Biology of *Larinus cur tus* Hochhut (Coleoptera: Curculionidae), a European Weevil for Biological Control of Yellow Starthistle, *Centaurea solstitialis* L. (Asteraceae), in the United States

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The biology of the weevil *Larinus cur tus* Hochhut was studied in the field in northern Greece and in the laboratory in Rome, Italy, and in Thermi, near Thessaloniki, Greece. The species is univoltine, and adults overwinter in ground litter. Eggs are inserted into the flowers of yellow starthistle (*Centaurea solstitialis* L.) where the larvae feed mainly on developing achenes, destroying an average of over 96% of the seeds in infested flowerheads. Overwintered adults lived up to 84 days, females laid up to 70 eggs each, eggs hatched $\pm 4.2 \pm 0.6$ days after being laid, larvae required 17 to 20 days to develop through the four instars, and pupal development required 4 to 5 days under laboratory conditions.

Six percent of 360 seedheads collected on July 13 and 28, 1988 were infested with *L. cur tus* larvae and up to 89% of the larvae were parasitized. The species is recommended for the biological control of *C. solstitialis* in the United States.

**Key Words:** *Larinus cur tus*; insects; weevil; *Centaurea solstitialis*; weed; biological control.

INTRODUCTION

Yellow starthistle (YST), *Centaurea solstitialis* (Asteraceae), native to southern Europe and parts of western Asia (Maddox, 1981), has been accidentally introduced into several parts of the world, including the United States. It is spreading at an alarming rate in pastures, vineyards, and other habitats (Maddox and Mayfield, 1985), especially in the western United States. Maddox et al. (1985) reported 0.48 million hectares were infested in California in 1958 and this increased to 3.2 million hectares by 1985. Lacey (1989) reported that infestations in Idaho, Oregon, and Washington increased 315% between 1978 and 1988.

Because chemical control of the weed is not feasible nor environmentally sound, the weed has become a target for biological control. Because YST is an annual that propagates exclusively by seeds, seed feeders are desirable control agents. Promising biocontrol agents have been identified by Sobhian and Zwölfer (1985), Clement and Mimmochi (1988), and Clement (1990). A gall fly from Italy, *Urophora jaculata* Rondani, misidentified as *U. sirunaseva* Hering (White and Clement, 1987), was released in the United States between 1969 and 1977, but did not become established. To date, four other insect species, *Bangasternus orientalis* Capion (Coleoptera: Curculionidae), *U. sirunaseva* Hering (Diptera: Tephritidae), *Eustenopus villostus* (Boheman) (Coleoptera: Curculionidae), and *Chaetorellia australis* Hering (Diptera: Tephritidae) originating from Greece have been released and established in the United States (Fornasari et al., 1991; Turner et al., 1994).

Additional seed-destroying and rosette-feeding insects will probably be needed to control YST over the range of habitats in which it is a problem. *Larinus cur tus* (Coleoptera: Curculionidae) is one such natural enemy that was released for biological control of YST in the United States during 1992 and 1993. The establishment of the species has been confirmed (Turner et al., 1994). Information on the biology, distribution (Fig. 1), and host range of this species was summarized by Sobhian and Zwölfer (1985). This species is univoltine and overwinters as adults among debris on the soil surface. In a field experiment carried out in Greece with seven plant species, including safflower, artichoke, and sunflower, *L. cur tus* attacked exclusively *C. solstitialis* (Gropp et al., 1990). The weevil oviposits into the flowerheads of YST and its larvae feed on achenes. In this paper, we present further information on the biology and phenology of *L. cur tus*, and its interspecific interactions with co-occupants of YST capitula, which should be of interest to biological control workers and other scientists.

**MATERIALS AND METHODS**

**Field Studies**

Field observations were made in northern Greece during 1986 to 1989. In 1988, naturally growing wild YST
plants at the University Farm in Thernvi, near Thesaloniki, were inspected two or three times per week (starting on May 1) to determine the appearance of the first overwintered adults. To determine the best time for collecting adults in the field, YST plants in a field at Oreokastro (6 km north of Thessaloniki) were inspected during early (hottest hours) and late afternoon.

