



Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider

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ABSTRACT

Animals use chemical cues for signaling between species. However, anthropogenic chemicals might interrupt this natural chemical information flow, with potential impacts on predator–prey interactions. Our goal was to explore how Buccaneer® Plus, a common herbicide similar to Round-up® (active ingredient glyphosate), affected the interactions between intraguild predators. The wolf spider *Pardosa milvina* (Hentz, 1844) is numerically dominant in agricultural systems across the eastern United States, and often falls prey to or competes with the larger wolf spider, *Hogna helluo* (Walckenaer, 1837) and/or the carabid beetle, *Scarites quadricaps* (Chaudoir, 1843). We tested the effects of chemical cues from these intraguild predators and exposure to herbicide on the activity, emigration, and survival of *P. milvina* using a full-factorial laboratory experiment. Both predator cues and herbicide led to a decrease in movement by *P. milvina*. However, although *H. helluo* cues alone decreased movement, *S. quadricaps* cues only decreased movement when combined with herbicide. These results indicate that predation risk and herbicide application likely interact in complex ways to affect the movement of a major arthropod predator in agricultural systems, and thus may have complex effects on the food web.

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1. Introduction

Chemical cues are important for signaling both within and between species. Intra-specific chemical cues (pheromones) are used for advertising territories (Sillero-Zubiri and Macdonald, 1998), locating mates (Byrne and Keogh, 2007) and recognizing and communicating with kin (Howard and Blomquist, 2005). Inter-specific chemical cues (kairomones) are used by prey to avoid predators (Grostal and Dicke, 1999) or conversely by predators to locate prey (Koivula and Korpimäki, 2001). Therefore, factors that affect the abilities of animals to detect these natural chemical cues could have impacts at both population and community levels.

There is a growing body of evidence that suggests anthropogenic chemicals often interrupt this natural chemical information flow between animals (reviewed in Lurling and Scheffer (2007); Klaschka (2008)). For instance, Scott et al. (2003) found that in the presence of cadmium (a common trace metal contaminant) juvenile rainbow trout are unable to sense chemicals released by injured conspecifics. Furthermore, Mandrillon and Saglio (2007)

found that toad tadpoles did not respond to chemical signals from a crayfish predator when those cues were combined with greater than 0.01 mg L^{−1} of the herbicide amitrole. Detailed studies of the impacts of anthropogenic chemicals on natural chemical signaling are overwhelmingly biased towards aquatic systems (over 80% of studies conducted – Lurling and Scheffer, 2007; Boyd, 2010). Only a few studies have addressed the effects of anthropogenic chemicals on natural chemical signaling in terrestrial systems, despite their potential importance in these systems (e.g. Komez et al., 2001; Salerno et al., 2002; Griesinger et al., 2011).

Modern agriculture involves the application of chemicals to manage insects, fungi, weeds and bacteria (Pimentel, 2005). Specifically the use of glyphosate-based herbicides in agriculture has increased rapidly worldwide with the development of genetically modified resistant crops (Woodburn, 2000; Lundgren et al., 2009). Early studies of the effects of glyphosate-based herbicides on arthropods revealed only minor effects on a few species (Hassan et al., 1988; see Giesy et al. (2000) for a review; Haughton et al., 2001a,b), thus these researches attributed changes in terrestrial arthropod activity and density after exposure to these herbicides in the field as indirect effects resulting from changes in plant structure (Haughton et al., 1999, 2001a; Giesy et al., 2000; Bell et al., 2002). However, more recent studies have shown that glyphosate-based herbicides can directly influence the behavior or survival of

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some predatory terrestrial arthropods (Michalkova and Pekar, 2009; Schneider et al., 2009; Benamú et al., 2010; Evans et al., 2010). For example, exposure to glyphosate-based herbicides alters activity patterns of carabid beetles and wolf spiders (Michalkova and Pekar, 2009; Evans et al., 2010), has negative effects on prey consumption and reproduction in orb spiders (Benamú et al., 2010) and reduces survival in lacewings and wolf spiders (Schneider et al., 2009; Evans et al., 2010).

