

## Field Evidence of an Airborne Enemy-Avoidance Kairomone in Wolf Spiders

Kenneth W. Schonewolf · Ryan Bell · Ann L. Rypstra ·  
Matthew H. Persons

Received: 19 September 2005 / Revised: 10 November 2005 /  
Accepted: 3 February 2006 / Published online: 23 May 2006  
© Springer Science + Business Media, Inc. 2006

**Abstract** *Hogna helluo*, *Pardosa milvina*, and *Trochosa ruricola* are co-occurring species of wolf spiders within agricultural fields in the eastern USA. The largest species, *H. helluo*, is a common predator of the two smaller species, *P. milvina* and *T. ruricola*. *H. helluo* frequently resides within soil fissures where *P. milvina* and *T. ruricola* may be attacked when they enter or walk near these fissures. We tested the ability of *P. milvina* and *T. ruricola* to avoid *H. helluo*-containing burrows by detecting airborne enemy-avoidance kairomones associated with *H. helluo*. To simulate soil fissures and control for visual and vibratory means of predator detection, we baited funneled pitfall traps with one of the following ( $N = 20$  traps/treatment): (1) blank (empty trap); (2) one house cricket (*Acheta domesticus*); (3) one adult female *H. helluo*; and (4) one adult male *H. helluo*. Over two separate 3-d periods, we measured pitfall capture rates of *P. milvina* and *T. ruricola* as well as other incidentally captured ground-dwelling arthropods. During the day, male *P. milvina* showed significant avoidance of pitfall traps baited with *H. helluo* of either sex but showed no avoidance of empty traps or those containing crickets. At night, male *T. ruricola* showed a qualitatively similar pattern of avoiding *H. helluo*-baited traps, but the differences were not statistically significant. We found no evidence that other ground-dwelling arthropods either avoided or were attracted to *H. helluo*-baited traps. This study suggests that an airborne enemy-avoidance kairomone may mediate behavior among male *P. milvina* in the field.

**Keywords** Semiochemical · Lycosidae · Airborne · Chemical cue · Antipredator · Predation risk

---

K. W. Schonewolf · R. Bell · M. H. Persons (✉)  
Department of Biology,  
Susquehanna University, Selinsgrove, PA 17870, USA  
e-mail: persons@susqu.edu

A. L. Rypstra  
Department of Zoology,  
Miami University, Hamilton, OH 45011, USA

## Introduction

Semiochemicals among insects have been studied extensively, but comparable studies among spiders have received less attention (Francke and Schulz, 1999; Dicke and Grostal, 2001; Schulz, 2004). Of the studies documenting behavioral responses to chemical cues among spiders, the vast majority (reviewed in Schulz, 2004) have examined pheromones rather than kairomones or other types of semiochemicals. Spider kairomones documented so far are of two types: foraging kairomones used to locate prey and enemy-avoidance kairomones used by prey species to avoid predators (Ruther et al., 2002). Foraging kairomones are known among ant-hunting spiders (Zodariidae) (Allan et al., 1996), jumping spiders (Salticidae) (Clark et al., 2000; Jackson et al., 2002), and wolf spiders (Lycosidae) (Persons and Uetz, 1996; Punzo and Kukoyi, 1997; Persons and Rypstra, 2000; Hoefler et al., 2002). Enemy-avoidance kairomones have been found in bowl-and-doily spiders (Linyphiidae) (Suter et al., 1989), but have been studied more extensively in the Lycosidae (Punzo, 1997; Persons et al., 2001).

*Pardosa milvina* (Araneae, Lycosidae, Hentz, 1844) is a small wolf spider that exhibits a range of antipredator behaviors, such as freezing, vertical climbing, and reduced activity, when exposed to silk and excreta from the larger syntopic wolf spider *Hogna helluo* (Araneae, Lycosidae, Walckenaer, 1837) (Persons and Rypstra, 2001; Persons et al., 2001, 2002; Barnes et al., 2002; Lehmann et al., 2004). In these studies, *P. milvina* was in direct contact with substrates containing deposits from *H. helluo*, and, therefore, the methods allowed for the possibility that *P. milvina* gained information about *H. helluo* via tactile cues. However, one of the experiments revealed that *P. milvina* avoids substrates that have been occupied by *H. helluo* even before coming into physical contact with them (Persons and Rypstra, 2001). This finding suggests that *P. milvina* may be able to detect an airborne cue released by *H. helluo*.

*Pardosa milvina* can be expected to gain significant benefits by being able to detect airborne enemy-avoidance kairomones of *H. helluo*. *H. helluo* is a common ground-dwelling spider in agricultural systems in the eastern USA (Marshall et al., 2002) and a facultative burrower that often hides in soil fissures during the day when *P. milvina* is most active (Walker et al., 1999; Marshall et al., 2002). Many members of the genus *Pardosa*, including *P. milvina*, are active diurnal foragers that range widely across the ground surface and use soil fissures similar to those used by *H. helluo* as temporary retreats (Walker et al., 1999; Samu et al., 2003). Thus, it would be advantageous for *P. milvina* to detect volatiles emitted either directly from *H. helluo* or the material it leaves behind so that it could avoid entering burrows or soil fissures occupied by *H. helluo* as it navigates the landscape.

