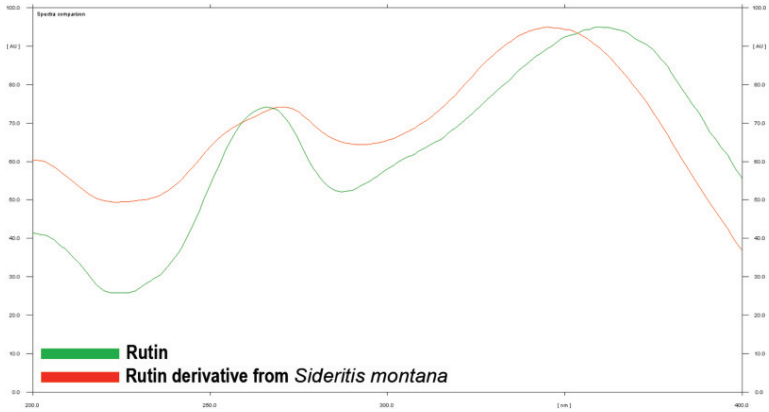

Figure 11. Rutin derivative *in situ* UV spectra of standard and compound separated from the analyzed sample.

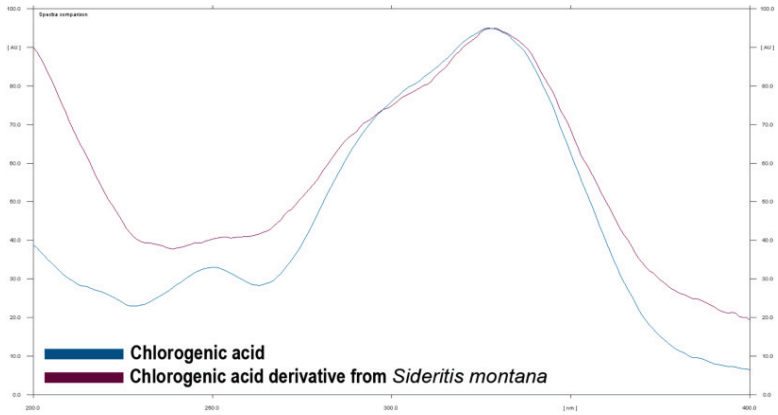

Figure 12. Chlorogenic acid derivative *in situ* UV spectra of standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of the root, stem and leaf of *Sideritis montana* species and the preliminary TLC analysis of *Sideritis montanae herba* polyphenols were achieved. The root has circular shape and secondary structure due to the presence of two meristems (phellogen and libero-ligneous cambium). The aboveground stem has four-rib quadratic shape and secondary structure due to the intrafascicular libero-ligneous cambium. Leaf's limb has hypostomatic bifacial dorsiventral structure. From the 13 characteristic bands, two main polyphenolic components were identified by TLC: one rutin derivative and one chlorogenic acid derivative.

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HISTO-ANATOMICAL AND PRELIMINARY PHYTOCHEMICAL RESEARCHES ON *PAEONIA PEREGRINA* MILL. (*PAEONIACEAE*) SPECIES

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Keywords: *Paeonia peregrina Mill., histo-anatomy, polyphenols, thin layer chromatography*

ABSTRACT

The paper contains the histo-anatomical researches on the root, stem and leaf of Paeonia peregrina Mill. species, as well as the preliminary phytochemical analysis of the polyphenols content from the aerial parts (Paeoniae peregrinae herba). Of the 10 specific bands for the polyphenolic components, highlighted in the thin layer chromatogram, one caffeic acid derivative was identified.

INTRODUCTION

Paeonia peregrina Mill., Common peony (*Paeoniaceae*), is a herbaceous perennial species, which blooms in May–June, spontaneous in meadows and forest edges, in Southeastern Europe and Turkey (Ciocârlan 2000).

The vegetal products (roots, flowers, seeds) obtained from *Paeonia* species contain various active principles: monoterpenoids (An et al. 2006, He et al. 2013, Kostova et al. 1998, Osaka et al. 2008, Shi et al. 2016b), triterpenoids, gallic and catechic tannin, flavonoids, aromatic and aliphatic acids (Ivanova et al. 2002, Ogawa et al. 2015, Picerno et al. 2011, Shu et al. 2014), lignans (Liu et al. 2015).

The extracts of *Paeonia* sp. exhibit useful pharmacological actions, such as: neuroprotective in Parkinson and Alzheimer diseases (Kim et al. 2014, Liu et al. 2015, Sevim et al. 2013), immunomodulatory and anti-inflammatory in Chinese and Bulgarian medicine (Jiang et al. 2011, Nikolova and Ivanovska 1998), antioxidant, antifungal (Picerno et al. 2011), antibacterial (An at al. 2006, Ivanova et al. 2002), antiallergic (Shi et al. 2016a), hepatoprotective against oxidative stress (Ahmad and Tabassum 2013, Ko et al. 2016), androgen modulators (Washida et al. 2009), antiproliferative effect against BT 483 human breast cancer cells and OVCA 429 human ovarian cancer cells (Li et al. 2014), inhibitory effect on Cu^{2+}-induced low-density lipoprotein oxidation (Ogawa et al. 2015).

In the specialty papers, there are some data concerning *P. peregrina* histo-anatomy (Mereacre et al. 2015) and chemical composition (Nikolova and Ivanovska 1998). The aim of our paper was the histo-anatomical investigation of the root, stem and leaf of the above-mentioned species and the preliminary analysis of the polyphenols content from the aerial parts (*Paeoniae peregrinae herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested from *P. peregrina* plants in blossom, in May 2016, from the surroundings of Pleniţa village, Dolj County (southwestern Romania).

The fixation and preservation of tuberized 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 stains: 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) and then photographed using a Sony DSLR-A380 digital system adapted to the microscope.

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

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was performed on the aerial parts of *P. peregrina* species (*Paeoniae peregrinae 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 *Paeoniae peregrinae 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

Tuberized root

In cross-section, tuberized root has circular shape and secondary structure due to the presence of the two meristematic secondary zones: subero-phellodermic and libero-ligneous cambium. From the outside towards the inside of tuberized

root, the following histological sequence was highlighted. Periderm is made of suber, phellogen and phelloderm. Suber is made of 4–5 layers of flattened cells impregnated with suberin. From point to point, suber is exfoliated. Subero-phellodermic cambium consists of a single layer of anterior-posterior flattened cells, with thin walls, the radial slightly undulated. Phelloderm is made of 2–3 layers of cells with cellulosic thin walls. Many usins are found into the cortical parenchyma of the primary structure. Conductive tissues are disposed in two concentric rings. Phloem forms a thin, external ring, made of sieve tubes, phloem parenchyma and annex cells. Libero-ligneous cambium is found between xylem and phloem tissues. Xylem forms the internal ring made of metaxylem vessels of different sizes, placed on radial rows in a mass of libriform tissue, pushing to the center protoxylem vessels with small diameter. Secondary xylem is accompanied by libriform tissue. In longitudinal-radial sections, xylem vessels present reticulate and spiral thickenings. Protoxylem is accompanied by xylem parenchyma. Medullary rays are multicellular, uni-, bi- or multi-seriate, cellulosic, both in the phloem and xylem tissue rings. Medullary parenchyma is absent (Figures 1–3).

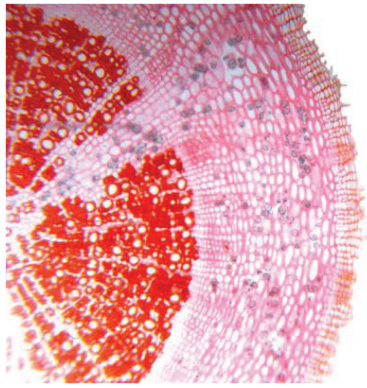

Figure 1. Cross-section through *P. peregrina* tuberized root: overview (Congo red–chrysoidine staining, ×40).

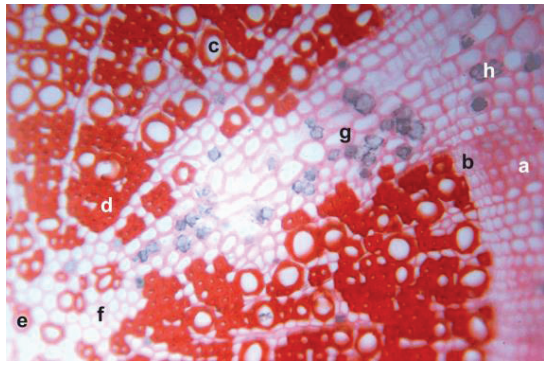

Figure 2. Cross-section through *P. peregrina* tuberized root: (a) secondary phloem tissue; (b) libero-ligneous cambium; (c) secondary xylem vessel; (d) libriform tissue; (e) protoxylem; (f) xylem parenchyma; (g) medullary ray; (h) ursins (Congo red–chrysoidine staining, ×100).

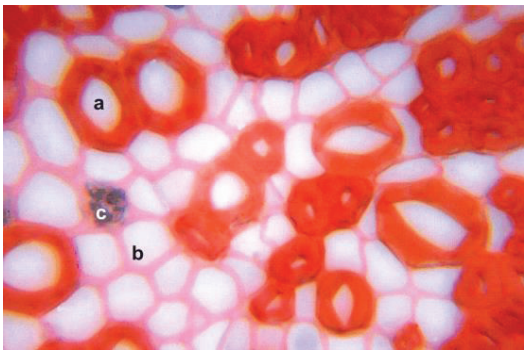

Figure 3. Cross-section through *P. peregrina* tuberized root: (a) secondary xylem vessel; (b) xylem parenchyma; (c) ursin (Congo red–chrysoidine staining, ×400).

Aboveground stem

In cross-section, the aboveground stem has circular shape modified by some ribs and secondary structure generated by intrafascicular libero-ligneous cambium. Epidermis shows heterodiametric cells with thickened outer wall covered by a thick cuticle with toothed relief. Epidermal cells are tangential elongated with thin radial walls and thick tangential external and internal walls. Stomata are evidenced from place to place. Cortex is made up of two areas. The outer area consists of 2–3 layers of angular collenchyma. Opposite to the ribs are 5–6 layers of angular collenchyma. The inner zone of the cortex is well represented, of parenchyma type with ursins. Conducting tissues are organized into numerous collateral-open libero-ligneous fascicles with various sizes. At the phloem pole, the libero-ligneous fascicles are flanked by one cap of sclerenchyma tissue. Libero-ligneous cambium has intra-fascicular disposition. Phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. Secondary xylem is made up of metaxylem vessels with different calibers scattered into well-represented libriform and xylem tissue. In longitudinal-radial sections, xylem vessels have spiral and reticulate thickenings. Primary xylem is poorly represented, being made of some protoxylem vessels and xylem parenchyma. Large medullary rays are multicellular, pluriseriate, cellulosic. Medullary parenchyma is well developed, of meatus type (Figures 4–6).

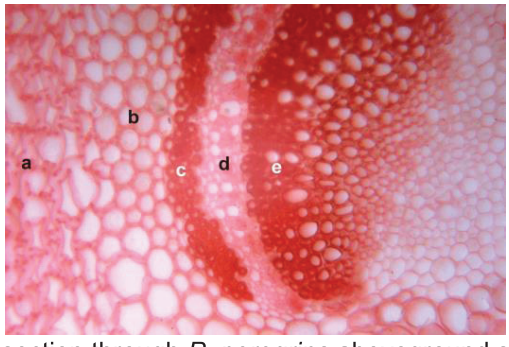

Figure 4. Cross-section through *P. peregrina* aboveground stem: (a) angular collenchyma; (b) cortical parenchyma; (c) sclerenchyma cap; (d) phloem tissue; (e) xylem tissue (Congo red–chrysoidine staining, ×100).

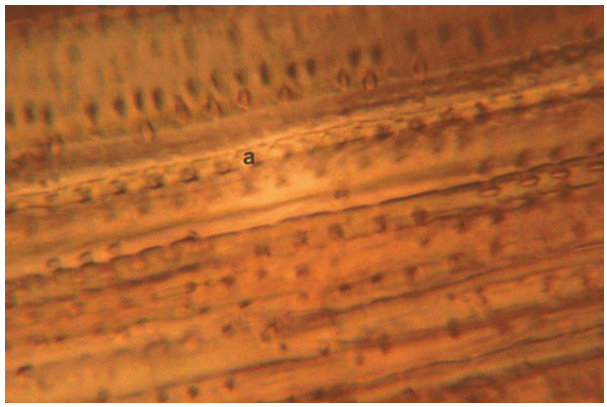

Figure 5. Longitudinal-radial section through *P. peregrina* aboveground stem: (a) reticulate vessel (Congo red–chrysoidine staining, ×400).

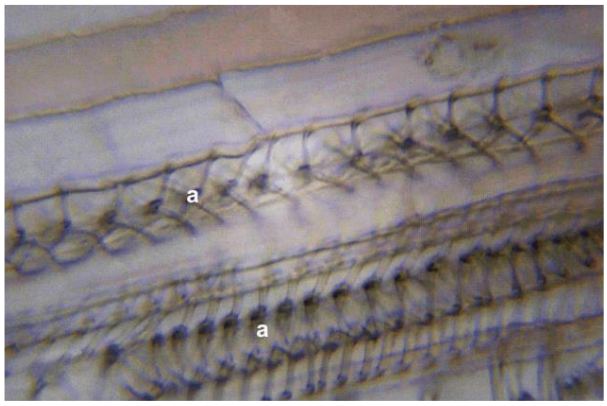

Figure 6. Longitudinal-radial section through *P. peregrina* aboveground stem: (a) spiral vessel (Congo red–chrysoidine staining, ×400).

Leaf

Leaf's limb

In cross-section, from the outside to the inside of leaf's limb, the following histological sequence is highlighted. A single layer of upper epidermis is made of flattened large cells with thickened tangential external and internal walls and thin radial walls. External walls are bulged and covered by a thick cuticle with dentate relief. Mesophyll is organized in a single layer of palisade parenchyma made of small slightly elongated cells, rich in chloroplasts, as well as of 4–5 layers of lacunose parenchyma composed of disorderly arranged small cells with aeriferous spaces. Many small libero-ligneous fascicles, each surrounded by an assimilatory fascicular sheath and numerous ursins, are found into the mesophyll. Mesophyll has bifacial 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. Many stomata are found at this level. The median rib looks as a prominent trough on abaxial side. The outer epidermis consists of small cells, slightly anterior-posterior flattened, with external wall covered with dentate relief cuticle. At the

adaxial pole, there are rare unicellular digitate tector hairs. Angular collenchyma is found under the epidermis, at both adaxial and abaxial poles. In the central zone is located a single libero-ligneous conducting fascicle disposed in a mesophyll mass rich in usins. Into the libero-ligneous fascicle, xylem vessels are seriate disposed and the medullary rays are multicellular, uniseriate, cellulosic. The leaf's limb has hypostomatic bifacial dorsiventral structure (Figures 7 and 8).

Figure 7. Cross-section through *P. peregrina* leaf's limb: overview (Congo red–chrysoidine staining, ×40).

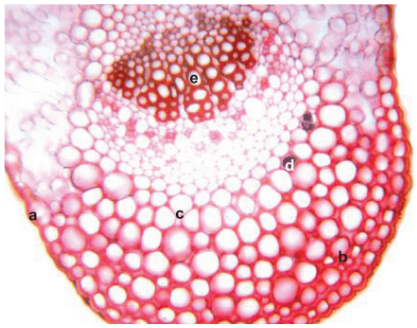

Figure 8. Cross-section through *P. peregrina* leaf's limb: (a) epidermis; (b) angular collenchyma; (c) leaf's parenchyma; (d) ursin; (e) conducting fascicle (Congo red–chrysoidine staining, ×100).

Petiole

Petiole has corded shape, with flat adaxial side modified by two wings. In cross-section, from the outside to the inside of petiole, the following histological sequence is evidenced. A single epidermal layer consists of heterodiametric small cells with thin radial walls and thickened tangential external and internal walls. External wall is covered by a dentate cuticle. Stomata are found from point to point. Subepidermal angular collenchyma is organized into 2–3 layers. Many libero-ligneous fascicles with various sizes are found into the fundamental parenchyma; they are arranged on a semicircle and flanked by one sclerenchyma cap, at the periphloemic side (Figure 9).

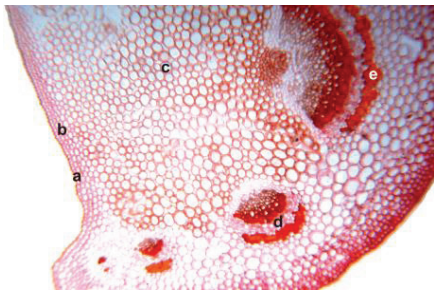

Figure 9. Cross-section through *P. peregrina* petiole: (a) epidermis; (b) angular collenchyma; (c) fundamental parenchyma; (d) conducting fascicle; (e) sclerenchyma cap (Congo red–chrysoidine staining, ×40).

TLC analysis

The experimental data on the preliminary TLC analysis of polyphenols from *Paeoniae peregrinae herba* are presented in Figures 10–12. Caffeic acid derivative (R_f 0.59) was identified in an amount of 64.5 mg/100 g of dried vegetal product.

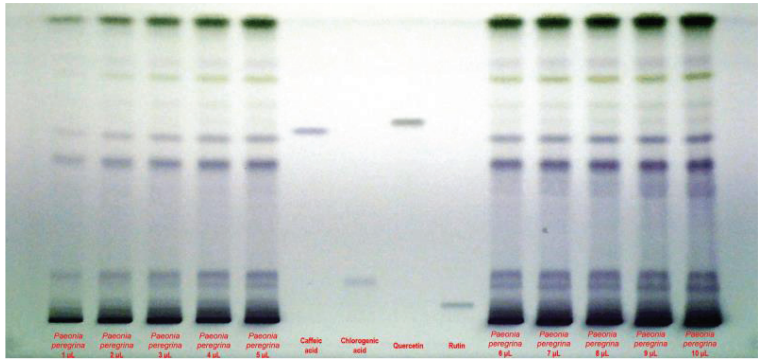

Figure 10. TLC chromatogram of polyphenols from *Paeoniae peregrinae herba* methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

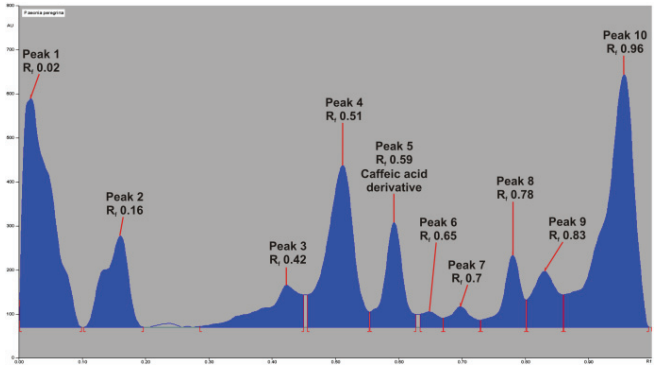

Figure 11. Densitogram of polyphenols (UV 254 nm) separated from *Paeoniae peregrinae herba* methanolic extract.

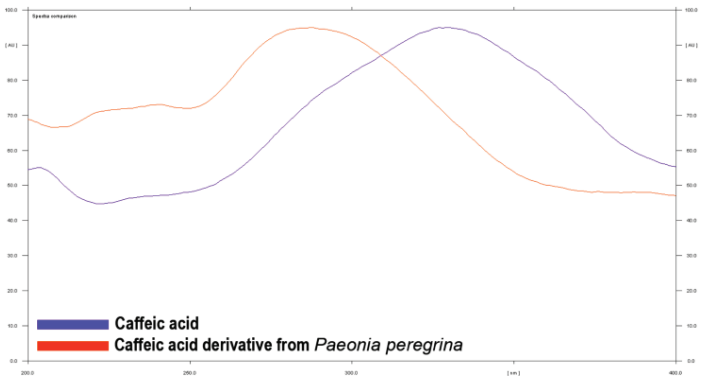

Figure 12. Caffeic acid derivative *in situ* UV spectra of standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of the root, stem and leaf of *Paeonia peregrina* species and the preliminary TLC analysis of *Paeoniae peregrinae herba* polyphenols were accomplished. Tuberized root has circular shape and secondary structure due to the presence of the two meristematic secondary zones (subero-phellodermic and libero-ligneous cambium). Aboveground stem has circular shape modified by some ribs and secondary structure generated by intrafascicular libero-ligneous cambium. The leaf's limb has hypostomatic bifacial dorsiventral structure. Petiole has corded shape, with flat adaxial side modified by two wings. Of the 10 specific bands for the polyphenolic components, highlighted by TLC technique, one caffeic acid derivative was identified.

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PRELIMINARY STUDY REGARDING PHYTOPATOGENIC MOULDS OF SWEET POTATO TUBERS AND BIOLOGICAL CONTROL PERSPECTIVES

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Keywords: sweet potato, phytopathogenic moulds, biocontrol bacteria

ABSTRACT

The sweet potato (Ipomoea batatas) is a less known vegetable for Romanian growers and consumer. This plant is a member of the morning glory family, taxonomic classified as Convolvulaceae. Originated from Central America and north-west part of Latin America, this vegetable is considered to be a thermophilic plant, drought tolerant, with moderate pedologic requirements. The plants grow well on medium fertile soils, well drained with loose structure. Thereby, the sandy soils of southern Oltenia region should offers favorable pedoclimatic conditions for sweet potato crop. In our study we isolated and identified various fungal phytopathogens isolated from Romanian produced sweet potato and we searched for autochthones bacterial strains with antifungal properties in order to develop biological control means against these moulds.

INTRODUCTION

The *Ipomoea batatas* plant is a member of the *Convolvulaceae* family, thermophilic, drought tolerant, with moderate pedologic requirements. As it grow well on medium fertile soils, well drained with loose structure, the sandy soils of southern Oltenia region can offer favorable pedoclimatic conditions for sweet potato crop.

Although the sweet potato (*Ipomoea batatas*) is a less known vegetable for Romanian growers and consumer, the tubers are very well appreciated due to their nutritional value. The sweet potatoes are a valuable source of nutrients and soluble fibers (Burri, 2011).

Most of the cultivars of sweet potato have white, yellow or orange pulp, but there are also some with purple flesh. The varieties with colorful pulp are rich in beta-carotene and antioxidants. Sweet potatoes contain vitamin A, B complex, vitamin C, minerals (Ca, K, P, Na, Mg, Fe, Zn), fatty and amino acids (http://www.healthfame.com/2013/06/purple-sweet-potato-nutrition.html).

Due to its highly nutritional value, the sweet potato tubers are easily affected by various plant pathogens, including fungi and bacteria. As this vegetable is les common for the Romanian growers, it is recommended to evaluate the

phytosanitary risks that can occur in locally farms were sweet potatoes are produced.

One of the purposes of the present study was to evaluate the phytopathogenic spectrum that can occur on *Ipomoea batatas* tubers produced in Romania. Another aim of this study was to identify and select autochthon beneficial bacteria with antimicrobial potential against the identified sweet potato pathogens.

