Field and laboratory observations of the life history of the Swiss biotype of *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae)


**Summary**

The Italian biotype of ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae), is considered the most important biological control agent for the suppression of tansy ragwort, *Senecio jacobaea* L. (Asteraceae), in Mediterranean climates. Repeated attempts to introduce this beetle into colder climates have failed to establish populations capable of weed control. The spread of tansy ragwort into the northern Rocky Mountains of Montana prompted a reexamination of the cold-adapted biotype of *L. jacobaeae* reported from Switzerland. A detailed field and laboratory examination of *L. jacobaeae* life history from naturally occurring populations in central Europe was conducted. Adult flea beetles, first collected in late June, started oviposition 2 weeks after emergence from pupae and reached peak oviposition rate after 4 weeks. Over-wintering occurs in the egg stage, as diapause delayed hatch until spring. Larvae initially fed within the leaf tissues in early spring and moved into the root crowns later in the season. Over 70% of second-year plants dissected were infested with larvae in naturally occurring field populations. *L. jacobaeae* from Switzerland were found to be phenologically adapted to continental climates and were released in Montana starting in autumn 2002.

**Keywords:** *Senecio jacobaea*, Montana, ragwort flea beetle.

**Introduction**

The ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae), is a specialist herbivore that feeds on the invasive weed tansy ragwort, *Senecio jacobaea* L. (Asteraceae), a biennial herb native to Eurasia (Frick, 1970). *L. jacobaeae* populations originating in Italy are considered the cornerstone of the successful biological control of tansy ragwort in the Pacific Northwest west of the Cascade Mountains (Hawkes and Johnson, 1978; McEvoy and Coombs, 1999). *S. jacobaea* population densities were reduced by 99% in the first 4 years near Fort Bragg, California as a result of root feeding by *L. jacobaeae* larvae (Hawkes and Johnson, 1978). The discovery of a major infestation of tansy ragwort in northwestern Montana rekindled interest in the biological control of this noxious weed. The previous success of *L. jacobaeae*, along with its compatibility with other biological control agents released in Montana (Hawkes and Johnson, 1978; Markin, 2003), made introduction of this agent a high priority. However, repeated attempts to establish populations from Oregon into Montana from 1998 to 2002 met with little success (Markin, 2003). Italian *L. jacobaeae* adults collected from Oregon emerge from pupation in the spring, aestivate throughout the summer and await autumn rains to start reproduction and larval development. Adaptations for Mediterranean habitats may limit the ability of the Italian strain to increase populations in colder climates.

This study was initiated to explore the suitability of populations of *L. jacobaeae* from north-western Switzerland for introduction in Montana. Comparisons of the autumn-breeding Italian and summer-breeding Swiss flea beetle strains were conducted under laboratory and greenhouse conditions in Rome, Italy.
Field and laboratory observations of the life history of the Swiss biotype of *Longitarsus jacobaeae*

(Frick, 1971; Frick and Johnson, 1972, 1973; Windig, 1991). However, over-wintering traits were never field-validated, and the mechanisms of winter survival of the Swiss strain were merely speculated (Windig and Vrieling, 1996). Further, descriptions of egg diapause may have contained experimental artifacts because of interbreeding of strains in the laboratory (Frick and Johnson, 1972). Our first objective was to determine the over-wintering life stage of the Swiss flea beetles in their native habitat and to clarify the patterns of egg diapause under controlled conditions. We then examined the distribution and development of Swiss *L. jacobaeae* under field and greenhouse conditions to determine if their life history traits may better suit this strain to the short summer/cold winters found in Montana’s continental climate.

**Materials and methods**

Studies on the ecology and seasonal phenology of *L. jacobaeae* were conducted during the summers of 2000 through 2002 at four field sites in Switzerland: L’Himelette, St. Imier, Mervilier, and Mettembert (Table 1). Sites were located on gently to moderately steep, south to south-western facing slopes in seasonally grazed pastureland. The plant community was dominated by grasses, with mixed forbs occupying less than 35% cover. Tansy ragwort occurred in scattered patches at densities of up to 30 plants per square metre. Snow cover varied between years but extended into mid-March only at the high-elevation L’Himelette site when spring phenologies were observed in 2001. Laboratory experiments were conducted at the Biological Control Containment Facility at Montana State University, Bozeman, MT. After approval for release was granted for the Swiss populations in 2002, further garden and greenhouse experiments were conducted at the USDA Forest Service-Rocky Mountain Research Station in Bozeman, Montana.

