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Comparison of the suitability of three pest leafminers (Diptera: Agromyzidae) as hosts for the parasitoid *Dacnusa sibirica* (Hymenoptera: Braconidae)

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Abstract. The suitability of *Liriomyza sativae* Blanchard, *L. trifolii* (Burgess) and *L. bryoniae* (Kaltenbach) as hosts of the solitary larval-pupal parasitoid *Dacnusa sibirica* Telenga was studied. This parasitoid is used as a biological control agent against *L. trifolii* and *L. bryoniae*. The parasitoid laid eggs in *L. sativae*, but no adult parasitoids emerged from the puparia. In contrast, *D. sibirica* adults emerged from the puparia of *L. trifolii* and *L. bryoniae*, and there was no significant difference in emergence rate, female developmental time, or sex ratio when parasitizing these two host species. The parasitoid adults that emerged from the *L. bryoniae* puparia were significantly larger than those from *L. trifolii* puparia. In conclusion, *D. sibirica*, a useful biological control agent of *L. trifolii* and *L. bryoniae*, cannot control *L. sativae*.

INTRODUCTION

Dacnusa sibirica Telenga (Hymenoptera: Braconidae) is a solitary larval-pupal parasitoid that attacks *Liriomyza* (Diptera: Agromyzidae) pests (Minkenberg & van Lenteren, 1986). It is used as a biological control agent for the serpentine leafminer, *L. trifolii* (Burgess), tomato leafminer, *L. bryoniae* (Kaltenbach) and pea leafminer, *L. huidobrensis* (Blanchard) in Europe (Malais & Ravensberg, 1991), and is effective against *L. trifolii* in Japan (Ozawa et al., 2001).

Similar to *L. trifolii* and *L. huidobrensis*, the vegetable leafminer, *L. sativae* Blanchard, is indigenous to the New World and causes serious damage to vegetables and ornamentals (Spencer, 1973). This leafminer extended its geographical range to Africa and Asia in the 1990s (Iwasaki et al., 2000) and coexists with *L. trifolii* and *L. bryoniae* on tomato plants in Japan (Abe & Kawahara, 2001). The spread of *L. sativae* is considered to have been caused by the world-wide trade of crops. To determine whether *D. sibirica* can be used as a biological control agent against *L. sativae*, the host suitability of *L. sativae* was compared with that of *L. trifolii* and *L. bryoniae.*

MATERIAL AND METHODS

Insects and plants

All insects were maintained and tested at 25°C under a 15L : 9D photoperiod. Laboratory cultures of Japanese populations of *L. sativae*, *L. trifolii*, and *L. bryoniae* were established from larvae collected in Joyo (Kyoto Prefecture), Itoman (Okinawa Prefecture) and Kyoto (Kyoto Prefecture), respectively. Kidney bean, *Phaseolus vulgaris* L., was used as a host plant for leafminer colony maintenance and experiments (for rearing details see Abe, 2001). A laboratory culture of *D. sibirica* on *L. trifolii* was established from individuals that were reared at Arysta LifeScience Co., Ltd (Tokyo).

Emergence rate, developmental time and sex ratio

After emergence, female wasps of *D. sibirica* were placed with one male in a glass tube (8 mm diameter, 4 cm high) for mating. One day later, they were used in the experiments. The oviposition rate of *D. sibirica* is highest on the second day after emergence at 25°C (Minkenberg, 1990).

Dacnusa sibirica females prefer first and second instar larvae of *Liriomyza* spp. for oviposition (Malais & Ravensberg, 1991). Therefore, *P. vulgaris* plants, with two true leaves, was infested with 2-day-old (first instar) larvae of each *Liriomyza* species as described in Abe (2001). The base of the stem of each plant was immersed in water in a 10-ml glass vial; the vial was attached to the inside of the bottom of a cylindrical glass tube (6.4 cm diameter, 22 cm high). The top of the tube was covered with organdy and the bottom with Kimwipe®. Undiluted honey was streaked on the inside of the tube as a food source for the wasps. Just after transferring a plant into a tube, one mated female adult of *D. sibirica* was released in the tube and allowed to oviposit in the *Liriomyza* larvae for 24 h before removal. Ten wasps were used for each *Liriomyza* species. There was no significant difference in the mean number of host larvae per replicate among the three species (Table 1, ANOVA, F = 0.540, d.f. = 2, P > 0.05). After removal of the wasps, the immature *Liriomyza* were reared and the emergence of *D. sibirica* wasps was recorded. The adult wasps that emerged were sexed and the lengths of the hind tibia (as indices of body size) of 15 randomly chosen wasps of each sex of each species were measured to the nearest 6.7 µm under a binocular microscope. After adult emergence,

TABLE 1. Effects of host species on emergence rate and sex ratio of *Dacnusa sibirica.* Mean ± SE. Means with the same letter were not significantly different from each other in the same column (n = 10).