Since many of the interactions among terrestrial arthropod predators that inhabit agroecosystems are mediated by chemical cues (Dicke and Grostal, 2001), the potential for herbicides to disrupt natural information transfer and alter food web dynamics is great (Lurling and Scheffer, 2007). Studies have revealed that glyphosate can inhibit olfaction in fish and thus potentially interfere with their normal reactions to chemical signals (Tierney et al., 2006, 2007). Furthermore, commercially formulated glyphosate-based herbicides can obstruct natural chemical signaling in amphibians and spiders (Relyea, 2005; Griesinger et al., 2011). For example, a recent study revealed that a commercial formulation of a glyphosate-based herbicide reduced the efficacy of the airborne pheromones employed by virgin female wolf spiders attempting to attract mates (Griesinger et al., 2011). Here we explore the influence of that same herbicide formulation on the manner in which the spider responds to substrate borne cues from its intraguild predators. Changes in activity can affect an animal's susceptibility to predation (Persons et al., 2001) and the likelihood that it will colonize or remain in highly disturbed agroecosystems where herbicide use is common (Brust, 1990; Bishop and Riechert, 1990; Halaj et al., 2000; Thorbek and Bilde, 2004). Thus, we designed our study to examine the effects of herbicide, by itself and in combination with predator chemical signals, on the movement patterns and the propensity of an agrobiont predator to leave a specific area.

1.1. Study system

The focal species for these experiments was *Pardosa milvina* (Araneae: Lycosidae), a numerically dominant, epigeal generalist arthropod predator in agricultural fields throughout eastern North America (Young et al., 1989; Draney, 1997; Marshall and Rypstra, 1999; Marshall et al., 2002). The sensitivity of *P. milvina* to its chemical environment has been very well-documented; it uses both air- and substrate-borne cues to find mates and detect predators (Searcy et al., 1999; Persons and Rypstra, 2001; Rypstra et al., 2003; Wilder and Rypstra, 2004; Schonewolf et al., 2006) and alters its activity in the presence of a commercial formulation of a glyphosate-based herbicide (Evans et al., 2010). Two larger predatory arthropod species coexist with *P. milvina*: the wolf spider, *Hogna helluo* (Araneae: Lycosidae) (300–800 mg), and the ground beetle *Scarites quadricaps* (Coleoptera: Carabidae) (500 mg) (Snyder and Wise, 1999; Marshall et al., 2002). The response of *P. milvina* to silk, feces and other excreta left in an area occupied by *H. helluo* is fine tuned; their anti-predator behaviors are gauged appropriately to the risk based on the size, sex and hunger level of *H. helluo* that produced the cues (Persons and Rypstra, 2001; Bell et al., 2006). Although we have observed *S. quadricaps* to prey on *P. milvina* in the laboratory, nothing was known about the importance of chemicals in mediating their interactions prior to this study. Nevertheless, given the sensitivity of *P. milvina* to substrate-borne chemical information, we expected that they would be able to detect chemical cues through feces or other excreta from *S. quadricaps*. In addition, previous research has demonstrated that all three of these species alter aspects of their activity on surfaces treated with glyphosate (Evans et al., 2010) and that the same herbicide formulation interferes with signaling between male and female *P. milvina* (Griesinger et al., 2011). Therefore, it seemed likely

that herbicide would influence its natural ability to detect and respond appropriately to chemical cues from its predators.

We hypothesize that a commercially formulated herbicide with the active ingredient glyphosate affects the activity of *P. milvina* and interferes with their reaction to substrate-borne chemical cues from either *H. helluo* or *S. quadricaps*. Here we report experiments that explore the effect of chemical cues from the larger predators, each alone and accompanied by herbicide, on activity level and the propensity for *P. milvina* to leave a closed arena.