**Field observations**

Adult emergence from pupation was determined through weekly vacuum samples carried out in Switzerland prior to and during the adult emergence from pupation in June–July 2001. The first field-collected adult ragwort flea beetles were used to determine the starting date of oviposition from different populations. Adults were sexed, and paired beetles were placed in 1-L clear plastic cylinders with fresh-cut *S. jacobaea* leaves inserted in 2-cm cubes of moistened floral foam placed on top of a 90-mm filter paper. Five replicates were used for each population. Every 2 to 3 days, beetles were transferred to clean containers with fresh food, and cylinders, leaves, paper, and foam were inspected for eggs.

The spatial and temporal aspects of *L. jacobaeae* larval biology of Swiss populations were investigated in 2001. Plant samples were initially collected March 15 and then, starting April 15, every 2 weeks from the four sites. The last sampling period was July 27 at the lower elevation sites and August 11 at L’Himelette. For each site, ten samples were collected along a wandering transect starting at a randomly selected point in the pasture. Samples included a tansy ragwort plant and all of the soil and other plants within a 10-cm radius. Samples were removed using a spade and hand trowel and stored individually in a plastic bag at 2°C until dissection.

*S. jacobaea* plants from each sample were categorized into five demographic groups: seedling (<5 leaves), rosette, multiple rosettes (attached to a single root crown), bolting, and flowering. Plants were dissected, and the locations of larvae and determination of the larval instars were recorded. Larval instars were determined according to the morphology and color of the anal sclerites and the color and width of the head capsule (Newton, 1933; Windig, 1991).

**Laboratory experiments**

Two experiments were conducted during the winter of 2002 to 2003 to investigate adult emergence from pupation. The first experiment utilized neonate larvae from St. Imier inoculated on plants maintained in the greenhouse, while the second experiment was conducted at ambient outdoor temperatures with eggs from Mettembert.

Tansy ragwort plants were grown from seeds for 3 months in 3-L plastic pots in the greenhouse with a 14-h photoperiod, and average temperatures of 25°C (day) and 20°C (night). Medium-sized rosettes (average of 17.7 leaves) were randomly assigned *L. jacobaeae* density treatments (0, 5, 10, 15, 20 or 30 eggs or larvae per plant) in a complete block design with five replicates. An additional treatment of five eggs or larvae was initiated as part of each block to be destructively sampled

**Table 1.** First 2001 vacuum collections of adult *Longitarsus jacobaeae* and onset of oviposition for field-collected adults from four field sites in Switzerland.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates</th>
<th>Elevation (m)</th>
<th>Adult emergence</th>
<th>Oviposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mettembert</td>
<td>47°24' N, 7°20' E</td>
<td>640</td>
<td>23 June</td>
<td>2 July</td>
</tr>
<tr>
<td>Mervilier</td>
<td>47°24' N, 7°18' E</td>
<td>660</td>
<td>29 June</td>
<td>4 July</td>
</tr>
<tr>
<td>St. Imier</td>
<td>47°09' N, 6°59' E</td>
<td>800</td>
<td>7 July</td>
<td>10 July</td>
</tr>
<tr>
<td>L’Himelette</td>
<td>47°08' N, 7°01' E</td>
<td>1160</td>
<td>13 July</td>
<td>17 July</td>
</tr>
</tbody>
</table>
to determine larval developmental stage. Larvae were transferred directly to plants; while cold-treated eggs (minimum 60 days at 2°C) were transferred to the base of the rosettes on blotter paper and covered with moist peat moss.

Plants for the second experiment were acclimatized for 3 weeks (average 10°C) until the season’s first persistent snowfall on December 2, 2002. Plants were moved to prepared garden plots immediately after application of egg treatments, covered with snow, and maintained outdoors until July when larval development was nearing completion.

After the larvae from destructive samples from each experiment reached the late third instar and pre-pupal stages, all test plants were placed in opaque emergence cages. As adult beetles moved into clear collection vials, the date of emergence was recorded. At the end of the experiment, the bags were inspected for adult beetles that had not moved into the vials.

The time required for eclosion of eggs of the Swiss biotype from the St. Imier population was investigated to determine the period of egg diapause. Five replicates of ten apparently viable eggs from a single collection date were placed on filter paper in 90 mm diameter Petri dishes loosely sealed with paper towels. Dishes were kept moist by applying water on the outer portion of the paper towel and stored in plastic bags to maintain humidity.

Treatments varied in length of exposure to cold. The zero day treatment was immediately placed in the growth chamber held at a constant temperature (20.0 ± 0.5°C) with a 14-h photophase. All other treatments were cooled to 2 ± 1°C and stored for 20 to 180 days without light. At 20-day intervals, dishes were removed from cold storage and placed in the growth chamber. Eggs were observed daily for hatch or signs of mortality, and all hatched and dead eggs were removed. Remaining viable eggs were returned to the growth chamber.

