MONITORING THE INFLUENCE OF MAIN ENVIRONMENT FACTORS OVER THE FOLIAR LIMB STOMATAS OF SEVERAL VEGETAL SPECIES FROM THE CRIŞUL NEGRU RIVER COURSE, NV ROMANIA

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Abstract
The purpose of the present studies is monitoring – for a ten months period (fall of the 2008 and spring of 2009) – the exterior aspects of the foliar limbs epidermises of some nettle (Urtica dioica L.) and blackberry (Rubus fruticosus L.) leafs, taken from four sites from the Crişul Negru River course, from its spring (Băiţa Plai area) and all the way to its exit from the country (Ant area), into a sites heavily mined of outcrop rocks, for identifying the possible pollutions with heavy metals (Cu, Cd, Ni, Co, Zn), comparatively with the ones identified at the control batch, located on the course of the Crişul Repede River, towards Bulz area. The resulted data have been correlated with the chemical indexes found in the same locations, for monitoring the heavy metal pollution. At the end of the monitoring we were able to conclude that, from the point of view of the superior and inferior epidermises aspect, at both species, we did not find any changes characteristic to polluted areas, the differences toward the control, being assigned to climatic and geographic factors.

Key words: pollution, stomata, Crișul Negru, *Urtica, Rubus*

INTRODUCTION

The leaf is a vegetative organ of the cormophytes, being a side expansion of the stem, together forming an offshoot (Deliu, 1999), having mainly an assimilating role. The foliar limb is the most important part of the leaf, structurally as well as functionally.

The foliar limb's structure is the result of the action of a group of internal and external factors, during plants phylogeny but also ontogeny (Toma and Rugină, 1998). There are edaphic, biotic and anthropic factors which influence the plants' organism (Botnariuc and Vădineanu, 1982). The light is the most important factor among pedo-climatic factors influencing the structure of the foliar limb. As a result, there are variations of the leaf's structure from the same species or even at the same individual, dependent on lighting. In this way, the leafs with strong lighting are small, rigid, thick, intensely green, having a thicker cuticle, a larger number of stomata, developed palisade tissue, denser nervures with numerous conducting vessels, wider sclerenchymatic areas, unlike the ones in the shadow which are larger, thinner, light green, with thin epidermic layer, few chloroplasts, nervures with fewer vessels and reduced mechanical tissue (Deliu, 1999).

Along with factors independent from the human activity, the anthropic factors, among which pollution, have drastic consequences over the structure and implicitly over the vegetal organs functioning, respectively vegetal organisms, dependent on its nature (Mudd and Kozlowski, 1975; Günthardt-Goerg and Vollenweider, 2007).

Pollution with heavy metals is among the most dangerous pollutions, its removal being a difficult issue, implying very expensive and long term actions. High concentrations of heavy metals can generate sometimes, lethal effects on plants (Mendelssohn et al., 2001).

At plants, organisms which cannot move on their own from the pollute areas, the morph-anatomic changes are frequently an expression of the physiologic adapting to different environmental factors (Barnes et al., 1997). The width of the adaptive changes, of morph-anatomic and physiologic degree is related to the tolerance degree of the respective vegetal species. Morphologic indicators can be used in quantifying the phenotypical plasticity of the regeneration and comparison between species.

Stomata placement, their density and dimensions, their osteole's opening degree, the epicuticular wax type, the topography of the cuticule's surface, the type and density of the tector hairs, the distribution of the guard cells of the stomata, are just as much structural characteristic of the leaf, parameters which can expose differences in some plant species sensibility not only at climacteric factors, but also at different pollutants, especially atmospheric ones (Evans, 1984; Nouchi, 1992; Viskari, 2000).

Due to the fact that there are numerous factors affecting the density and dimension of the stomata, determining the exact reason for the occurred morphologic changes at this level can only be made in association with other anatomic, physiologic, physic, biochemical etc. determinations.

In present studies, we intend to monitoring, in August 2009 - September 2010 period, foliar limb morphologic aspects of *Urtica* and *Rubus* plants leafs which provided from Crişul Negru, from its source, to the border with Hungary (Băița Plai, Fânațe, Borz and Ant area), biological research being made in parallel with chemical determinations of heavy metals in soil, water and vegetation in that area, located in a mining area, compared with the same parameters identified in the Cri□ul Repede (Bulz area – which was the control).

MATERIAL AND METHOD

Among herbaceous species which can be found on the Crişul Negru and Crişul Repede Valley course, at Bulz (control batch), we have chosen the nettle (*Urtica dioica* L.), and among the shrub species, we have chosen the blackberry (*Rubus fruticosus* L.).We have chosen the two species, a herbaceous one and a ligneous one also from ecological reasons, because, Rijmenams (1983), studying the distribution between *Urtica dioica* L. and *Rubus fruticosus* L. in some areas in Belgium, observed that, the two plant species behave, obviously, antagonistically, in matters of reactions towards the ecologic factors. Generally, identifying numerous nettle specimens in a certain perimeter represents an indicator of the presence in that soil of the nitrates or phosphates, resulted as animal or human residues, but this was not the object of our study.