*Trochosa ruricola* (Araneae, Lycosidae, De Geer, 1778) is also a co-occurring agrobiontic wolf spider species intermediate both in size and abundance relative to *P. milvina* and *H. helluo*. Anecdotal field observations suggest that *T. ruricola*, unlike *P. milvina*, prefers burrowing or hiding under objects during the day and actively forages at night. Field and laboratory observations of *T. ruricola* indicate that they are also commonly preyed upon by *H. helluo* (personal observation) and may also benefit substantially from early detection of *H. helluo* via an airborne cue.

In an open plot field study, we tested the ability of *P. milvina* and *T. ruricola* to detect airborne enemy-avoidance kairomones associated with *H. helluo*. By

enclosing *H. helluo* in traps that allowed volatiles to escape but prevented free-ranging *P. milvina* and *T. ruricola* from having any direct contact cues, we were able to use differences in the rates at which these smaller wolf spider species were captured in the traps as evidence of either or both species using airborne information to avoid the predator. We also measured trapping frequencies of other incidentally captured ground-dwelling arthropods to assess if these other species can detect airborne volatiles from *H. helluo* and to control for the possibility that these other captured arthropods might serve as an attractant or deterrent for *P. milvina* and *T. ruricola*.

## Methods and Materials

Adult male and female *H. helluo* were collected in late May 2003 from mixed corn and alfalfa fields in White Deer Township, Union County, PA, USA. They were maintained in individual opaque plastic containers (10.5-cm diam  $\times$  7.5 cm deep) with a 1-cm-deep layer of moistened peat moss covering the bottom. Prior to being used as stimuli within pitfall traps, *H. helluo* were maintained at 22°C with a 14:10 hr light/dark cycle. When in the laboratory, spiders were fed with two to four adult house crickets *Acheta domesticus* (L.) (Orthoptera: Gryllidae) each week.

In an 8-ha fallow cornfield at Susquehanna University Ecology Research Center, Selingsgrove, Snyder County, PA, USA, a grid of 96 live-fall pitfall traps was established (12 columns  $\times$  8 rows). This site was selected because of the high population density of *P. milvina* and *T. ruricola*. The rows and columns of traps were 12.5 m from each other. An individual trap consisted of a vertically oriented 0.7-cm-thick polyvinyl chloride (PVC) pipe (10.1-cm diam  $\times$  18 cm deep) that was inserted into the soil flush with the ground surface. A plastic cup (9.8-cm diam  $\times$  17 cm deep) was then inserted within the PVC sleeve and pushed to the bottom of the hole. Each pitfall trap was then capped with a small smooth plastic funnel (10-cm diam at top, tapering to an opening 2-cm diam at the bottom). The lip of the funnel was flushed with the ground and with the edge of the cup making it possible for wolf spiders and other small crawling arthropods to get in but not get out of the trap. This design also prevented spiders from viewing the contents of the cup prior to being trapped. Seismic detection of *H. helluo* from outside the trap was also highly unlikely because there was a small gap between the inner cup and outer PVC sleeve that would have impeded transmission of *H. helluo* movement across the barrier. Seismic information was further limited by restricting *H. helluo* to small containers that minimized their movement during the testing period. The relatively small funnel opening effectively prevented large predatory species from physically entering the trap and potentially preying on the smaller spiders.

We checked the empty traps in the grid for 3-d prior to the experiment to determine baseline trapping rates of *P. milvina* and to test for possible trap site effects. Traps remained open for an 8-hr sampling period (0900–1700 hr). A  $\chi^2$  analysis was performed on the 80 traps with the highest capture rates by using individual trap as the treatment. We found no significant difference in the total number of captured *P. milvina* spiders across traps ( $\chi^2 = 84.683$ ,  $df = 79$ ;  $C_v = 100.749$ ,  $P = 0.25$ ;  $N = 670$ ). We caught 503 males, 153 females, and 14 unsexed juveniles. All 80 traps were then paired (highest and lowest, second highest and

second lowest, third highest and third lowest, etc.) to further minimize potential trap bias effects. We sequentially assigned each of these 40 paired pitfall traps to each of the assigned treatments and ran a second  $\chi^2$  test by using each of the 40 pairs of traps as a separate treatment. We found no significant difference in capture rates of *P. milvina* among these pairs, indicating no confounding trap bias among unbaited traps by treatment pairing ( $\chi^2 = 9.317$ ,  $df = 39$ ;  $C_v = 54.572$ ,  $P > 0.999$ ;  $N = 670$ ).

