Behavioral Response to Larval Tracks and the Influence of Tracks on Intraguild Scavenging by Coccinellid Larvae

Susan E. Moser · Kenneth F. Haynes · John J. Obrycki

Abstract A paired design was used to determine that *Harmonia axyridis* 4th instars were not influenced by the presence of conspecific larval tracks, but well-fed *H. axyridis* 4th instars spent less time on plants that contained tracks left by *Coleomegilla maculata* 4th instars. To determine if the presence of larval tracks influences intraguild scavenging by *H. axyridis* 4th instars, dead 4th instars were placed in Petri dishes that contained or did not contain larval tracks. The presence of larval tracks did not influence the feeding frequency or the amount of time before feeding. However, larvae dragged their pygopod on dish surfaces more frequently if the dish contained larval tracks. In addition, starved *H. axyridis* larvae were more likely to feed on the prey and dragged their pygopod less frequently than well-fed larvae.

Keywords Competition · foraging behavior · chemical signaling · larval behavior · interspecific interactions · intraguild predation

Introduction

Coccinellid larvae not only respond to chemical cues left by their prey, but also release chemicals as they walk (Marks 1977; Růžička 1997). Larvae periodically

S. E. Moser · K. F. Haynes · J. J. Obrycki
Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY 40546, USA

Present Address:
S. E. Moser (✉)
USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory c/o Insectary, Iowa State University, Ames, IA 50011, USA
e-mail: Susan.Moser@ars.usda.gov

mark the plant surface with a secretion from their pygopod or anal disc (Marks 1977; Laubertie et al. 2006) as they move on plant surfaces (Marks 1977; Ferran and Dixon 1993; Doumbia et al. 1998). These secretions contain non-volatile hydro-carbons which may aid in adhesion to plant surfaces (Spiegler 1962; Hodek 1973; Kosaki and Yamaoka 1996) but also may act as a signal to identify previously searched areas; *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) larvae spend less time on previously searched plants (Marks 1977). Scent-marking is well known among insect parasitoids (Price 1970; van Dijken et al. 1992; Höller and Hörmann 1993; Bernstein and Driessen 1996), but has rarely been examined for insect predators (Marks 1977; Nakashima et al. 2002).

Intraguild predation occurs between organisms that compete for and feed on the same host and also prey on each other (Polis et al. 1989). Intraguild predation and cannibalism frequently occur among predaceous coccinellid larvae (Hodek and Honêk 1996; Lucas 2005). Because larval tracks may indicate that a coccinellid larva is nearby, it is likely beneficial to avoid oviposition in areas containing larval tracks. The presence of larval tracks has resulted in lower oviposition rates by female coccinellids (Table 1), chrysopids (Růžička 2001) and aphid parasitoids (Nakashima and Senoo 2003). Fourth instar *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) and 4th instar *Cycloneda limbifer* Say (Coleoptera: Coccinellidae) also spend less time in areas that contain tracks left by conspecific larvae (Rutledge et al. 2008; Růžička and Zemek 2008). Detection of larval tracks by other larvae may also influence the likelihood of intraguild predation occurring between coccinellid larvae in the field. For example, an immature may avoid an area if it detects that another larva is present. Alternatively, it may orient towards the track and actively pursue the larva.

Harmonia axyridis Pallis (Coleoptera: Coccinellidae) is a predatory, polyphagous species native to western Asia that can be found in diverse habitats (Krafsur et al. 1997; Yasuda and Ohnuma 1999; Specty et al. 2003; Brown et al. 2008). Now distributed throughout North America, *H. axyridis* has rapidly become one of the most abundant coccinellid species within many agricultural systems (LaRock and Ellington 1996; Brown and Miller 1998; Colunga-Garcia and Gage 1998; Cottrell and Yeargan 1998; Michaud 2002; Brown 2003; Brown et al. 2008). Its establishment has been correlated to the decline of several native coccinellid species (LaMana and Miller 1996; Michaud 2002; Harmon et al. 2007; Brown et al. 2008; Hessler and Kieckhefer 2008; Koch and Galvan 2008; Pell et al. 2008). It is a highly successful cannibal and intraguild predator on coccinellid eggs and larvae (Cottrell and Yeargan 1998; Snyder et al. 2000; Cottrell 2004; Snyder et al. 2004; Moser and Obrycki 2009). Larvae of *H. axyridis* have morphological adaptations (e.g. spines along dorsal abdominal surface, size of mandibles) (Dixon 2000; Yasuda and Kimura 2001; Michaud and Grant 2003) and behavioral adaptations (e.g. increased aggression with decreased prey availability) (Michaud and Grant 2003), which may aid *H. axyridis* larvae during larval attacks. In Belgium, 32% ($n=28$) of the field caught *H. axyridis* larvae tested positive, using GC-MS to detect genus-specific alkaloids, for feeding on several heterospecific coccinellid larvae (Hautier et al. 2008). In laboratory studies, *H. axyridis* are able to complete development when fed other coccinellid larvae (Wagner et al. 1999; Yasuda and Ohnuma 1999; Snyder et al. 2000; Sato and Dixon 2004; Pell et al. 2008; Moser and Obrycki 2009). Therefore, rather than avoiding tracks left by conspecifics and heterospecifics, it may

Table 1 Species of Coccinellidae that avoid oviposition (X) due to the presence of conspecific and heterospecific larval tracks

