



## Morphological injury of cut-leaf teasel, *Dipsacus laciniatus* L. (Dipsacaceae) induced by the eriophyid mite *Leipothrix dipsacivagus* Petanovic et Rector (Acari: Eriophyoidea)

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To cite this article: I. Pecinar , B. Stevanovic , B.G. Rector & R. Petanovic (2009) Morphological injury of cut-leaf teasel, *Dipsacus laciniatus* L. (Dipsacaceae) induced by the eriophyid mite *Leipothrix dipsacivagus* Petanovic et Rector (Acari: Eriophyoidea), Journal of Plant Interactions, 4:1, 1-6, DOI: [10.1080/17429140802361015](https://doi.org/10.1080/17429140802361015)

To link to this article: <https://doi.org/10.1080/17429140802361015>



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Published online: 20 Feb 2009.



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ORIGINAL ARTICLE

**Morphological injury of cut-leaf teasel, *Dipsacus laciniatus* L. (Dipsacaceae) induced by the eriophyid mite *Leipothrix dipsacivagus* Petanovic et Rector (Acari: Eriophyoidea)**

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(Received 25 April 2008; final version received 22 July 2008)

The eriophyid mite *Leipothrix dipsacivagus* Petanovic et Rector provokes severe malformations to its host plant, cut-leaf teasel (*Dipsacus laciniatus* L.), in the field. These injuries were examined at the morpho-anatomical level in infested plants collected in the field and experimentally infested plants kept under controlled laboratory conditions. A number of symptoms were observed including reduced growth, internode shortening, leaf rolling and wrinkling, and shrunken inflorescences. After severe attack, the leaves of bolted plants became chlorotic with necrotic spots and started to wilt. Young rosettes died. Morphometric analysis revealed significant differences between infested and healthy field-collected bolting plants. Infested plants were significantly shorter than healthy plants, with smaller leaves and flower heads. Striking injuries were also observed on young leaves of experimentally infested plants. It is expected that further investigations will elucidate the full extent of the damage to aboveground parts of *D. laciniatus* due to infestation by *L. dipsacivagus*.

**Keywords:** biological control of weeds; Eriophyidae; plant-mite interaction

**Introduction**

Eriophyoid mites are obligate plant feeders (Lindquist and Oldfield 1996). All aboveground plant organs may be attacked by eriophyoid mites. Eriophyoid species are usually restricted to a narrow range of acceptable host-plant species. The majority have been reported from a single host species or a few species within a single genus (Oldfield 1996). Since they tend to be specialized feeders they can make ideal biological control agents of weeds, being safe to release on weeds in new areas, even where closely related plants may be important crops (Rosenthal 1996), and causing morphological and physiological damage to their hosts. Plant damage may be recognizable to the trained eye. Host-plant symptoms are roughly classified into galls, which are localized growths of the host plant in reaction to mite attack (in some cases mite galls may cause considerable disturbance in the growth and development of the plant), distortions without producing new or abnormal structures, toxemia, or other nondistortional feeding effects. Morphological malformations of weed plant organs induced by eriophyoid mites are well known and many have been described in detail (Boczek and Petanovic 1996; Craemer et al. 1996; Petanovic 1996).

The eriophyid mite *Leipothrix dipsacivagus* Petanovic et Rector was described after field collection in Serbia, Bulgaria, and France in 2005 (Petanovic and Rector 2007). Mites were collected from both upper and lower leaf surfaces of common teasel and cutleaf

teasel (*Dipsacus fullonum* L. and *D. laciniatus* L. [Dipsacaceae], respectively) where they were associated with rust-like symptoms and wrinkles on the longitudinal folds of leaves. Dense populations of mites induced serious malformations to *D. laciniatus* plants that were observed in an abandoned field at Bojcinska šuma in northern Serbia, approximately 30 km west of Belgrade (N 44°47.765', E 20°05.955'). After this first record on *D. laciniatus*, the mite has subsequently been found at more localities in Serbia and in Bulgaria on *D. laciniatus* and at one location each in France and Italy on *D. fullonum* (Petanovic and Rector 2007; unpublished data). The symptoms provoked by this mite on both host plants in all locations were always similar; namely, plants attacked by the mite suffered stunted growth due to shortened internodes, wrinkled leaves, and deformed, closely packed flower-heads. In addition, injuries were observed in epidermal cells and tissues of leaf mesophyll (Pecinar et al. 2007).

