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ANATOMICAL INJURIES CAUSED BY *LEIPOTRIX DIPSACIVAGUS* PETANOVIC & RECTOR ON CUT-LEAF TEASEL, *DIPSACUS LACINIATUS* L. (DIPSACACEAE)

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Abstract — The present study highlights some conspicuous structural malformations of the native Eurasian plant *Dipsacus laciniatus* L. (Dipsacaceae) caused by infestation with a newly determined eriophyid mite, *Leipothrix dipsacivagus* (Petanovic & Rector, 2007). The most striking structural changes, induced by mite feeding were evident in the stunted appearance of infested plants and conspicuous injuries to their leaf tissues. The significant damage it causes to *D. laciniatus* recommends the narrow host-range mite *L. dipsacivagus* as a potential agent for biological control of this plant, which is widespread everywhere in the lowlands of Europe and is listed as an invasive and noxious weed in the USA.

Key words: *Dipsacus laciniatus, Leipothrix dipsacivagus,* anatomical features, tissue injuries, eriophyid mites

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INTRODUCTION

The Eurasian species cut-leaf teasel, *Dipsacus laciniatus* (Dipsacaceae), is widespread in Serbia in wetlands and in disturbed areas such as roadside ditches and clear-cuts of alluvial forest. It was introduced to North America as early as the 1700's, probably together with common teasel or accidentally with other plant material from Europe. Extremely rapid spreading has been observed in the last 20-30 years, almost certainly due to the great work of constructing the inter-state highway system. Cut-leaf teasel has been listed as a noxious species in five states and as an invasive weed in several states and national parks of the USA (Rector et al., 2006). As an alien invasive plant, *D. laciniatus* has become the target of a classical biological control program in the USA.

D. laciniatus grows as an herbaceous biennial plant with a basal rosette in the first year. During the second year, it sends out a tall, flowering stalk and dies after flowering. The flowering plant is straight, erect, single from the base, but branching in the apical part. The rosette leaves are more or less lanceolate with small spines, especially on the lower midrib. Flowering stems have large, pinnate, opposite leaves, joined at the base and surrounding the stem to form cups which hold water. The stem and leaves are remarkably prickly. The plant's flowers are small and packed in a dense terminal cylindrical cluster, i.e., in oval-shaped heads.

Dense colonies of an eriophyid mite were observed for the first time on *D. laciniatus* in Northern Serbia in 1999. Later, mites of the same species were found on *D. laciniatus* in Bulgaria and on *D. fullonum* in France, both in 2005. Finally, this mite was determined as a new species, *Leipothrix dipsacivagus* (Petanovic and Rector, 2007).

Investigation of mite morphological and biological characteristics was carried out along with monitoring of host plant injuries in field-collected specimens from native habitats. Preliminary host-specificity tests indicated that the host range of *L. dipsacivagus* is limited mainly to *Dipsacus* spp. (Stoeva et al., 2007). Description of infested plants and quantification of morphological alternations and anatomical damage in particular (as well as some physiological or biochemical changes) can therefore be considered extremely important and essential for nomination and usage of the given eryophid mite as a biological control agent.

The aim of this study was to establish anatomical alterations and damage to the leaves of *D. laciniatus* in its native habitats caused by infestation with the eriophyid mite *L. dipsacivagus*. The results of anatomical analyses could help to elucidate the host plant-mite relationship, and make the mite a candidate for biological control of *D. laciniatus* invasion. In general, data concerning details of plant anatomical injuries induced by mite feeding are still very scarce.

MATERIAL AND METHODS

Plants were collected in the field from a 0,1 ha area of an abandoned cultived field at the Petrovčić location (44^0 47.765', 20^0 0.5955' E) near Belgrade in October of 2005. The collected plant material was fixed in 50% alcohol and deposited in the herbarium of the Institute of Botany and Jevremovac Botanical Garden, Faculty of Biology, University of Belgrade, as well as at the Institute of Entomology, Faculty of Agriculture, University of Belgrade, Belgrade.

Our anatomical analyses dealt with flower stem leaves of infested and non-infested plant specimens collected in the field. First, the above-ground parts of collected plants were examined under a stereomicroscope in the laboratory to confirm their healthy or infested status. Plant material was then fixed in 50% ethyl alcohol and prepared for anatomical analysis using the standard paraffin method for light microscopy (Ruzin, 1999). Leaf slides 3-5 μm thick were sectioned using a sliding microtome and stained with both safranin and hematoxylin. Permanent slides of infested (30 samples) and non-infested (30 samples) plant leaves were observed and analyzed under a light microscope (LEICA DMLS) and photographed with a CANON PowerShot S50 camera.