The experiment was begun with one of the following stimuli placed in the bottom of each pitfall trap: (1) no added stimulus (negative control,  $N = 20$ ); (2) one adult *A. domesticus* (positive control,  $N = 20$ ); (3) one adult female *H. helluo* ( $N = 20$ ); and (4) one adult male *H. helluo* ( $N = 20$ ). The traps were baited by placing the stimulus arthropod in a plastic vial (4.5-cm diam  $\times$  8 cm deep) that contained a folded moist paper towel as a water source. We then covered the vial with two layers of fiberglass screening and secured it with a rubber band. Each vial was placed in its assigned treatment trap. The fiberglass screening allowed any volatiles present to emanate and simultaneously served to further obscure visual detection of the stimulus from directly above. Each negative control trap contained a vial, moist paper towel, rubber band, and fiberglass screening, but was otherwise empty. All traps were live traps and devoid of preservatives or other chemicals.

The experiment was carried out from June 17 to 23, 2003. There was no rain during this period. Traps remained open daily during an 8hr period (0900–1700 hr). All traps were checked and emptied of arthropods three times daily during the daylight hours of June 17–19. Traps were checked at 1100, 1400, and 1700 hr. For June 21–23, traps were checked three times at night during an 8-hr sampling period from 1900 to 0500 hr. Traps were checked at 2300, 0200, and 0500 hr, primarily to assess *T. ruricola* trap catch rates, but all arthropods found in the traps were recorded during this period. One-way analyses of variance (ANOVAs) were used to analyze the differences in the total number of *P. milvina* and *T. ruricola* captured among the four trap treatments during the day and night, respectively. We then used a Tukey *post hoc* comparison of means test to compare significant differences among treatments. Additional arthropods caught incidentally in the traps were recorded and identified at least to class and, in some cases, to species. When sample sizes permitted, these taxa were also subjected to statistical analysis across treatments. For all arthropods other than *P. milvina* and *T. ruricola*, sample sizes were insufficient for ANOVA. Instead, captures from individual traps were pooled by treatment and subjected to a  $\chi^2$  analysis when possible. When sample sizes allowed, we also used binomial tests to compare the total number of spiders caught across all treatments during the day vs. the night for each age and sex category of *P. milvina* and *T. ruricola*. Using a binomial test, we also compared the number of males vs. the number of females captured for both diurnal and nocturnal testing periods.

## Results

There was a significant effect of treatment on the total number of *P. milvina* per trap during the day (ANOVA,  $F = 9.549$ ,  $P < 0.001$ ,  $N = 519$ ,  $\beta = 0.998$ ; Tables 1 and 2). Fewer males were captured in traps with *H. helluo* (ANOVA,  $F = 11.084$ ,  $P < 0.001$ ,  $N = 374$ ,  $\beta = 1.00$ ; Table 1), but there was no difference in female *P. milvina* captures among treatments (ANOVA,  $F = 1.548$ ,  $P = 0.209$ ,  $N = 140$ ,  $\beta = 0.383$ ; Table 1).

**Table 1** Number of *Pardosa milvina* and *Trochosa ruricola* (mean ± SE) caught per treatment<sup>a</sup> per trap, June 17–23, 2003, Snyder County, PA, USA<sup>b</sup>

Sex	Blank	Cricket	Female <i>Hogna helluo</i>	Male <i>Hogna helluo</i>	F	P
Diurnal (N)						
Total <i>P. milvina</i> (519)	9.0 ± 0.76a	7.9 ± 0.73a	4.65 ± 0.72b	4.4 ± 0.78b	9.55	<0.001
Male <i>P. milvina</i> (374)	7.0 ± 0.71a	5.65 ± 0.61a	2.9 ± 0.51b	3.15 ± 0.53b	11.08	<0.001
Female <i>P. milvina</i> (140)	1.95 ± 0.34	2.0 ± 0.39	1.5 ± 0.31	1.1 ± 0.31	1.55	0.21
Nocturnal (N)						
Total <i>T. ruricola</i> (217)	0.51 ± 0.06	0.52 ± 0.08	0.38 ± 0.06	0.40 ± 0.06	1.19	0.31
Male <i>T. ruricola</i> (187)	0.48 ± 0.06	0.44 ± 0.07	0.28 ± 0.05	0.36 ± 0.06	2.24	0.08
Female <i>T. ruricola</i> (27)	0.03 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0.03 ± 0.02	1.49	0.22

<sup>a</sup> Treatments were empty trap (negative control), *Acheta domesticus* house crickets (positive control), female *H. helluo*, and male *H. helluo*.

<sup>b</sup> Letters after means indicate significant differences between treatments based on Tukey *post hoc* test. Numbers in parentheses indicate sample size.

Sample sizes for *P. milvina* at night (Table 3) were too small to analyze statistically for age and sex effects by using ANOVA; however, by using a  $\chi^2$  test, we did find that significantly fewer *P. milvina* were captured at night in traps baited with *A. domesticus* compared to all other groups (Table 3), but we found no significant avoidance of traps baited with *H. helluo*.