Common teasel and cut-leaf teasel are invasive weeds in the USA that were most likely introduced in colonial times in contaminated seed of the obsolete minor crop cultivated teasel, *D. sativus* (L.) Honck. Invasive teasels are currently the target of an American classical biological control program. Biological control candidates currently under study include eriophyid mites, insects, and fungi. These two teasels are native to Europe and are commonly found in open, sunny, poorly drained habitats along roadsides and railroads, as well as in meadows,

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pastures, and heavily disturbed areas. They can form dense, thorny stands and they disperse by seed. Large plants are capable of producing tens of thousands of seeds. These teasels are both biennials, passing their first year as rosettes before bolting, reproducing, and dying in the second year (Rector et al. 2006). Bearing in mind its host specificity (Stoeva et al. 2008) and its impact on the target plant, *L. dipsacivagus* is considered a good candidate for biological control of invasive teasels (Petanovic and Rector 2007).

The purpose of this study was to monitor, at the morpho-anatomical level, injuries to *D. laciniatus* caused by *L. dipsacivagus* infestation in both field-collected plants and plants experimentally infested under controlled laboratory conditions.

## Material and methods

### Experiments on field-collected plants

Bolting *D. laciniatus* plants were collected in 2005 and 2006 from an area of approximately 0.1 ha, on abandoned agricultural land at Bojcinska šuma, in northern Serbia, approximately 30 km west of Belgrade (N 44°47.765', E 20°05.955'). Both symptomatic and apparently healthy plants were returned to the laboratory and examined under a stereo-microscope to check for the presence or absence of mites. Mite specimens were mounted on slides using Heinze's and/or Keifer's media (Amrine and Manson 1996). Species identification was accomplished using a phase-contrast microscope under magnification of 1000–1250 ×.

Thirteen morphological traits were measured for morphometrical analysis from 15 plants each of field-collected infested and mite-free *D. laciniatus*. The traits measured were: HP, total height of plant; NRSH, non-ramified stem height; THS, total height of synflorescence; IL, internode length in synflorescence zone; NPB, number of the primary branches of synflorescence; LPB, primary branch length; TNF, total number of flower heads; NSB, number of secondary branches; LSL, length of non-ramified stem leaf; WSL, width of non-ramified stem leaf; LRL, length of leaf in ramified zone; WRL, width of leaf in ramified zone; and AFH, apical flower head height.

Plants that had obvious signs or symptoms of attack by other herbivores (e.g., insects or spider mites) were not analyzed in this study in order to minimize the possibility of confounding damage by other herbivores with damage by *L. dipsacivagus*. In addition, leaf samples were sent to the Dipartimento di Scienze e Technologie Agroambientali in Bologna, Italy, to test for the presence of phytoplasmas (a group of plant pathogens commonly associated with the Eriophyidae). These tests were negative (Bertacini and Duduk; personal communication). Infection of field collected plants by other plant pathogens that

did not show signs (e.g., virus) cannot be ruled out, however.

