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EFFECT OF CONSPECIFIC AND HETEROSPECIFIC FECES ON FORAGING AND OVIPOSITION OF TWO PREDATORY LADYBIRDS: ROLE OF FECAL CUES IN PREDATOR AVOIDANCE

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Abstract—Growing evidence suggests a flow of chemical information from higher to lower trophic levels that affects foraging and oviposition of 'prey' in response to potential risks from predators. This was investigated in two species of ladybird predators of aphids, *Harmonia axyridis* and *Propylea japonica*. *H. axyridis* is known to be the stronger intraguild predator and *P. japonica* to be the more frequent intraguild prey in interactions of these two species. These ladybirds share aphid prey on mugworts, hibiscus, and Italian ryegrasses in fields of northern Japan but largely avoid each other on the same plant. Fecal cues of these ladybird predators were found to contribute in their assessment of predation risk from conspecific and heterospecific competitors in common habitats. Gravid females of *H. axyridis* reduced rates of feeding and oviposition when exposed to feces of conspecifics, but not when exposed to feces of *P. japonica.* In contrast, gravid females of *P. japonica* reduced feeding and oviposition when exposed to feces of both *H. axyridis* and its own species. Females of both ladybird species exhibited similar behavior in response to water extracts of feces. For *P. japonica*, the influence of heterospecific feces was greater than that of conspecific feces. Our results demonstrate that feces of ladybirds contain odors that have the potential to deter the feeding and oviposition activities of conspecific as well as heterospecific ladybirds. Such deterrence allows these insects to avoid predation risk. Differences in responses of the two predators are discussed.

Key Words—Foraging behavior, ladybird predators, aphid prey, predator avoidance, feces, chemical cues.

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INTRODUCTION

Ladybird predators foraging in aphid patches exploit the rising phase of aphid populations for oviposition in order to maximize fitness (Kindlmann and Dixon, 1993; Dixon, 1997; Agarwala and Bardhanroy, 1999). Aphid patches attract several species of predators. Because of the temporary nature of aphid colonies in space and time (Dixon, 1998) and depletion of the prey population caused by predation, ladybird predators often face competition for limited food and risk of intraguild predation (Agarwala and Dixon, 1992; Rosenheim et al., 1995; Hironori and Katsuhiro, 1997). Eggs and young larvae are particularly vulnerable to cannibalism (Agarwala, 1991; Lucas et al., 1997) and interspecific predation (Agarwala et al., 1998; Obrycki et al., 1998a,b; Phoofolo and Obrycki, 1998; Yasuda and Ohnuma, 1999). How do foraging ladybirds decide which patches to attack and which to avoid? Species that can assess predation risk and avoid predator-rich patches would increase their fitness for survival and reproduction (Krebs and Davies, 1987; Polis et al., 1989; Grostal and Dicke, 2000; Dicke, 2000). The role of female ladybirds is assumed to be important in assessing risk as they determine the site of oviposition (Gutierrez et al., 1984; Dixon, 2000).

Oviposition-deterring semiochemicals have been reported in a number of aphidophagous predators such as chrysopids (Růžička, 1994, 1996, 1997a, 1998), coccinellids (Růžička, 1997b; Doumbia et al., 1998; Hemptinne et al., 1998, 2001; Hemptinne and Dixon, 2000; Yasuda et al., 2000), and cecidomyiid (Růžička and Havelka, 1998). The effects of these semiochemicals are associated with either larval tracks of conspecifics (Yasuda et al., 2000; Hemptinne et al., 2001), heterospecifics (Růžička, 1997b), or both (Růžička, 2001), or possibly feces (Doumbia et al., 1998). Semiochemicals present in the larval tracks of ladybird beetles contain certain alkanes or their mixtures that deter conspecific females of *Coccinellia septempunctata* L., *Adalia bipunctata* (L.) (Hemptinne et al., 2001), and *Harmonia axyridis* Pallas (Yasuda et al., 2000) from laying eggs in aphid colonies already attacked by larvae. Another study demonstrated that females of different coccinellid species vary in their oviposition-deterrence response to larval tracks of different predator species, thereby suggesting that larval tracks might contain different semiochemicals or different mixtures (Růžička, 2001). These semiochemicals, however, do not affect the time spent by female beetles in searching, as evident by the similar frequency of their fecal spots between clear substrate and those contaminated with larval tracks (Růžička, 2001).

According to Dicke and Grostal (2001), of all chemical information gathered by animals, cues about predation risk are of special significance because predation risk usually has important and immediate consequences on fitness. Feces from herbivorous insects are known to either deter predators (Whitman et al., 1990; Olmstead, 1994; Müller and Hilker, 1999) or to be exploited by predators and parasitoids (Grewal et al., 1993). Feces from herbivorous insects are also known to deter conspecifics from oviposition (Renwick and Radke, 1980; Prokopy et al., 1984; Hilker, 1985; Hilker and Klein, 1989; Anderson et al., 1993). Insect feces contain chemicals derived from the insect's food, and some of these may be volatiles that may be recognized from a distance (Aegolopoulos et al., 1995). Feces of carnivorous insects are produced in small quantity and may contain protein metabolites that could be effective oviposition deterrents for species that serve as prey of these predators (Nolte et al., 1994; Kats and Dill, 1998; Grostal and Dicke, 2000). In northern Japan (Tsuruoka, Yamagata Prefecture), *Harmonia axyridis* Pallas and *Propylea japonica* (Thunberg) are the primary ladybird predators of aphids on mugworts, *Artemisia vulgaris* var. *indica*, hibiscus, *Hibiscus syriacus*, and Italian ryegrass, *Lolium multiforum*, but the two predators mostly avoid co-occurring in the same patch. Thus, these ladybirds feed on the same prey aphid species yet tend to be spatially separated. This could be adaptive as the co-occurrence of differently sized larvae often leads to interspecific predation (Agarwala and Dixon, 1992; Rosenheim et al., 1995; Lucas et al., 1997), and, in the laboratory study, eggs and larvae of *P. japonica* are preyed upon by *H. axyridis* (Agarwala, unpublished). *H. axyridis* is a large and polyphagous predator with larvae that can complete development on aphids and on many nonaphid insect prey, whereas *P. japonica* is a smaller and more specialized predator of aphids and some coccids (Hodek and Honek, 1996; Dixon, 2000). We hypothesized that the smaller ladybird species might be able to assess predation risk in its environment and avoid interaction with the larger species. We, therefore, investigated the effects of fecal cues of the two species of ladybird predators on conspecific and heterospecific females. Because of their poor dispersal ability, larval ladybirds likely stay and complete their development within a patch, and, therefore, their feces likely accumulate on plants in the patch. Feces may also accumulate from ovipositing females when they stay in a patch for a certain length of time. This accumulating feces might contain substances that can affect aggregation and oviposition of conspecifics and heterospecifics in the competitive environment of patchy resources.

