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Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival

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Abstract Humans commonly apply chemicals to manage agroecosystems. If those chemicals influence the behaviour or survival of non-target arthropods, the food web could be altered in unintended ways. Glyphosate-based herbicides are among the most ubiquitous pesticides used around the world, yet little is known about if and how they might affect the success of terrestrial predatory arthropods in agroecosystems. In this study, we quantified the effects of a commercial formulation of a glyphosate-based herbicide on the activity of three predatory arthropod species that inhabit agricultural fields in the eastern United States. We also measured the survival of the most common species. We tested the reactions of the wolf spider, *Pardosa milvina*, to either direct application (topical) or contact with a treated substrate (residual). We quantified the reactions of a larger wolf spider, *Hogna helluo*, and a ground beetle, *Scarites quadriceps*, to a compound (topical plus residual) exposure. *Pardosa milvina* reduced locomotion time and distance under topical herbicide exposure, but increased speed and non-locomotory activity time on exposed substrate. Both *H. helluo* and *S. quadriceps* increased non-locomotory activity time under compound herbicide exposure. Over a period of 60 days post-exposure, residually exposed *P. milvina* exhibited lower survivorship compared to topically exposed and control groups. Thus, exposure of terrestrial arthropods to glyphosate-based herbicides affects their behaviour and long-term survival. These results suggest that herbicides can affect arthropod community dynamics separate from their impact on the plant community and may influence biological control in agroecosystems.

Keywords Glyphosate · Herbicide · Agroecosystem · Activity · Survival · Wolf spider · Biocontrol · Ground beetle

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Introduction

Glyphosate-based herbicides are the most commonly used herbicides worldwide, largely because they are considered effective at killing weeds, safe to users, and viewed to be minimally harmful to the environment (Pan 1996; Baylis 2000; Woodburn 2000; Lundgren et al. 2009). Thus far, the few existing studies on terrestrial arthropods reveal few lethal effects of exposure to these herbicides (Bramble et al. 1997; Giesy et al. 2000; Haughton et al. 2001a; Lindsay and French 2004; Michalková and Pekár 2009; Benamú et al. 2010). However, the increasingly heavy use of these herbicides, facilitated by the introduction of genetically modified resistant crop plants, has contributed to the decline of agrobiont arthropod diversity in recent decades (Cane and Tepedino 2001; Benton et al. 2003; Thorbek and Bilde 2004). Early studies suggest that herbicide application drives changes in arthropod population dynamics via changes in vegetation structure (Brust 1990; Haughton et al. 1999, 2001b, 2003; Bell et al. 2002; Roy et al. 2003; Jackson and Pitre 2004) and availability of alternative prey (Asteraki et al. 1992).

With the widespread and frequent application of glyphosate-based herbicides, it is unlikely that many inhabitants of agroecosystems are able to escape some sort of exposure; if animals are not sprayed directly during application, they are likely to end up contacting a sprayed surface shortly thereafter. Evidence is emerging that contact with these herbicides affects a variety of aspects of the biology of the predatory arthropods in these managed habitats. For example, recent studies have found detrimental effects of exposure to a glyphosate formulation on the development and reproduction of both the predatory lacewing, *Chrysoperla externa* (Neuroptera, Chrysopidae), (Schneider et al. 2009) and the web spider, *Alpaida veniliae* (Araneae, Araniedae) (Benamú et al. 2010); both species are common in South American agroecosystems. In contrast, in a study of the effects of the residues of a glyphosate herbicide on the predatory, defensive, locomotory, and reproductive behaviour of spiders and beetles common in European agroecosystems, the only response documented was slight shift in beetle activity (Michalková and Pekár 2009). Thus, although there are some potentially important sublethal impacts of these herbicides, not surprisingly their impact seems to vary with exposure modality and the species studied. Nevertheless, all of these authors suggest that further study is required of a broader array of responses in a larger number of species before predictions can be made regarding how the repeated application of glyphosate-based herbicides might be affecting the arthropod community structure and its role in the food web (Schneider et al. 2009; Michalková and Pekár 2009; Benamú et al. 2010).