2. Materials and methods

2.1. Collection and maintenance of arthropods

We conducted the following experiment between September and October of 2007 using animals we collected from corn and soybean fields at the Miami University Ecology Research Center (Oxford, OH). These fields were treated twice annually with the same formulation of herbicide as used in our study; however, we did not collect any arthropods within 3 d after herbicide application in the field, and did not use them in experiments until 2 months after the date of last herbicide application. We maintained all animals in a laboratory environmental room at 25 °C, 55–60% RH and a 13:11 L:D cycle. We housed all animals in translucent plastic containers (8 cm diameter × 5 cm high for each *P. milvina* and 12 cm diameter × 10 cm high for each *H. helluo* and *S. quadricaps*) with a moistened layer of 50:50 peat moss and soil mix covering the bottom (2 cm deep for *P. milvina* and *H. helluo*, and 4 cm deep for *S. quadricaps*, as this species burrows). We fed all animals a weekly diet of two appropriately sized crickets (*Acheta domesticus*). To control for possible effects of age or sex of *P. milvina* in this experiment, we selected only penultimate or adult females from the laboratory pool of collected individuals and distributed them randomly among the treatments. We also only used female *H. helluo* at the penultimate molt or adulthood because they are significantly larger than males, differ in morphology and tend to consume more prey (Walker and Rypstra, 2002); however, male and female *S. quadricaps* are similar in size and morphology so we did not sort them by sex.

2.2. Herbicide preparation

We used the commercially formulated herbicide Buccaneer® Plus, also known as Roundup II original®, created by the Monsanto Company, St. Louis, Missouri, USA (United States Patent US4528023). This herbicide contains the active ingredient glyphosate (480 g L⁻¹) in the form of isopropylamine salt, and an added polyethoxylated tallowamine (POEA) surfactant. Before applying the herbicide to the substrate we diluted it to 2.5%, which was within the manufacturer's recommended levels of 0.625–5%. We used a spray rate of 127.4 mL m⁻² (or 15.3 kg a.i. ha⁻¹ of glyphosate), which was the minimum necessary to gain a complete and uniform coverage of the areas for the laboratory container with filter paper.

2.3. Experimental design

Predators leave substrate-borne cues behind as they occupy an area (e.g. feces and other excreta, for spiders, silk). We measured the effects of these cues separately and combined with herbicide on the activity and emigration of *P. milvina* in the laboratory. We employed a full factorial design of six treatments, each with a sample size of *n* = 20. *P. milvina* were randomly assigned to one of the six following treatments: (1) *H. helluo* cues/water, (2) *H. helluo*

cues/herbicide, (3) *S. quadriceps* cues/water, (4) *S. quadriceps* cues/herbicide, (5) no cues/water, or (6) no cues/herbicide.

2.4. Arena preparation

We conducted each trial in a cylindrical plastic test arena 15 cm in diameter with filter paper on the bottom. The arena was divided into quadrants: alternating wet papers (herbicide or water) with dry papers (predator cues or blank paper), such that for each treatment two quadrants were covered with water or herbicide and two quadrants were covered with no cues or predator cues (Fig. 1). This simulated *P. milvina* encountering herbicide and/or predator cues in the field directly after herbicide spraying has occurred. Each arena had four 1 cm diameter escape holes (one per quadrant) that were opened during the second part of each trial.

2.5. Collection of predator cues and application of herbicide

For the treatments with predator cues, we allowed *H. helluo* or *S. quadriceps* to deposit cues on a piece of filter paper for 24 h prior to running the trials where we exposed *P. milvina* to these cues. To control for differences in cue signatures by hunger, we fed each of these predators one 16 mm cricket 15 min prior to beginning their deposition of cues. Directly before the trials, we removed each predator from the deposition container, cut its cue paper into four pieces and placed two of these in non-adjacent quadrants within the test arena. We sprayed a separate semicircle of clean filter paper with 1.25 mL of either herbicide (2.5% glyphosate solution applied at a rate of 127.4 mL m⁻²) or water using an airbrush sprayer, cut it into two pieces and added these to the other two quadrants (Fig. 1).

