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Endoparasitoid Assemblage of the Pea Leafminer, *Liriomyza huidobrensis* (Diptera: Agromyzidae), in Southern Ontario

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ABSTRACT The pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), is an important pest of leafy greens in the Holland Marsh, Bradford, Ontario, and was first discovered in this area in 1998. A 2-yr study was undertaken to document the parasitoid complex of this recent addition to leafminer guild in Ontario and examine endoparasitoids reared from pea leafminer pupae collected from eight host plants: celery (*Apium graveolens* L.), spinach (*Spinacia oleracea* L.), lettuce (*Lactuca sativa* L.), mustard greens (*Brassica juncea* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.), and gai lan (*Brassica alboglabra* L.). Members of three families of Hymenopteran parasitoids were identified: *Chrysocharis oscinidis* Ashmead (Eulophidae), *Halticoptera circulus* (Walker) (Pteromalidae), and three species in the Braconidae, including two unidentified *Dacnusa* spp. and one unidentified *Opius* sp. Mortality and parasitism rates of pea leafminer pupae were higher in 2002 than 2003 and varied according to host plant. Parasitism rates ranged from 4 to 27% in 2002 and 4% to 13% in 2003. The parasitoid complex differed among host plants, and there were differences in the range of host plants on which parasitoids were found to parasitize pea leafminers.

KEY WORDS endoparasitoids, *Halticoptera circulus*, *Chrysocharis oscinidis*, *Dacnusa* spp., *Opius* sp.

THE PEA LEAFMINER, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), is an important agricultural pest. First described in Argentina in 1926 (Blanchard 1926) and endemic to the neotropics (Spencer 1973), this small fly has spread to Canada, Europe, the Middle East, South Africa, Sri Lanka, and Indonesia (Weintraub and Horowitz 1995, Shepard et al. 1998, Scheffer et al. 2001). Damage to host plants occurs by stippling, i.e., adult feeding and oviposition holes on leaf surfaces, and by larval mining of spongy mesophyll tissue (Spencer 1973, Parrella et al. 1985, Civelek et al. 2002). Leafminer damage can cause reduced photosynthetic ability, wilting and premature death of leaves, reduced plant growth, death of young seedlings, and cosmetic damage, resulting in reduced yields and marketability (Spencer 1973, Parrella 1987). Pea leafminer is a highly polyphagous leafminer and has hosts in at least 15 plant families, including Alstroemeriaceae, Apiaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae, Liliaceae, Linaceae, Oxalidaceae, Polemoniaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae, and Violaceae (Spencer 1973, 1990, Martin et al. 2005a).

In Canada, pea leafminer was first identified from a greenhouse near Kettleby, Ontario, in 1998, and the following year serious damage was observed in several

field crops in the area (Graeme Murphy, personal communication; McDonald et al. 2000). Since its discovery, it has become a well established, but geographically restricted, pest causing considerable economic losses, particularly for growers of spinach, *Spinacia oleracea* L., Asian leafy vegetables such as gai lan, *Brassica alboglabra* L., and celery, *Apium graveolens* L. (Martin et al. 2005a).

Pea leafminer has shown a tendency to develop insecticide resistance (Weintraub and Horowitz 1995), and thus alternate methods for its control have been the subject of much interest (Weintraub and Horowitz 1995, Rauf et al. 2000, Civelek et al. 2002). Biological control agents, including the parasitic wasps, *Diglyphus isaea* (Walker) and *Dacnusa sibirica* Telenga, and the nematode *Steinernema feltiae* (Filipjev), have been used to manage *Liriomyza* spp. populations in both greenhouse and field crops (Murphy and LaSalle 1999, Williams and Walters 2000).