Species	Avoid oviposition	Track-laying species	Reference
Adalia bipunctata	X	*Adalia bipunctata*	Doumbia et al. 1998
		Adalia decempunctata	
		Coccinella septempunctata	
Aphidecta obliterata		*Adalia bipunctata*	Oliver et al. 2006
	X	*Aphidecta obliterata*	
Ceratomegilla undecimnotata	X	*Ceratomegilla undecimnotata*	Růžička 2003
Cheilomenes sexmaculata	X	*Ceratomegilla undecimnotata*	Růžička 2006
	X	*Cheilomenes sexmaculata*	
	X	*Cycloneda limbifer*	
		Harmonia dimidiata	
Coccinella septempunctata	X	*Coccinella septempunctata*	Růžička 2001
		Cycloneda limbifer	
		Hippodamia (Semiadalia) undecimnotata	
		Leis dimidiata	
Cycloneda limbifer		*Coccinella septempunctata*	Růžička 2001
	X	*Cycloneda limbifer*	
	X	*Hippodamia (Semiadalia) undecimnotata*	
	X	*Leis dimidiata*	
Harmonia axyridis		*Coccinella septempunctata*	Yasuda et al. 2000
	X	*Harmonia axyridis*	
Hippodamia convergens	X	*Coleomegilla maculata*	Michaud and Jyoti 2007
	X	*Hippodamia convergens*	
Hippodamia (Semiadalia) undecimnotata		*Coccinella septempunctata*	Růžička 2001
		Cycloneda limbifer	
	X	*Hippodamia (Semiadalia) undecimnotata*	
	X	*Leis dimidiata*	

benefit *H. axyridis* larvae to use larval tracks as a pathway to find other coccinellid larvae.

The objective of this study was to determine if the behavior of *H. axyridis* larvae is influenced by the presence of conspecific and heterospecific larval tracks. We hypothesized that the time larvae spent on plants would be influenced by the presence of tracks, and that this response would be dependent on an individual's satiation level. Furthermore, we hypothesized that the presence of larval tracks would affect larval movement and aggressiveness (measured by feeding frequency and time before feeding) when larvae were paired with alternative prey items.

Materials and Methods

Insect and Plant Rearing

The North American coccinellid, *Coleomegilla maculata* DeGeer was used to obtain heterospecific tracks because it co-occurs in agricultural habitats with *H. axyridis* (Cottrell and Yeargan 1998; Musser and Shelton 2003; Moser and Obrycki 2009) and several studies have found that *H. axyridis* will prey upon the eggs, larvae, and pupae of *C. maculata* in the laboratory (Cottrell and Yeargan 1998; Phoofolo and Obrycki 1998; Moser and Obrycki 2009). Adult *C. maculata* were collected from overwintering sites at the University of Kentucky Spindletop Research Farm, Lexington, KY and *H. axyridis* adults were collected from overwintering sites in urban environments within Lexington, KY during the fall 2007 and spring 2008. Females were separated into individual Petri dishes (Fisherbrand®; polystyrene; 100×15 mm; Fisher Scientific Company L.L.C.; Pittsburgh, PA.) and were provided cotton soaked with water and an *ad libitum* supply of *Acyrthosiphon pisum* Harris (Homoptera: Aphididae). Faba bean, *Vicia faba* L. (Fabales: Fabaceae) were used to rear *A. pisum*; these beans were planted in 110 mm diameter pots (Kord products; Toronto, Canada) with Promix BX® soil (Premier® Horticulture Inc.; Quakertown, PA.), watered daily, and grown under greenhouse conditions (25–29°C; L:D 14:10 h).

After laying an egg mass, females were placed into new dishes. Larvae from the same egg mass were reared within the dish until 2nd instar eclosion, at which time larvae were separated and reared individually. Larvae were provided an *ad libitum* supply of *A. pisum* daily until 4th instar eclosion. Within 30 h of 4th instar eclosion, larvae were placed into experimental assays. All coccinellid life stages were maintained at $20.0±1.0°C$ with a photoperiod 16:8 h (L:D). Voucher specimens of the adults were deposited in the University of Kentucky Insect Museum, Department of Entomology, University of Kentucky, Lexington, KY.

Experimental Design

Time Spent on Plants A paired design was used to compare the time *H. axyridis* larvae spent on plants containing tracks to time spent on plants without tracks (controls). Two bean plants containing two fully-expanded opposite leaves were used in each bioassay. Plants were paired based on the similarity of leaf size. Each plant was cut at the plant base <5 min before the bioassay. To prevent rapid wilting, plants were vertically placed within separate water-filled glass test tubes and each replicate was completed within 45 min period of excision. Preliminary experiments indicated that plants would maintain turgidity for the entire test period. Plants remained vertical by inserting moist cotton between the tube and the plant stem. The moistened cotton also provided a surface for larvae to walk on. To ensure that each plant was approximately the same height, each stem was cut above the paired leaves. The exposed plant was 8 cm in height; the paired leaves were 5 cm above the cotton base and the top of the cut stem was 2.5 cm above the leaves.

Plants were placed side-by-side on opposite ends of an open-sided plastic test tube holder (10×10×5 cm) so that the leaves of each plant did not touch. The holder

was then placed within a water-filled (7 cm depth) rectangular plastic container; the water was level with the cotton base of each test tube and did not come in contact with the plant stem or leaves. With a small paintbrush, a putative track-laying larva was randomly placed on one of the plant stems and allowed to move freely on the plant for 30 min. The water in the plastic container served as a barrier to prevent larvae from moving off the plant during the 30 min period, after which the track-laying larva was removed from the plant with a paintbrush and the test tube holder, containing both the treated and untreated plants, was removed from the water-filled container. The treated plant was examined for frass, and if found, frass was removed from the plant with a paintbrush.