At sampling the vegetal material used for the accomplishment of the vegetal biology studies we respected the standard rules for collecting the vegetal material (Andrei and Paraschivoiu, 2003), establishing fixed points for sampling the assays from the Crişul Negru River (western Romania), using the Global Positioning System (GPS) (Table 1), in the areas: Băița Plai (upstream), Fânațe (near tailing pond see Filip et al., 2009), Borz (site ROSCI0061 Nature 2000 in Crişul Negru narrow path) (http://www.apm-bihor.ro/SituriNatura2000/Situri.Nat.2000.htm), Ant (downstream, at the river's exit from Romania, towards Hungary), and as control we chose a site in Bulz area, placed on the banks of the Crişul Repede, considered as unpolluted (site Natura 2000, ROSCI0062 Crişului Repede narrow path -Pădurea Craiului).

Table 1

GPS coordinates at sampling sites.

Coordinates	**Bulz (*control*)**	**Băița Plai**	**Fânațe**	**Borz**	**Ant**
Latitude (N)	46°28'55"	46°28'55"	46°30'16"	46°40'22"	46°39'45"
Longitude (E)	22°40'28"	22°36'12"	22°32'16"	22°11'32"	21°28'8"
Altitude (m)	366	483	355	210	98

The researched area is located on a bordering river between Romania and Hungary that has its springs into a sites heavily mined of outcrop rocks with a high content of heavy metals. The main pollution sources are represented by the gold, silver, copper, lead, zinc, molybdenum, bromide mining activities from Băița Plai, situated at the headwaters of Crişul Negru River, and where are important outcrops of metal sulphur compounds.

Sampling exactly from these sites, with an error of maximum 1 m, was made at every field trip; when choosing the specimens from which we sampled leafs (vegetative organs, subjects for vegetal biology studies) we identified the individuals with medium high stalks, among that population (meaning that we did not choose neither the highest stalk individuals nor the shorter stalk ones); we also respected the leaf's position on the stalks, meaning the median part of the plant, and at the foliar limb level we respected the standards described by Andrei and Paraschivoiu (2003). The research was made in the fall of 2008, which was an extended one, allowing us to sample vegetal material even at the beginning of December, and also in the spring of 2009. The number of sampled assays for the studies regarding the foliar limb morphology was of 30 from each sampling site, meaning a total of 150 assays.

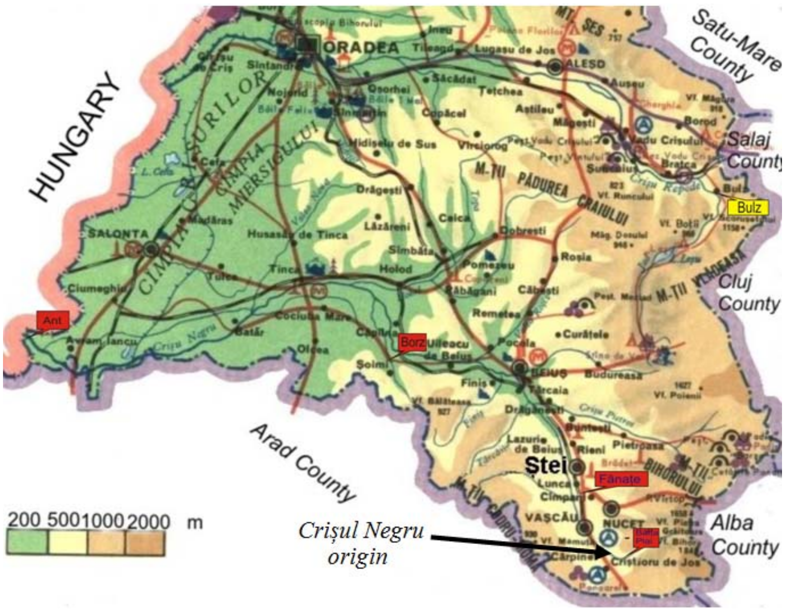

Fig. 1. Location of sampling sites along the Crişul Negru River: Băița Plai (the river origin, in mining area), Fânațe, Borz, Ant, and Crişul Repede River: Bulz – control area.

At both nettle and blackberry, in the spring of 2009 we have sampled leafs from several young specimens, which were in a intensive growth phase, and which at the end of July most of them reached, the growth indexes indentified in August 2008, thus showing us that from the ecological factors point of view, the two years, 2008 and 2009 have been similar. Marking the epidermises was made monthly, for 10 months, both in autumn (August, September, November and December) and also in spring (March, April, May, June and July). In the current account, the data is presented as a result of the statistic processing on periods, respectively the fall of 2008, and the spring of 2009. Presenting all the charts with the statistic processing of the data would have overcrowded the current account, and that is why we stood at presenting strictly the results connected to the purpose of our project, the fundamental research not being a major objective for it.