Because pea leafminer has only recently been identified in the Holland Marsh area, there is no existing information about its parasitoid complex in Ontario. This report details a 2-yr study to determine the species composition of the endoparasitoid assemblage of pea leafminer in the Holland Marsh and to study the influence of pea leafminer host plant on parasitism rates and relative abundance of parasitoids. Endoparasitoids, specifically koinobionts, were the subject of this study because of their degree of host specificity

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(Hawkins 1994), and thus high potential as biological control agents of pea leafminer (Debach and Rosen 1991).

Materials and Methods

Parasitoid Assemblage. In both 2002 and 2003, plant and insect samples were collected at the University of Guelph Muck Crops Research Station, Kettleby, Canada (44°5' N, 79°35' W). In 2002, collections were made in research plots established for a study on pea leafminer host preference (Martin et al. 2005b), which included the following host plants: celery cultivar Florida 683; pea, *Pisum sativum* L. cultivar Bolero; potato, *Solanum tuberosum* L., cultivar Shepody; spinach cultivar Unipack 12; gai lan or Asian broccoli, *B. alboglabra*, cultivar Guy lon; and lettuce, *Lactuca sativa* L., cultivar Ithaca M.I. Onion, *Allium cepa* L., cultivar Fortress, was also present in the field plot but was not used in this study. Host plants were arranged in a randomized complete block design with four replications. Each block contained six beds (5 m long by 0.9 m wide), with one host plant grown per bed according to recommended between- and within-row spacings (Ontario Ministry of Agriculture and Food 2002). Celery was planted on 16 July, pea and potato on 1 August, and gai lan, lettuce, and onion on 8 August. Field populations of pea leafminer were monitored throughout the season with yellow sticky cards to determine the occurrence of peak population and to trigger collection of specimens. On 26 September 2002, 4 mature plants were sampled from each plot for all hosts, except pea, where 16 plants per plot were sampled to compensate for small plant size. For the period 19–26 September 2002, sticky trap captures of pea leafminer averaged 285.8 adults per trap per week. Plants were transported to the laboratory at the University of Guelph and laid on trays covered with a sheet of wax paper and a thin layer of white sand to facilitate pupation and collection of pupae. Pupation trays were held at $22 \pm 4^\circ\text{C}$ with 16 L:8 D for 72 h. All pupae, to a maximum of 50 from each tray, were placed into individual sealed cells of clear plastic cell trays (Bio-Serv, Frenchtown, NJ), held at $22 \pm 4^\circ\text{C}$ and 16 L:8 D, and monitored daily for emergence of pea leafminer and parasitoids. After emergence, parasitoids were stored in a freezer before pinning.

In 2003, collections were made from plots established for insecticide efficacy experiments (Hallett and Heal 2004a–d, Hallett et al. 2004) that included five host crops: celery, spinach, romaine lettuce, cultivar Parris Island 318 M.I., and mustard greens, *Brassica juncea* L., cultivar Savanna Hybrid. For each host plant, plots were arranged in a randomized complete block design with four to six treatments, each replicated four times. Pea leafminer populations were monitored for the duration of the growing season using yellow sticky cards. On 20 September 2003, four plants were collected from the outer rows of each of the untreated control plots and transported to the university. For the period 12–20 September 2003,

sticky trap captures of pea leafminer averaged 336.9 adults per trap per week. Each plant was placed in a pupation tray as described above, covered with a lid, and held at $22 \pm 4^\circ\text{C}$ with 16 L:8 D for 144 h. The time allowed for pupation was longer than in 2002 to obtain sufficient numbers of pupae. Pupae and parasitoids were treated as described above.

Parasitoid specimens were air dried, mounted on points, and identified to family before sending to the Canadian National Collection (CNC) of Insects, Arachnids, and Nematodes, Ottawa, Ontario, Canada, for identification by Drs. Gary Gibson (Pteromalidae), Henri Goulet (Braconidae) and John Huber (Eulophidae). Voucher specimens of all species and genera collected have been deposited with the CNC.