2.6. Trials

For each trial we placed a female *P. milvina* under a clear vial in the center of the arena and allowed her to acclimate for 5 min, then we removed the vial and the trial began. We recorded each spider from above during the 15 min activity trial using a video camera, and measured activity levels using an automated digital data collection system (VideoMex V, Columbus Instruments, Columbus, OH, USA). The VideoMex reports the time spent ambulatory (time

during which the movement is more than one body length per second), time spent moving in place (which includes smaller motions such as waving legs or rotating but not relocating) and total distance travelled. In addition we calculated average speed as time spent ambulatory divided by distance traveled. After the 15 min activity trial, we recaptured the spider under a vial and allowed it 5 min to re-acclimate. We then removed paper barriers from the emigration holes, allowing the spider to leave the arena. We monitored the spider for another 15 min and recorded if and when it left the container through the emigration holes.

2.7. Data analysis

We used either a natural log or square root transformation to obtain normal distributions and homoscedasticity for each activity measure (time spent ambulatory, time spent moving in place, distance traveled and average speed). We then used a two-way ANOVA (analysis of variance) with predator cues and herbicide as factors to compare the above activity variables between the six treatments (exposure to cues from the predators *H. helluo*, *S. quadriceps* or no cues, crossed with exposure to herbicide or water). Three of the measures, time spent ambulatory, distance travelled and average speed were not independent of each other as the first two were used to calculate the third. Therefore, significance for these three measures was assessed at $p < 0.0167$ after the institution of a traditional Bonferroni correction. Movement in place was independent of the other three measures and was therefore assessed at the usual $p < 0.05$. Tukey post-hoc tests were used to compare treatments for any variables that were found significant. We additionally addressed the question of whether spiders showed a preference that differed by treatment for the “wet” parts (paper covered with water or herbicide) vs. the “dry” parts of the arena (blank paper or covered with *S. quadriceps* or *H. helluo* cues). To do this, we first subtracted the time spent in behaviors on the wet parts of the substrate from time spent on the dry parts of the substrate. Then, we used a two-way ANOVA with predator cues and herbicide as factors to compare between treatments the resulting dry–wet substrate values for each of the activity variables. Finally, we pooled by treatment measures of each activity variable on the wet and dry parts of the substrate. We then used paired *t*-tests to investigate whether behaviors differed on wet vs. dry substrate regardless of treatment. When testing between dry and wet parts of the arena, as previously, we used a traditional Bonferroni correction to control for non-independence between the variables time spent ambulatory, distance travelled and average speed, resulting in a significance level of $p < 0.0167$. We next explored the effects of treatment on whether or not *P. milvina* emigrated in the time allotted for a trial (yes or no) using a Logistic model (Menard, 2002) with presence or absence of predator cues and presence of herbicide or water as factors. Finally, we measured time to emigration and compared it between treatments using a Proportional Hazards analysis (Cox, 1972), where we censored individuals that did not emigrate within the 15 min trials. All statistical analyses were conducted using JMP 7.0 (SAS institute Inc.).

3. Results

Predator cues significantly influenced the distance *P. milvina* traveled, their time spent ambulatory and their average speed, whereas herbicide alone only impacted motion in place (Table 1, Fig. 2). *P. milvina* spent significantly less time ambulatory, spent less time moving in place, traveled more slowly, and covered less distance when exposed to *H. helluo* cues, regardless of the presence of herbicide. In fact, these spiders nearly halved their average speed and time spent ambulatory and travelled about a third of

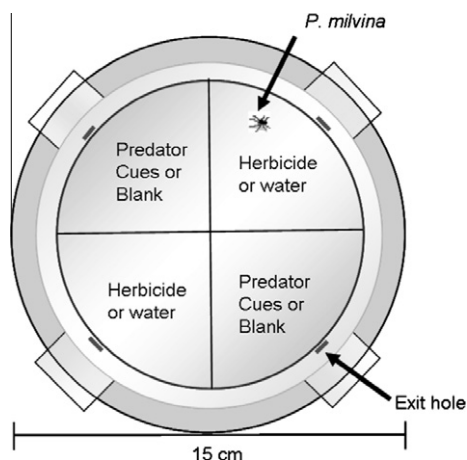


Fig. 1. Laboratory arena for exposing *Pardosa milvina* to herbicide and/or predator cues. Filter paper pieces with herbicide or water are alternated by those with predator cues (*Hogna helluo* or *Scorites quadriceps*) or blank paper. For example, in the *H. helluo*/herbicide treatments, there were two pieces of filter paper with *H. helluo* cues alternating with two that were covered with herbicide. Four exit holes located around the arena were blocked during the activity part of the trials but were opened afterwards to allow emigration to be measured.