Two experimental *H. axyridis* larvae were individually placed with a small paintbrush on the cotton at the base of the treated and untreated plants. A video camera was used to determine time spent by the experimental larvae on the plants. Timing for each larva began once the larva walked onto the plant surface (forelegs and middle legs touching the plant) and ended when the larva walked back onto the cotton base (forelegs and middle legs touching the cotton). The bioassay continued for 15 min or until both larvae left the plant surfaces. Each bioassay was replicated with different larvae and plants 35 times.

Six separate assays were conducted to determine if *H. axyridis* fourth instars detect chemical tracks left by conspecific larvae (bioassays 1–4) or by *C. maculata* larvae (bioassays 5 and 6), and to determine if satiation level influences the response. In bioassays 1 and 2, third instar *H. axyridis* were used to supply the tracks, and in bioassays 3 and 4, fourth instar *H. axyridis* supplied the tracks. The observed larvae were supplied with an *ad libitum* supply of aphids (bioassays 1, 3, 5) or aphid prey was removed about 24 h prior to the assay (bioassays 2, 4, 6). The data were not normally distributed; therefore Wilcoxon signed-rank matched pair tests were used to compare the time *H. axyridis* larvae spent on plants containing tracks to plants that did not contain tracks for each assay (JMP 7.0.1, SAS Institute 2007).

Tracks and Intraguild Scavenging A factorial design was used to examine how larval tracks influence 4th instar *H. axyridis* behavior when alternative prey is present. Treatments were selected based on the trends observed in the first study; treatments included track presence (present or absent) and satiation level for different prey types (larvae fed or starved prior to encountering alternative prey). Alternative prey items included larvae that were killed by freezing and then thawed to room temperature (about 5 min). Individual prey items were placed in the middle of the base of a Petri dish (100×15 mm) that contained tracks and in dishes that did not contain larval tracks. In dishes containing tracks, the prey item (3rd instar *H. axyridis*, 4th instar *H. axyridis*, or 4th instar *C. maculata*) was the same species and instar that left the tracks. Tracks were obtained by placing a larva (3rd instar *H. axyridis*, 4th instar *H. axyridis*, or 4th instar *C. maculata*), starved for 24 h, within the Petri dish for 3 h. Prior to each bioassay, the experimental larva was *ad libitum* fed *A. pisum* or starved for 24 h. The experimental larva was placed in the Petri dish and a video camera was used to record foraging behavior (time before feeding on the prey, time spent stationary, pygopod dragging, and backward walking) for 1 h or until the observed larva physically encountered the prey item. There were eight treatments and each treatment was replicated 8 times; a total number of 64 larvae were used.

Fig. 1 Mean difference (± SE) in time (s) spent on plants between 4th instar *H. axyridis* exposed to plants ▶
with and without larval tracks. The mean difference was obtained by subtracting the time spent by 4th
instar *H. axyridis* on plants that contained tracks from the time the paired larvae spent on plants that did
not contain tracks (*n*=35); if positive, more time was spent on plants that did not contain tracks and if
negative, more time was spent on plants that contained tracks. Six bioassays were conducted, each differed
by the larvae that left the track, or by the satiation level of the observed larvae; (**A**) tracks were left by 3rd
instar *H. axyridis* and observed larvae were fed an *ad libitum* supply of *Acyrthosiphon pisum* prior to the
bioassay, (**B**) tracks were left by 3rd instar *H. axyridis* and observed larvae were starved for 24 h prior to
the bioassay, (**C**) tracks were left by 4th instar *H. axyridis* and observed larvae were fed an *ad libitum*
supply of *A. pisum* prior to the bioassay, (**D**) tracks were left by 4th instar *H. axyridis* and observed larvae
were starved for 24 h prior to the bioassay, (**E**) tracks were left by 4th instar *C. maculata* and observed
larvae were fed an *ad libitum* supply of *A. pisum* prior to the bioassay, and (**F**) tracks were left by 4th
instar *C. maculata* and observed larvae were starved for 24 h prior to the bioassay.

Pygopod dragging occurred when the pygopod was positioned and pulled along
the surface for 1–3 s. Backward walking is characterized as a cessation of forward
motion, attachment of the pygopod to the surface, and a backward movement of the
abdomen towards the pygopod. This movement results in the abdomen curving into
an arc-shape, away from the surface and a dragging of the front legs along the
surface. The pygopod is then released and the abdomen is lengthened. Backward
movement may be repeated several times before the larvae turns and walks with a
new heading.

An analysis of variance, using the factors of track presence and satiation level for
each prey item was compared for each of the following responses: time before
feeding on the prey item, time spent stationary, and time spent moving backwards. A
Tukey HSD analysis was used to compare means within significant effects and non-
significant interactions were removed from the model. A $\log_e$ transformation was
used to normalize the time data and x + 1 was added to stabilize the variance prior to
the analyses. Nominal logistic regressions were performed to compare the binomial
responses of feeding frequency, backward walking and pygopod dragging using the
same factors (track presence and satiation level for each prey item). If the effect of
satiation level for each prey type was significant, a separate Chi-square test was used
determine if satiation level or prey type (species and instar) affected the response. A
likelihood-ratio chi-square test for goodness of fit compared the occurrence of
pygopod dragging and backward walking based on location within the dish (lid,
base, or both). The statistical software used was JMP 7.0.2 (SAS Institute 2007). All
analyses used a significance level of $\alpha = 0.05$.