Parasitism and Mortality Rates. Pea leafminer mortality rates were calculated for each plant sampled by dividing the number of pupae from which no insect emerged plus pupae that produced parasitoids by the total number of pupae collected. The rate of parasitism was determined by dividing the number of emerged parasitoids by the total number of emerged insects (i.e., pea leafminer plus parasitoids), thereby eliminating any pea leafminer that died before emergence (Harding 1965, Trumble and Nakahihara 1983). The proportion of mortality caused by parasitism was determined by dividing the number of emerged parasitoids by the total number of dead pupae (i.e., number of pupae from which no insect emerged plus the number of pupae that produced parasitoids). Data for mortality and parasitism rates and the proportion of mortality caused by parasitism were subjected to arcsine square-root transformation followed by one-way analysis of variance (ANOVA) using PROC GLM (SAS Institute 2001). The assumptions of ANOVA were verified using PROC UNIVARIATE before analyses. Outliers (one each in the 2002 and 2003 mortality data and one in the 2003 parasitism rate data), which resulted from low pupal sample sizes on individual plants, were removed to achieve normal distributions before analyses. When significant models were found, means separations were performed using Fisher protected Tukey's (honestly significant difference [HSD]) tests, with $\alpha = 0.05$.

Distribution Among Hosts. Distribution of parasitoid taxa by host plant was calculated by dividing the number of parasitoids belonging to one genus by the total number of parasitoids collected for that host. Distribution of parasitoid taxon by host plant was examined by dividing the number of parasitoids of a particular taxon collected from a given host plant by the total number of parasitoids of that taxon for that year.

Distribution of parasitoid taxa by host plant for both years was subjected to χ^2 goodness-of-fit contingency table analysis using PROC FREQ (SAS Institute 2001) followed by pairwise χ^2 tests, with $\alpha = 0.05$, to compare distributions of each parasitoid taxon among host plants and the parasitoid complex present on each host plant.

Table 1. Number of pea leafminer and parasitoid adults emerged from pea leafminer pupae collected in Sept. 2002 and 2003 at the Holland Marsh, Kettleby, Canada

Year	No. pea leafminer pupae collected	No. emerged pea leafminers	No. emerged parasitoids			
			Pteromalidae	Braconidae		Eulophidae
			<i>H. circulus</i>	<i>Dacnusa</i> spp.	<i>Opius</i> sp.	<i>C. oscinidis</i>
2002	4,250	2,110	226	35	30	0
2003	3,261	2,463	64	135	1	1

Results and Discussion

Parasitoid Assemblage. Five species of parasitoids were collected from pea leafminer pupae: *Halticoptera circulus* (Walker) (Pteromalidae), an *Opius* sp. and two unique *Dacnusa* spp. (all Braconidae), and a single specimen of *Chrysocharis oscinidis* Ashmead (Eulophidae) (Table 1). These results represent the first records of parasitoids of *L. huidobrensis* in Ontario. Given that this study was conducted just 4 and 5 yr after the discovery of *L. huidobrensis* in Ontario, it is possible that more parasitoids will adopt pea leafminer as a host in the future. *H. circulus* and the *Dacnusa* spp. were abundant in both years. Both *C. oscinidis* and *H. circulus* have been observed previously on *Liriomyza* spp. in Florida and California (Trumble and Nakakihara 1983, LaSalle and Parrella 1991, Schuster and Wharton 1993). *Dacnusa* spp. are well-known parasitoids of *Liriomyza* spp. and are available commercially in North America and Europe for control of leafminers. *Dacnusa siberica* is often sold packaged together with the ectoparasitoid *Diglyphus isaea*. If the *Dacnusa* spp. were introduced to the Bradford area through a commercial biological control program, *D. isaea* may also be present in the Holland Marsh, but our study was not directed at collection of this ectoparasitic species. We chose to examine endoparasitoids rather than ectoparasitoids in this study because endoparasitoids, and koinobionts in particular, are considered to have narrow host ranges. Koinobionts live in their host while the host continues to feed and grow; many koinobionts kill their host at the pupal stage and pupate within the host remains (Godfray 1994). This life history strategy demands a prolonged intimate host interaction, and thus, prolonged exposure to the host's immune system (Hawkins 1994).