MATERIALS AND METHODS

Biological Material

Two Korean varieties of *Ipomoea batatas*, Chestnut sweet potato and Pumpkin sweet potato were analyzed in this study. The tubers were produced in 2015 growing season at the Research – Development Center for Plant Growth on Sandy Soils (CCDCPN) Dăbuleni, Dolj County, Romania, and provided by Dr. Drăghici Reta, with the courtesy of Dr. Diaconu Aurelia the manager of the research center.

Phytopathogenic fungi

Tubers with phytopathogenic infections (figure 1) were transferred into the laboratory and placed in moist chamber to facilitate the growth of the plant pathogenic microorganisms. The direct isolation technique was applied, and samples of mycelia or spores grown on potato tubers were transferred on potato-dextrose-agar (PDA) medium. After inoculation, the samples were encoded and incubated at 26°C. Repeated plating on PDA growth media was carried out in order to have pure microbial cultures.

The isolated fungi were taxonomic identified using classical microbiological technique, based on cultural and microscopic traits (Samson and van Reenen-Hoekstra, 1988). The macroscopic characteristics were analyzed in pure cultures obtained on PDA. The microscopic examination was performed on young, actively growing cultures. The microscope slides were performed, by placing the specimen in a drop of distilled water or lactophenol cotton blue stain.

Figure 1. Sweet potatoes infected with phytopathogenic fungi.

Beneficial bacteria

Soil samples from sweet potato nurseries of CCDCPN Dăbuleni were analyzed in order to isolate indigenous bacterial strains with antagonistic activity against *Ipomoea batatas* phytopathogens. One gram of soil sample was distributed in 10ml of sterile phosphate saline buffer (PBS) followed by one hour incubation at

100 rpm rotary shaking. Subsequently, 100 µl supernatant was plated with a Drigalsky ooze on Luria Bertani agar (LBA) medium and incubated at 28°C. After 24-48 hours of incubation single bacteria colonies were passed on fresh LBA medium. Routinely, the strains were grown on LBA media at 28°C.

Isolated bacterial strains were microbiologic characterized for Gram reaction, KOH 3% reaction and fluorescence in UV light on King B medium.

Antagonism evaluation

Dual-culture assay was used to evaluate the antagonistic activity of bacterial isolates against sweet potato fungal pathogens. The test was performed on PDA media. Each plate was inoculated with two bacterial strains, streaked at 2 cm from the center of the plate. Mycelia plugs (5mm diameter) were taken from fresh fungal cultures and placed in the middle of the PDA Petri dish. The plates were incubated at 27ºC and analyzed periodically up to 10 days. Control plates inoculated only with the pathogenic fungi were simultaneously prepared. The antagonistic activity of the bacterial strains was calculated according to Islam et al. (2009).

RESULTS AND DISCUSSIONS

The spectrum of phytopathogens in Romanian produced sweet potato was analyzed only on post-harvest tubers.

The identified moulds found on sweet potato tubers were common phytopathogens of *Pythium* (figure 2), *Botrytis* (figure 3) and *Fusarium* (figure 4) genera. It is considered that *Pythium* sp. infections were caught in the field, before storage, and root rot continued during transit and storage.

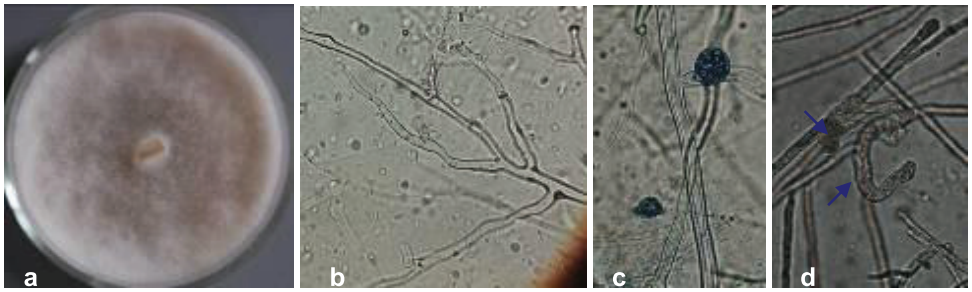

Figure 2. *Pythium* sp. culture on PDA medium (a), aseptate mycelium (b) sporangia (c), oogonum and antheridia (d).

Botrytis cinerea was identified on Pumpkin variety of sweet potato, causing gray mould of the tubers. On PDA medium, the fungus developed abundant conidiophores and conidia of grey color, and black sclerotia. Pathogen identification was carried out based on the culture morphology, conidiophores morphology and branching, and conidial shape and distribution.

Botrytis cinerea is a common plant pathogen all over the world and it is mentioned to cause gray moldiness of sweet potatoes stored roots, in North America (Agriculture Handbook no.165 1960), Africa (1994) and Asia (International Potato Center 1989).

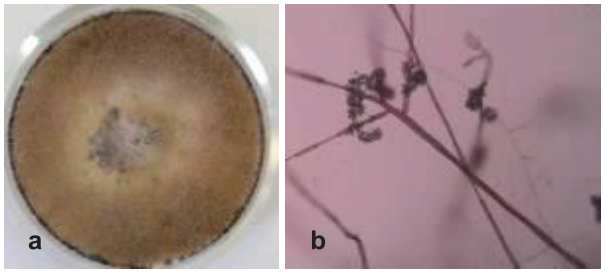

Figure 3. *Botrytis cinerea* isolated from Pumpkin variety of sweet potato
a- Gray mould on PDA medium, b- monopodial branched conidiophore with conidia

Fusarium species were identified mostly on Chestnut variety of sweet potato, causing surface rot of roots. Two of the *Fusarium* isolates (Chestnut 2b and 2a) were similar to *F.oxysporum* and *F. semitectum*, respectively. Both species can develop micro- and macroconidia, and clamidospores. In *F. oxysporum* the chlamydospores can be terminal or intercalary disposed. In *F. semitectum* chained chlamydospores can be found in the hyphae, and single chlamydospores inside conidia (Ingle and Rai, 2011).

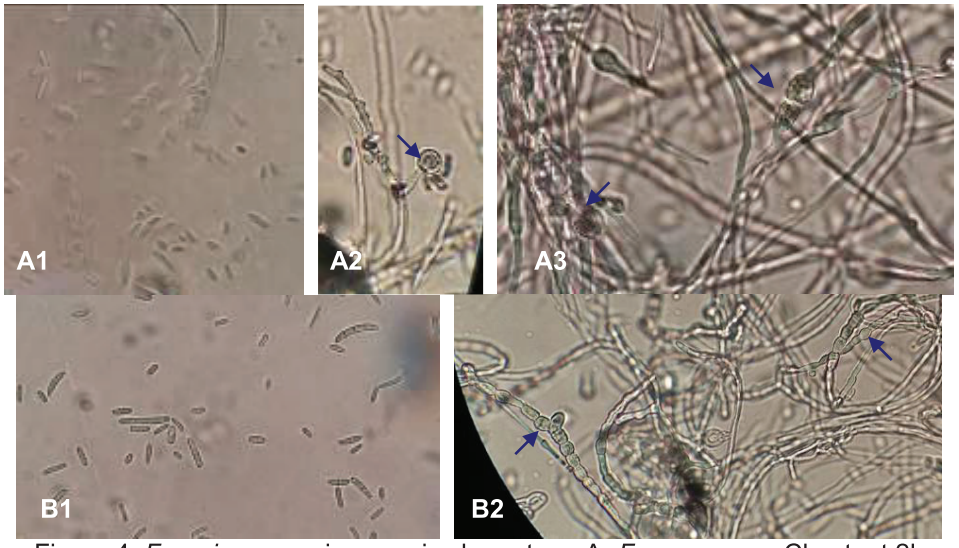

Figure 4. *Fusarium* sp. microscopic characters: A- *F. oxysporum* Chestnut 2b micro- and mesoconidia (A1), terminal chlamydospore (A2), intercalary disposed chlamydospores in hyphae (A3); B- *F. semitectum* Chestnut 2a micro- and macroconidia (B1), and chained chlamydospores in hyphae (B2).

Tubers placed in moist chamber were rapidly depreciated by *Rhizopus* soft rot. The fungus was identified due to the symptoms produced on the roots, correlated with the cultural characters on PDA medium and microscopic traits (figure 5).

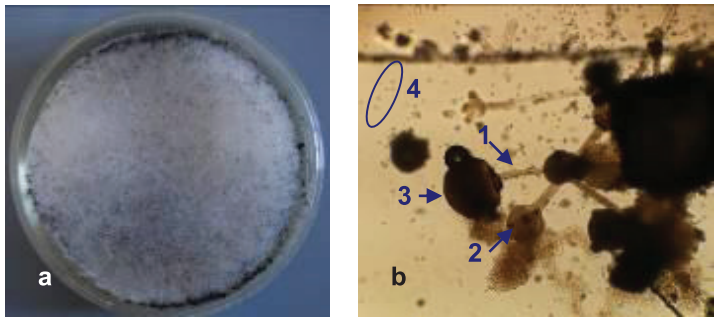

Figure 5. *Rhizopus stolonifer* (syn. *R. nigricans*) isolated from sweet potato tubers a. Fungal growth on PDA medium; b. Microscopic aspects of *Rhizopus* sporangiospores (1), collumella (2), sporangium (3) and sporangiospores (4).

The long storage at low temperature, high humidity level and lack of ventilation, also favor the development of other moulds, such as *Epicoccum* sp. storage rot, and various species of *Penicillium* blue-mould (figure 6).

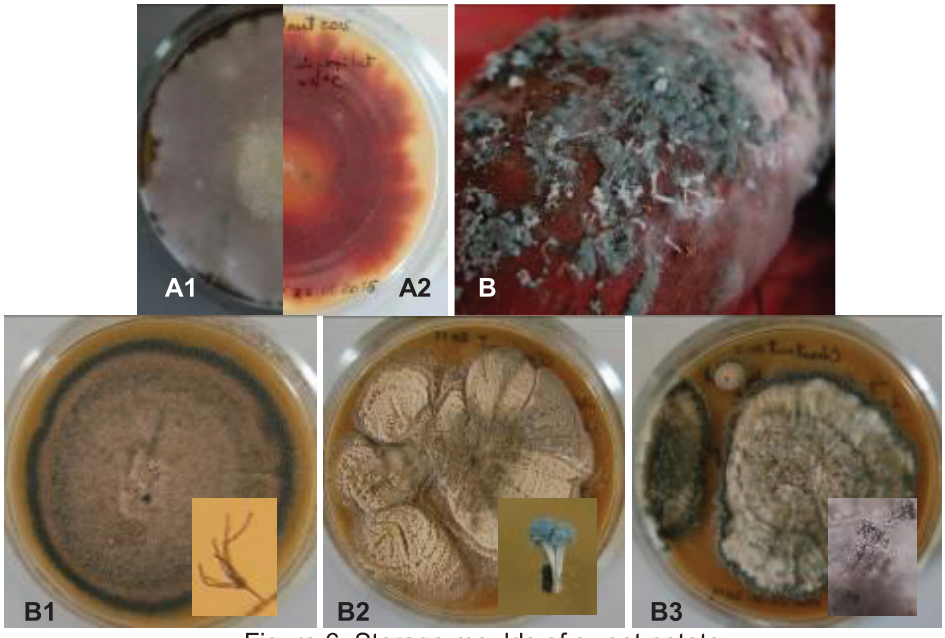

Figure 6. Storage moulds of sweet potato:
A. Epicoccum sp. fungal growth on PDA medium, ventral (A1) and dorsal view (A2) B. Blue-mould rot caused by various *Penicillium* species: B1- *Penicillium* with monoverticillate conidiophores, B2- *Penicillum* growing coremia (synnemata), and B3- *Penicillium* with terverticillate conidiophores.

Due to the identified spectrum of moulds it is recommended to manage the storage condition after harvest according to the needs of the sweet potato tubers. Otherwise, fungal pathogens, like *Rhizopus* sp. and *Penicillium* spp., as we

identified, can rapidly damage the stored products, especially if the tubers suffered cold or freezing stress, associated with humid and poorly ventilated deposit.

To reduce the proliferation of the phytopatogenic moulds of sweet potato, we isolated and purified 22 autochthon bacterial strains. Twelve of the isolated strains were Gram positive bacteria (Dj.s1a1, Dj.s1a3, Dj.s1b1, Dj.s1b2, Dj.s1c1, Dj.s1d1, Dj.s1e1, Dj.s2a2, Dj.s2b1, Dj.s2c2, Dj.s2d2, Dj.ss1e), and the other ten strains were Gram negative (Dj.s1a2, Dj.s1c2, Dj.s1c3, Dj.s1d2, Dj.s1e2, Dj.s2a1, Dj.s2c1, Dj.s2d1, Dj.s2e1, Dj.s2e2). Grown on King B medium, two bacterial strains produced fluorescent pigments in UV light, Dj.s2a1 and Dj.s2d1.

In order to select indigenous bacterial strains with inhibitory activity against *Ipomoea batatas* phytopathogens we evaluated the antagonism activity (figure 7) against the isolated moulds, by double-culture technique (table 1).

Table 1

Antagonistic potential of autochthon bacterial strains against *Ipomoea batatas* phytopathogens

Bacterial isolates	Bacterial inhibitory efficacy against *Ipomoea batatas* moulds evaluated after 3 days of co-cultivation at 27°C					
	Pythium sp.* isolated from Chestnut variety	*Fusarium* sp. isolated from Chestnut variety	*Fusarium oxysporum* Chestnut 2b	*Fusarium semitectum* Chestnut 2b	*Botrytis cinerea* isolated from Pumpkin variety	*Fusarium* sp. isolated from Pumpkin variety
Dj.s1a1	31,8%	53,3%	37,5%	70%	85%	58,3%
Dj.s1a2	31,8%	13,3%	N.A.	N.A.	N.A.	N.A.
Dj.s1a3	27,3%	0%	41,7%	25%	40%	37,5%
Dj.s1b1	31,8%	33,3%	52,2%	70%	85%	62,5%
Dj.s1b2	13,6%	0%	45,8%	25%	85%	0%
Dj.s1c1	4,5%	6,7%	37,5%	25%	65%	37,5%
Dj.s1c2	36,4%	13,3%	37,5%	20%	35%	54,2%
Dj.s1c3	31,8%	0%	37,5%	25%	40%	58,3%
Dj.s1d1	50%	53,3%	50%	50%	85%	58,3%
Dj.s1d2	27,3%	26,7%	37,5%	65%	N.A.	54,2%
Dj.s1e1	0%	0%	41,7%	40%	75%	45,8%
Dj.s1e2	59%	33,3%	29,2%	35%	85%	45,8%
Dj.ss1e	13,6%	53,3%	29,2%	0%	55%	29,2%
Dj.s2a1	18,2%	20%	29,2%	20%	65%	33,3%
Dj.s2a2	18,2%	0%	29,2%	20%	50%	0%
Dj.s2b1	31,8%	53,3%	45,8%	55%	85%	50%
Dj.s2c1	31,8%	13,3%	33,3%	15%	0%	37,5%
Dj.s2c2	31,8%	0%	29,2%	25%	40%	25%
Dj.s2d1	18,2%	26,7%	37,5%	30%	45%	37,5%
Dj.s2d2	22,7%	0%	0%	20%	40%	33,3%
Dj.s2e1	75%	66,7%	58,3%	50%	85%	54,2%
Dj.s2e2	75%	33,3%	50%	30%	85%	50%

* = Bacteria inhibitory activity against *Phytium* sp. was evaluated after 24hours of co-cultivation. N.A.= the information is not available.

Bacteria inhibitory activity against *Ipomoea batatas* moulds was evaluated using the efficacy formula presented by Islam et al. (2009). Six bacterial strains, Dj.s1a1, Dj.s1b1, Dj.s1d1, Dj.s2b1, Dj.s2e1, Dj.s2e2, were appreciated to have more than 50% efficacy in reducing phytopathogenic growth, and a large spectrum of antagonistic activity.

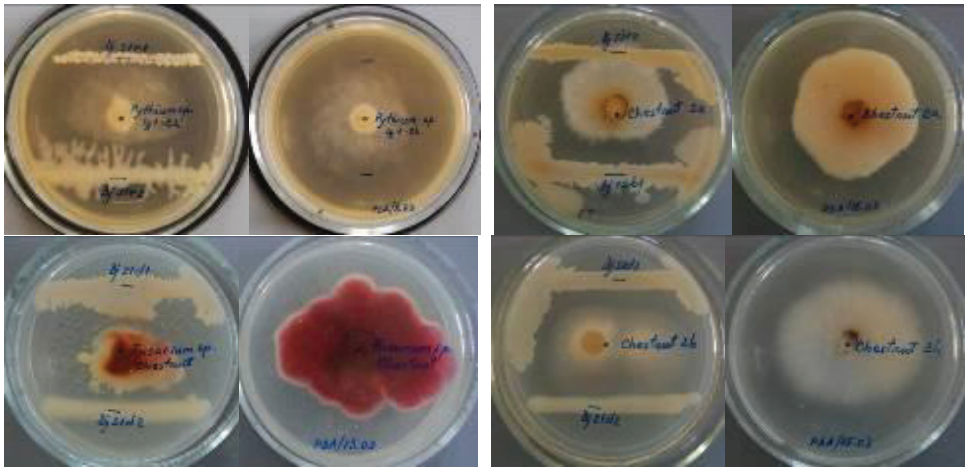

Figure 7. Bacterial inhibitory activity against *Ipomoea batatas* moulds evaluated after 3 days of co-cultivation at 27°C.

Microscopic analysis of clear inhibition zones developed by some of the bacterial strains against *Botrytis cinerea* revealed swelling of the hyphae and cell membranes destruction due to osmotic stress caused by bacterial antifungal metabolites (figure 8).

Figure 8. Swelling or cell walls and plasma membranes destruction of *B. cinerea* hyphae caused by the antifungal metabolites excreted by Dj.s1b1 bacterial strain.

Similar aspects caused by *Bacillus* spp. lipopeptide antibiotics and fungal cell wall degrading enzymes were also reported on *Botrytis cinerea* mould (Sicuia, 2012). In *Fusarium graminearum*, treatments with iturin A or plipastatin A caused cell walls and plasma membranes destruction of hyphae and conidia (Gong et al. 2015).

CONCLUSIONS

Common phytophatogenic moldiness were detected on sweet potato tubers autochthonous produced, such as *Pythium* sp., *Fusarium* spp. and *Botrytis cinerea* moulds. Fungal pathogens, like *Rhizopus* sp. and *Penicillium* spp. were also identified to damage stored tubers, especially if the roots suffered cold or freezing stress, associated with humid and poorly ventilated deposit conditions.

However, six autochthon bacterial strains, Dj.s1a1, Dj.s1b1, Dj.s1d1, Dj.s2b1, Dj.s2e1, Dj.s2e2, were appreciated to have more than 50% efficacy in reducing phytopathogenic growth, and a large spectrum of antagonistic activity against *Pythium* sp., *Fusarium* spp. and *Botrytis cinerea* moulds.

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FLORISTIC RESEARCH IN THE MIDDLE BASIN OF THE RIVER OLTEȚ, AT TETOIU VILLAGE

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Keywords: *flora, species of wild plants, rare and endangered species, regeneration of flora, preservation of species.*

ABSTRACT

This paper presents the species of plants from the flora in the Middle Basin of the river Olteţ, some of them rare, endemic and endangered, existing besides the common species. Identification of the species of plants on the territory of the village contributes to the floristic characterization of the whole basin of the river Olteţ, in point of altitude, soil and the intervention of the anthropic factor. From the hydrophilic species which form associations along the riverbank to the associations of xerophytes on the high hills there is a diversity of species, some of which are presented in this paper.

INTRODUCTION

The village of Tetoiu in the Vâlcea county is located 90 km from the town of Râmnicu Vâlcea and 60 km from the town of Craiova, on the platform of the Olteţ river in the Getic Piedmont. The relief includes the Olteţ meadows, valleys and hills.

Reducing exploitation of some meadows and the consumption of spring water has made it possible for the species Orchis laxiflora Lam. to be found in several points of the Dealul Viilor village (Vineyard Hill), along with Anthoxanthum odoratum L., Lysimachia nummularia L., Juncus effusus L., Juncus inlexus L. Species like Humulus lupulus L. and Cucubalus baccifer are growing in the Olteţ riverside coppice, whose exploitation has been reduced, and the Erytronium dens-canis L. subsp. Niveum (Baumg.) Buia et Păun , Vinca minor L. and Convallaria majalis L. can be found in the woody associations of Quercus frainetto Ten., Quercus cerris L. and Ouercus robor L.

Shrubs like Rubus caesius L., Rosa canina L., Crataegus monogyna Jacq. are expanding over some grasslands where grazing is no longer intensive. This is the place where the plant species reach maturity, producing stolons and seeds.

MATERIAL AND METHODS

For research we have studied a great number of scientific papers on the nearby areas, the region of Oltenia or the country's territory, written by P. Enculescu (1924), Flora R.S.R (1952-1976), M. Păun (1964), M. Păun, Gh.

Popescu (1971), Gh. Popescu (1988) Ciocîrlan (2009). We have conducted research for the present paper by doing field work along several years, systematic observation of the species, watching their appearance and development depending on each year's climatic conditions. During research we have noticed modifications in the exploitation of the land, which brought about changes in the floristic composition of vegetal associations, through the appearance or disappearance of some species, through variations of the frequency and density of bioforms. Research in the Basin of the Olteţ river has also been done by M. Păun, Gh. Popescu, D. Cârţu and others. The closest areas surveyed are Bălceşti-Goruneşti and the „La Cismaru" woods at Ţepeşti.

RESULTS AND METHODS

The species included in this paper are presented in phylogenetic order, grouped by families, mentioning the site where they have been found, their biological form and origin.

Fam. Ranunculaceae Adans.

Anemone ranunculoides L. f. biflora - Piscul lui Ţâpan, (Tapan Peak), the Chirca Forest G., Eur.

Figure 1. Anemone ranunculoides L. f. biflora
(photo: M. Burduşel, April 2014)

Fam. Cannabaceae Martinov

Humulus lupulus L. – Zăvoi Cişmea (Fountain Grove), Zăvoi Punte (Bridge Grove), H., Euras., N. Am.

Fam. Urticaceae Juss.

Parietaria officinalis L. - Zăvoi Cişmea (Fountain Grove), Zăvoi Şasa (Sasa Grove), H., Centr. Eur.- submedit.

Fam. Caryophyllaceae Juss.

Gypsophila muralis L. – La Perieţeanu, Valea Roşcăi (Rosca Valley), la Gutui (the Quinces), T., Euras.

Dianthus armeria L. – La Gîldan, Dealul viilor (Vineyard Hill), Valea Roşcăi (Rosca Valley), T., Eur.

Cucubalus baccifer L. – Zăvoi Cişmea (Fountain Grove), H., Euras.

Agrostemma githago L. – La Gutui (the Quinces), la Teteş, T., Cosm.

Lychnis coronaria (L.) Desr. – La Mărgineni, La Rudării, H., Centr. SE Eur.

Fam. Rosaceaea Adans.

Potentilla micrantha Ramond ex DC – Piscul lui Ţâpan (Tapan Peak), Paciuroaica, H., Centr. Eur.-submedit.

Aremonia agrimonoides (L.) DC – Chirca, Stupină (the Apiary), Valea Noii (the Noia Valley), H., Centr. Eur.-submedit.

