Relative abundance of parasitoids reared during field exposure of sentinel larvae of the leafminers *Liriomyza trifolii* (Burgess), *L. sativae* Blanchard, and *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae)

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Abstract

In order to examine the relative abundance of indigenous parasitoids against three leafminer species, *Liriomyza trifolii*, *L. sativae* and *Chromatomyia horticola*, kidney bean plants infested with larvae of each species were exposed in vegetable gardens or grassland to different seasonal conditions (spring, summer and fall). Abundant parasitoid species were *Chrysocharis pentheus*, *Diglyphus isaea* and *D. minoeus* in spring or fall, and *Neochrysocharis formosa*, *D. albiscapus* and *D. pusztensis* in summer. Also, abundant parasitoid species varied among the different field conditions; however, within the same season and field the dominant parasitoid species were similar in all three leafminer species tested. Our findings indicate that the dominant parasitoid species did not exhibit any strong preference in the field between the three host species, namely *L. trifolii*, *L. sativae* or *C. horticola*.

Key words: Leafminer; *Liriomyza trifolii*; *Liriomyza sativae*; *Chromatomyia horticola*; parasitoid

INTRODUCTION

The agromyzid leafminers *Liriomyza trifolii* (Burgess) and *L. sativae* Blanchard are serious pests of vegetables and ornamental crops around the world. In Japan, they were first recorded in 1990 and 1999, respectively (Saito, 1993; Iwasaki et al., 2000). In addition, an indigenous leafminer, *Chromatomyia horticola* (Goureau), has been an economically important pest of garden pea, lettuce and Japanese radish since the late 1990s (Saito, 2004). All three leafminers have developed high levels of resistance to a range of chemical insecticides (e.g. Parrella and Keil, 1984; Saito et al., 1992; Saito, 2004; Ohta et al., 2005; Tokumaru et al., 2005), but insecticides do eliminate local parasitoids, leading to pest resurgence, suggesting that parasitoids may be important natural enemies of leafminers under insecticide-free conditions (e.g. Oatman and Kennedy, 1976; Schuster and Price, 1985; Saito et al., 1996; Saito, 2004). These findings clearly demonstrate that strategies such as conservation and enhancement of local parasitoids

are essential for successful integrated leafminer management.

In Japan, more than 30 species of parasitoids have been recorded from agromyzid leafminers (Kamijo, 1978; Takada and Kamijo, 1979; Konishi, 1998). Most parasitoids of agromyzid leafminers are thought to be polyphagous, but mechanisms for host preference and selection by leafminer parasitoids are poorly understood (Murphy and LaSalle, 1999). The objective of this study was to evaluate the relative abundance of indigenous parasitoids on *L. trifolii*, *L. sativae* and *C. horticola* by exposing kidney bean plants infested with sentinel leafminer larvae to naturally occurring parasitoid species under different field and seasonal conditions. The results are discussed in terms of host, habitat and seasonal preferences of the commonly found leafminer parasitoids.

MATERIALS AND METHODS

Leafminer laboratory cultures. A laboratory culture of *L. trifolii* was established from a popula-

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tion collected in Hamamatsu, Shizuoka, Japan in April 1991, a culture of *L. sativae* was established from a population collected in Iwata, Shizuoka, Japan in June 2004, and a *C. horticola* culture was established from a population collected in Iwata, Shizuoka, Japan in April 2005. The cultures were maintained on the primary leaves of kidney bean (*Phaseolus vulgaris* L.). Pots of kidney bean plants were prepared for exposure experiments as follows; about 100 adults of each leafminer species were released into plastic cages (38×40×30 cm) containing 20 seedlings of kidney bean bearing primary leaves, with one seedling per pot (9 cm diameter). After incubation for one day at 25±1°C and 16L:8D photoperiod, the kidney bean plants were removed and kept in a separate cage for three days under the same conditions described until eggs hatched. Kidney beans infested with approximately 30–50 individuals of 1st instar leafminer larvae per primary leaf were used in field exposure experiments.

Exposure experiments. Experiments were carried out in spring (May), summer (August to September) and fall (November) in 2005. Three experimental fields, two vegetable gardens for private household use and an area of grassland, were established in and around Shizuoka University, located in Ohya, Shizuoka, Japan. In Garden A (30×50 m), vegetables such as garden pea (*Pisum sativum* L.) were grown. In Garden B (40×40 m), vegetables such as radish (*Raphanus sativus* L.), tomato (*Lycopersicon esculentum* Mill.) and komatsuna (*Brassica rapa* L. “Nothovar”) were grown. In Gardens A and B, there were pre-existing infestations of *C. horticola* and *L. sativae*, respectively, but no insecticides were used for leafminer control. The grassland area (50×50 m) contained weeds, mostly Compositae and Gramineae such as tall goldenrod (*Solidago altissima* L.) and southern crabgrass (*Digitaria ciliaris* (Retz.) Koel.). No leafminer infestations were observed in the grassland area. The spring survey was carried out in Garden A and grassland, and the summer and fall surveys in Garden B and grassland.