Askew and Shaw (1986) argued that this extended contact encourages evolutionary adaptations to the host's immune response, causing the parasitoid to have a narrow host range caused by its need for specialized adaptations. Specialized natural enemies are desirable as classical biological control agents, because of low potential for impacts on nontarget species, and because they tend to be density-dependent, and thus may be able to regulate even low levels of the target pest (Debach and Rosen 1991).

Parasitism and Mortality Rates. Mortality rates of pupae were significantly higher in 2002 than in 2003 ($F = 154.47$; $df = 1,162$; $P < 0.0001$), as were parasitism rates ($F = 9.21$; $df = 1,162$; $P = 0.0028$; Table 2). Mortality rates ranged from 39.2 to 59.7% in 2002 and 17.0 to 33.1% in 2003. Parasitism rates ranged from 3.8% to 27.3% in 2002 and 4.4% to 13.4% in 2003. The proportion of mortality attributable to parasitism averaged 15.7% in 2002. Despite low rates of parasitism in 2003, mortality attributable to parasitism averaged 26.3%. Rates of both parasitism and mortality attributable to parasitism reported herein represent minimum estimates of parasitism, because they are based only on numbers of emerged adult parasitoids and do not account for the presence of larval or pupal parasitoids that died within pea leafminer pupae.

In both years, samples were taken from plots in which no insecticides were applied; however, the highest parasitism rate observed was only 27.3%. This value is not comparable to previous field studies in which parasitism rates on various *Liriomyza* leafminers exceeded 80% (Poe and Montz 1981, Trumble and Nakakihara 1983). Rates of parasitism vary with host species, parasitoid species, season, and the application of pesticides (Poe and Montz 1981). In heavily

Table 2. Mean \pm SE mortality and parasitism of pea leafminer pupae from different host plants host plants in Sept. 2002 and 2003 at the Muck Crops Research Station, Kettleby, Canada

Year	Host plant	Mortality rate (mean \pm SE)	Parasitism rate (mean \pm SE)	Mortality caused by parasitism (mean \pm SE)
2002	Celery	0.574 \pm 0.038a	0.273 \pm 0.027a	0.320 \pm 0.067a
	Lettuce	0.597 \pm 0.034a	0.188 \pm 0.034a,b	0.138 \pm 0.023b,c
	Spinach	0.529 \pm 0.028a	0.175 \pm 0.015a,b	0.189 \pm 0.018a,b
	Pea	0.480 \pm 0.032a,b	0.099 \pm 0.013b,c	0.150 \pm 0.026b,c
	Gai lan	0.494 \pm 0.026a,b	0.063 \pm 0.020c,d	0.067 \pm 0.022c
2003	Potato	0.392 \pm 0.029b	0.038 \pm 0.011d	0.079 \pm 0.028c
	Celery	0.316 \pm 0.042a	0.134 \pm 0.029a	0.282 \pm 0.058a
	Lettuce	0.281 \pm 0.028a	0.082 \pm 0.014a,b	0.245 \pm 0.044a
	Spinach	0.331 \pm 0.026a	0.092 \pm 0.014a,b	0.210 \pm 0.027a
	Mustard greens	0.170 \pm 0.015b	0.044 \pm 0.007b	0.313 \pm 0.049a

Means in the same year and column followed by the same letter are not significantly different: Tukey's HSD, $\alpha = 0.05$. Untransformed means are presented.

sprayed areas away from field borders, parasitism rates may drop to near zero (Poe and Montz 1981). Parasitism rates of *L. huidobrensis* by *D. isaea* in Israel were significantly lower in celery plots treated with either cyromazine or abamectin compared with control plots (Weintraub and Horowitz 1995, Weintraub 1999, 2001, Civelek et al. 2002). Application of insecticides to neighboring plots at our study site and to surrounding commercial fields may have contributed to the low parasitism rates observed, particularly in 2003. Parasitism rates may also be low because of relatively low adoption of pea leafminer as a host by endemic parasitoids in the 5 yr since its discovery; parasitism rates may increase in the future as pea leafminer becomes exposed to more parasitoids over both space and time.

