Impact of larval and adult feeding of *Psylliodes chalcomera* (Coleoptera: Chrysomelidae) on *Centaurea solstitialis* (yellow starthistle)


Summary

The flea beetle, *Psylliodes chalcomera* (Illiger) (Coleoptera: Chrysomelidae), is a promising candidate biocontrol agent for yellow starthistle, *Centaurea solstitialis* L., a weed of primary importance in the western USA. Two sets of trials were performed to evaluate the impact of insect feeding at larval and adult stages. The larval transfer test showed a significant impact of larval feeding on yellow starthistle, although limited to seed production. *P. chalcomera* adult feeding was negatively influenced by the presence of other individuals on the same substrate. On the other hand, the individual feeding rates, especially for females, suggest a potential defoliation impact. Comparing the outcome of both experiments with the infestation rates observed in the field, *P. chalcomera* shows potential to damage yellow starthistle, although more studies are needed to assess the impact of such damage.

Keywords: impact assessment, flea beetle, feeding damage.

Introduction

In 2001, a population of the flea beetle *Psylliodes chalcomera* (Illiger) (Coleoptera: Chrysomelidae) was observed developing on yellow starthistle, *Centaurea solstitialis* L. (Asteraceae: Cardueae), in the vicinity of the village of Volna, Krasnodar territory, southern Russia. This insect is known to attack *Carduus* species (Dunn and Rizza, 1976; Dunn and Campobasso, 1993), but it had not been previously observed feeding on species in the genus *Centaurea*. Subsequent behavioral and molecular genetic studies showed that the population associated with yellow starthistle was biologically different from populations associated with *Onopordum* sp. in Russia and *Carduus* sp. in Italy (De Biase et al., 2003, 2004). Consequently, we are evaluating individuals from this population to determine if they would be suitable candidates for the biological control of yellow starthistle. An important part of this evaluation is to assess the potential impact that the insect can have on the target plant (Balciunas and Coombs, 2004).

Yellow starthistle is an important rangeland weed in the western USA, especially in regions with a mild moist winter and dry summer (Mediterranean climate; Maddox, 1981; Sheley et al., 1999). It is a winter annual forb native to southern Europe and southwestern Asia. Seeds germinate in the late fall or early spring, rosettes develop until late spring and then plants bolt and flower until they senesce due to lack of moisture or freezing. *P. chalcomera* has one generation per year.
(Dunn and Rizza, 1976; Cristofaro et al., 2004). Overwintering adults feed on yellow starthistle foliage in the early spring and oviposit on or near the plants. Larvae tunnel in the leaf midribs and young stems and exit the plant to pupate in the soil. Emerging adults feed briefly, mate and then aestivate and hibernate until the following spring.

Methods

Insects

Experiments on the impact of larval and adult feeding were carried out utilizing 65 adults of *P. chalcocoma*, collected near Volna (45°07′36″N; 36°41′35″E; altitude 16 m) on March 28 to 31, 2005. Based on our previous experience, adults collected at this time of year should be completing diapause and ready to begin feeding and ovipositing.

In the laboratory, the insects were placed in a 3-l glass beaker with crumpled paper and fresh yellow starthistle leaves at room temperature (18°C to 25°C) and with a 16:8 L/D cycle (natural and artificial light combined) for a week to allow complete reactivation after diapause. During this time mating was often observed.

Females and males were then separated. Males were kept in the same beaker described above, while females were individually placed in Petri dishes (25°C, 16:8 L/D cycle) with small bouquets of fresh yellow starthistle leaves with the aim to select ovipositing individuals. Laid eggs were collected daily, counted and placed in sterile Petri dishes over wet filter paper to allow hatching.

After 1 week, ovipositing females were put back to the common container with males, where the insects were allowed to feed on fresh yellow starthistle leaf bouquets replaced every other day.

Larval transfer

The larval transfer test was carried out on early bolting, US biotype, greenhouse grown potted yellow starthistle plants. Two treatments and one negative control were set up transferring 10, 20 and 0 larvae per plant, respectively, with ten replicates each. Before the start of the experiment, plant height, root-crown diameter and number of internodes were recorded for each plant.

First-instar larvae that emerged from the eggs maintained on filter paper were transferred with a fine brush onto leaf axils, where larvae are known to enter the plant under natural conditions. To help the access of the larvae into the stem tissue, a small opening through the leaf tricomes was dug at each leaf axil with a sharp instrument. When enough neonate larvae were available, the transfers were done simultaneously on each replicate.