Oviposition-deterring properties of feces in aphidophagous ladybirds have not been studied previously. Therefore, we first ascertained that feces of the two species accumulate on different plant species where they often forage for aphids. We also determined the rate of fecal production by larvae and adults and the length of time that feces remain on plants. We then exposed adult female ladybirds to larval and adult feces and their water extracts to determine effects on prey consumption, oviposition, and behavior.

METHODS AND MATERIALS

Incidence of Ladybirds and Their Fecal Deposits in Fields. To ascertain the incidence of the two species and their feces in fields, aphid-infested leaves of three hosts plants ($N = 100$ per species)—mugworts, hibiscus, and Italian ryegrass—were sampled in an area of about 1.5 km^2 around the premises of the Yamagata University at Tsuruoka, Japan. Leaves containing aphids, larvae, and/or adults of *H. axyridis* and *P. japonica* and their fecal deposits were collected individually in 9-cm-diam. Petri dishes and brought to the laboratory. Feces from these leaves were collected separately and the wet weight recorded within 3 hr of collection. It was possible to identify feces of the two ladybird species in the field because of the shape and size. *H. axyridis* excreted feces as pellets that were longer and thicker in comparison to the smaller pellets excreted by *P. japonica*. The numbers of aphids of each species and the active stages of ladybird predators present in the samples ($N = 100$ leaves per plant species) were also recorded.

Laboratory Protocol. Fourth instars and adult females of *P. japonica* and *H. axyridis* were used in experiments. Each stock culture was kept in 9-cm-diam. Petri dishes with fresh bean leaves, damp paper tissue, and a piece of corrugated filter paper and fed with pea aphids, *Acyrthosiphon pisum* (Harris). Insects were kept at $22 \pm 1°C$ and a photoperiod of 16L:8D. Two-day-old fourth instars or 18- to 20-day-old adult females were starved for 24 hr in order to induce similar levels of hunger. Feces of larvae or adults of the two species were distinguished as 1, 5, or 10 days old after their deposition and obtained from the stock culture. Ladybirds often deposited feces as pellets on bean leaves or tissue and filter papers in Petri dishes. One-day-old feces were easily collected with the tip of a fine paintbrush, but 5- and 10 day old feces were dry and often fixed to surfaces. These were removed by applying a little pressure with the tip of a needle or a pair of fine forceps at the base of fecal deposits.

Experiments were done either in Petri dishes (5 or 9 cm diam.) or on potted plants. Dishes were lined with filter paper before use. Individual 16-day-old bean plants, *Vicia faba*, about 15 cm in height, bearing 6–7 leaves and grown in 9-cm-diam. pots, were used. A 9-cm-diam. filter paper was placed on the soil around the base of each bean plant. A grease band around the base of the stem, near the soil, of each plant prevented larvae or adults from leaving. A ventilated plastic cylinder was placed over each plant to isolate it from adjacent ones. Live adult pea aphids were offered to larvae and adult ladybirds, 40 per larva or adult female to *H. axyridis* and 20 per larva or adult female to *P. japonica*, on leaves of the bean plant, both in Petri dishes and on potted plants (mean fresh weight $\pm$ SE of adult pea aphid $= 2.70 \pm 0.08$ mg; $N = 24$). In a separate study, these numbers of aphids were determined to meet the optimal daily requirements for larvae and adults of the two ladybird species (data unpublished). Any ladybirds or aphids that dropped off the potted plants were placed back on during observations at periodic intervals.

Rates of Deposit of Feces by Larvae and Adult Ladybirds on Optimal Diets. Fourth instars and adult females of the two species were kept individually in

9-cm-diam. Petri dishes or released at 1 cm above the grease bands of potted plants ($N = 10$). These were offered pea aphids as described. The fecal pellets that were deposited in 24 hr were separately collected from each dish and potted plant and weighed on a microbalance. The number of aphids eaten by individual larvae and adults during the same period in each treatment was recorded.

Length of Time Feces Remain on Plants. To determine how long feces remained on plants after being deposited by beetles, 30 larvae of each species were individually released at the bases of infested potted plants. After 24 hr, larvae were removed from all plants. Fecal pellets were collected separately from 10 plants each with feces of *H. axyridis* and *P. japonica*. The remaining plants were left undisturbed and examined for fecal remains after 5 and 10 days. On each occasion, fecal pellets were collected from 10 plants each for each species. All feces were weighed on the day of collection within 3 hr.

Effects of Feces in Combination with Prey and Host Plant on Behavior of Female Ladybirds. Ten gravid females of both *P. japonica* and *H. axyridis* were kept individually with 1-, 5-, or 10-day-old feces of larvae or adults of their own or the other species. Each female of both species was kept with a bean leaf infested with an optimal number of pea aphids and evenly contaminated with 1 mg of 1-, 5-, or 10-day-old feces. In fields, nearly the same amount of feces per leaf was found on the three plant species (Table 1 below). The numbers of aphids eaten and eggs laid by the females were recorded after 24 hr.

Influence of Feces on Behavior of Female Ladybirds. To evaluate whether feces of conspecifics or heterospecifics influence encounter rates of female ladybirds and their reactions to feces, two experiments were carried out. Individual *H. axyridis* and *P. japonica* females were kept with 1 mg of 1-day-old feces of larvae or adults of their own or the other species in 5-cm-diam. Petri dishes. Each experiment, one on *H. axyridis* females and the other on *P. japonica* females, consisted of four treatments. In each treatment, 10 females were exposed to one kind of feces only. Thus, eight treatments were set up. Feces were placed on a piece of 1-cm-diam. filter paper and kept in the center of the Petri dish. The numbers of encounters made and reaction to feces (avoidance, i.e., backing off or moving away after approaching feces, no reaction) of their own versus the other species for both larvae and adults, or feeding by a female beetle in 5 min were analyzed with χ^2 test.

Effectiveness of Fecal Cues in Water-Extract on Oviposition by Female Ladybirds. To examine whether chemical cues from the feces of larvae and adults were effective in influencing the oviposition by female beetles, water extracts of feces were used. To control for the effect of water, a control of sterile distilled water was used. One-day-old feces of larvae and adults of the two species (10 mg each) were homogenized in 1 ml of sterile distilled water in a clean dry glass tube. The mixture was allowed to precipitate for 1 hr before the supernatant was collected. In each replicate, 200 μl of this supernatant was transferred by a micropipet to the center of a 5-cm-diam. Whatman filter paper and placed in the center of a 9-cm-diam. Petri dish. In a trial study, supernatant prepared from this ratio (10 mg/ml) was found to be oviposition deterrent in both ladybird species. Ten *P. japonica* and 10 *H. axyridis* females were kept individually with water extracts of feces of larvae or adults of their own or the other species and provided 20 (*P. japonica*) or 40 (*H. axyridis*) adult pea aphids. Ten females of each species were kept individually with the control extract. Thus, for each ladybird species, 50 females were used in five replicates. The number of eggs laid by individual females was recorded after 24 hr. Bean leaves were not used for providing aphids in order to exclude the possible influence of host plant volatiles.