*Larinus curtus* adults must feed on YST flowers to assure development of their ovaries and egg production (Zwölfer, personal communication). Therefore, it was assumed that the first series of YST flowers opening would escape oviposition by *L. curtus*. To verify this, three samples (n = 100 each) of YST seedheads, consisting of the first, second, and third series of flowerheads to appear in the field, were collected on June 30 and July 5, 1988 near Oreokastro and examined for the presence of *L. curtus*. To determine the rate of seed consumption by *L. curtus* larvae, 500 field-collected seedheads were dissected and the numbers of seeds in attacked seedheads containing only adults, pupae, and mature larvae were recorded and compared with the number of seeds in 10 uninsestated seedheads. (Seedheads not infested by natural enemies were extremely rare.)

To determine the rate of larval parasitism and the larval instars attacked by parasitoids, two samples of seedheads were collected on July 13 and 28, 1988 near Oreokastro. On each date, one sample of mature, dry seedheads and one sample of green seedheads in the postflowering stage were collected (n = 60 to 100). The seedheads were dissected and the numbers of living and parasitized larvae were recorded.

**Laboratory Studies**

The biology and phenology were studied in the quarantine laboratory in Rome, Italy, during 1987 to 1989. The insects used in these studies originated from natural populations near Thessaloniki, Greece.

**Egg.** Observations on the preeclosion period and the degree of fertility were made with 183 eggs, less than 5 h old. The eggs were measured under a stereomicroscope and then placed in 35-cm³ plastic cups with a layer of moistened plaster of Paris on the bottom and kept in an incubator at a constant temperature of 27 ± 0.5°C and 60 to 70% relative humidity in the dark. The numbers of hatched and infertile eggs were recorded daily.

**Larva, pupa, and adult.** Larval development was studied by transferring 400 neonate larvae to YST flowers and dissecting the flowerheads at regular intervals. Number, instar, and size of larvae as well as pupae found were recorded. To verify the time required for development from egg to pupa and adult, six potted YST plants were caged with adults, using black Tulle sleeve cages. Forty adults were caged on each plant (sex ratio 1:1) for 5 h. Flowers in which oviposition occurred were labeled and then dissected at various intervals to follow the development of the immature stages. Six hundred-eleven flowerheads or seedheads were dissected. Pupae found inside cells were checked daily to determine the time needed for development to adult.

**Fertility and longevity of adults.** Adult longevity and fertility were studied in a quarantine glasshouse under natural light. Temperature and humidity were recorded
with an hygrothermograph. Overwintered adults were field-collected at Oreokastro, Greece, as soon as they appeared on YST in June. Ten weevil pairs were kept separate (one male and one female per cage) in transparent plexiglass tubes, 7 cm in diameter and 10 cm long, and covered with a fine-mesh nylon screen on top to allow air circulation. Three YST flowers were placed in each tube; the stems were inserted into a water-filled vial through a hole at the bottom of the cage. Flowers were replaced daily, and the number of eggs, as well as dead adults, was recorded. Dead females were dissected to record the number of eggs remaining in their ovarioles.

**Oviposition into infested and uninfested flowers.** During 1989, an experiment at the University Farm of Thessaloniki was carried out to determine whether ovipositing *L. curtus* females would discriminate against flowers containing larvae of *B. orientalis* or *C. australis*. *Centaurea solstitialis* rosettes (*n* = 21) were field-collected on April 10 and transplanted into three screen cages (1 × 1 × 1 m, seven plants per cage) erected on May 12, before the plants had produced flower buds. Ten unsexed *B. orientalis* adults were put into one of the cages on May 30, while *C. australis* adults (five females and two males on June 26, five females and four males on June 30, and four females and five males on July 1) were released into the second cage. The sex ratio of field-collected *B. orientalis* adults during late May and early June was 1:1 (Sobhian et al., 1992). Thus, it was expected to have released five females in the cage, sufficient for obtaining a large number of eggs on the plants. The third cage contained uninfested plants. Depending on availability, three to five flowers infested with *B. orientalis* and the same number of uninfested flowers were offered to two pairs of *L. curtus* for oviposition (replicated 10 times). One-liter transparent plastic containers with two 5-cm holes covered with screen cloth were used as test containers. Bouquets of infested flowers and uninfested flowers, kept in vials with water, were placed in the containers. The bouquets were replaced daily and checked for eggs.