Here we report the effects of exposure to a commercial formulation of glyphosate-based herbicide (Buccaneer Plus©, Monsanto, St. Louis, MO) on three of the most abundant predatory arthropods in agroecosystems of eastern North America. We selected one diurnal wolf spider species (Lycosidae) and explored the impact of topical (being hit directly by a droplet of herbicide) versus residual (walking in an area recently sprayed with herbicide) herbicide exposure on its activity and long-term survival. We then selected two primarily nocturnal species, one larger wolf spider (Lycosidae) and one beetle (Carabidae), and documented any shifts in their activity in response to a compound (simultaneous topical and residual) herbicide exposure. We tested the hypotheses that all exposure pathways would elicit changes in the behaviour of each of these species, as well as affect the long-term survival rate of the diurnal species.

Study species

The small wolf spider *Pardosa milvina* (Araneae: Lycosidae), weighing in at approximately 20 mg, is the numerically dominant epigeal predator in agroecosystems of eastern North America, reaching densities as high as 100 individuals/m^2 in soybean fields in southwest Ohio (Marshall and Rypstra 1999; Marshall et al. 2000, 2002). The chemical sensitivity of this species has been well-documented; it can glean sophisticated information regarding mate quality and the specific nature of predation risk from air- and substrate-borne chemicals (Searcy et al. 1999; Persons et al. 2001; Rypstra et al. 2003; Schonewolf et al. 2006). Thus, we predicted that this species would easily detect and react to contact with the herbicide by altering its activity. We also explored if and through what exposure modality herbicide might impact survival in this species.

Two other common epigeal predatory arthropods co-occur with *Pardosa milvina* across the growing season: the large wolf spider *Hogna helluo* (Araneae: Lycosidae) (approximately 400 mg) and the ground beetle *Scarites quadriceps* (Coleoptera: Carabidae) (approximately 400 mg). Both of these species maintain densities of approximately 1–2 individuals/m^2. Compared to *P. milvina*, less is known about the chemical awareness of *H. helluo* and *S. quadriceps*, but there is documented evidence of chemically mediated predator/prey detection in many lycosids and carabids (Kielty et al. 1996; Persons and Rypstra 2000; Eiben and Persons 2007). Therefore, we predicted that that these larger predatory arthropods would also be able to detect herbicide and, in so doing, would alter their activity upon exposure.

Methods

Collection and maintenance

We collected all specimens from corn and soybean fields at the Miami University Ecology Research Center (Oxford, Butler County, Ohio, USA). Adult female *Pardosa milvina* were collected during May 2005 and all experiments with them were completed by 15 June 2005. We collected and ran experiments with adult *Scarites quadriceps* between March and June 2007, and with adult female *Hogna helluo* between September 2007 and January 2008. When not involved in experimentation, animals were housed individually in cylindrical plastic containers with a mixture of moist potting soil and peat moss in a 2–3 cm layer on the bottom. The containers in which we housed *P. milvina* and *S. quadriceps* were 8 cm in diameter with 5 cm walls and those for *H. helluo* were 12 cm in diameter with 8 cm walls. When in the laboratory, all animals were maintained in an environmental chamber at 24°C on a 13:11 light:dark cycle. We fed *S. quadriceps* and *H. helluo* two crickets (*Acheta domesticus*), each approximately 0.6 cm in length, and *P. milvina* two smaller crickets (0.3 cm in length) per week. Prior to each trial with *P. milvina*, we provided the spider with four crickets and allowed it to feed for 24 h, after which we removed any remaining prey items and held the spider with access to water but no additional food for 5 days. *Scarites quadriceps* and *H. helluo* were fed 48 h prior to trials.

Herbicide preparation

In all experiments, we used a commercially formulated herbicide solution (Buccaneer Plus©) containing 41% (480 g/l) glyphosate (*N*-(phosphonomethyl)glycine) isopropylamine salt and 59% other ingredients, including a polyethoxylated tallowamine (POEA) surfactant. The recommended volume of formulated solution for field application is 2.34–4.68 l/ha, which equals 0.23–0.46 ml/m^2. It is recommended that this volume be applied in a diluted solution of approximately 93.54–374.16 l/ha, which equates to a concentration range of 0.625–5%. For all experiments, we diluted the commercial formulation with distilled water to a concentration of 2.5% (12 g/l of the glyphosate salt), as would be sprayed most commonly in agricultural fields. At this concentration, a field application rate of 9.35–18.7 ml/m^2 is recommended.

