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ANATOMICAL ALTERATIONS OF *GALIUM MOLLUGO* L. LEAVES CAUSED BY ERIOPHYOID MITE *ACULUS ANTHOBIUS* (NAL.)

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Anatomical alterations of leaves of weed plant *Galium mollugo* L. (Rubiaceae) caused by eriophyoid mite *Aculus anthobius* (Nal.) are described and quantified. Symptoms can be considered as leaf rolling (galls). Anatomical alterations are expressed as destructions of epidermal cells of upper and under surface of leaves and they can be classified as toxemias.

KEY WORDS: *Galium mollugo, Aculus anthobius,* anatomical alterations, eriophyoid mites.

INTRODUCTION

Species of the genus *Galium* are on the list for biological control, especially *G. aparine* L. that is included in European crop weeds control programs. *G. aparine* is increasing agricultural problem in western USA (WHITSTON, 1991), while *G. spurium* has become a major weed of field crops in Alberta and other prairie provinces of Canada (MALIK & VANDEN BORN,1988). Both of plant species as well as G. *mollugo* are indigenous to Europe, but introduced to North America.

According to ROSENTHAL (1996) eriophyoid mites are ideal biological control agents against plant pests and they have been seriously considered for the biological control of weeds since the 1970's.

Eleven species of eriophyoids have been recorded from *Galium* spp. (AMRINE & STASNY, 1994). Five species have been recorded until now in Serbia & Montenegro (Table I).

Table I. Eriophyoids registered on *Galium* spp. in Serbia & Montenegro (PETANOVIĆ & STANKOVIĆ,1999, PETANOVIĆ, unpublished)

Eriophyoid species	Plant species	Relation to the host	Reference
Aceria galiobia (Can.)	*Galium mollugo* L	flower galls	JANEŽIĆ,1982
			PETANOVIĆ & STANKOVIĆ,1999
	G. verum L.		BOCZEK & PETANOVIĆ,1996
			PETANOVIĆ & STANKOVIĆ, 1999
Aculus anthobius (Nal.)	*G. verum* L.	flower deformation and leaf rolling	PETANOVIĆ & STANKOVIĆ,1999
	G. vernum Scop		PETANOVIĆ, 1998
	G. rubioides L.		PETANOVIĆ, 1998
	G . mollugo L.		PETANOVIĆ, unpublished (loc. Krnule-Vladimirci,Beograd)
Cecidophyes galii (Karp.)	*G. mollugo* L.	leaf curling	JANEŽIĆ,1977,1982
	G. aparine L.		JANEŽIĆ,1977,1982
			BOŽA,1983
			PETANOVIĆ & STANKOVIĆ, 1999
			BOŽA,1983
	G. verum L.		JANEŽIĆ,1977,1982
			BOŽA,1983
	G. kitabelianum Roem et Schult		PETANOVIĆ, unpublished loc.: Mt.Bukulja
Epitrimeru umbonis Boczek	*G. rubioides* L.	leaf and shoot rust	PETANOVIĆ, 1998
Tegoprionus dentatus (Nal.)	*G. verum* L.	vagrant	PETANOVIĆ, 1999

Aculus anthobuius (Nal.) has been reported only from type locality Loraine, France (NALEPA ,1892) and Finland (LINDROTH, 1904). It was described form *G. verum* (Rubiaceae) causing greening of blossoms. *G. sylvaticum*, *G. uliginosum* and *G. boreale* have been mentioned as alternate hosts (AMRINE & STASNY, 1994). According to FARKAS (1965) *A. anthobius* is distributed through out the Europe. Besides greening of blossoms symptoms have also been described as bulging of leaves and flower deformation (DAVIS *et al.* 1982).

Recently, (CRAEMER *et al*.,1999) described new species, *Cecidophyes rouhollahi* Craemer from *G. aparine* in France. It was possible to keep a colony of the mite on *G. spurium* in the greenhouse throughout the year. *C. rouhollahi* is morphologically and biologically close to *C. galii*. Symptoms, i.e. rolling of leaves are similar too. *A. anthobius* superficially causes the same type of alterations to the host plant.

Morpho-anatomical alterations of weed host plants have been investigated till now only on two species, *Euphorbia segierana* (Neck) (MIHAJLOVIĆ, 1996) and *Cirsium arvense* (L.) Scop. (RANČIĆ, 2003).