Host species	Number of host larvae exposed	Emergence rate of *D. sibirica* (arcsine transformed)	Sex ratio of *D. sibirica* (arcsine transformed)
L. sativae	30.8 ± 3.8a	0(0)a	–
L. trifolii	33.9 ± 3.6a	0.50 ± 0.09 (45.1 ± 5.4)b	0.49 ± 0.10 (44.4 ± 7.7)a
L. bryoniae	28.7 ± 3.2a	0.66 ± 0.05 (55.1 ± 2.9)b	0.50 ± 0.06 (43.3 ± 3.8)a

TABLE 2. Effects of exposure to *Dacnusa sibirica* on pupal mortality of *Liriomyza sativae.* Mean ± SE. Means were not significantly different from each other in the same column (n = 10).

	Number of puparia	Pupal mortality of *L. sativae* in puparia without exit holes (arcsine transformed)
Treatment	30.8 ± 3.8	0.26 ± 0.04 (30.3 ± 2.7)
Control	31.3 ± 3.5	0.19 ± 0.04 (25.2 ± 2.4)

host puparia with no exit holes were dissected under a binocular microscope to record mortality of *Liriomyza* species and larval, pupal, or adult mortality of *D. sibirica*. As a control, 10 *P. vulgaris* plants infested with *L. sativae* were prepared and reared without a wasp. After adult emergence, mortality of *L. sativae* was recorded.

Parasitization of *L. sativae*

As in the previous experiment, 2-day-old larvae of *L. sativae* were exposed to a mated female of *D. sibirica* for 24 h in a cylindrical glass tube. The mean (± SE) number of *L. sativae* larvae per wasp was 43.6 ± 4.6 (n = 10). After removal of the wasp from the tube, the *L. sativae* larvae were reared for two days. Then each *L. sativae* larva was transferred into a drop of Ringer's solution on a glass slide and dissected with two minute pins under a microscope. The number and developmental stage of *D. sibirica* found in the *L. sativae* larvae were recorded.

Data analysis

Arcsine transformed data of emergence rate and sex ratio in *D. sibirica* were analyzed with Scheffé's multiple comparison test (Scheffé, 1953) and one-way ANOVA, respectively. Effects of exposure to *D. sibirica* on pupal mortality of *L. sativae* were also analyzed with one-way ANOVA after arcsine transformation. Effects of host species on body size and developmental time of *D. sibirica* were analyzed using Scheffé's multiple comparison test. The significance level for all statistical tests was set at P = 0.05.

RESULTS

Emergence rate, developmental time and sex ratio

Except for seven *L. bryoniae* larvae, all *Liriomyza* larvae formed puparia. No dead larvae, pupae, or adults of *D. sibirica* were found in host puparia without exit holes. These puparia contained only dead host pupae. Adults of *D. sibirica* did not emerge from the *L. sativae* puparia (Table 1). The pupal mortality of *L. sativae* did not differ significantly between treatment and control (Table 2, ANOVA, F = 1.925, d.f. = 1, P > 0.05). In contrast, adults of *D. sibirica* emerged from the *L. trifolii* and *L. bryoniae* puparia (Table 1). Neither the emergence rate (number of adult wasps that emerged / host puparium) of *D. sibirica* (Table 1, ANOVA, F = 2.653, d.f. = 1, P > 0.05) nor the sex ratio (Table 1, ANOVA, F = 0.009, d.f. = 1, P > 0.05) differed significantly between *L. trifolii* and *L. bryoniae*. Hind tibial lengths of *D. sibirica* that emerged from *L. bryoniae* puparia were significantly longer than those from *L. trifolii* puparia (Table 3, Scheffe's multiple comparison test, P < 0.05). Table 4 shows total developmental times, from oviposition to adult emergence of both sexes of *D. sibirica* raised on *L. trifolii* and *L. bryoniae*. The total developmental time of male *D. sibirica* was significantly shorter than that of female *D. sibirica* in each host species (Scheffe's multiple comparison test, P < 0.05). The total developmental time of male *D. sibirica* that emerged from *L. trifolii* puparia was significantly shorter (albeit less than one day) than that from *L. bryoniae* puparia (Scheffe's multiple comparison test, P < 0.05). However, no significant difference was found in total developmental time of female *D. sibirica* between the two host species (Scheffe's multiple comparison test, P > 0.05).