This first phase of the test was carried out in laboratory at 18°C to 26°C and 16:8 L/D cycle (natural and artificial light combined). Two weeks after the transfer of the first larva, each plant was enclosed in a 60- by 23-cm fine (1 mm) nylon mesh cage, supported by an inner aluminum frame and fastened around the outside of the pot with a 3-cm wide elastic band. The pots were then moved to a shade house outside the laboratory (6°C to 34°C min/max, 18.7°C mean temperature, 14:10 to 15:9 L/D conditions; April to June, 2005).

Forty days after transfer of the last larva, the cages were inspected to recover emerged adults. Such inspections were repeated every other day until the 60th day after the transfers, when all successfully developed adults were considered to have emerged. Cages were then removed and pot soil inspected for dead adults or pupae. At this time, corresponding to the full flowering stage, half the plants from each treatment (*n = 5*) were harvested, carefully cleaned and weighed, and the number and stage of the flower buds, root-crown diameter and plant height were also recorded. Harvested plants were then dissected to estimate the mean number of galleries per plant, their position and average length. After dissection, the material from each plant was put together in paper bags, dried at 65°C for 72 h in a ventilated stove and weighed.

The remaining plants (*n = 5* for each treatment) were left undisturbed to allow the completion of the life cycle and seed production. When all flower heads were senescent, plants were harvested, measured, dissected and weighed following the above described methods. In addition, the seeds from ten mature flower buds sampled from each plant were collected, counted and separated into mature and immature. A germination test in Petri dishes was carried on sub-samples of mature and immature seeds from each plant.

Adult feeding

The aim of this experiment was to assess the feeding impact of variable numbers of *P. chalcocoma* adults on fresh cut yellow starthistle leaves by measuring the leaf area consumed per day. In addition, we wanted to investigate if the presence of one or more other individuals of the same or opposite sex on the same substrate would determine any significant increase or reduction of the amount of leaf tissue consumed in a specific time by an adult. The following combinations of males (M) and females (F) were tested: 1F, 2F, 5F, 1M, 2M, 5M, 1F + 1M, 2F + 2M and 1F + 1M. Each treatment was replicated ten times, except 5M (five replicates) and 5F (three replicates).

The trials were carried out in sterile 10-cm diameter Petri dishes in a climatic cabinet (25°C, 50% to 60% RH, 16:8 L/D cycle). The insects were allowed to feed on one single freshly cut yellow starthistle leaf, laid on a moist disk of filter paper for 24 h. The Petri dishes
also contained a moist sterile cotton plug (approximately 1 cm³), a substrate to encourage oviposition.

Before each trial, leaves were carefully washed, rinsed and scanned into a TIF format digital image using a desktop flatbed scanner. At the end of each 24 h testing session, the leaves were again scanned. After each test, insects were placed back in a common container for at least one full day before insects were randomly selected for the next feeding trial. Eggs were collected daily, counted and placed in sterile Petri dishes over wet filter paper to allow hatching.

**Image analysis**

Before performing image analysis, each image was edited with Photoshop LE v.5.0 (Adobe Systems, San Jose, CA) to colour the background and the eaten areas with two uniform tints (respectively, black and red). Such edits were achieved selecting background and eaten areas of the image with the magic wand tool, adjusting the tolerance level to fit their full width and finally coloring them with the paint bucket tool.

Next, the amount of leaf area eaten by adults of *P. chalcomera* was evaluated utilizing public domain software (Image v.4.0.3.2 beta for Windows, National Institute of Health, Bethesda, MD). Scanned leaf image dimensions were converted from pixels into square centimetres using the calibration function of the software and using a strip of scaled paper that was included in all scans as reference (O’Neal *et al.*, 2002).

Once calibrated, the red channel of each image was first inverted to negative and then converted to black

![Figure 1](image-url)

**Figure 1.** The length of galleries and the number of tunnels made by larvae of *Psylliodes chalcomera* when 0, 10 or 20 first-instar larvae were transferred onto yellow starthistle (mean ± 95% CI).
and white with the threshold function of the program. The threshold level was manually adjusted to fit the actual eaten area that was finally measured with the measure function of the software.