For studying the stomata, replicas of the foliar epidermis surface were made, by marking them, resulting moulds on 2% colodium films (solved in a mix, with equal parts of ethyl alcohol and ether); the colodium solution has been applied on the dry surface of the leaflets, in a thin layer (Andrei and Paraschivoiu, 2003). The mould represents the negative of the epidermis' surface. The samplings were made from the superior epidermis, and also from the median area's inferior one from the blackberry and nettle leafs' half foliar limbs, located in the middle of the stalk.

The evaluation of the stomata number dimensions and opening degree of the osteole was made by a 40X objective and with an ocular micrometer with an amplifying power of 7X (280X). The pictures were taken by the microscope's digital camera. The micrometric index was calculated using Andrei and Paraschivoiu (2003) method. The dimensions of the stomatic cells were determined through measuring their lengths and width. For each sample three readings were made, and the data were processed mathematically and statistically using the *t* test.

Reporting the data measured through biometry – representing the stomata density, their dimensions regarding length and width, but also the osteole opening diameter – from the leafs collected from Băi□a, Fâna□e, Borz and Ant was made in comparison with the leafs sampled from Bulz area (control), whose biometrical values were considered to be 100%.

The resulted moulds were examined at the MOTIC microscope, type: BA 200, CCD digital camera microscope, 1/3 CCD sensor, 712*582 pixels and analogue and digital output.

Analyzing the obtained data was made in correlation with the anatomic, physiologic changes (assimilating pigments determination), with the quantitative values of the heavy metals identified in the water, vegetation and soil (Fodor et al., unpublished data) from the same location made in the area.

Taking the statistic data was made using SPSS for Windows, version 17.0 (SPSS Inc.)

RESULTS AND DISCUSSIONS

a) The study of the foliar limbs stomata of the nettle plants, from the perimeter included in the study

From the microscopic analysis of the nettle leafs *(Urtica dioica* L.*)* foliar limbs marks, results the fact that, this species has *hypostomatic leafs* (the stomata are present only at the level of the inferior epidermis). The *superior* epidermis has polygonal cells, slightly prolonged, with straight walls (Fig. 2 A). Instead, the *inferior* epidermis of the foliar limbs is made up of epidermal cells with undulated walls, having an irregular contour, in which one can identify anomocitic stomata, whose guard cells cannot be distinguished from the epidermal ones (Fig. 2 B).

At both epidermis level one can identify urticant hairs (especially on the nervures), very long with end point (Fig. 2 C) and simple tector hairs, unicellular, in diverse dimensions, cytolytic (Fig. 2 C, E), having a vesicular basis and sharp point, and also secretor hairs made up of two components, namely: the hair's foot, on one hand, and on the other hand the secretor cell (bi- or tetra cellular), located on its upper side, of much smaller dimensions comparatively to the tector ones (Fig. 2 F).

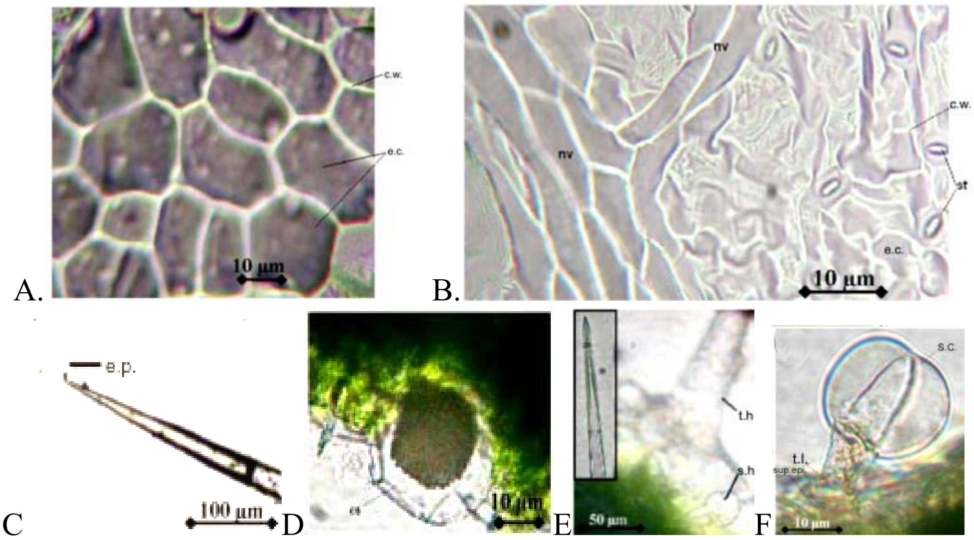

Fig. 2. Nettle (*Urtica dioica* L.) foliar limbs surfaces, sampled from the banks of the Crişul Negru; aspects of the superior epidermis (A) and of the inferior epidermis with stomata (B); of the urticant hairs (C); cistolite (D); tector hairs (E); secreting hairs (F) (e.c. –epidermal cell; e.p. – endpoint ; c.w. – cell wall; cs - cistolite; h.l.. – hair leg; nv – nervure; st – stomata; s.c. – secretor cells; sup.epi. – superior epidermis; t.h. – tector hair) (original).