Sorbus domestica L. – La Rudării, Pădurea lui Traian Doctorul, (Doctor Traian Forest) Ph., Atl.-medit.

Fam. Fabaceae Lindl. (Leguminoasae Adans.)

Chamaecytisus hirsutus (L.) Link. – La Drumul Satului (the Village Road), la Pârâu (the Brook), la Teteş, Ph., Eur. Centr.

Vicia lathyroides L. – La Saivane (the Livestock Shelters), Chirca, în Vălcea (the Vale), T., Eur.

Vicia pisiformis L. – La Paciuroaica, la Teteş, H., Eur.

Lathyrus aphaca L. – La Perieţeanu, la Punte (the Bridge), la Cişmea (the Fountain), T., Medit.

Figure 2. Lathyrus aphaca L.
(photo: M. Burduşel, June 2012)

Fam. Lythraceae J.St. Hil.

Lythrum virgatum L. – Zăvoi Olteţ (Oltet Grove), Cişmea (the Fountain), H., Euras. Cont.

Fam. Euphorbiaceae Juss.

Euphorbia lingulata Heuffel – Chirca, Şerbăneşti, H., Balc.

Fam. Apiaceae Lindl. (Umbelliferae Juss.)

Orlaya grandiflora (L.) Hoffm. – Ududoiul lui Predeşel (Predesel's Creek), Surupină (the Apiary), T., Submedit.

Fam. Apocynaceae

Vinca minor L. – La Cetate în Pârâu (the Citadel at the Brook), Saivane (Livestock Shelters). Ch., Centr. Eur.-medit.

Fam. Oleaceae Hoffmanns et Link

Fraxinus ornus L.- Stupină (the Apiary), Sub Vii (the Vineyard), Ph., Submedit.

Fam. Boraginaceae Adans.

Cynoglossum hungaricum Simonk. – Pădure Pârâu (the Brook Woods), la Cişmea (the Fountain), Ht., Pont.

Fam. Lamiaceae Martinov (Labiatae Adans.)

Prunella laciniata L. - Dealul Viilor (Vineyard Hill), Şerbăneşti, la Rudării, H., Centr. Eur.-medit.

Fam. Solanaceae Adans.

Hyoscyamus niger L. – La Perieţeanu, la Punte(the Bridge), Ht., Euras.

Fam. Valerianaceae Batsch

Valerianella locusta (L.) Latter. Em. Betcke – Pârâu-salcîmi (the Brook Acacias), Vălcea (the Vale), T., Eur.

Fam. Asteraceae Matinov (Compositae Adans.)

Anthemis tinctoria L. subsp. Tinctoria – La Groapă (the Pit), pădurea lui Traian Doctorul (Doctor Traian's Forest),H., Euras.cont

Echinops exaltatus Schrad. (E. commutatus Jur.) – Chirca, H., Centr. eur.

Fam. Liliaceae Adans.

Erythronium dens-canis L. subsp.niveum (Baumg.) Buia et Păun – Piscul lui Ţâpan (Tapan Peak), Chirca, Vălceaua Şetrarului (Setraru Vale), Saivane (Livestock Shelters), Valea Roşcăi (Rosca Valley), G., V. and SV. Ro. End.

Figure 3. Erythronium dens-canis L. subsp. niveum
(photo: M. Burduşel, March 2015)

Ornithogalum pyramidale L. (O. brevistylum Wolfner) – Chirca, Drumul Satului(Village Road), Valea Roşcăi (Rosca Valley), peste Şasa, G., Centr. Eur.

Convallaria majalis L. – Piscul lui Ţâpan (Tapan Peak), Vălceaua Şetrarului (Setrar Vale), Vălceluşe (the Vales), G., Eur.

Fam. Orchidaceae Adans.

Orchis laxiflora Lam. subsp. elegans (Heuff.) Soo – Dealul Viilor (Vineyard Hill) (Ududoiul lui Predeşel – Predesel's Creek)), Valea Roşcăi (Rosca Valley), G., Pont.-pan.

Figure 4. Orchis laxiflora Lam. subsp. elegans
(photo: M. Burduşel, June 2013)

Neottia nidus-avis (L.) Rich. – Piscul lui Ţâpan Tapan Peak), Şerbăneşti, G., Euras.

Platanthera bifolia (L.) Rich. – Pădurea lui Traian Doctorul (Doctor Traian's Forest), Chirca, G. Euras

Fam. Poaceae Barnhart (Gramineae Adans.)

Apera spica-venti (L.) Beauv.subsp. spica-venti – Drum Cişmea (Fountain Road), peste Pârâu (over the Brook),T., Euras

THE FLORA ANALYSIS

Analysis of the species by origin shows the following situation of the floristic specimens: Eur.=6, Euras.=6, Centr.Eur.-submedit.=3, Centr.Eur.-medit.=2,Balc.=2, Centr. Eur =2, N. Am. =1, Cosm.=1, Centr. SE Eur.=1, Eur. Centr.=1, Atl.-medit=1, Medit.=1, V and SV. Ro. End.=1, Pont.=1, Pont.-pan=1, Euras. Cont=1

CONCLUSIONS

The wild flora on the territory of Tetoiu village includes species from several families, with a different frequency and density from one habitat to another.

Several of these species need protection by reducing the exploitation of these habitats.

In this way their extinction is avoided in places where there is a small number of specimens and their reappearance is ensured in places where their disappearance was noticed during research over the years.

Implementation of certain protective measures by reducing the exploitation of some species and of some habitats where these species are growing would make possible an increase in the number of specimens.

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TARAXACUM OFFICINALE WEBER - A PLANT FOR PHYTOREMEDIATION

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Keywords: *Taraxacum officinale; heavy metals; radionuclides; cell*

ABSTRACT

Previous researches on Taraxacum officinale leaves point out that this is a phytoremediatory species, being capable to absorb the pollutant exogenous particles from the environment (air, water and soil). In this study, it was established the content in heavy metals (spectrometry method), as well as the radionuclide content of the soil (0-20 cm level) and radionuclides from Taraxacum officinale leaves, through Duggan method (gamma spectrometry). Through electron microscopy analysis, it was established the penetrance of the exogenous particles in the parenchyma of the leaf (at stomata level). It was also established the interaction of the exogenous particles with the cell organelles.

INTRODUCTION

At the end of the 19th century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species used to accumulate a big amount of metals in their leaves (Baumann, 1885, in Lasat, 2002). Ample researches performed with different species point out that many plant, bacteria or fungi species possess the capacity to adsorb a great amount of toxic substances (heavy metals, radionuclides, a/o) from the environment. In 1935, Byers reported that plants from the *Astragalus* genus were able to accumulate up to 0.6 % selenium in dry shoot biomass (Lasat, 2002). Rascio (1977) reported tolerance and high Zn accumulation in the shoots of *Thlaspi caerulescens*. The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunamyia (1980) and Chaney (1983). The first field trial on Zn and Cd phytoextraction was conducted in 1991 (Baker et al.). Despite subsequent reports claiming the identification of Co, Cu, and Mn hyperaccumulators, the existence of plants hyperaccumulating metals other than Cd, Ni, Se and Zn has been questioned and requires additional confirmation (Salt et al., 1995). Lead is also a main contaminant for soil. Several

species manifest accumulating properties for lead in soil (*Ambrosia artemisiifolia*, *Carduus nutans*, *Commelina communis*, a/o (Lasat, 2002).

In the last decade, extensive research has been conducted to investigate the biology of metal phytoextraction. Despite significant success, our understanding of the plant mechanisms that allow metal extraction is still emerging. In addition, relevant applied aspects, such as the effect of agronomic practices on metal removal by plants are largely unknown. It is conceivable that maturation of phytoextraction into a commercial technology will ultimately depend on the elucidation of plant mechanisms and application of adequate agronomic practices. Natural occurrence of plant species capable of accumulating extraordinarily high metal levels makes the investigation of this process particularly interesting.

Previous researches point out that the *Taraxacum officinale* (common dandelion) species presents the ability to absorb different exogenous particles from the environment, especially at the level of the leaves. The leaves being disposed at the soil surface, through lower epidermis, which is in direct contact with the soil surface, can absorb different exogenous particles from water, soil and air (Corneanu et al., 2000). For these properties, this species is supposed to be proper for the decontamination of many areas (Corneanu et al. 2011). In this study, it was investigated the cell wall penetration by exogenous particles, as well as the interaction of the cell organelles with exogenous particles. They penetrate the leaf through the epidermal cells or at the stomata level. We mention that this herbaceous species was used, together with other species, for the decontamination processes at Chernobyl Atomic Power Plant (Ukraine).

MATERIAL AND METHODS

The study site is located in Gorj County, Romania in the vicinity of ThermoElectric Power Plant (TEPP) Rovinari, on a sterile waste dump (Lat: 44°54'59.30"N, Long: 23° 7'49.54"E, Alt: 166m) . The Control site is located on the Motru River, at the edge of a poplar belt (Lat: 44°34'41.83"N, Long: 23°26'52.03"E, Alt: 108 m).

Biological material. The investigation of the ultrastructural features of the leaf were performed on mature plants of dandelion (at flowering), developed in the Control area (Arginesti - Picu), as well as on the plants developed on the sterile waste dump from Rovinari near TEPP-Rovinari.

Soil analysis was performed on samples from the 0-20 cm depth of the A horizon. Both soil samples were characterized in terms of basic chemical properties (pH, content in organic C, nitrogen, mobile phosphorus and potassium). **The amount of heavy metals** from the soil was determined by atomic absorption spectrometry in air-acetylene flamer in hydrochloric solution resulted by digestion of soil samples in HClO4-HNO3 mixture.

The radionuclide activity was performed on soil and plant samples through gamma spectrometry (Duggan 1996).

The electron microscopy investigations were performed using the classical method. Pieces of about 1 mm^3 size (provenance from the middle of the leaf) were infiltrated and embedded in EPON 812. Seriated sections of 80-90 nm thick, contrasted with uranyl acetate and lead citrate, were analyzed at a TEM JEM-JEOL 1010 (*Babeş-Bolyai* University, Electron Microscopy Centre).

RESULTS AND DISCUSSIONS

The analysis of the main chemical characteristics and the amount of heavy metals of the soil samples.

Arginteşti-Picu (Control) – profile located on the bank of the Motru River, where the soil has a very low content of organic matter (1.01% organic carbon), total nitrogen (0.106%), mean amount of mobile phosphorus (31.0 mg/kg) and mobile potassium (169.0). The reaction of the soil is slightly alkaline (pH= 7.48). The content in heavy metals is within normal limits for all analyzed elements, except for Ni, with a higher value, but without inducing environmental problems (Table 1).
Rovinari – Sterile waste dump- profile on a sterile material extremely low in organic matter (0.73% organic carbon), total nitrogen (0.065%) and soluble potassium (89.0 mg / kg) and medium supplied with soluble phosphorus (20.0 mg / kg). The reaction of the substratum is slightly alkaline (pH = 8.03).The content of heavy metals indicates the increase of Cd and Ni, up to the caution thresholds (Table 1).

Table 1

The amount of heavy metals in the soil (mg/kg soil) in the two considered sites

Heavy metal	Control Argineşti - Picu	Sterile waste dump, Rovinari	Normal content in soil
Zn	99.0	104.0	100.0
Cu	16.3	16.5	20.0
Fe	17,331.0	20,525.0	*
Mn	321.0	445.0	900.0
Pb	16.9	17.1	20.0
Ni	30.6	43.4	20.0
Cr	10.8	15.5	30.0
Co	11.7	14.6	*
Cd	1.13	1.37	1.0

*No available data

The amount of radionuclides in the soil and in the dandelion plant.

The analysis of the radionuclide activity identified radionuclides belonging to the three natural series, primary radionuclides that are not part of these (K-40 si Be-7) and an artificial radionuclide (Cs-137) (Table 2).

The soil samples contain both natural and artificial radionuclides whose concentration is above the limit of detection of the measuring apparatus. The concentration of the natural radionuclides is limited to those that are found in the soil in Romania (Chiosila et al.1994). An importantly determined radionuclide was the Pb-210 whose concentration in the soil samples ranged between 20.8 Bq/kg (Control) and 47.7 Bq/kg and is considered a radionuclide with great radiotoxicity. Cs-137 concentration, whose origin is the accident at Chernobyl, varied between 56.9 Bq/kg and 19.9 Bq/kg. In most parts of Romania, the content soil in Cs-137 has fallen below 1Bq/kg over the past decade, but there are regions in the area where the values are still high (Chiosilă 2004).

Table 2

The radionuclide activity in soil (Bq/kg) and in plant (Bq/kg fresh mass) in the two stations on the Jiu river valley (A horizon, depth level 5-20 cm)

Radionuclide	Soil			Dandelion plant	
	Arginesti-Picu	Rovinari sterile waste	Limit content in Romania	Arginesti-Picu	Rovinari sterile waste
Th 234/U 238	< 7.13	45.4 ± 2.19	25.0	<4.62	<5.68
Ra-226	22.5 ± 21.4	29.9 ± 1.3	38.0	<1.77	0.73 ± 0.37
Pb-210	20.8 ± 2.36	47.7 ± 3.95	20.0-40.0	3.8± 0.82	11.2 ± 1.29
Bi-214	22.3 ± 1.61	27.4 ± 1.41	20.0-40.0	<1.7	<1.92
Pb-214	22.9 ± 1.11	30.4 ± 1.27	20.0-40.0	<1.8	<2.41
U-235	3.18 ± 0.95	4.0 ± 0.46	2.0	<0.65	<0.92
Ac228/Th232	30.8 ± 2.27	34.8 ± 2.56	39.0	<3.22	<3.94
Pb-212	24.1 ± 1.16	46.7 ± 1.53	20.0-50.0	*	*
K-40	512.9 ± 30.7	539.9 ± 27.6	540.0	170.3± 13.9	151.3 ± 14.6
Be-7	< 10.7	< 14.3	*	<110	<135
Cs-137	56.9 ± 3.19	19.9 ± 27.6	*	1.85± 0.19	<1.22

*No available data; < Under the detection limit

In the case of plant samples, the only ones whose radionuclide concentration was above the limit of detection of the measuring apparatus are: Ra-226, Pb-210 (which is part of the category of radionuclides with very high radiotoxicity), the primary radionuclide K-40 and the artificial radionuclide Cs-137. In the dandelion plants harvested from the sterile waste dump, the concentration of Ra-226 was 0.73 Bq/kg and of Pb-210 was 11.2 Bq/kg, significantly higher than in the Control plants. These values indicate that the plants manage to focus the aforementioned radionuclides that come from the soil in which they grow.

The lead implication in the phytoremediation process was pointed out by other researchers. Thus, Rădulescu et al. (2013) made investigations regarding the heavy metal accumulation in the leaves of *Cardus nutans* L. and *Taraxacum officinale* L., in an area from Năvodari (Romania), with a pollution of different nature: industrial activity, domestic pollution, a/o. In the leaves of the two species, there is accumulated a high level of lead, copper and other heavy metals. They consider that the two species can be used as bio-indicators for “actual pollution degree of the environment with heavy metals”, especially with copper (Rădulescu et al. 2013). Also, the climatic factors, botanical structure and mineral composition are responsible for the variation in the content of some heavy metals, such as: Cu, Ni, Pb, Zn, Cr, and Cd. Keane et al. (2001) performed an experiment using 29 *Taraxacum officinale* (dandelion) populations from the mid-western part of the United States, regarding their resistance to eight metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) present in the environment and leaves. They detected “a positive significant correlation between soil concentrations of each metal and measures of PM10 at a site, signifying that airborne particulate matter is a good indicator of soil metal contamination’’. Similarly, Rosselli et al. (2006) analyzed the contents of Cd, Cu and Zn in the leaves of common dandelion developed in three different sites, and established that “Cd and Cu concentrations in *Taraxacum* leaves lay under the threshold of those which are admitted by Swiss law for heavy metal contents in food, whereas Zn concentration was slightly above”. Because the dandelion leaves

are used usually as salad, Giacomino et al. (2016) analyzed the concentration of Cd, Cr, Cu, Fe, Mn, Pb, and Zn in 12 sites in the province of Cune (Piedmont, Italy). They recommend that “a caution should be used in consuming spontaneously growing vegetables”. For this reason, dandelion will be used as food in the affected areas, with prudence.

Ultrastructural features of the dandelion leaves.

Previous researches point out that the dandelion adsorbed the exogenous matter from the environment (Corneanu et al. 2010). Exogenous particles from the environment penetrate the cell wall and are spread in the parenchyma through aeriferous circulating system or through the cell wall.

(A) Control plants. In the Control plants, leaves present the normal ultra-structural features. The epidermal cells are covered with a thin cuticle, pellicular cytoplasm and a few cellular organelles. In the palisade parenchyma cells, there are some types of substances, of exogenous origin, or synthesized in the leaf. The substances of exogenous origin present a different shape and size. They can be: (a) electron-dense particles that penetrate trough epidermis, present free in cytoplasm, vacuole or chloroplast; (b) granular particles, met especially in the vacuole; (c) particles of acicular shape, grouped in fascia, met in the aeriferous circulatory system, in the vacuole on the tonoplast surface, on the inner part of the parenchyma cell, near the mitochondria and chloroplast. The substances of endogenous origin, synthesized in the cell: (a) granular substances, medium electron-dense, synthesized in the cytoplasm or chloroplast; (b) an organic substance, intracellularly synthesized, usually of spherical shape or mushroom shape with a diameter of 6-11 μm. This endogenous amorphous substance is present in the cell vacuole, sometimes in relation to the chloroplast and the acicular exogenous substances. The chloroplast contains few plastoglobules, the stroma thylakoids and practically there are no starch grains. The mitochondria present a lax matrix and a few cristas. Near the chloroplast and mitochondria, there are a few peroxisomes. The nucleus presents the heterochromatin dispersed in it. Sometimes, in the cytoplasm around the nucleus, there are present some electron-dense particles. The central cylinder is made of ligneous cells, liberian cells and fundamental parenchyma. Lacuna parenchyma is made of cells with unregulated contour, in which there are many substance types: (a) electron-dense particles present in the vacuole and chloroplast; (b) granular particles present in the vacuole; (c) acicular substances present in the vacuole or near cell organelles; (d) granular, medium electron-dense substances; (e) organic substance of different shapes disposed between the cell organelles. In some chloroplast from the lacuna parenchyma, there were met starch grains. The lower epidermis is covered with a thin cuticle. Sometimes, there were met electron-dense particles traversing the cell wall or present in the chloroplast. The exogenous particles can penetrate the leaf at stomata level.

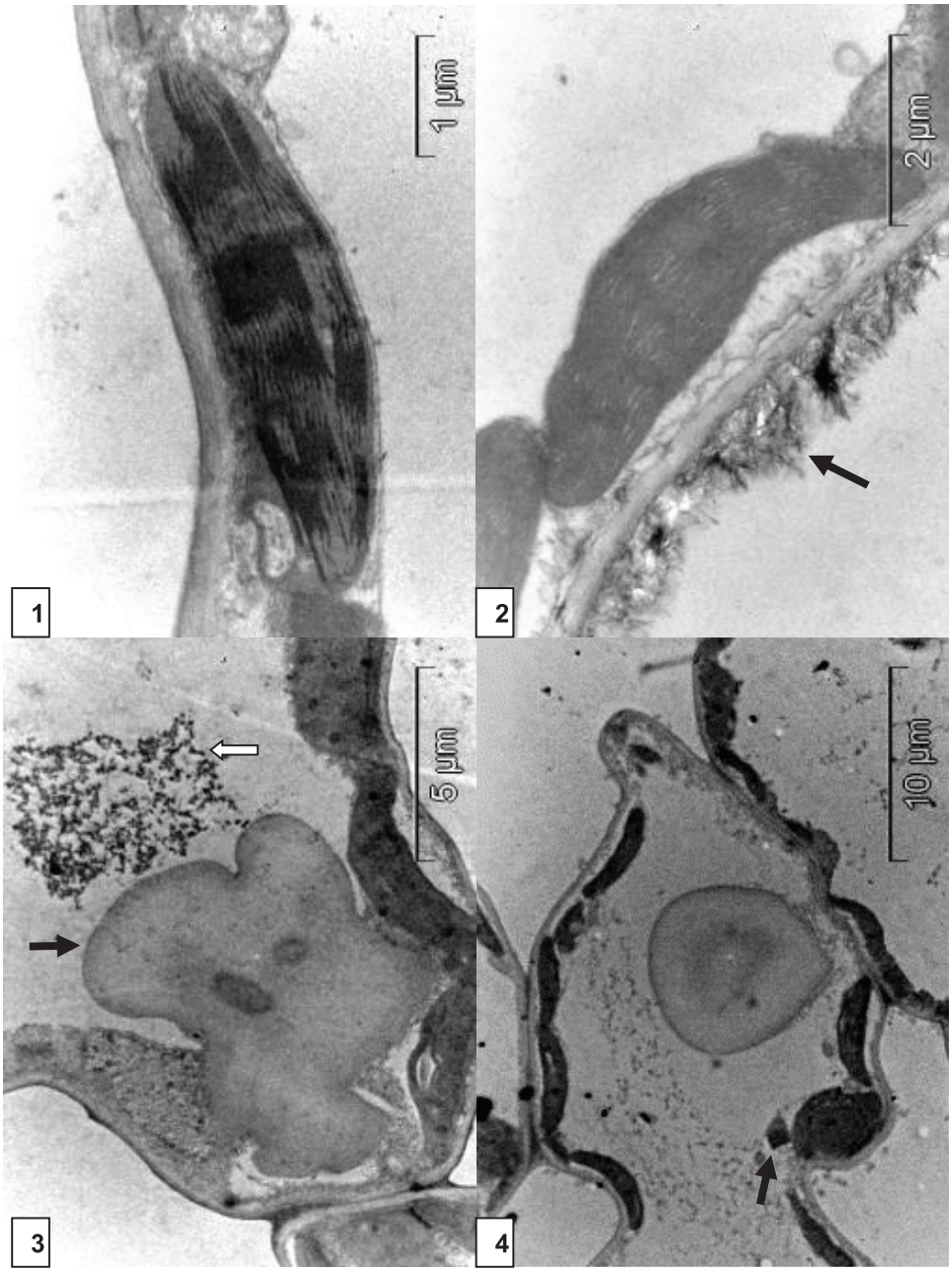

Plate 1. *Taraxacum officinale*, Control. Fig. 1. Chloroplast from palisade parenchyma; Fig. 2. Acicular shape particles on cell wall (→); Fig. 3. Amorphous (endogenous matter →) and granular particles (exogenous particles⇒); Fig. 4. Parenchyma cell with peroxisomes (→) and other inclusions.