Bearing in mind that description of morphoanatomical alterations of plants is crucial for understanding host plant phytophagous agent relationship which is important for the potential use in biological weed control, the aim of the study was to describe and quantify such alterations of *G. mollugo* leaves induced by *Aculus anthobius* infestation.

MATERIALS AND METHODS

Anatomy of leaves was described from permanent microscopic preparations. Slides were prepared using standard method for light microscopy (JENSEN,1962, BLAŽENČIĆ,1988). Cross sections of plant material (5-7 μm thick) have been stained afterwards with saphranine and alcian blue. Preparations have been observed under LEICA DMLS microscop. Photographs have been taken using LEICA DC 300 digital camera. In order to define main differences on the anatomical level following characters have been measured: thickness of leaf, high of epidermal cells of upper surface of leaf, thickness of mesophyll and high of epidermal cells of undersurface of leaf. Measurements have been taken using software package IM 1000, comparatively for four zones of each leaf: the leaf base, central part close to the main vein and the zone of the main vein. 45 measurements have been done on 15 uninfested and 15 infested leaves. Statistical analysis has been done using programs package SPSS 8.0. For each of mentioned characters main parameters of descriptive statistics have been calculated: mean, minimum, maximum and standard deviation. Significant differences between infested and uninfested plants were strengthened carrying out unifactorial analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Uninfested leaves of *G. mollugo* are symmetrical dorsoventraly with the prominent main vein on cross sections. (Figs.1 and 2). Parameters of descriptive statistics of the main investigated characteristics are presented in Table II. Meso-

Table II. Uninfested *Galium molugo* leaves-descriptive statistics

	Minimum	Maximum	Mean	Std. Deviation
Epidermal layer of upper surface of leaf-leaf base	9.80	23.00	15.45	2.87
Mesophyll-leaf base	33.90	76.50	52.20	10.47
Epidermal layer of under surface of leaf- leaf base	5.30	17.50	10.54	2.67
Epidermal layer of upper surface of leaf-central part	9.90	22.40	16.21	3.59
Mesophyll- central part	42.90	99.50	73.37	11.22
Epidermal layer of under surface of leaf- central part	5.40	18.80	10.94	2.91
Epidermal layer of upper surface of leaf- close to vein	9.60	25.40	16.97	3.47
Mesophyll- close to vein	51.00	108.70	80.73	13.47
Epidermal layer of under surface of leaf- close to vein	5.60	23.80	12.16	4.57
Epidermal layer of upper surface of leaf- main vein	9.00	34.00	16.74	4.96
Mesophyll-main vain	99.90	197.40	152.21	25.64
Epidermal layer of under surface of leaf- main vain	8.60	35.30	19.91	6.60

phyll is 50-80μm high and clearly differentiated into palisade and spongy tissues. Epidermal layer of upper and under surface of leaves are about 15-16μm, and 10 -12μm , respectively, except close to the main vein were epidermal cells are about 20μm high.

Edges of infested leaves express rolling galls (Figs. 3 and 4). Parameters of descriptive statistics for the main characters of infested leaves are presented on Table III. Infested leaves are significantly thinner in comparison with uninfested except in the zone of the main vein.Mesophyll is about 30-60μm high. Epidermal layer of undersurface on the edges of leaves and in the middle is significantly thinner (average high about 6-8μm and the minimum of 2-3μm). Epidermal layer of the undersurface of leaves is thinner only on edges . In the zone of the main vein epidermal cells are significantly larger in comparison with uninfested leaves (average high of infested epidermis account to 20μm and of uninfested 16μm). In the zones with destructed epidermal cells (on edges and fragmentarily in the middle of leaf) structure of mesophyll has been disarranged, especially if both epidermal layers are destructed. The mentioned disarrangement express itself in smaller mesophyll cells, larger intercellular spaces, loosing differentiation to spongy and palisade tissue and in drying of leaves.

Table III. Infested *Galium molugo* leaves- descriptive statistics

	Minimum	Maximum	Mean	Std. Deviation	Stat. Sign. Diff.
Epidermis of upper surface –leaf base	2.30	18.40	8.48	4.70	+
Mesophyll- leaf base	12.40	52.70	33.68	11.52	+
Epidermis of under surface - leaf base	2.20	12.60	6.48	3.28	+
Epidermis of uppersurface-central part	2.30	59.00	16.34	8.34	nssd
Mesophyll-centarl part	41.10	89.70	60.66	9.66	+
Epidermis undersurface-central part	2.10	24.20	7.98	5.74	+
Epidermis of upper surface-close to vein-close to vain	7.50	26.30	15.38	4.34	nssd
Mesophyll-close to vain	43.90	98.50	65.49	15.36	+
Epidermis of undersurface-close to vain	1.90	28.30	12.03	6.15	nssd
Epidermis of upper surface-main vain	10.40	35.40	21.86	5.37	+
Mesophyll-main vain	90.50	223.80	148.79	34.73	+
Epidermis of under surface- main vain	15.40	51.60	29.37	8.85	+