Urticant hairs are unicellular and have large dimensions; they have a multicellular foundation base, where an “endpoint” cell is set, with a dilated base, the cell houses formic acid; in the apical area, when touching, the urticant nettle hair fractures in diagonal, hurting the skin, spilling its vacuolar content of sodium formiate, histamine and acetylcholine, generating a sting sensation into the penetrated skin (Deliu, 2000). Just like the blackberry thorns, the urticant nettle hairs are actually an under epithelial emerging. In comparison with the control assays – nettles sampled from Bulz- the secondary nervures’ average length and width from the leafs of the samples risen in Băi□a Plai and Borz areas, as well as the average width of the inferior epidermis’ cells for the leafs sampled from Ant have shown superior values in comparison with the control, highly significant from the statistic data processing perspective (Table 2 and 3). These increases can be explained taking into account that at Borz and Ant have been found leafs with the biggest dimensions. As for the nervures, on the inferior side of the leaf, they have been slightly distinguishable, without any projections on the superior side of the leaf, only on their inferior side, fact explained anatomically through the lack of certain collenchymas layers over the vascular bundle, existing at the nettle plants’ leafs sampled from the Crişul Negru valley (Petruț et al., unpublished data).

Table 2

Biometrical data determined at the foliar limbs epidermises level of the nettle specimens leafs *(Urtica dioica* L.*)*, from the areas: Bulz *(control)*, Băița Plai, Fânațe, Borz and Ant, in the months August - December, year 2008 (cel - cells; dens - density; epi - epidermal; L – length; sec. nv – secondary nervure; SD – standard deviation; st - stomata; p – significance threshold; w - width).

Type of epidermis	**Biometrisation**	**Site (locality)**								
		Bulz *(control)*	**Băița Plai**		**Fânațe**		**Borz**		**Ant**	
		mean ± SD	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*
Superior epidermis	L. epi. cel. (µm)	*51.39±9.58*	34.21±4.60	***	43.42±8.13	**	36.93±2.70	***	27.41±1.37	***
	w.epi.cel. (µm)	*37.81±4.98*	18.29±1.28	***	22.36±4.08	***	30.51±6.95	*	20.55±1.61	***
Inferior epidermis	L. sec. nv. (µm)	*388.25±53.96*	474.97±58.7	***	284.76±83.4	**	436.99±19.1	***	355.82±20.78	*
	w. nv. nv. (µm)	*328.11±61.87*	411.95±50.0	***	221.68±57.5	***	378.76±61.8	***	271.88±44.46	***
	L.epi.cel. (µm)	*48.70±1.01*	22.69±0.95	***	29.62±3.48	***	27.92±0.96	***	49.05±7.32	-
	w. epi. cel.(µm)	*19.37±0.84*	13.57±0.88	***	14.17±1.35	***	13.62±1.47	***	29.82±2.37	***
	st. dens. (nr.)	*11.63±0.49*	8.96±0.81	***	15.04±1.20	***	6.79±0.88	***	8.88±0.95	**
	L.st.cel. (µm)	*27.42±0.91*	19.85±0.97	***	19.47±1.64	***	19.29±0.78	***	21.18±0.78	**
	w. st. cel. (µm)	*18.64±0.64*	9.95±4.09	***	8.66±0.95	***	18.23±0.89	-	18.83±0.85	-

Note: * - significance tendency ($p < 0.1$), ** - significant ($p < 0.05$), *** - very significant ($p < 0.01$) towards the control.

Table 3

Biometrical data determined at the foliar limbs epidermises level of the nettle *(Urtica dioica* L.*)*, sampled from: Bulz *(control)*, Băi□a Plai, Fânațe, Borz and Ant, in the months March - July, year 2009 (cel-cells; dens-density; epi-epidermal; L – length; sec. nv – secondary nervure; SD – standard deviation; st - stomata; p – significance threshold; w - width).