Table 1ANOVA and Cox Proportional Hazards test results measuring the effects of predator cues and herbicide on the activity and emigration of *P. milvina*.

	Ambulatory (s) ^b (p-value)	Movement in place (s) ^{a,b} (p-value)	Distance (cm) ^c (p-value)	Avg. speed (cm s ⁻¹) ^c (p-value)	Emigration (yes/no) (p-value)	Time to emigration (p-value)
Model	$F_{5,119} = 7.83$ $p < 0.001$	$F_{5,119} = 2.54$; $p = 0.032$	$F_{5,114} = 7.34$ $p < 0.001$	$F_{5,114} = 6.24$; $p < 0.001$	$\chi^2_5 = 24.51$ $p < 0.001$	$\chi^2_5 = 17.33$ $p = 0.004$
Predator	<0.001	0.048	<0.001	<0.001	<0.001	<0.001
Herbicide	0.105	0.014	0.512	0.470	0.043	0.343
Pred × Herb	0.025	0.871	0.056	0.228	0.022	0.243

Significant model *p* values (in bold) for time spent ambulatory, distance travelled and average speed were assessed at $p < 0.0167$ after a traditional Bonferroni correction was instituted for non-independence of these measures. All *p* values for portions of models significant at $p < 0.05$ are also in bold.

^a Movement in place was independent of the other three measures and was therefore assessed at the usual $p < 0.05$.

^b Square root transformation of data.

^c Natural log transformation of data.

the distance compared to control spiders not exposed to predator cues (Fig. 2). In contrast, when exposed to *S. quadricaps* cues alone, *P. milvina* showed no statistically significant difference in activity compared to control spiders. Interestingly, there was a significant predator by herbicide interaction in the ANOVA that was caused by the fact that they decreased their time spent ambulatory when exposed to *S. quadricaps* cues and herbicide together (Table 1, Fig. 2A). Thus, while there was a difference in the response to *S. quadricaps* vs. *H. helluo* cues when each was presented with water, that difference disappeared in the presence of herbicide (Fig. 2).

Finally, treatment had no effect on the time that *P. milvina* spent performing any of the activity measures on the dry vs. wet parts of the arena, but the spiders appeared to have a consistent preference for movement on the dry parts (Table 2). Paired *t*-tests comparing the dry vs. wet parts of the arena (pooled among treatments) confirmed that spiders did in fact spend significantly more time moving, both ambulatory and in nonforward motion, on dry parts of the arena. Conversely, spiders moved more quickly on the wet portions of the arena, but there was no difference in the distance that the spiders travelled by substrate type (Table 2).

Whether or not a spider emigrated from the arena was significantly impacted by treatment (Table 1; Fig. 3). Predator cues, herbicide and the interaction between the two were all significant factors affecting whether a spider emigrated or not (Table 1). Overall, it appears that herbicide reduced the propensity of *P. milvina* to emigrate from the arenas, when it was presented along with predator cues (Fig. 3). Spiders in the *H. helluo* and herbicide combined treatment only emigrated 43% of the time, whereas spiders in the *S. quadricaps* and water treatment emigrated 100% of the time. The other treatments were intermediate (Fig. 3). Out of the spiders that emigrated, there was a significant difference among treatments for time to emigration (Table 1; Fig. 3). According to Likelihood ratio tests these differences among treatments were driven by presence of predator cues (Table 1). Spiders in both of the treatments exposed to *H. helluo* cues took the longest to emigrate, spiders exposed to herbicide and *S. quadricaps* cues combined were intermediate and the other three groups emigrated much more quickly (Fig. 3). There was no significant impact of herbicide on time to emigration or an interaction between predator cues and herbicide (Table 1).

