

## Interactions of Native and Non-Native Lady Beetle Species (Coleoptera: Coccinellidae) With Aphid-Tending Ants in Laboratory Arenas

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**ABSTRACT** Interactions between lady beetles and the European fire ant (*Myrmica rubra* L.) tending potato aphids [*Macrosiphum euphorbiae* (Thomas)] were compared in the laboratory. Lady beetle species native to North America (*Coccinella trifasciata* perplexa Mulsant, *Coleomegilla maculata lengi* Timberlake, *Hippodamia convergens* Guérin-Ménéville) and non-native species of Palearctic origin [*Coccinella septempunctata* L., *Harmonia axyridis* (Pallas), *Hippodamia variegata* (Goeze), *Propylea quatuordecimpunctata* L.] were evaluated. *Harmonia axyridis* consumed a significantly greater number of aphids compared with all other species but *C. septempunctata*. Ant stings affected *H. variegata* and *C. septempunctata* to a greater extent than other species. Ants showed a significantly greater amount of aggression toward *H. convergens* and *H. variegata* compared with *P. quatuordecimpunctata*. *P. quatuordecimpunctata*, *C. trifasciata*, and *H. axyridis* reacted significantly less to ants compared with *H. variegata*, *H. convergens*, *C. maculata*, and *C. septempunctata*. Differences in interactions with natural enemies may explain, in part, the successful establishment of some non-native coccinellids in new habitats.

**KEY WORDS** coccinellid, ant, native, non-native, natural enemies

Some ant species tend plant-feeding insects, such as aphids, mealybugs, and scale insects, to exploit their sugary excrement known as “honeydew” as a food source (Auclair 1963, Way 1963, Buckley 1987, Völkl et al. 1999). Tending ants may move aphids to shelter them from unfavorable environmental conditions and clean them of debris such as their own sticky excrement, accumulations of which can promote fungal growth (Hölldobler and Wilson 1990, Gonzalez Hernandez et al. 1999). Ants may also provide protection to aphids from predators and parasites (Bartlett 1961, Way 1963, Buckley 1987, Vinson and Scarborough 1989, Charles 1993, Reimer et al. 1993, Jahn and Beardsley 1994, Gonzalez Hernandez et al. 1999).

Generally considered beneficial because they feed on plant pests (Hodek 1973, Gordon 1985), lady beetles have been intentionally introduced to new locations worldwide for biological control in agricultural crops (Gordon 1985, Dreistadt and Flint 1996, Koch 2003). They have also been unintentionally introduced through plant exports and other cargo (Chantal 1972, Schaefer et al. 1987, Day et al. 1994). Reductions in native lady beetle numbers have been correlated with introductions of non-native lady beetles (Elliot et al. 1996, Brown and Miller 1998, Colunga-Garcia and Gage 1998, Michaud 2002, Brown 2003, Turnock et al.

2003, Alyokhin and Sewell 2004). It is believed that some non-native lady beetle species may outcompete native species for food (Michaud 2002). Because many lady beetle species feed primarily on plant-feeding insects, such as aphids, at least during part of their life cycle (Gordon 1985, Hodek and Honěk 1996), their competitive abilities may be in part determined by their interactions with tending ant species (Bartlett 1961, Vinson and Scarborough 1989, Hanks and Sadof 1990, Jahn and Beardsley 1994, Sloggett et al. 1998, Sloggett and Majerus 2000).

Many assessments of the relationships between lady beetle species have been made by measuring relative abundances (Elliot et al. 1996, Brown and Miller 1998, Colunga-Garcia and Gage 1998, Michaud 2002, Brown 2003, Turnock et al. 2003, Alyokhin and Sewell 2004, Finlayson et al. 2008), intraguild predation (Takahashi 1989, Elliot et al. 1996, Hough-Goldstein et al. 1996, Hironori and Katsuhiko 1997, Cottrell and Yeargan 1998, Yasuda and Ohnuma 1999, Dixon 2000, Kajita et al. 2000, Sakuratani et al. 2000, Lynch et al. 2001, Yasuda et al. 2001, Michaud 2002, Brown 2003, De Clercq et al. 2003, Yasuda et al. 2004), and direct competition (Dixon 2000, Michaud 2002, Yasuda et al. 2004) between lady beetle species. There has been little examination of indirect interactions that may influence lady beetle populations. Although a number of studies have documented differences in numbers of lady beetles and/or their prey in environments with

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and without ants (Chapin 1966, Bradley 1973, Bhatkar 1982, Jiggins et al. 1993, Sloggett et al. 1998, Dutcher et al. 1999, Corbara et al. 1999, Sloggett et al. 1999, Sloggett and Majerus 2000, Kaplan and Eubanks 2002), few studies have assessed differences between lady beetle species in their interactions with ants that might favor the survival of one species over another. We conducted laboratory studies with three native and four non-native lady beetle species presented with aphid prey that were protected by ants. We evaluated prey consumption and interactions with ants to determine whether different interactions with natural enemies may differentially affect the survival of different lady beetle species.