Type of epidermis	**Biometrisation**	**Site (locality)**								
		Bulz *(control)*	**Băi□a Plai**		**Fânațe**		**Borz**		**Ant**	
		mean ± SD	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*
Superior epidermis	L. epi. cel. (µm)	49.12±6.12	32.59±7.76	***	42.77±1.55	***	34.60±1.42	***	26.08±0.78	***
	w.epi.cel. (µm)	32.05±1.65	18.06±0.72	***	21.42±0.98	***	27.35±1.04	**	19.50±0.93	***
Inferior epidermis	L. sec. nv. (µm)	321.78±12.92	300.65±12.90	*	279.54±31.12	***	364.07±17.24	***	327.03±16.32	-
	w. nv. nv. (µm)	263.75±16.34	226.78±12.12	***	221.57±20.02	***	311.22±12.94	***	200.43±16.32	***
	L.epi.cel. (µm)	38.97±0.98	20.73±0.73	***	25.13±0.98	***	21.37±1.91	***	45.97±1.99	**
	w. epi. cel.(µm)	19.20±0.73	10.68±0.96	***	13.21±2.09	***	11.94±1.00	***	27.75±1.00	**
	st. dens. (nr.)	11.83±0.75	9.17±0.41	**	15.17±0.75	**	6.83±0.41	***	9.00±0.00	**
	L.st.cel. (µm)	19.53±1.69	18.90±0.93	*	18.90±0.00	*	18.90±0.00	*	19.22±0.77	-
	w. st. cel. (µm)	15.15±0.98	10.68±0.96	***	8.13±0.98	***	17.70±0.93	**	18.00±0.99	**

Note: * - significance tendency ($p < 0.1$), ** - significant ($p < 0.05$), *** - very significant ($p < 0.01$) towards the control.

On the other hand, *the stomata density*, has shown improvements towards the control, at the level of the leaf surface sampled from Fâna□e, meaning an inverse ratio to the leafs' dimensions, highly significant data from a statistic point of view; at Fâna□e – in this time of the year – have been identified the nettle specimens with the smallest leafs. After Deliu (2000), the stomata density increases with the decrease of the foliar surface even if, the stomata number – on surface unit – is genetically determined, still, it may vary – between certain limits – dependent on the environment conditions where the plant lives.

On the other hand, it is well known that, the CO_2 level from the atmosphere influences the morphology and anatomy of the leaf. The stomata, who controls the gas exchange and the water loss from plants, especially from leafs, are affected by the CO_2 concentration from the atmosphere, as well as by the water deficit, high temperatures etc. Thus, the CO_2 increase from the atmosphere leads to the contraction of the stomata conductibility **(**Field et al., 1995) and of the osteole's opening **(**Radoglou and Jarvis**,** 1990 a and b) and/or to the decrease of the stomata density. But, such a response of the stomata, in relation with the CO_2 concentration from the atmosphere, is not a generalized one. After Berryman and its collaborators (1994), many plants show a decrease of the stomata density, while Radoglou and Jarvis (1990 b) or Estiarte and its collaborators (1994) announced an increase of the stomata frequency, once with the increase of the CO_2 concentration. Also Nighat and its collaborators (2000) have described at the *Ruellia tuberose* the decrease of the stomata density, of their dimensions and the osteole's diameter, both at the stomata from the abaxial side and at the ones from the adaxial side, in the prior to flowering, flowering and post flowering period, being under the polluting action of the coal smog. Similar aspects have also been described by Jensen and Kozlowsky, in the year 1975, at the *Fraxinus americana.*

In our case, it is also important that, the leafs' stomata of the nettle plants sampled from Borz and Ant were more deepened in the epidermis, not being at the same level with the epidermal cells, fact - usually - met at the plants living in dry areas, this deepening of the stomata being an adaptation of the plants to the dry atmosphere, determining a diminishing of the excessive evapoperspiration produced at the foliar stomata level and protecting the plant against the water losses, respectively against fading. We mention that, Ant area is located in a plain area, where climate is drier.

The positive or negative differences, compared to the control plants, have been generally very significant from a statistic point of view, at most of the indexes measured through biometry (Table 2).

In the spring of 2009, have generally been registered, morphological aspects of the nettle plants foliar limbs similar to the ones identified in the fall of 2008, mentioning that the average of the indexes measured through biometry, respectively the average values of the two epidermises cell dimensions (length and width), of the stomata cells and especially distance between nervures have been more reduced (Table 3) at the plants grown until July, compared to the ones measured through biometry in the second part of the year 2008 (Table 2), and that is because, in the first case the plants were in a intensive growth phase, and in the second part of the year, the growth was less emphasized or even null.

As a result of the statistic processing of the raw data, we observed that, from the growth indexes point of view measured by us through biometry at the nettle plants (as well as at the blackberry ones), in the two years when these parameters have been studied, the obtained results have been relatively similar, because at the end of July 2009 we identified plants with similar morph-anatomic aspects with the ones from August 2008. A significant difference, in comparison with 2008 (when we noticed significant growths in this case towards the then control) (Table 2), was noticed related to the average distance length between the secondary nervures, at the nettle leaves sampled from Ant, whose average value, in the period March-July 2009, recorded increases, in comparison with the respective parameter identified at the control (leaflets grown on plantlets, in Bulz area), the differences between them being statistically insignificant (Table 3).