4. Discussion

The results from this experiment confirm that *P. milvina* wolf spiders are sensitive to their chemical environment. Most importantly, although herbicide did not detectably affect the strong response of *P. milvina* to chemical cues from the co-occurring wolf spider, *H. helluo*, it altered their response to cues from *S. quadricaps* beetles. Specifically, while there does not appear to be a response

to *S. quadricaps* information when it was presented with water, when it was presented along with herbicide, there was a reduction in time spent ambulatory making it statistically indistinguishable from the reactions to *H. helluo*. Since we know that the response to *H. helluo* cues is protective but costly because it reduces foraging and reproductive success (Persons et al., 2002), these results suggest that the response to *S. quadricaps* cues with herbicide is inappropriate and might have implications for success of individual *P. milvina* at the time of spraying.

These results underscore the need to explore the reactions of animals to the combinations of stressors they may experience in a natural situation. We might have concluded that *P. milvina* did not detect or respond to *S. quadricaps* cues at all had we not looked for the interaction with herbicide and we might have concluded that this spider did not respond to herbicide at all had we only explored its response to *H. helluo* cues. Exploring these effects together reveals a synergistic effect that is potentially costly to *P. milvina*. This study adds to a body of work that reveals that there can be strong and unexpected interactions between natural and human induced stressors that need to be explicitly explored (Sih et al., 2004; Relyea, 2005).

P. milvina responded to predator cues alone in a way that was dependent on the predator to which they were being exposed. In the presence of cues from the wolf spider *H. helluo*, they exhibited a very strong reduction in activity and subsequently emigrated from the container less often. In contrast, in the presence of cues from *S. quadricaps*, they emigrated more often but we did not detect any significant shifts in activity. The difference in the strength of *P. milvina*'s reaction to its two intraguild predators could be explained by the silk laid down by *H. helluo* as it travels, which likely provides an additional tactile cue that is not present from *S. quadricaps*. However, we also suspect that *H. helluo* is a larger threat to *P. milvina* in nature than is *S. quadricaps*, and its cues would thus be expected to induce a stronger response. The specificity with which *Pardosa* detects the size, sex, and hunger levels from *H. helluo* cues suggests that it has been an important predator. Since most large wolf spiders, like *H. helluo*, are sit-and-wait predators that only lunge when their motion-based vision reveals prey (Persons and Uetz, 1996; Rovner, 1996), it can easily be seen that a reduction in activity would be protective. On the other hand, *S. quadricaps* appear to be more active but, at the same time, more cumbersome hunters (personal observation, K.W.) and so *P. milvina* might be able to escape this predator by simply running away. Thus, although it is highly likely that *P. milvina* are able to detect the chemical cues of *S. quadricaps*, there is presumably no adaptive need to shift their activity level in response. Instead, they may leave the area altogether, or if they stay, simply become more visually aware so that they are prepared to escape if and when they see this predator. These differential responses to the two predators by *P. milvina* are not unexpected as predator specific avoidance