Results

Time Spent on Plants Well-fed 4th instar *H. axyridis* did not differ in time spent on
plants due to the presence of 3rd instar *H. axyridis* tracks (mean ± SE used
throughout; tracks present: 396±75 s, control: 368±66 s) (df = 1, *N*=35, W_+ = 18.0,
P=0.70; Fig. 1a). Starved 4th instars also spent a similar amount of time on plants
that contained 3rd instar *H. axyridis* tracks (276±52 s) compared to plants that did
not contain tracks (176±42 s; df = 1, *N*=35, W_+ = −91.5, *p*=0.12; Fig. 1b). Time
spent on plants containing 4th instar *H. axyridis* tracks by well-fed (348±59 s) and
starved individuals (275±47 s) was also similar to time spent on plants without
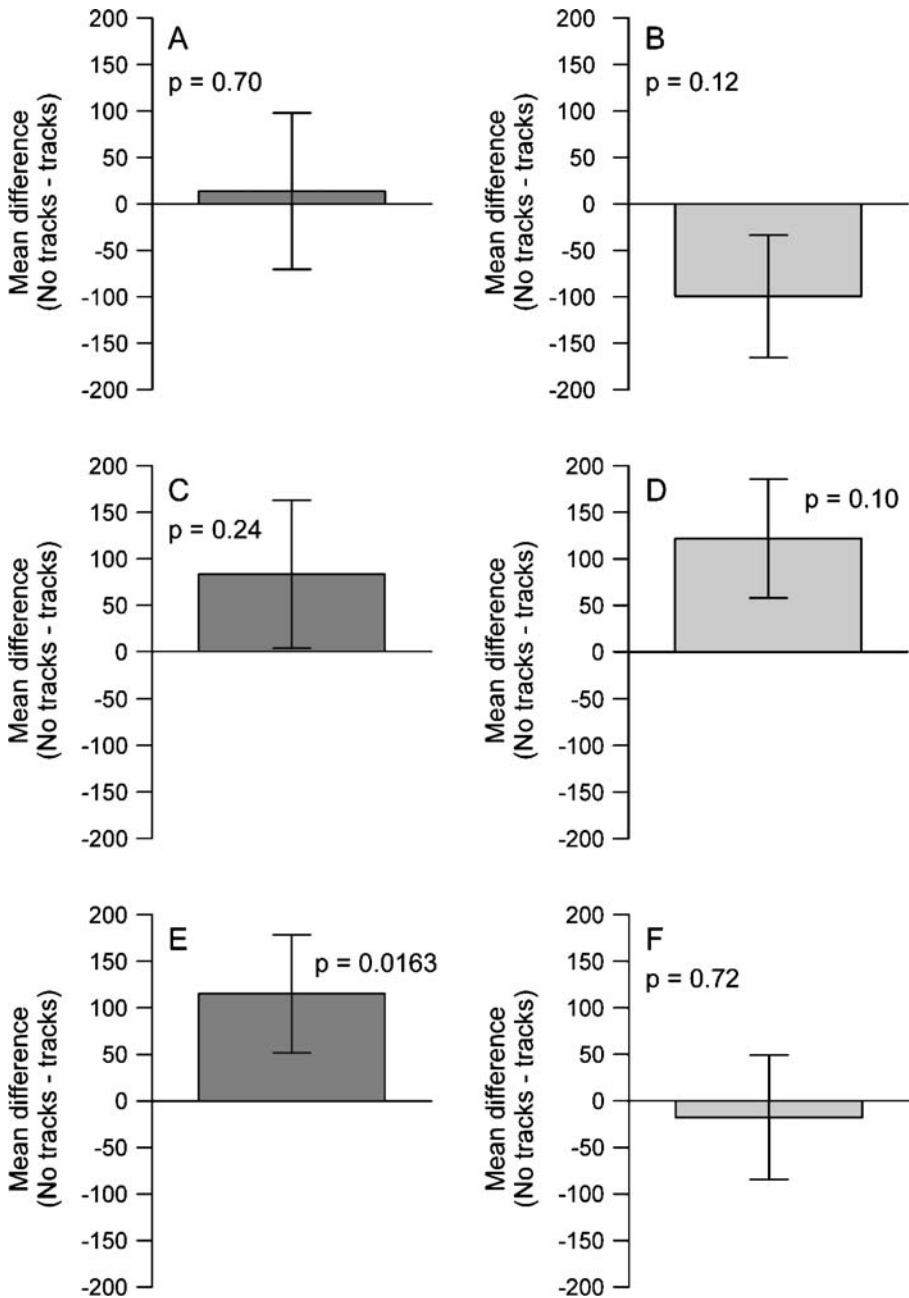
tracks (well-fed: 432 ± 53 s; starved: 397 ± 57 s; well-fed: df $= 1$, $N=35$, $W_+ = 63.5$, $P=0.24$; starved: df $= 1$, $N=35$, $W_+ = 95.5$, $P=0.10$; Fig. 1c, d). Well-fed individuals spent significantly less time on plants containing *C. maculata* larval tracks (251 ± 55 s) in comparison to plants that did not contain tracks (366 ± 57 s; df $= 1$, $N=35$, $W_+ = 137.5$, $P=0.016$; Fig. 1e). However, there was no difference in time spent on

Table 2 The number of *Harmonia axyridis* 4th instars that fed on the prey item, moved backwards, or dragged their pygopod in the presence or absence of larval tracks when fed an *ad libitum* supply of *Acyrthosiphon pisum* or starved for 24 h prior the bioassay