A total of 597 *P. milvina* were captured during diurnal and nocturnal sampling periods (Tables 2 and 3). Significantly more (519 vs. 78) spiders were captured during the day than during the night (binomial test;  $P < 0.001$ ) supporting our general field observations that *P. milvina* is active diurnally. Over the entire diurnal sampling period, 519 *P. milvina* were caught (374 males, 140 females, and 5 juveniles too young to accurately determine sex; Table 2). In comparison, over the entire nocturnal sampling period, 78 *P. milvina* were caught (12 males, 26 females, and 40 juveniles; Table 3). Although *P. milvina* is generally diurnal, we found significant differences in capture rates among adult males, adult females, and juveniles during the day and night. We captured significantly fewer juveniles during the day than at night (binomial test;  $P < 0.001$ ). Significantly fewer females were captured than males during the day (binomial test;  $P < 0.001$ ), but at night, this trend was reversed with significantly fewer males captured than females (binomial test;  $P = 0.05$ ).

There was no significant effect of treatment on the total number of *T. ruricola* per trap during the night (ANOVA,  $F = 1.186$ ,  $P = 0.314$ ,  $N = 217$ ,  $\beta = 0.310$ ; Table 1). Adult male capture rates did not vary significantly among treatments (ANOVA,  $F = 2.238$ ,  $P = 0.083$ ,  $N = 187$ ,  $\beta = 0.558$ ; Tables 1 and 3), but fewer males were found in the *H. helluo*-baited traps. Fewer female *T. ruricola* were caught during the trapping period, and we found no difference in female trapping rates among treatments (ANOVA,  $F = 0.725$ ,  $P = 0.548$ ,  $N = 27$ ,  $\beta = 0.175$ ; Tables 1 and 3).

**Table 2** Diurnal captures of arthropod and gastropod taxa in pitfall traps by treatment, June 17–19, 2003, Snyder County, PA, USA<sup>a</sup>

Taxon	Treatment					
	Blank	Cricket	Female <i>H. helluo</i>	Male <i>H. helluo</i>	$\chi^2$ <sup>b</sup>	<i>P</i>
Order Araneae						
Family Lycosidae						
Male <i>P. milvina</i>	140	113	58	63	50.62	<i>P</i> < 0.001
Female <i>P. milvina</i>	39	44	33	24	6.34	0.05 < <i>P</i> < 0.1
Juvenile <i>P. milvina</i>	1	1	2	1	*	
<i>P. milvina</i> total	180	158	93	88	49.45	<i>P</i> < 0.001
<i>Pardosa saxatilis</i>	0	0	1	0	*	
<i>Allocosa funerea</i>	3	3	3	0	*	
<i>Schizocosa avida</i>	1	0	1	1	*	
<i>T. rucicola</i>	0	0	1	2	*	
<i>Pirata</i> spp.	1	1	0	0	*	
<i>Gladicosa</i> spp.	0	0	1	1	*	
Family Thomisidae						
<i>Xysticus gulosus</i>	7	9	10	10	0.67	0.95 < <i>P</i> < 0.975
Male <i>Xysticus ferox</i>	32	25	22	25	2.08	0.50 < <i>P</i> < 0.75
Female <i>X. ferox</i>	22	14	11	12	5.07	0.10 < <i>P</i> < 0.25
<i>X. ferox</i> total	54	39	33	37	6.20	0.10 < <i>P</i> < 0.25
Family Gnaphosidae						
<i>Zelotes</i> sp.	7	5	4	6	0.91	0.95 < <i>P</i> < 0.975
Family Clubionidae						
<i>Clubiona</i> sp.	0	2	0	2	*	
Family Salticidae						
<i>Phidippus audax</i> juvenile	0	0	1	0	*	
Family Dictynidae sp.	4	1	0	0	*	
Order Opiliones	3	2	1	3	*	
Order Coleoptera						
Family Carabidae						
<i>Scarites subterraneus</i>	2	2	3	5	*	
Family Curculionidae						
Family Chrysomelidae	11	3	6	5	5.56	0.1 < <i>P</i> < 0.25
Order Hymenoptera						
Family Pompiliidae	13	23	17	17	2.91	0.25 < <i>P</i> < 0.50
Order Collembola sp.	0	1	0	0	*	
Order Hemiptera						
Family Pentatomidae						
<i>Acrosternum</i> sp.	0	1	0	0	*	
Order Orthoptera						
Family Gryllidae						
<i>Gryllus pennsylvanicus</i>	2	1	0	0	*	
Class Gastropoda	0	2	2	0	*	

<sup>a</sup>Treatments were empty trap (negative control), *A. domesticus* house crickets (positive control), female *H. helluo*, and male *H. helluo*.

<sup>b</sup> $\chi^2$  tests were completed only for taxa where the expected frequencies of the treatments were greater than 5.

A total of 220 *T. ruricola* were captured during diurnal and nocturnal sampling periods (Tables 2 and 3). The species was caught in higher numbers during the night than during the day (binomial test;  $P < 0.001$ ; Tables 2 and 3). Only three *T. ruricola* were caught over the entire 3-d diurnal period (Table 2), quantitatively supporting our hypothesis that *T. ruricola* is largely nocturnal. Over the entire nocturnal sampling period, 217 *T. ruricola* were caught (187 adult males, 27 adult females, and 3 late-instar juvenile females; Table 3). During nocturnal sampling, we captured more males than females (binomial test;  $P < 0.001$ ).