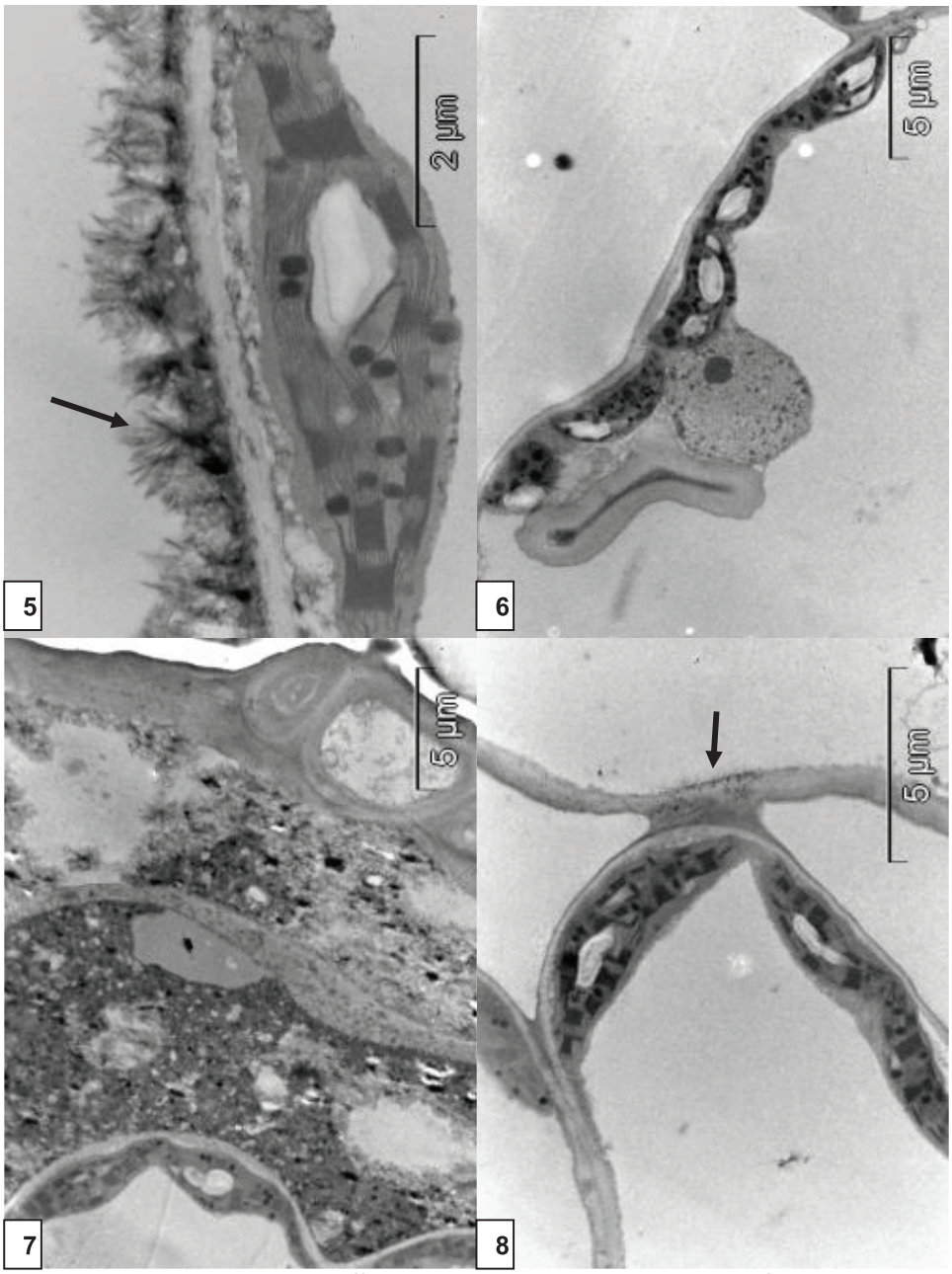

Plate 2. *Taraxacum officinale*, sterile waste dump. Fig. 5. Chloroplast with one starch grain and acicular substance (→); Fig. 6. Amorphous substance, nucleus and chloroplasts with starch grains;Fig. 7. A destroyed cell, near conducting vessels. Fig. 8. Acicular substance in the cell wall (→).

(B) Plants developed on the sterile waste dump from Rovinari. In the plants developed on the sterile waste dump, in cells, there is accumulated a bigger amount of exogenous particles (represented by heavy metals and radionuclides), especially in the cells situated near the circulatory system. The cells from the circulatory system and the acicular particles play a role in the elimination of the cell rests.

Similar to the Control case, the exogenous particles from the environment penetrate the cuticle and epidermal cell and pass into the leaf. The cells from the palisade parenchyma present a cytoplasm with cell organelles disposed parietally, in the vacuole being present a granular matter and electron-dense exogenous particles. The chloroplasts present a developed granal system, a few plastoglobules and 1-2 starch grains. Sometimes, in the near cytoplasm, near the chloroplast there are present acicular structures. In the parenchyma cells (palisade and lacuna), there are present some substance types; (a) exogenous, granular, electron-dense particles, present in the vacuole and in the chloroplast, near the stroma thylakoids; thin-granular substance, present in the vacuole; (c) acicular substance, in the aeriferous space, on outer cell wall; (d) a hyaline substance synthesized probably in the cell, situated near the nucleus having a cremwurst shape, circle shape situated in the vacuole near the chloroplast, ring shape, in the vacuole, a/o.

The central cylinder presents a double importance: (a) the classical role for the transport of intracellular substances, (b) transporting of the cell rests. Probably, the accumulation of the substances induced one of these actions.

The exogenous particles accumulate in a great amount, cross the apoptosis process and are destroyed. Thus, near the conducting vessels, there are parenchyma cell with a destroyed content (Figs. 5, 6). The parenchyma from the dandelion leaf is made up of a few cell layers; some conducting vessels can come up near the epidermal cells. Thus, it is facilitated the process of apoptosis and the exchange of substance.

The common dandelion is a phytoaccumulator species. Previous investigations evidenced its possibility to adsorb and accumulate different radionuclides. In this process, some cells are destroyed (Fig. 7). There probably exists a genetic determinism for these two processes:

(1) The accumulation of radionuclides or heavy metals takes place in the vessels from the central cylinder, in the parenchyma cells situated near the conducting vessels, or in some spaces of the aerial circulating system from this region.
(2) The accumulation process is followed by an apoptosis process having as result the destruction of these deposits.

Bini et al. (2012) analyzed the leaf ultrastructure in plants cultivated on metal-contaminated soil or in pots amended with compost. Bini et al. (2000, 2008) reported that metal uptake and translocation to the aerial parts were reduced, except for Zn, which manifests an antagonist effect with other metals. Cr is accumulated in the root and acts as a barrier against Cr translocation (Bini et al. 2008).

CONCLUSIONS

Taraxacum officinalis (dandelion) is a phytoaccumulator species for some heavy metals and radionuclides. This species can vegetate in regions affected by extractive and energetic industries.

In the dandelion leaves, there can be adsorbed some heavy metals from the environment, such as: lead, cooper, nickel, chromium, a/o, as well as radionuclides, such as: Ra-226, Pb-210, Cs-137. As the dandelion can adsorb soil and air pollutants from the environment, it can be used for cleaning up the soil. In the same time, it is recommended to be used as food with prudence.

The dandelion leaves present some structural features as species for phytoremediation. The epidermis is covered by a thick cuticle and facilitates the penetration of exogenous particles from the environment. The exogenous particles can also penetrate in the leaf at the stomata level. In the parenchyma tissue, it is present an aerial conducting system that facilitates the transport of the exogenous and endogenous particles in the plant. In cells of the leaves there are exogenous and endogenous particles, involved in the apoptosis processes and in the transport of the rests outside the cell. As a result of the accumulation of a lot of exogenous substances from the environment, some parenchyma cells are destroyed. The circulatory system and acicular exogenous substances are involved in the elimination of the cell remains at outer.

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THE AFFECTION OF ORAL CAVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Keywords: *systemic lupus erythematosus, lesions, ulceration*

ABSTRACT

This paper presents Systemic lupus erythematosus (SLE), which is a chronic inflammatory disorder with multisystem involvement due to autoimmune phenomena, generated by the presence and persistence of autoantibodies in excess.The multitude of clinical and biological manifestations, caused by autoantibodies cause multisistemic damage, evolving towards the emergence of organ insufficiencies. Skin lesions and mucous membranes, especially the oral mucosa, is a frequent manifestation of SLE and require early diagnosis and effective treatment in patients with SLE, systemic treatment to prevent complications.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory disorder with multisystem involvement due to autoimmune phenomena, generated by the presence and persistence of autoantibodies in excess. The multitude of clinical and biological manifestations, caused by autoantibodies cause multisistemic damage, evolving towards the emergence of organ insufficiencies.

Epidemiological evidence of the disease is variable depending on age, race, sex and geographic area. Thus, the incidence of SLE presented globally in literature is between 1-23 per 100,000 inhabitants/year, with variations depending on geographic region, race and ethnicity, as the United States, being 5.1 per 100,000 inhabitants and in Europe between 2-5 per 100,000 inhabitants (Danchenko et al. 2006).

The prevalence in women is nine times higher than men, frequent childbearing age, age of onset is 16-55 years, criminalizing hormonal factor in the pathogenesis of the disease. Thus citing that 60% of patients SLE onset is between 16-55 years, 20% had onset before 16 years and 15% after 55 years.

The symptoms in men are dominated by an onset in an earlier age than in women, with serosity, with lower phenomena of photosensitivity, causing a higher mortality, while the onset in the elderly persons is marked by pulmonary events, sicca syndrome, musculoskeletal and serosities manifestations (Eular. 2015).

Black persons are three times more affected than whites, the genetic variability plays an important role in the disease. SLE manifest itself by specific symptoms: impaired musculoskeletal, mucocutaneous, cardiac, hematologic, renal,

pulmonary, nervous system. The prevalence of oral damage in patients with SLE is uncertain but may vary between 9-45% in patients with SLE and from 3-20% in patients with chronic skin damage. It also reported an incidence of oral damage in juvenile-onset of SLE 0.3-0.9 per 100,000 population, with a prevalence of 0.3-8.8 per 100,000 inhabitants, with a peak at puberty [Lourenço SV et al. 2006]. It cited in medical literature, a positive correlation between impaired systemic and cutaneous manifestations in SLE, LES without skin's manifestation has a higher incidence in systemic involvement; skin lesions of SLE, allowing early diagnosis of the disease, before systemic damage, while those without skin's lesions are later diagnosed, being in a more advanced stage of the disease (Direkrit Chiewchengchol et al. 2014). The prevalence of oral damage in patients with SLE is uncertain but may vary between 9-45% in patients with SLE and from 3-20% in patients with chronic skin damage. It also reported an incidence of oral damage in juvenile-onset of SLE 0.3-0.9 per 100,000 population, with a prevalence of 0.3-8.8 per 100,000 inhabitants, with a peak at puberty (Lourenço et al. 2006).

Mucocutaneous damages are represented by malar rash, photosensitivity (erythema following exposure to sunlight). Discoid lupus and ulcerations are included in ACR criteria for the diagnosis of this disease.

Skin lesions in SLE can be specific or nonspecific (and common with the other diseases). Specific injuries are: acute injuries (malar rash, generalized erythema, bullous SLE), subacute cutaneous lupus, chronic lupus (localized discoid, generalized discoid) profundus lupus. Nonspecific injuries are: panniculitis, urticarial lesions, vasculitis, livedo reticularis, oral lesions, alopecia, skin disorders (psoriasis, lichen planus, porphyria), hypertrophic lupus (Verrucous), alopecia, Raynaud syndrome.

Muco-cutaneous lesions are important for rapid diagnosis of the disease, can be the first manifestation of the disease, particularly juvenile-onset SLE, while in adults, may be limited only in skin manifestations. (Chiewchengchol et al. 2015).

The study lesions in juvenile-onset SLE (JSLE) and LES in adults showed that the frequency of skin lesions is higher in JSLE. Damage is described oral tongue, mouth, lips and palate. Damage mucosa ulceration disease appears as painless, oral, nasopharyngeal or vaginal petechiae on the palate, gums and even the tongue, which in turn evolve to erosion of nasal septum perforation and its. These lesions must be differentiated from lichen planus, oral candidiasis, aphthous stomatitis, herpes lesions in Behcet's syndrome, leukoplakia and neoplastic lesions.

Oral mucosal lesions are of two types, with specific histological changes LES - discoid lesions and aphthous ulcers. Specific lesions of lupus begin with solitary erythema and patches before developing hemorrhage, with a curb reticulata. Usually the lesions are painless and located on the palate. Instead, nonspecific aphthous ulcers are usually painful, with multiple lesions in the mouth, lips and nasal septum with a bleeding tendency. Nasopharyngeal and oral ulcers occur during active disease and remission of the disease disappears.

The most prevalent lesion was ulcers (28.1%) and most often involved were labial and buccal mucosa (Khatibi M et al. 2012).

Oral manifestations of erythematosus discoid lupus are referred to as "oral discoid lesions" and this occurs in about 20% of patients. They can take place without the involvement of developing skin lesions or skin lesions before. Discoid lesions on the oral mucosa are most frequently labial, buccal mucosa and purple

edge. Typical cases of oral discoid lesions are clinically characterized by the presence of white papules, central erythema area demarcation ribbed white.

Histologically, there were described parakeratosis or ortokeratoza, acanthosis, epithelial atrophy, vacuolar degeneration of the basal membrane basal keratinocytes necrosis, thickening of the basal membrane.

The injuries situated on the border of purple lips (especially the lower lip), deserve special attention because they can be with or without epithelial dysplasia.

Other secondary orofacial signs can be: burning mouth, dry mouth disease, salivary gland (focal necrosis of the parotid gland), descuamativa gingivitis which can lead to increased incidence of dental caries and the occurrence of candidiasis, particularly when administering immunosuppressive drugs (1. Aline Lauria Pires Abrão et al.).

MATERIAL AND METHODS

The study group included 21 patients from some dental offices witch presented oral changes.

Patients were selected on clinical criteria: subjective (itching gums, burning, pain when chewing and abnormal mastication), goals (state of oral hygiene, oral lesions, tooth mobility, gingival, the presence of periodontal pockets, indicating papillary bleeding).

There were not included in the study pregnant women or people who have not agreed to participate in the study. Participants were aged between 15-45 years, during which require more attention to possible complications of the oro-dental device.

Findings periodontal status was achieved by general and local clinical examination and by the examination of patients medical records and observations recorded in the personal file.

RESULTS AND DISCUSSIONS

Mucocutaneous manifestations is the second ACR criteria, the frequency of occurrence in SLE diagnosis after haematological one, 60-85% of patients with SLE developing mucocutaneous manifestations.

Data from the literature have reported a positive correlation between impaired systemic and cutaneous manifestations in SLE, but no studies contradict this hypothesis, SLE without skin showing a higher incidence in systemic involvement. Skin lesions allow early diagnosis of the disease, before systemic damage, while those without skin lesions are diagnosed later, in more advanced stage of the disease. (Chiewchengchol et al. 2014)

The prevalence of oral damage in patients with SLE is uncertain but may vary between 9-45% in patients with SLE and from 3-20% in patients with chronic skin damage. It also reported an incidence of juvenile-onset SLE 0.3-0.9 per 100,000 populations, with a prevalence of 0.3-8.8 per 100,000 inhabitants, with a peak at puberty (Lourenço et al. 2006).

It noticed a predominance of patients in urban areas in women, which can be explained both by addressing increased by specialist medical services, patients in urban areas and by the economic and social higher in urban areas and predominant disease as specified in studies in women.

All participants were aged 15-45 years, with predominant injuries especially in women, ages 15-19, were correlated with the onset of oral lesions at

an early age, the absence of lesions in men in debut it is shown that onset is more common in men by poliserositis. Thus this oral lesions in young patients requires special attention in the early diagnosis of SLE with the initiation of systemic specific therapy to avoid complications (Kelley 2008).

Labial mucosa and oral lesions were more common compared to gingivitis and associated manifestations.

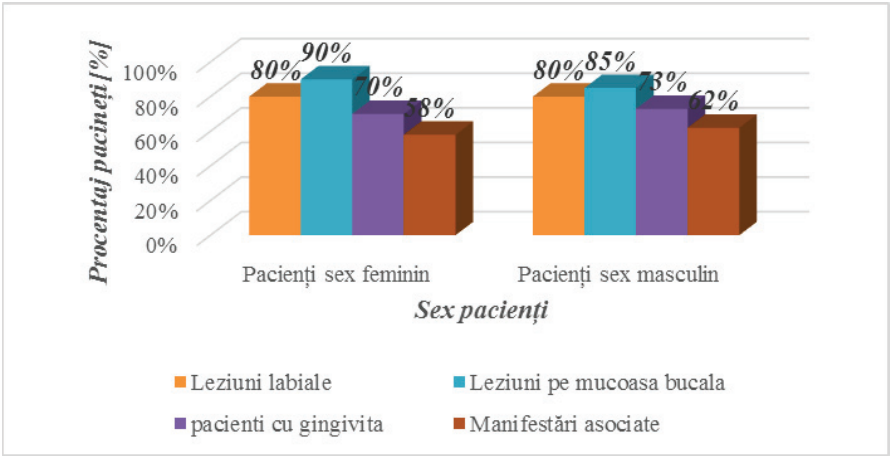

Figure 1. The distribution by gender of oral mucosa lesions.

The reasons of presentation were different. Most cases presenting gingival bleeding - in July, followed by pain - 6 cases. After a thorough examination of the participants in the study group and analysis of medical records of patients, the results obtained from history taking, we recorded a variety of oral amendments in the presence of SLE.

Odonto-periodontal examination included a clinical examination of the oral cavity which consisted of dry mucosal surfaces presence that may be associated with burning mouth. Xerostomia constant irritate oral soft tissues, causing inflammation and pain. SLE patients with xerostomia are more prone to periodontal infections and tooth decay. It mentions the presence of numerous carious lesions.

In most cases the presence of gingival inflammation was present plaque. The intensity varies gingival inflammation and the presence of dental plaque and tartar. I encountered the following issues: localized and generalized gingivitis on incentives to marginal gingiva and interdental papillae, red, soft consistency, easy bleeding, reduced amount of dental plaque and tartar.

Results of the oral hygiene condition: plaque index has an average value of 1.5, with a minimum of 0 and a maximum of 3.0.

In patients studied we found the presence of proper oral hygiene at a rate of 71.43% and satisfactory, 19.03% respectively, present oral lesions being influenced by the state of hygiene.

Depending on the clinical form of chronic periodontitis, inflammatory lesions found were low, moderate or severe intensity, manifested by: papillary

inflammation and moderate marginal, shallow pockets (4-6 mm), medium (7-8 mm) or high (> 8 mm), inflammatory exudate present or absent in periodontal pockets, tooth mobility pathological varying degrees.

In some patients discoloration, volume, consistency gum inflammation associated with gum disease were not visible on inspection and inflammation was inferred by bleeding index, which was dominant in the group 64.5%.

In young patients, we found generally localized presence of gingival retraction, smaller (4-6 mm) compared with patients over age 40 who had the most extensive gingival teeth with values exceeding 6 mm.

CONCLUSIONS

Oral lesions in SLE are important events that cause addressability patients to dental offices. Lesions may hasten the diagnosis of patients.

Knowing the changes that occur in the periodontal tissues after bacterial infection, new therapeutic strategies should be inclined to modulate the action of the body's response to pathogens.

In conclusion, early diagnosis and effective treatment in patients with SLE, systemic treatment may hasten developing complications.

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All authors contributed equally to the study and they have the same rights.

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THE ASSESSMENT OF NUTRITION STATUS IN PATIENTS WITH TYPE 1 DIABETES MELLITUS DIAGNOSED OVER THAN 25 YEARS

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Keywords: *obesity, type 1 diabetes mellitus, dyslipidemia*

ABSTRACT

For normal function, the human body requires a constant supply of energy intake is achieved through food principles (Jensen et al. 2012). Being a body warm-blooded, that lacks the storage capacity of the heat and the possibility of transforming other forms of external energy source unique and indispensable to the survival of human remains energy produced by dissolution of chemical bonds in the structure of food health of each individual, often measured by what is called an optimal nutritional status derived from balance achieved between supply and nutritional intake (Camina-Martín et al. 2015). The existence of a state of optimal nutrition promotes the growth and development of the organism, maintain health, enable work daily and participates protect the body from various diseases assaults (Elmadfa and Meyer 2014).

INTRODUCTION

Nutritional status assessment is made on subjective data (hard working, self-esteem weight etc.) and on clinical data endpoints (anthropometric indices, functional etc.) or laboratory (bioelectric impedance, densitometry, biochemical tests etc.) (Jeernov et al. 1990). The objective of this study was to assess nutritional status in a group of patients with type 1 diabetes diagnosed over than 25 years ago.

MATERIAL AND METHODS

We studied a group of 44 patients with T1DM older than 25 years, hospitalized in the Diabetic Clinic Center (Phylantropia Hospital Craiova). We analyzed the following history, clinical and paraclinical dates: the oldness of diabetes mellitus, personal history, blood pressure, palpation of pulse at the level of dorsal artery of foot, posterior tibial artery, popliteal artery and femoral artery, the vessels auscultation from the base of the neck, glycaemia, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin dose, electrocardiogram, cardiologic examination, ecocardiography and coronarography at the indication of the cardiologist, Eco Doppler vascular, neurologic examination, CT and RMN at the indication of the neurologist.

RESULTS AND DISCUSSIONS

From the 44 patients included in the study, 14 patients (31,81%) were female and 30 patients (68,19) were male.

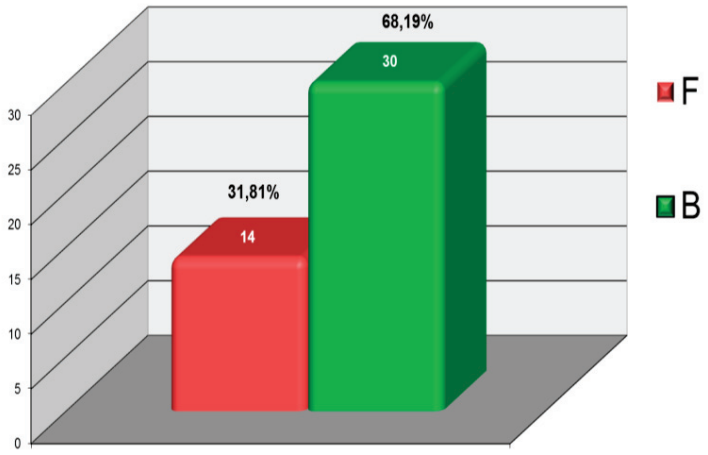

Figure 1. Distribution by gender of studied patients.

Concerning the age of patients, 2 patients (4,54%) were between 30-40 years, 12 patients (27,27%) were between 41-50 years, 15 (34,09%) patients were between 51-60 years and 15 patients (34,09%) over 60 years.

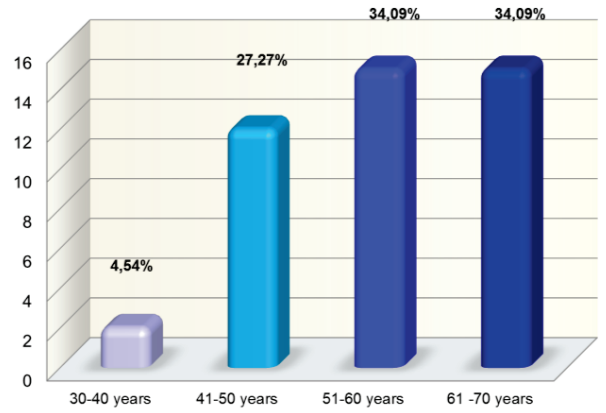

Figure 2. Age decades of studied patients.