**Field infestation rates**

*P. chalcomera* field infestation rates were assessed in May 2001 near Volna and Primorski (45°15'08" N; 36°52'17"E; elevation 5 m) on yellow starthistle, Italian thistle, *Carduus pycnocephalus* L., and nodding or musk thistle, *Carduus thoermerii* Weinm. In May and June 2003, two samples were repeated on the same population of yellow starthistle at Volna location only. At each sampling, 20 to 65 whole plants from dense patches were harvested, dissected and the numbers of larvae were recorded. In 2003, the crown diameter and height of the plants were also measured, and their fresh weight was evaluated.

**Statistical analysis**

Data were analyzed using analysis of variance for classified effects or linear regression for quantitative effects. The relationship between total gallery length and number of seeds per capitulum was modeled by using least squares nonlinear regression with the Weibull equation using the quasi-Newton estimation method in the computer program Statistica (release 5.1, StatSoft Inc., Tulsa, OK).

**Results and discussion**

**Larval transfer experiment**

Establishment of larvae, as measured by the number of tunnels, was the same whether ten or 20 larvae were transferred to yellow starthistle plants; however, the total length of tunnelling increased with the number of larvae transferred (Fig. 1). Relatively few larvae were able to complete their development up to the adult stage: A total of five and seven adults were recovered, respectively, from 20- and 10-larvae treatments, which was only 2.5% and 7% survivorship, respectively. Although some larval mortality was expected, due to the experimental conditions that required ‘overcrowding’ larvae on the plants to obtain the maximum impact, an additional cause of mortality can probably be ascribed to the dispersal behaviour of newly transferred larvae. We observed some larvae moving away from the intended insertion point on the plant and they fell from the plant and possibly died.

Larval damage (total tunnel length) did not affect wet weight, dry weight, number of buds, number of mature flowers, number of senescent flowers or total number of capitula. This is probably because (1) these plant characters are influenced by plant growth that occurred before the insect damage occurred, (2) the plants were able to compensate for the damage or (3) the damage was too small to affect the plant. However, feeding by *P. chalcomera* larvae significantly reduced the number of seeds produced per capitulum (whether or not dry

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**Figure 2.** The relationship between total gallery length, made by *Psylliodes chalcomera* tunneling in yellow starthistle, which increased with greater numbers of larvae, and the number of seeds per mature capitulum.
weight was included as a covariate). The data were fit by the Weibull equation \( Y = c \times \exp\left(-(X/a)^b\right) \), where \( c = 31.786 \pm 3.548 \) (SE), \( a = 3.839 \pm 2.465 \), \( b = 1.188 \pm 1.175 \), \( R = 0.830 \). The equation predicts that 8 cm of larval tunnelling is able to reduce seed production by 90% and 14 cm by 99% (Fig. 2). This suggests that the larval damage reduced the plant’s ability to provide nutrients to developing seeds. The number of capitula per dry weight of plant decreased slightly with increasing length of larval tunnelling (slope = -0.098 \pm 0.035 SE; \( F_{(1, 27)} = 8.0; P < 0.009 \)), suggesting that larval damage decreased resources available to develop capitula.

**Adult feeding experiment**

Females feed more than males [area eaten (1F vs 1M): females = 0.416 \pm 0.058 (SE), males = 0.027 + 0.007 cm\(^2\), \( F_{(1, 18)} = 43.7, P < 0.0001 \); and feeding scars per individual: females = 11.700 + 1.627 (SE), males = 2.300 + 0.517, \( F_{(1, 18)} = 30.33, P < 0.0001 \)] (Fig. 3). Feeding per insect was not affected by crowding at the observed levels for either males or females, when each sex was analyzed alone (slope of linear regression for area eaten or number of feeding scars per individual, \( P > 0.05 \)). However, there was a significant interaction between sex and number of insects for both response variables that was related to a tendency of feeding by females to decrease with crowding while feeding by males remained constant.

**Field infestation rates**

The infestation rate of yellow starthistle plants observed in southern Russia ranged from 36% to 90% of yellow starthistle plants sampled in May or June in 2001 and 2003 (Table 1). The number of larvae per infested plant ranged from 2.3 to 6.9. No larvae were observed in nearby plants of *C. pycnocephalus* at the Volna site, but 20% of *C. thoermari* were infested at the Primorskyi site (Fig. 4). Although *C. thoermari* plants were larger than yellow starthistle, the number of larvae per infested plant was lower. These results suggest that either *C. thoermari* is a more suitable host than *C. pycnocephalus* or that the insect populations on

![Figure 3. The effect of crowding different numbers of adult males and females on mean feeding rates per individual of *Psylliodes chalcomera* on leaves of yellow starthistle during 24 h (1M one male, 1F one female, 2F2M two females with two males etc.)](image-url)
Table 1. The infestation rate and the number of larvae of *Psylliodes chalcomera* per infested plant species observed at two sites in southern Russia (±SE).

