Biological Control of Alfalfa Blotch Leafminer (Diptera: Agromyzidae) in Ontario: Status and Ecology of Parasitoids (Hymenoptera: Braconidae, Eulophidae) 20 Years After Introduction

George E. Heimpel
University of Minnesota

Francois Meloche
Agriculture and Agri-Food Canada

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BIOLOGICAL CONTROL OF ALFALFA BLOTCH LEAFMINER (DIPTERA: AGROMYZIDAE) IN ONTARIO: STATUS AND ECOLOGY OF PARASITOIDS (HYMENOPTERA: BRACONIDAE, EULOPHIDAE) 20 YEARS AFTER INTRODUCTION

George E. Heimpel1 and Francois Meloche2

ABSTRACT

Two European parasitoid species were released in Ontario during the late 1970's to control the alfalfa blotch leafminer, Agromyza frontella (Rondani) (Diptera: Agromyzidae). One of these, Dacnusa dryas (Nixon) (Hymenoptera: Braconidae), rapidly became established and the other, Chrysocharis liriomyzae (= C. punctifacies) (Delucchi) (Hymenoptera: Eulophidae) was never recovered in Ontario. In 1999, we found both D. dryas and C. liriomyzae parasitizing first-generation A. frontella in Ontario in 1999. The combined parasitism rate for both species as revealed by larval dissections was 97.5% by the end of the first A. frontella generation. Of the adult parasitoids reared, 86% were D. dryas and 14% were C. liriomyzae. Most parasitized larvae contained a single unencapsulated (i.e., healthy) larva, along with one or more encapsulated eggs. No larvae were encapsulated, but the overall egg encapsulation rate was 47%. By the end of the first A. frontella generation, 86% of parasitized hosts contained at least one unencapsulated parasitoid and could therefore produce an adult parasitoid, and 12% of parasitized hosts escaped parasitism by containing only encapsulated parasitoids. The sex ratio of D. dryas was even at emergence, but strongly female-biased in sweep samples from the field. Egg loads of D. dryas females were all greater than zero and as high in the field as our highest laboratory estimates, suggesting that egg availability does not limit fitness under the conditions that we observed in the field.

The alfalfa blotch leafminer, Agromyza frontella (Rondani) (Diptera: Agromyzidae) is a pest of European origin that was discovered in Massachusetts in the late 1960's (Miller and Jensen 1970; Drea and Hendrickson 1986). It spread throughout at least 16 northeastern states in the United States and eastern Canada during the 1970's (Harcourt et al. 1987). Fourteen parasitoid species were imported for its control during the 1970's in the United States, but only three became established in North America: Dacnusa dryas (Nixon) (Hymenoptera: Braconidae), Chrysocharis liriomyzae (= C. punctifacies) (Delucchi) (Hymenoptera: Eulophidae), and Miscogaster hortensis Walker (Hymenoptera: Pteromalidae) (Drea and Hendrickson 1986). Both

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1Dept. of Entomology University of Minnesota, St. Paul MN 55108, USA.
2Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Center, Central Experimental Farm, K.W. Neatby, room 3017, Ottawa, Ontario K1A 0C6, Canada.
D. dryas and C. liriomyzae are credited with controlling A. frontella populations in the United States (Drea and Hendrickson 1986), and D. dryas alone is credited with controlling A. frontella in Canada (Harcourt et al. 1988). Both species are solitary larval-pupal endoparasitoids. Daecnusa dryas females oviposit mainly in 1st and 2nd instar hosts (Harcourt et al. 1986, Guppy et al. 1988), while C. liriomyzae oviposits mainly in 2nd and 3rd instars (Drea and Hendrickson 1986). Daecnusa dryas is known only from A. frontella in Europe but has been reared from Liriomyza trifoliiarum Spencer, a serpentine leafminer of alfalfa native to the United States (Hendrickson 1979). Chrysocharis liriomyzae has a much broader host range than D. dryas in its native Europe (Hansson 1985; Drea et al. 1982) and has also been reared from L. trifoliiarum in the United States (Hendrickson 1979).