Statistical analysis was conducted using Minitab® v. 12 (Minitab Inc., 1998) with α = 0.05. One-way analysis of variance (ANOVA) was used to compare plant demographic groups for larval feeding damage and infestation rates. Tukey’s pairwise comparisons of demographic groups were analyzed using a family α = 0.05 (individual α = 0.0066). For both the garden and greenhouse adult emergence experiments, data were pooled across all inoculation levels and tested for normality with the Anderson–Darling normality test. The non-parametric Kruskal–Wallis test was then used to compare egg and larval inoculation treatments for rates of adult emergence. In the egg diapause experiment, the mean egg hatch from each replicate represented samples of the larger population and were normally distributed (A² < 0.603, df > 4, P > 0.05). We compared cold treatments using the calculated parameters of each replicate in the one-way ANOVA model. Simple linear regression analysis was used to describe change in eclosion rates. Comparison of regression lines were accomplished using paired t tests.

Results

Field observations

Adult flea beetles emerged in early summer in the 2001 field surveys in Switzerland (Table 1). The first adult L. jacobaeae flea beetles were collected during the third week of June at 600 m sites. Adult emergence from higher elevations was delayed approximately 1 week for every 200 m of increased elevation (Table 1). Newly emerged adults continued to be collected for a period of 2 to 3 weeks at low and medium elevations. Beetles from the highest elevation site emerged over a longer period, possibly due to variation in egg hatch caused by delayed snowmelt in microhabitats shaded by nearby conifer trees. First adult emergence from the St. Imier and L’Himelette sites occurred approximately 120 days after the collections of neonate larvae in March 2001. Our results are at least a month later than those reported by Frick (1971), but beetles transported from Switzerland to Rome, Italy, and Albany, CA, may have started development sooner due to exposure to lower elevations and earlier warm weather.

Adult flea beetles collected in Switzerland began oviposition within 2 weeks of the first adult collections (Table 1). Populations from higher elevations were first collected later in the season and had a corresponding later onset of oviposition. After the initial onset of oviposition for each population, additional collections of adults commenced oviposition immediately following transport to the laboratory.

Larval feeding damage was apparent in 72% of all plants dissected from Swiss field sites in 2001, although larvae were recovered from only 37% of these plants (Figure 1). Larval presence varied significantly among plant demographic groups (F₄,₉₀₉ = 5.40, P < 0.001), with seedlings and flowers having fewer larvae than rosettes, multiple-rosettes and bolting plants (Figure 1). Seedlings comprised 15% of the plants examined but were damaged significantly less than other plants despite their availability in all sampling periods (Figure 1). Only first-instar larvae were recovered from seedlings, possibly because these plants were too small to sustain larval development beyond this stage. Bolting plants occurred later in the season (May 1 at lower elevations) and were predominantly infested with third larval instars. Flowering plants were first collected on June 28 at lower elevations, coinciding with the beginning of adult emergence at these sites (Table 1).

Larvae of the Swiss L. jacobaeae had a distinct spatial distribution pattern within the plant that changed over time as the larvae and plants developed (Figure 2). Almost all early-season larvae (94.7%) were found in the basal rosette leaves of S. jacobaea. Freshly hatched larvae entered the plant through the leaf blade and fed...
Field and laboratory observations of the life history of the Swiss biotype of *Longitarsus jacobaeae*

between the epidermal layers as they moved toward the leaf veins and petiole. Once the first instars reached a leaf vein, they continued to feed downward to the base of the petiole. It was common to find more than one larva in a single leaf, with their feeding tunnels intertwined in the petiole. Later-season larvae moved into the upper root crown (Figure 2). A small percentage (6.1%) of the larvae of all instars was found within the base of stems of bolting plants; however, there were no larvae or signs of larval feeding damage in stems above the lowest stem leaf or approximately 2.5 cm above the basal rosette. The majority of third-instar larvae (83.5%) were found within the root crown. Larval feeding on the root crown occurred in the root cortex. Larvae were rarely collected from the roots (0.43% of total), and most damage to roots occurred only 1 cm away from the root crown as third-instar larvae exited the plant for pupation in late spring.

**Laboratory experiments**

*L. jacobaeae* larvae raised under ambient conditions in the garden in Montana completed their development 130.7 ± 4.8 days (*n* = 41) after snowmelt, with mean
adult emergence on July 20, 2003. Adult emergence occurred over a period of 3 weeks (Figure 3), and the time required for emergence did not differ between egg-density treatments (Kruskal–Wallis test, $H = 1.71, df = 4, P = 0.788$). There were also no significant differences in percent of adult emergence from different-density egg inoculation treatments (Kruskal–Wallis test, $H = 3.90, df = 4, P = 0.450$). Development rates observed in Montana were slightly longer than the average development rates observed in Switzerland but were well within the range observed at higher elevation sites.

