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Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems

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ABSTRACT

Chemical communication is important to many arthropod species but the potential exists for anthropogenic chemicals to disrupt information flow. Although glyphosate-based herbicides are not acutely toxic to arthropods, little is known regarding their effects on natural chemical communication pathways. The wolf spider, *Pardosa milvina*, is abundant in agroecosystems where herbicides are regularly applied and uses air- and substrate-borne chemical signals extensively during mating. The aim of this study was to examine effects of a commercial formulation of a glyphosate-based herbicide on the ability of males to find females. In the field, virgin females, when hidden inside pitfall traps with herbicide, attracted fewer males than females with water. Likewise females in traps with a ring of herbicide surrounding the opening were less likely to attract males than those in traps surrounded by water. We explored the reaction of males to any airborne component of the herbicide in a laboratory two-choice olfactometer experiment. When no female pheromones were present, males were equally likely to select herbicide or water treated corridors and they all moved through the apparatus at similar speeds. When female pheromones were present, the males that selected control corridors moved more slowly than those that selected herbicide and, if we control for the initial decision time, more males selected the control corridors over the herbicide. These data suggest that glyphosate-based herbicides are “info-disruptors” that alter the ability of males to detect and/or react fully to female signals.

1. Introduction

Many organisms, including most arthropods, use chemical messengers to mediate their interactions with the environment (Dicke and Takken, 2006; Hoffmann et al., 2006; Symonds and Elgar, 2008). These infochemicals can be critical to setting a developmental trajectory, avoiding predation, finding food, identifying social groups, and assessing mates (Dicke and Takken, 2006; Symonds and Elgar, 2008). Recently attention has been focused on the potential for the substances that humans release into natural systems to act as “info-disruptors” and interfere with the ability of animals to accurately detect, interpret, and respond to their chemical environment (Lurling and Scheffer, 2007; Klaschka, 2008, 2009). Although heretofore little attention has been paid to this potential side effect of anthropogenic compounds, evidence is emerging that the phenomenon is widespread in both aquatic and terrestrial systems (Zhou et al., 2005; Desneux et al., 2007; Lurling and Scheffer, 2007; Polnert et al., 2007; Cook and Moore, 2008; Klaschka, 2008).

Herbicides with glyphosate as their active ingredient are routinely applied to gardens and agricultural systems worldwide (Pan, 1996; Baylis, 2000; Woodburn, 2000). While glyphosate itself is regarded as safe due to its low lethal toxicity to animals and low rates of runoff (Rueppel et al., 1977; Smith and Oehme, 1992; Giesy et al., 2000), some commercial formulations, especially those with polyethoxylated tallowamine (POEA) surfactants, have much more damaging impacts (Edginton et al., 2004; Howe et al., 2004; Wang et al., 2005; Bringolf et al., 2007). Although glyphosate-based herbicide formulations have strong effects on zooplankton and amphibians (Chen et al., 2004; Howe et al., 2004; Relyea, 2005; Battaglin et al., 2009), most studies of terrestrial arthropods have not revealed any direct effects (Bramble et al., 1997; Haughton et al., 1999, 2001a; Giesy et al., 2000; Lindsay and French, 2004). Nevertheless, recent studies show that glyphosate-based herbicides produce some behavioral shifts in wolf spiders (Araneae: Lycosidae) and ground beetles (Coleoptera: Carabidae) (Michalková and Pekár, 2009; Evans et al., 2010) and the consumption of

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exposed prey reduces the reproductive success of lacewings (Neuroptera; Chrysopidae) and orb-weaving spiders (Araneae; Araneidae) (Schneider et al., 2009; Benamú et al., 2010). Clearly there is a need for further exploration into the scope of the nonlethal effects of these herbicides on terrestrial arthropods.

The aim of this study was to determine if a commercial formulation of a glyphosate-based herbicide could be acting as an "info-disruptor" (sensu Lurling and Scheffer, 2007) and interfere with natural chemical communication in a terrestrial arthropod predator. We elected to study the diurnal wolf spider species, *Pardosa milvina* (Araneae: Lycosidae) because it is common in agricultural fields (Marshall and Rypstra, 1999; Marshall et al., 2002), and females alter their locomotion when exposed to these herbicides (Evans et al., 2010). Both male and female *P. milvina* garner sophisticated information regarding potential mates using chemical information (Searcy et al., 1999; Rypstra et al., 2003, 2009; Schonewolf et al., 2006). In particular, airborne pheromones are important in attracting males to females (Searcy et al., 1999), and cues deposited on substrates allow males to evaluate females and decide whether to commence courtship (Rypstra et al., 2003, 2009). Given the importance of chemical information to mate location and assessment in *P. milvina*, we deemed it likely that this anthropogenic substance would influence the ability of males to identify and approach females