The largest distances between nervures were identified at the nettle foliar limbs sampled from Borz area (not in Băița Plai, like in 2008), data who proved to be statistically significant in comparison with the control (Table 3), even if the largest cells of the superior epidermis were found at the control lot (the nettle leaves sampled form Bulz) (Table 3); as for the inferior epidermis cell dimensions, except the leafs sampled from Ant, all the other leaves, from Băița Plai, Fânațe, respectively Borz have had lower values, at this parameter, both concerning the positive differences and the negative ones, data who compared to the control, have been highly significant from the statistic processing perspective (Table 3). Just like in the fall of 2008, in the spring and summer of 2009, the average of the inferior epidermis stomata density of the foliar limbs from the Fâna□e area was the only value superior to the control (regarding the stomata frequency), instead, at these leafs we found the smallest stomata, compared to the control, the differences being highly significant statistically (Table 3).

The distances between the nervures and the dimensions of the stomata cells were the parameters whose registered values at different sampling spots from Crişul Negru were the closest to the ones marked at the control (nettle leaves from Crişul Repede, Bulz area), except the ones from Borz and Ant area (where Crişul Negru exits Romania), where the differences compared to the control were sometimes even insignificant, in the other cases (Băița and Fânațe), the differences were significant compared to the control, from a statistic point of view (Table 3).

b) The study regarding the foliar limbs stomata of the blackberry plants, obtained from the studied perimeters

After analyzing the optic microscopy images of the blackberry leafs foliar limbs, we can observe that, similar to the nettle, stomata are placed only at the inferior epidermis level (hypostomatic) (Fig. 3 A and B).

The superior epidermis of the blackberry leafs contained cells with irregular contour, with cellular walls moderately undulated (Fig. 3 A). The *inferior* epidermis of the foliar limb is also made up of cells whose walls are undulated, but it also has anomocitic stomata (Fig. 3 B). On both epidermises it has heteromorph cells, of different sizes, always larger on the superior side than the ones from the inferior side. At the level of the limb one can observe long, unicellular, without branches (Fig. 3 C) tector hairs, with its origins into the epidermal cells and also several thorn like emergences, having their basis into the under epidermal stratums (Fig. 3 C). Epidermal cells have simple or ursine oxalate crystals.

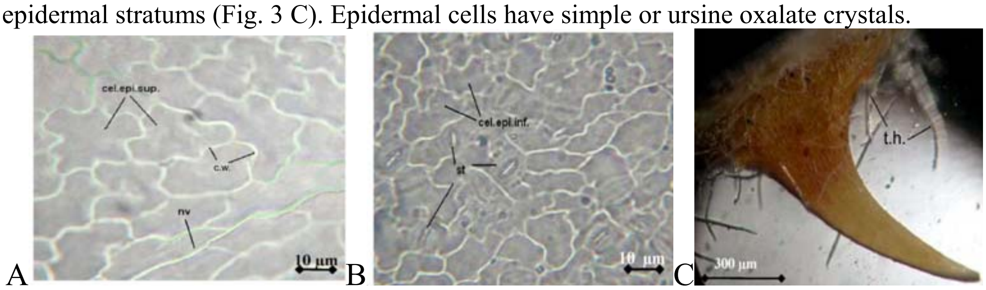

Fig. 3. Aspects of the superior epidermis (A), of the inferior epidermis with stomata (B) and of the thorn like under epidermal emergence (C), identified at the level of the foliar limbs of several blackberry specimens (*Rubus fruticosus* L.*)* sampled from the banks of the Crişul Negru: - tector hair; B - thorn like under epidermal emergence (c.w. – cell wall; cel.epi.sup. – cells of superior epidermis; cel.epi.inf. – cells of inferior epidermis; nv - nervure; st - stomata; t.h. – tector hair)

If at the nettle we observed certain increases compared to control regarding the values of the parameters measured through biometry, at the *blackberry* the situation was reversed, meaning that most of the morphologic parameters registered at the foliar limbs level of the leaves sampled from the banks of the Crişul Negru (Table 4 and 5), from the four sampling spots, respectively Băița Plai, Fânațe, Borz or Ant have marked superior values compared to the ones met at the control batch (leaves sampled from blackberry plants grown in the Bulz area) (Table 4), except the dimension of the inferior epidermis' cells – but excepting those from the Băița Plai area.

At Bulz were observed the densest nervures, with smaller distances between them (Table 4), but also less emphasized, being thinner.

At the control' blackberry leafs (sampled from Bulz area) the epidermal cells were uniform and located at the same level with the stomatic cells; instead, at leafs sampled from Borz and Ant – the same as for the nettle – the stomata were encrypted. This situation may be due to the drier climate found downstream Crişul Negru, in the plain area.

Although the foliar limb dimensions, superior epidermis cell size, including the size of the stomatic ones have had higher values for the blackberry leaves sampled from Ant, the stomata density showed significant negative differences from a statistic point of view (Table 4). If we add the fact that, at such leaves the stomata were encrypted, it is possible that the reduced frequency of the stomata from the foliar limb level might represent another adapting of these plants to draught, climacteric characteristic typical for the area of the Western Plain of Romania.