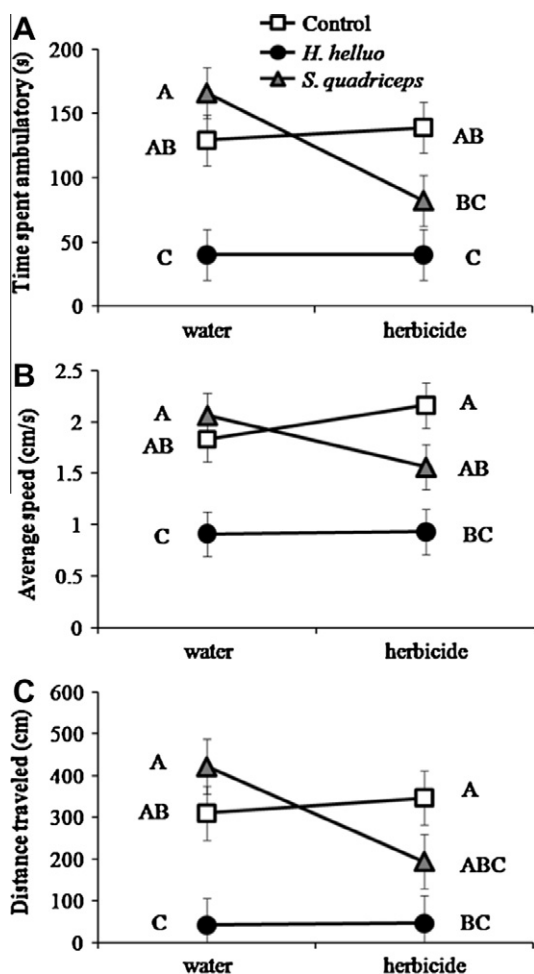


Fig. 2. Activity of *Pardosa milvina* when exposed to herbicide or water and/or predator cues (*Hogna helluo*, *Scarites quadriceps* or no cues) in 15 min laboratory trials. Bars indicate \pm SE. (A) Time spiders spent ambulatory in seconds. (B) Average speed across the trial in cm s^{-1} . (C) Distance spiders traveled in centimeters. Letters on the figures show comparisons between treatments according to Tukey post-hoc tests, where treatments with the same letter are not significantly different.

behavior by prey is common in a variety taxa, including insects (Ferris and Rudolf, 2007), amphibians (Relyea, 2001), and mammals (Seyfarth et al., 1980).

We were surprised that there was no detectable response of *P. milvina* to herbicide alone in these studies, especially in light of the fact that Evans et al. (2010) reported that this same herbicide formulation reduces *P. milvina* activity. We suspect that the answer lies in the differential responses observed here to wet and dry surfaces (Table 2). Specifically, *P. milvina* seem to be more active on dry surfaces than wet and all of our arenas were a combination of both. However, Evans et al. (2010) used designs with the either the entire arena wetted (half with water, half herbicide) or the entire arena dry (before and after exposure to herbicide). In this way the response they observed to herbicide would not be confounded by the separate response to a moist surface. Since moisture reduces the efficacy of predator cues (Wilder et al., 2005), the mixed design we used was necessary to address the interaction of herbicide with predator cues. Additionally since farmers would likely apply herbicide when it was dry and because the plants in the fields would intercept some of the herbicide, the soil surface would likely be a patchwork of dry and herbicide wetted areas, more analogous to the design we used in this study. Nevertheless, this unexpected result points to another factor contributing to the complexity of understanding if and how anthropogenic stressors impact the activities of terrestrial animals.

Table 2

Activity measures divided by substrate type (dry^a – wet^b) for *P. milvina* in response to predator cues and herbicide.

	Ambulatory (s)	Movement in place (s)	Distance (cm)	Speed (cm s^{-1})
Model (F; p-value) ^c	$F_{5,119} = 0.83$; $p = 0.53$	$F_{5,119} = 0.34$; $p = 0.89$	$F_{5,100} = 1.36$; $p = 0.25$	$F_{5,98} = 0.83$; $p = 0.53$
Model (t; p-value) ^d	$t_{119} = 5.70$; $p < 0.0001$	$t_{119} = 10.71$; $p < 0.0001$	$t_{100} = 0.93$; $p = 0.1766$	$t_{98} = -5.58$; $p < 0.0001$
No cues water	15.95 ± 7.98	267.15 ± 57.66	4.57 ± 6.10	-0.80 ± 0.26
No cues herbicide	23.25 ± 7.98	212.05 ± 57.66	6.77 ± 6.10	-1.13 ± 0.25
<i>H. helluo</i> water	12.63 ± 8.18	223.16 ± 59.16	4.41 ± 6.26	-0.83 ± 0.32
<i>H. helluo</i> herbicide	2.85 ± 7.98	188.25 ± 57.66	-3.41 ± 6.10	-0.60 ± 0.32
<i>S. quadriceps</i> water	26.95 ± 7.98	206.95 ± 57.66	8.07 ± 6.10	-0.96 ± 0.26
<i>S. quadriceps</i> herbicide	24.68 ± 8.18	195.00 ± 59.16	13.85 ± 6.26	-1.51 ± 0.32

Significant model *p* values (in bold) for time spent ambulatory, distance travelled and average speed were assessed at $p < 0.0167$ after a traditional Bonferroni correction was instituted for non-independence of these measures. Movement in place was independent of the other three measures and was therefore assessed at the usual $p < 0.05$.

^a Indicates measurements on pieces of filter paper covered with water or herbicide in a trial.