Second-generation St. Imier beetle larvae placed directly on greenhouse plants completed their development in an average of 80.6 ± 0.1 days ($n = 61$) after inoculation. These beetles emerged over a period of 21 days (Figure 3). There was no significant differences in percent of adult emergence among treatments (Kruskal–Wallis test, $H = 1.91, df = 4, P = 0.752$). The time required for complete larval development corresponded with observations by Frick (1971), who found that laboratory-reared larvae required an average of 80 days to complete development.

The Swiss ragwort flea beetles have been reported to have a facultative egg diapause (Frick, 1971). In our study, eggs of the Swiss biotype hatched after an average of 65.1 ± 2.9 days without exposure to cold temperature when kept at a constant 20°C (Figure 4), but the eclosion period was reduced after exposure to cold temperatures. The time required for eggs to hatch was negatively correlated with time of exposure to cold over the first 60 days ($y = -0.733x + 67.068, R^2 = 0.938, P \leq 0.001$). Time to eclosion continued to decrease for cold treatments of 80 days or more but at a more gradual rate ($y = -0.0516x + 19.995, R^2 = 0.532, P < 0.001$). We estimated that diapause was completed after 69 days of cold exposure at the point that the

![Figure 3](image-url)

**Figure 3.** Adult *Longitarsus jacobaeae* emergence from the populations raised in the greenhouse (from St. Imier, $\bar{x} = 11.6 \pm 0.8$ days, $n = 61$ adults) and under open-field conditions at Bozeman, MT, in 2003 (from Mettembert, $\bar{x} = 6.7 \pm 0.1$ days, $n = 41$ adults).

![Figure 4](image-url)

**Figure 4.** Mean incubation period (per replicate) for Swiss *Longitarsus jacobaeae* eggs at 20°C with 14 h photophase after removal from cold treatments (2 ± 2°C). The regression lines are significantly different ($t = 17.31, df = 46, P < 0.0001$).
regression lines intersect (Figure 4; significant change in regression lines: $t = 17.31, df = 46, P < 0.0001$).

**Discussion**

We found that Swiss populations of *L. jacobaeae* have several life history traits that make them suitable candidates for biological control in cold continental climates. Adult beetles are reproductively active throughout the summer. They lay diapause eggs that persist through the winter and are ready to hatch in early spring. The larvae attack the rosette plants just as they are recovering from winter stress. Larvae of Swiss *L. jacobaeae* inhabit fresh leaves in early development and then move into the root crowns during the second and third instars. Damage caused by larval feeding affects plant growth and may reduce reproductive output of their host plant. Large numbers of larvae have been recorded from tansy ragwort in open-field host tests and can cause mortality at high densities (Hawkes, 1968; Puliafico, 2003).

The ragwort flea beetles collected in Switzerland differed in several important life history traits from those originally studied by Frick (1971). No signs of over-wintering larvae or pupae were found during extensive field collections and observations during the 3 years of this study. Larvae of the Swiss populations demonstrated distinct spatial partitioning of their host plants as they develop, with first and early second-instar larvae almost exclusively inhabiting the foliage and above-ground portions of the plant and most third-instar larvae found only in the root crowns. Finally, adult emergence was recorded at the end of June and first 2 weeks of July in their native habitats in Switzerland. Almost all of the differences between our results and those originally published by Frick (1971) can be attributed to laboratory artifacts caused by raising cold-adapted Swiss flea beetles under conditions better suited for their Mediterranean counterparts from Italy. We also found strong evidence of egg diapause broken by extended cold treatment. Crossbreeding of Swiss and Italian strains (Frick and Johnson, 1972) may have contributed to some of the inconsistencies between our egg eclosion data and those previously reported. Much of the confusion caused by these laboratory results has been repeated throughout the subsequent literature (e.g., Hawkes and Johnson, 1978; Windig, 1991; Coombs et al., 1999). Clarification of Swiss *L. jacobaeae* life history traits will improve the ability to establish these beetles for biological control, while increasing our understanding of their potential impact on tansy ragwort infestations in colder continental climates.

**Acknowledgements**

The authors would like to acknowledge the technical assistance of A. de Meij, Y. Wang, E. Reneau, M. Statsney, S. Teyssiere and A. Ordoniz. Thanks to K. Marske, M. Julien, and P. Hatcher for comments on an earlier draft. This study was funded by the Montana Noxious Weed Trust Fund and the USDA Forest Service-Rocky Mountain Research Station.

**References**