A number of other arthropods were captured in the pitfall traps (Tables 2 and 3). In most cases, there were too few individuals to analyze statistically by treatment. Of those species with sufficient sample size for analysis, we found no differences in trapping rate across treatments (Tables 2 and 3). However, in males of the crab spider *Xysticus gulosus* (Keyserling, 1880), there appeared to be a nonsignificant trend to avoid *H. helluo*-baited traps (Table 3).

## Discussion

Our results suggest that both sexes of *H. helluo* release an airborne kairomone that enables male *P. milvina* to detect and avoid *H. helluo* during the day, but we found no evidence that *P. milvina* or *T. ruricola* avoid *H. helluo* at night. Our data indicate that the detection by *P. milvina* is not very fine-tuned. Although we found significantly fewer male *P. milvina* in traps baited with *H. helluo*, *P. milvina* did not discriminate between male and female predators. In addition, they were not attracted to or repulsed by whatever cues might be released by adult crickets during the day. Although *T. ruricola* showed no statistically significant trap avoidance, the number of males found in *H. helluo*-baited traps was 19–40% lower than the number in cricket-baited or control traps, respectively.

Our finding that male, but not female, *P. milvina* detect and avoid *H. helluo* may be a statistical artifact because, throughout the study, male capture frequency was much higher than females and resulted in greater statistical power ( $\beta = 1.00$  for males vs. 0.383 for females). This higher capture frequency suggests that males of *P. milvina* tend to be more active than females overall, as may be true of most wolf spiders (Hallander, 1967; Cady, 1984; Persons, 1999; Persons and Uetz, 1999). However, in laboratory studies, no differences in male and female *P. milvina* activity were detected (Walker and Rypstra, 2003), suggesting that pitfall traps may be an indicator of *P. milvina* space use rather than activity level *per se*, or that laboratory findings are not representative of movement patterns of spiders under field conditions. Notably, other studies have shown that an airborne pheromone attracts *P. milvina* males to female conspecifics (Searcy et al., 1999). In addition, males use pheromones to identify the mating status of females, whereas females assess male quality by using primarily visual cues (Rypstra et al., 2003). Thus, it may be that males are more sensitive to chemical cues in general and, specifically, more sensitive to airborne cues than are females.

Female *H. helluo* show significantly higher attack frequencies on *P. milvina* than male *H. helluo* (Walker and Rypstra, 2002; Lehmann et al., 2004); therefore, females pose a much greater predation risk. It was therefore surprising that *P. milvina* was unable to discriminate between male and female *H. helluo*. If an airborne sex

**Table 3** Nocturnal captures of arthropod and gastropod taxa in pitfall traps by treatment, June 21–23, 2003, Snyder County, PA, USA<sup>a</sup>

Taxon	Treatment					
	Blank	Cricket	Female <i>H. helluo</i>	Male <i>H. helluo</i>	$\chi^2$ <sup>b</sup>	<i>P</i>
Order Araneae						
Family Lycosidae						
Male <i>P. milvina</i>	4	1	0	7	*	
Female <i>P. milvina</i>	8	2	10	6	5.38	0.10 < <i>P</i> < 0.25
Juvenile <i>P. milvina</i>	11	5	13	11	3.6	0.25 < <i>P</i> < 0.50
<i>P. milvina</i> total	23	8	23	24	11.19	0.01 < <i>P</i> < 0.025
Male <i>T. ruricola</i>	57	53	34	43	6.86	0.05 < <i>P</i> < 0.10
Female <i>T. ruricola</i>	4	9	10	4	4.56	0.10 < <i>P</i> < 0.25
Juvenile <i>T. ruricola</i>	0	0	2	1	*	
<i>T. ruricola</i> total	61	62	46	48	4.17	0.10 < <i>P</i> < 0.25
<i>A. funerea</i>	0	1	0	0	*	
<i>Sch. avida</i>	0	0	0	2	*	
Male <i>H. helluo</i>	1	0	0	0	*	
Thomisidae						
<i>X. gulosus</i>	15	19	6	11	7.27	0.05 < <i>P</i> < 0.10
Juvenile <i>Xysticus</i> spp.	5	5	7	10	2.48	0.25 < <i>P</i> < 0.50
<i>Xysticus</i> spp. total	20	24	13	21	3.33	0.25 < <i>P</i> < 0.50
Family Linyphiidae						
Ergioninae	4	2	3	2	*	
Gnaphosidae						
<i>Zylothes</i> sp.	0	1	0	0	*	
Order Coleoptera						
Family Chrysomelidae						
Unidentified flea beetle spp.	29	35	28	37	1.82	0.50 < <i>P</i> < 0.75
Family Carabidae	1	1	0	1	*	
<i>S. subterraneus</i>	1	1	0	1	*	
Order Orthoptera						
Family Gryllidae						
<i>G. pennsylvanicus</i>	8	10	5	6	2.03	0.50 < <i>P</i> < 0.75
Family Acrididae	3	1	1	2	*	
Order Hymenoptera						
Family Formicidae	7	6	9	11	1.79	0.50 < <i>P</i> < 0.75
Family Apiidae						
<i>Apis mellifera</i>	0	2	1	1	*	
Collembola	1	0	2	2	*	
Order Dermaptera						
Family Forficulidae	0	2	0	1	*	
Class Diplopoda	0	0	1	2	*	
Class Chilopoda	0	0	1	0	*	
Class Isopoda spp.	9	2	2	3	*	
Class Gastropoda: slug	0	1	0	0	*	
Class Clitellata						
Family Lumbriculidae						
<i>Lumbricus</i> spp.	1	0	1	1	*	