Statistical Analyses. Results of paired treatments (e.g., for production of feces by larvae or adult females in Petri dishes and on potted plants) were analyzed by using the Student's t test. Results of multiple comparisons, including amount of fecal deposits onto three plant species in fields, length of time feces remained on plants, and effects of larval and adult feces or their water extracts on prey consumption or oviposition of conspecific and heterospecific female ladybirds were analyzed by one-way or two-way analyses of variance (ANOVA). Significant differences between means of different treatments were established with the Scheffé multiple range test. Results of behavioral responses by female ladybirds to conspecific and heterospecific feces and proportions of plants visited by only one or both ladybird species in fields were analyzed by a χ^2 test. The level of significance for all statistical tests was $P = 0.05$.

RESULTS

Incidence of Ladybirds and Fecal Deposits in Fields. In fields, 37–53% of the aphid-infested leaves of the three host plant species had deposits of ladybird feces. The ratio of ladybird predators to aphids per leaf of a host plant species was higher for mugwort, and its leaves were contaminated with greater amounts of feces in comparison to aphid-infested leaves of hibiscus and Italian ryegrasses (Table 1; total feces: $F_{2,297} = 6.73$, $P = 0.001$; feces per leaf contaminated: $F_{2,129} = 6.49$, $P = 0.002$). For the three host plant species, the proportion of plants with only *P. japonica* or only *H. axyridis* was either equal to (hibiscus) or significantly higher than (mugworts and Italian ryegrass) those with both species (Figure 1).

Rates of Deposit of Feces by Larvae and Adult Ladybirds on Optimal Diets. In both species, fourth instars produced more feces per aphid consumed than did adult females (Figure 2; *H. axyridis*: potted plants: $t_{18} = 3.49$, $P < 0.001$, Petri dishes: $t_{18} = 2.88$, $P < 0.01$; *P. japonica*: potted plants: $t_{18} = 3.86$, $P < 0.001$, Petri dishes: $t_{18} = 2.13$, $P < 0.01$). The number of aphids consumed by a fourth instar or an adult female after 24 hr was nearly the same on potted plants and in Petri dishes (Table 2; *H. axyridis*: larvae: $t_{18} = 0.66$, $P > 0.05$; adults: $t_{18} = 0.70$, $P > 0.05$; *P. japonica*: larvae: $t_{18} = 0.91$, $P > 0.05$; adults: $t_{18} = 2.52$, $P > 0.05$). Larvae

TABLE 1. RATIO OF LADYBIRD LARVAE AND ADULTS TO APHIDS AND AMOUNT OF FECES COLLECTED FROM APHID-INFESTED LEAVES OF THREE HOST PLANT SPECIES IN FIELDS (100 LEAVES)

Host plants and aphids[a]	Ratio of ladybirds to aphids	Leaves with ladybird (total)	Feces collected per leaf (mean ± SE, mg)[b]	
			Feces (%)	Contaminated
Mugworts (*Macrosiphoniella yomogifoliae* Shinji)	0.03	53	63.75 ± 0.64(a)	1.18 ± 0.04(a)
Hibiscus (*Aphis gossypii* Glover)	0.021	41	43.60 ± 0.55(b)	1.06 ± 0.04(a)
Italian ryegrasses (*Sitobion avenae* Takahashi)	0.008	37	35.30 ± 0.50(b)	0.95 ± 0.05(b)

[a] Aphid species in parenthesis.
[b] Dissimilar letters in parenthesis following means in a column indicate significant difference at $P < 0.05$; Scheffé's multiple range test.

of the two ladybird species deposited similar amounts of feces in Petri dishes and on potted plants, but adult females deposited more feces in dishes than on plants (Table 2; *H. axyridis*: larvae: $t_{18} = 0.48$, $P > 0.05$; adults: $t_{18} = 4.49$, $P = 0.001$; *P. japonica*: larvae: $t_{18} = 1.49$, $P > 0.05$; adults: $t_{18} = 5.94$, $P = 0.001$). These results suggest that some feces deposited on plants by adult females (but not larvae) drop to the soil or the lower parts of the plants.

Length of Time Feces Remain on Plants. The wet weight of the feces deposited by beetles in a 24-hr period declined in the days thereafter (amount of fecal material remaining for *H. axyridis*: after 1 day = 1.66 ± 0.08 mg, after 5 days = 0.71 ± 0.05 mg, after 10 days = 0.23 ± 0.03; *P. japonica*: after 1 day = 0.72 ± 0.03 mg, after 5 days = 0.33 ± 0.03 mg, after 10 days = 0.13 ± 0.02 mg). The reduction in the wet weight of feces remaining was statistically significant for both species (*H. axyridis*: $F_{2,27} = 247.08$, $P < 0.001$; *P. japonica*: $F_{2,27} = 104.70$, $P < 0.001$) and might be attributed to two factors: (1) the drying of feces [*H. axyridis*: dry weight of feces (mg) = 0.90 × fresh weight of feces (mg), $N = 25$; *P. japonica*: dry weight (mg) = 0.86 × fresh weight (mg), $N = 25$], and (2) dislodgement as aphids and predators walked over the material (during the period ladybird larvae remain on plants) that caused some feces to fall to the soil or the lower parts of the plants.

Effects of Feces in Combination with Prey and Host Plant on Behavior of Female Ladybirds. Females modified their foraging and oviposition in response to fecal cues of larval and adult stages. *Propylea japonica* females consumed significantly fewer aphids and laid fewer eggs in the presence of 1- or 5-day-old