Distribution Among Hosts. Mortality rates were significantly different among host plants (2002: $F = 5.58$; $df = 8, 84$; $P = 0.0002$; 2003: $F = 4.40$; $df = 10, 60$; $P = 0.0073$), as were parasitism rates (2002: $F = 16.09$; $df = 8, 85$; $P < 0.0001$; 2003: $F =$; $df = 10, 60$; $P < 0.0105$; Table 2). In both years, mortality rates and parasitism rates were higher on celery, lettuce, and spinach than on other host plants. Host plant effects on parasitism rates have been shown in other locales, particularly when overall parasitism rates are high (Shepard et al. 1998, Rauf et al. 2000). In 2002, leafminers in potato had the lowest rate of parasitism, which is comparable with results observed in Indonesia (Rauf et al. 2000). In this study, differences in parasitism rates among host plants do not seem to be related to observed differences in pea leafminer egg densities among hosts (Martin et al. 2005b). Significant differences among host plants in the proportion of mortality attributable to parasitism were observed in 2002 ($F = 7.29$; $df = 8, 83$; $P < 0.0001$) but not in 2003 ($F = 1.10$; $df = 10, 61$; $P = 0.3565$; Table 2). In 2002, higher proportions of mortality were attributable to parasitism on celery and spinach than on potato and gai lan.

Significant differences were found among host plants in the type and abundance of parasitoid taxa present in both 2002 ($\chi^2 = 137.93$; $df = 10$; $P < 0.0001$) and 2003 ($\chi^2 = 84.38$; $df = 9$; $P = 0.0091$; Fig. 1). In 2002, *H. circulus* was the dominant parasitoid species on celery and spinach, accounting for >95% of parasitism. *H. circulus* and *Opius* sp. were the main species found on broccoli and pea, whereas *H. circulus* and *Dacnusa* spp. were most common on lettuce and potato. In 2003, *H. circulus* was again the most dominant parasitoid on spinach, but *Dacnusa* spp. were the dominant parasitoids on celery, lettuce, and mustard greens. In 2003, *Opius* sp. and *C. oscinidis* were found only on mustard greens.

Distributions of parasitoids among host plants differed significantly among parasitoid taxa in 2002 ($\chi^2 = 137.93$; $df = 10$; $P < 0.0001$) and 2003 ($\chi^2 = 84.38$; $df = 9$; $P = 0.0091$), and only *H. circulus* parasitized pea leafminer pupae on all hosts examined (Fig. 2). In both years, significantly more *H. circulus* emerged from pea leafminer pupae collected from spinach than from any other host plants. In 2002, *H. circulus* was more abundant on celery than pea; significantly fewer *H. circulus* emerged from pupae collected from potato and gai lan