Statistically significant differences ($p < 0.5$) between uninfested and ifested leaves are presented in last column with mark +; nssd-no statistically significant differences between uninfested and infested leaves

Differences between measured values of investigated characters of uninfested and infested leaves of *G. mollugo* leaves are summarized on Fig. 5.

The mite *Aculus anthobius* is causative agent of leaf rolling of the host plant *Galium mollugo.* The symptom, can be superficially considered as leaf gall. Alterations on anatomical level are expressed as destructions of epidermal cells of upper and under surface of leaf as the consequence of mite feeding. According to classification of symptoms given by WESTPHAL & MANSON (1996) these symptoms can be considered as toxemias. Leaf rolling supply refuge for reproduction and feeding of eriophyoids and very often mites establish prominent colonies within. Dense population of mites provokes a large number of dead cells. In place of them thick layer of calose and lignin substances are accumulated. These symptoms are also expresed as russetig (ROYALTY & PERRING,1996).

In order to separate the mite impact on the host , description and quantification of similar leaf rollings caused by othes species of eriophyoids is needed.

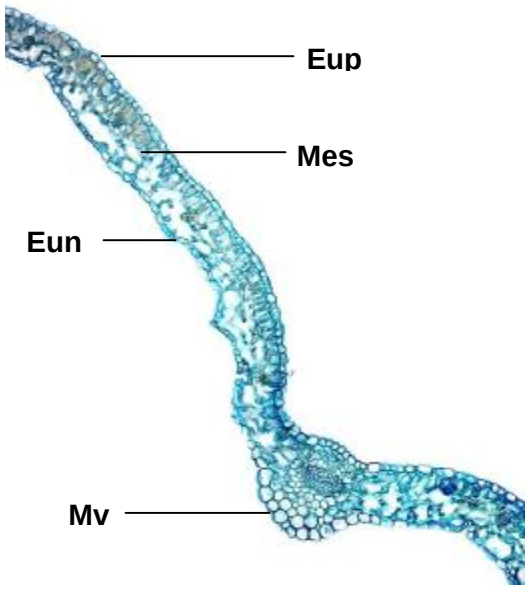

Fig 1. Cross section of uninfested leaf of *Galium mollugo*(x 50). Eup-upper epidermis; Eun-under epidermis; Mes-mesophyll; Mv-main vein

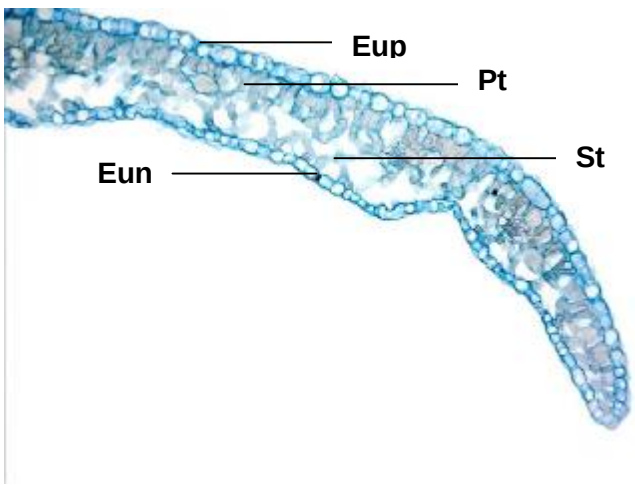

Fig 2. Cross section of uninfested leaf of *Galium mollugo*(x 200); Eup-upper epidermis; Eun-under epidermis; Pt-palisade tissue; St-spongie tissue;

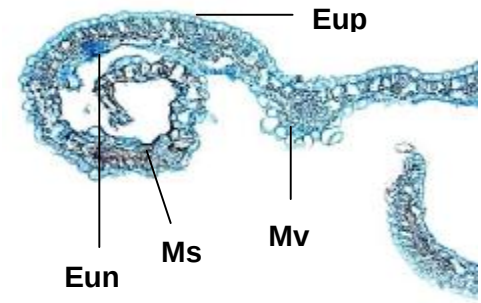

Fig 3. Cross section of infested leaf of *Galium mollugo* (x 50); Eup-upper epidermis; Eun-under epidermis; Ms-mesophyll; Mv-main vein