### Materials and Methods

**Study Species.** We chose seven aphidophagous lady beetles species that are abundant in Maine (Finlayson et al. 2008) for this study. Three of those are native to the state: the three-banded lady beetle *Coccinella trifasciata perplexa* Mulsant, the twelve-spotted lady beetle *Coleomegilla maculata lengi* Timberlake, and the convergent lady beetle *Hippodamia convergens* Guérin-Méneville. *C. trifasciata* is native from Labrador south to New Jersey and west to California and Alaska (Gordon 1985). The native range of *C. maculata* is restricted to eastern North America from Ontario to Georgia and west to Texas and Minnesota (Gordon 1985). *H. convergens* is a widespread species, with its native range from British Columbia and Ontario south to South and Central America and the Antilles (Gordon 1985).

We used four non-native lady beetles: the seven-spotted lady beetle *Coccinella septempunctata* L., the multicolored Asian lady beetle *Harmonia axyridis* (Pallas), the variegated lady beetle *Hippodamia variegata* (Goeze), and fourteen-spotted lady beetle *Propylea quatuordecimpunctata* L. All four species are Palearctic in origin and were both intentionally and inadvertently introduced in North America. *C. septempunctata* has been established in North America since 1973 (Angalet and Jacques 1975) and in the eastern United States since 1979 (Angalet et al. 1979). The first established population of *H. axyridis* in North America was documented 1988 (Chapin and Brou 1991, Tedders and Schaefer 1994) and now this species occurs throughout much of the continental United States (Koch 2003). *H. variegata* is currently widespread throughout northeastern North America (Gordon and Vandenberg 1991, Wheeler 1993, Wheeler and Stoops 1996, Hoebeke and Wheeler 1996, Ellis et al. 1999, Cormier et al. 2000). The first established population of *P. quatuordecimpunctata* was found in Quebec in 1968 (Wheeler 1990). In Maine, it was first documented in 1988 in Kennebec, Penobscot, and Aroostook Counties, where it is believed to have expanded its range from existing populations in Quebec (Wheeler 1990).

The European red ant, *Myrmica rubra* L. is a Palearctic species native to Europe and northern Asia (Elmes 1975, Collingwood 1979, Elmes et al. 1999,

Czechowski et al. 2002). It was first documented in the United States in 1908 in Forest Hills, MA (Wheeler 1908) and has since been observed in the United States in Maine, New Hampshire, Massachusetts, Rhode Island, New York, New Jersey, Pennsylvania, and Washington, DC, and in Canada in Nova Scotia, New Brunswick, Québec, and Ontario (Grodén et al. 2005). *Myrmica rubra* is known to commonly tend aphid colonies in its native range (Seifert 1996) and in Maine (Garnas 2005). In Maine, it is highly aggressive and known to have a profound impact on insect communities, including decreases in native ants and increases in plant-feeding insects (Garnas 2005). Therefore, we decided to use *M. rubra* as a model species to test the comparative ability of different lady beetle species to secure aphid prey in the presence of tending ants.

We chose the potato aphid, *Macrosiphum euphorbiae* (Thomas), to serve as the ant-tended prey. *M. euphorbiae* is native to North America and common in Maine (Blackman and Eastop 1984). As a generalist, it is known to feed on >200 varieties of plants including potato (*Solanum* sp.), apple (*Malus* sp.), *Aster* sp., rose (*Rosa* sp.), and ragweed (*Ambrosia* sp.) (Blackman and Eastop 1984). It is also known to be tended by *M. rubra* (C.F., unpublished data) and is a common prey item for many lady beetle species (Shands et al. 1972, Gordon 1985, Hodek and Honěk 1996).

**Insect Origins and Maintenance.** We collected adult lady beetles 48–72 h before test initiation in Orono, ME, from a variety of habitats: mixed shrub (*Solidago* sp., *Rubus* sp., *Prunus* sp., *Rosa* sp., *Cornus sericea*, *Alnus* sp.), apple (*Malus* sp.), grain (*Hordeum* sp., *Avena* sp.), mixed organic crops (*Solanum lycopersicon*, *Allium* sp., *Brassica* sp., *Pisum* sp., *Phaseolus* sp.), and field (*Phleum pratense*, *Trifolium* sp., *Cirsium* sp., *Vicia* sp., *Fragaria* sp.). Captured beetles were housed in Percival I-33VL Intellus environmental chambers (Percival Scientific, Perry, IA) at 20°C and 16 (light):8 (dark)-h photoperiod and provided with water, but no food, for 48 h before test initiation.

We collected 10 ant nests, each containing a queen and from 300 to 500 workers, from an area of known infestation in suburban Bar Harbor, ME (latitude: 44.385904, longitude: -68.209514), on 14 June 2006. Ants were housed in the laboratory in plastic containers (125 cm long, 67 cm wide, 15 in cm tall). To prevent ants from escaping, container walls were coated with Fluon (ACG Chemicals Americas, Bayonne, NJ). For shelter, each nest was provided with a potato plant (15-cm-diameter pot) and an inverted peat pot (10 cm diameter), under which a moist sponge supplied a constant supply of water. Twice a week, each nest was provided with six drosophila larvae, 0.5 g of granulated sugar, and 2.0 g of chopped, boiled eggs.