Behavioural assay

We quantified the effects of herbicide on the activity of all three species in cylindrical plastic arenas with filter paper covering the bottom. In all cases, we placed the arena under a video camera in an isolated booth, which was connected to an automated digital-data collection system in a nearby laboratory room (Videomex-V, Columbus Instruments, Columbus, Ohio, USA). At the beginning of a session, we sequestered the subject under a glass vial in the center of the arena and allowed it to acclimate. After the acclimation period, we lifted the vial to release the subject and activated the video system to monitor the arena for 30 min. This system allowed us to quantify the following features of the specimen's activity: (a) total distance travelled during the session, (b) time spent in locomotory activity (movement that required a shift of the image of at least one body length), (c) time spent in non-locomotory activity (movement of the legs or rotation of the body that changed the shape of the image but did not displace it on the screen), and (d) mean speed (distance divided by locomotory time). For trials where the arena was subdivided into regions, these measures were quantified by region. All arenas and vials were cleaned, wiped with ethanol, and allowed to dry before each use. We discarded all filter paper after one use. No animal participated in more than one trial.

Pardosa milvina: residual exposure

We monitored the activity of 30 randomly selected female *Pardosa milvina* in plastic cylindrical arenas, 20 cm in diameter with 8 cm walls. Two semicircles of filter paper, one sprayed with 1.5 ml distilled water and the other with 1.5 ml field-prepared herbicide (equivalent to 95.54 ml/m^2), covered the floor of the arena. Preliminary trails revealed that 1.5 ml was the minimum amount of liquid we could reliably spray to ensure full coverage of the filter paper. We left a 1 cm gap between the two regions, made slightly wider in the center, to allow us to introduce the spider in a neutral area (see Persons et al. 2001 for diagram). The paper was placed in the arena immediately after spraying and just prior to introducing the spider. We sequestered the spider under a 3 cm diameter glass vial in the center of the arena for a 15 min acclimation period before release. The data collection system quantified the activity of the spider for the subsequent 30 min but summarized the data for the herbicide and water areas separately.

We verified that the amount of time that the spiders occupied each half of the arena was normally distributed using the Wilk-Shapiro normality statistic, and compared the time spent on control area to the time spent on the herbicide treated area using a paired *T*-test. Because differences in residence time could drive differences in time spent in other activities, we calculated the proportions of the total time that the spider spent engaged in locomotory or non-locomotory activity on each half of the arena. We transformed these proportions by taking the arcsine of the square root to achieve normality (Neter et al. 1985), and compared values for the control side to herbicide treated side using paired *T*-tests. We also compared the mean speed of the spider in each section of the arena with a paired *T*-test. All these analyses were conducted in JMP 7.0.

Pardosa milvina: topical exposure

To control for individual reactions to handling, we quantified the activity of each spider twice; once before and once after exposure to either distilled water ($n = 15$) or field-prepared herbicide ($n = 30$). In all cases we covered the bottom of the arena with one clean dry piece of filter paper and allowed the spider a 15 min acclimation period before release. After its initial run, we anesthetized each spider using CO_2 and applied a 0.5 µl droplet of either field-prepared herbicide solution or distilled water to the dorsal surface of the abdomen. We allowed the spider 15 min to fully recover from anaesthesia. We then returned it to the center of the arena, where it was held under the vial for an additional 15 min before release. We used MANOVA with repeated measures as available in JMP 7.0

to evaluate the effects of anesthetization and handling (time effect) and to determine if the application of the herbicide solution affected the distance travelled, time spent in locomotory or non-locomotory activity, and mean speed differently than the application of distilled water (time × treatment interaction).