TABLE 3. Effects of host species on hind tibial length of *Dacnusa sibirica*. Mean ± SE. Means with the same letter were not significantly different from each other (n = 15).

Host species	Sex	Hind tibial length (mm)
L. trifolii	Male	0.494 ± 0.010a
	Female	0.508 ± 0.008a
L. bryoniae	Male	0.587 ± 0.009b
	Female	0.593 ± 0.013b

TABLE 4. Effects of host species on developmental time of *Dacnusa sibirica.* Mean ± SE. Means with the same letter were not significantly different from each other.

Host species	Sex	Number of individuals	Total developmental time from oviposition to adult emergence (day)
L. trifolii	Male	86	15.6 ± 0.1a
	Female	67	17.0 ± 0.1c
L. bryoniae	Male	99	16.3 ± 0.1b
	Female	89	16.9 ± 0.1c

Parasitization of *L. sativae*

Eggs of *D. sibirica* were found in *L. sativae* larvae in each replicate, and first instar larvae of the wasp were found in six replicates. The mean (± SE) parasitization rate and egg hatchability for the ten replicates were 0.61 ± 0.08 and 0.30 ± 0.10, respectively. Superparasitism occurred in three replicates. The rates of superparasitism (number of hosts containing more than one *D. sibirica* / number of hosts containing any *D. sibirica*) were 0.06, 0.17, and 0.17.

DISCUSSION

Our results reveal that *D. sibirica* cannot complete its development in *L. sativae*. Females of *D. sibirica* oviposited in *L. sativae* larvae and some of these eggs hatched. However, no adult wasps emerged from the puparia. Moreover, parasitization by *D. sibirica* did not significantly influence the mortality of *L. sativae* pupae. Thus, *L. sativae* is not a suitable host for *D. sibirica*. Further study is required to clarify how *L. sativae* kills the immature stages of *D. sibirica*.

In contrast, *L. trifolii* and *L. bryoniae* are equally suitable as hosts for *D. sibirica*, because *D. sibirica* adults emerged from the puparia of both species, with no significant difference in emergence rate, female developmental time, or sex ratio between the two host species. Adult wasps that emerged from *L. bryoniae* puparia were significantly larger than those from *L. trifolii* puparia. Host pupae can be regarded as fixed host resource available for the development of *D. sibirica* offspring. The pupa of *L. bryoniae* is larger than that of *L. trifolii* (Abe, in press.). Thus, the difference in body size of *D. sibirica* adults may be caused by the difference in pupal size of the two host species.

Generally, large female parasitoids have higher fitness than small females (King, 1987; Visser, 1994). Under field conditions, such a relationship between female size and fitness has been demonstrated in some parasitoids (Visser, 1994; Kazmer &

Luck, 1995; West et al., 1996; Bennett & Hoffmann, 1998; Ellers et al., 1998). Thus, in order to estimate the fitness of parasitoids, size has been investigated in a number of studies of biological control and evolutionary ecology (Roitberg et al., 2001). Since size and fecundity in *D. sibirica* females are positively related (Croft & Copland, 1993), *L. bryoniae* would be a more suitable host than *L. trifolii* for *D. sibirica*.

Among the three *Liriomyza* species studied, *L. sativae* and *L. trifolii* are more closely related to one another than they are to *L. bryoniae*, judging from their external morphology (Spencer, 1973). This phylogenetic relationship is supported by mitochondrial sequence data (Miura et al., 2004). Moreover, the close relationship between *L. sativae* and *L. trifolii* is indicated by the production of hybrids under laboratory conditions (Tokumaru & Abe, in press). The present results show that, unlike *L. trifolii* and *L. bryoniae*, *L. sativae* is not a good host for *D. sibirica*. Thus, phylogenetic relationship and suitability for parasitoid survival and development are not congruent among the host species used in this study

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