Daecnusa dryas and C. liriomyzae were released in 1977 and found established in 1978 in Delaware (Hendrickson and Plummer 1983). From the established populations, additional releases were made in 1978 in New Jersey, New York, Ohio and Pennsylvania (Hendrickson and Barth 1979). By 1981, both species were established in 13 states (Hendrickson and Plummer 1983). In Canada, both parasitoids were introduced between 1978 and 1980 (release sites were in Prince Edward Island, Ontario and Quebec; Guppy et al. 1984), but only D. dryas was recovered (Guppy et al. 1984). Daecnusa dryas was subsequently released at multiple sites throughout Ontario (Harcourt et al. 1986). In Ontario, A. frontella is no longer considered a pest in regions where it was originally established, and the last survey of A. frontella parasitoids in their areas of release was conducted in the mid-1980's (Guppy et al. 1988, Harcourt et al. 1988). Harcourt et al. (1988) showed that in eastern Ontario D. dryas was the key factor in controlling A. frontella by 1985. Chrysocharis liriomyzae was recovered on Prince Edward Island in 1988 (J. Huber, pers. comm.) following the initial releases but was never detected in A. frontella parasitism and population dynamics studies in Ontario (Guppy and Meloche 1987, Harcourt et al. 1988).

Agronomy frontella has recently spread westward to Wisconsin, Minnesota (Hutchison et al. 1997), Illinois, and North Dakota in the United States (Venette et al. 1999), and Manitoba in Canada (Lundgren et al. 1999). Daecnusa dryas has not been reported from any of these sites, but C. liriomyzae has been reared from A. frontella in Wisconsin (T. J. Davis and D. B. Hogg, unpubl. data) and Minnesota (G.E. Heimpel, unpubl. data), where A. frontella has been a minor pest during its invasion.

Here, we confirm the presence of D. dryas in an area where it had established in Ontario in the early 1980's and provide the first report of C. liriomyzae in Canada. We also report on the abundance and levels of parasitism and encapsulation of D. dryas and C. liriomyzae and provide data on the dynamics of sex ratio and egg load in D. dryas.

**MATERIALS AND METHODS**

**Collection site.** Parasitoid collections were done in two adjacent alfalfa fields at an Agricultural Experiment station of Kemptville college (University of Guelph campus) located in Winchester, Ontario; coordinates N45°03.580', W75°20.239'. Samples from the 2 fields were processed separately, but no significant differences were found in any of the parameters measured, so the data from the two sites are pooled here. All sampling was done on May 27 and June 3–June 6, 1999; the latter of these dates corresponded with the end of the first A. frontella generation. The alfalfa stands were mature and had a
moderate infestation of *A. frontella*; no quantification of *A. frontella* densities was attempted.

**Sweep samples and mine collections.** Twenty-one groups of 100 sweeps were done with a standard 38 cm sweep net using pendulum swings on 3–6 June 1999. The number of *D. dryas* adults captured per 100 sweeps was recorded. Individuals were aspirated into 9-dram plastic aspirator vials that contained streaks of honey and were placed in a cooler with ice packs. After each day of sampling, vials containing parasitoids were placed in plastic boxes containing moist paper towels and stored in a refrigerator at approximately 4°C. Vials were transported to St. Paul, Minnesota, USA, by air (USDA-APHIS importation permit # 37044).

Leaf mines containing 3rd-instar ABLM larvae were collected on 27 May and 4–6 June 1999. Fifty alfalfa trifoliates containing mines and 3rd instar *A. frontella* were collected on 27 May as a preliminary sample, and approximately 5,000 mined trifoliates were collected on 3–6 June. Trifoliates were placed in groups of approximately 1,000 into ziplock plastic bags (2-liter size) for storage and transport of *A. frontella*. Following methodologies developed by Drea et al. (1982), a piece of moist paper towel was placed into the bags and they were partially filled with air before being closed.