Pardosa milvina: survival

Each *P. milvina* from both the residual and topical experiments was returned to its respective container and maintained under standard laboratory conditions for 60 days after its trial. At the twice weekly feeding times, we recorded whether the spider was alive or not. We compared survival of the spiders exposed to herbicide via walking on exposed substrate (residual exposure), spiders exposed directly to distilled water (topical exposure controls) and spiders exposed directly to herbicide (topical exposure treated) using a Kaplan–Meier product limit estimator as available in JMP 7.0.

Scarites quadriceps and *Hogna helluo*: compound exposure

We monitored the activity of these larger species in cylindrical plastic arenas 24 cm in diameter with 10 cm walls. To provide a flat surface, we covered the bottom of the arenas with a layer of Plaster of Paris. We controlled for individual responses to handling by quantifying activity of each animal twice; control animals were exposed to distilled water in two consecutive trials, whereas treatment animals were exposed to distilled water in the first trial and herbicide in the second trial ($n = 12$ per treatment for *H. helluo*; $n = 11$ per treatment for *S. quadriceps*). Prior to its initial trial, we placed an animal in a 3 cm diameter glass vial, whereupon we applied a 2 μl droplet of distilled water via micropipette to the dorsal surface of the abdomen (*H. helluo*) or thorax-abdomen juncture (*S. quadriceps*). These species were large and slow enough that we did not have to anesthetize them to apply the treatment as we had done with *P. milvina*. We then applied 5 ml of distilled water to the filter paper using an airbrush (the amount determined to completely cover the area). We placed the animal in the center of the arena under a vial for a 10 min acclimation period, released it, and recorded its activity for 30 min. Next, we removed the subject from the arena, returned it to its vial, and cleaned the arena. We then repeated the procedure, exposing the same individual to either distilled water (control) or field-prepared herbicide solution (treatment). We used MANOVA with repeated measures as available in JMP 7.0 to evaluate the effects of handling the animal a second time (time effect) and to determine if the application of the herbicide solution affected the distance travelled, time spent in locomotory or non-locomotory activity, or mean speed differently than the application of distilled water (time × treatment interaction).

Results

Pardosa milvina: residual exposure

Pardosa milvina spent approximately 30% less time on the herbicide treated side of the arena than on the side to which only distilled water had been applied (Table 1). Interestingly, *P. milvina* spent a lower proportion of time in locomotory activity and covered less distance but moved significantly faster on the herbicide as compared to the water treated side of the arena (Table 1). The proportion of time spent in non-locomotory activity (movement that did not lead to a change in position) was not significantly different at the 0.05 level, but there was a tendency for the spiders to engage in this activity more often on the herbicide side of the arena (Table 1).

Pardosa milvina: topical exposure

Overall, handling of *P. milvina* (anesthetization and application of either water or herbicide) caused spiders to move shorter distances, spend less time in locomotory activity, and more time in non-locomotory activity, but had no effect on speed (Tables 2, 3; Fig. 1). Most of the reduction in distance and locomotory time was due to shifts in spiders treated with herbicide; exposure to herbicide reduced the distance travelled and time in locomotory activity by about one-third, whereas essentially no shift in these metrics was observed for water-treated spiders (Tables 2, 3; Fig. 1a, b). Exposure to herbicide did not affect the time spent in non-locomotory activity differently than exposure to water, and neither treatment had any impact on mean speed (Tables 2, 3; Fig. 1c).

Pardosa milvina: survival

There was a significant treatment effect on survival over the 60 days post-exposure ($\chi^2 = 10.90$, $p = 0.004$). Spiders exposed topically to herbicide or water survived equally as well (Fig. 2). However, spiders in the residual exposure study died at a higher rate, even though only half the arena was treated with herbicide and each spider was in the arena for only 30 min (Fig. 2).