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Host plant</th>
<th>Infestation rate</th>
<th>No. larvae/infested plant</th>
<th>No. plants sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volna</td>
<td>10 May 01</td>
<td><em>C. solstitialis</em></td>
<td>38%</td>
<td>2.33 ± 0.44</td>
<td>40</td>
</tr>
<tr>
<td>Volna</td>
<td>10 May 01</td>
<td><em>Carduus pycnocephalus</em></td>
<td>0%</td>
<td>0%</td>
<td>20</td>
</tr>
<tr>
<td>Primorski</td>
<td>11 May 01</td>
<td><em>C. solstitialis</em></td>
<td>36%</td>
<td>2.71 ± 0.48</td>
<td>50</td>
</tr>
<tr>
<td>Primorski</td>
<td>11 May 01</td>
<td><em>Carduus thoermeri</em></td>
<td>20%</td>
<td>1.20 ± 0.20</td>
<td>50</td>
</tr>
<tr>
<td>Volna</td>
<td>19 May 03</td>
<td><em>C. solstitialis</em></td>
<td>63%</td>
<td>4.34 ± 0.54</td>
<td>65</td>
</tr>
<tr>
<td>Volna</td>
<td>16 June 03</td>
<td><em>C. solstitialis</em></td>
<td>90%</td>
<td>6.91 ± 0.63</td>
<td>48</td>
</tr>
</tbody>
</table>

Figure 4. The frequency distribution of number of larvae of *Psylliodes chalcomera* attacking some Cardueae plants in the field at sites in southern Russia.

Figure 5. The relationship between number of larvae of *Psylliodes chalcomera* per plant of yellow starthistle and plant height in the field at sites in southern Russia (sampled in May and June 2003).
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these two plants may be genetically different. The latter theory is supported by observations in laboratory host plant specificity experiments and analysis of molecular genetics, which indicate at least three host-associated genetically isolated populations of this morphological species (Cristofaro et al., 2004; De Biase et al., 2003, 2004).

The number of larvae per plant increased with plant size, as represented by either plant height or root diameter (Fig. 5). Although plants were taller on the June sample than in May [64.2 ± 2.0 (SE) vs 34.1 ± 1.4 cm], sample date did not significantly affect the relationship between the number of larvae and plant height. The best-fitting regression equation was $Y = -1.01 (±0.90 \text{ SE}) + 0.111(±0.018) \times X$, where $X =$ plant height (cm) ($F_{1,111} = 39.00$, $P = 0.0001$), but the regression only explained about a quarter of the variation. Because larvae were probably too small to significantly affect plant height at the time of sampling, the results suggest that larger plants either attract higher rates of infestation or that they are able to support more larvae (which may be cannibalistic). If this tendency of finding more larvae on larger plants holds true, it is an attractive property for a prospective biological control agent because higher numbers are probably necessary to impact larger plants.

Conclusions

Feeding damage caused by larvae at the levels that we observed in the laboratory, where they produced one to five galleries and zero to two adults per plant, was not sufficient to reduce plant size of yellow starthistle plants. However, larval damage greatly reduced the number of seeds per capitulum and the number of capitula per plant biomass, which is very interesting damage to inflict on an annual plant. Transfer of about 12 larvae observed in the field was much lower (generally less than four larvae); however, it is not known whether larvae in laboratory to evaluate the larval feeding impact with infestation rates similar as the ones recorded in natural conditions.

Although adult females fed more (11.0 scars, 0.41 cm² per day) than males (2.3 scars, 0.03 cm²) and, in the field, feeding is likely to occur during 2 to 3 months in the spring, unless they have gregarious behaviour or preferentially attack young plants, this level of damage is not likely to significantly affect plant growth. Our crowding experiment showed that the adults feed less when crowded, and they generally have not been observed to aggregate on plants in the field. However, preference for or impact on young rosettes has not been studied.

References