For each leafminer species, five pots of infested kidney beans were placed in trays (35×46×5 cm), with one pot per tray. For experimental exposure, 15 trays (5 per leafminer species) were placed in each of the experimental areas. To catch *Liriomyza* pupae, filter paper was placed on the soil surface of

each pot and trays were covered with gauze (mesh 0.2 mm). The kidney bean plants were exposed for one week in the spring and summer surveys, and for two weeks in the fall survey. After each exposure, leaves containing parasitoids and leafminers were removed and *Liriomyza* leafminer pupae in the trays were collected. The insect samples were placed on dry paper towels and incubated in plastic cups (12 cm diameter, 10 cm height) with mesh lids and maintained at 25±1°C and 16L:8D photoperiod. After one month, emerged parasitoid and leafminer adults were counted, and parasitoids were identified using taxonomic keys by Konishi (1998).

RESULTS

Results of the spring survey are shown in Table 1. In Garden A, six parasitoid species were identified. Of these, *Chrysocharis pentheus* (Walker) was the dominant parasitoid from all three leafminer species, and *Diglyphus isaea* (Walker) and *Neochrysocharis formosa* (Westwood) were commonly found in all leafminer species. In grassland, eight parasitoid species were identified. *N. formosa* had the highest abundance of all leafminer species, while the number of *D. isaea* was high from *L. trifolii* and *C. horticola*, and *Opius* sp. showed high abundance in *L. sativae*. Leafminer species other than the species tested were collected from almost all of the samples, because leafminer species naturally occurring in the field study area appeared to lay eggs on the exposed kidney beans.

Results of the summer survey are shown in Table 2. In Garden B, nine parasitoid species were obtained. Of these, *D. pusztenensis* (Erdős & Novicky) was the most abundant in *L. trifolii* and *C. horticola*, while *N. formosa* was the most abundant in *L. sativae*, and high numbers were reared from *L. trifolii* and *C. horticola*. Also, many adults of *L. sativae* were collected from trays with samples of *L. trifolii* and *C. horticola* exposed in Garden B. In grassland, six parasitoid species were obtained and the abundance of *D. albiscapus* Erdős and *N. formosa* was high in all three leafminer species.

Results of the fall survey are shown in Table 3. *D. isaea* and *D. minoensis* (Walker) were the two dominant parasitoids of all three leafminer species in Garden B and grassland, respectively. However,

Table 1. Number and percent composition (in parenthesis) of parasitoids and leafminers obtained in the spring survey

Parasitoid and leafminer	Garden A			Grassland		
	<i>L. trifolii</i>	<i>L. sativae</i>	<i>C. horticola</i>	<i>L. trifolii</i>	<i>L. sativae</i>	<i>C. horticola</i>
Parasitoid						
Braconidae						
<i>Dacnusa sasakawai</i> Takada	3 (0.5)					
<i>Opius</i> sp.				3 (0.5)	42 (32.1) [†]	12 (14.5)
Eulophidae						
<i>Chrysocharis pentheus</i> (Walker)	245 (42.5) [‡]	202 (48.0) [‡]	31 (44.9) [‡]	27 (4.8)	12 (9.2)	3 (3.6)
<i>Diglyphus albiscapus</i> Erdős	5 (0.9)	1 (0.2)	2 (2.9)	37 (6.6)	1 (0.8)	1 (1.2)
<i>Diglyphus isaea</i> (Walker)	162 (28.1) [†]	73 (17.3)	17 (24.6) [†]	181 (32.1) [†]	10 (7.6)	13 (15.7) [†]
<i>Hemiptarsenus varicornis</i> (Girault)				10 (1.8)		
<i>Neochrysocharis formosa</i> (Westwood)	131 (22.7)	117 (27.8) [†]	7 (10.2)	265 (46.8) [‡]	65 (49.6) [‡]	41 (49.4) [‡]
<i>Neochrysocharis</i> sp.	31 (5.4)	25 (5.9)	12 (17.4)	38 (6.7)	1 (0.8)	10 (12.0)
<i>Pnigalio katonis</i> (Ishii)				2 (0.4)		3 (3.6)
Unknown species		3 (0.7)		1 (0.2)		
Total number	577	421	69	564	131	83
Leafminer						
<i>Liriomyza trifolii</i> (Burgess)	14			37	46	39
<i>Liriomyza sativae</i> Blanchard		23	1	17	136	26
<i>Chromatomyia horticola</i> (Goureau)		1	6			31
Total number	14	24	7	54	182	96

For each leafminer species, parasitoid species causing the highest (‡) and second highest (†) percent composition are indicated.