<sup>a</sup>Treatments were empty trap (negative control), *A. domesticus* house crickets (positive control), female *H. helluo*, and male *H. helluo*.<sup>b</sup> $\chi^2$  tests were completed only for taxa where the expected frequencies of the treatments were greater than 5.

pheromone of *H. helluo* were used as an enemy-avoidance kairomone by *P. milvina*, sex discrimination would appear to be readily achievable. Because volatiles from adult male or adult female *H. helluo* elicited similar avoidance responses by *P. milvina*, this suggests that the kairomone is not a sex pheromone.

Although it has been shown that *P. milvina* can recognize substrate-borne cues from prey items (Hoeffler et al., 2002), we uncovered no evidence that *P. milvina* responded either positively or negatively to the crickets contained in our traps during the day. We did find a significant tendency for *P. milvina* to avoid cricket-baited traps at night. The adult crickets that we used were much larger than those consumed by *P. milvina*. If *P. milvina* detected the crickets, and was able to determine their size, then they may have avoided them based on this criterion alone. Previous studies have demonstrated that *P. milvina* can estimate the size of *H. helluo* based on substrate-borne chemical cues alone by responding to the quantity of silk and excreta produced (Persons and Rypstra, 2001). Alternatively, *P. milvina* may not have had sufficient experience with crickets of this size and species to respond strongly to their emissions one way or another. Previous studies have found that *H. helluo* are attracted to volatiles from *P. milvina* but apparently not *A. domesticus* (Persons and Rypstra, 2000).

Although male *T. ruricola* and male *X. gulosus* showed the same general pattern as *P. milvina* with respect to trap capture rates, differences did not reach the level of statistical significance. This could be attributable to low statistical power. As an alternative hypothesis, *T. ruricola* and *H. helluo* are both nocturnally active and are closer in size to one another. As such, *H. helluo* may not present as great of a predation risk to *T. ruricola* as it does to *P. milvina*. *Xysticus gulosus* is smaller than *T. ruricola* and well within the prey size range of *H. helluo*. However, unlike *T. ruricola*, we have never observed *X. gulosus* predation by *H. helluo* in the field. Although we found no evidence that *Xysticus* spp. respond to *H. helluo* volatiles, there is field evidence that some *Xysticus* species are capable of detecting volatile chemicals (Aldrich and Barros, 1995).

*Pardosa milvina* failed to show significant avoidance of *H. helluo* at night. This also could have been attributable to a lack of statistical power because far fewer *P. milvina* were captured at night. Alternatively, diurnal and nocturnal conditions may have influenced kairomone production and persistence by *H. helluo*. Previous studies have found that *P. milvina* antipredator responses are reduced when they encounter *H. helluo* silk and excreta that have been wetted, but that subjecting *H. helluo* silk and excreta to heat has no effect on *P. milvina* response (Wilder et al., 2005). Based on these findings, dew at night may have reduced the effectiveness of any volatile kairomone produced by *H. helluo* resulting in less effective avoidance by *P. milvina* males, whereas the higher temperatures and sunlight during the day likely had no negative effect on *P. milvina* responsiveness to this kairomone. However, our use of moist paper towels in this study likely minimized extreme differences in ambient trap humidity levels during the day vs. the night, but humidity levels outside of the trap probably varied considerably.

We cannot completely rule out the hypothesis that *P. milvina* could have been attracted to insects or spiders captured in the traps other than *H. helluo*. Another alternative hypothesis is that differential predation by trapped larger spiders or ground beetles could have skewed the capture frequencies across treatments. However, both of these alternative interpretations of biased trap capture rates are

