Host-specificity testing on *Leipothrix dipsacivagus* (Acari: Eriophyidae), a candidate for biological control of *Dipsacus* spp.

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Summary

*Leipothrix dipsacivagus* Petanovic & Rector is the first eriophyid mite recorded from hosts in the genus *Dipsacus* L. and is considered a potential candidate for biological control of invasive teasels (*Dipsacaceae*). Host-specificity testing on *L. dipsacivagus* (Acari: Eriophyidae) was carried out under insectary conditions for 4 months, from 19 April until the end of August, 2006. The laboratory colony of *L. dipsacivagus* was descended from mites collected on *Dipsacus laciniatus* L. in Klokotnitsa, Bulgaria. They were tested in choice and no-choice tests on *Dipsacus fullonum* L., *Knautia arvensis* L., *Cephalaria* sp. and *Scabiosa* sp. In the choice experiments, individual *D. laciniatus* plants infested with *L. dipsacivagus* were placed in cages with one to two plants of each test species. There were four replicates. Data were recorded at 10, 20, 30, 40, 60 and 90 days after infestation of test plants. After 10 days, only *D. laciniatus* was infested. After 20 days, mites were vagrant on all the plants except *Scabiosa*, and colonies were established on all *Dipsacus* plants. Some *Cephalaria* and *Scabiosa* plants had vagrant mites at days 20 and 30, respectively, but all of these were dead by days 30 and 40, respectively. By day 60, all *Knautia* plants were colonized, although these colonies later died. The mite successfully colonized only *D. laciniatus* and *D. fullonum* and temporarily colonized *K. arvensis*. In the no-choice tests, each of four plants of a given test species was infested with five mites/plant in a single cage. There were three replications (cages) for each test-plant species. Mites on plants other than *Dipsacus* spp. began to die after 10 days. Reproducing populations of *L. dipsacivagus* established only on *D. laciniatus* and *D. fullonum*.

**Keywords**: teasel, invasive plants, mites, *Dipsacaceae*.

Introduction

*Leipothrix dipsacivagus* Petanovic & Rector is the first eriophyid mite recorded from hosts in the genus *Dipsacus* L. (Petanovic and Rector, 2007). This species was first collected in Serbia in 1999 but was misidentified (Petanovic, 2001). It was subsequently collected during surveys conducted in Serbia, Bulgaria and France in 2005, described as a new species (Petanovic and Rector, 2007), and is now a candidate for biological control of invasive teasels (*Dipsacus* spp., *Dipsacaceae*) in the USA. According to Petanovic and Rector (2007), the mite occurs on both the upper and lower leaf surfaces of *Dipsacus* spp. as a vagrant causing rust-like symptoms, wrinkles on the longitudinal folds of the leaves, ‘witches broom’ of the plant (i.e. reduced internode length and deformed leaves), stunting, delayed flowering and malformation of the flower heads. Analyses of symptomatic plant tissues for presence of microbial plant pathogens were negative (Petanovic and Rector, unpublished data). A study of the injuries caused by the mite (Pecinar et al., 2007) showed that the injury to the leaves is conspicuous at the morphological and physiological as well as the anatomical level.

Eriophyid mites are frequently considered to be promising candidates for weed biological control due to their high host specificity, rapid life cycle and severe damage to their host plants (Littlefield and Sobhian, 2000; Rancic and Petanovic, 2002; Sobhian et al., 2004). In one study, more than 80% of eriophyid
species occurring on weeds were monophagous (Boczek and Petanovic, 1996), which is a key criterion for a successful weed biological control agent. A critical part of any biological control program is to test the host range of candidate agents to establish the level of risk posed to non-target plants and ensure that agent releases will not do more harm than good (Cullen and Briese, 2001).

The purpose of the present paper is to report on initial results concerning host-specificity testing of the new eriophyoid mite Leipothrix dipsacivagus, a potential candidate for biological control of Dipsacus spp., in the USA.

Methods

Origin and maintenance of test population

Leipothrix dipsacivagus individuals were collected from cutleaf teasel, Dipsacus laciniatus L., plants in a field near Klokotnitsa, Bulgaria (42°00.43'N, 25°27.41'E) and brought to the insectary of the Department of Entomology, Agricultural University in Plovdiv, Bulgaria. Species identification was made by R. Petanovic, Department of Entomology, Faculty of Agriculture, University of Belgrade, Serbia. The original mite colony was set up in August 2005 and maintained under insectary conditions on potted D. laciniatus plants.