We obtained potato aphids from a colony maintained in our laboratory. The colony was originally founded by aphids collected from potato (*Solanum tuberosum*, Family: Solanaceae) fields in Presque Isle, ME, and maintained for at least 20 generations on excised potato foliage in the laboratory. The colony was housed in Percival I-33VL Intellus environmental

chambers (Percival Scientific, Inc., Perry, Iowa) at 20°C and 16 (light):8 (dark)-h photoperiod.

**Lady Beetle Consumption of Potato Aphids.** To assure that the different beetle species used in these experiments would indeed feed on the species of aphid provided, we conducted feeding trials. In each trial, we placed a single lady beetle in a 100 by 15-mm polystyrene petri dish with an excised leaf infested with 10 late-instar aphid nymphs. Housed in a separate petri dish, we added the lady beetle to the petri dish containing the aphids by quickly exchanging lids between the two petri dishes when the lady beetle was on the lid. After 24 h, we recorded the number of surviving aphids. We conducted five trials with each lady beetle species.

**Ant-Aphid-Lady Beetle Interactions in Laboratory Arenas.** From 15 June to 6 July 2006, we conducted 20 trials for each lady beetle species. Before trials, we transferred 10 adult aphids to the main stem of potato plants using a soft-bristled paintbrush (these plants were different than plants used for nesting that were already in enclosures). Aphid numbers were counted every other day until reproduction was documented by the presence of nymphs. After 1 wk, aphid numbers reached at least 20 individuals per plant, and plants were introduced to ant enclosures in an area opposite the plant used for nesting. Once ants were observed tending aphids (in contact with aphids, sometimes moving aphids, but not consuming aphids), a single lady beetle was introduced. Each of the 10 ant nests was used in random order twice with an individual of each lady beetle species. Each trial contained only one beetle, and individual beetles were not reused. The lady beetle was transferred from the petri dish in which it was held by allowing it to crawl upon the end of a paintbrush. It was placed on the potato plant 5–10 cm above the aphid colony. We observed and documented ant and lady beetle behavior, including aphid consumption, for 20 min.

Based on preliminary observations, we divided interactions between lady beetles and ants into separate aggressive and reactive behavioral elements. We used the number of times ( $f$ , frequency) each element occurred during each trial to calculate modified aggression and reactions scores where elements were weighted based on energetic investment (Carlin and Hölldobler 1986, Holway et al. 1998, Suarez et al. 1999, Garnas et al. 2007). The aggression score was used to compare differences in ant aggression toward different lady beetle species and calculated according to the following formula:

$$\text{Aggression Score} = -1 \times f_a + 1 \times f_b + 2 \times f_c + 3 \times f_d + 4 \times f_e + 5 \times f_f$$

where  $f$  refers to the frequency at which a particular behavioral element was observed in a trial, and subscript letters refer to the following behavioral elements: a, avoiding; b, prolonged antennation; c, opening mandibles; d, chasing; e, grasping/biting; f, stinging.

Similarly, the reaction score was used to compare lady beetle response to ant aggression and calculated according to the following formula:

$$\text{Reaction Score} = -1 \times f_A + 1 \times f_B + 2 \times f_C + 3 \times f_D + 4 \times f_E + 5 \times f_F + 6 \times f_G$$

where  $f$  refers to the number of times a particular behavioral element was observed in our trials, and subscript letters refer to the following behavioral elements: A, continuing behavior previous to contact; B, changing movement (behavior altered from previous activity); C, pulling in legs/antennae; D, preening; E, turning on back/flailing legs/fluttering wings; F, backing away/running away; G, flying away.

**Lady Beetle Tolerance of Ant Stings.** Different species of beetles seemed to exhibit different reactions to stings by *M. rubra* during the trials described above. Therefore, we also subjected beetles to intentional sting trials with agitated ants to compare the effects of ant stings. As described above with behavioral trials, a lady beetle was transferred from the petri dish in which it was held by allowing it to crawl on the end of a paintbrush. It was transferred to a location near the ant nest and in the immediate proximity (within 1.5 cm) of patrolling ants by allowing it to crawl from the paintbrush into the observation arena. We tested 20 individuals of each species, with one beetle per trial, and each of the 10 ant nests used in random order twice with different individuals of each lady beetle species. We recorded the number of stings and removed lady beetles after having been stung, on average, 14 times (range: 10–20). Because we had a limited ability to control stings (the ants clung and continued stinging when we removed the lady beetles), a higher level of precision in obtaining stings was not possible. Throughout the course of the study, we held 20 additional individuals of each species under identical conditions with the exception that they were not introduced to ants and thus were not stung. These beetles served as controls. All beetles were held for 72 h of observation after exposure to ant stings, or not stung, in the case of control beetles, and their behavior was documented. Beetles were provided with moisture and held in individual petri dishes in Percival I-33VL Intellus environmental chambers at 20°C and 16 (light):8 (dark)-h photoperiod.

We assigned each beetle a response score based on its activity during the 72-h observation period. The value of the response score increased as the effects observed increased in intensity from no effect, to a behavioral effect, a physical effect, and death, where 0 = active or active when prodded; 1 = inactive or slow when prodded; 2 = impaired ambulatory locomotion, wings stretched out, or flips on back; or 3 = dead. When several effects of varying intensity were documented for a given beetle, the score assigned reflected only the observation with the highest value during the 72-h period.