### Experiments on laboratory-reared plants

In a separate test, laboratory-reared plants were sown from field-gathered seed into plastic containers and kept in a growth chamber to avoid contamination by mites or insects. Plants were grown under temperatures between 19.8 and 21.4°C, with a light:dark cycle of 16:8 h and relative air humidity of 40%. The seedlings were transplanted to plastic pots and held in closed glass containers with relative air humidity of 72.5%. After the plants had developed their first two leaves, ten *L. dipsacivagus* females collected from the field were transferred to each of 15 plants using an entomological needle under a stereo-microscope. Since not all infested plants were successfully colonized, only ten plants were included in the ultimate analysis. Cohorts of the infested mites were chosen at random and mounted on slides to confirm that they were *L. dipsacivagus*. Ten young control plants were kept under the same conditions as the infested plants without being infested. Plants were observed over time to compare changes in morphology between infested and control plants. Changes in mite density were calculated at six, seven, and eight weeks after infestation in order to compare the influence of mite population growth on the development of symptoms. The leaf area was measured and mites were counted on five randomly selected 1 cm<sup>2</sup> cuttings to estimate total mite number for the entire leaf surface.

On laboratory-grown plants the following morphological features were measured in the rosette stage: LL1, length of first leaf; WL1, width of first leaf; LL2, length of second leaf; WL2, width of second leaf; LL3, length of third leaf; WL3, width of third leaf; LL4, length of fourth leaf; WL4, width of fourth leaf; LL5, length of fifth leaf; and WL5, width of fifth leaf.

The mean values and standard deviations of all measurements were calculated for each plant and one-way analysis of variance (ANOVA) was performed to test the differences between means of infested and control plants. The statistical analysis software package SPSS for Windows, Standard Version (SPSS INC., 1999) was used. Scanning electron microscopy (SEM, JOEL JSM-6460 LV) analyses were also carried out in order to compare the surface structures of leaves of laboratory-grown control and infested plants. A similar analysis was attempted for field-collected leaves but was not logistically practicable.

## Results

### Morphological comparisons of field-collected plants

'Healthy' plants (asymptomatic and without mites found on them) in their native habitat were on

Table 1. Basic statistical data for 13 morphological traits of *Dipsacus laciniatus* plants non-infested and infested by *Leipothrix dipsacivagus* under field conditions. Bold values indicate significant differences ( $p < 0.05$ ).

Trait	Healthy plants ( $n = 15$ ) Mean $\pm$ SD (cm)	Infested plants ( $n = 15$ ) Mean $\pm$ SD (cm)	ANOVA $p$ -value
HP	137.5 $\pm$ 14.0	55.0 $\pm$ 10.1	<b>&lt;0.00001</b>
NRSH	85.8 $\pm$ 8.0	35.4 $\pm$ 9.8	<b>&lt;0.00001</b>
THS	51.7 $\pm$ 19.8	19.6 $\pm$ 6.7	<b>&lt;0.00001</b>
IL	15.8 $\pm$ 3.8	4.4 $\pm$ 1.2	<b>&lt;0.00001</b>
NPB	4.9 $\pm$ 2.3	3.6 $\pm$ 1.7	0.19385
LPB	35.0 $\pm$ 11.3	11.7 $\pm$ 5.6	<b>&lt;0.00001</b>
TNF	12.7 $\pm$ 8.0	10.3 $\pm$ 5.9	0.53448
NSB	7.6 $\pm$ 5.3	7.3 $\pm$ 4.1	0.87883
LSL	29.5 $\pm$ 6.5	21.6 $\pm$ 6.1	<b>0.00252</b>
WSL	7.4 $\pm$ 2.5	5.9 $\pm$ 1.2	<b>0.04844</b>
LRL	14.2 $\pm$ 3.0	11.1 $\pm$ 2.7	<b>0.00624</b>
WRL	6.3 $\pm$ 2.0	4.8 $\pm$ 2.2	0.07077
AFH	4.9 $\pm$ 0.7	3.5 $\pm$ 1.0	<b>0.00013</b>

HP, total height of plant; NRSH, non-ramified stem height; THS, total height of synflorescence; IL, internodes length in synflorescence zone; NPB, number of the primary branches of synflorescence; LPB, primary branch length; TNF, total number of flower heads; NSB, number of secondary branches; LSL, length of non-ramified stem leaf; WSL, width of non-ramified stem leaf; LRL, length of leaf in ramified zone; WRL, width of leaf in ramified zone; AFH, apical flower head height.

average approximately 140 cm tall with internodes approximately 16 cm long. Lateral branches rose an average of 86 cm above the level of the soil and primary branches averaged 35 cm in length. Non-ramified stem leaves of these plants were narrowly ovate and on average approximately 30 cm long and 7 cm wide at their widest point. The ovoid flower heads were approximately 5 cm tall with small, whitish, densely packed flowers. Each individual plant produced approximately 13 flower heads.

