Overwintering Ability of *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) in Southern Ontario, Canada

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**ABSTRACT**

Survival after cold chilling and spring emergence of pea leafminer, *Liriomyza huidobrensis* (Blanchard), were evaluated in both the field and laboratory in 2001 and 2002. Spring emergence was evaluated through the use of traps placed in various fields and hoop-houses that had high populations of pea leafminers the previous season. No pea leafminer adults were captured in spring emergence cages during either year. To evaluate cold chilling in the laboratory, a factorial design with three temperatures (approximately −5, 0, and 5°C), eight exposure periods (0, 1, 2, 4, 8, 16, 32, and 64 d), and four replicates was employed. Pupal mortality increased as the length of exposure increased, with no survival after 64 d of chilling at any temperature. Chilling exposure periods in the laboratory were compared with the number of days per month that soil temperatures in the study area fell below 5, 0, and −5°C. No field-collected pupae exposed to winter field conditions survived until the spring. The pea leafminer seems to be unable to overwinter in southern Ontario, except in protected areas.

**KEY WORDS** pea leafminer, *Liriomyza huidobrensis*, overwintering, cold chilling, emergence

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The pea leafminer, *Liriomyza huidobrensis* (Blanchard), is a highly polyphagous pest that was first found in southern Ontario at the Holland Marsh in 1998 (McDonald et al. 2000). Pea leafminer can cause direct damage to the photosynthetic tissue of host plants caused by larval leaf mining and esthetic damage caused by oviposition and feeding punctures produced by adult females (Spencer 1973). Local crops experiencing damage include lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), celery (*Apium graveolens* L.), Asian crucifers (*Brassica* spp.), greenhouse ornamentals, greenhouse cucumbers (*Cucumis sativus* L.), and onions (*Allium cepa* L.).

Cold hardness or tolerance refers to the capacity of an organism to withstand exposure to low temperature with varying survival depending on species, developmental stage, season, nutritional status, and duration of exposure (Lee 1989). It is unknown whether pea leafminer experiences diapause (Zhao and Kang 2000); however, pupae and adults of *Liriomyza* spp. are more likely to survive adverse winter conditions than eggs or larvae within plant residue (MacDonald and Walters 1993). Despite sporadic adult sticky card captures in late May and June, pea leafminer populations peak during late August or early September in the Holland Marsh region (44°51′ N, 79°60′ W) of southern Ontario. Late season population peaks and crop damage may reflect a lack of overwintering ability with population replenishment from greenhouses in the spring or very low winter survival.

Knowledge of the overwintering location of pea leafminer will help determine control measures that can be implemented in the spring to prevent field populations from reaching economically damaging levels. The purpose of this research was to determine if field-collected pea leafminer pupae were capable of surviving temperatures comparable with winter soil temperatures at the Holland Marsh and to determine if pea leafminer overwinter in the field and/or in unheated hoop-houses.

**Materials and Methods**

**Insect Collection.** Mature celery, cultivar ‘Florida 683’, plants with extensive pea leafminer damage were collected from field plots at the University of Guelph Muck Crops Research Station, Kettleby, Ontario, Canada, on 25 September 2001 and 30 September and 7 October 2002. Plants were suspended by the base from metal racks over waxed paper. Mature larvae emerged from the leaf tissue and dropped to the waxed paper where they pupated. Three to 4 d after suspension of the celery, the pupae were collected and placed into a cooler at 9°C to minimize further development. Collections in 2002 resulted in few pupae from celery; therefore, pupae were collected concurrently from spinach.

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Soil Temperatures. Average daily soil temperature at 5 cm depth was obtained from the Muck Crops Research Station weather station data for the years 1998–2002. The number of days per month that soil temperatures fell below 5, 0, and −5°C were calculated for comparison with exposure periods used in controlled environment trials.

Controlled Environment Trials. In 2001, the experimental set up was a factorial design with three temperatures (−5, 0, and 4°C), eight exposure periods (0, 1, 2, 4, 8, 16, 32, and 64 d), and four replicates of each treatment. Each treatment replicate consisted of 20 pupae placed into a ventilated, 7-dram vial containing moist filter paper. A similar design was employed in 2002, with three temperatures (−6.5, 0, and 7.5°C), eight exposure periods (0, 1, 2, 4, 8, 16, 32, and 64 d), two hosts (spinach and celery), and two replicates of each treatment. Temperatures in 2002 varied from those in 2001 because of differences in equipment capabilities. In both years, control pupae were held at 24 ± 1°C with a 16-h photoperiod. At the end of each exposure period, treatment vials were placed into the same conditions as the control vials. Eight weeks after completion of the final exposure period, the number and sex of emerged adults in each vial were determined using a dissecting microscope (×25). In 2002, the prevalence of pea leafminer parasitization was higher than in 2001; therefore, the number of emerged parasitoids was determined.