After 25 years of evolution diseases only one pacient (2,27%) presented denutrition, 15 pacients (34,09%) normal weight, 20 patients (45,45%) overweight and 8 patients (18,18%) presented different degrees of obesity.

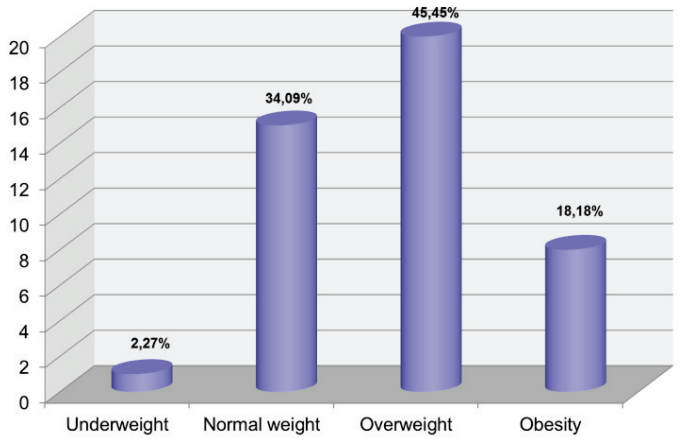

Figure 3. Nutritional status of studied patients

Studing the association between nutrition status and macrovascular complications, arterial hypertension and dyslipidemia we obtained the following dates:

Table 1

Nutrition status and macrovascular complications, arterial hypertension and dyslipidemia

	Arterial hypertension	Dyslipidemia	Chronic ischemic heart disease	Stoke	Diabetic obliterant artheriopathy
Normal weight (15 patients)	5 (33,33%)	6 (40%)	5 (33,33%)	1 (6,66%)	8 (53,33%)
Overweight (20 patients)	9 (45%)	9 (45%)	8 (40%)	2 (10%)	6 (30%)
Obesity (8 patients)	7 (87,5%)	8 (100%)	6 (75%)	1 (12,5%)	3 (37,5%)

Drawing a parallel between insulin doses and patients nutrition status results a mean of insulin necessary wich is 36,06 UI in normal patients, 50,25 UI in overweight patient and 58,37 UI in obese patients.

CONCLUSIONS

Overweight was met with a big frequence in patients with T1DM after 25 years of evolution, obesity was also met, but in a smaller percentage.

Evaluating the corelation between nutrition status with the association of arterial hypertension, dyslipidemia, chronic ischemic heart disease we observed a big frequence in overweight and obese patients.

Regarding stroke and diabetic obliterant arteriopathy we didn't find important differences between normal, overweight and obese patients.

Insulin necessary grows with BMI value.

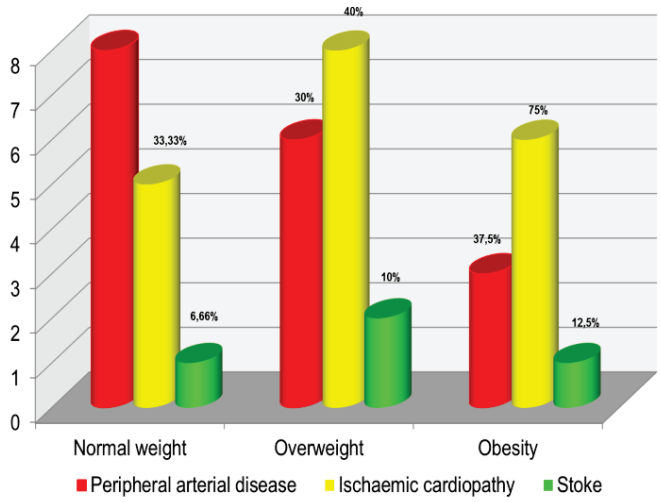

Figure 4. Corelation between nutritional status and diabetes macrovascular complications.

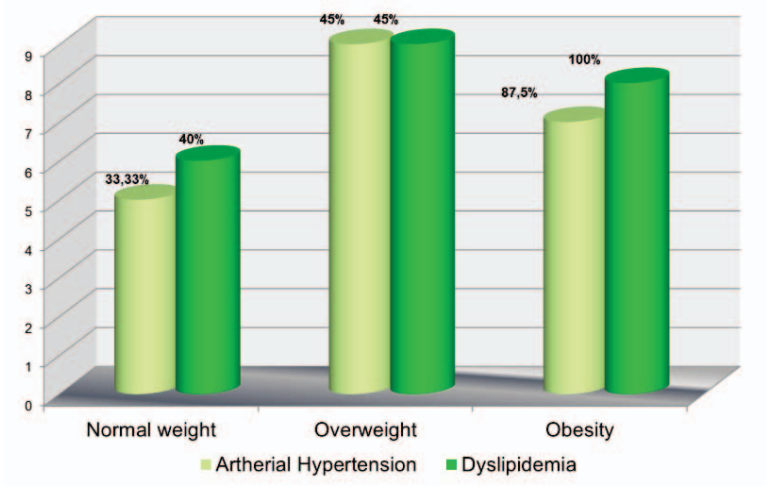

Figure 5. Correlation between nutritional status and the presence of hypertension.and dyslipidaemia in type 1 diabetes mellitus.

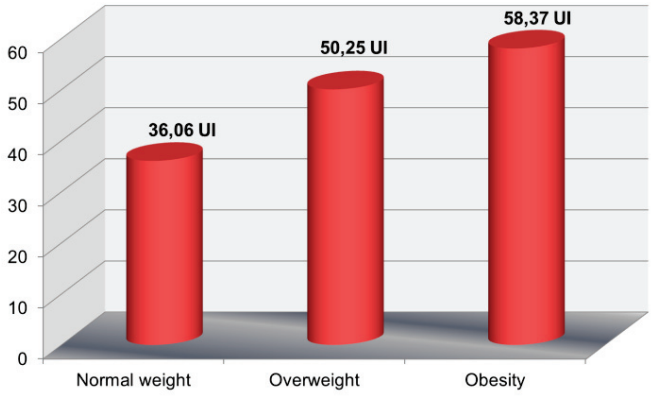

Figure 6. The correlation between insulin dose and nutritional status.

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All the authors contributed equally to the study and they have the same rights.

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MONITORING THE MINING MOTH OF CHESTNUT CAMERARIA OHRIDELLA DESCHKA-DIMIĆ IN THE GREEN AREAS OF CARACAL

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Keywords: *Cameraria ohridella* Deschka-Dimić, *monitoring*

ABSTRACT

In our country's fauna,numerous animal species including insect species have entered accidentally due to climate change, migration. They have adapted to climatic conditions of our country. Current researches are based on studying new species due to their unforeseen development with serious ecological, biological, economic and social repercussions. Humanity begins to be aware of keeping under control the invasive species.

INTRODUCTION

Green areas provide relaxation and health sources answering the needs of relaxation and recreation of modern man who is more and connected to urban centers (Purcelean and Cocalcu 1969).

Ornamental species found in the green areas have a series of pests and disease. In our country, in 1996 has been identified a new mining pest – chestnut moth - *Cameraria ohridella* Deschka-Dimić. During the last 4 years this species spread rapidly in the south of the country and causes protection problem to the ornamental, wild or horse chestnut - *Aesculus hippocastanum* L., as a decorative element in parks, on sidewalks and mainly around green areas in urban centers (Oltean 2005, Șandru 1998; Racosy 1999; Perju and Oltean 2002; Bădescu 2003).

Damages caused to the chestnut trees were investigated by other authors a well (Paschorn-Walcihek 1994) and in our country (Vișoiu and Poșta 2000).

Thus, the biocenotic balance of ornamental chestnut was disturbed by the larvae that feed on the parenchyma of the leaf epidermis (Oltean et al. 2006).

Because it produces premature defoliation and creates problems of protection it is necessary to know the morphological characteristics, particularities of biological cycle, biology, ecology, attack method.

MATERIALS AND METHODS

In 2016 across Central Park of Caracal, Olt country surveys were conducted to monitor the species *Cameraria ohridella* Deschka-Dimić, where this pest was reported and identified by the characteristics of mines formed.

Researches were conducted over several trees where the presence of this pest was reported.

To monitor the population of horse-chestnut leaf miner (*Cameraria ohridella* Deschka-Dimić) pheromone traps were used with sexual attractant 37-atraCAM (figure.1), synthesized by the Research Institute ”Raluca Ripan” Cluj Napoca, since 1998 (Oltean et al., 2006).

Monitored chestnut trees were selected from four different areas in the park, we placed three traps on each tree, pheromone bait being changed every six weeks.

a b

Figure 1: a. Pheromone trap with sexual attractant pheromone (orig.)
b. Detailed trap for adults (orig.)

To follow the biological cycle of the species,we took 10 leaves from each monitored point from the first half of May till September.The leaves were examined to establish the species stages of development and to determine the state of the biological stage: larva , pupa and adult. (figure. 2)

Figure 2. Leaves gathered to establish the stages of development (orig.).

RESULTS AND DISCUSSIONS

Since May of 2016 a series of observations and measurements have been organized with the purpose to elucidate some aspects of monitoring, biology, ecology and control the species *Cameraria ohridella* Deschka-Dimić ,in green areas of Caracal.

To determine the flight dynamics of adult *Cameraria ohridella* Deschka-Dimić, a number of 9 pheromone bait traps were installed on April 29, 2016, before the first flight of the hibernating generation (figure. 3).

Figure 3. Pheromone trap installed in the ornamental chestnut (orig.).

Traps reading was performed weekly. Pheromone bait capsule was changed every six weeks, this operation was performed on: June 19 th, July 30 th, September 10 th. The first flight of hibernating adult generation in 2016 in Caracal occurred on May 5 th, when the first shots were obtained in the pheromone traps. (Table 1).

Table 1

Number of adults (male) *Cameraria ohridella* Deschka-Dimić caught with specific sexual pheromone attractant traps 37-atraCAM, (Caracal 2016)

Hibernated generation		1st Generation		2nd Generation		3 Generation	
Period	No.of insects	Period	No.of insects	Period	No.of insects	Period	Nr.of insects
5.05-8.05	104	6.06-12.06	361	15.07-24.07	601	28.08-4.09	288
9.05-15.05	81	13.06-9.06	401	25.07-31.07	566	5.09-11.09	154
16.05-22.04	37	20.06-6.06	64	1.08-7.08	484	12.09-18.09	96
23.05-29.05	102	27.06-3.07	40	8.08-14.08	700		
30.05-5.06	81	04.07-0.07	8	15.08-21.08	170		
		11.07-4.07	54	22.08-27.08	316		
Total/ Generation 2.05-5.06	405	6.06-14.07	928	15.07-27.08	2837	28.08-18.09	535

Hibernating generation adults' flight was took place during a period of five weeks, including the end of April - the first decade of July, the entire period 405 adults were seized, representing an average of 50,6 adults/traps.

First generation adults' flight took place for a period of six weeks, when 928 adults were captured, average number of catches performed by a trap is of 116 adults/trap.

Second generation adults' flight took place for a period of 6 weeks and 2837 adults were captured, the flight of the third generation took place for a period of 3 weeks and there was the lowest number of 535 adults captured.

CONCLUSIONS

Cameraria ohridella Deschka-Dimić is a pest of ornamental chestnut trees in the area of Caracal park. They used working methods of counting adults using pheromone traps with specific sexual pheromone 37-atraCAM to track their biological cycle.

The number of adults and development stages were influenced by climatic variations registered in 2016.

In 2016 the maximum number of adults captured was recorded in the second generation (2837 copies).

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EVALUATION OF THE ATTACK OF THE PATHOGEN *XANTHOMONAS CAMPESTRIS PV. JUGLANDIS (PIERCE) DYE.* AT CERTAIN GENOTYPES OF WALNUT TREE IN VÂLCEA AREA

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Keywords: *walnut tree, bacteria, attack, selection, variety*

ABSTRACT

In 2015, at the University of Craiova - Research and Development Station for Fruit Growing Vâlcea, there were conducted research studies on the reaction of some autochthonous and foreign varieties and selections of walnut tree to the attack of the pathogen Xanthomonas campestris pv. juglandis (Pierce) Dye., responsible for bacterial blight. The research aimed to establish the degree of attack (DA%) of the pathogen at each genotype according to the evolution of the climatic factors that induce variations in the evolution of the disease.The studies were conducted under conditions of natural infection, in two different periods (June and September), at the level of leaves and fruits, at same fruit trees (three repetitions per tree).

INTRODUCTION

Xanthomonas campestris pv. juglandis (Pierce Dye, 1978) synonymous *Xanthomonas juglandis* (Pierce) Dowson, later called *Xanthomonas arboricola pv. juglandis* Vauterin 1995 and Moragrega 2012; Dye, 1980 is the pathogen that limits walnut world production up to 50-60% depending on the variety and environmental conditions (Payne and Johnson 1981 and Garden 1986).

Mulrean and Schroth 1982 state that the strongest attacks occur in areas with high temperatures and numerous rainfalls, while Belisario 1997 reported that this disease was identified in many countries.

Hajri 2012, on the basis of molecular analysis undertaken using AFLP, established that walnut bacteriosis is determined by two different strains: vertical oozing canker (VOC) and apical necrosis (WB). Pereira 2015 announced that the complete genome of the bacterium was isolated and revealed, and Vauterin 1995 reclassified the genus *Xanthomonas* based on DNA studies of 183 races.

In 2001, Scortichini revealed 61 species obtained from plants from different collections. Shami 2013 specifies that there is a whole variety of *Xanthomonas arboricola juglandis* within the territory of Iran, but they found many similarities among them.

Xanthomonas campestris pv. juglandis is an anaerobic, gram negative, mesophilic, with more than 20 pathovars bacteria (Mazzagalia 2013). It appears in the shape of a cane, 1.5 - 3μ long and 0.3 - 5 μ thick and it does not form spores

(Baicu and Săvescu 1978). The infections are favoured by wet weather and occur by stomatically or through injuries. In spring, primary infections occur when temperature reaches 12-14°C and the duration of incubation is 10-12 days.

In this paper, we present the behaviour of 26 genotypes of walnut tree: 6 selections (3 of autochthonous origin and 3 of foreign origin) and 20 varieties (9 of foreign origin and 11 of autochthonous origin) to the attack of the pathogen *Xanthomonas campestris pv. juglandis* (Pierce) Dye under the climatic conditions registered at the Research Station for Fruit Growing Vâlcea, in 2015.

MATERIAL AND METHOD

The experiment was made in a 20-year plantation where trees are planted at distances of 9 x 8m. The biological material was represented by 26 walnut genotypes (19 genotypes with terminal fructification and 7 genotypes with lateral fructification).

The observations were made under the conditions of natural infection in the field and it was followed the evolution of the attack of the pathogen *Xanthomonas campestris pv. juglandis (Pierce) Dye*, at the level of the leaves and fruits in two periods, respectively June and September. There were analysed 200 organs (leaves and fruits) for each genotype. The estimation of the attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* was made based on frequency (F%), intensity (I%) and degree of attack (DA%), according to the methodologies used in Forecasting and Warning Stations of the National Phytosanitary Network (M.A.I.A. - Forecasting and Warning Methods, 1980).

The attack frequency (F%) of a pathogen is the relative number of the attacked plants or plant organs reported to the total number of studied organs or plants. The frequency value is determined by direct observations on the total number of organs being calculated according to the following formula:

$F\% = \frac{n \times 100}{N}$,

where: n= total number of attacked plants or organs; N= total number of studied plants or organs.

The attack intensity (I%) refers to the degree of disease of a plant or an organ.

Intensity was calculated based on the formula:

$I\% = \frac{i \times f}{n}$,

where: i - represents the grade or percentage of coverage in a scale from 0 to 6; f - represents the number of attack cases for each grade; n = total number of attack cases.

The degree of attack (DA %) represents the attack level at all the plants (attacked and non-attacked) within a certain surface or at all the organs of a plant. The degree of attack is rendered by the relation:

$DA\% = \frac{F\% \times I\%}{100}$,

where: F% - represents the attack frequency; I% - represents the attack intensity.

RESULTS AND DISCUSSIONS

Temperature, precipitation amount and relative humidity are factors the action of which directly impacts the evolution dynamics of any pathogen. The data presented in Table 1 illustrate the direct influence of the climatic conditions

registered in 2015 on the incidence of the attack of *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the 26 studied walnut genotypes. It is noticed that, in the respective year, the evolution of the climatic conditions was less favourable for the pathogen, because, during the periods of maximum vulnerability to infections, relative humidity was low on the background of increased monthly mean temperatures and reduced and unevenly distributed precipitation amounts.

As a result of the evolution of the climatic factors in the two periods (June and September), the attacks on the leaves and fruits have relatively low values regardless of the genotype and type of fructification (Tables 2, 3, 4, 5, 6, 7, 8 and 9).

From the data rendered in Table 2, which emphasize the attack on the leaves of certain autochthonous varieties and selections, it results that the most significant value of the degree of attack (DA%) was recorded in case of the selection HC O3 (2.07%) followed by the selection HC O2 (1.55%).

Regarding the pathogen attack on the leaves of foreign varieties and selections, as shown in Table 3, the attack values ranged between 0.00% in case of the selections J. Mandshurica and J. Sieboldiana and 2.04% at Payne variety.

The low values of the degree of attack are induced by its low frequency and intensity as a result of the reduced precipitation amount during May (12.44 mm). At the same time, it can be noticed that, in case of foreign selections and varieties where the attack on the leaves is present, the levels were slightly higher compared to those on the autochthonous ones.

Regarding the attack on the fruits of certain autochthonous genotypes in June, there were recorded values between 0.14% at Jupâneşti variety and 1.89% at HC O3 selection. It may be noticed that in case of the Romanian genotypes, at the first determination, the attack on the fruits is somewhat lower compared to that on the leaves. The response of certain foreign genotypes to the attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye* on the fruits in June is rendered in Table 5. It can be observed that, in case of fruits, there were recorded attack values between 0.00% (J. Mandshurica and J. Sieboldiana) and 2.53% at Payne variety, variety that also recorded the highest value of the attack on the leaves.

The values of the degree of attack (DA%) on the leaves recorded at the Romanian genotypes in September (Table 6) are low, ranging between 0.09% at Valrex variety and 2.59% at VL 102 H selection.

There can be also noticed that the selections HC O2 and HC O3, characterized by lateral fructification, registered somewhat higher values of the attack of 2.02%, respectively 1.96% compared to the rest of the genotypes, in case of which the values of the degree of attack were well below 1%. In case of the foreign genotypes (Table 7), the attack on the leaves in September reached the value of 2.59% at Payne variety, while other three varieties (Hartley, Adams 10, Vina) registered values higher than 1%.

The values of the degree of attack (DA%) on fruits registered in September (Tables 8 and 9) show that the evolution of the bacterium *Xanthomonas campestris pv. juglandis (Pierce) Dye.* was not favoured by the climatic conditions registered in the summer months of 2015, so that the attack was almost stopped.

At the Romanian genotypes (Table 8), the frequency of the attack on fruits (F%) had values ranging between 5.15% at Unival variety with terminal fructification and 23.6% at the selection HC O2 with lateral fructification.

Table 1

Climatic conditions registered at the Meteorological Station Rm. Vâlcea, in 2015

Year	Month	Monthly mean temperature °C	Precipitation (amount) mm	Relative humidity %
2015	January	1.1	36.5	80.3
	February	1.7	37.2	80.3
	March	6.4	49.6	71.6
	April	10.9	42.8	55.3
	May	17.2	12.4	70.0
	June	19.8	28.6	68.3
	July	24.0	36	58.4
	August	21.9	89.2	67.4
	September	18.2	139.4	76.4

Table 2

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the leaves of certain autochthonous varieties and selections of walnut tree in June

No.	Specification	Origin	Fructification type	June 2015		
				F %	I %	DA %
1	Germisara	Romania	Terminal	6.83	5.52	0.38
2	Jupânești	Romania	Terminal	2.91	2.75	0.08
3	Velnița	Romania	Terminal	5.41	4.39	0.23
4	Sibișel 44	Romania	Terminal	6.08	4.04	0.49
5	Valcor	Romania	Terminal	3.75	2.14	0.16
6	Valrex	Romania	Terminal	3.33	2.56	0.17
7	Valmit	Romania	Terminal	4.08	3.67	0.29
8	Valcris	Romania	Terminal	3.41	2.91	0.19
9	Unival	Romania	Terminal	2.91	3.86	0.22
10	Valstar	Romania	Terminal	2.50	4.01	0.20
11	HC O2	Romania	Lateral	14.16	14.64	2.07
12	HC O3	Romania	Lateral	15.50	10.04	1.55
13	Sarmis	Romania	Terminal	3.66	5.08	0.37
14	VL 102 H	Romania	Terminal	5.33	4.15	0.44

Table 3

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the leaves of certain foreign varieties and selections of walnut tree in June

No.	Specification	Origin	Fructification type	June 2015		
				F %	I %	GA %
1	Franquette	France	Terminal	4.50	4.06	0.18
2	J. Mandshurica	France	Terminal	0.00	0.00	0.00
3	J. Sieboldiana	France	Terminal	0.00	0.00	0.00
4	J. Nigra	France	Terminal	9.66	5.49	0.50
5	Hartley	USA	Lateral	11.00	7.30	0.80
6	Adams 10	USA	Terminal	11.50	6.70	0.77
7	Serr	USA	Lateral	13.66	6.60	0.90
8	Vina	USA	Lateral	14.66	6.18	0.90
9	Manregian	USA	Terminal	8.33	7.39	0.61
10	Pedro	USA	Lateral	11.50	6.49	0.74
11	Idaho	USA	Terminal	16.00	9.25	0.93
12	Payne	USA	Lateral	15.91	15.91	2.04

Table 4

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the fruits of certain autochthonous varieties and selections of walnut tree in June

No.	Specification	Origin	Fructification type	June 2015		
				F %	I %	GA %
1	Germisara	Romania	Terminal	4.83	1.82	0.17
2	Jupânești	Romania	Terminal	4.83	1.97	0.14
3	Velnița	Romania	Terminal	5.50	2.23	0.24
4	Sibișel 44	Romania	Terminal	4.83	2.36	0.22
5	Valcor	Romania	Terminal	4.33	1.72	0.14
6	Valrex	Romania	Terminal	5.00	1.77	0.17
7	Valmit	Romania	Terminal	4.33	2.09	0.18
8	Valcris	Romania	Terminal	4.50	2.11	0.18
9	Unival	Romania	Terminal	4.16	1.96	0.16
10	Valstar	Romania	Terminal	4.83	2.58	0.24
11	HC O2	Romania	Lateral	20.33	0.27	1.88
12	HC O3	Romania	Lateral	19.00	9.95	1.89
13	Sarmis	Romania	Terminal	4.50	2.20	0.19
14	VL 102 H	Romania	Terminal	6.50	3.76	0.24

Table 5

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the fruits of certain foreign varieties and selections of walnut tree in June