**Statistical Analyses.** Data normality was tested using the Wilk-Shapiro test (PROC UNIVARIATE; SAS Institute, Cary, NC). Frequency data that were not nor-

mally distributed were transformed using  $\sqrt{X + 0.001}$  transformations (Zar 1999). Data from the aphid feeding trials, consumption during behavioral trials, and ant sting trials were transformed using rank transformations (Conover and Iman 1981). Means and SEs reported in this paper were calculated from the untransformed data.

Mean numbers of potato aphids consumed by different lady beetle species were compared by one-way analysis of variance (ANOVA; PROC GLM; SAS Institute). We used a split-plot ANOVA (PROC MIXED; SAS Institute), with beetle species as the plots and behaviors as the subplots, to compare behaviors among different lady beetle species, conducting a separate test for all ant behaviors and for all beetle behaviors. When interactions between beetle species and behavior were statistically significant, we conducted additional one-way ANOVAs followed by Tukey mean separation tests (PROC GLM; SAS Institute), comparing the frequencies with which different lady beetle species displayed each behavior.

Aggression and reaction scores were compared among the tested lady beetle species using one-way ANOVAs followed by Tukey mean separation tests (PROC GLM; SAS Institute). To determine whether there was a relationship between aggression and reaction scores or between aphid consumption during trials and aggression/reaction scores, we used correlation analysis (PROC CORR; SAS Institute).

The number of stings received by different lady beetle species was compared using one-way ANOVA (PROC GLM; SAS Institute). To determine whether different lady beetles responded differently to being stung by ants, we used a split plot ANOVA (PROC MIXED; SAS Institute) with lady beetle species as the plots and exposure status to ant stings (stung experimental beetles and not stung control beetles) as the subplots. When interactions between beetle species and sting status were statistically significant, we conducted additional one-way ANOVAs followed by Tukey mean separation tests (PROC GLM; SAS Institute) comparing differences among the species separately for stung beetles and control beetles. To determine whether being stung made a difference for each species, the mean scores for the stung beetles were also compared with the mean scores for the control beetles (PROC TTEST; SAS Institute).

## Results

**Verification of Lady Beetle Consumption of Potato Aphids.** Lady beetles consumed, on average,  $8.46 \pm 0.34$  (SE) potato aphids during the 24-h trial period (Table 1). There was no difference among the different species (ANOVA,  $df = 6,28$ ,  $F = 1.17$ ,  $P = 0.3478$ ).

**Ant-Aphid-Lady Beetle Interactions in a Laboratory Arena.** Different lady beetle species were found to interact differently with ants. When considering ant behaviors, the main effect of species (ANOVA,  $df = 6,114$ ,  $F = 43.14$ ,  $P < 0.0001$ ) and ant behavior

**Table 1.** Mean no. ( $\pm$ SEM) of aphids (*M. euphorbiae*) consumed (of 10) after 24 h with one each of seven lady beetle species ( $n = 5$ )

	Lady beetle species	Consumption
Native	<i>C. trifasciata</i>	$7.00 \pm 0.6999$
	<i>C. maculata</i>	$9.20 \pm 0.5107$
	<i>H. convergens</i>	$8.60 \pm 0.4775$
Non-native	<i>C. septempunctata</i>	$7.60 \pm 0.8199$
	<i>H. axyridis</i>	$9.60 \pm 0.4229$
	<i>H. variegata</i>	$9.20 \pm 0.4091$
	<i>P. quatuordecimpunctata</i>	$8.00 \pm 0.6849$

(ANOVA,  $df = 6,798$ ,  $F = 134.20$ ,  $P < 0.0001$ ) were both significant, as were interactions between species and ant behavior (ANOVA,  $df = 36,798$ ,  $F = 14.34$ ,  $P < 0.0001$ ). Thus, one-way ANOVAs were conducted comparing the different lady beetle species for each ant behavior (Table 2). There were differences between beetle species in four ant behaviors: prolonged antennation, biting, grasping, and stinging. *C. trifasciata* received a significantly higher frequency of prolonged antennation from ants compared with *H. axyridis*; however, there were no differences among the other beetle species. *H. convergens*, *H. variegata*, and *C. maculata* received significantly higher frequencies of ant biting, grasping, and stinging compared with *C. trifasciata* and *P. quatuordecimpunctata*.

Because the interaction between lady beetle species and lady beetle behavior was highly significant (ANOVA,  $df = 48,1064$ ,  $F = 11.74$ ,  $P < 0.0001$ ), we conducted one-way ANOVAs comparing the different lady beetle species for each lady beetle behavior (Table 3). We found differences between beetle species in five behaviors: continuing behavior previous to contact, pulling in legs/antennae, turning on back, flailing legs, and running away. When confronted with ants, *C. septempunctata* continued its behavior previous to contact to a significantly greater extent compared with *H. convergens*, *H. variegata*, and *C. maculata*. *H. variegata* pulled in its legs and antenna significantly more frequently than *H. axyridis* and *P. quatuordecimpunctata*. Similarly, *H. variegata*, *C. maculata*, and *H. convergens* turned on their backs significantly more frequently than did *H. axyridis*, *C. trifasciata*, and *P. quatuordecimpunctata* and flailed their legs significantly more frequently compared with *C. trifasciata* and *P. quatuordecimpunctata*. *H. convergens*, *C. septempunctata*, and *C. maculata* ran away significantly more frequently compared with *C. trifasciata* and *P. quatuordecimpunctata*. The following lady beetle behaviors did not differ between beetle species: changing movement, preening, fluttering wings, and flying away. Although lady beetles did back away from ants during preliminary observations, that behavior was not observed during trials; thus, it was not included in the split-plot ANOVA comparing lady beetle species and lady beetle behaviors described above.