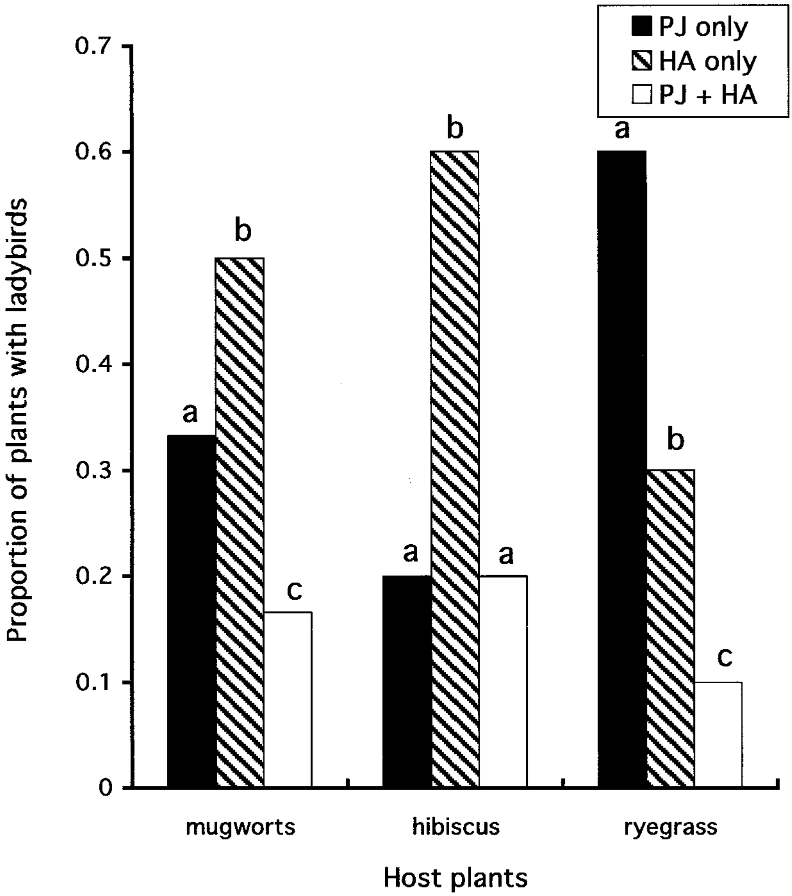

FIG. 1. Proportion of feces-contaminated plants of the three plant species ($N = 20$ plants per species) that harbored larvae and/or adults of only *P. japonica* (PJ), only *H. axyridis* (HA), or both predators. Bars of a host plant with different letters indicate significant differences at $P < 0.05$ (χ^2 test).

H. axyridis feces of both larval and adult stages than in the presence of feces of larvae and adults of their own species (Table 3; Figure 3a,b). However, prey consumption and oviposition by *P. japonica* females in the presence of 10-day-old feces did not differ depending on whether the feces were from conspecifics or from *H. axyridis*.

Harmonia axyridis females consumed signficantly fewer aphids and laid fewer eggs in the presence of 1-day-old feces of their own species than they did in the presence of *P. japonica* feces. This was true for feces of both larvae and adult

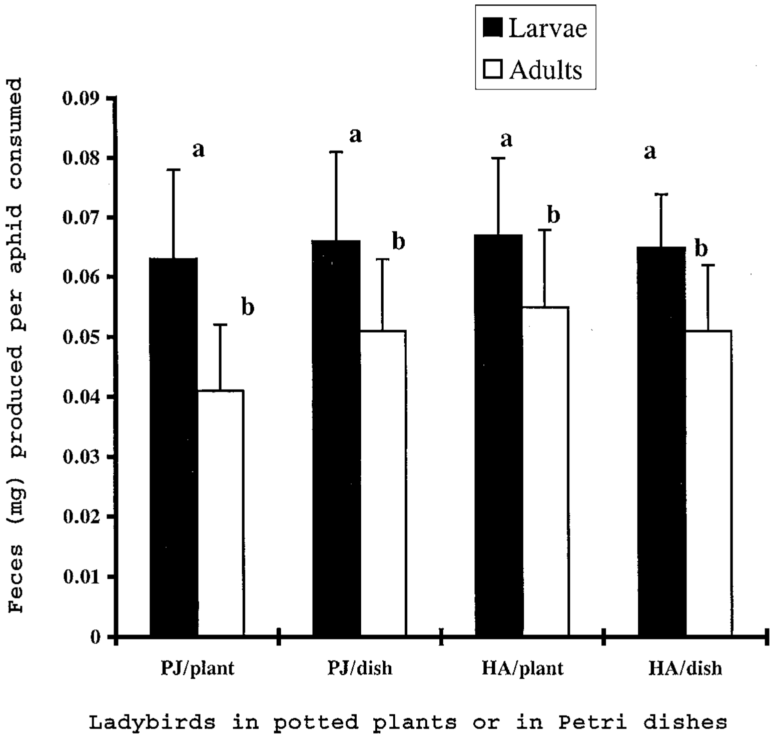

FIG. 2. Wet weight (mean ± SD) of feces produced per aphid consumed by a fourth instar or an adult female of *P. japonica* (PJ), or *H. axyridis* (HA) in 24 hr when kept on an optimal diet of the pea aphid either in 9-cm-diam. Petri dishes or on potted plants ($N = 10$). Bars of a treatment with different letters indicate significant difference at $P < 0.05$ (Student's t test).

beetles (Table 4; Figure 4a,b). Prey consumption and oviposition by *H. axyridis* females did not differ in the presence of 5- or 10-day-old feces of their own species versus those of *P. japonia*.

Influence of Feces on Behavior of Adult Ladybirds. Ladybird females did not differ in their encounter rate with feces of their own versus the other species for either larval or adult feces (*P. japonica* female: larval feces: $\chi^2 = 0.82$, $P > 0.05$; adult feces: $\chi^2 = 0.03$, $P > 0.05$; *H. axyridis* female: larval feces: $\chi^2 = 0.22$, $P > 0.05$; adult feces: $\chi^2 = 0.00$, $P > 0.05$). However, the nature of responses to the feces differed. In *P. japonica*, females avoided feces of both larval and adult *H. axyridis* upon contact more frequently than they did feces of conspecifics (larval feces: $\chi^2 = 36.38$, $P < 0.001$; adult feces: $\chi^2 = 41.78$, $P < 0.001$; Figure 5a). In contrast, *H. axyridis* females avoided conspecific feces more frequently than

TABLE 2. PEA APHIDS EATEN AND FECES COLLECTED AFTER 24 HOURS FROM FOURTH INSTARS OR ADULT FEMALES OF *H. axyridis* OR *P. japonica* KEPT IN PETRI DISHES OR ON POTTED PLANTS ($N = 10$)[a]

	Aphids eaten (N)		Feces collected (mg)	
Treatment	Potted plants	Petri dishes	Potted plants	Petri dishes
H. axyridis				
Larvae	27.10 ± 1.06(a)	28.20 ± 1.53(a)	1.68 ± 0.14(a)	1.79 ± 0.16(a)
Adults	32.10 ± 1.59(a)	33.90 ± 1.57(a)	1.29 ± 0.13(a)	1.58 ± 0.13(b)
P. japonica				
Larvae	13.60 ± 0.89(a)	12.50 ± 0.75(a)	0.73 ± 0.02(a)	0.83 ± 0.05(a)
Adults	14.20 ± 2.39(a)	15.80 ± 0.73(a)	0.58 ± 0.05(a)	0.84 ± 0.05(b)

[a] Dissimilar letters in parenthesis following means in a row for each experiment indicate significant difference at $P < 0.05$, Student's t test.

P. japonica faces (larval feces: $\chi^2 = 21.51$, $P < 0.001$; adult feces: $\chi^2 = 21.64$, $P < 0.001$; Figure 5b).

The contrast was also apparent in the number of encounters to which there were no reactions. In *P. japonica*, females failed to react to conspecific feces upon encounter more frequently than they did to *H. axyridis* (Figure 5a; larval feces: $\chi^2 = 28.74$, $P < 0.001$; adult feces: $\chi^2 = 35.62$, $P < 0.001$). In *H. axyridis*, however, females failed to react to feces of *P. japonica* more frequently than they did to feces of conspecifics (Figure 5b; larval feces: $\chi^2 = 23.70$, $P < 0.001$; adult feces: $\chi^2 = 23.96$, $P < 0.001$). Few encounters of females of either species resulted in feeding on conspecific feces.