^b Indicates measurements on pieces of filter paper that was blank or covered with predator cues.

^c ANOVA testing for differences by treatment in *P. milvina*'s preference for dry vs. wet substrate.

^d Paired *t*-tests testing for *P. milvina*'s overall preference of dry vs. wet substrate using data pooled by treatment.

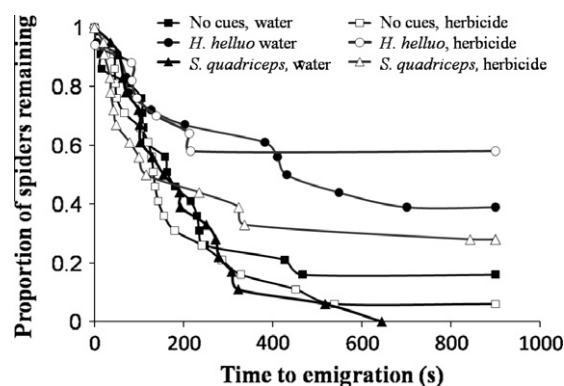


Fig. 3. Proportion of *P. milvina* remaining in the container over time when exposed to predator cues (*H. helluo*, *S. quadriceps*, or none) and/or herbicide or water in 15 min laboratory trials. During the trials, spiders were given the opportunity to emigrate out of any of four possible holes equally spaced around the container.

Our contrived measure of emigration does not suggest that *P. milvina* are likely to leave areas where stressors are introduced, at least in the short run. Indeed, almost 60% of the animals remained when they were in an environment with both herbicide and *H. helluo* cues. This result is consistent with those of a field study of Buddle and Rypstra (2003), which found no effect of the presence of *H. helluo* on the emigration of *P. milvina*. However, we do know that the presence of *H. helluo* or its cues affect habitat selection of *P. milvina* within a field (Rypstra et al., 2007). *P. milvina* can be characterized as a versatile predator well adapted to these highly disturbed agricultural systems (Marshall and Rypstra, 1999). Since any field at any time is likely to be a patchwork of predators or herbicide or both, the scale at which these processes affect *P. milvina* distribution is likely to be within the field. Perhaps more importantly, the dramatic decrease in activity in the presence

of *H. helluo* cues is likely to result in an increase in exposure to the herbicide when it is also present. Since Evans et al. (2010) revealed that 30 min in a container where half the bottom was treated with this same glyphosate formulation was sufficient to reduce survival over a subsequent 30 d period, encountering herbicide along with these predator cues is likely to enhance mortality.

These results suggest that a commercially formulated glyphosate-based herbicide and predator cues collectively change the movement of an important arthropod predator. Changes in *P. milvina*'s behavior induced by these chemical stressors might have important impacts at both the population and community levels. For example, *P. milvina* is a vagile species able to track habitat quality (e.g. prey abundance) and colonize areas accordingly (Walker et al., 1999; Marshall et al., 2000). Therefore, any factors that lead to changes in movement in this species could impact its ability to forage on insect prey as well as colonize new habitat patches.

Ultimately, these results contribute to growing knowledge of how anthropogenic and natural chemical cues might interact to mediate behavior in an understudied terrestrial system. Additionally, the results of these experiments suggest directions for further research. In this experiment, our application rate was higher than that which would likely be found in a real situation unless the herbicide was improperly applied e.g. spray rate was not properly controlled (Akihiro et al., 2008), or where pooling occurred in lower areas of the field. We used this higher than normal application rate to ensure complete coverage of the substrate in the experiment and to look for initial effects as this is the first study to address how *P. milvina* responds to predator cues in the presence of herbicide. Now that we have evidence that high levels of herbicide can influence the way that *P. milvina* responds to predator cues, we can use more realistic levels in further experiments to find the lowest levels that still have an effect. Furthermore, some interesting questions are raised for additional studies on animals dealing with chemical stressors in terrestrial systems: what mechanisms lead to different effects of anthropogenic chemicals on an animal's responses to different predators? And do these chemicals change an animal's reactions to natural chemical cues other than those from predators? (e.g. prey cues, or cues from conspecifics)

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