At Borz and Ant the sampling was made around 11:00 a.m., and afterwards were taken the samples from Băița Plai and Fânațe – we have identified stomata with their alveoli open, with an average opening of 3,5 – 4.0 µm, reason why their width was slightly larger, compared to the other stomata; still, the largest stomata were identified at the blackberry leafs from plants grown on the Crişul Negru banks, in Ant area, with dimensions between 17 - 20 µm, which means that the opening of the osteole is not the only reason for the growth of the stomata's dimensions, this situation being also due to the leafs dimensions which were larger in Ant area (Table 4). In this respect, in the case of an SO_2 and O_3 pollution, Varshney and Garg (1980) noticed at species from the genus *Cicer, Phaseolus, Vigna* and *Dolichos* significant differences of diminishing the foliar surface and the biomass of the polluted leafs, as well as the chlorophyll content, compared to control, the leafs of the same species but unaffected by the pollution with these substances, which in our case was not observed (Blidar et al., unpublished data).

As a result of the entire biometric data processing registered every month, in the **period March – July 2009**, we concluded that, in the case of the *blackberry*, the biggest differences between control (leafs sampled in Bulz) and the leafs sampled from different spots on the banks of the Crişul Negru, from Bihor county, highly significant statistically were identified regarding the distance between the secondary nervures, in the case of those from Băița Plai, fact due to dense nervures met at the foliar limbs belonging to control leafs, which were in the same time less emphasized, slightly visible at the microscope (Table 5). A similar situation to the one met at the blackberry leafs sampled in the period of March-July 2009, was also observed in the fall of 2008, with slight differences regarding raw values, these ones being lower in spring – summer, compared to fall (Table 4 and Table 5), instead they are statistically insignificant data, except the following cases: in 2009, the superior epidermis' cell length showed increases (20%) compared to control at Borz not only Ant and the cell density was higher at Borz (the positive difference having a significance tendency toward the control) not only at Fânațe (Table 5). The largest stomatic cells in the case of the blackberry leafs studied, just like in autumn, the leafs from Ant were measured through biometry, but we should mention that, in Ant there were also the largest foliar limbs, reason why the superior epidermis cells showed highly significant increases from the statistic processing perspective, compared to control.

Table 4

Biometrical data determined at the blackberry leafs *(Rubus fruticosus* L.*)* foliar limb epidermis' level, from the areas: Bulz, Băița Plai, Fânațe, Borz, Ant, in the months August - December, year 2008 (cel - cells; dens - density; epi - epidermal; L - length; sec. nv – secondary nervure; SD – standard deviation; st - stomata; p – significance threshold; w – width).

Type of epidermis	**Biometrisation**	**Site (locality)**								
		Bulz *(control)*	**Băița Plai**		**Fânațe**		**Borz**		**Ant**	
		mean ± SD	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*
Superior epidermis	L. epi. cel. (μm)	*28.35±0.00*	30.23±3.49	-	18.42±1.23	***	29.93±1.50	-	27.41±1.37	*
	w.epi.cel. (μm)	*19.22±0.72*	18.00±0.63	-	17.37±0.98	*	27.91±1.95	***	22.65±0.81	**
Inferior epidermis	L. sec. nv. (μm)	*263.73±40.34*	486.08±29.4	***	379.77±23.4	**	348.9±21.61	***	375.42±26.68	***
	w. nv. nv. (μm)	*200.43±15.22*	353.43±22.2	***	348.68±20.5	***	316.76±31.7	*	251.78±34.43	***
	L.epi.cel. (μm)	*24.57±0.00*	23.94±1.44	-	23.63±3.40	-	19.92±0.96	***	19.01±0.62	*
	w. epi. cel. (μm)	*18.00±0.00*	19.53±0.91	-	14.18±3.35	***	13.62±1.47	***	17.22±0.57	*
	st. dens. (nr.)	*14.17±0.38*	14.33±0.48	-	15.04±1.20	*	15.79±0.88	**	12.28±0.73	**
	L.st.cel. (μm)	*12.29±0.97*	11.97±0.91	-	10.48±1.64	**	12.22±0.58	-	18.18±0.48	***
	w. st. cel. (μm)	*10.10±0.94*	9.78±0.74	-	8.66±0.95	***	9.23±0.39	*	17.13±0.54	***

Note: * - significance tendency (p < 0.1), ** - significant (p < 0.05), *** - very significant (p < 0.01) towards the control.

Table 5

Biometrical data determined at the blackberry *(Rubus fruticosus* L.*)* leafs foliar limbs epidermis' level, sampled from: Bulz *(control),* Băița Plai, Fânațe, Borz and Ant, in the months Mach - July, year 2009 (cel-cells; dens-density; epi-epidermal; L-length; sec. nv – secondary nervure; SD – standard deviation; st - stomata; p – significance threshold; w – width).