Table 2. Number and percent composition (in parenthesis) of parasitoids and leafminers obtained in the summer survey

Parasitoid and leafminer	Garden B			Grassland		
	<i>L. trifolii</i>	<i>L. sativae</i>	<i>C. horticola</i>	<i>L. trifolii</i>	<i>L. sativae</i>	<i>C. horticola</i>
Parasitoid						
Braconidae						
<i>Dacnusa sasakawai</i> Takada	3 (4.5)					
<i>Opius</i> sp.	1 (1.5)			7 (12.7)		1 (1.8)
Eulophidae						
<i>Asecodes delucchii</i> (Boucek)				1 (1.8)	1 (1.9)	1 (1.8)
<i>Chrysocharis pentheus</i> (Walker)	4 (6.0)	6 (18.8) [†]	17 (14.5)	5 (9.1)		
<i>Diglyphus albiscapus</i> Erdős	14 (20.9) [†]		7 (6.0)	26 (47.3) [‡]	30 (56.6) [‡]	18 (32.1) [†]
<i>Diglyphus isaea</i> (Walker)	2 (3.0)					
<i>Diglyphus pusztenis</i> (Erdős & Novicky)	21 (31.3) [‡]	3 (9.4)	37 (31.6) [‡]			
<i>Hemiptarsenus varicornis</i> (Girault)	4 (6.0)	1 (3.1)	1 (0.9)			
<i>Neochrysocharis formosa</i> (Westwood)	14 (20.9) [†]	18 (56.3) [‡]	29 (24.8) [†]	12 (21.8) [†]	21 (39.6) [†]	36 (64.3) [‡]
<i>Neochrysocharis</i> sp.	4 (6.0)	4 (12.5)	26 (22.2)	4 (7.3)	1 (1.9)	
Total number	67	32	117	55	53	56
Leafminer						
<i>Liriomyza trifolii</i> (Burgess)	25			51	2	
<i>Liriomyza sativae</i> Blanchard	223	569	541		12	
<i>Chromatomyia horticola</i> (Goureau)			4			7
Total number	248	569	545	51	14	7

For each leafminer species, parasitoid species causing the highest (‡) and second highest (†) percent composition are indicated.

Table 3. Number and percent composition (in parenthesis) of parasitoids and leafminers obtained in the fall survey

Parasitoid and leafminer	Garden B			Grassland		
	<i>L. trifolii</i>	<i>L. sativae</i>	<i>C. horticola</i>	<i>L. trifolii</i>	<i>L. sativae</i>	<i>C. horticola</i>
Parasitoid						
Braconidae						
<i>Opius</i> sp.				6 (23.1) [†]		8 (24.2) [†]
Eulophidae						
<i>Asecodes erxias</i> (Walker)					1 (33.3) [†]	
<i>Diglyphus albiscapus</i> Erdős				1 (3.8)		
<i>Diglyphus isaea</i> (Walker)	23 (74.2) [‡]	1 (33.3) [‡]	37 (84.1) [‡]			
<i>Diglyphus minoeus</i> (Walker)		1 (33.3) [‡]	5 (11.4) [†]	19 (73.1) [‡]	2 (66.7) [‡]	25 (75.8) [‡]
<i>Hemiptarsenus varicornis</i> (Girault)	7 (22.6) [†]	1 (33.3) [‡]				
<i>Neochrysocharis formosa</i> (Westwood)	1 (3.2)		2 (4.5)			
Total number	31	3	44	26	3	33
Leafminer						
<i>Liriomyza trifolii</i> (Burgess)	10			149	1	
<i>Liriomyza sativae</i> Blanchard	22	10	5		33	1
<i>Chromatomyia horticola</i> (Goureau)			2			
Total number	32	10	7	149	34	1

For each leafminer species, parasitoid species causing the highest (‡) and second highest (†) percent composition are indicated.

small numbers of leafminers and parasitoids were reared and this was reflected in findings such as only four parasitoid species being collected from both areas and only three parasitoids emerging from two samples of *L. sativae*.

Our results demonstrated that the dominant and common parasitoids obtained from the three leafminer species differed between fields and/or seasons; however, within the same of the leafminer species tested in field exposure.