Prey item[a]		Satiation[b]	Fed on prey		Moved backward		Dragged pygopod	
Species	Instar		No tracks ($n=8$)	Tracks ($n=8$)	No tracks ($n=8$)	Tracks ($n=8$)	No tracks ($n=8$)	Tracks ($n=8$)
H. axyridis	3rd	Starved	8	8	3	4	0	1
	4th	Starved	8	8	3	2	0	1
	4th	Well-fed	5	5	6	3	1	4
C. maculata	4th	Well-fed	5	2	5	2	3	5

[a] The prey item is the same species and instar that left the track

[b] Larvae were fed an *ad libitum* supply of *Acyrthosiphon pisum* or starved for 24 h prior the bioassay

plants due to *C. maculata* track presence if the observed larvae were starved (df = 1, $N=35$, $W_+ = 21.5$, $P=0.72$; Fig. 1f).

Tracks and Intraguild Scavenging The number of *H. axyridis* 4th instars that fed on the prey (77%, $n=64$) was not influenced by the presence of larval tracks ($\chi^2 = 0.54$, df = 1, 4, $P=0.46$; Table 2). The satiation level of the observed larvae impacted feeding on the prey items (feeding frequency: $\chi^2 = 29.84$, df = 3, 4, $P<0.0001$; time before feeding: $F=9.00$, df = 1, 4, $P<0.0001$); starved larvae were faster to contact prey and fed nearly twice as often compared to well-fed larvae (time before feeding: $F=9.00$, df = 1, 4, $P<0.0001$; feeding frequency: $\chi^2 = 27.62$, df = 1, $P<0.0001$; Tables 2 and 3). Larvae fed most often on conspecific prey items ($\chi^2 = 19.92$, df = 2,

Table 3 The mean (± SE) time (s) *Harmonia axyridis* 4th instars spent before feeding, remaining stationary, and moving backwards in the presence and absence of larval tracks ($n=64$). Different letters indicate a statistically significant difference within the column (Tukey HSD, $p<0.05$)

Prey item[a]		Satiation[b]	Before feeding		Stationary		Moving backward	
Species	Instar		No tracks	Tracks	No tracks	Tracks	No tracks	Tracks
			combined mean		combined mean		combined mean	
H. axyridis	3rd	Starved	635±254	1162±389	102±49	304±144	160±108	139±71
			899±234 ab		203±78 A		149±63	
	4th	Starved	794±468	383±311	142±91	10±7	298±194	10±7
			588±277 a		77±47 A		154±101	
	4th	Well-fed	2232±557	1831±563	1745±543	1645±559	249±179	21±17
			2031±386 bc		1695±377 B		135±92	
C. maculata	4th	Well-fed	2592±498	3157±398	1885±534	2793±408	171±99	0.3±0.3
			2874±317 c		2339±1380 B		85±52	

[a] The prey item is the same species and instar that left the track

[b] Larvae were fed an *ad libitum* supply of *Acyrthosiphon pisum* or starved for 24 h prior the bioassay

$P<0.0001$; Table 2). Larval tracks did not influence the time before the observed larvae contacted the prey ($F=0.03$, df = 1, 4, $P=0.87$; Table 3).

The time larvae spent stationary in the dish was not influenced by track presence ($F=0.86$, df = 1, 4, $P=0.36$). However, well-fed larvae spent more time (2017 ± 258 s) stationary within the dish compared to starved larvae (140 ± 46 s; $F=145.66$, df = 1, 4, $P<0.0001$; Table 3).

Similar numbers of individuals dragged their pygopod based on location in the dish (dish base = 27%, lid = 53%, both locations = 20%; $\chi^2 = 2.67$, df = 2, $P=0.26$). Pygopod dragging was observed more often in dishes that contained tracks compared to dishes that did not contain tracks ($\chi^2 = 5.48$, df = 1, 4, $P=0.0192$; Table 2). In addition, pygopod dragging was influenced by the satiation level of the observed larvae and the prey type within the dish ($\chi^2 = 13.76$, df = 3, 4, $P=0.0033$). Well-fed larvae dragged their pygopod more frequently compared to starved larvae ($\chi^2 = 11.51$, df = 1, $P=0.0007$; Table 2) and more often if the prey were *C. maculata* 4th instars ($\chi^2 = 9.15$, df = 2, $P=0.0103$; Fig. 2).

Backward walking occurred less often on the dish base (4%) rather than on the lid (48%) or both locations (48%, $n=28$; $\chi^2 = 14.73$, df = 2, $P=0.0006$). The number of individuals that moved backwards was not influenced by the treatments (track presence: $\chi^2 = 3.29$, df = 1, 4, $P=0.07$; satiation level and prey item: $\chi^2 = 3.29$, df = 3, 4, $P=0.50$; Table 2); however, the duration of time spent moving backwards was greater if the dish did not contain larval tracks (219 ± 72 s) compared to dishes that contained tracks (42 ± 20 s; $F=5.04$, df = 1, 4, $P=0.0285$). Larval satiation and prey type did not influence the time larvae spent moving backwards ($F=0.62$, df = 3, 4, $P=0.61$; Table 3).