**Parasitism rates.** We measured parasitism rates using dissections of *A. frontella* larvae and pupae and by rearing adult insects from *A. frontella* pupae. Thus, our potential sample was restricted to larval and larval-pupal endoparasitoids. *Agromyza frontella* larvae left the mines and pupated within the plastic bags. We dissected all 50 larvae from the 27 May sample within 24 h of collection from the field and a subset of 100 larvae and 100 pupae from the 3–6 June sample on 10 June after transport to Minnesota and refrigeration at 4°C. Larvae or pupae were placed in a drop of distilled water on a microscope slide and torn open using two pairs of fine forceps. Larval contents were viewed at 50X using a compound microscope and we recorded the number of parasitoid eggs or larvae per *A. frontella* larva or pupa as well as whether or not they were encapsulated. Characteristics of eggs, larvae and encapsulated eggs were based on previous observations of *Dacnusa dryas* by Meloche and Guppy (1990), but species identifications of immature parasitoids were not made.

We collected a sub-sample of approximately 2,000 pupae from the plastic bags and placed them into 116 plastic petri dishes (6 cm diameter) in groups ranging in number between 6 and 25. The dishes were stored at 22°C, 16:8 L:D, and 70% ± 10% R.H and checked for emergence of *A. frontella* flies or their parasitoids until no more emergence was observed.

**Dacnusa dryas sex ratios.** We scored the sex ratio from a sample of about 100 adult *D. dryas* collected during the sweep net collections. We also determined the sex ratio of a sample of 19 adult *D. dryas* reared from the larval collections within one day of emergence, and another group of 25 adults that were held for 7–10 days in 9-dram plastic vials at conditions described above with a streak of honey.

**Dacnusa dryas egg loads.** We recorded the number of mature eggs present in the ovaries for three groups of *D. dryas*: (i) 83 females from the sweep samples that were kept alive and chilled for 3 d prior to dissection, (ii) 8 females reared from the larval collections that had emerged from host puparia within 24 h, and (iii) 12 females reared from the larval collections that had emerged 7 to 10 days prior to being dissected. All females were provided with honey. Females were killed by freezing just prior to the dissections and were placed within a drop of distilled water on a microscope slide. The females were impaled with an insect pin through the thorax and the last abdominal segment was removed with a pair of fine forceps. The ovaries were teased
Table 1. Results of A. frontella larval and pupal dissections from samples collected as 3rd instar larvae on 5/27/99 and 6/3 – 6/6/99, along with percentages of A. frontella larvae and pupae yielding parasitoids vs. adult A. frontella.

<table>
<thead>
<tr>
<th>Host status</th>
<th>Collection date: 5/27/99 (n = 50)</th>
<th>Collection dates: 6/3–6/6/99 (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) No parasitoids present</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>(b) Only unencapsulated parasitoid eggs or larvae present</td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td>(c) Only encapsulated parasitoid eggs present</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>(d) Both encapsulated eggs and unencapsulated eggs or larvae present</td>
<td>0</td>
<td>99</td>
</tr>
<tr>
<td>Percent parasitism:</td>
<td>54%</td>
<td>98%</td>
</tr>
<tr>
<td>Percentage of hosts that can produce parasitoid adults:</td>
<td>48%</td>
<td>86%</td>
</tr>
<tr>
<td>Percentage of hosts escaping parasitism through encapsulation:</td>
<td>11%</td>
<td>12%</td>
</tr>
<tr>
<td>Percentage of hosts escaping parasitism:</td>
<td>52%</td>
<td>14%</td>
</tr>
<tr>
<td>(a + c)/n</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

out. Eggs were viewed at 50X and considered mature if they were fully chorionated and deemed to be of full size. As a measure of parasitoid size, the length of one hind tibia of each female was measured to the nearest 0.04 mm.