Flowers containing *B. orientalis* eggs (*n* = 40) were examined under a stereomicroscope and only those on which at least one *B. orientalis* larva had hatched and penetrated into the plant tissue were selected for the test. For *C. australis*, the presence of egg chorions under the bracts of a flower was used as an indication of infestation. The test with *B. orientalis* was started on July 3 and stopped on July 19; that with *C. australis* was started on July 12 and ended on July 19.

**RESULTS AND DISCUSSION**

**Field Observations**

**Life history.** During 1988, the first overwintered adults (Fig. 2) appeared on YST plants at the Thessaloniki University Farm on May 14. However, the appearance of adults on YST plants in the spring varied from year to year, probably due to temperature changes. On June 7, 1988, 110 adults (34 females and 76 males) were collected on YST plants near Oreokastro. All were found singly; no copulating pairs were observed. A second sample of 235 adults (96 females and 139 males) was collected at the same location on June 15 when copulating pairs were present. During 1989, only 1 adult was found on May 26 and 2 on June 15, during 90 and 120 min of search at the same location, but adults were common by the end of June. Early collections yielded more males than females, which indicates that the males probably appear on the plants earlier than females or are more active and therefore are more easily found. The sex ratio of field-collected adults was about 1:1 (*n* = 97) in late June and early July 1989. In two cases, males of *L. curtus* were found trying to copulate with *E. villosus* females that were preparing oviposition holes in YST flower-buds.

The last adults of the overwintered generation, a copulating pair, were observed on YST plants at the University Farm on July 28, 1988. The first adults of the new generation (*n* = 4) emerged on August 2, from a sample of about 1000 YST seedheads (early flowers) collected on June 13 at Oreokastro and kept in a screen bag in the laboratory. These data suggest that the life span of adults is about 1 year. Adults of the new generation were never observed feeding before the following spring. During the oviposition period, adults spent most of their time on YST flowers on which they fed, copulated, and oviposited. Observations during the hottest part of the day in July (over 35°C) showed that most of the adults left those flowers exposed to direct sun and rested in shady places, generally on the plants.

On July 30, at 08:30 h, the YST plants at the University Farm were checked for adults. Ten females and 13 males were found on or partly in the flowers. At 15:30 h on the same day, the same plants were checked. Only 2 females, 1 male, and 1 unsexed adult were found. There was little opportunity to collect adults during the warmest hours of the day when the temperature was over 30°C. On June 27, 1989, only 1 adult could be collected on YST plants (in Oreokastro) from 13:30 to 14:00 h, when the temperature was over 30°C, whereas 38 adults were collected from 19:40 to 20:10 h during the cool hours of the same day (24–25°C). However, when the temperature remained below 30°C, the adults stayed on or near flowers and flower buds and could be collected throughout the daylight hours. These data show that temperature influences the behavior of *L. curtus*; this is important for selecting the right time for field collections. *Larinus curtus* adults are good flyers and normally fly from one flower to another and from plant to plant.

**Oviposition behavior.** Before oviposition, females prepare a niche among the florets and then insert their
FIG. 2. Adult of *L. curtus* on yellow starthistle flowerhead.

FIG. 3. Protective cover on the egg of *L. curtus*. 
FIG. 4. Mature larva of *L. curtus* in an opened seedhead of yellow starthistle with head capsules of previous instars. Seeds have been completely destroyed.

FIG. 5. Pupa of *L. curtus* in an opened seedhead of yellow starthistle. All seeds have been consumed.
abdomen to lay an egg a few millimeters above the receptacle. The eggs are then covered with a protective case prepared with chewed florets and attached to an untouched floret. The preparation and oviposition take about 15 min. Not more than one egg per flowerhead was found.

On July 8, at 11:45 h, one female was found on a YST flower. Her abdomen was inserted into the flower so that only the thorax and head were visible. Five minutes later, she withdrew and put her head into the flower. After 8 min she turned around and put her abdomen again into the flower and stayed motionless for 5 min in this position (only the thorax and head were visible). Finally, she left the flower and walked down the stem a few centimeters, rested 3 to 4 minutes, and flew to the tip of a branch that did not have a flower. After a few seconds, she flew to an open flower on which there was a copulating pair, put her head into the flower, and appeared to be feeding or preparing another oviposition cell.