Effectiveness of Fecal Cues in Water Extracts on Oviposition by Female Ladybirds. Females of *P. japonica* laid significantly fewer eggs in treatments with water extracts of ladybird feces than in controls (Table 5). Among treatments, numbers of eggs laid were higher in the presence of water extracts of conspecific feces than in the presence of water extracts of *H. axyridis* feces ($F_{4,45} = 30.02$, $P < 0.001$). Females of *H. axyridis* laid more eggs both in the control and in treatments with water extracts of *P. japonica* feces than females in treatments with water extract of conspecific feces (Table 5; $F_{5,45} = 16.01$, $P < 0.001$).

DISCUSSION

Previous studies showed the adult ladybird beetles response to oviposition-deterring semiochemicals in larval tracks of conspecifics and heterospecifics (Hemptinne and Dixon, 2000; Yasuda et al., 2000; Růžička, 2001; Hemptinne et al., 2001) and the deleterious effects on the development of larger species by eating semiochemical-coated eggs of smaller ladybird species (Agarwala and Dixon, 1992; Agarwala et al., 1998). The results of this study have advanced the existing

TABLE 3. TWO-WAY ANOVA FOR MEAN NUMBER OF PEA APHIDS CONSUMED AND EGGS LAID BY *P. japonica* FEMALES PROVIDED WITH 1-, 5-, OR 10-DAY-OLD FECES FROM LARVAE OR ADULTS OF THEIR OWN SPECIES OR *H. axyridis*

		Feces of larvae or adults of conspecific and heterospecific ladybirds used					
		1 day old		5 days old		10 days old	
Source	*df*	*F*	*P*	*F*	*P*	*F*	*P*
Aphids consumed, main effects:							
Between ladybird species	1	28.90	<0.001	45.59	<0.001	0.13	0.724
Between ladybird stages	1	5.39	0.027	0.06	0.800	0.28	0.597
Interaction species × stages	1	0.004	0.947	1.62	0.132	0.008	0.930
Error	36						
Eggs laid, main effects:							
Between ladybird species	1	23.41	<0.001	78.78	<0.001	0.06	0.809
Between stages	1	1.64	0.132	1.97	0.123	3.59	0.072
Interaction species × stages	1	0.08	0.778	0.45	0.506	0.002	0.962
Error	36						

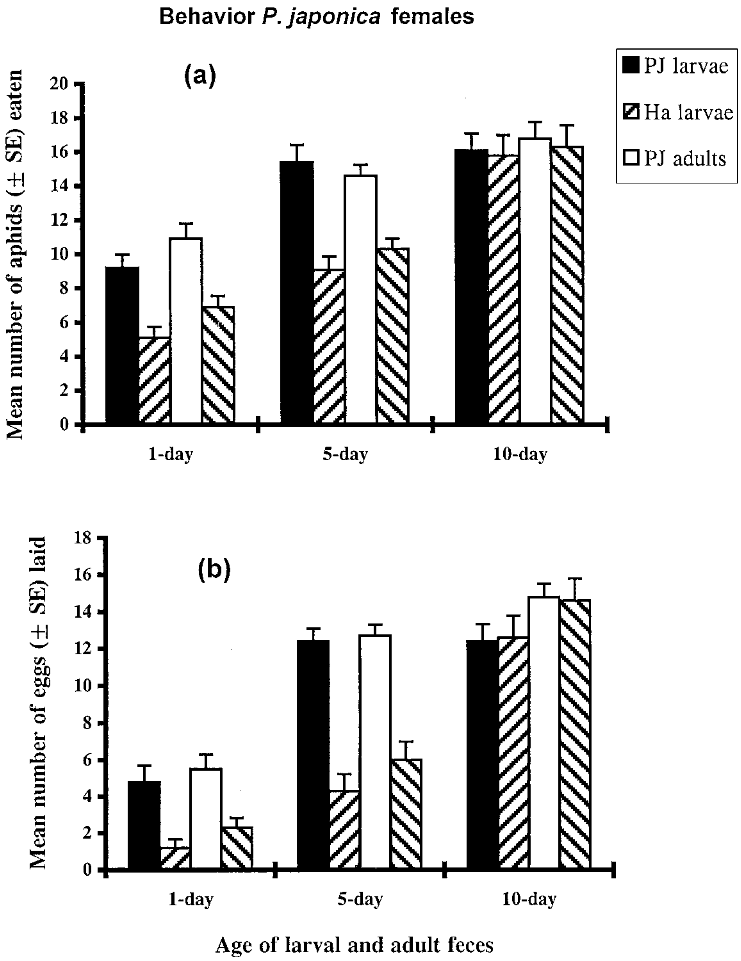

FIG. 3. Numbers (mean ± SE) of aphids consumed (a) and eggs laid (b) by a *P. japonica* (PJ) female when maintained on an optimal diet of pea aphids (20 aphids/female) while exposed to 1 mg of 1-, 5-, or 10-day-old feces of larvae or adults of conspecifics or *H. axyridis* (HA) ($N = 10$).

knowledge by identifying a new source of semiochemicals in ladybird feces and the effects of them on prey consumption and oviposition of female beetles. In this study, larvae and adults of *H. axyridis* and *P. japonica* foraged in the same habitats, but largely occupied mutually exclusive spaces. Feces accumulate on plants as the predators remain in patches of high prey density. Fourth instars produced more feces per prey consumed than did adult females, and larval feces accumulated in

TABLE 4. TWO-WAY ANALYSIS OF VARIANCE FOR MEAN NUMBER OF PEA APHIDS CONSUMED AND EGGS LAID BY *H. axyridis* FEMALES PROVIDED WITH 1-, 5-, OR 10-DAY-OLD FECES OF LARVAE OR ADULTS OF THEIR OWN SPECIES OR *P. japonica*

		Feces of larvae or adults of conspecific and heterospecific ladybird used					
		1 day old		5 days old		10 days old	
Source	*df*	*F*	*P*	*F*	*P*	*F*	*P*
Aphids consumed, main effects:							
Between ladybird species	1	161.05	<0.001	0.08	0.779	0.00	1.00
Between ladybird stages	1	6.61	0.014	0.02	0.898	1.49	0.131
Interaction species × stages	1	0.73	0.397	1.86	0.127	0.98	0.327
Error	36						
Eggs laid, main effects:							
Between ladybird species	1	24.51	<0.001	0.04	0.837	0.17	0.686
Between ladybird stages	1	1.31	0.122	0.05	0.820	0.49	0.489
Interaction species × stages	1	0.138	0.712	0.31	0.582	0.00	1.00
Error	36						