RESULTS

Sweep samples. The mean number of D. dryas adults per 100 sweeps was 36.8 ± 4.8 (S.E.M.; n = 21 sets of 100 sweeps). Counts of C. liriomyzae were not made from the sweep samples because we were unaware of the presence of this species at that time.

Parasitism rates. Of the 50 3rd-instar A. frontella larvae that were collected on 27 May, 27 contained a single parasitoid egg and of these, three were encapsulated (Table 1). The parasitism rate was therefore 54% on this date, with an egg encapsulation rate of 11%. There were no significant differences between the results of larval and pupal dissections from the 4–6 June larval collections, so we pool the results here. Of 200 larvae sub-sampled from collections on these dates, only five contained no parasitoids, so that the overall parasitism rate was 97.5%.

Of the parasitoids found during the dissections from the material collected 3–6 June, three were unencapsulated eggs, 158 were encapsulated eggs, and 172 were unencapsulated larvae. Unlike Meloche and Guppy (1990), we found no encapsulated parasitoid larvae. The relatively high representation of larvae in our sample may be due to the fact that the dissec-
Figure 1. Numbers of parasitoid larvae (all unencapsulated) and encapsulated eggs present in hosts that contained between one and five parasitoid individuals. Numbers above pairs of bars are sample sizes (number of hosts).

Data points were done 3 to 6 days after collections were made. The observed level of encapsulation may be artificially elevated for the same reason: by the time we did our dissections, a large fraction of the hosts contained parasitoid larvae, which would be expected to kill any other unencapsulated individuals present in the hosts. The distribution of parasitoid stages within hosts is typical for solitary parasitoids: a single first-instar larva and varying numbers of eggs (Fig. 1). The varying combinations of encapsulated eggs, unencapsulated eggs, and unencapsulated larvae can either lead to parasitoid survival (if at least one unencapsulated individual is present) or host survival (if only encapsulated individuals are present). The percentages of hosts that would be expected to produce adult parasitoids vs. adult *A. frontella* flies, based on these considerations, are presented in Table 1.

Out of 1,945 pupae that had been collected as 3rd instar larvae, 12 resulted in the emergence of *A. frontella* flies, 573 emerged as *D. dryas* adults (86% of adult parasitoids recovered), and 93 emerged as *Chrysocharis liriomyzae* adults (14% of adult parasitoids recovered). The remaining 1,267 pupae (65%) did not emerge and a dissection of a sub-sample of these pupae in November 1999 revealed that most puparia contained only dead insect remains and were therefore probably not in diapause.

**Dacnusa dryas sex ratios.** The sex ratio of *D. dryas* that were collected by sweep-netting as adults was significantly female-biased (*P < 0.001* using a log likelihood ratio test; Sokal and Rohlf 1981), while the sex ratios of *D.**
Table 2. Secondary sex ratios (proportion of adults that are males) and egg loads of *D. dryas* that were either field-collected as adults, or emerged in the laboratory from field-collected larvae and held for either 1 or 7–10 days. For the egg loads, means followed by a different letter are significantly different at P < 0.05 using a Tukey–Kramer means separation test.

<table>
<thead>
<tr>
<th>Dacnusa dryas group</th>
<th>Proportion males (n)</th>
<th>Egg load ± S.E.M.(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field-collected as adults</td>
<td>0.12 (95)</td>
<td>38.3 ± 1.4 (83)a</td>
</tr>
<tr>
<td>Emerged in lab, 1 d old</td>
<td>0.58 (19)</td>
<td>16.4 ± 2.2 (8)b</td>
</tr>
<tr>
<td>Emerged in lab, 7–10 d old</td>
<td>0.52 (25)</td>
<td>36.4 ± 2.6 (12)a</td>
</tr>
</tbody>
</table>

*dryas* that had been collected as larvae and reared to adulthood in the laboratory were not significantly different from 0.5 (Table 2). There was no significant difference between the sex ratios of 1 day-old and 7 to 10 day-old laboratory-reared *D. dryas* (Log likelihood $\chi^2 = 0.15$, $P > 0.5$), but the difference between field-collected and laboratory-reared sex ratios was highly significant (Log likelihood $\chi^2 = 28.1$, $P < 0.0001$). The hind tibia lengths (a measure of parasitoid size) did not differ significantly between the sexes in any of the three groups examined (t-tests, $P > 0.15$ for all analyses).