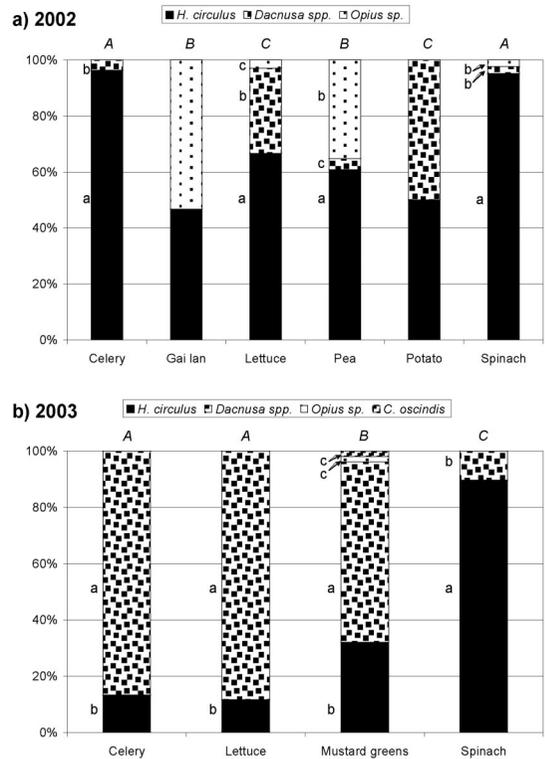


Fig. 1. Distribution of the pea leafminer parasitoid species, *H. circulus* (Walker), *Dacnusa* spp., *Opius* sp., and *C. oscinidis* Ashmead, among various host plants sampled from the Muck Crops Research Station, Kettleby, Canada, in (a) 2002 and (b) 2003. Capitalized letters above host plants indicate differences among hosts in species distributions; distributions within the same year with the same letter do not differ significantly, χ^2 goodness-of-fit tests, $\alpha = 0.05$. Lowercase letters within each host plant indicate differences between relative abundance of parasitoid species; species within the same host plant with the same letter do not differ significantly, χ^2 goodness-of-fit tests, $\alpha = 0.05$.

than from other plants. In 2003, *H. circulus* was more abundant on mustard greens than on celery and lettuce. In 2002, *Dacnusa* spp. were most abundant on lettuce, followed by potato; significantly fewer *Dacnusa* specimens emerged from pupae collected from celery, pea, and spinach than from these two hosts, and no *Dacnusa* emerged from pupae collected from potato and celery. In 2002, *Opius* sp. were most abundant on pea, followed by gai lan and then spinach and lettuce. No *Opius* emerged from pupae collected from potato and celery. In both years, relative abundance of *H. circulus* was higher on spinach than on lettuce, whereas *Dacnusa* spp. were less abundant on spinach than lettuce.

According to Hawkins (1994), only weak plant effects on parasitism should be observed in cultivated habitats because of the many plant characters that influence a parasitoid's ability to locate a suitable host,