By contrast, the field-collected *D. laciniatus* plants infested with *L. dipsacivagus* had smaller main stems compared to healthy plants. These plants were approximately 55 cm tall on average and the internodes were shorter than on healthy plants, approximately 4 cm long. The lateral branches of infested plants were 12 cm long on average and arose only 35 cm from the ground. The leaves were severely

deformed, longitudinally wrinkled, and chlorotic. Non-ramified stem leaves were 22 cm long and 6 cm wide; flower heads were 3.5 cm tall and each plant produced approximately 10 flower heads (Table 1, Figure 1).

Results of one-way ANOVA revealed significant differences between healthy and infested field-collected plants for the following traits: plant height (height of the main stem); height of the non-ramified part of the stem; height of synflorescence; internode length; length of the primary branches; number of secondary branches; length of nonramified stem leaves; width of non-ramified stem leaves; length of leaves in ramified zone; and height of the apical head (Table 1).

#### Morphological modifications of plants infested under laboratory conditions

Initial symptoms of damage to young rosette plants of *D. laciniatus* caused by *L. dipsacivagus* became apparent four weeks after infestation under experimental conditions. The leaves of these young plants began to show symptoms of chlorosis and russeting from the fourth to eighth week after infestation. Comparison of upper leaf surfaces of infested and control plants using SEM revealed damages to the leaf surface structure on infested leaves. Namely, the epidermal cells appear indistinct and wilted or necrotic with a wrinkled cuticle (Figure 2).

Results of measurements of length and width of first, second, and third leaves four weeks after infestation revealed significant differences between control and infested plants for the following traits: Length of first and second leaves, and length and width of third leaves. Results of measurements of length and width of first, second, third, and fourth leaves six weeks after infestation revealed significant

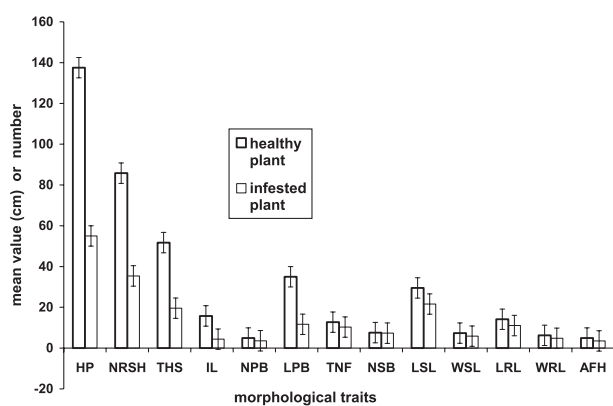


Figure 1. Comparison of various morphological traits of healthy *Dipsacus laciniatus* plants and plants naturally infested with *Leipothrix dipsacivagus* under field conditions, based on significantly different morphological traits (See Table 1 for legend). Bars indicate standard deviations from the means.