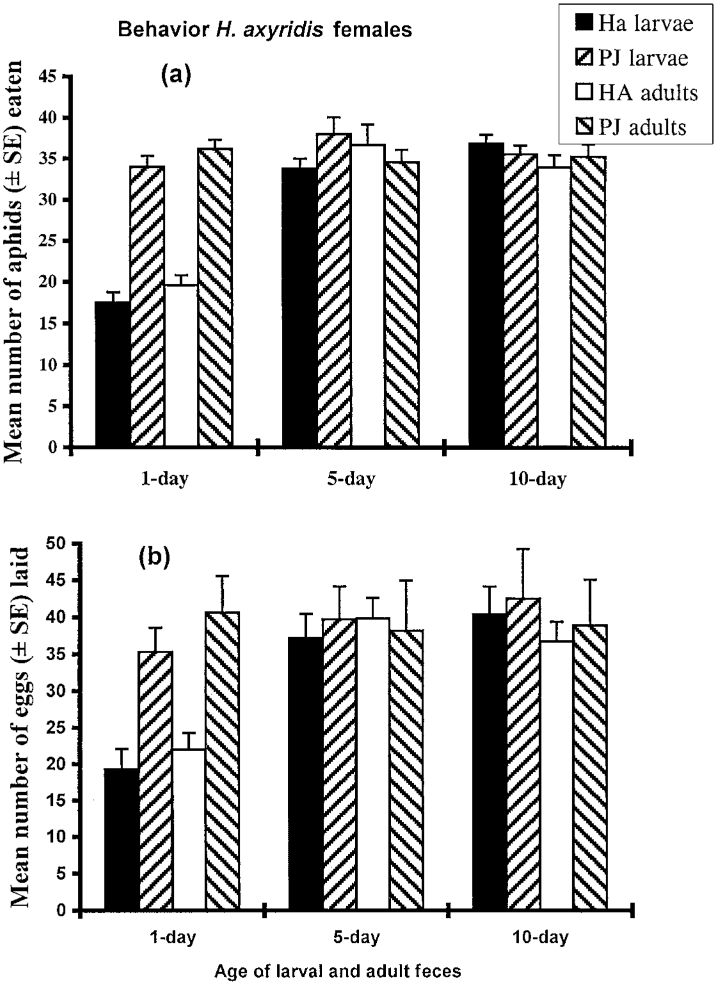

FIG. 4. Numbers (mean ± SE) of aphids consumed (a) and eggs laid (b) by a *H. axyridis* (HA) female when maintained on an optimal diet of pea aphids (40 aphids/female) while exposed to 1 mg of 1-, 5-, or 10-day-old feces of larvae or adults of conspecifics or *P. japonica* (PJ) ($N = 10$).

larger quantity on plants. These differences can be attributed to three factors: first, larvae are flat-bodied, adults are domed-shaped second, larvae stay longer than adults in a patch (Dixon, 2000); and finally, fourth instars have poorer food conversion efficiency than do adult females (Hodek and Honek, 1996). Results showed that fecal deposits on plants shrank in size with time, which may be

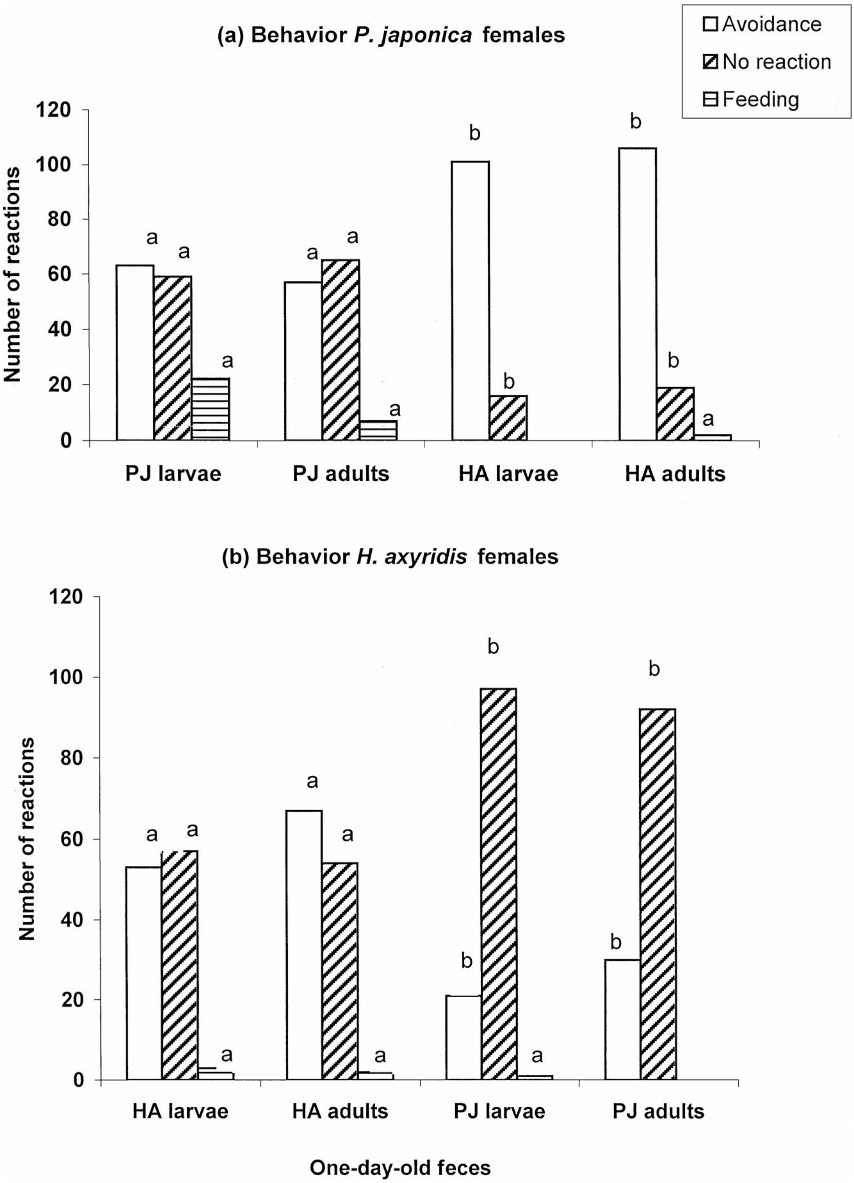

FIG. 5. Number of instances in which avoidance, no reaction, or feeding on fecal pellets occurred when a *P. japonica* (PJ) female (a) or *H. axyridis* (HA) female (b) encountered 1-day-old feces of larvae or adults of conspecifics and heterospecifics during 5 min of observation in 5-cm-diameter Petri dishes ($N = 10$). Similar bars with different letters in each graph indicate significant differences at $P < 0.05$ (χ^2 test).