Aggression scores were significantly different among the tested lady beetle species (ANOVA,  $df = 6,133$ ,  $F = 9.68$ ,  $P < 0.0001$ ; Table 4). *H. convergens* and

**Table 2.** Mean frequency ( $\pm$ SEM) of ant behaviors in response to discovery of a lady beetle scored in determination of aggression score ( $n = 20$ )

Lady beetle species		Ant behaviors			
		Avoiding	Prolonged antennating	Opening mandibles	Chasing
Native	<i>C. trifasciata</i>	0.25a $\pm$ 0.16	5.60a $\pm$ 1.36	0.45a $\pm$ 0.25	0.00a $\pm$ 0.00
	<i>C. maculata</i>	0.15a $\pm$ 0.11	2.20ab $\pm$ 1.38	1.45a $\pm$ 0.53	0.35a $\pm$ 0.25
	<i>H. convergens</i>	0.10a $\pm$ 0.07	2.65ab $\pm$ 0.94	0.55a $\pm$ 0.36	0.15a $\pm$ 0.15
Non-native	<i>C. septempunctata</i>	0.10a $\pm$ 0.07	4.50ab $\pm$ 1.09	2.10a $\pm$ 1.03	0.05a $\pm$ 0.05
	<i>H. axyridis</i>	0.10a $\pm$ 0.07	1.20b $\pm$ 0.49	0.10a $\pm$ 0.07	0.00a $\pm$ 0.00
	<i>H. variegata</i>	0.15a $\pm$ 0.11	4.90ab $\pm$ 1.75	0.75a $\pm$ 0.34	0.00a $\pm$ 0.00
	<i>P. quatuordecimpunctata</i>	0.05a $\pm$ 0.05	2.55ab $\pm$ 0.90	0.10a $\pm$ 0.07	0.00a $\pm$ 0.00
		$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$
		0.28 0.9462	3.04 0.0082	2.71 0.0611	1.67 0.1326
Native		Biting	Grasping	Stinging	
	<i>C. trifasciata</i>	3.05c $\pm$ 1.12	0.05c $\pm$ 0.05	0.35c $\pm$ 0.25	
	<i>C. maculata</i>	11.85ab $\pm$ 2.44	2.85ab $\pm$ 0.77	7.45ab $\pm$ 2.03	
Non-native	<i>H. convergens</i>	16.35a $\pm$ 3.78	4.50a $\pm$ 1.22	13.85a $\pm$ 3.28	
	<i>C. septempunctata</i>	4.25c $\pm$ 1.50	0.55bc $\pm$ 0.25	0.60c $\pm$ 0.29	
	<i>H. axyridis</i>	5.80bc $\pm$ 2.42	0.85bc $\pm$ 0.75	3.55bc $\pm$ 2.20	
	<i>H. variegata</i>	14.95a $\pm$ 2.70	6.10a $\pm$ 1.93	11.05a $\pm$ 2.26	
	<i>P. quatuordecimpunctata</i>	0.65c $\pm$ 0.28	0.05c $\pm$ 0.05	0.65c $\pm$ 0.28	
		$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$	
	10.14 <0.0001	11.02 <0.0001	13.05 <0.0001		

Letters associated with each mean are results of Tukey mean separation tests comparing beetle species for each behavior. For each behavior, means with the same letter are not significantly different.

*H. variegata* were exposed to significantly more ant aggression than all other species except *C. maculata*. *P. quatuordecimpunctata*, however, provoked the least amount of aggression. Similarly, there was significant variation in reaction scores among the tested lady beetle species (Table 4). Reaction scores for *H. variegata*, *H. convergens*, *C. maculata*, and *C. septempunctata* were significantly higher (ANOVA,  $df = 6,133$ ,

$F = 10.18, P < 0.0001$ ) than those for the other three species. There was also a strong positive correlation between aggression and reaction scores ( $r = 0.6196, P < 0.0001$ ).

Aphid consumption during behavioral trials differed between the different lady beetle species (Table 5; ANOVA,  $df = 6,133, F = 6.15, P < 0.0001$ ). *H. axyridis* consumed a significantly greater number of

**Table 3.** Mean frequency ( $\pm$ SEM) of lady beetle behaviors in response to ant attack scored in determination of reaction score ( $n = 20$ )