Hogna helluo: compound exposure

From the first to the second trials, the mean speed of *H. helluo* increased, but no other activity metric changed

Table 1 Activity metrics recorded (mean ± SE) for *Pardosa milvina* ($n = 30$) in a divided arena with distilled water applied to one side and herbicide applied to the other side

Behaviour	Water side	Herbicide side	$\|T\|$	p
Time spent in region (s)	1047.2 ± 60.3	753.2 ± 60.1	2.44	0.021
Total distance travelled (cm)	596.8 ± 100.9	549.4 ± 93.7	2.36	0.025
Proportion of time spent in locomotory activity	0.57 ± 0.03	0.43 ± 0.03	2.80	0.009
Proportion of time in non-locomotory activity	0.40 ± 0.04	0.60 ± 0.05	1.77	0.088
Speed (cm/s)	0.59 ± 0.10	0.92 ± 0.19	2.20	0.036

Mean (±SE) are given for two-sided paired T-test conducted on transformed data

Table 2 Mean (±SE) for activity metrics quantified before and after exposure to water or herbicide of three species of predatory arthropods

	Water			Herbicide		
	n	Before	After	n	Before	After
Pardosa milvina	15			30		
Distance travelled (cm)		1462.2 ± 205.4	1361.7 ± 276.3		1524.0 ± 217.2	1006.6 ± 173.2
Locomotory time (s)		475.7 ± 39.9	444.7 ± 28.2		564.7 ± 38.7	394.0 ± 39.4
Non-locomotory time (s)		437.5 ± 53.3	573.3 ± 40.3		499.4 ± 25.0	645.0 ± 41.6
Speed (cm/s)		3.3 ± 0.5	3.1 ± 0.6		3.0 ± 0.3	3.0 ± 0.3
Hogna helluo	12			12		
Distance travelled (cm)		1349.2 ± 416.5	1697.8 ± 454.7		1482.2 ± 414.5	1648.3 ± 398.2
Locomotory time (s)		445.3 ± 92.6	538.7 ± 83.7		560.3 ± 121.6	611.7 ± 108.0
Non-locomotory time (s)		801.6 ± 63.2	644.1 ± 55.6		722.8 ± 71.2	897.6 ± 88.8
Speed (cm/s)		2.5 ± 0.3	2.9 ± 0.4		2.3 ± 0.3	2.5 ± 0.3
Scarites quadriceps	11			11		
Distance travelled (cm)		3314.0 ± 648.1	3490.1 ± 650.3		3264.3 ± 706.8	3675.9 ± 830.1
Locomotory time (s)		1222.2 ± 122.1	1290.5 ± 127.6		1002.5 ± 138.8	1110.5 ± 135.5
Non-locomotory time (s)		193.8 ± 31.0	199.0 ± 40.0		151.8 ± 27.7	362.3 ± 80.5
Speed (cm/s)		2.7 ± 0.3	2.5 ± 0.3		3.2 ± 0.4	3.0 ± 0.4

significantly (Tables 2, 3). Similarly, herbicide had no differential effects on distance travelled, time in locomotory activity, or mean speed as compared to distilled water for this species (Tables 2, 3; Fig. 1d, e). However, herbicide and water had contrasting effects on non-locomotory activity; *H. helluo* exposed to distilled water spent less time in non-locomotory activity, whereas those exposed to herbicide spent more time in non-locomotory activity (Tables 2, 3; Fig. 1f).

Scarites quadriceps: compound exposure

From the first to the second trial, *S. quadriceps* exhibited no significant change in distance travelled, time spent in locomotory activity, or mean speed (Tables 2, 3). Likewise, herbicide exposure produced no differences in these locomotory measures when compared to distilled water (Tables 2, 3; Fig. 1 g, h). However, there was a significant overall increase in the time spent in non-locomotory activity in the second trial, which was primarily due to an increase in the time that beetles exposed to herbicide spent in non-locomotory activity (Tables 2, 3; Fig. 1i).