DISCUSSION

Two alien leafminers, *L. trifolii* and *L. sativae*, and a native leafminer, *C. horticola*, have become serious pests on vegetables and ornamental crops in Japan. Leafminers have a wide host range, and kidney beans exposed in our surveys had many eggs deposited on them by leafminer species other than the species being tested. However, the results of the exposure experiments were probably unaffected by such contamination, either because the exposure period had finished before eclosion of eggs laid by contaminating leafminer species, or because immature contaminating leafminer larvae were infrequently parasitized. Leafminer parasitoids are not reported to attack host eggs (Koniishi, 1998), and immature leafminer larvae are fre-

quently used for host-feeding but not oviposition by adults in the family Eulophidae (Sugimoto and Ishii, 1979), which was the dominant parasitoid family in our surveys.

Previous studies demonstrated that *D. isaea* and *D. minoeus* were abundant on *C. horticola* (Takada and Kamijo, 1979; Saito, 2004), while *N. formosa* was one of the most abundant parasitoid species reared from *L. trifolii* and *L. sativae* (Saito et al., 1996; Arakaki and Kinjo, 1998; Tokumaru et al., 2007). These findings may have been influenced by the seasonal conditions during which the recordings were made. Hondo et al. (2006) examined temperature tolerance and net reproductive rate at 15, 20, 25 and 30°C for several parasitoid species. From their results, the authors suggested that *D. isaea*, *D. minoeus*, *C. pentheus* and *D. pusztenensis* are more adapted to low temperatures, and *N. formosa* and *Hemiptarsenus varicornis* (Girault) are more adapted to high temperatures. In our exposure experiments, the dominant parasitoid species differed in each season: the abundant parasitoid species were *D. isaea*, *D. minoeus* and *C. pentheus* in spring or fall (cool seasons), and *N. formosa* in summer (hot seasons), except *D. pusztenensis*, which was more abundant in summer. However, *C. horticola* has been shown to be abundant in cooler seasons (Chiba, 1983), while the hosts, *L. trifolii* and

L. sativae, were found at high densities during warm and hot seasons (Kobayashi et al., 1992; Tokumaru and Abe, 2001). Thus, optimal temperatures for parasitoid development may account for the dominance of different parasitoid species during the three seasons.

Salvo et al. (2005) demonstrated that resource concentration in cultivated habitats has a beneficial effect on the richness and abundance of leafminer parasitoids. In our surveys, however, total numbers of species and individuals of parasitoids were similar between vegetable gardens and grassland within the same season. *C. pentheus*, *D. albiscapus*, *D. isaea*, *D. minoews* and *N. formosa* were relatively common in the grassland habitat with poor resources, but in contrast, *Asecodes delucchi* (Boucek), *A. erxias* (Walker), *Opius* sp. and *Pnigalio katonis* (Ishii) were less frequently recorded from grassland. Different habitat distributions of parasitoid species may be influenced by characteristics such as vegetation structure, temperature preferences, motility for host searching and host range, including non-leafminer hosts. In the fall survey, the numbers of species and individuals of parasitoids were lower than surveys in spring and summer, possibly due to reduced efficiency in host-searching under low temperatures and a decrease in parasitoid density following a decline of leafminer populations.

As mentioned previously, the distribution of parasitoid species varied between the different field and seasonal conditions; however, within a season and within the same experimental field, the dominant parasitoid species were similar for all leafminer species. The finding suggests that the most common leafminer parasitoid species are polyphagous and have no strong or obvious host preference between *L. trifolii*, *L. sativae* or *C. horticola*. Such polyphagous parasitoids have an important role in the dynamics of invasive leafminers. Murphy and LaSalle (1999) reported that invasive leafminer populations have sometimes been observed to decline naturally a few years after invasion, and the authors hypothesized that this could be due to the action of local natural enemies, particularly polyphagous parasitoids. In Japan, alien *L. trifolii* populations declined naturally in the initial area invaded, Shizuoka, four or five years after being first recorded in 1990 (Saito, 2006).

The results obtained in this survey provide infor-

mation for the future management of *Liriomyza* spp. For example, the parasitoid complex of *C. horticola* may be a useful tool for the biological control of *Liriomyza* leafminer populations in glasshouses, because the dominant parasitoid species exhibit similar abundance in field tests comparing *Liriomyza* spp. and *C. horticola*. Studies are underway on the possible augmentative biological control of *L. sativae* populations by parasitoids that emerge from *C. horticola* when infested garden pea plants are introduced into glasshouse tomato crops attacked by *L. sativae* (Doi et al., 2005; Baeza Larios and Ohno, 2007). This method has the advantage that *C. horticola* very rarely infests tomato crops.

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