Discussion

Coccinellid immatures may encounter a high incidence of cannibalism and intraguild predation (egg and larval predation) (Hodek and Honêk 1996); therefore, it is

Fig. 2 Percentage of 4th instar *H. axyridis* that dragged their pygopod in the presence of 3rd or 4th instar *H. axyridis* or *C. maculata* prey.
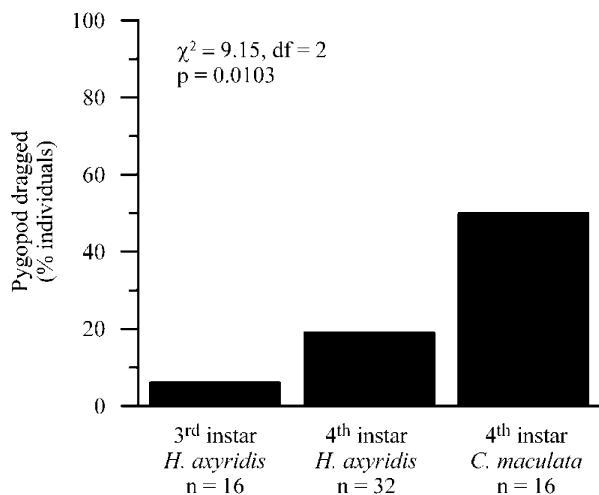
advantageous for larvae to detect tracks of other larvae and avoid areas containing tracks or to follow the track and eat the other larva. Detection and avoidance of conspecific tracks has been demonstrated with 4th instar *H. convergens* larvae (Rutledge et al. 2008) and 4th instar *C. limbifer* larvae (Růžička and Zemek 2008). However in this study, the presence of tracks only reduced the duration of time spent on plants containing *C. maculata* tracks by well-fed *H. axyridis* larvae. Contrary to our hypothesis, *H. axyridis* larvae did not spend less time on plants that contained conspecific tracks and the presence of tracks did not influence feeding on other coccinellid larvae. Although characteristics of larval movement were altered due to track presence, pygopod dragging increased and time spent moving backward decreased, conspecific larval tracks may play only a minor role in foraging behavior of *H. axyridis* 4th instars.

Well-fed *H. axyridis* larvae spent less time on plants containing *C. maculata* tracks, but starved individuals spent similar amounts of time on plants, regardless of track presence. Changes in behavior based on satiation are well known among coccinellid larvae. For example, increased aggression among *H. axyridis* larvae has often been documented to occur with increased hunger (Snyder et al. 2000; Burgio et al. 2002; Moser and Obrycki 2009). In this study, starved individuals fed on the prey more frequently than did satiated individuals. We also observed starved larvae to remain stationary for shorter durations compared to well-fed individuals, which is consistent with previous findings demonstrating that starved coccinellid larvae travel greater distances, walk at faster speeds, and remain stationary for shorter durations than well-fed larvae (Ng 1986).

Evidence for predator detection may be observed through changes in anti-predatory behaviors, such as changes in larval movement (Ferris and Rudolf 2007). We observed two distinctive larval movements in this study, backward walking and pygopod dragging. Backward walking is a previously undocumented behavior among coccinellid larvae and is likely related to surface adhesion and abiotic factors within the dish. Time spent walking backward was greatest on the dish lid compared to the dish base and by placing the pygopod firmly on the surface and walking back towards the pygopod, larvae may have increased adhesion to the Petri dish. Pygopod dragging may also increase adhesion to the walking surface; however, this behavior was observed by larvae walking on the dish base as well as by larvae walking on the dish lids' lower surface.

Larval tracks are released from the pygopod (Laubertie et al. 2006) and dragging of the pygopod on the surface of plants has been suggested to be an intentional marking behavior (Marks 1977). Larvae have been observed to drag their pygopod for brief periods along many plant surfaces, including leaf edges and vertically along plant stems (S. E. Moser, personal observation). In this study, *H. axyridis* 4th instars dragged their pygopod more often in dishes with tracks and dragging behavior increased if *C. maculata* 4th instars were present. By dragging their pygopod, *H. axyridis* 4th instars may be signaling the other larva or themselves to stay away. Organisms may signal when detecting potential predators as a means to deter predation (pursuit-deterrent signal, Woodland et al. 1980) and for identification purposes (scent matching hypothesis, Gosling 1990). In addition, well-fed *H. axyridis* larvae benefit less from intraguild predation with *C. maculata* compared

to starved *H. axyridis* larvae (Moser and Obrycki 2009) and well-fed larvae dragged their pygopod more often compared to starved larvae.

Individuals that signal conspecifics through marking may decrease intraspecific competition if conspecifics avoid these areas. However, these same signals may increase predation pressure by advertising their presence or, in the case of *H. axyridis* larvae, these signals may not be perceived or do not stimulate a response. The observed larvae were kept in the same dishes throughout the rearing process and were repeatedly exposed to their own tracks prior to the experiment. Constant exposure to compounds found in their own track may have led to sensory adaptations or habituation to tracks with similar chemical compositions, and thus lowering the response when encountering a conspecific track in the experiment. Similarly, *Adalia bipunctata* L. (Coleoptera: Coccinellidae) females avoid oviposition in areas containing conspecific larval tracks, but do not respond to tracks when previously exposed to them during rearing (Frechette et al. 2004). Furthermore, if larvae become habituated to these signals, such as during periods of high larval abundance, larvae may interact more frequently and intraguild predation may increase. Information regarding the reaction (or lack thereof) to a larva's own mark was not recorded, and further evidence is needed to determine if larvae detect their own marks and if they are able to distinguish their marks from marks left by conspecifics.