TABLE 5. EFFECT OF WATER EXTRACTS OF LARVAL AND ADULT FECES OF CONSPECIFICS AND HETEROSPECIFICS ON OVIPOSITION BY *P. janponica* AND *H. axyridis* ($N = 10$)[a]

Treatment	Eggs laid after 24 hr by female (MEAN ± SE) *P. japonica*	*H. axyridis*
Water extract		
P. japonica feces		
Adults	7.80 ± 0.95(a)	40.50 ± 2.58(a)
Larvae	7.30 ± 0.53(a)	37.50 ± 2.31(a)
H. axyridis feces		
Adults	3.70 ± 0.81(b)	23.30 ± 2.57(b)
Larvae	3.10 ± 0.67(b)	21.80 ± 1.73(b)
Water only (control)	14.80 ± 1.14(c)	38.90 ± 2.00(a)

[a] Dissimilar letters in parenthesis following means in a column indicate significant difference at $P < 0.05$; Scheffé's multiple range test.

attributed to the effects of drying and shaking of plants by foraging animals, with the consequence that some feces fall to the surrounding ground or lower parts of plants. This implies that recently produced and still wet and sticky feces of predators were most likely to be effective sources of signals to other predators. Similarly, wet feces of *Pieris* butterflies were found to contain more volatiles than dry feces and to be more helpful to parasitoids in host-searching (Aegolopoulos et al., 1995).

Our results further demonstrate that fecal cues of the larger species *H. axyridis* caused conspecific as well as *P. japonica* females to reduce their rates of feeding and oviposition. In contrast, fecal cues of the smaller species, *P. japonica,* affected rates of feeding and oviposition of conspecifc females but not *H. axyridis* females. Grostal and Dicke (1999, 2000) and Dicke and Grostal (2001) provided several examples from both invertebrates and vertebrates (including insects and mites) where potential prey avoid predation by reducing feeding and breeding upon perceiving kairomones contained in the feces of intraguild predators. Reduced feeding by ladybirds in the present study may be attributed to reduced foraging caused by avoidance of patches containing feces of predators.

Durations of the effectiveness of fecal cues differed between the two species. In the case of *H. axyridis*, 1-day and 5-day-old feces were avoided by *P. japonica* females but only 1-day-old feces were avoided by conspecific females. In *P. japonica*, only 1-day-old feces were avoided by conspecific females. The differences in avoidance responses may be attributed to the relative risk of predation. *H. axyridis* is a more generalist predator with higher voracity (more than twice that of *P. japonica*; Agarwala, unpublished), and its eggs and larvae are avoided by larvae and adults of *P. japonica* (Agarwala, unpublished). In contrast, *P. japonica* is a more specialist predator, and its eggs and larvae are not known to be toxic. By avoiding both conspecific and heterospecific fecal cues, *P. japonica* females seem to be seeking nearly enemy-free spaces that would enhance their fitness for foraging and oviposition. Lack of a avoidance response in *H. axyridis* females to feces of *P. japonica* suggests that larger ladybirds avoid conspecific interactions but not interspecific ones in which they are most likely to prevail as intraguild predators. A classical study of interactions involving the ladybird species of this study and *Coccinella septempunctata bruckii* in the presence of extraguild prey (aphids) (Sato, unpublished), as cited by Dixon (2000), suggests that larvae of *H. axyridis* survive better when kept with larvae of *P. japonica* and *C. septempunctata bruckii* than when kept with aphids alone. In marked contrast, larvae of *P. japonica* survive less well when kept with larvae of *H. axyridis* or *C. septempunctata bruckii* than with aphids alone. These results support the hypotheses that *H. axyridis* is a superior predator, that its larvae prevail in interspecific competition, and that its interactions with other species may often result in those species leaving the patch or plant rather than remaining to risk becoming intraguild prey.

H. axyridis is a polyphagous predator (Hodek and Honek, 1996) and is often the last ladybird to arrive and attack aphids in a patch because of its requirement for high prey density (Takahashi, 1989; Agarwala and Yasuda, 2001). Given the temporary nature of short-lived aphid colonies, growing larvae of *H. axyridis* may often face food scarcity. This could lead to intense intraspecific competition. Avoiding oviposition in sites already occupied by conspecifics, as suggested by our findings, could, therefore, be advantageous. Further study is now called for to identify the chemicals in feces that affect foraging and oviposition beahvior of *H. axyridis* and *P. japonica*.

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REFERENCES

AEGOLOPOULOS, N. G., DICKE, M., and POSTHUMUS, M. A. 1995. Role of volatile inforchemicals emitted by feces of larvae in host-searching behavior of parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae): a behavioral and chemical study. *J. Chem. Ecol.* 21:1789–1811.

AGARWALA, B. K. 1991. Why do ladybirds (Coleoptera: Coccinellidae) cannibalise? *J. Biosci.* 16:103–109.

AGARWALA, B. K. and BARDHANROY, P. 1999. Numerical response of ladybird beetles (Col., Coccinellidae) to aphid prey (Hom., Aphididae) in a field bean in north-east India. *J. Appl. Entomol.* 123:401–405.

AGARWALA, B. K. and DIXON, A. F. G. 1992. Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecol. Entomol.* 17:303–309.

AGARWALA, B. K. and YASUDA, H. 2001. Larval interactions in aphidophgaous predators: effectiveness of wax cover as defence shield of *Scymnus* larvae against predation from syrphids. *Entomol. Exp. Appl.* 100:101–107.

AGARWALA, B. K., BHATTACHARYA, S., and BARDHANROY, P. 1998. Who eats whose eggs? Intra versus interspecific interactions in starving ladybird beetles predaceous on aphids. *Ethol. Ecol. Evol.* 10:361–368.

ANDERSON, P., HILKER, M., HANSSON, B. S., BOMBOSCH, S., KLEIN, B., and SCHILDKNECHT, H. 1993. Oviposition deterring components in larval frass of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae): a behavioural and electrophysiological evaluation. *J. Insect Physiol.* 39:129–137.

DICKE, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: amultitrophic perspective. *Biochem. Syst. Ecol.* 28:601–617.

DICKE, M. and GROSTAL, P. 2001. Natural enemies detection *Annu. Rev. Ecol. Syst.* 32:1–23.

DIXON, A. F. G. 1997. Patch quality and fitness in predatory ladybirds. *Ecol. Stud.* 130:205–223.

DIXON, A. F. G. 1998. Aphid Ecology: An Optimisation Approach. Chapman & Hall, London.

DIXON, A. F. G. 2000. Insect Predator–Prey Dynamics: Ladybird Beetles and Biological Control. Cambridge University Press, Cambridge.

DOUMBIA, M., HEMPTINNE, J.,-L., and DIXON, A. F. G. 1998. Assessment of patch quality by ladybirds: role of larval tracks. *Oecologia* 113:197–202.

GREWAL, P. S., GAUGLER, R., and SELVAN, S. 1993. Host recognition by entomopathogenic nematodes: Behavioural response to contact with host feces. *J. Chem. Ecol.* 19:1219–1231.

GROSTAL, P. and DICKE, M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: A case for acarine interactions. *Behav. Ecol.* 10:422–427.