unlikely. First, we found no evidence for differential trapping rates by treatment for species other than *P. milvina*. Second, because the traps were designed to capture small wolf spiders, few larger potential predators were able to physically enter the traps. Third, because we cleared the traps frequently, arthropod densities never were sufficiently high to encourage predation. We also visited traps with sufficient frequency to observe any predation when it did occur. Only two instances of predation were found. This occurred twice in the control traps, and these were omitted from the analysis because the spiders could not be positively identified with respect to age, sex, or *Pardosa* species. Only six species found in the traps were capable of preying upon *Pardosa*: one beetle species, the carabid *Scarites subterraneus* (Fabricius, 1775); two species of crab spider, *Xysticus ferox* (Hentz, 1847) and *X. gulosus* (Keyserling, 1880); and three wolf spiders, *H. helluo* (Walckenaer, 1837), *Schizocosa avida* (Walckenaer, 1837), and *T. ruricola*. *Trochosa ruricola* and *S. subterraneus* occurred in sufficiently low numbers during the day as to be unlikely to have had a significant predation effect (three *Trochosa* during the day and 12 *Scarites* during the day). *Schizocosa avida* also occurred only in very low numbers during the day and night, and only a single *H. helluo* was captured making both unlikely to bias the results. The crab spiders, although numerous, were never larger than *Pardosa*, and, more importantly, they were either equally distributed across all treatment groups or showed trends toward the same capture bias as *Pardosa*, and thus, if anything, would underestimate the magnitude of the capture bias effect because predation would skew it in the opposite direction as what the results indicate. Furthermore, during trap checks, we found no evidence of intraguild predation occurring between *P. milvina* and either of the *Xysticus* spp.

Research on airborne chemical communication in spiders is in its infancy. Currently, there are few studies of communication among spiders via airborne pheromones (Tietjen, 1979; Watson, 1986; Willey and Jackson, 1993; Searcy et al., 1999; Papke et al., 2001; Becker et al., 2005) and even fewer that test for airborne chemical cues among different species of spider (Persons and Rypstra, 2000; Jackson et al., 2002; Li and Lee, 2004; Kasumovic and Andrade, 2004). Our study provides evidence that wolf spiders can and do use an airborne enemy-avoidance kairomone within an ecologically relevant context. We suggest that future studies of interspecific interactions among spiders should consider volatile cues as a possible mediator of observed behavioral responses.

**Acknowledgments** We thank Ashley Boyer and Daisy Conduah for their help in setting up pitfall traps and collecting spiders used in this study. This research was funded by NSF grants DBI 0216776 to M. Persons and DBI 0216947 to A. Rypstra.

## References

- ALDRICH, J. R. and BARROS, T. M. 1995. Chemical attraction of male crab spiders (Araneae, Thomisidae) and kleptoparasitic flies (Diptera, Milichiidae and Chloropidae). *J. Arachnol.* 23:212–214.
- ALLAN, R. A., ELGAR, M. A., and CAPON, R. J. 1996. Exploitation of an ant chemical alarm signal by the zodariid spider *Habronestes bradleyi* Walckenaer. *Proc. R. Soc. Lond. Ser. B* 263:69–73.

- BARNES, M. C., PERSONS, M. H., and RYPSTRA, A. L. 2002. The effect of predator chemical cue age on antipredator behavior in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *J. Insect Behav.* 15:269–281.
- BECKER, E. RIECHERT, S., and SINGER, F. 2005. Male induction of female quiescence/catalepsis during courtship in the spider, *Agelenopsis aperta*. *Behaviour* 142:57–70.
- CADY, A. B. 1984. Microhabitat selection and locomotor activity of *Schizocosa ocreata* (Walckenaer) (Araneae: Lycosidae). *J. Arachnol.* 11:297–307.
- CLARK, R. J., JACKSON, R. R., and CUTLER, B. 2000. Chemical cues from ants influence predatory behavior in *Habrocestum pulex*, and ant-eating jumping spider (Araneae, Salticidae). *J. Arachnol.* 28:309–318.
- DICKE, M. and GROSTAL, P. 2001. Chemical detection of natural enemies by arthropods: An ecological perspective. *Ann. Rev. Ecol. Syst.* 32:1–23.
- FRANCKE, W. and SCHULZ, S. 1999. Pheromones, pp. 197–261, in D. Barton, K. Nakanishi, O. Meth-Cohn and K. Mori (eds.). *Comprehensive Natural Products Chemistry*, Vol. 8. Elsevier, Amsterdam.
- HALLANDER, H. 1967. Range and movements of the wolf spiders *Pardosa chelata* (O. F. Miller) and *P. pullata* (Clerck). *Oikos* 18:360–364.
- HOEFLER, C. D., TAYLOR, M., and JAKOB, E. M. 2002. Chemosensory response to prey in *Phidippus audax* (Araneae, Salticidae) and *Pardosa milvina* (Araneae, Lycosidae). *J. Arachnol.* 30:155–158.
- JACKSON, R. R., CLARK, R. J., and HARLAND, D. P. 2002. Behavioural and cognitive influences of kairomones on an araneophagic jumping spider. *Behaviour* 139:749–775.
- KASUMOVIC, M. M. and ANDRADE, C. B. 2004. Discrimination of airborne pheromones by mate-searching male western black widow spiders (*Latrodectus hesperus*): species- and population-specific responses. *Can. J. Zool.* 82:1027–1034.
- LEHMANN, L., WALKER, S. E., and PERSONS, M. H. 2004. The influence of predator sex on chemically-mediated antipredator response in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *Ethology* 110:1–17.
- LI, D. and LEE, W. S. 2004. Predator-induced plasticity in web-building behaviour. *Anim. Behav.* 67:309–318.
- MARSHALL, S. D., PAVUK, D. M., and RYPSTRA, A. L. 2002. A comparative study of phenology and daily activity patterns in the wolf spiders *Pardosa milvina* and *Hogna helluo* in soybean agroecosystems in southwestern Ohio (Araneae: Lycosidae). *J. Arachnol.* 30:503–510.
- PAPKE, M. D. RIECHERT, S. E., and SCHULZ, S. 2001. An airborne female pheromone associated with male attraction and courtship in a desert spider. *Anim. Behav.* 61:877–886.
- PERSONS, M. H. 1999. Hunger effects on the foraging responses to perceptual cues in immature and adult wolf spiders (Lycosidae). *Anim. Behav.* 57:81–88.
- PERSONS, M. H. and RYPSTRA, A. L. 2000. Preference for chemical cues associated with recent prey in the wolf spider *Hogna helluo* (Araneae: Lycosidae). *Ethology* 106:27–35.
- PERSONS, M. H. and RYPSTRA, A. L. 2001. Wolf spiders show graded antipredator behavior in the presence of chemical cues from different sized predators. *J. Chem. Ecol.* 27:2493–2504.
- PERSONS, M. H. and UETZ, G. W. 1996. Wolf spiders vary patch residence time in the presence of chemical cues from prey (Araneae, Lycosidae). *J. Arachnol.* 24:76–79.
- PERSONS, M. H. and UETZ, G. W. 1999. Age and sex-based differences in the foraging decisions of wolf spiders. *J. Insect Behav.* 12:723–736.
- PERSONS, M. H., WALKER, S. E., RYPSTRA, A. L., and MARSHALL, S. D. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae: Lycosidae). *Anim. Behav.* 61:43–51.
- PERSONS, M. H., WALKER, S. E., and RYPSTRA, A. L. 2002. Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *Behav. Ecol.* 13:386–392.
- PUNZO, F. 1997. Leg autotomy and avoidance behavior in response to a predator in the wolf spider, *Schizocosa avida* (Araneae, Lycosidae). *J. Arachnol.* 25:202–205.
- PUNZO, F. and KUKOYI, O. 1997. The effects of prey chemical cues on patch residence time in the presence of chemical cues from prey (Araneae, Lycosidae). *Bull. Br. Arachnol. Soc.* 10:323–326.
- RUTHER, J. MEINERS, T., and STEIDLE, J. L. M. 2002. Rich in phenomenon—lacking in terms. A classification of kairomones. *Chemoecology* 12:161–167.
- RYPSTRA, A. L., WIEG, C., WALKER, S. E., and PERSONS, M. H. 2003. Mutual mate assessment in wolf spiders: differences in the cues used by males and females. *Ethology* 109:315–325.