Lady beetle species		Lady beetle behaviors				
		Continuing behavior previous to contact	Changing movement	Pulling in legs/antennae	Preening	Turning on back
Native	<i>C. trifasciata</i>	1.15ab $\pm$ 0.45	1.90a $\pm$ 0.50	1.10ab $\pm$ 0.37	0.20a $\pm$ 0.20	0.00d $\pm$ 0.00
	<i>C. maculata</i>	0.25b $\pm$ 0.18	0.65a $\pm$ 0.21	0.60ab $\pm$ 0.17	0.05a $\pm$ 0.05	1.15ab $\pm$ 0.27
	<i>H. convergens</i>	0.45b $\pm$ 0.22	3.00a $\pm$ 0.61	0.85ab $\pm$ 0.21	0.00a $\pm$ 0.00	0.85bc $\pm$ 0.27
Non-native	<i>C. septempunctata</i>	2.35a $\pm$ 0.62	2.90a $\pm$ 0.71	1.15ab $\pm$ 0.79	0.10a $\pm$ 0.10	0.35cd $\pm$ 0.15
	<i>H. axyridis</i>	1.20ab $\pm$ 0.47	1.05a $\pm$ 0.34	0.45b $\pm$ 0.20	0.00a $\pm$ 0.00	0.05d $\pm$ 0.05
	<i>H. variegata</i>	0.35b $\pm$ 0.17	3.15a $\pm$ 0.61	1.60a $\pm$ 0.37	0.00a $\pm$ 0.00	2.45a $\pm$ 0.66
	<i>P. quatuordecimpunctata</i>	1.40ab $\pm$ 0.82	1.05a $\pm$ 0.36	0.40b $\pm$ 0.27	0.05a $\pm$ 0.05	0.00d $\pm$ 0.00
		$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$
		3.48 0.0032	1.00 0.4281	2.63 0.0194	0.56 0.7627	15.12 <0.0001
Native		Flailing legs	Fluttering wings	Backing away	Running away	Flying away
	<i>C. trifasciata</i>	0.00c $\pm$ 0.00	0.00a $\pm$ 0.00	0.00a $\pm$ 0.00	0.60de $\pm$ 0.31	0.00a $\pm$ 0.00
	<i>C. maculata</i>	1.85a $\pm$ 0.46	0.15a $\pm$ 0.08	0.00a $\pm$ 0.00	2.65abc $\pm$ 0.50	0.20a $\pm$ 0.12
Non-native	<i>H. convergens</i>	1.25ab $\pm$ 0.38	0.05a $\pm$ 0.05	0.00a $\pm$ 0.00	3.75a $\pm$ 0.58	0.00a $\pm$ 0.00
	<i>C. septempunctata</i>	0.55bc $\pm$ 0.26	0.05a $\pm$ 0.05	0.00a $\pm$ 0.00	3.30ab $\pm$ 0.80	0.20a $\pm$ 0.12
	<i>H. axyridis</i>	0.25bc $\pm$ 0.25	0.05a $\pm$ 0.05	0.00a $\pm$ 0.00	1.05cde $\pm$ 0.32	0.05a $\pm$ 0.05
	<i>H. variegata</i>	2.45a $\pm$ 0.82	0.00a $\pm$ 0.00	0.00a $\pm$ 0.00	1.75bcd $\pm$ 0.58	0.00a $\pm$ 0.00
	<i>P. quatuordecimpunctata</i>	0.00c $\pm$ 0.00	0.00a $\pm$ 0.00	0.00a $\pm$ 0.00	0.25e $\pm$ 0.18	0.05a $\pm$ 0.05
		$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$	$F_{6,133} P$
	9.98 <0.0001	1.41 0.2163	NA NA	11.08 <0.0001	1.8 0.1037	

Letters associated with each mean are results of Tukey mean separation tests comparing beetle species for each behavior. For each behavior, means with the same letter are not significantly different.

NA, not applicable.

**Table 4.** Aggression and reaction scores (mean  $\pm$  SEM) from behavioral trials with different lady beetle species and *M. rubra* ( $n = 20$ )

Lady beetle species		Aggression score	Reaction score
Native	<i>C. trifasciata</i>	17.35bc $\pm$ 4.89	8.45b $\pm$ 2.01
	<i>C. maculata</i>	90.20ab $\pm$ 19.94	29.05a $\pm$ 3.65
	<i>H. convergens</i>	140.40a $\pm$ 29.97	34.60a $\pm$ 3.54
Non-native	<i>C. septempunctata</i>	26.70bc $\pm$ 6.62	27.90a $\pm$ 5.36
	<i>H. axyridis</i>	39.85bc $\pm$ 20.54	9.60b $\pm$ 2.92
	<i>H. variegata</i>	130.75a $\pm$ 20.20	34.85a $\pm$ 7.22
	<i>P. quatuordecimpunctata</i>	8.10c $\pm$ 2.55	3.80b $\pm$ 1.46

Letters associated with each mean are results of Tukey mean separation tests comparing beetle species for each score. For each score, means with the same letter are not significantly different.

aphids compared with all other species but *C. septempunctata*. When considering all species, there was a significant negative correlation between aphid consumption and aggression score ( $r = -0.3251$ ,  $P < 0.0001$ ) and between aphid consumption and reaction score ( $r = -0.1882$ ,  $P = 0.0260$ ).