Leaf tissue morphometry was performed with the IM 500 software package by measuring: total leaf thickness (LT), height of epidermal cells of the upper (UEH) and lower (LEH) leaf side, thickness of the mesophyll (MT) and that of the upper (UPT) and lower (LPT) palisade tissue, and thickness of the spongy (ST) tissue. Statistical analysis was done using SPSS 10.0 program package One-factor analyses of variance (ANOVA) was used to establish significant differences between the leaf parameters of infested and non-infested plants.

At the same time, semi-permanent slides of mites were made using common methods of light microscopy. Here, mites were embedded in Heinze's medium and/or Keifer's medium (Amrine and Manson, 1996). Identification was done using a phase contrast microscope with magnification of 1000–1250x.

RESULTS AND DISCUSSION

Infested plants exhibited reduced and spindly growth (up to 0.5 m high), shortening of internodes of the flower stalk, and delayed flowering caused by mite development on them. In contrast, non-infested (healthy) plants attained normal size (often more than 2 m high), with much longer internodes (Fig. 1). In addition, the flower stalk leaves of infested plants were wrinkled, with either chlorotic spots and/or reddish brown discoloration, and the inflorescence was reduced in size (Fig. 2).

Changes of leaf anatomy were as conspicuously evident between infested and non-infested plants. The basal, rosette leaves of non-infested (healthy) *D. laciniatus* plants are of dorsiventral structure, while the flower stalk leaves, are of isobilateral organization, both of them being amphistomatous. The lower side of stem leaves is densely covered with uni- and multicellular, usually lignified, unbranched simple hairs as well as with scattered glandular hairs. The stomata are at the level of epidermal cells. The mesophyll is differentiated into several layers of palisade tissue and 1-3 layers of spongy tissue (Fig. 3B). Palisade tissue is developed towards both surfaces, arranged in 2-3-layered upper and 1-2 layered lower palisade parenchyma. Cells of the first layer of upper palisade tissue are elongated and tightly packed, whereas fairly large intercellular spaces are usually present among cells of other layers of palisade tissue. There are also intercellular spaces among cells of the

Fig. 1. Non infested (left) and infested (right) *D. laciniatus* from an abandoned cultivated field in Petrovčić**.**

Fig. 2. Infested plant with modified inflorescence and some discolored leaves (in the lower part of the stem)

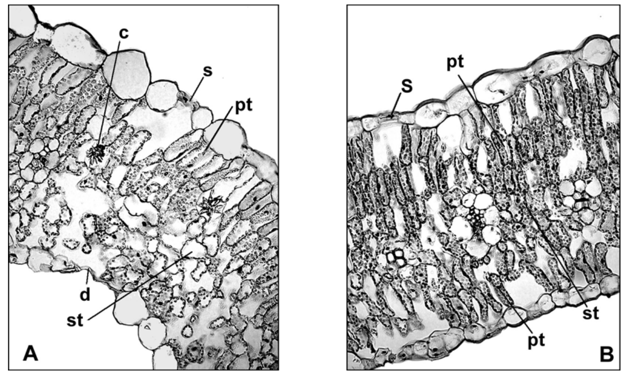

Fig. 3. Cross section of infested (A) and non-infested (B) leaf of *Dipsacus laciniatus* (x 200). PT – palisade tissue, ST – spongy tissue, C – crystal, D – damage, S – stoma.

spongy tissue. The main leaf vein is composed of parenchyma and 3–4 collateral vascular bundles. In general, crystals are sparsely present and clustered.

The curled flower-stem leaves of infested plants exhibit not only significant cellular injuries, but also changes of mesophyll organization. To be specific, the mesophyll of infested leaves consists of 2-3 layers of palisade parenchyma and several layers of non-specifically differentiated spongy parenchyma (Fig. 3A). In addition, the epidermal cells of such leaves are enlarged (hypertrophied) in relation to those of non-infested plant leaves.

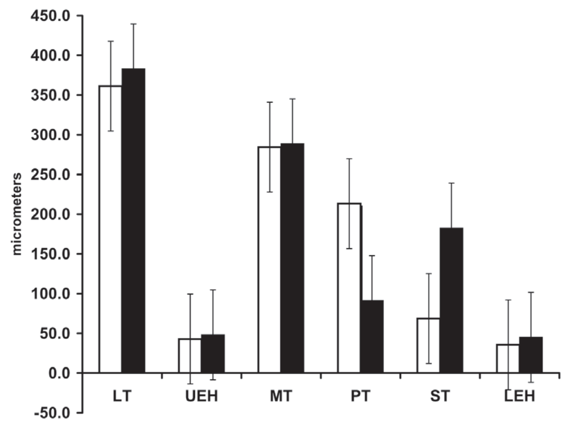

Fig. 4. Comparative data of some leaf parameters (in µm, with standard error) in non-infested (open bars) and infested (dark bars) plants from field. LT – total leaf thickness, UEH – upper epidermis height, MT – mesophyll thickness, PT – palisade tissue thickness, ST – spongy tissue thickness, LEH – lower epidermis height