- SAMU, F., SZIRANYI, A., and KISS, B. 2003. Foraging in agricultural fields: local 'sit-and-move' strategy scales up to risk-averse habitat use in a wolf spider. *Anim. Behav.* 66:939–947.
- SCHULZ, S. 2004. Semiochemistry of spiders, pp. 110–150, in R. T. Cardé, and J. G. Millar (eds.). *Advances in Insect Chemical Ecology*. Cambridge University Press, Cambridge, UK.
- SEARCY, L. E., RYPSTRA, A. L., and PERSONS, M. H. 1999. Airborne chemical communication in the wolf spider *Pardosa milvina*. *J. Chem. Ecol.* 25:2527–2533.
- SUTER, R. B., SHANE, C. M., and HIRSCHMEIER, A. J. 1989. *Frontinella pyramitela* detects *Argyrodes trigonum* via cuticular chemicals. *J. Arachnol.* 17:237–240.
- TIETJEN, W. J. 1979. Tests for olfactory communication in four species of wolf spiders (Araneae, Lycosidae). *J. Arachnol.* 6:197–206.
- WALKER, S. E. and RYPSTRA, A. L. 2002. Sexual dimorphism in trophic morphology and feeding behavior of wolf spiders (Araneae: Lycosidae) as a result of differences in reproductive roles. *Can. J. Zool.* 80:679–688.
- WALKER, S. E. and RYPSTRA, A. L. 2003. Sexual dimorphism and the differential mortality model: is behaviour related to survival? *Biol. J. Linn. Soc.* 78:97–103.
- WALKER, S. E., MARSHALL, S. D., and RYPSTRA, A. L. 1999. The effects of feeding history on retreat construction in the wolf spider *Hogna helluo*. *J. Arachnol.* 27:689–691.
- WATSON, P. J. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Linyphia litigiosa* (Araneae, Linyphiidae). *Science* 223:219–221.
- WILDER, S. M., DEVITO, J., PERSONS, M. H., and RYPSTRA, A. L. 2005. The effects of moisture and heat on the efficacy of chemical cues used in predator detection by *Pardosa milvina* (Araneae, Lycosidae). *J. Arachnol.* 33:857–861.
- WILLEY, M. B. and JACKSON, R. R. 1993. Olfactory cues from conspecifics inhibit the web-invasion behavior of *Portia*, a web-invading, araneophagic jumping spider (Araneae: Salticidae). *Can. J. Zool.* 71:1415–1420.