**Lady Beetle Tolerance of Ant Stings.** Although there were no significant differences ( $df = 6,133$ ,  $F = 2.18$ ,  $P = 0.9912$ ) in the number of stings received by each beetle species (mean = 14.40 stings, SE = 0.2422), the main effect of species (ANOVA,  $df = 6,114$ ,  $F = 10.94$ ,  $P < 0.0001$ ) and sting status (ANOVA,  $df = 1,133$ ,  $F = 119.10$ ,  $P < 0.0001$ ) were both significant, as were interactions between species and sting status (ANOVA,  $df = 6,133$ ,  $F = 14.98$ ,  $P < 0.0001$ ). For the lady beetles exposed to ant stings, response scores were significantly different among the species ( $df = 6,133$ ,  $F = 6.45$ ,  $P < 0.0001$ ; Table 6), with *H. variegata* and *C. septempunctata* having significantly higher scores than *H. axyridis*, *H. convergens*, and *C. maculata*. Control lady beetles not exposed to ants showed similar response scores (mean score = 0.2643, SE = 0.039;  $df = 6,133$ ,  $F = 1.14$ ,  $P = 0.3444$ ). When comparing beetles exposed to ant stings to the unexposed beetles of the same species, the former showed significantly higher response scores in five beetle species: *C. septempunctata*, *C. trifasciata*, *H. convergens*, *H. variegata*, and *P. quatuordecimpunctata*. There were no differences when comparing beetles stung to those not stung in the remaining two species: *C. maculata* and *H. axyridis*.

**Table 5.** Consumption (mean  $\pm$  SEM) of aphids by different beetle species during behavioral trials with *M. rubra* ( $n = 20$ )

Lady beetle species		Consumption
Native	<i>C. trifasciata</i>	0.45bc $\pm$ 0.2112
	<i>C. maculata</i>	0.02c $\pm$ 0.1094
	<i>H. convergens</i>	0.35bc $\pm$ 0.1500
Non-native	<i>C. septempunctata</i>	1.50ab $\pm$ 0.5104
	<i>H. axyridis</i>	2.00a $\pm$ 0.6407
	<i>H. variegata</i>	0.35c $\pm$ 0.2209
	<i>P. quatuordecimpunctata</i>	0.40bc $\pm$ 0.1522

Letters associated with each mean are results of Tukey mean separation tests comparing aphid consumption for each beetle species. Means with the same letter are not significantly different.

From the greatest to the least effects, these five species were *H. variegata*, *C. septempunctata*, *C. trifasciata*, *P. quatuordecimpunctata*, and *H. convergens*. The dominant condition (observed in 10 of 20 trials) in experimental trials for *H. variegata* was "impaired ambulatory locomotion," i.e., legs appearing to be non-functional and dragging behind the beetles. This condition was also observed seven times with *P. quatuordecimpunctata*, three times each with *C. maculata* and *C. trifasciata*, two times with *C. septempunctata*, in one trial with *H. convergens*, but in no trials with *H. axyridis*. This condition was not observed in control trials with any of the seven species.

## Discussion

Three lady beetle species, *H. convergens*, *H. variegata*, and *C. maculata*, received the most aggressive behaviors from ants (biting, grasping, and stinging) more frequently than the other beetle species tested. *C. trifasciata*, *P. quatuordecimpunctata*, and *C. septempunctata* were generally documented to receive these behaviors the least. *H. axyridis* was generally intermediate between these two groups. As expected, these patterns are reflected in aggression scores (Table 4).

Lady beetle behaviors in response to ant aggression (Table 3) followed a similar pattern to the groupings observed with ant behaviors. When there were differences between lady beetle species for a given behavior, *H. convergens*, *H. variegata*, *C. maculata*, and/or *C. septempunctata* generally had a higher frequency of reactive behaviors compared with *C. trifasciata* and/or *P. quatuordecimpunctata*. *H. axyridis* was generally intermediate between these two groups. Again, these general groupings based on differences in lady beetle species considering individual behaviors were reflected in overall reaction scores (Table 4). Reaction scores for *H. variegata*, *H. convergens*, *C. maculata*, and *C. septempunctata* were significantly greater than those for *H. axyridis*, *C. trifasciata*, and *P. quatuordecimpunctata*.

It seems intuitive that high frequencies of aggression from ants would result in higher frequencies of reactive behaviors from lady beetles. It is interesting to note that, despite the fact that *H. convergens* and *H. variegata* had the highest aggression scores, *C. septempunctata* had the highest reaction score relative to its aggression score. When interpreting these results, we must consider how the reaction by lady beetles might affect aggression by the ants. The most frequent reaction by *C. septempunctata* to ant aggression was to run away. Two possible explanations of why ant aggression does not escalate in this species are that running away is an effective method of avoidance or that ants stop perceiving the retreating lady beetle as a threat. However, *H. convergens* ran away as much as *C. septempunctata*, yet ants were much more aggressive toward it. *C. septempunctata* has been shown to deter attacks by ants chemically. *C. septempunctata* was shown to use reflex bleeding to deter attacks by *Formica polyctena* when it used the ants' odor trails to locate aphid prey (Bhatkar 1982). Also, Tursch et al.