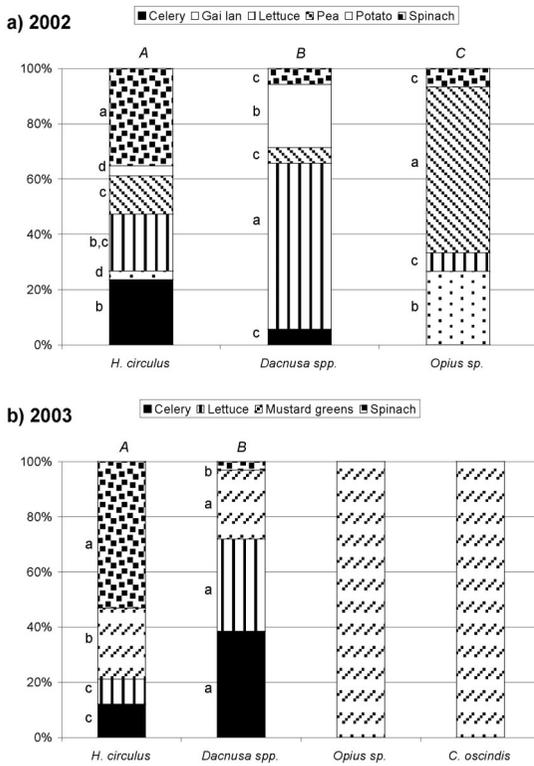


Fig. 2. Distribution of individuals among host plants for the pea leafminer parasitoids *H. circulus* (Walker), *Dacnusa* spp., *Opilus* sp., and *C. oscinidis* Ashmead, sampled from the Muck Crops Research Station, Kettleby Canada, in (a) 2002 and (b) 2003. Capitalized letters above species bars indicate differences among species in their host plant distributions; distributions within the same year with the same letter do not differ significantly, χ^2 goodness-of-fit tests, $\alpha = 0.05$. Lowercase letters within each parasitoid species indicate differences between relative abundance of individuals on each host plant; host plants within the same species with the same letter do not differ significantly, χ^2 goodness-of-fit tests, $\alpha = 0.05$. Note that in 2003, only single specimens of *Opilus* sp. and *C. oscinidis* were collected and thus could not be subjected to statistical analysis.

such as plant chemistry, size, structure, and habitat (Godfray 1994). For practical purposes, chemistry, size, and structure can be regarded as consistent within a treatment plant, and thus, variation in parasitism rates with host plant is a valid consideration. Our data support the assertion that plant type influences parasitism rates and parasitoid distributions. The parasitoid complex was consistent on spinach in both years, and the relative abundances of *H. circulus* and *Dacnusa* spp. were consistent on spinach and lettuce in both years.

In conclusion, leafminer populations are generally kept under economically damaging thresholds by their naturally occurring parasitoids (Oatman and Johnson 1981). For parasitoid populations to be effective, pesticide use must be reduced (Rauf et al. 2000) on crops that are impacted by pea leafminer and on surrounding crops or weeds that might act as shel-

ter or food for adult parasitoids (Genung 1981). In the absence of pesticides, leafminers experience higher rates of parasitism in cantaloupes (*Cucumis melo* L.), lettuce, and spring alfalfa (*Medicago* spp. L.) (Hills and Taylor 1951, Jensen and Koehler 1970). In California, *Liriomyza sativae* Blanchard only reached economically damaging levels when its parasitoids were killed off by pesticides (Oatman and Kennedy 1976, Johnson et al. 1980). The Holland Marsh is an intensely farmed region, and there are many other pests that require chemical control measures. Even if not specifically targeted at the pea leafminer, pesticide use in field vegetable crops is unlikely to permit existing parasitoid populations to increase to levels sufficient for control, as evidenced by the low parasitism rates seen in this study.

Because effective field control of pea leafminer by their parasitoids is unlikely in the Holland Marsh, other options must be explored. Martin et al. (2005a) found that pea leafminer were unable to overwinter under conditions typical of Ontario winters and that they were most likely overwintering in greenhouses and reinfesting neighboring vegetable fields each summer. Thus, if pea leafminer can be effectively controlled in greenhouses, field infestations could be lessened or avoided altogether. The parasitoids *D. sibirica*, *Opilus pallipes* Wesmael, and *D. isaea* have been used successfully to control greenhouse populations of pea leafminer, and *D. isaea* has been shown to be compatible with common chemical control programs for other pests used in greenhouses (Kaspi and Parrella 2005). Understanding parasitoid complexes is crucial when considering any control method for *Liriomyza* (Palumbo et al. 1994). Further study of the parasitoid complex of pea leafminers in the Holland Marsh is warranted to understand further the assemblage dynamics including species level identifications, target life stage(s), distribution and specialization of the parasitoids, parasitoid assemblage size (i.e., the number of parasitoid species attacking the host; Hawkins 1994), parasitoid overwintering ability, relative susceptibility to insecticides (Schuster and Price 1985), and ease of rearing. It is desirable to use a diversity of parasitoid species in controlled releases (Shepard et al. 1998), because of varying plant preferences, life cycle lengths, synchronization with the target pest, and winter survival of different parasitoid species. Braconid and pteromalid parasitoids of the pea leafminer in Ontario were found to be better adapted than their host to cold chilling (Martin et al. 2005a) and may prove to be effective biological control agents. Of the parasitoids identified herein, *H. circulus* seems to have the greatest potential as a biological control agent of pea leafminer because of its abundance and parasitism of pea leafminers on all host plants examined.

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