Type of epidermis	**Biometrisation**	**Site (locality)**								
		Bulz *(control)*	**Băița Plai**		**Fânațe**		**Borz**		**Ant**	
		mean ± SD	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*	mean ± SD	*p*
Superior epidermis	L. epi. cel. (μm)	25.13±0.98	26.08±0.78	-	18.30±0.93	***	30.54±0.73	***	35.48±0.78	***
	w.epi.cel. (μm)	17.70±0.93	17.40±0.73	-	16.77±0.81	**	18.45±0.75	*	22.60±0.00	***
Inferior epidermis	L. sec. nv. (μm)	232.07±16.37	395.47±26.06	***	274.27±16.32	***	300.65±17.36	***	295.38±25.85	***
	w. nv. nv. (μm)	189.92±0.04	247.92±12.94	***	242.60±32.69	***	284.83±28.31	***	226.78±12.94	***
	L.epi.cel. (μm)	23.23±0.98	22.92±0.78	-	22.92±1.43	-	21.33±0.98	**	19.80±0.99	***
	w. epi. cel. (μm)	18.30±0.93	18.0±0.73	-	13.87±1.03	***	13.22±1.23	***	17.97±1.57	-
	st. dens. (nr.)	14.67±0.52	14.50±0.55	-	15.33±0.52	**	14.33±0.52	*	12.50±0.84	***
	L.st.cel. (μm)	11.96±0.99	11.03±0.77	*	10.08±0.98	**	11.62±0.78	-	18.90±0.00	***
	w. st. cel. (μm)	9.77±0.77	9.14±0.77	-	8.45±1.04	**	9.08±0.78	*	17.40±0.73	***

Note: * - significance tendency (p < 0.1), ** - significant (p < 0.05), *** - very significant (p < 0.01) towards the control.

Because the quantitative values of the heavy metals (Cu, Cd, Ni, Co, Zn), measured both from the waters of the two rivers (Fodor et al., unpublished data) or from the fish living in these waters, namely the common minnow (*Phoxinus phoxinus)* and the carnivorous European chub (*Leuciscus cephalus)* (Petrovici and Pacioglu, 2010), and from the nearby (maximum 1 m) soils or from the leafs studied by us didn't show values to overcome normal ones, the differences identified by us were thought to be caused by environment factors typical for every area separately and by the ontogenetic phase in which the plants were.

In the period 2004-2006, the surface water chemistry measurements suggest high values for copper in the upstream sections as a direct influence of mining activities, with mean values of 10.2 μgg-1 (Josan et al., 2003) upstream Site Băita Plai and with mean values between 20-30 μgg-1 for downstream sites of tailing ponds at Băita Plai or with values in the range of 50-60 μgg-1 at Fânate (Filip et al., 2009). For these metals the concentration at downstream sites is much lower indicating a possible dilution and eventually their uptake by other ecological sectors of the river through a biotic processes, by the benthic and hyporheic sediments, as it has been suggested for the river Aries (Marin et al., 2010) (like other species of vertebrates or invertebrates, sediments etc) then in the upstream sites (Josan et al., 2003; Filip et al., 2009).

CONCLUSIONS

1. As a result of the research regarding the exterior aspect of the leaf's foliar limb epidermis, made at the two plant species, herbaceous (nettle) or ligneous (blackberry), we may conclude that, the differences – which were statistically significant – regarding the epidermal or stomatic cells dimension, distance between nervures, stomata density are due to the ontogenetic phase in which the specimens were in at the sampling moment or the different climate conditions, according to the area where they grew (mountain - plain). It is impossible to find - in the same calendar period – similar morphologic (or hysto-anatomic) aspects, at the specimens sampled from area with different altitude, like for example the stomata adaptations, through their encrypting into the epidermis, at the nettle and blackberry leafs belonging to specimens grown in the Ant (plain) area. Important for our research is the fact that, we did not meet at the studied plants' leafs morphological aspects, characteristic for some polluted areas.

2. The data registered at the beginning of the year, namely March – July 2009, comparatively to the ones registered in the fall, showed average values for the epidermal cells dimensions, but especially for the smaller distances between nervures, less variations were observed at the stomatic cells dimensions and the stomata density on the epidermis' surface was slightly higher, for both studied plants, a normal situation from a ontogenetic point of view.

Acknowledgments

The studies were made within the RO-2006/018 - 446.01.01.01.10, PHARE 2006 cross-border cooperation project, called "Monitoring the radioactivity and the heavy metals content the main environment factors and their influence over the ecosystems from the Cri□ul Negru River route in the Bihor - Bekes euro region", coordinated by the Environmental Protection Agency Bihor (APM). The authors are grateful to Alin Mo□, Eniko Bereczki, Adriana Calapod, Dorina Radove□ for their support in the project development and providing working equipment.

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