Thus, the leaves of infested plants were thicker (382.8 μm) than leaves of non-infested plants (361.2 μm). This difference was realized primarily due to the wide, diffuse, non-specifically differentiated spongy tissue (192.5 μm) and enlarged upper (48.1 μm) and lower (45.1 μm) epidermal cells of infested plant leaves in comparison to the the narrower layer of spongy tissue (68.7 μm) and smaller upper (43.0 μm) and lower (35.6 μm) epidermal cells of healthy plant leaves (Table 1). It can be stated that the most important aspects of structural differentiation between non-infested and infested plant leaves are those related to thickness of the spongy and palisade tissue, height of epidermal cells of the lower leaf side, total leaf thickness, and height of epidermal cells of the upper leaf side in that order of significance (Table 1 and Fig. 4).

Table 1. Leaf anatomy parameters (in μm) of non-infested and infested plants.

	Non-infested Mean±SD	Infested Mean±SD	p
LT	361.2±15.9	382.8±25.6	**0.00000***
UEH	43.0±6.9	48.1±8.8	**0.015***
UEL	52.7±16.4	66.8±20.6	**0.005****
MT	284.5±15.5	288.6±14.6	0.482 ns
UPL	136.9±13.0	91.2±29.8	**0.00000***
LPT	76.4±9.9	0	**0.00000***
ST	68.7±14.1	192.5±51.4	**0.00000***
LEH	35.6±8.7	45.1±9.9	**0.00000***
LEL	42.7±9.07	53.6±14.6	**0.001****

LT – total leaf thickness, UEH – upper epidermis height, UEL – upper epidermis length, MT – mesophyll thickness, UPT – upper palisade tissue thickness, LPT – lower palisade tissue thickness, ST – spongy tissue thickness, LEH – lower epidermis height, LEL – upper epidermis length; ns-not significant, *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$

The investigations confirmed a considerable impact of the mite *L. dipsacivagus* on its host plant at both the morphological and the anatomical level. The observed morphological and anatomical deformations of leaves of *D. laciniatus* were consequences of feeding activity of the eriophyid mite *L. dipsacivagus*. Feeding of mite and the presence of dense mite population on the lower leaf surface initiated leaf curling and distortion and/or undulation of the leaf blade, thus provoking deformations of lower mesophyll tissue. In these grooves, the mesophyll cells became compromised and/or disorganized in various ways, leaving large intercellular spaces among them (Pećinar et al., 2006 a,b,c). Similar damages of mesophyll layers were recorded in feeding interaction of *Aceria anthocoptes* with the leaves of *Cirsium arvense* (Rančić, 2003, 2006) and in mesophyll cells of citrus leaves infested with the mite *Phyllocoptruta oleivora* (Royalty and Perring, 1988, 1996). The most striking anatomical change induced by mite feeding in infested plants, was the enlargement of epidermal cells, primarily on the lower leaf side. Similar alterations were present in epidermal cells of lower leaf side of *Euphorbia seguierana* infested with *Aculops euphoriae* (Mihajlović, 1996).

The present results indicate that the new eriophyid mite *Leipothrix dipsacivagus* can be nominated as a good potential agent for biological control of the cut-leaf teasel *D. laciniatus*. In addition, further investigations might confirm the mite's potential for the biological control of some other invasive teasels, particularly *D. fullonum,* since *L. dipsacivagus* was found on both upper and lower leaf surfaces of *D. fullonum* as a vagrant, causing rust-like symptoms, longitudinal wrinkling of the leaves, "witches'-broom" of this species, its stunted development, and delayed flowering (Petanović and Rector, 2007).

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АНАТОМСКЕ ПРОМЕНЕ УЗРОКОВАНЕ АКТИВНОШЋУ *LEIPOTRIX DIPSACIVAGUS* PETANOVIC & RECTOR НА ЧЕШЉУГИ, *DIPSACUS LACINIATUS* L. (DIPSACACEAE)

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У раду су описане и квантификоване промене на листовима *Dipsacus laciniatus L.* (Dipsacaceae) проузроковане инфестирањем ериофидном грињом *Leipotrix dipsacivagus* (Acari: Eriophyoidea). Инфестиране биљке су нижег стабла, мање величине цвасти, "рђасте", као и са значајним променама у ткивима листова. Уочени утицај ериофидне гриње представља могућност њене примене као потенцијалног агенса биолошке борбе против зељасте коровске биљке *D. laciniatus*.