Test plants

Several closely related plant species from the family Dipsacaceae were chosen: Dipsacus fullonum L., Knautia arvensis L., Scabiosa sp. and Cephalaria sp. Plants of D. laciniatus, the original host of the colony, were used as a control. The test plants were grown in plastic pots 8 cm in diameter from field-collected seed. Plants were used in tests after they had formed their first two foliar leaves. Test plants were inspected to ensure that they were in healthy condition at the time of testing.

Host-specificity testing

The host-specificity tests were conducted under laboratory conditions from 19 April until the end of August, 2006. Two types of cages made from clear, plastic panels with fine nylon mesh tops were used for the experiments: small (20 × 20 × 40 cm) and large (20 × 40 × 40 cm). During the tests, there was approximately 16 h of light per day in the insectary with temperatures of approximately 22°C during the day and 15°C at night. Relative humidity was 50% to 60% in the insectary and 70% to 80% within the plastic cages. Two different tests were designed for studying the host-specificity: choice tests and no-choice tests.

Choice test – Free migration was allowed from any infested D. laciniatus plant to other plants in the same cage. In each cage, one pot with an infested D. laciniatus plant was placed together with either three or seven pots of a test plant species, depending on the size of the cage. Plants were arranged in a randomized design within the test cages (four pots in the small and eight pots in the large cages). Pots were arranged such that the leaves of adjacent test plants within each cage were touching to facilitate migration of the mites between plants. For each plant species, four replications were made with each cage representing a replication. The plants were checked regularly under a stereo microscope to monitor the migration of the mites. At 10, 20, 30, 40, 60 and 90 days, the number of test plants with mites present was recorded. The infestation was recorded as the number of infested plants in each cage and the percentage of infestation was calculated.

No-choice test – Potted plants of D. fullonum, K. arvensis, Scabiosa sp. and Cephalaria sp., were tested. D. laciniatus plants were tested in separate cages as a control. Five adult mites were transferred under a stereo microscope to the leaves of each test plant, using a fine brush. For each replicate, four plants of one test species were arranged in a small cage with three replications per species. The plants were checked under a stereo microscope regularly. At 10, 20, 30, 40, 60 and 90 days, the infestation of test plants was recorded.

In both, the choice and no-choice tests migration, reproduction and feeding damage were recorded in addition to the presence of mites.

Results and discussion

Choice test

The initial results from the choice tests are presented in Table 1 and Fig. 1. By the tenth day after the beginning of the experiment, migration was observed only on D. laciniatus plants. After 20 days, migration was observed to all test species, except for Scabiosa sp. At that time, all plants in all control cages were 100% infested, while in the test cages, infestation varied from 0% on Scabiosa sp. to 50% on D. fullonum plants. After 30 days, 100% of the D. fullonum plants were infested, and after 60 days, 100% of the K. arvensis plants were infested. Migration to the other two species, Cephalaria sp. and Scabiosa sp., was observed at 20 and 30 days, respectively.

Soon after migrating, most of the mites on plants other than Dipsacus spp. died. By the 30th day on Cephalaria sp. and by the 40th day on Scabiosa sp., all the mites on these plants were dead. No further migration was observed to any of the test plants of these two species.

Besides the differences in the duration of dispersal, differences in the development of the mite depending on the host plant species were also noted. On the control (D. laciniatus), the mite began reproducing after 10 days, while on D. fullonum and K. arvensis plants, mites were reproducing after 20 days and 30 days, respectively. After 30 days, there were no differences in...
the activity of the populations on these two test-plant species and the control. The mite was reproducing and increasing its population density on D. laciniatus, D. fullonum and K. arvensis plants (with one exception) until the end of the experiment.

Feeding damage was observed only on plant species on which the mite successfully reproduced (i.e., D. laciniatus, D. fullonum and K. arvensis). For all three of these plant species, the oldest leaves of the rosettes wilted and became chlorotic and eventually withered. This feeding damage to rosette leaves under laboratory conditions was quite different from the damage to bolting and flowering Dipsacus spp. plants, observed by the authors in the field in 2004–2006, near the villages of Klokotnitsa (42°00.43′N, 25°27.41′E), Lozen (42°37.57′N, 23°30.34′E), Gorski Izvor (42°00.93′N, 25°26.14′E) and Dalbok Izvor, Bulgaria (42°11.34′N, 25°04.81′E; Stoeva et al., unpublished data) nor like that reported by Petanovic and Rector (2007). This could be due to physiological differences between rosettes and bolting or flowering plants or due to fundamental differences between field and insectary conditions.