GROSTAL, P. and DICKE, M. 2000. Recognising one's enemies: A functional approach to risk assessment by prey. *Behav. Ecol. Sociobiol.* 47:258–264.

GUTIERREZ, J., BAUMGAERTNER, J. U., and SUMMERS, C. G. 1984. Multitrophic models of predator–prey energetics. *Can. Entomol.* 116:923–963.

HEMPTINNE, J.-L. and DIXON, A. F. G. 2000. Defence, oviposition and sex: semiochemical parsimony in two species of ladybird beetles (Coleoptera, Coccinellidae)? A short review. *Eur. J. Entomol.* 97:443–447.

HEMPTINNE, J.-L., LOGANY, G., and DIXON, A. F. G. 1998. Mate recognition in the two-spot ladybird beetle, *Adalia bipunctata*: role of chemical and behavioural cues. *J. Insect Physiol.* 44:1163–1171.

HEMPTINNE, J.-L., LOGANY, G., DOUMBIA, M., and DIXON, A. F. G. 2001. Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Chemoecology* 11:43–47.

HILKER, M. 1985. Larvenkot als Eiablage-Deterrens bei *Spodoptera littoralis*. *Naturwissenschaften* 72:485–486.

HILKER, M. and KLEIN, B. 1989. Investigation of oviposition deterrent in the larval frass of *Spodoptera littoralis* (Boisd.). *J. Chem. Ecol.* 15:929–938.

HIRONORI, Y. and KATSUHIRO, S. 1997. Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. *Entomophaga* 42:153–163.

HODEK, I. and HONEK, A. 1996. Ecology of Coccinellidae. Kluwer Academic, Dordrecht, The Netherlands.

KATS, L. B. and DILL, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecol. Sci.* 5:361–394.

KINDLMANN, P. and DIXON, A. F. G. 1993. Optimal foraging in ladybird beetles (Coleoptera: Coccinellidae) and its consequences for their use in biological control. *Eur. J. Entomol.* 90:443–450.

KREBS, J. R. and DAVIES, N. B. 1987. An Introduction to Behavioural Ecology. Blackwell Scientific, Oxford.

LUCAS, E., CODERRE, P., and BRODEUR, J. 1997. Instar-specific defense of *Coleomegilla maculata lengi* (Col.: Coccinellidae): influence on attack success of the intraguild predator *Chrysoperla rufilabris* (Neur.: Chrysopidae). *Entomophaga* 42:3–12.

MÜLLER, C. and HILKER, M. 1999. Unexpected reactions of a generalist predator towards defensive devices of cassidine larvae (Coleoptera, Chrysomelidae). *Oecologia* 118:166–172.

NOLTE, D. I., MASON, J. R., EPPLE, G., ARONOV, E., and CAMPBELL, D. L. 1994. Why are predator urines aversive to prey? *J. Chem. Ecol.* 20:1505–1516.

OBRYCKI, J. J., GILES, K. L., and ORMORD, A. M. 1998a. Experimental assessment of interactions between larval *Coleomegilla maculata* and *Coccinella septempunctata* (Coleoptera: Coccinellidae) in field cages. *Environ. Entomol.* 27:1280–1288.

OBRYCKI, J. J., GILES, K. L., and ORMORD, A. M. 1998b. Interactions between an introduced and indigenous coccinellid species at different prey densities. *Oecologia* 117:279–285.

OLMSTEAD, K. L. 1994. Waste products as chrysomelid defenses, pp. 311–318, *in* P. H. Jolivet, M. L. Cox, and E. Petitpierre (eds.). Novel Aspects of the Biology of Chrysomelidae. Kluwer Academic, Dordrecht, The Netherlands.

PHOOFOLO, M. W. and OBRYCKI, J. J. 1998. Potential for intraguild predation and competition among predatory Coccinellidae and Chrysopidae. *Entomol. Exp. Appl.* 89:47–55.

POLIS, G. A., MYERS, C. A., and HOLT, R. D. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annu. Rev. Ecol. Syst.* 20:297–330.

PROKOPY, R. J., ROITBERG, B. D., and AVERILL, A. L. 1984. Resource partitioning, pp. 301–330, *in* W. J. Bell, and R. T. Carde (eds.). Chemical Ecology of Insects. Chapman & Hall, London.

RENWICK, J. A. A. and RADKE, C. D. 1980. An oviposition deterrent associated with frass from feeding larvae of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Environ. Entomol.* 9:318–320.

ROSENHEIM, J. A., KAYA, H. K., EHLER, L. E., MAROIS, J. J., and JAFFEE, B. 1995. Intraguild predation among biological control agents: theory and evidence. *Biol. Control* 5:303–335.

RŮŽIČKA, Z. 1994. Oviposition-deterring pheromones in *Chrysopa oculata* (Neuroptera: Chrysopidae). *Eur. J. Entomol.* 91:361–370.

RŮŽIČKA, Z. 1996. Oviposition-deterring pheromone in Chrysopidae (Neuroptera): Intra- and interspecific effects. *Eur. J. Entomol.* 93:161–166.

RŮŽIČKA, Z. 1997a. Persistence of the oviposition-deterring pheromone in *Chrysopa oculata* (Neur.: Chrysopidae). *Entomophaga* 42:109–114.

RŮŽIČKA, Z. 1997b. Recognition of oviposition-deterring allomones by aphidophagous predators (Neuroptera: Chrysopidae, Coleoptera: Coccinellidae). *Eur. J. Entomol.* 94:431–434.

RŮŽIČKA, Z. 1998. Further evidence of oviposition-deterring allomone in chrysopids (Neuroptera: Chrysopidae). *Eur. J. Entomol.* 95:35–39.

RŮŽIČKA, Z. 2001. Oviposition responses of aphidophagous coccinellids to tracks of ladybird (Coleoptera: Coccinellidae) and lacewing (Neuroptera: Chrysopidae) larvae. *Eur. J. Entomol.* 98:183–188.

RŮŽIČKA, Z. and HAVELKA, J. 1998. Effects of oviposition-deterring pheromone and allomones on *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). *Eur. J. Entomol.* 95:211–216.

TAKAHASHI, K. 1989. Intra- and interspecific predations of lady beetles in spring alfalfa fields. *Jpn. J. Appl. Entomol. Zool.* 57:199–203.

WHITMAN, D. W., BLUM, M. S., and ALSOP, D. W. 1990. Allomones: chemicals for defense, pp 289–351, *in* D. L. Evans, and Schmidt, J. O. (eds.) Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators. State University of New York Press, Albany, New York.

YASUDA, H. and OHNUMA, N. 1999. Effect of cannibalism and predation on the larval performance of two ladybird beetles. *Entomol. Exp. Appl.* 93:63–67.

YASUDA, H., TAKAGI, T., and KOGI, K. 2000. Effects of conspecific and heterospecific larval tracks on the oviposition behavior of the predatory ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Eur. J. Entomol.* 97:551–553.