The first flower on which it was assumed that the female had oviposited was dissected under a stereomicroscope. An egg was found in a small, round, thin-walled cell (Fig. 3) among the outer rows of florets. The apical part of the cell was covered with a black mucus-like substance. Examination of 26 flowers containing eggs showed that the females always prepare an egg cell and cover the top of it with a dark brown to black substance. This egg cell probably serves for protection. The neonate larva leaves the egg cell through its base and starts feeding on the white, soft achenes.

No larvae were found in the first series of flowerheads collected on June 30, whereas 2% of the second series and 12% of the third series of flowers were infested by *L. curtus*.

**Seed consumption.** Larvae fed inside the seedheads, mainly on achenes and receptacles, and also consumed some of the pappus hairs. Only the basal portion of the receptacle remained, which protected the pupa. The total number of seeds in 19 seedheads infested only by *L. curtus* was 40 (\(\bar{x} 21 \pm 2.4\)), whereas the total number of seeds in 10 uninested seedheads, from a sample of 500, was 537 (\(\bar{x} 53.7 \pm 10.4\)). Comparing the two means, the total rate of seed consumption per larva per attacked seedhead was over 96%. Because YST propagates exclusively by seeds, this damage should be very effective in reducing the propagation of the plant. Mature larvae of *U. sirunasevera* and *L. curtus* were occasionally found in the same YST seedhead.

**Larval parasitism.** Larvae were attacked by unidentified parasitoids. In two samples of green seedheads in postflowering stage collected on July 13 and 28 (n = 100 and 60), 13 living and 2 parasitized larvae were found. In two samples of mature (dry) seedheads collected on the same dates and at the same location (n = 100), 1 living and 8 parasitized larvae were found. The rate of parasitization of larvae in green and mature seedheads was thus about 13 and 89%, respectively. These figures suggest that the parasitoids mainly attack mature larvae.

**Laboratory Studies**

**Egg.** Eggs are glossy, milky-white, and oval. The average width was 0.83 ± 0.032 mm and average length was 1.3 ± 0.066 mm (n = 20). The time from oviposition to eclosion was on average 4.2 ± 0.56 days (n = 183). Eighty-five percent of eggs hatched under these conditions.

**Larva, pupa, and adult.** *Larinus curtus* had four larval instars under laboratory conditions (Fig. 4). Development from egg (n = 40) to the pupal stage took on average 23.2 ± 1.5 days (n = 32), with a mortality of 20% (\(\bar{x} 23 \pm 2^\circ\)C; 60 ± 17% RH). Development from first instar larvae to the pupal stage took 17 to 20 days (n = 32). Newly formed pupae (Fig. 5) were white and became light yellow after 24 h. Forty-four hours after pupation the pupae were dark yellow and 24 h later melanization of the eyes had started. Development from pupae (n = 20) to the adult stage took on average 4.4 ± 0.5 days (n = 19) (\(\bar{x} 22 \pm 5^\circ\)C; 61±18% RH). Mortality during the development of pupae to adults was 5%. Newly emerged adults were yellowish and melanization occurred within 36 h (Fig. 2). Development from oviposition to emergence of adults took about 28 days under the above-mentioned conditions.

**Longevity and fertility.** A test was started on July 26 and ended on October 17, 1989, when the last adult, a male, died. At \(\bar{x} 20 \pm 5^\circ\)C (range 7–32°C) and relative humidity of \(\bar{x} 61 \pm 17%\) (range 26–87%), the females laid on average 1.33 ± 1.02 eggs per day, ranging from zero to five eggs per day. The rate of fertile eggs was 60.7%. Females stopped ovipositing on an average 12.8 ± 7 days before they died (Table 1). Eggs laid were fertile throughout the entire oviposition period, except for a few unfertile eggs laid shortly before females died. The last fertile egg was laid on September 6 and the adults died during September and October, 1989. Considering that these adults had emerged during August, 1988, their longevity was about 13 months. The longevity of the males was slightly higher than that of the females. Under laboratory conditions, the mean longevity of field-collected, overwintered beetles was 44.3 ± 14.7 days for females (n = 10), ranging from 13 to 60 days, and 46.3 ± 21.6 (n = 10) days for males, ranging from 16 to 77 days.