Discussion

All three of the agrobiont predatory arthropod species we tested displayed some activity shifts in response to herbicide. *Pardosa milvina* avoided surfaces where herbicide had been applied when they could, and moved less when in contact with the herbicide (Table 1; Fig. 1). Both of the larger predators, *Hogna helluo* and *Scarites quadriceps*, increased the amount of time spent in non-locomotory activity after a combined topical and residual exposure (Fig. 1). This reaction verifies that they detected the herbicide and perhaps were rotating, pivoting in place or preening more frequently. To the extent that these behavioural shifts may take away from the normal activities of these three predators, such as foraging or searching for mates, their success in an agroecosystem could be altered

Table 3 Results of repeated measures MANOVA for activity metrics quantified including the effects of time (i.e. simple handling effects) and time × treatment (differential effects of water or herbicide)

	Time			Time × treatment		
	df	*F*	*p*	df	*F*	*p*
Pardosa milvina	1,44			1,44		
Distance travelled (cm)		10.01	0.002		4.94	0.032
Locomotory time (s)		10.01	0.003		4.79	0.034
Non-locomotory time (s)		10.43	0.002		0.01	0.911
Speed (cm/s)		0.32	0.573		0.5	0.484
Hogna helluo	1,22			1,22		
Distance travelled (cm)		2.57	0.123		0.32	0.575
Locomotory time (s)		1.98	0.173		0.17	0.687
Non-locomotory time (s)		0.02	0.901		5.80	0.025
Speed (cm/s)		4.41	0.048		0.49	0.493
Scarites quadriceps	1,20			1,20		
Distance travelled (cm)		0.19	0.288		0.19	0.666
Locomotory time (s)		0.09	0.205		0.09	0.772
Non-locomotory time (s)		5.53	0.029		5.53	0.029
Speed (cm/s)		2.29	0.147		0.06	0.816

and, if so, herbicide could have subtle unintended effects on the agricultural food web.

We also found that a brief residual herbicide exposure depressed *Pardosa milvina* survivorship over a period of 60 days post-exposure, while topical exposure did not (Fig. 2). To our knowledge, these findings represent the first evidence for direct effects of field-prepared glyphosate-based herbicide exposure on the survival of a terrestrial arthropod over an ecologically long time frame. Importantly, our findings demonstrate that the lethal effects of exposure to this herbicide vary based on the mode of the exposure. In our topical exposure trials, herbicide contacted only the dorsal abdominal surface, and the droplet tended to bead up rather than spread across the cuticle even though the formulation included a surfactant effective in dispersing the agent on plant leaves. However, walking on a surface coated with herbicide may have been more likely to result in its entering the spider's digestive or respiratory system and ultimately having a toxic effect. The spider may have ingested herbicide if it applied its mouthparts to the damp surface to drink or if it preened its tarsi in an attempt to remove the strange chemical. Additionally, the ventral surface of the spider, where the book lungs are located, may come in contact with a treated surface and allowed the herbicide to enter the body. Either of these pathways would put the herbicide in contact with epithelial surfaces, which are susceptible to damage by glyphosate-based herbicides in amphibians (Edginton et al. 2004). Even though further study is required to understand the precise mechanism that produced the survival patterns we documented here, our results suggest that those studies will need to pay attention both to the mode of exposure and the time frame over which the animals are monitored in order to reveal ecologically relevant patterns.

We observed a general decrease in locomotory activity by *Pardosa milvina* upon both residual and topical exposure to herbicide, but an increase in speed on herbicide treated surfaces and an increase in non-locomotory activity after topical treatment. This reduced locomotory activity could drive increased residence time in herbicide patches, which our survival results suggest would negatively affect long-term survival. However, the increased speed over substrates laden with herbicide could allow *P. milvina* to spend less time on the contaminated substrate and thus minimize their exposure and their risk. In the presence of predator information, *P. milvina* reduce their speed and spend more time immobile and in non-locomotory activity (i.e. spend a smaller proportion of residence time in locomotion) and these behavioural shifts translate directly into longer survival in the presence of an actual predator (Persons et al. 2001). Thus, the shift in activity in response to herbicide could affect how susceptible they are to predation by sit-and-wait predators. Thus, future research should explore the mutual effects of herbicide on predators and prey in order to determine how these animals might balance short term (i.e. avoiding predation) versus long term (i.e. toxicity of the herbicide) survival risks.