**Dacnusa dryas egg loads.** Egg loads of 1 day-old *D. dryas* females that had been collected in the field as larvae and reared to adulthood in the laboratory were significantly lower than egg loads of females that had been collected in the field as adults or 7 to 10 day-old females that had been reared to adulthood in the laboratory (Table 2). Egg load was positively correlated with hind tibia length for females that had been collected as adults as well as 7 to 10 day-old females that had been collected as larvae and allowed to emerge in the laboratory (Fig. 2). The lack of a significant correlation between hind tibia length and egg load in the 1 day-old laboratory-reared group may be an artifact of low sample size.

**DISCUSSION**

*Dacnusa dryas* was introduced into Canada in 1977–1980 and we have shown that it is still present in Ontario. Parasitism rates were similar to, or higher than those reported in the early and mid 1980’s from various sites in Ontario (Harcourt et al. 1986, 1988), but our sweep-net catches were lower than those reported by Guppy et al. (1988) from the same period. To our knowledge, our recovery of *Chrysocharis liriomyzae* constitutes the first record in Ontario for this species. *Chrysocharis liriomyzae* was released in Canada alongside *D. dryas* in 1978–1980 (Guppy et al. 1984), and only two adult specimens were recaptured on Prince Edward Island in 1988 (J. Huber, pers. comm.). Whether the *C. liriomyzae* that we found stem from these Canadian introductions or from releases in the United States (Hendrickson and Barth 1979) is unclear.

**Parasitism and refuges from parasitism.** Our data on parasitism rates come both from dissections and from rearing insects to adulthood. While we were not able to distinguish between *D. dryas* and *C. liriomyzae* immature stages from the dissections, it was possible to count parasitoid eggs and larvae, and to determine whether immature parasitoids were encapsulated (Meloche and Guppy 1990). Dissection data revealed that the parasitism rate increased from approximately 50% to almost 100% between 27 May and 4–6 June (Table 1). This observation is consistent with other data showing that *A. frontella* appear in the field as adults before *D. dryas* (Guppy
Figure 2. Correlation between *D. dryas* hind tibia length and egg load of females that were either collected in the field as adults (filled circles; $r^2 = 0.13$; $P < 0.001$; egg load = $1.7 \text{(hind tibia length)} - 16.5$), or reared to adulthood in the laboratory and held 1 day (open squares; $r^2 = 0.19$; $P > 0.25$) or 7–10 days prior to dissection (open triangles; $r^2 = 0.55$; $P < 0.01$; egg load = $2.4 \text{(hind tibia length)} - 40.7$). Mean values for the 3 groups of parasitoids are compared in Table 2.

et al. 1988) and suggests that this difference in emergence times leads to a temporal (early season) refuge from parasitism. Such refuges may increase the stability of the host-parasitoid system by decreasing the likelihood of host extinction (e.g. Hassell 1978, Murdoch 1990), but they may also increase the size of average or equilibrium host populations (Murdoch 1990, Hochberg and Hawkins 1992) and diminish the probability of successful biological control (Hawkins et al. 1993). The temporal refuge from parasitism enjoyed by *A. frontella* is apparently not sufficient to disrupt biological control, however. Indeed, one of the hallmarks of the success of this biological control project is the synchrony of *D. dryas* with the distinct generations of *A. frontella* (Drea and Hendrickson 1986, Guppy et al. 1988, Harcourt et al. 1988).