Females oviposited in open flowers at a temperature of 26 to 27°C. Usually one egg per flowerhead was laid. However, when sufficient numbers of flowers were not available more than one egg (up to five) was laid in one flower. The long oviposition period of the weevil covers almost the entire flowering period of its host plant. Other studies (Fornasari, unpublished data) showed that *L. curtus* preferred North American plants to control plants from Greece for oviposition.
TABLE 1

Results of Fecundity and Longevity Tests on *Larinus curtus* Conducted in Rome from July 26 to October 17, 1989

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Mean No. eggs laid per % per day (n)**</th>
<th>Total No. eggs laid per %</th>
<th>Percentage fertile eggs</th>
<th>No. days with no oviposition before death</th>
<th>No. eggs in ovarioles at dissection</th>
<th>Longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.86 ± 0.91 (21) [0-3]</td>
<td>18</td>
<td>44.4</td>
<td>10</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>1.17 ± 0.75 (4) [0-2]</td>
<td>7</td>
<td>57.1</td>
<td>24</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>1.00 ± 0.59 (7) [0-2]</td>
<td>9</td>
<td>66.6</td>
<td>3</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>1.03 ± 0.74 (39) [0-3]</td>
<td>40</td>
<td>40.0</td>
<td>11</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>1.59 ± 1.09 (46) [0-4]</td>
<td>70</td>
<td>0.0</td>
<td>9</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>1.59 ± 1.04 (37) [0-3]</td>
<td>59</td>
<td>74.6</td>
<td>13</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>1.40 ± 1.12 (47) [0-5]</td>
<td>66</td>
<td>86.4</td>
<td>8</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>8</td>
<td>0.71 ± 0.76 (28) [0-2]</td>
<td>19</td>
<td>8.9</td>
<td>27</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>1.64 ± 0.69 (33) [0-3]</td>
<td>54</td>
<td>85.2</td>
<td>7</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>10</td>
<td>1.59 ± 1.28 (44) [0-5]</td>
<td>70</td>
<td>77.1</td>
<td>15</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>Mean**</td>
<td>1.34 ± 1.02 (30) [0-5]</td>
<td>49.5 ± 21.5</td>
<td>60.7</td>
<td>12.8 ± 7.391</td>
<td>0.5 ± 1.269</td>
<td>46.8 ± 22.4</td>
</tr>
</tbody>
</table>

** Oviposition days.
* Oviposition period very short; see column for oviposition period.
† Died very early.
* Calculated on daily observations and not averages.

Mating was observed only during June and July. The duration of copulation was very variable (a few minutes to four hours). Many attempts to copulate were observed before actual mating occurred.

*Host selection.* In the oviposition experiment offering flowerheads infested with *B. orientalis* larvae and insect-free flowers, *L. curtus* apparently did not discriminate between infested and healthy flowers, with 46 and 47 eggs being laid, respectively. Dissection of the flowerheads showed that at the time they open and are used by *L. curtus* for oviposition, *B. orientalis* larvae are feeding mainly in the bracts rather than in the flowerheads. The distribution of eggs on the flowerheads used in the test was quite variable. Between 2 and 6 eggs were laid in a flowerhead, whereas 1 or 2 of the flowerheads remained uninested. The distribution of eggs under natural conditions is probably different. In 31 flowerheads infested with *C. australis* eggs, 5 eggs were laid, and in 31 uninsected flowerheads only 2 eggs were laid. Again, there was no indication that *L. curtus* females preferred uninsected flowerheads for oviposition.

Considering the information presented, the information reported by Groppe et al. (1990) on host specificity, and the extensive host specificity tests carried out by Fornasari and Turner (1994), we conclude that *L. curtus* is a promising agent for biological control of *C. solstitialis*. Also, other unpublished studies by Fornasari showed that North American plants were even preferred by *L. curtus* to the European control plants from Greece. The long oviposition period of the weevil covers almost the entire flowering period of its host plant. Since YST propagates only by seeds and up to 96% seed consumption by one larva in a flowerhead is very effective in reducing seed production, it is expected that in the absence of its parasitoids, once introduced in North America, the weevil should build up high population densities and might play a major role in reducing weed density. The results of the study also lead us to the assumption that several biological control agents that attack the flowerheads in various developmental stages (such as *U. sirius*, *B. orientalis*, *C. australis*, and *E. villosus*) are essential for biological control of YST.

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