Often referred to as an aggressive intraguild predator, *H. axyridis* larvae frequently attack other coccinellid species (Dixon 2000; Yasuda and Kimura 2001; Michaud and Grant 2003; Moser and Obrycki 2009) and unlike species that avoid tracks left by other larvae (Rutledge et al. 2008; Růžička and Zemek 2008); *H. axyridis* did not avoid areas marked by conspecifics. *Harmonia axyridis* larvae often gain fitness benefits from feeding on coccinellid larvae and the lack of response by *H. axyridis* larvae in areas containing tracks may be of particular benefit if prey abundance is low (Wagner et al. 1999; Snyder et al. 2000; Sato and Dixon 2004; Moser and Obrycki 2009). Coccinellid populations can be greatly impacted by intraguild predation and competition with other coccinellids. The establishment of *H. axyridis* in new geographic areas has frequently been correlated with declines of native coccinellid species. By not responding to tracks, *H. axyridis* larvae may interact with other larvae more frequently in comparison to other coccinellid species.

In summary, larval tracks are composed of non-volatile chemical secretions released by coccinellid larvae and the presence of larval tracks frequently results in avoidance behavior of adults and larvae (Table 1). Avoidance of areas containing tracks can reduce intraspecific interactions; however, aggressive predators, such as *H. axyridis* larvae, may not avoid areas containing tracks. In this study, exposure to conspecific and heterospecific larval cues rarely resulted in avoidance behavior and the presence of tracks did not decrease intraguild scavenging by *H. axyridis* larvae. *Harmonia axyridis* larvae may interact frequently with other coccinellid larvae in the field because they lack the avoidance response observed in other coccinellid species.

References

Bernstein C, Driessen G (1996) Patch-marking and optimal search patterns in the parasitoid *Venturia canescens*. J Anim Ecol 65:211–219

Brown MW (2003) Intraguild responses of aphid predators on apple to the invasion of an exotic species, *Harmonia axyridis*. BioControl 48:141–153

Brown MW, Miller SS (1998) Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. Entomol News 109:136–142

Brown PMJ, Roy HE, Rothery P, Roy DB, Ware RL, Majerus MEN (2008) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. Biocontrol 53:5–21

Burgio G, Santi F, Maini S (2002) On intra-guild predation and cannibalism in *Harmonia axyridis* (Pallas) and *Adalia bipunctata* L. (Coleoptera: Coccinellidae). Biol Cont 24:110–116

Colunga-Garcia GM, Gage SH (1998) Arrival, establishment, and habitat use of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. Environ Entomol 27:1574–1580

Cottrell TE (2004) Suitability of exotic and native lady beetle eggs (Coleoptera: Coccinellidae) for development of lady beetle larvae. Biol Cont 31:362–371

Cottrell TE, Yeargan KV (1998) Intraguild predation between an introduced lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), and a native lady beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae). J Kan Entomol Soc 71:159–163

Dixon AFG (2000) Insect predator-prey dynamics: ladybird beetles and biological control. Cambridge University Press, Cambridge

van Dijken MJ, van Stratum P, van Alphen JJM (1992) Recognition of individual-specific marked parasitized hosts by the solitary parasitoid *Epidinocarsis lopezi*. Behav Ecol Sociobiol 30:77–82

Doumbia M, Hemptinne JL, Dixon A (1998) Assessment of patch quality by ladybirds; role of larval tracks. Oecologia 13:197–202

Ferran A, Dixon AFG (1993) Foraging beaviour of ladybird larvae (Coleoptera: Coccinellidae). Eur J Entomol 90:383–402

Ferris G, Rudolf VHW (2007) Responses of larval dragonflies to conspecific and heterospecific predator cues. Ecol Entomol 32:283–288

Frechette B, Dixon AFG, Alauzet C, Hemptinne JL (2004) Age and experience influence patch assessment for oviposition by an insect predator. Ecol Entomol 29:578–583

Gosling LM (1990) Competitor assessment by scent matching—an experimental test. Behav Ecol Sociobiol 26:415

Harmon JP, Stephens E, Losey J (2007) The decline of native coccinellids (Coleoptera: Coccinellidae) in the United States and Canada. J Insect Conserv 11:85–94

Hautier L, Grégoire J, Schauwers J, Martin GS, Callier P, Jansen J, Biseau J (2008) Intraguild predation by *Harmonia axyridis* on coccinellids revealed by exogenous alkaloid sequestration. Chemoecology 18:191–196

Hessler LS, Kieckhefer RW (2008) Status of exotic and previously common native coccinellids (Coleoptera) in South Dakota landscapes. J Kan Ent Soc 81:29–49

Hodek I (1973) Biology of Coccinellidae. Academia, Prague and W. Junk, The Hague

Hodek I, Honêk A (1996) Ecology of Coccinellidae. Dordrecht, The Netherlands, Kluwer Academic Publishers, Boston, MA

Höller C, Hörmann R (1993) Patch marking in the aphid hyperparasitoid, *Dendrocerus carpenter*: the information contained in patch marks. Oecologia 94:128–134

Koch RL, Galvan TL (2008) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. Biocontrol 53:23–25

Kosaki A, Yamaoka R (1996) Chemical composition of footprints and cuticular lipids of three species of lady beetles. Jpn J Appl Entomol Zool 40:47–53

Krafsur ES, Kring TJ, Miller JC, Nariboli P, Obrycki JJ, Ruberson JR, Schaefer PW (1997) Gene flow in the exotic colonizing ladybeetle *Harmonia axyridis* in North America. Biol Cont 8:207–214