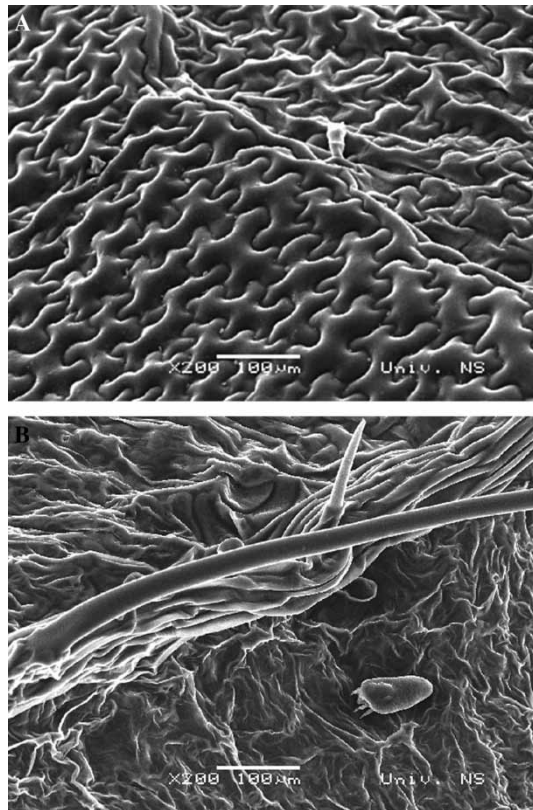


Figure 2. Scanning electron micrographs of upper leaf surfaces of laboratory-grown *Dipsacus laciniatus* plants: (a) control (without treatment); (b) eight weeks after infestation with *Leipothrix dipsacivagus* (mite visible at lower right).

differences between control and infested plants for the following traits: Length and width of first, third, and fourth leaves, and length of second leaves. Results of measurements of length and width of first, second, third, fourth, and fifth leaves eight weeks after infestation revealed significant differences between control and infested plants for the following traits: length and width of the first, second, third, and

fifth leaves, and width of fourth leaves (Table 2). Generally speaking, the average length and eventually width of infested leaves were significantly smaller than for leaves of control plants. Changes in the plant appearance were in accordance with the changes in mite density and the duration of infestation. After six weeks, the mite populations reached their peak, followed by a decrease in density as a consequence of emigration from heavily infested plants. Changes in means of certain morphological traits that were significantly different for infested and control plants under experimental conditions appeared to be correlated to duration of infestation and mite density (data not shown).

### Discussion

The malformations of the aboveground parts of *D. laciniatus* were shown here to be a specific response by this plant to the feeding activity of the herbivorous eriophyid mite *L. dipsacivagus*. The most discernible effects, observed on bolted plants under field conditions, were stunted growth, shortened internodes, smaller leaves, and abnormal proliferation of deformed flower heads. Statistical analysis revealed that nine morphometric traits out of 13 were significantly different between 'healthy' and infested plants.

Results of the experiments conducted under laboratory conditions demonstrated the impact of mite feeding as slowed development of the plant and reduction in the average length and width of the leaves. Injuries observed on leaves and petioles could also be attributed to mites feeding on the plant. Superficial symptoms can be described as 'russeting', according to the classification of Westphal and Manson (1996).

The most obvious injuries were observed in the structure and shape of the lower and upper epidermis. Damage to the epidermal cells ranged from distortion

Table 2. Comparison of rosette leaf measurements four, six, and eight weeks after infestation for laboratory-grown *Dipsacus laciniatus* plants infested with *Leipothrix dipsacivagus* and control plants that were not infested. Significance determined by ANOVA: n.s. = not significant; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .

	Four weeks after infestation			Six weeks after infestation			Eight weeks after infestation		
	Control plants	Infested plants	Significance	Control plants	Infested plants	Significance	Control plants	Infested plants	Significance
LL1	6.6±0.5	3.0±1.0	***	5.2±0.7	0	***	6.8±0.8	1.3±1.4	***
WL1	1.1±0.1	1.0±0.1	n.s.	1.3±0.1	0	***	1.4±0.2	0.6±0.6	**
LL2	4.5±0.5	2.7±1.2	***	8.9±0.4	3.8±0.6	*	9.3±0.8	0	***
WL2	1.0±0.1	0.6±0.1	n.s.	2.1±0.2	1.1±0.2	n.s.	2.0±0.2	0	***
LL3	1.6±1.4	0	**	16.2±1.2	3.1±0.9	***	18.2±1.7	4.6±2.7	***
WL3	0.3±0.3	0	**	2.8±0.8	0.8±0.4	***	3.0±0.3	1.4±0.8	*
LL4				10.3±1.6	0.5±0.5	***	19.4±2.3	3.8±2.5	***
WL4				1.9±0.5	0.3±0.3	***	2.8±0.6	1.0±0.4	n.s.
LL5							3.8±2.7	0	***
WL5							0.8±0.6	0	**