Of the activities we monitored in our experiments with *H. helluo* and *S. quadriceps*, only the time spent in non-locomotory activity was affected by herbicide treatment; both species spent more time in non-locomotory activity upon exposure to herbicide than when treated with water. With these larger arthropods, leg/antenna displacement due to preening was difficult to quantify via our automated tracking system; thus the majority of non-locomotory activity we observed should be attributed to pivotal movement. We do not know exactly how this activity might relate to the normal functioning of these species and further investigation is required to ascertain if and how this shift in behaviour influences the success of these animals in the field.

Although our field-prepared herbicide solution concentration, at 2.5% commercial formulation by volume, was well within the calculated range based on manufacturer recommendations for control of annual weeds in agricultural fields (Buccaneer Plus® label), our application rates per unit area were higher than recommended because of our priority to completely cover filter paper surfaces and thus disallow unexposed refugia. Nevertheless, the high water solubility, veritable ubiquity in North American streams (Kolpin et al. 2006), and variable deposition across agricultural fields (Feng et al. 1990) of glyphosate-based herbicide formulations suggest a potential for exposure to

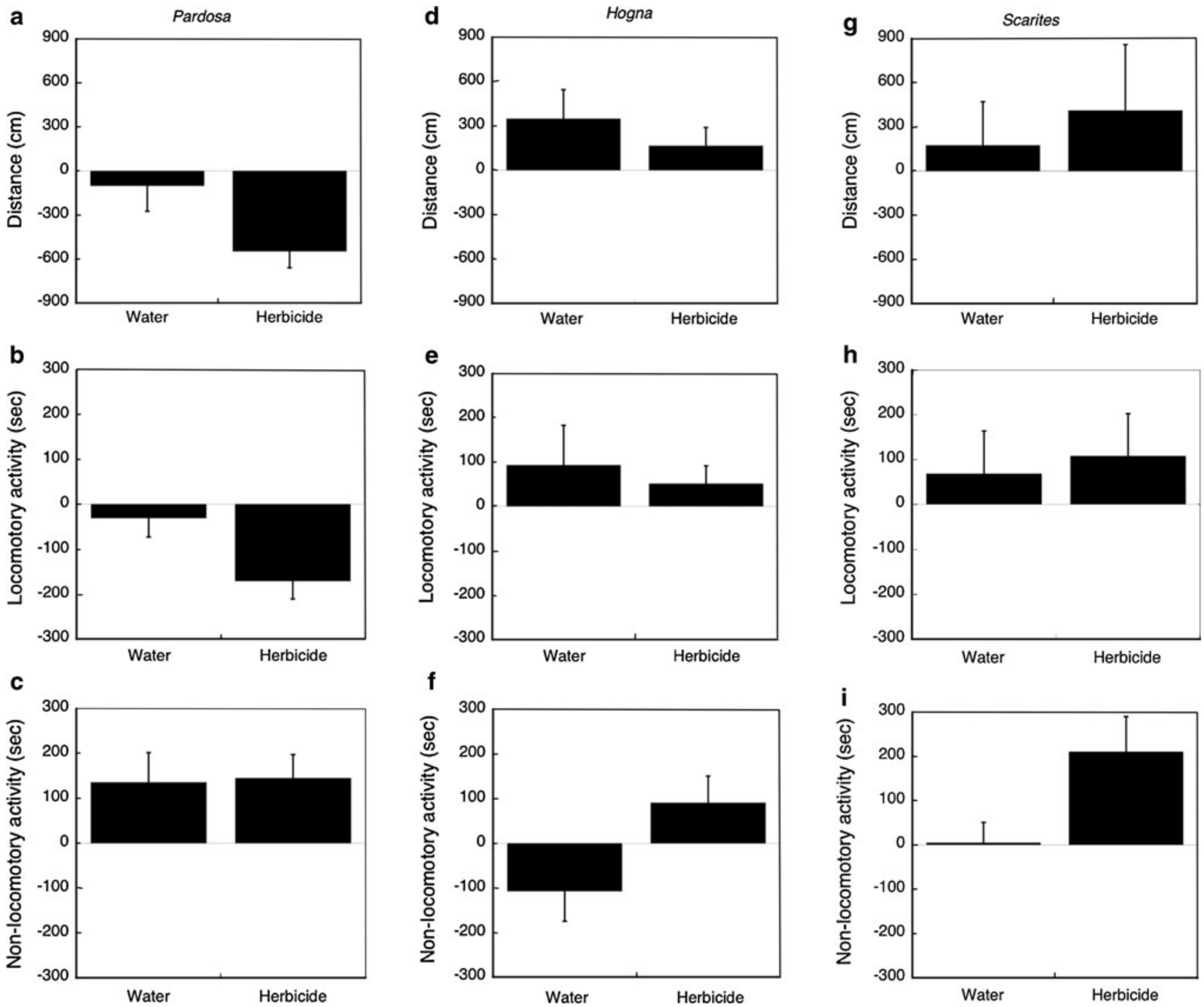

Fig. 1 Shifts in activity levels of animals exposed to either distilled water or herbicide (after exposure–before exposure) including the total distance travelled, time spent in locomotory activity (movement that displaced the animal more than a body length) and time spent in non-locomotory activity (rotating or shifts of position that did not displace the animal). **a–c** *Pardosa milvina* exposed topically to either water or herbicide in between trials. **d–f** *Hogna helluo* and **g–i** *Scarites quadriceps*, both of which experienced a compound exposure to either water or herbicide in the second trial (see text for full description)

volumes above label recommendations. Now that we have identified specific behavioural responses to the herbicide at this level, an exploration of the effects of different doses, varying both by concentration and application rate, is in order.