On Scabiosa sp. and Cephalaria sp., although mites had migrated, they did not establish populations. Approximately 20 to 25 days after migration, the mobile forms had died. No eggs or immature stages were found on these plants. There was some evidence of feeding, although the leaves did not become chlorotic or desiccated.

An additional experiment was conducted for the test plant species on which development of mite populations had established. From the cages, housing choice tests involving D. fullonum and K. arvensis plants, the D. laciniatus plant, which had been the plant originally infested with mites in the choice-test cages, was removed. The cages were left with only the test plants (D. fullonum or K. arvensis), onto which mites had migrated and established populations. On the D. fullonum plants, mite populations continued to develop, whereas on K. arvensis, all mites died 30 days after removal of the D. laciniatus plants.

The development of the population on K. arvensis plants in the choice tests (when the test plants were touching the infested D. laciniatus plant) and the cessation of population development after removal of the D. laciniatus plant could be explained as a result of induction of a state of central nervous excitation (Marohasy, 1998) by the presence in the two Dipsacus spp. (but not in K. arvensis), of volatiles or other compounds that stimulate mite feeding or that are necessary for mite reproduction.

The results from the choice test showed that L. dipsacivagus migrates, feeds, reproduces and establishes sustained populations on D. laciniatus and D. fullonum, while it can feed temporarily on K. arvensis but cannot sustain itself on this host in the absence of D. laciniatus. Neither Cephalaria nor Scabiosa proved suitable as hosts to L. dipsacivagus in this experiment.

### No-choice test

Ten days after the beginning of the no-choice experiments, in which all plants were directly infested (five mites per plant), there were still live mites in all replications on all the tested plants (Table 1 and Fig. 1). Mites began to die after 10 days on some of the Scabiosa sp. and Cephalaria sp. plants and after 20 days on K. arvensis plants. By the 30th day, all the mites on all the plants of these species were dead. There was

#### Table 1. Results of host-specificity testing with Leipothrix dipsacivagus at lab conditions at the Agricultural University-Plovdiv in 2006.

<table>
<thead>
<tr>
<th>Test plant species</th>
<th>No of plants tested</th>
<th>No of infested plants (days after infestation)</th>
<th>Feeding damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days 20 days 30 days 40 days 60 days 90 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Test 1. Choice test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipsacaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. laciniatus L. (control)</td>
<td>20</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Dipsacus fullonum L.</td>
<td>20</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>K. arvensis L.</td>
<td>20</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Scabiosa sp.</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cephalaria sp.</td>
<td>20</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><strong>Test 2. No-choice test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipsacaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. laciniatus L. (control)</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Dipsacus fullonum L.</td>
<td>12</td>
<td>9 (3)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>K. arvensis L.</td>
<td>12</td>
<td>8 (4)</td>
<td>–</td>
</tr>
<tr>
<td>Scabiosa sp.</td>
<td>12</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>Cephalaria sp.</td>
<td>12</td>
<td>12</td>
<td>–</td>
</tr>
</tbody>
</table>

Number in brackets shows the number of plants with dead mites.

* All mites are dead.
Host-specificity testing on *Leipothrix dipsacivagus*

**Figure 1.** Infestation of *Leipothrix dipsacivagus* onto test plants in choice and no-choice tests under laboratory conditions.

![Choice test graph](image)

![No-choice test graph](image)

no reproduction on these species, although there was some evidence of feeding. In contrast, on *D. laciniatus* and *D. fullonum* plants, there were eggs and immatures present on the tenth day and the population density was increasing. After 40 days, feeding damage was observed on these two species as described from the choice test.

The results from the no-choice test show that the mite *L. dipsacivagus* reproduces and establishes populations on *D. laciniatus* and *D. fullonum* plants, both of which are noxious weeds in the USA, and cannot colonize the confamilial species *K. arvensis*, *Scabiosa* sp. or *Cephalaria* sp. These preliminary results are promising, in that the effective host range of this mite appears to be restricted well within the family Dipsacaceae, if not within the genus *Dipsacus*. This would bode well for the prospects of this mite as a candidate for biological control of invasive teasels in the New World, as there are no native or economically important members of the family Dipsacaceae there.

Due to ambiguities in the results of choice tests of *L. dipsacivagus* on *K. arvensis*, further no-choice testing using larger initial infestations are under way. In addition, testing remains for the remainder of the host-specificity test list for this biological control candidate, which comprises approximately 40 species, mostly outside the Dipsacaceae, including rare and/or threatened native American plants in closely related families (e.g. Caprifoliaceae).

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References