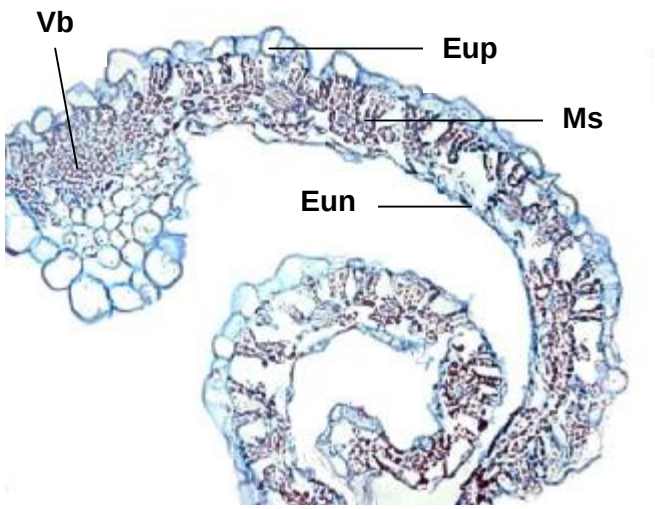

Fig 4. Cross section of infested leaf of *Galium m*ollugo(x 200); Eup-upper epidermis; Eun-under epidermis; Ms-mesophyll; Vb-vascular bundle.

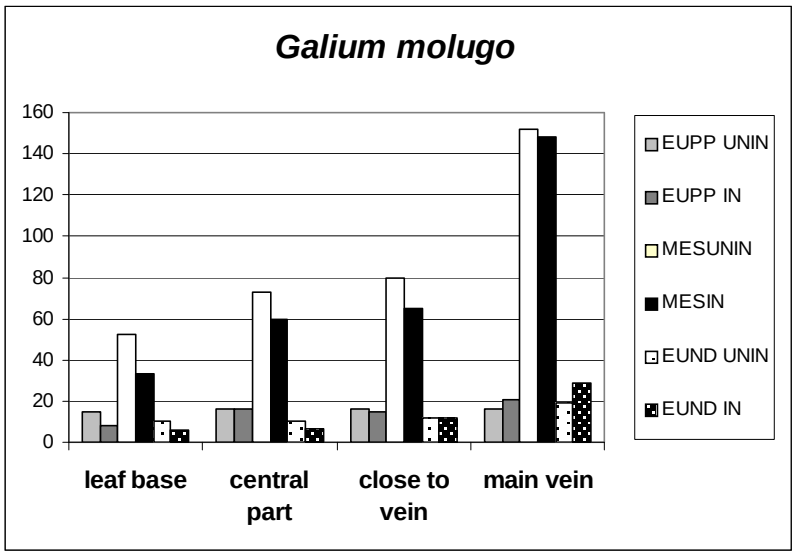

Fig 5. Comparative data of quantitative parameters of infested and uninfested leaves; EUPP UNIN-upper surface epidermis of uninfested leaves; EUPP IN-upper surface epidermis of infested leaves; MESUNIN-mosophyll of uninfested leaves; MESIN-mesophyll of infested leaves; EUND UNIN-undersurface epidermis of uninfested leaves; EUND IN-undersurface epidermis of infested leaves.

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АНАТОМСКЕ ПРОМЕНЕ ЛИСТОВА *GALIUM MOLLUGO* L. ПРОУЗРОКОВАНЕ ЕРИОФИДОМ *ACULUS ANTHOBIUS* (NAL.)

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И з в о д

У раду су описане и квантификоване промене на листовима коровске врсте *Galium mollugo* L. (Rubiaceae) проузроковане исхраном ериофидне гриње *Aculus anthobius* (Nal.) (Acari:Eriophyoidea). Симптоми који се манифестују увијањем ивица листова се могу уврстити у промене типа гала. На анатомском нивоу промене се манифестују у виду деструкције епидермалних ћелија лица и наличја листа које се могу према садашњој класификацији (Westphal & Manson,1996) сматрати токсемијама.

Указано је на потребу да се опишу и друге сличне промене изазване другим врстама ериофида на истој и /или сродним врстама рода *Galium* ради јасног разграничења утицаја на биљку домаћина.

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