Another refuge from parasitism that is available to many host larvae is encapsulation of parasitoid eggs (Godfray and Hassell 1991). Meloche and Guppy (1990) found that between 0 and 16% of *D. dryas* eggs were encapsulated by *A. frontella* larvae between 1983 and 1987 in Ontario. The total encapsulation rate in our study was 47%, but this value may be artificially inflated for reasons discussed above. In addition to the potential for experimental bias, the differences in the encapsulation rate between our
data set and that of Meloche and Guppy (1990) could be due either to an increased ability to encapsulate \textit{D. dryas} eggs by \textit{A. frontella} larvae and/or to encapsulation of \textit{C. liriomyzae} or other parasitoids. However, a large percentage of the encapsulated eggs were found in hosts that also contained an unencapsulated parasitoid larva, so that the effective refuge from parasitism (i.e., the fraction of parasitized hosts that contained only encapsulated parasitoids) was not greater than 12\% (see Table 1).

Overall parasitism levels were similar to those reported by Harcourt et al. (1988) for \textit{D. dryas} alone in Ontario, higher than those reported by Harcourt et al. (1986) for \textit{D. dryas} alone attacking second-generation \textit{A. frontella} in Ontario, and higher than those reported for Hendrickson and Plummer (1983) for \textit{D. dryas} and \textit{C. liriomyzae} combined in Delaware. In our data set, parasitism by \textit{D. dryas} greatly exceeded that of \textit{C. liriomyzae}. The dominance of \textit{D. dryas} could either be attributed to the fact that it has presumably been established in Ontario for longer than \textit{C. liriomyzae}, or to intrinsic differences between the species. Life table studies by Harcourt et al. (1988) demonstrated that \textit{D. dryas} can control \textit{A. frontella} without contributions from other parasitoids, and very high rates of \textit{A. frontella} parasitism by \textit{C. liriomyzae} alone have been found in Wisconsin as well (T. J. Davis, D.B. Hogg and J. L. Wedberg, unpubl. data). Both species together have also contributed to the successful control of \textit{A. frontella} in Delaware (Hendrickson and Plummer 1982). Thus, it is likely that these species are compatible and can control \textit{A. frontella} alone or together.

**Sex ratio and egg load.** As did Guppy et al. (1988), we found that the sex ratio of \textit{D. dryas} at emergence was 1:1. The highly female-biased sex ratio from the sweep samples (see Table 1) must therefore indicate either differential adult mortality or emergence patterns of the sexes in the field and/or sex-specific sampling bias. We found no evidence for sex-specific differences in adult mortality in the laboratory. Since many of the releases of \textit{D. dryas} were conducted using adults from sweep-net samples (Hendrickson and Barth 1979), these results suggest that female-biased samples were likely introduced at many sites.

The average egg load (number of mature eggs present in the ovaries) of \textit{D. dryas} females reared in the lab increased between days 1 and 7, indicating that this species is synovigenic (Jervis et al. 2001). Field-caught females had egg loads that were similar to the 7-day value, and all contained at least 12 eggs (see Fig. 2). These relatively high egg loads suggests that the fitness of the \textit{D. dryas} females that we collected was limited by the number of suitable hosts encountered, not by the egg supply. A complete lack of egg-depletion is rare and has been reported for only one parasitoid in the field (Heimpel and Rosenheim 1998, Rosenheim et al. 2000), but is expected to occur when host availability is very low (Rosenheim 1996, Sevenster et al. 1999). We do not have direct data on host density at our field site, but the high parasitism rates that we found during the time that we collected adult parasitoids suggest that the ratio of adult parasitoids to suitable (i.e., unparasitized) hosts may have been very high. Parasitism rates were substantially lower just one week prior to this sample (see Table 1) and it is possible that \textit{D. dryas} used a higher proportion of their egg complement during that period. Harcourt et al. (1988) suggested that attributes contributing to the spectacular success of \textit{D. dryas} as a biological control agent include dispersal and colonization ability, a narrow host range, synchrony with host populations, and appropriate climatic requirements. Our egg load data suggest that high potential fecundity can likely be added to this list.
ACKNOWLEDGMENTS

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LITERATURE CITED