No.	Specification	Origin	Fructification type	June 2015		
				F %	I %	DA %
1	Franquette	France	Terminal	10.33	1.66	0.17
2	J. Mandshurica	France	Terminal	0.00	0.00	0.00
3	J. Sieboldiana	France	Terminal	0.00	0.00	0.00
4	J. Nigra	France	Terminal	9.33	4.58	0.42
5	Hartley	USA	Lateral	13.66	9.57	1.30
6	Adams 10	USA	Terminal	12.00	5.72	0.68
7	Serr	USA	Lateral	13.66	3.89	0.53
8	Vina	USA	Lateral	20.33	6.69	1.36
9	Manregian	USA	Terminal	11.00	3.63	0.61
10	Pedro	USA	Lateral	13.33	6.13	1.21
11	Idaho	USA	Terminal	10.00	7.03	0.70
12	Payne	USA	Lateral	19.00	11.35	2.53

Table 6

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the leaves of certain autochthonous varieties and selections of walnut tree in September

No.	Specification	Origin	Fructification type	September 2015		
				F %	I %	DA %
1	Germisara	Romania	Terminal	6.58	5.75	0.38
2	Jupânești	Romania	Terminal	3.25	3.30	0.10
3	Velnița	Romania	Terminal	6.25	4.10	0.25
4	Sibișel 44	Romania	Terminal	6.91	5.20	0.36
5	Valcor	Romania	Terminal	4.41	2.94	0.13
6	Valrex	Romania	Terminal	4.05	2.14	0.09

7	Valmit	Romania	Terminal	4.66	3.75	0.17
8	Valcris	Romania	Terminal	4.08	27.8	0.11
9	Unival	Romania	Terminal	3.75	2.32	0.09
10	Valstar	Romania	Terminal	3.16	4.42	0.14
11	HC O2	Romania	Lateral	15.50	13.04	2.02
12	HC O3	Romania	Lateral	16.50	11.92	1.96
13	Sarmis	Romania	Terminal	4.16	5.56	0.23
14	VL 102 H	Romania	Terminal	13.80	18.81	2.59

Table 7

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the leaves of certain foreign varieties and selections of walnut tree in September

No.	Specification	Origin	Fructification type	September 2015		
				F %	I %	DA %
1	Franquette	France	Terminal	5.66	4.16	0.29
2	J. Mandshurica	France	Terminal	0.00	0.00	0.00
3	J. Sieboldiana	France	Terminal	0.00	0.00	0.00
4	J. Nigra	France	Terminal	11.50	5.25	0.60
5	Hartley	USA	Lateral	12.16	11.62	1.41
6	Adams 10	USA	Terminal	8.66	8.99	1.78
7	Serr	USA	Lateral	15.16	6.95	0.05
8	Vina	USA	Lateral	16.33	11.17	1.82
9	Manregian	USA	Terminal	9.60	8.94	0.86
10	Pedro	USA	Lateral	12.80	7.41	0.94
11	Idaho	USA	Terminal	8.50	9.75	0.83
12	Payne	USA	Lateral	13.80	18.81	2.59

Table 8

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the fruits of certain autochthonous varieties and selections of walnut tree in September

No.	Specification	Origin	Fructification type	September 2015		
				F %	I %	DA %
1	Germisara	Romania	Terminal	6.65	1.66	0.22
2	Jupânești	Romania	Terminal	6.00	2.42	0.29
3	Velnița	Romania	Terminal	6.80	1.75	0.25
4	Sibișel 44	Romania	Terminal	7.65	1.99	0.30
5	Valcor	Romania	Terminal	5.30	2.25	0.25
6	Valrex	Romania	Terminal	6.00	1.65	0.15
7	Valmit	Romania	Terminal	5.30	2.34	0.24
8	Valcris	Romania	Terminal	6.30	2.03	0.25
9	Unival	Romania	Terminal	5.15	2.77	0.28
10	Valstar	Romania	Terminal	6.00	2.07	0.25
11	HC O2	Romania	Lateral	23.60	8.81	2.07
12	HC O3	Romania	Lateral	21.00	9.67	2.03
13	Sarmis	Romania	Terminal	6.50	2.88	0.37
14	VL 102 H	Romania	Terminal	7.50	3.69	0.55

Table 9

The attack produced by *Xanthomonas campestris pv. juglandis (Pierce) Dye.* on the fruits of certain foreign varieties and selections of walnut tree in September

No.	Specification	Origin	Fructification type	September 2015		
				F %	I %	DA %
1	Franquette	France	Terminal	13.00	3.34	0.43
2	J. Mandshurica	France	Terminal	0.00	0.00	0.00
3	J. Sieboldiana	France	Terminal	0.00	0.00	0.00
4	J. Nigra	France	Terminal	12.00	3.86	0.46
5	Hartley	USA	Lateral	16.00	3.93	0.62
6	Adams 10	USA	Terminal	14.00	3.29	0.46
7	Serr	USA	Lateral	16.00	6.48	1.03
8	Vina	USA	Lateral	23.00	5.07	1.16
9	Manregian	USA	Terminal	13.30	4.66	0.61
10	Pedro	USA	Lateral	16.00	6.50	1.04
11	Idaho	USA	Terminal	12.00	8.68	1.04
12	Payne	USA	Lateral	20.60	15.68	3.23

The attack intensity (I%) was between 1.65% at Valrex variety and 9.67% at the selection HC O3. The low values of the frequency and intensity of the attack led to low values of the degree of attack ranging between 0.15% at Valrex variety with terminal fructification and 2.07% at the selection HC O2.

Regarding the foreign genotypes (Table 9), it is observed the same trend, the values of the degree of attack (DA%) on the fruits varying between 0.00% at the selections J. Mandshurica and J. Sieboldiana and 3.23% at Payne variety characterized by lateral fructification.

Analysing the evolution of the attack of the pathogen *Xanthomonas campestris pv. juglandis (Pierce) Dye.*, under the climatic conditions of 2015, there were found insignificant differences between the studied autochthonous and foreign genotypes, however in favour of the autochthonous genotypes.

CONCLUSIONS

The climatic conditions of the year 2015 registered at the Research Station for Fruit Growing Vâlcea did not favour the evolution of the attack of the bacterium *Xanthomonas campestris pv. juglandis (Pierce) Dye.*, responsible for bacterial blight, one of the most serious diseases of the walnut tree.

The studied autochthonous genotypes behaved slightly different to the attack on the leaves and fruits at different periods, depending on the type of fructification.

The most resistant autochthonous genotype to the attack on the leaves in June was Valcor variety characterized by terminal growth, while in September, it was Jupâneşti variety with a degree of attack (GA%) of 0.10%.

The most resistant autochthonous genotype to the attack of the bacterial blight on the fruits was Jupâneşti variety with the lowest degree of attack both in June and in September.

The attack frequency and intensity of *Xanthomonas campestris pv. juglandis (Pierce) Dye*. on leaves and fruits, regardless of the determination moment, registered low values in case of foreign genotypes, as well.

The values of the degree of attack (DA%), at some foreign genotypes, were slightly higher than those registered at the autochthonous ones.

Among the foreign genotypes, we mention Hartley, Adams 10 and Payne varieties, which were characterized by a weaker reaction to the attack on the leaves and fruits.

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THE ROLE OF INFLAMMATION IN OBESITY

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ABSTRACT

Obesity is a chronic disease which is defined as the excessive accumulation of body fat, with adverse implications on the health status resulting in the decrease in life expectancy and/or the emergence of severe health disorders.

INTRODUCTION

Increased intra would increase the risk of morbidity for different diseases, fact established by numerous recent studies.

The consequences of obesity have visibility throughout the body, obese patients presenting an increased risk of developing a number of serious complications. Overall mortality increases with increasing body weight. The level of mortality is low for values of the BMI of between 18.5 and 25 kg/m^2. Instead, the relative risk of early mortality is double in both sexes when the BMI exceeds 30 kg/m^2 (Ahima et al. 2000, Becker et al. 2001, Blaj et al. 2002).

Mediators of inflammation are involved in physiological processes that obesity, especially abdominal causes an increased cardiovascular risk. Adipocytokines stimulates the hepatic synthesis of acute phase proteins (CRP, fibrinogen), visceral adipose tissue is most likely subclinical inflammation headquarters (Fauci et al. 2008, Kelly et al. 2001, Libby et al. 2007, Maggio et al. 2006).

In recent years epidemiological studies have confirmed that patients with elevated basal plasma levels of CRP are at increased risk of coronary heart disease and myocardial infarction (Fernandez-Real et al. 2003, Klein et al. 2008, Klein 2008).

Thus, CRP is a risk factor for coronary heart disease indirectly. hs-CRP is a strong predictor of future cardiovascular events and recommended AHA 2003 between inflammatory markers currently used, highly sensitive C-reactive protein is only approved for clinical use. Decision intervals are set for cardiovascular risk assessment as recommended by the CDC/AHA:

- <1 mg/l - low risk;
- 1 to 3 mg/l - moderate risk;

• > 3 mg/l - increased risk (Bahceci et al. 2004, Museteanu et al. 2009, Ridker 2003).

MATERIAL AND METHODS

The present study was conducted on 172 obese patients who were hospitalized in the Emergency County Hospital Craiova in the period January 2015-March 2016.

Dosage selective high C-reactive protein was performed in the laboratory with the help Synevo and Inorganic Analytical Chemistry Laboratory at the University of Craiova, Faculty of Science, Department of Chemistry.

latex immunoturbidimetric method

Benchmarks - <0.5 mg/dL.

The limit of detection - 0.0425 mg/dL (4.05 nmol/L)

Limitations and interference

Increases CRP is a nonspecific response to inflammation and infection. To assess the risk cardiac recommend using a different type of test - ultra-sensitive CRP ("high-sensitive CRP"), which can detect lower levels of CRP2, (Becker et al. 2001, Wendt et al. 2002).

Epidemiological studies carried out in recent years have confirmed that patients with elevated baseline plasma CRP levels have an increased risk of coronary heart disease and myocardial infarction.

Were divided into 3 groups according to the degree of obesity (obesity group 1 patients with grade 1, group 2 patients with obesity grade 2 and grade 3 obesity lot 3).

Statistical test used was the Student's t for highlighting the difference between the averages selective high levels of protein C in patients in the 3 groups, the ANOVA was used to compare averages between the three groups, obtaining a value of p = 0.0014 which showed that the average of the three groups differ in a statistically highly significant.

RESULTS AND DISCUSSIONS

Epidemiological studies in recent years have confirmed that patients with elevated baseline plasma CRP levels have an increased risk of coronary heart disease and myocardial infarction.

Prospective showed that C-reactive protein is an important predictor of cardiovascular events in healthy individuals not only but especially in patients with stable angina or acute coronary syndromes with or without ST segment-T (Eckel et al. 2002, Fernandez-Real et al. 2003). The level of protein C has been shown to be useful for risk stratification on recurrent ischaemia and death of patients with stable angina and unstable, those who have made percutaneous transluminal angioplasty, and for assessing the risk of myocardial infarction, stroke, peripheral arterial disease and sudden death (Klein 2008, Libby et al. 2007, Paveliu 2002).

If values > 10 mg/L should be considered non-cardiovascular cause. Distribution of hs CRP values on the lots is shown in Table 1.

Table1

Distribution of hs CRP values batch

hs CRP	Lot 1	Lot 2	Lot 3	Total
Nr	89	61	22	172
Minim	0.3	0.4	0.5	0.3
Maxim	8	8.5	7.8	8.5
Medie	2.39	3.38	4.00	2.95
Dev.std.	2.02	2.26	2.39	2.23
CV	84.48%	66.70%	59.79%	75.51%
Test Student	L1-L2	L1-L3	L2-L3	ANOVA
p	0.00567	0.00175	0.28478	0.00146

Table 2

Distribution of hs CRP values batch

hs CRP	<1	1-3	>3	Total
Lot 1	30	33	26	89
Lot 2	10	19	32	61
Lot 3	4	4	14	22
Total	44	56	72	172

• patients in group 1: 30 patients (33.71%) presented hs CRP <1 mg/L (low risk), 33 patients (37.08%) had hs CRP 1-3 mg/L (hazard moderate) and 26 patients had hs CRP> 3 mg/L;

• in group 2, 10 patients (16.39%) had low cardiovascular risk, 19 patients (31.15%) moderate risk and 32 patients (52.46%) increased cardiovascular risk;

• in group 3, 4 patients (18.18%) had low cardiovascular risk, 4 patients (18.18%) moderate risk and 14 patients (63.64%) increased cardiovascular risk.

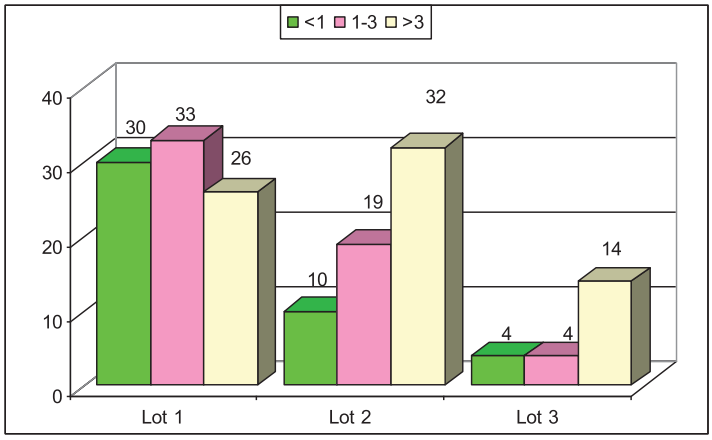

Fig. 1 Distribution of hs CRP values batch.

Hs-CRP correlation painful BCI

If the 60 patients with CAD painful included in the study were found the following (Table 3, Fig. 1):

- 2 patients (3.33%) presented hs CRP <1 mg/L (low risk);
- 17 patients (28.33%) had hs CRP 1-3 mg/L (moderate risk);
- 41 patients (68.33%) having hs CRP> 3 mg/L (high risk).

Table 3

The correlation between hs CRP levels and presence of painful BCI

hs CRP	<1	1-3	>3	Total
BCId+	2	17	41	60
BCId-	42	39	31	112
Total	44	56	72	172

Patients with CAD painless showed the following values of hs CRP (tab. 4, fig. 2):

- 4 patients (9.76%) with hs CRP <1mg/L (low risk);
- 14 patients (34.15%) with hs CRP 1-3 mg/L (moderate risk);
- 23 patients (56.10%) with hs CRP> 3 mg/L.

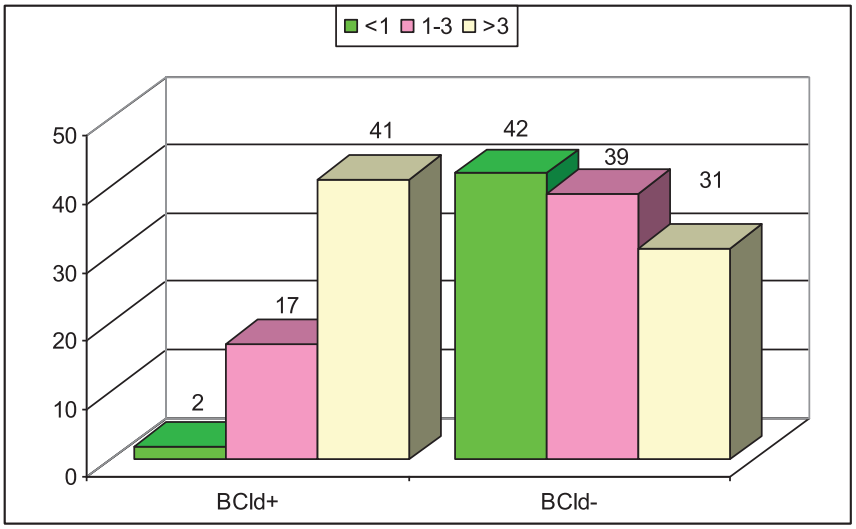

Fig.2. Correlation Level 2 prot C disease – coronary.

Table 4

BCI hs CRP level correlation painless

hs CRP	<1	1-3	>3	Total
BCId+	4	14	23	41
BCId-	40	42	49	131
Total	44	56	72	172

The prevalence of moderate risk/increased cardiovascular events increases significantly (p <0.001) with the degree of obesity and especially with the increase in CFA (Table 5).

Table 5

The relationship between the level of hs CRP and the degree of obesity

CFA	Obesity	hs CRP			
		<1	1-3	>3	Total
CFA 1	Grd. I	30	19	10	59
CFA 2	Grd. II	14	25	37	76
CFA 3	Grd.III	0	12	25	37
Total		44	56	72	172

CONCLUSIONS

Being the second cause of preventable death (after smoking), obesity is a major public health problem. The prevalence of obesity and overweight increased in almost all countries and age groups in the world and the economic cost of obesity is estimated that 2-7% of all health spending**.**

Given the fact that now, more than 1 billion people have excess body weight (overweight) and over 300 million are obese, over the next two decades the number could double, which would lead to a significant increase associated pathology and a shorter average lifespan of obese patients 8-10 years, I considered necessary to study obesity and etiopathogenic factors involved in the occurrence.

The prevalence of moderate risk/increased cardiovascular events (assessed by measuring hs CRP) increased significantly (p <0.001) with the degree of obesity (especially with increasing CFA) and its association with endocrine-metabolic changes.

Of the 89 patients with grade 1 obesity (group 1), 30 patients (33.71%) had had values of hs CRP <1 mg/L (low risk), 33 patients (37.08%) had values hs CRP 1-3 mg/L (moderate risk) and 26 patients had hs CRP> 3 mg/L;

Long-term objectives of prevention of cardiovascular diseases occurring in the context of obesity are reducing body weight and reducing the risk factors. To achieve this we advocate combining a diet low in fat, well dosed physical exercise and use of pharmacological preparations with as few side effects.

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PRL SECRETING PITUITARY TUMOR

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Keywords: *hyperprolactinemia, pituitary microadenoma, atypical clinical picture*

ABSTRACT

Case study on a patient 44 years, which raised atypical clinical diagnosis, differential diagnosis and treatment, which had numerous hospitalizations following which he confirmed the diagnosis of hyperprolactinemia.

INTRODUCTION

This paper studies the case of a patient of 44 years, residing in urban areas, hospitalized in the Endocrinology Clinic Emergency County Hospital Craiova. The patient is hospitalized for major weight gain, high leg edema, swelling of the hands bilateral dorsal face, amenorrhea.

The patient had a month ago in an inpatient department of internal diseases for the same symptoms. After carrying out a clinico-biological balance completely internal diseases department, was excluded etiology of liver, renal or cardiac edema, the patient being guided by the service of endocrinology.

In endocrinology clinic patient has conducted further investigations.

• The patient's medical history relevat - family history: hypertensive mother.

• Physiological personal history: menarche at 15 years, a birth.

• Bradimenoree patient had undergone treatment for that mcu injectable progesterone.

• At 25 years had a normal birth, then the cycle was irregular.

• At 30 years and has installed amenorrhea.

• Previous medical history are insignificant.

• Regarding working conditions, the patient says he worked 19 years in the Chemical Plant in Craiova toxic environment with the objective Cr.Exam revealed:

• Height 1.62 m

• 115 kg weight, BMI 44,5kg/m^2 (obesity grade 3)

• Paleness, cold

• Conjunctival tissue, excess fat in the mood gynoid, lower limb edema, bilateral and on the dorsal hands

• Pain in the lumbar spine

• Normal breathing apparatus
• Cardiovascular: blood pressure 110/70 mm Hg, AV 90 beats per minute, rhythmic heart sounds
• Digestive tract: liver to 2 cm below the lip
• Central nervous system: in normal
• Palpable thyroid

Laboratory investigations conducted revealed:
hypercholesterolemia (cholesterol 256 mg/dL)
hypertriglyceridemia (triglycerides 268.5 mg/dL)
prolactinemie important - micro IU Prolactin 124 200/mL (normal 127-637micro UI/mL), prolactin micro IU after PEG 109 654/mL.
Complete blood counts and biochemical tests were normal rest.
Hormonal dosages, except for prolactin, have been located in physiological limits, as follows:
TSH Micro 2.26/mL (0.27 to 4.2 micros normal/mL)
0.931 micro FSH (follicular phase normal 3.5 to 12.5 micro IU/mL, 1.7 to 7.7 micro UI luteal phase/mL, 25.8 to 134.8 micro IU menopause/mL)
hGH = 0.93 ng/mL (normal <10)
dehydroepiandrosterone sulfate (DHEA-S) = 228.1 microg/dL (normal 60.9 to 337 microg/dL)
17-OH progesterone 0.5 ng/ml (normal follicular phase 0.1 to 0.8 ng/mL, luteal phase 0.6 -2.3 ng/mL, 0.3 to 1.4 ng/mL ovulation, menopause from 0.13 to 0.51 ng/mL).
EKG- sinus rhythm without repolarization change.

MATERIAL AND METHOD

Dosage prolactin and other hormonal dosage was performed in the laboratory with the help Synevo and Inorganic Analytical Chemistry Laboratory at the University of Craiova, Faculty of Science, Department of Chemistry.
Method - Immunochemical with detection by electrochemiluminiscenta (plates) (ECLIA) (Prazers et al. 2003).
Reference values - are dependent on age and sex.

RESULTS AND DISCUSSIONS

Radiography heart-lung: no injuries evolutionary pleural lung, heart leveled.
Radiography of skull profiles: to Turkic showed a much larger, changing the contour-neregulat-, the bottom saddle is much lowered sphenoid sinuses, clinoidele previous hypertrophied, weathered, rear clinoidele reduced to scrap by bone osteolysis, sphenoid sinuses shrunk more.
MRI skull: highlights macronodul intrapituitara pituitary necrotic displacement, especially the left side, mass effect on the walls of the lodge saddlery, embedding left cavernous structures, pituitary stem oblique, right-hand moved to the midline, with symmetrical optic chiasm Figure 2 (Hanache et al. 2001).
Eye examination: showed a normal appearance of the fundus and normal visual acuity.

Figure 1.Radiografie of its Turkish.

Thyroid ultrasound - tiroida normal, the heterogeneous structure, fine nodular.

Following investigations carried out and the diagnosis of MACROPROLACTINOM established for that treatment with Dostinex 2 capsules 3 days; and spironolactone 2 capsules 2 times per day -6 weeks.

A-conducted clinical biological reassessment after 2 months of treatment, the disease having a slight improvement in terms of serum prolactin values slightly down from the previous determinations (115 600 IU micro/mL).

Due to the large volume of tumor, with possible evolution of the optic chiasm and consequently the risk of vision impairment, the patient is offered admission to hospital of neurosurgery Bagdasar Arseni to punish surgical tumor hipofizare.

The particular circumstances

- The reason that led the patient to the doctor was sudden and significant increase in weight, Musculoskeletal pain, peripheral edema and large (Brada et al. 2002, Molitch 2005).

• Atypical onset of disease evolution galactorrhea without hyperprolactinemia feature (Dorshkind et al. 2000, Pena et al. 2001).