LL<sub>1</sub>, length of 1st leaf; WL<sub>1</sub>, width of 1st leaf; LL<sub>2</sub>, length of 2nd leaf; WL<sub>2</sub>, width of 2nd leaf; LL<sub>3</sub>, length of 3rd leaf; WL<sub>3</sub>, width of 3rd leaf; LL<sub>4</sub>, length of 4th leaf; WL<sub>4</sub>, width of 4th leaf; LL<sub>5</sub>, length of 5th leaf; WL<sub>5</sub>, width of 5th leaf.

to complete necrosis depending on the density of the mite population. Mites fed by sucking the contents of epidermal cells, causing the leaves to become deformed and shrunken. It is not known whether they produce any toxins while feeding. Russeting symptoms are caused by other species of free-living eriophyid mites on their host plants, e.g., *Aculus schlechtendali* (Nalepa) on apple leaves (Easterbrook and Fuller 1986; Kozłowski 1998), *Aculops lycopersici* (Masse) on tomato leaves (Royalty and Perring 1988; Haque and Kawai 2002), *Aculus fockeui* (Nal. et Trt.) on plum leaves (Suski and Badowska-Czubik 1995), and *Aceria anthocoptes* (Nal.) on Canada thistle leaves (Rancic et al. 2006). According to Suski and Badowska-Czubik (1995) dense populations of *A. fockeui* on plum (400 per leaf) caused shortening of internodes, scorching of apical shoots and death of the apical meristem. Similarly, infestation of *A. lycopersici* caused great injury to tomato plants, with large numbers of leaves turning brown and drying up; the number of leaves, plant height and the diameter of the main stem of these plants all decreased as a result of that infestation (Haque and Kawai 2002). In our experiments, feeding on *D. laciniatus* by adults and immatures of *L. dipsacivagus* under laboratory conditions brought about severe damage leading to necrosis, premature senescence of young rosette leaves, gradual weakening, and plant death a few months after infestation (the first infested plant died five weeks after infestation, followed by others). Such heavy infestations were obtained under controlled laboratory conditions meant to minimize generation time and optimize population growth, including low solar radiation, high humidity, and the absence of predators or competitors for food and space. Nonetheless, it is reasonable to assume that infestation of plant rosettes could result in similar intensity of morphological damage under field conditions. Indeed, considerable damage caused by the eriophyid mite *L. dipsacivagus* has been observed on mature plants in the field.

According to Rector et al. (2006), besides host specificity as a key criterion of biological control candidates (BCC) for the target plant, BCCs that attack the taproot or the rosette hold the greatest promise for biological control of invasive teasels. The eriophyid mite *L. dipsacivagus* caused serious damage to young rosettes under laboratory conditions in addition to remarkable damage to bolting plants in the field.

First results obtained by Stoeva et al. (2008) also indicate high host specificity for this species. Bearing in mind the specific trophic niche of this mite (it has been observed feeding only on epidermal cells of the leaf lamina and flowerhead involucre), absence of competition with other biological control agents may also become a positive trait of this mite species, depending on which other agents might be released.

Further studies of anatomical injury and physiological alterations induced by *L. dipsacivagus* will seek to explain the precise causes of these malformations of *D. laciniatus* tissues under field conditions. Elucidation of the effects on plant physiology could have important implications on strategies for the eventual deployment of *L. dipsacivagus* as a biological control agent of *Dipsacus* spp. in the USA, especially regarding potential interactions with other proposed biological control agents.

### Acknowledgements

We are grateful to Milos Bokorov (University of Novi Sad, Serbia) for help in preparation of SEM photographs. We are also indebted to anonymous reviewers for improvement of the manuscript. This study was supported in part by the Serbian Ministry of Science and Environment Protection (grants # 143006B and # 143015).

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