Field Survival. One hundred field-collected pupae were placed into each of four ventilated 7-dram plastic vials containing moist filter paper. Two vials were placed into each of two 1-liter plastic containers filled with Styrofoam packing chips. Two 1-cm-diameter holes were made in the bottom of each plastic container to allow for ventilation and drainage. The containers were buried in the field at the Muck Crops Research Station on 15 October 2002 to an approximate depth of 10 cm, where they would experience similar soil temperatures as naturally occurring pea leafminer pupae. The containers were removed on 25 March 2002, placed into a growth chamber at 24 ± 2°C with a 16-h photoperiod, and monitored weekly for emergence. One vial flooded and was not placed into the growth chamber.

Leafminer Emergence. In 2001, emergence traps were made using black plastic oil funnels (30 cm diameter) spray-painted white to reduce abnormal heating of the covered soil and premature emergence of soil-dwelling insects. Two ventilation holes (1.5 cm diameter) were melted in the funnel neck in offset positions and covered with fine mesh. These ventilation holes prevented condensation on the interior surface that might inhibit the upward movement of emerged pea leafminer adults. The top of each funnel was closed with a rubber stopper containing a 5-mm hole. A 5 cm length of rigid plastic tubing was inserted into the upper surface of the rubber stopper, and a 7-dram inverted vial was placed over the tubing, thereby trapping any insects that emerged from the covered soil.

All emergence traps were situated at the Holland Marsh, Ontario, Canada, in fields where pea leafminer populations were identified the previous season. In April, at each of three locations, 5 emergence traps were set inside hoop-houses, 5 traps around hoop-houses, 5 traps around other structures, and 20 traps in fields. Yellow sticky card traps were placed near field emergence traps to monitor adult flight. Sixteen emergence traps were also established at the Muck Crops Research Station in a celery plot that had high infestations in 2000. At this site, traps were distributed evenly between plots where celery had been mowed and residue had either been left or cultivated to a depth of 10–15 cm in the fall of 2000. All traps were monitored twice weekly until early July.

In 2002, emergence cages were established at the Muck Crops Research Station in celery plots that experienced heavy pea leafminer infestation in 2001. Emergence cages consisted of four wooden corner posts ≈30 cm tall, supporting white floating row cover. All edges of the row cover were buried in the soil, and each cage covered an approximate area of 1 m². Within each cage, two yellow sticky cards were clipped onto stakes at a height of 20 cm. Soil preparation during the fall of 2001 consisted of three treatments: (1) celery mowed and residue left; (2) celery mowed and residue cultivated to a depth of 10–15 cm; and (3) celery not mowed. Six emergence cages per treatment were established in mid-April. Staked sticky cards were placed within close proximity to observe adult flight and all traps were monitored every 1–2 wk until late June.

Statistical Analysis. Percent survival and corrected percent mortality were determined for each treatment where percent survival = (no. of emerged adults/([total no. of pupae − no. parasitoids emerged]) × 100 and corrected percent mortality = ([survival of control − survival of treatment]/survival of control) × 100 (Zhao and Kang 2000). Corrected percent mortality and the proportion of parasitoids emerged were analyzed by analysis of variance (ANOVA; PROC GLM; SAS Institute 1999) after arcsine transformation. Two basic assumptions of ANOVA, i.e., independent treatment and model effects and random, independent, and normally distributed errors, were verified before analysis. For each temperature, percent mortalities of the exposure periods were ranked using Tukey’s honestly significant difference (HSD) test. In all cases α = 0.05, and actual, rather than transformed, data are presented.

Results

Soil Temperatures. In all years for which data were complete, soil temperatures at 5 cm depth fell below 5°C for 145–173 d and below 0°C for 102–143 d of the year at the Muck Crops Research Station. Soil temperatures at this depth very rarely fell below −5°C (i.e., 0–4 d/yr).

Controlled Environment Trials. A significant time × temperature interaction (F = 2.44; df = 12.60; P = 0.0118) indicated that mortality of pea leafminer...
at a given temperature varied with length of exposure in 2001; therefore, mortality data were analyzed separately for each temperature. At 0 °C (F = 21.53; df = 6.18; P < 0.0001) and 4°C (F = 33.55; df = 6.18; P < 0.0001), pupal mortality was significantly higher after 32 and 64 d of exposure than during exposure periods of 16 d or less (Fig. 1). Exposure periods of 16 d or longer at −5°C (F = 34.99; df = 6.18; P < 0.0001) had significantly higher levels of pupal mortality than periods of 8 d or less. At −5°C, 100% of pea leafminer pupae died after 16 d of exposure, whereas this mortality level was not reached until 64 d at either 0 or 4°C.