• Significant increases in prolactin values over a thousand times higher than normal

• Rest hormonal dosage were within normal limits

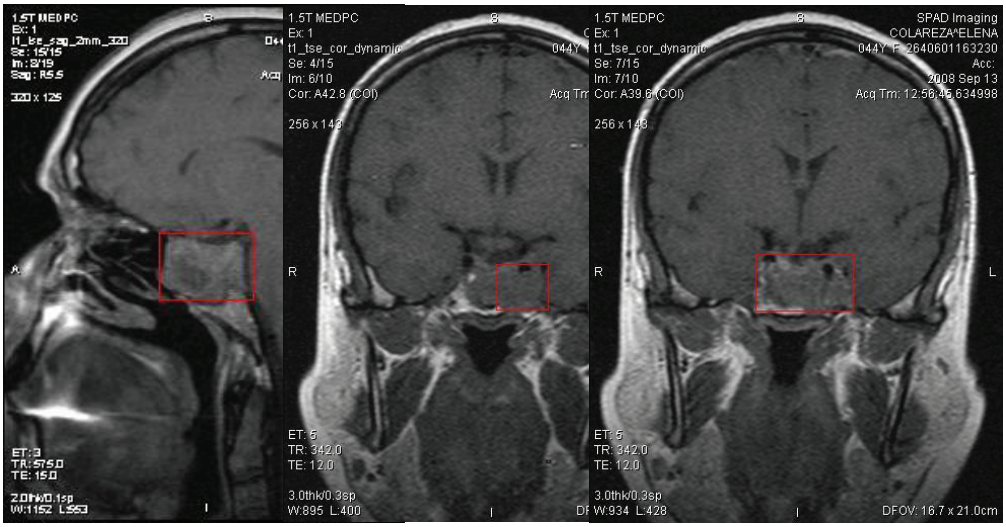

Figure 2. MRI's prolactinoma Images.

CONCLUSIONS

This case study was conducted because of atypical symptoms present at an adult woman without specific hormonal disorders.

Lack of specific galactorrhea hyperprolactinemia or lack of specific symptoms pituitary tumor presence, made the patient to be admitted to the service of rheumatology and internal diseases, with late diagnosis of endocrine pathology.

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FRUITS VARIABILITY OF *Setaria glauca* (L)P.B. WEED FROM THE ECOLOGY OF MAIZE CROP

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Keywords: S.glauca, variability, spike- ear, spikelets, maize

ABSTRACT

*Of annual monocots from maize crop, yellow foxtail experienced a pronounced adaptability. Here, every year the competition takes place in which maize can't express its productive potential. In determining weed management proved useful some degree of variability. They might be considered essential because the weed prove adaptation to various environmental conditions. In the study plant had spikes 7.6-9.0 cm long, they formed 201-250 spikelets who weighed 0.3-0.4 g, having absolute mass (thousand spikelet weight-TSW) 1.5-2.0 g. Spikelet had the upper glume 2.4-2.5 mm and down glume 1.4-1.5 mm. Spikelet was 2.8-2.9 mm long, 1.4-1.5 mm wide and the bristles 6-7 mm. The correlations obtained had increasing and positive trends. Were significant links between: spike length with spikelets weight (r = 0. 747***), between the number of spikelets with absolute spikelet weight (r = 0.307**), and between spikelet dimensions (length and width, r = 0.333***). Between length of the upper and the lower glume was positive but an-significant correlation (r = 0.182). The results showed that the weed has high capacity to adapt to maize crop ecology.*

INTRODUCTION

Species *Setaria glauca* (L)P.Beauv. [pro syn *S.pumila* (Poir)R. Et Sch, *S.lutescens* (Weig)EC Hibb, *Panicum glaucum* L, *P.pumilum* Poir ie, SETGL code Bayer, yellow foxtail] occur each year in spring crops (Chirilă 1998), including maize (Wang & Dekker 1995). The name comes from the plant of *Setaria* hairs (Păunescu 1996; 2000) that resembles the light autumn with silk (*seta*- silk). These hairs are between fertile branches bearing spikelets, around the ear, which gives the bristles of general appearance. The plant is annual (Anghel et al. 1975; Clayton et al. 1994; Kucera 1998), and requires relatively high temperatures for vegetation (Manoliu et al. 1996; Pensiero 1999). From an agricultural perspective, *S.glauca* has a high capacity to compete, especially its high density it expresses (Béres & Sarda 1999; Pensiero 2003). Twinning node seedlings quickly formed, with many shoots/tillers, then gradual warming of the environment, face-paced streching strains (Păunescu 2000). At the top of each stem form a spike panicle of different lengths (3-10 cm). Spike catch a variety of spikelets which is surrounded by red-yellowish bristles (Barkworth et al. 2003; Espejo Rerna et al. 2000). Denticulated thirst they were on the edge facing the top. An ear can form 60-180 kernels both on

the main stem and the tillers (Quattrocchi 2000; Wu et al. 2006). Spikelet has a glume on the domed top, 5 ribs and covers about ½ the length of the fruit (Păunescu 1996). Down glume is much smaller. Spikelet has 3.0-3.5 mm length, while caryopsis is 2.0-3.5 mm lung and 1.5-2.2 mm wide, being dressed in palea. *S.glauca* has the usual forms namely ploidy 2n = 36 and 2n = 72 being spread all over the world. But recently they found shapes with smaller sets of chormozones, 2n = 18 and 2n = 24.

In this study was obtained variability of the main characters of spike: absolute length, number of spikelets/spike, spikelets weight/spike, thousand spikelets weight (TSW), upper glume length, down glume length, spikelet length, spikelet width, and bristles length.

MATERIALS AND METHODS

Measurements were made in late August, the last three years, on the *S.glauca* plants. They were randomly chosen areas under maize in the Southern Highlands. From infested areas have been harvested 100 strains of *S.glauca* after zig-zag method with a stop every 20-25 metres. Thus, spike were cut, was placed one by one separately in the bag and then were brought to the laboratory. Measurements and determinations of spike included: absolute length, number of spikelets/ spike, spikelets weight/spike and thousand spikelets weight (TSW). For spikelets were determined: the length of the upper and lower glume, width and length of spikelet, the bristles length. Expression of variability of analyzed characters was done with polygon frequency (histogram). Evolution of a histogram values established by drawing class intervals, or as absolute values as such. The histogram of specific character od each weed revealed the modal value (higher frequency) and variation limits concerned. Further, between the main characters were established some correlations considered important. Such correlations may be important in developments of these characters by observing the trend in the studied ecotype. In drawing graphs was using Excel program. Further, given the multiplicity of measurements performed statistical processing was done to them. It used analysis of variance- Anova test. Absolute values were considered as strings processed change. The indices calculated were: media ($\bar{a} = \frac{\Sigma x}{n}$), variance ($s^2 = \frac{1}{n-1}\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]$), standard error ($s = \sqrt{S^2}$) and variation coefficient ($s\ \% = \frac{S}{\bar{a}} . 100$).

RESULTS AND DISCUSSIONS

Variability of spike characters. A *S.glauca* normal ear is between 3 and 10 cm (often less than 3 cm and more than 12 cm). Its shape is cylindrical, compact, with numerous spikelets with red-brown or yellow bristles (Fairbrothers, 1979). Ear length of weed from maize crop was between 3.1 and 12.0 cm. Its frequency was different and specific (fig. 1).

Thus the largest amplitude had a 7.6-9.0 cm of ear lengths (35%). This was followed by 6.1-7.5 cm ears long (25%) and those with 9.1-10.5 cm (20%). Reduced frequency was of 3.1-4.5 cm (3%) and 10.6-12.0 cm (2%). Number of spikelets/ear is variable, by some authors of 60-180 pieces. The mesurements showed that the ears were formed between 50 and 350 spikelets. The highest frequency have ears to 201-250 spikelets/ear (34%)., followed by 251-300 (23%) and those with 151-200 (21%).

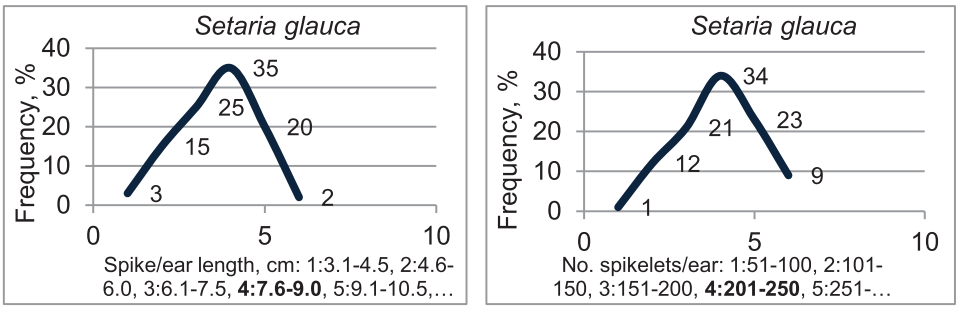

Fig. 1. Frequency evolution of ears length (left) and of spikelets no./ear

S. glauca ear showed that it can form a lot diversity of spikelets number. Their weight from the ear was between 0.1 and 0.8 g (fig. 2). Variability of spikelets weight was great and described a specific curve. The highest frequency had for ears with 0.3-0.4 g (27%). These were followed by those with 0.4-0.5 g (22%). 1% of spikes had values below 0.1 g and 3% had ears with 0.7-0.8 g. The absolute mass (TSW) of these spikelets followed a more uniform distribution. TSW values were scored between 1.0 1nd 3.5 g. They dominated at range 1.5 to 2.0 g. 3% 0.5-1.0 g values were, and 1% were between 3.0 and 3.5 g TSW.

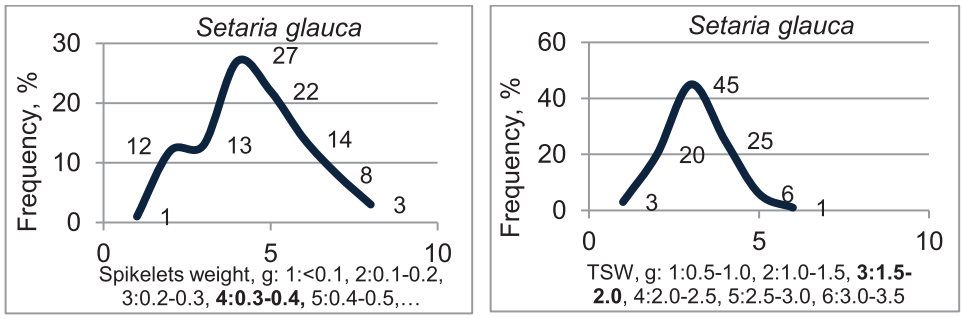

Fig. 2. Frequency evolution of spikelets weight (left)
and TSW- thousand spikelets weight

Variability of spikelets characters. Spikelets are an important component in the composition of *S.glauca* ears. They are oval-eliptic, thick convex plane is covered with glume and lemma (Kucera, 1998). The upper glume covers ½ of the ordinary. Spikelet was measured the length of the two glume (fig. 3).

Upper glume was of 1.6-2.7 mm lengths. The highest frequency had a 2.4-2.5 mm (43%), followed by those with 2.2-2.3 mm (24%) and 2.0-2.1 mm (18%). Lower glume (lemma) measured between 1.0 and 2.3 mm. Greater frequency had spikelets with 1.4-1.5 mm (32%). They followed those with 1.6-1.7 mm (25%) and 1.8-1.9 mm (16%).

Spikelets of *S.glauca* have different dimensions. Their length may be around 3 mm, usually between 3.0-3.5 mm. From the measurements was obtained values between 2.0 and 3.1 mm (fig. 4). Higher frequency had of 2.8-2.9 mm spikelets (42%), followed by one with 3.0-3.1 mm (25%). The short acconted for smaller shares. Spikelets width was between 1.0 and 2.1 mm. Most spikelets had

a thickness of 1.4-1.5 mm (46%), and those with 1.2-1.3 mm (25%) and 1.6-1.7 mm (17%). Bristles that accompany the entire length of ears, have certain lengths. The data shows the length between 5 and 9 mm (fig. 5). Have dominated the 6 mm (35%), followed by the 7 mm (31%) and 8 mm (22%). Bristles than 5 mm was 11%, and the 9 mm, 1%.

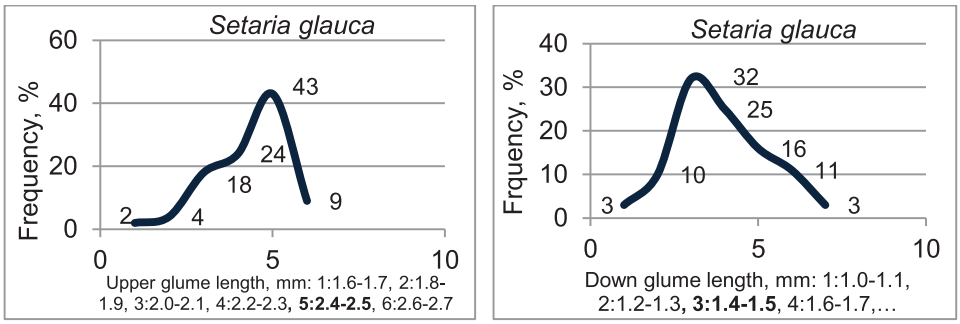

Fig. 3. Frequency evolution of glume length (left) and glumes width (lemma)

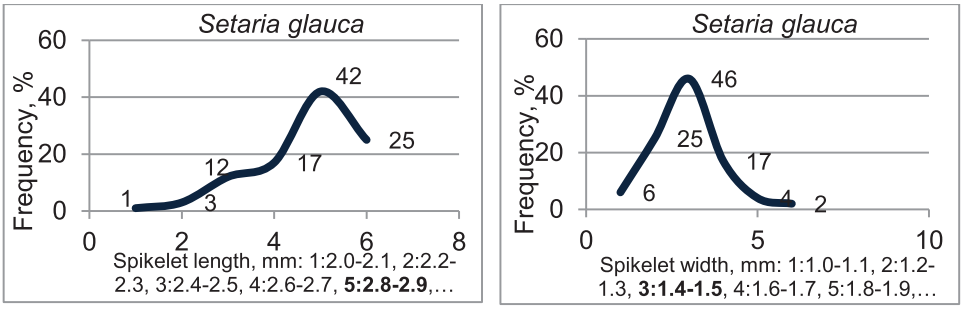

Fig. 4. Frequency evolution of spikelet length (left) and spikelet width

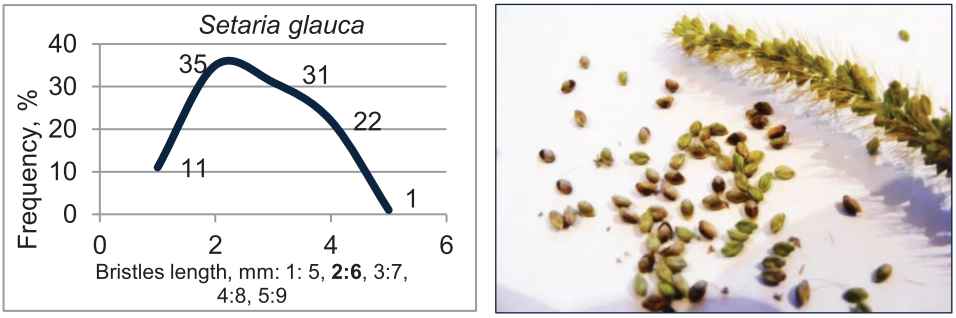

Fig. 5. Frequency evolution of bristles length.
Photo: ear, spikelets and bristles of *S.glauca*

Correlations between the main characters. Overall, positive correlations were obtained with different growth trends. Thus, between ear length and spikelets weight/ear it was very obvious correlation (r = 0.747***) (fig. 6). If the ear was longer, the number of spikelets formed is higher. The relationship between the

number of spikelets/ear and spikelets TSW was distinctly positive significant (r = 0.307**). The correlation shows that it is not mandatory to have more spikelets with more TSW.

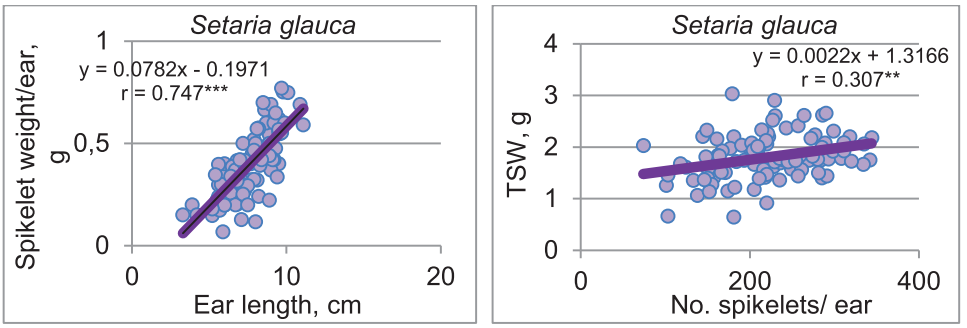

Fig. 6. Correlations between ear length with spikelets weight (left) and between spikelets number and TSW, *S.glauca* weed

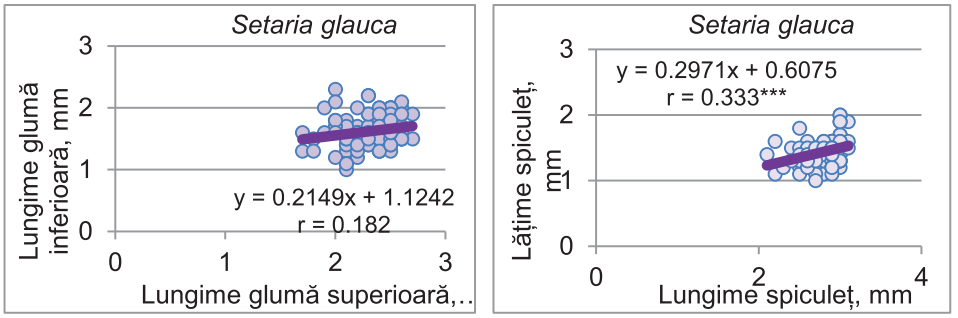

Fig. 7. Correlations between of upper glume with down glume (left) and between spikelets length with spikelet width, *S.glauca* weed

The relationship between the length of upper and lower glume was positive, but slightly upwards (r = 0.182) (fig. 7). It is possible that the two parts of *S.glauca* spikelets there isn't a certain affinity and dependence respectively. Spikelets dimensions: length and thickness showed a positive relationship, very close (r = 0.333***). Spikelets were longer and more widthness. These dimensions of the *S.glauca* spikelets could increase by the maize crop conditions like level of technology.

Statistical analysis of the variability of S.glauca fruits. Were calculated for each character analyzed media (a), variance (s^2), standard error (s) and coefficient of variation (CV,%). Historical estimates made have highlighted the characteristic values of species S.glauca grown in the maize crop. The values obtained were characteristic. Thus the ear average was 7.73 cm length, 221 spikelets/ear, 0.41 g their weight/ear, and 1.8 g of TSW (table 1).

Spikelets of S.glauca had average values of 2.3 mm length for upper glume, 1.6 mm for lower glume, 2.8 mm of spikelet length, 1.4 mm for spikelet width, and 6.7 mm for bristle length (table 2).

Table 1

Statistical indices of *S.glauca* ears

Indices	Length of ear, cm	No. of spikelets	Weight of spikelets, g	TSW, g
Media, ā	**7.73**	**221.4**	**0.4071**	**1.803**
Variance, s^2	2.382	3678	0.0261	0.1876
Std. error, s	1.543	60.65	0.1615	0.4331
Coef. var. %	**19.97**	**27.39**	**39.67**	**24.02**

Table 2

Statistical indices of *S.glauca* spikelets

Indices	Length of upper glume, mm	Length of lower glume, mm	Length of spikelet, mm	Width of spikelet, mm	Length of bristle, mm
Media, ā	**2.306**	**1.622**	**2.795**	**1.438**	**6.67**
Variance, s^2	0.0032	0.0698	0.0465	0.0371	0.951
Std.error, s	0.0565	0.2642	0.2157	0.1927	0.975
Coef. var. %	**2.45**	**16.29**	**7.72**	**13.40**	**14.62**

Other aspects of S.glauca fruit variability. Data obtained could express the specific way in which weed has adapted Southern ecology of maize crop. The mean and variability of the characters studied, it can be said that are characteristic. The ear length was 3.3- 11.1 cm, bristles length 5- 8 mm, the number of spikelets/ ear 74- 344 (table 3). Spikelet upper glume length of 1.0- 2.3 mm, the spikelet length of 2.1- 3.1 mm, the spikelet width of 1.0- 2.0 mm and the weight of spikelets/ ear of 0.127- 0.770 g. Thousand of spikelets weight was 1.065- 2.904 g.

Table 3

Values of fruits characters variability for *Setaria glauca (L)P.Beauv.* weed

No.	Fruits characters	Literature	Determinations
1.	Ear length, cm	-	3.3 – 11.1
2.	Bristles length, mm	-	5 - 8
3.	No. of spikelets/ ear	60 - 180	74 - 344
4.	Upper glume length, mm	-	1.7 – 2.7
5.	Lower glume length, mm	-	1.0 – 2.3
6.	Spikelet length, mm	3.0 – 3.5	2.1 – 3.1
7.	Spikelet width, mm	-	1.0 – 2.0
8.	Spikelet weight/ ear, g	-	0.127 – 0.770
9.	TSW- thousand spikelets weight, g	-	1.065 – 2.904

The coefficent of variation (CV%) was low for legth of upper glume (2.45%), length of spikelet (7.72%). The middle CV was for width of spikelet (13.40%), length of bristle (14.62%), Length of lower glume (16.29%) and length of ear (19.97%). Big CV was for TSW (24.02%), no. of spikelets/ ear (27.39%) and weight

of spikelets (39.67%). It notes that the diversity of *S.glauca* fruits characters dressed specific issues, possibly by new adapting on this maize crop in southern territory.

CONCLUSIONS

A common species that cause significant damage of crops is *S.glauca* (L)P.Beauv. The weed is widespread in the southern territory, because this ecotype may be well adapted by its special biology to the maize crop. Weed fruits characters have been less studied, especially when they are competing with a crop plant, like maize.

The data could be useful in developing appropriate management principles. In order to control through proper management is good to know as many characters. It was found that a species expressing widest variability may find it easier means of control and stop eventually. Studies of variation in species *S.glauca* refer to ear and spikelet of weed. The way how the characters have expressed variability could express a certain ecotype od the study area (table 4 and table 5).

We have had ears long enough, and bristles also longer. Another new determined characters were: spikelet dimensions 2.0- 3.1 mm length and 1.0- 2.1 mm width, no. of caryopsis/ spikelet 51- 350 pieces, spikelet weight/ ear <0.1- 0.8 g, and TSW 0.5- 3.5 g.