**Table 6.** Mean scores ( $\pm$ SEM) for observations of different beetle species after having been stung by ants ( $n = 20$ )

	Lady beetle species	Experimental	Control	<i>t</i>	<i>P</i>
Native	<i>C. trifasciata</i>	1.00ab $\pm$ 0.1622	0.15 $\pm$ 0.0819	-6.40	<0.0001
	<i>C. maculata</i>	0.45bc $\pm$ 0.1846	0.40 $\pm$ 0.1124	0.66	0.5181
	<i>H. convergens</i>	0.40bc $\pm$ 0.1338	0.10 $\pm$ 0.0688	-2.75	0.0128
Non-native	<i>C. septempunctata</i>	1.10a $\pm$ 0.1433	0.25 $\pm$ 0.0993	-8.06	<0.0001
	<i>H. axyridis</i>	0.25c $\pm$ 0.0993	0.35 $\pm$ 0.1094	1.45	0.1625
	<i>H. variegata</i>	1.40a $\pm$ 0.2224	0.30 $\pm$ 0.1277	-5.79	<0.0001
	<i>P. quatuordecimpunctata</i>	1.00abc $\pm$ 0.2176	0.30 $\pm$ 0.1051	-3.75	0.0014

Letters associated with experimental means are results of Tukey mean separation tests comparing beetle species for experimental results. Means with the same letter are not significantly different. *t* and *P* values refer to comparisons between experimental treatments and controls for each species.

(1971) found that *M. rubra* would not drink from water to which a defensive alkaloid produced by *C. septempunctata* had been added. It is possible that in this study behavioral activity documented for *C. septempunctata* in reaction scores coincided with chemical production that deterred further aggression by ants.

In the presence of ants, *H. axyridis* consumed more aphids than all other species except *C. septempunctata* (Table 5). There were no differences among lady beetle species in feeding trials conducted in petri dishes in the absence of ants. *H. axyridis* received a moderate level of aggression from ants but had a relatively low reaction score compared with the other lady beetle species. This might reflect a better relative ability of *H. axyridis* to forage successfully on ant-protected aphids. Similarly, Dutcher et al. (1999) found that *H. axyridis* withstood fire ant (*Solenopsis invicta* Buren) aggression more successfully than *H. convergens*. Although *H. convergens* was forced from plants housing its aphid prey and *S. invicta*, *H. axyridis* was able to remain. Alternatively, *H. axyridis* might have been a better forager in the relatively complex environment of our laboratory arenas, whereas that advantage disappeared in a simpler environment of petri dishes. *H. axyridis* is also a more voracious predator compared with *Cycloneda sanguinea* L. (Michaud 2002). Furthermore, in this study, *H. axyridis* seemed to be highly tolerant of ant venom (Table 6).

The negative correlations between aphid consumption and aggression/reaction scores are also not surprising. One function of aphid-tending ants is to protect aphids from predators (Bartlett 1961, Way 1963, Buckley 1987, Vinson and Scarborough 1989, Jahn and Beardsley 1994). Aggression from ants should thwart predators from taking aphids. Time spent reacting to ant aggression would reduce time available for foraging and feeding.

Mean scores for ant sting trials for five of the seven lady beetle species tested were significantly greater than each of these species' associated control trials. In two species, *H. axyridis* and *C. maculata*, ant venom was not documented to have any effects in this study. Exocrine glands in ants, such as the venom, Dufour, and mandibular glands, are known to contain a variety of compounds that are used externally (Cavill and Robertson 1965). Although many of the constituents of *M. rubra*'s

venom, Dufour, and mandibular gland excretions have been identified (Cammaerts-Tricot et al. 1976, Morgan et al. 1977, Evershed et al. 1981, Cammaerts et al. 1981a, Cammaerts et al. 1981b, Evershed et al. 1982, Attygalle et al. 1983b, Cammaerts 1984, Cammaerts 1992), little is known about the effects of *M. rubra* envenomization on insects. Determinations of the effects of these compounds have been limited primarily to uses for communication with conspecifics such as in trail and foraging area delineation (Cammaerts-Tricot et al. 1976, 1981a, b; Attygalle et al. 1983a; Cammaerts 1984, 1992) and worker recruitment to foraging sites (Cammaerts-Tricot et al. 1976, 1981a, b; Cammaerts 1978; Attygalle et al. 1983a).

Aggression scores were higher in species that seem to have more exposed parts on which ants could grasp. When *H. convergens*, *H. variegata*, and *C. maculata* were observed to pull in their legs, their concealment was incomplete and ants could still bite them. However, when *H. axyridis*, *C. septempunctata*, *C. trifasciata*, and *P. quatuordecimpunctata* pulled in their legs, their appendages appeared to be completely retracted and ant aggression subsided. Therefore, although interactions between ants and lady beetles may be behavioral and/or chemical, there may also be a physical component, ants acting as opportunists, grabbing what is available, causing aggression to escalate, or walking away when all parts are concealed.

Differences documented between lady beetle species may put some of them at a competitive advantage over others. In a number of earlier studies, *H. axyridis* has been found to be a superior competitor compared with other lady beetle species because of intraguild predation (Hironori and Katsuhiko 1997; Yasuda et al. 2001, 2004), prey utilization (Michaud 2002), and tolerance of pathogenic microorganisms (Saito and Björnson 2006). It seems that it also performs better in the presence of an aggressive aphid-tending ant, *M. rubra*. Although this study did not provide evidence that successful non-native species invariably have a competitive advantage over native species when dealing with an aggressive enemy, the differences between species provide further evidence that different lady beetle species have very different competitive abilities that may contribute to their successes or failures in new habitats.

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