We elected to use a common commercial formulation of a glyphosate-based herbicide because we were ultimately interested in understanding how these spray events impact the ecology of the animals living in the fields. Glyphosate itself has a strong propensity for adsorption to soil components, metabolism by bacteria, and/or inactivation by inorganic components (Rueppel et al. 1977). Despite these properties, short-term collections of glyphosate-based herbicides in saturated soil or moist plant litter may still provide enough exposure to affect behaviour and survival particularly when POEA surfactants are included in the formulation (Wang et al. 2005). In fact, these surfactants are often more toxic to organisms than the glyphosate itself (Adams et al. 1997; Perkins et al. 2000; Edginton et al. 2004; Howe et al. 2004; Bringolf et al. 2007), and they vary widely in chemical nature and concentrations among commercial herbicide formulations (Pan 1996). Obviously, we do not know the relative roles of the various components included in the formulation in eliciting our results. Our work is a first step to understanding how herbicides might impact the agricultural ecosystem in ways other than through the plants they are intended to kill.

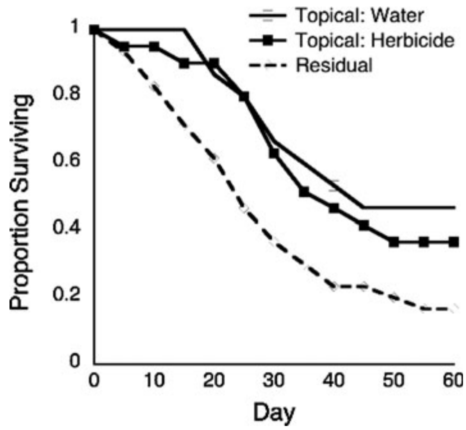

Fig. 2 Proportion of *Pardosa milvina* surviving over a 60-day period after experimentation. Spiders exposed to surfaces with herbicide applied (residual exposure) exhibited significantly lower survival than both the control and topically exposed spiders

Our findings contribute to the growing body of work demonstrating that the arthropod predators inhabiting agroecosystems around the world exhibit subtle shifts in behaviour and reproduction during or after exposure to herbicide (Schneider et al. 2009; Michalková and Pekár 2009; Benamú et al. 2010). These studies have implications for arthropod community dynamics, and ultimately biological control, in agroecosystems. Shifts in locomotion and associated behaviours in predatory arthropods may drive changes in colonization rates that affect predator population dynamics, foraging success and reproductive rates. These changes may in turn lead to changes in the community structure of these predators, altering their collective ability to exert top-down effects on herbivorous pest communities. A better understanding of agroecosystem community dynamics as affected by direct exposure to glyphosate-based herbicides requires future comparative research on effects on behaviour and survival among carabids, lycosids, and their common prey groups.

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