Table 4

Structure and analysis values of *S.glauca* ears

Ear length, cm	No. of spikelets	Spikelet weight/ear, g	TSW, g
3.1-4.5 3%	51-100 1%	<0.1 1%	0.5-1.0 3%
4.6-6.0 15%	101-150 12%	0.1-0.2 12%	1.0-1.5 20%
6.1-7.5 25%	151-200 21%	0.2-0.3 13%	**1.5-2.0 45%**
7.6-9.0 35%	**201-250 34%**	**0.3-0.4 27%**	2.0-2.5 25%
9.1-10.5 20%	251-300 23%	0.4-0.5 22%	2.5-3.0 6%
10.6-12.0 2%	301-350 9%	0.5-0.6 14%	3.0-3.5 1%
		0.6-0.7 8%	
		0.7-0.8 3%	

Table 5

Structure and analysis values of *S.glauca* spikelets

Upper glume length, mm	Down glume length, mm	Spikelet length, mm	Spikelet width, mm	Bristles length, mm
1.6-1.7 2%	1.0-1.1 3%	2.0-2.1 1%	1.0-1.1 6%	5 11%
1.8-1.9 4%	1.2-1.3 10%	2.2-2.3 3%	1.2-1.3 25%	**6 35%**
2.0-2.1 18%	**1.4-1.5 32%**	2.4-2.5 12%	**1.4-1.5 46%**	7 31%
2.2-2.3 24%	1.6-1.7 25%	2.6-2.7 17%	1.6-1.7 17%	8 22%
2.4-2.5 43%	1.8-1.9 16%	**2.8-2.9 42%**	1.8-1.9 4%	9 1%
2.6-2.7 9%	2.0-2.1 11%	3.0-3.1 25%	2.0-2.1 2%	
	2.2-2.3 3%			

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FRUITS DIVERSITY OF A NEW *Bromus secalinus* L. ECOTYPE FROM WHEAT CROP

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Keywords: *B.secalinus, diversity, fruits, winter wheat crop*

ABSTRACT

*Among the new species of weeds in wheat has spread Bromus secalinus L. (Hurlubert, 1955; Thompson et al. 1990). Weed harm the crop (Medlin et al. 2005), and so you can control, some studies of variability were needed. The way the plant grows here reflects the specific ecotype existence at a time or moment. From analysis and measurements showed that the panicle was 14-16 cm long, 7 nods of branches formed with a total of 31-38 spikelets. Kernels from a panicle weighted 2.5-3.0 g, and the weight of a thousand kernels (TKW) was 11-13 g. Spikelets had a length of 1.7-1.8 cm, on formed by 6 caryopses, and had upper glumes of 6 mm and the lower glumes 5 mm, with 5 mm aristae hair/ awn. An average of spikelet weighted 1.0-1.2 g. Between the different characters were established significant positive correlations: 0.415*** between panicle length and nr. of total spikelets from panicles, 0.847*** between spikelet length and no. of kernels, 0.659*** between total no of spikelets and kernels heavy from panicle, and 0.216* between panicle kernels weight and TKW. The present study demonstrated the wide possibilities that adapted this weed in winter wheat.*

INTRODUCTION

Species *B.secalinus* L. (pro syn *Serrafalcus secalinus* Bab, BROSE code Bayer, bromegrass, rye brome, chest or chess) has spread little time (Spalton, 2001) also in the South's white luvicsoils. The plant is an winter annual species (Chirilă, 2001), a port up to 100 cm in height surpassing wheat. The damage they cause are 28-48 % of 40-50 density per sq.m. (Prodan & Buia, 1958; Nikitin, 1983). Ecotype existing in the area could show some morphological characters that can express its adaptation to new culture conditions (Anghel et al. 1975). Adaptability can be observed by conducting studies of diversity (Schlichting, 1986). In general, the panicle lengths 8-17-21 cm is looking "open". Panicle has branches scattered knots, rising. Spikelets are almost compresses, hairless to puberule. The glumes are oval, the upper 5-8 mm length with 5-7 ribs, and lower glumes were 4.0-5.5 mm with 3-5 ribs. Spikelets length is 11-20 mm, with more flowers. Spikelets were 1-2 cm long and 6-10 mm wide, hairy and awns 1-5 mm in length, or may be missing. Each spikelet has more kernels. Mature beans are hulled deep, gooved and

section takes the form of the letter ”U”. The feed grains of *B.secalinus* are toxic (en.wikipedia.org).

Research conducted to establish the diversity of plant included: i)the panicle characters by length, number of nodes, number of spikelets, weight kernels and thousand grains mass (TGW), ii)the spikelet characters by length, number of kernels, length of upper glume and down glume, specific spikelet weight and awn length.

MATERIALS AND METHODS

Measurements were made in June (Gubanov et al. 2002) in the past 3 years on *B.secalinus* plants scattered in different areas with winter wheat crops, in the middle of Argeș County. They were chosen randomly among weed precincts, with 100 strains of *B.secalinus*. Each of stems were cut by panicle formed, after which they were brought to the laboratory. Measurements and determinations of panicle included: absolute length, number of nodes with branches, total spikelets, total weight of the kernels and TGW. For spikelets were measured length, number of kernels, glume length of the two (upper and lower), the total mass and length of awns.

The express the diversity of characters analyzed using an appropriate statistical method and frequency-specific polygon, or histogram (Ceapoiu 1968; Iosifescu et al. 1985). Evolution ranges of values established either by class or by absolute values as such. The specific of each character ecotype analyzed revealed the modal value (higher frequency) and variation limits concerned. Among the most important characters were established 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 variance analysis and the variation namely rows. 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 panicle characters. The literature shows that the *B.secalinus* panicle lengths of 8-21 cm, with nods and branches spread out and upward (Manoliu et al. 1996). Measurements showed that the new species ecotype had panicle length between 8 and 20.5 cm, but the frequency of such distribution was different lengths and specifications-figure 1 and photo 2. The highest frequency had a panicle 14-16 cm long (32 %), followed by those with 16 to 18 cm (24 %), and 12-14 cm (22 %). Short panicles, 8-10 cm were 3 % and those over 20 cm, 2 %.

The number of nodes in a panicle was between 5 and 8, and differ in their frequency distribution. The panicle with the highest frequency were those with 7 nods (49 %). However, 99 % of the panicles were between 6 and 8 nods. Number of spikelets from a panicle was variable-figure 2. Dominated spikelets were those of 31-38 pieces (48 %). Limit the number of spikelets in a panicle was between 15 and 57.

Kernels from a panicle showed different total weights i.e. between 1.50g and over 4.51 g. The highest frequency had between 2.51 and 3.00 g (29 %),

followed by those with 2.01-2.50 g (21 %) and 3.10-3.50 g (22 %). *B.secalinus* absolute kernels ranged between 7.3 and 19.7 g. The highest frequency had the kernels TGW with 11-13 g-figure 3.

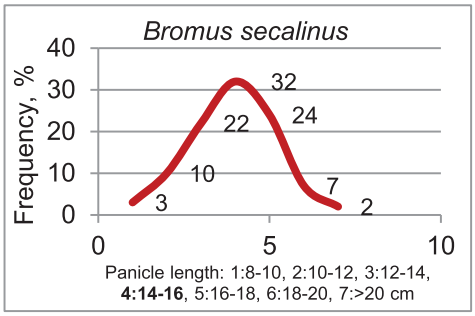
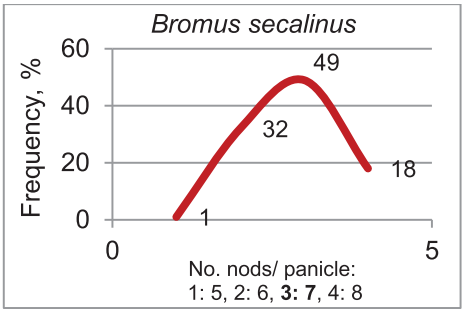


Figure 1. Frequency of *B.secalinus* panicle length.
Photo 1: *B.secalinus* from winter wheat crop (original).



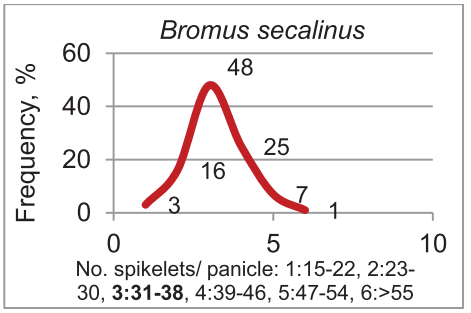
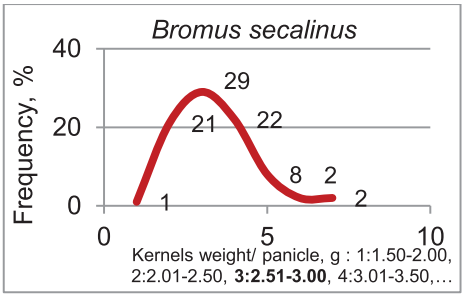


Figure 2. Evolution of nods number (left) and spikelets number from the panicle.



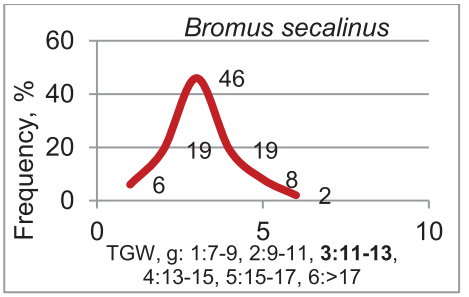


Figure 3. Evolution of caryopsis weigth from a panicle (left) and TGW of *B.secalinus.*

Diversity of spikelets characters. From what is known, it has spikelet 1.1-2.0 cm length, with glumes 5-8 mm the upper and 4.0-5.5 mm the lower. The awns have 1-5 mm, and the number of grains contained between 5 and 15. The determinations showed that the *B.secalinus* spikelets had lengths between 1.3 and 2.3 cm. Greater frequency had a spikelet of 1.7-1.8 cm length (40 %). Lengths of

1.5-1.6 cm were 28 %, and 1.9-2.0 cm 21 %. Spikelets weight fluctuated between 0.07 and 0.22 g while the modal value was 0.11-0.12 g (43 %)-figure 4.

Spikelet formed kernels between 4 and 10. The higher frequency has a spikelet with 6 kernels (37 %). Spikelets were very close to that contained 7 caryopses (30 %)-figure 5.

Spikelet glumes had a specific variability. Thus the upper glume have lengths of 5-8 mm, and the lower glume have between 4 and 6 mm. The higher frequency have been higher than 6 mm glume (69 %), and 5 mm below glume (53 %)-figure 6.

Awns of a spikelet can sometimes be missing or may have lengths of 1-5 mm long. The determinations made in the middle of spikelet, awns had between 1 and 7 mm long. The highest frequency had a 5 mm length of awns-figure 7.

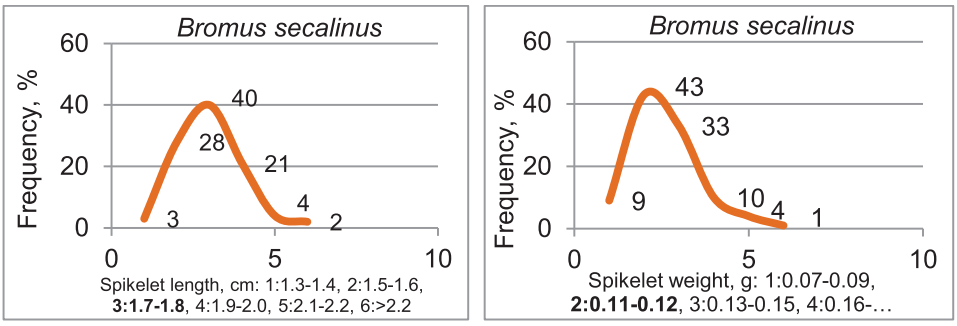


Figure 4. Evolution of spikelets length (left) and of spikelets weight/ panicle

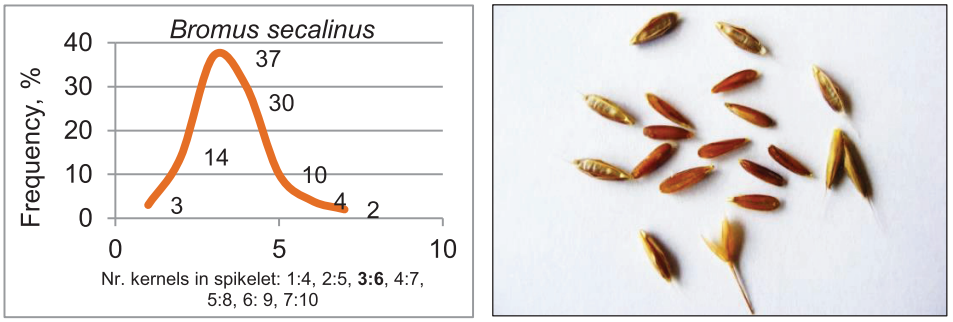


Figure 5. Evolution of caryopsis number from a spikelet.
Photo 3: caryopsis of *B.secalinus* (original)

Correlations between different characters. From the study of the correlation between the length of panicle and number of spikelets from panicle resulted in a favorable situation. The correlation coefficient, positive, had the r = 0.415***. The link between the length of spikelet and number of kernels from a spikelet was formed closer and feature r = 0.847***-figure 8. Both correlations show that the weed had a very good adaptability under consideration.

Between spikelets number from panicle and total caryopsis weight also achieved very positive and significant correlation (r = 0.659***). In another correlation between the weight of panicle and TGW achieved a positive correlation,

but with a lower insurance, r = 0.216*-figure 9. The explanation could be that the plant forms kernels, but depend more on their total weight than absolute.

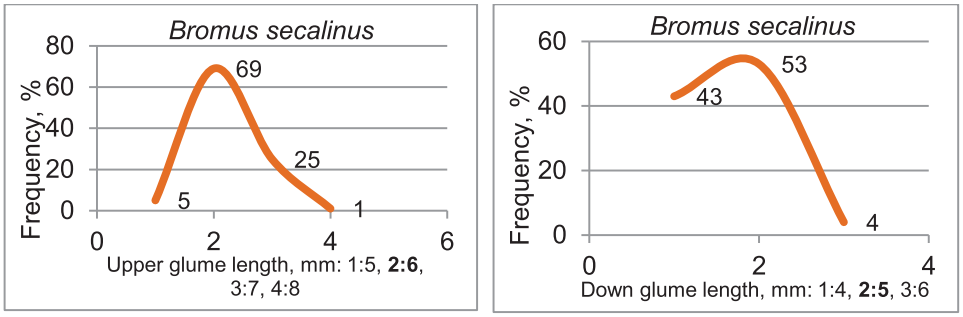


Figure 6. Evolution of upper glume length (left) and down glume length of *B.secalinus* spikelets.

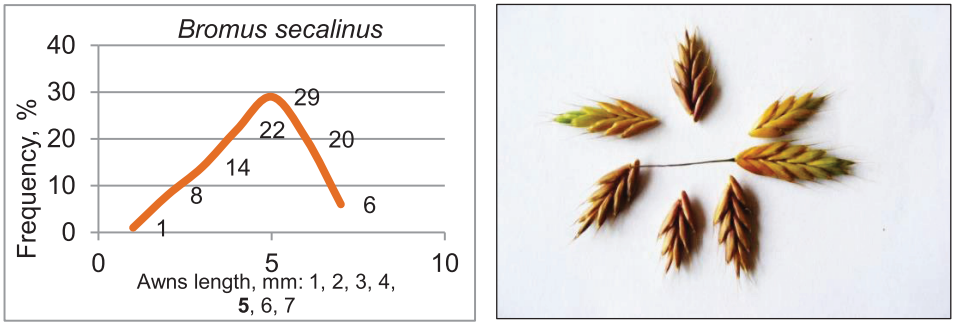


Figure 7. Evolution of awns length from *B.secalinus* spikelets.
Photo 4: awns of spikelets (original).

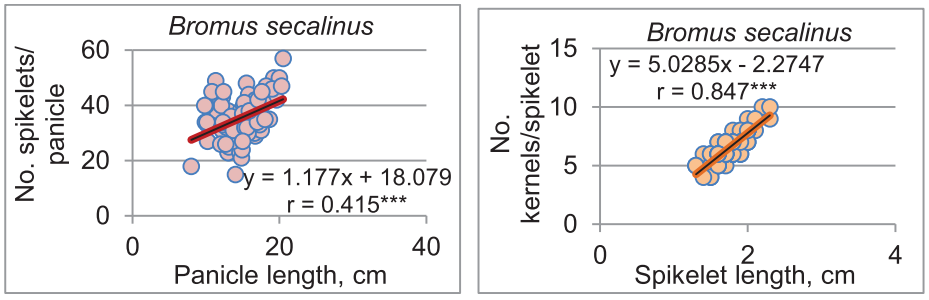


Figure 8. Correlation between the panicle length and spikelets number from panicle (left), and correlation between the spikelet length and caryopsis number from spikelets.

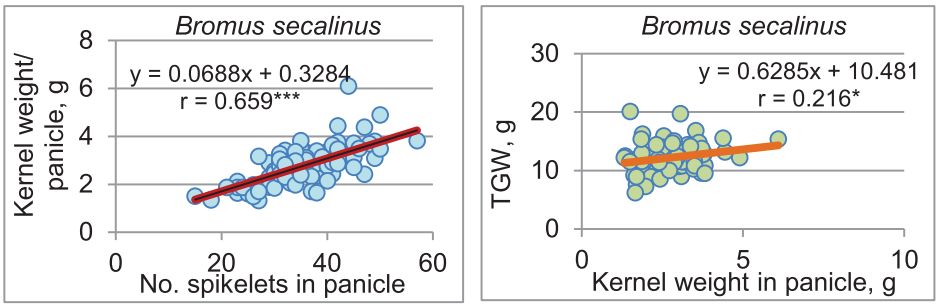


Figure 9. Correlation between spikelets number from panicle and caryopsis weight from panicle (left), and correlation between caryopsis weight from panicle and TGW.

Statistical indices of weed diversity. From the statistical values that have emerged have highlighted specific new *B.secalinus* ecotype that has spread to southern-table 1 and table 2. The length of panicle was14.9 cm and formed 6.8 nods and 35.6 spikelets. The total weight of kernels was 2.78 g, and TGW 12.23 g. Spikelet had a length of 1.75 cm, contained 6.56 kernels, had upper glume 6.2 mm, lower 4.6 mm and the spikelet weight was 0.1295 g.

Table 1

Statistic indices of *B.secalinus* panicles

Indices	Length, cm	No. of nods	No. of spikelets	Total caryopsis weight, g	TKW, g
Media, ā	**14.90**	**6.84**	**35.62**	**2.78**	**12.23**
Variance, s^2	6.67	0.52	53.57	0.58	4.96
Std. error, s	2.58	0.72	7.32	0.76	2.23
Var. coef, %	**17.32**	**10.54**	**20.55**	**27.50**	**18.21**

Table 2

Statistic indices of *B.secalinus* spikelets

Indices	Length, cm	No. of caryopsis	Upper glume length, mm	Down glume length, mm	Spikelet weight, g	Awn length, mm
Media, ā	**1.75**	**6.56**	**6.22**	**4.61**	**1.295**	**4.54**
Variance, s^2	0.04	0.61	0.79	0.32	796.7	1.91
Std. error, s	0.20	0.78	0.89	0.57	28.23	1.38
Var. coef, %	**11.44**	**11.93**	**14.29**	**12.29**	**21.18**	**30.42**

Other aspects of B.secalinus fruit diversity. Data obtained could express the specific way in which weed has adapted Southern ecology of winter wheat crop. The mean and variability of the characters studied, it can be said that are characteristic. The panicle length was 8.0-20.5 cm, no. of ramifications from panicle 5-8, no. spikelets/ panicle 15-57 (table 3). Spikelet length of 1.4-2.3 cm, no. caryopsis/ spikelet 4-10, upper glume length 5-8 mm, down glume length 4-6 mm, and spikelet weight of 0.07-0.22 g. Caryopsis weight/ panicle was 1.32-6.10 g, TKW-thousand kernels weight of 6.24-20.13 g, and awn length 1-7 mm.

Variation coefficient (VC) of these determinations showed to be small, middle to big values. Thus no. of nods had 10.54% VC, spikelet length 11.44%, no. of caryopsis 11.93%, down glume length 12.29% and upper glume length 14.29% were small VC. TKW with 18.21%, spikelet weight of 21.18% and no. of spikelets/ panicles of 20.55 were middle. Total caryopsis weight of 27.50% and awn length 30.42% were big.

Table 3

Values of fruits characters diversity for *B.secalinus L.* weed

No.	Fruits characters	Literature	Determinations
1.	Panicle length, cm	18 - 21	8.0 – 20.5
2.	No. ramifications/ paniclel	-	5 - 8
3.	No. spikelets/ panicle	-	15 - 57
4.	Spikelet length, cm	1.0 - 2.0	1.4 – 2.3
5.	Spikelet width, cm	0.6 - 1.0	-
6.	No. caryopsis/ spikelet	-	4 - 10
7.	Upper glume length, mm	5 - 8	5 - 8
8.	Down glume length, mm	4 - 5.5	4 - 6
9.	Spikelet weight, g	-	0.07 - 0.22
10.	Caryopsis weight/ panicle, g	-	1.32 - 6.10
11.	TKW- thousand kernels weight, g		6.24 - 20.13
12.	Awn length, mm	1 - 5	1 - 7

CONCLUSIONS

A new species that cause significant damage of winter crops is *B.secalinus*. The weed is widespread in the southern territory, because this ecotype may be well adapted by its special biology to the winter wheat crop. Weed fruits characters have been less studied, especially when they are competing with a crop plant, like wheat and anotheor winter crops.

Table 4

Structure and analysis values of *B.secalinus* panicles

Panicle length, cm	No. of nods	Total no. of spikelets	Total caryopsis weight, g	TKW, g
8-10 3%	5 1%	15-22 3%	1.50-2.00 1%	7-9 6%
10-12 10%	6 32%	23-30 16%	2.01-2.50 21%	9-11 19%
12-14 22%	**7 49%**	**31-38 48%**	**2.51-3.00 29%**	**11-13 46%**
14-16 32%	8 18%	39-46 25%	3.01-3.50 22%	13-15 19%
16-18 24%		47-54 7%	3.51-4.00 8%	15-17 8%
18-20 7%		>55 1%	4.01-4.50 2%	>17 2%
>20 2%			>4.51 2%	

The data could be useful in developing appropriate management principles. In order to control through proper management is good to know as many characters. It was found that a species expressing widest variability may find it

easier means of control and stop eventually. Studies of variation in species *B.secalinus* refer to panicle and spikelet of weed. The way how the characters have expressed diversity could express a certain ecotype of the study area (table 4 and table 5).

We have had panilce long enough, and spikelet also longer. Another new determined characters were: no. ramifications/ panicle 5-8, no. spikelets/ panicle 15-57, no. caryopsis/ spikelet 4-10, upper and lower glume quite normal, spikelet weight of 0.07-0.22 g, caryopsis weight/ panicle 1.32-6.10, TKW 6.24-20.13 g, and awn length 1-7 mm.

Table 5

Structure and analysis values of *B.secalinus* spikelets

Spikelet length, cm	No. of caryopsis	Upper glume length, mm	Down glume length, mm	Spikelets weigth, g	Awn length, mm
1.3-1.4 3%	4 3%	5 5%	4 43%	0.07-0.09 9%	1 1%
1.5-1.6 28%	5 14%	**6 69%**	**5 53%**	**0.10-0.12 43%**	2 8%
1.7-1.8 40%	**6 37%**	7 25%	6 4%	0.13-0.15 33%	3 14%
1.9-2.0 21%	7 30%	8 1%		0.16-0.18 10%	4 22%
2.1-2.2 4%	8 10%			0.19-0.21 4%	**5 29%**
>2.2 2%	9 4%			>0.21 1%	6 20%
	10 2%				7 6%

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