LaMana ML, Miller JC (1996) Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in Oregon. Biol Cont 6:232–237

LaRock DR, Ellington JJ (1996) An integrated pest management approach emphasizing biological control for pecan aphids. Southwest Entomol 21:153–166

Laubertie E, Martini X, Cadena C, Treilhou M, Dixon AFG, Hemptinne JL (2006) The immediate source of the oviposition-deterring pheromone produced by larvae of *Adalia bipunctata* (L.) (Coleoptera, Coccinellidae). J Insect Behav 19:231–240

Lucas É (2005) Intraguild predation among aphidophagous predators. Eur J Entomol 102:351–364

Marks RJ (1977) Laboratory studies on plant searching behaviour by *Coccinella septempunctata* L. larvae. Bull Entomol Res 67:235–241

Michaud JP (2002) Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. Environ Entomol 31:827–835

Michaud JP, Grant AK (2003) Intraguild predation among ladybeetles and a green lacewing: do the larval spines of *Curinus coeruleus* (Coleoptera: Coccinellidae) serve a defensive function? Bull Entomol Research 93:499–505

Michaud JP, Jyoti JL (2007) Repellency of conspecific and heterospecific larval residues to *Hippodamia convergens* (Coleoptera: Coccinellidae) ovipositing on sorghum plants. Eur J Entomol 104:399–405

Moser SE, Obrycki JJ (2009) Competition and intraguild predation among three species of coccinellids (Coleoptera: Coccinellidae). Ann Entomol Soc Am 102:419–425

Musser FR, Shelton AM (2003) Factors altering the temporal and within-plant distribution of coccinellids in corn and their impact on potential intra-guild predation. Environ Entomol 32:575–583

Nakashima Y, Senoo N (2003) Avoidance of ladybird trails by an aphid parasitoid *Aphidius ervi*: active period and effects of prior oviposition experience. Entomol Exp Appl 109:163–166

Nakashima Y, Teshiba M, Hirose Y (2002) Flexible use of patch marks in an insect predator: effect of sex, hunger state, and patch quality. Ecol Entomol 27:581–587

Ng SM (1986) The geotactic and phototactic responses of four species of aphidophagous coccinellid larvae. In: Hodek I (ed) Ecology of Aphidophaga. Kluwer Academic Publishers Group, Academia, Prague, pp 57–68

Oliver TH, Timms JEL, Taylor A, Leather SR (2006) Oviposition responses to patch quality in the larch ladybird *Aphidecta obliterata* (Coleoptera: Coccinellidae): effects of aphid density, and con- and heterospecific tracks. Bull Entomol Res 96:25–34

Pell JK, Baverstock J, Roy HE, Ware RL, Majerus MEN (2008) Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. Biocontrol 53:147–168

Phoofolo MW, Obrycki JJ (1998) Potential for intraguild predation and competition among predatory Coccinellidae and Chrysopidae. Entomol Exp Appl 89:47–55

Polis GA, Myers CA, Holt R (1989) The evolution and ecology of intraguild predation: competitors that eat each other. Annu Rev Ecol Syst 20:297–330

Price PW (1970) Trail odors: recognition by insects parasitic on cocoons. Science 170:546–547

Rutledge C, Eigenbrode SD, Ding H (2008) A plant surface mutation mediates predator interference among ladybird larvae. Ecol Entomol 33:464–472

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

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 (2003) Perception of oviposition-deterring larval tracks in aphidophagous coccinellids *Cycloneda limbifer* and *Ceratomegilla undecimnotata* (Coleptera: Coccinellidae). Eur J Entomol 100:345–350

Růžička Z (2006) Oviposition-deterring effects of conspecific and heterospecific larval tracks on *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). Eur J Entomol 103:757–763

Růžička Z, Zemek R (2008) Deterrent effects of larval tracks on conspecific larvae in *Cycloneda limbifer*. Biol Cont 53:763–771

Institute SAS (2007) SAS User guide: statistics. Cary, NC

Sato S, Dixon AFG (2004) Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. Agric Forest Entomol 6:21–24

Snyder WE, Joseph SB, Preziosi RF, Moore AJ (2000) Nutritional benefits of cannibalism for the lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae) when prey quality is poor. Environ Entomol 29:1173–1179

Snyder WE, Clevenger GM, Eigenbrode SD (2004) Intraguild predation and successful invasion by introduced ladybird beetles. Oecologia 140:559–565

Specty O, Febvay G, Grenier S, Delobel B, Piotte C, Pageaux J, Ferran A, Guillaud J (2003) Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae): comparison between natural and substitution prey. Arch Insect Biochem Physiol 52:81–91

Spiegler PE (1962) The origin and the nature of the adhesive substance in larvae of the genus *Chrysopa* (Neuroptera: Chrysopidae). Ann Entomol Soc Am 55:69–77

Wagner JD, Glover MD, Mosely JB, Moore AJ (1999) Heritability and fitness consequences of cannibalism in larvae of the ladybird beetle *Harmonia axyridis*. Evol Ecol Res 1:375–388

Woodland DJ, Jaafar Z, Knight ML (1980) The pursuit deterrent function of alarm signals. Am Nat 115:748–753

Yasuda H, Ohnuma N (1999) Effect of cannibalism and predation on the larval performance of two ladybird beetles. Entomol Exp App 93:63–67

Yasuda H, Kimura T (2001) Interspecific interactions in a tri-trophic arthropod system: effects of a spider on the survival of larvae of three predatory ladybirds in relation to aphids. Entomol Exp Appl 98:17–25

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