Mortality data in 2002 were analyzed separately for each temperature because of a significant time × temperature interaction (F = 5.82; df = 12.61; P < 0.0001). At 7.5°C (F = 18.64; df = 6.23; P < 0.0001), mortality levels after 32 and 64 d of exposure were significantly higher than after 16 d or less (Fig. 2). A host effect at 7.5°C (F = 8.36; df = 1.23; P = 0.0082) was caused by a significantly higher percent mortality of pupae reared from spinach (50.8 ± 8.39) than from celery (30.6 ± 9.57). Mortality data at 0°C were analyzed separately for each host because of a significant host × time interaction (F = 3.54; df = 6.13; P = 0.0266). In celery, pupal mortality was the same after 16 and 64 d at 0°C (F = 10.59; df = 6.6; P = 0.0056); however, pupal mortality at 64 d was significantly higher than that at 1, 2, 4, 8, and 32 d of exposure. At 0°C, in spinach (F = 16.12; df = 6.6; P = 0.0018), pupal mortality at 32 and 64 d of exposure was significantly higher than in the remaining treatments. At −6.5°C (F = 27.42; df = 5.19; P < 0.0001), mortality at 16, 32, and 64 d of exposure was similar and significantly higher than at 8 d or less (Fig. 2). A host effect observed at −6.5°C (F = 7.17; df = 1.19; P = 0.0149) was caused by a significantly higher percent mortality of pupae reared from spinach (76.0 ± 7.97) than from celery (57.3 ± 11.71).

Emerged parasitoids belonged to the families Braconidae and Pteromalidae (Hymenoptera). The proportion of pupae from which parasitoids emerged was analyzed separately for each temperature because of a significant time × temperature interaction (F = 2.61; df = 12.68; P = 0.0064). At 7.5°C (F = 5.24; df = 6.19; P = 0.0025), a greater proportion of parasitoids emerged at 64 d of exposure than in the remaining treatments (Fig. 3). Parasitoid emergence data at 0°C were analyzed separately for each host because of a significant host × time interaction (F = 4.73; df = 6.13; P = 0.0091). A greater proportion of parasitoids emerged from pupae reared from spinach (F = 34.25; df = 6.6; P = 0.0002) after 32 d of exposure compared with any other treatment, but the model was insignificant for celery (F = 0.84; df = 7.6; P = 0.5924). At −6.5°C, exposure time was not a significant variable for parasitoid emergence. A significant host effect at 7.5 (F = 16.18; df = 1.19; P = 0.0007), 0 (F = 11.50; df = 1.13; P = 0.0048), and −6.5°C (F = 18.14; df = 1.19; P = 0.0004) showed that a greater proportion of parasitoids emerged from pea leafminer pupae reared from spinach than from celery (Table 1).

**Field Survival.** No adults emerged when overwintered vials were placed into a growth chamber at 24°C with a 16-h photoperiod.
Leafminer Emergence. No pea leafminers were captured in emergence traps or cages at any location. Pea leafminers were sporadically captured on sticky traps beginning on 4 May 2001 at the Muck Crops Research Station, as well as around and inside other local hoop-houses. In 2002, adult pea leafminer were first captured at the Muck Crops Research Station and a local hoop-house on 22 May.

Discussion

Pea leafminer pupae present in fields at the Muck Crops Research Station in November would be members of the overwintering population, because host plants are absent and air temperatures are below estimated developmental thresholds (MacDonald and Walters 1993). These pupae would experience be-
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to emerge as adults, indicating an absence of diapause. Diapause induction would be expected by October because of reduced photoperiod and low temperatures in Ontario, and insufficient chilling would have occurred in the field by that time to satisfy potential diapause requirements. Although it is not known whether chilled individuals completed development in the same length of time as controls, emergence of adults from pupae held at 24°C indicated that they were not in a state of diapause when collected. Pea leafminer pupae in chilled conditions apparently enter a state of quiescence, which is reversed on return to warm temperatures.

Braconid and pteromalid parasitoids of the pea leaf-
miner are better adapted than their host to cold chilling. High rates of parasitism during the cold chilling study in 2002 indicated that, as the chilling period was extended, the proportion of parasitoids emerging in-

Table 1. Percent of pea leafminer pupae from which parasitoids emerged when held in the laboratory at varying temperatures

<table>
<thead>
<tr>
<th>Host</th>
<th>Percent (mean ± SE) of pupae from which parasitoids emerged</th>
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<tbody>
<tr>
<td></td>
<td>7.5°C</td>
</tr>
<tr>
<td>Spinach</td>
<td>53.8 ± 6.3a</td>
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<tr>
<td>Celery</td>
<td>14.9 ± 7.3b</td>
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</table>

Pupae were collected from two host plants at the Muck Crops Research Station, Kettleby, Ontario, Canada, Sept. 2002. Means in the same column followed by the same letter are not significantly different; ANOVA and Tukey’s HSD comparisons of means, α = 0.05.
creased because of reduced survival of the pea leafminer. Host plant effects on parasitism rates require further study, but may be caused by differences in plant architecture, nutrient availability, or phytochemistry (Dicke 1999).

The pea leafminer is unable to overwinter outside protected areas in Ontario for extended periods of time and should therefore be conservatively classified as having an opportunistic survival (Bale 1996) in this region. Pest management practices in the spring should be focused on preventing the spread of pea leafminer from heated greenhouses where populations can occur throughout the year.

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