With the studies reported herein, we tested the hypothesis that a commercial formulation of a glyphosate-based herbicide affects the response of males to female chemical signals and thus influences mate location in the wolf spider, *Pardosa milvina*. Using pitfall traps in the field, we documented the success with which males found virgin females in the presence of herbicide. Our previous study revealed that this herbicide reduced locomotory activity of *Pardosa* females (Evans et al., 2010); here we examined whether it affected the male's response to female signals by altering pheromone production or male behavior. We predicted that herbicide would either influence whether or not a female attracted a male, which would alter the probability of mating, or the number of males drawn to a particular female, which would alter the landscape for sexual selection. We also explored the relative impact of air- and substrate-borne components of the herbicide on males in the laboratory using a modified two-choice olfactometer. We reasoned that, if males detected an airborne component of the herbicide, they would choose an alternative route, especially when female pheromones were present. Whereas, if males primarily detected herbicide by direct contact, then they would approach, but not cross, a surface treated with herbicide. Any shift in the behavior of males as they search for females could impact mating opportunities, reproductive success, and the intensity of sexual selection in this species

2. Methods

2.1. Animal maintenance

Pardosa milvina were collected as juveniles from corn and soybean fields at the Ecology Research Center (ERC) of Miami University, Butler County, Oxford, OH, USA, from April through July of 2009 and 2010. Spiders were housed individually in translucent plastic cups (8 cm diameter with 5 cm walls) containing a thin layer of a 50:50 peat moss:soil mixture and fed two crickets (*Acheta domesticus*), each approximately half the size of the spider, weekly. All spiders were held in an environmentally controlled room at 25 °C with a 13L:11D photoperiod until at least one week after they molted to adulthood and, thus, we were certain that they were sexually mature virgins. No spider participated in more than one trial.

2.2. Herbicide preparation

We used a commercial formulation of the herbicide, Buccaneer® Plus, which is registered with the United States Environmental Protection Agency (US-EPA) as Roundup® II Original. This herbicide is manufactured by Monsanto, St Louis, MO, USA (United States Patent US4528023). As provided, this herbicide contains 41% (480 g L^{-1}) glyphosate (N-(phosphonomethyl)glycine) isopropylamine salt and 59% other ingredients, including a polyethoxylated tallowamine (POEA) surfactant. Monsanto recommends that this herbicide be applied in a solution diluted to a concentration between 0.625% and 5%. For the pitfall experiment, we diluted it with distilled water to a concentration of 2.5% (12 g L^{-1} of the glyphosate salt). We selected this concentration because it was the same as what had been sprayed on the agricultural fields of the ERC in recent years and had been shown to affect locomotory activity in *Pardosa* in previous studies (Evans et al., 2010). For the olfactometer experiment, we diluted it to a concentration of 1.6% (7.68 g L^{-1} of the glyphosate salt) so that we could apply the same volume of herbicide per unit area as we used in the pitfall experiment (2.4×10^{-4} mL cm^{-2}) but also fully cover the target surface evenly.

2.3. Pitfall experiment

In the first week of July 2009, we established a 5 × 5 grid of 25 pitfall traps, each 10 m from the closest neighbor, in a no-till soybean field at the ERC. Traps consisted of a translucent plastic cup (8.3 cm diameter with 14.7 cm walls) placed so that the rim was flush with the soil surface leaving no gaps between the cup and the soil. Inside each cup, we placed a mesh-capped vial (2.3 cm diameter with 3.8 cm walls) containing a circle of filter paper (2 cm diameter). We surrounded each pitfall cup with a 3 cm ring of filter paper. Each trap was shaded with a square piece of cardboard held 7–8 cm above the cup by four pieces of dowel rod.

Traps were randomly assigned to one of five treatments. Control cups contained an empty vial and filter paper; a female was sequestered in the vial inside the trap for all other treatments (as in Searcy et al., 1999). In one pair of treatments we applied 5 μL of either distilled water or herbicide solution to the filter paper inside the vial with the female. In another two treatments, we applied 0.926 mL of either distilled water or herbicide solution to the ring of filter paper surrounding the cup. Traps were established at 10:00 h and left for 7 h; females were removed at 17:00 h and the number of male *P. milvina* captured was recorded (as in Searcy et al., 1999). We randomly assigned the treatments to traps in the grid for five separate experimental runs (on July 9, 14, 16, 20 and 24 of 2009) ($n = 25$). Traps were left empty with cups removed for at least 2 d between runs.

We categorized traps as having captured zero, one, two, or three or more males. We used a three-way log-linear analysis to determine if the number of males was dependent on herbicide presence (or not) or herbicide location (inside or outside of the trap) (Sokal and Rohlf, 1995). Where there was a significant interaction, we explored differences between captures in the presence or absence of herbicide with planned contrasts.

2.4. Olfactometer experiment

We examined the potential for cues from the herbicide to interfere with the airborne pheromones of females using a two-choice olfactometer (Fig. 1). The olfactometer was constructed from Plexiglas with 5 cm walls covered with opaque white paper to eliminate visual disruptions. An entry area was open at the top so that air could escape; this was also where the males were introduced into the apparatus (Fig. 1). The entry area bifurcated into two 5 cm wide corridors with transparent colorless Plexiglas tops.

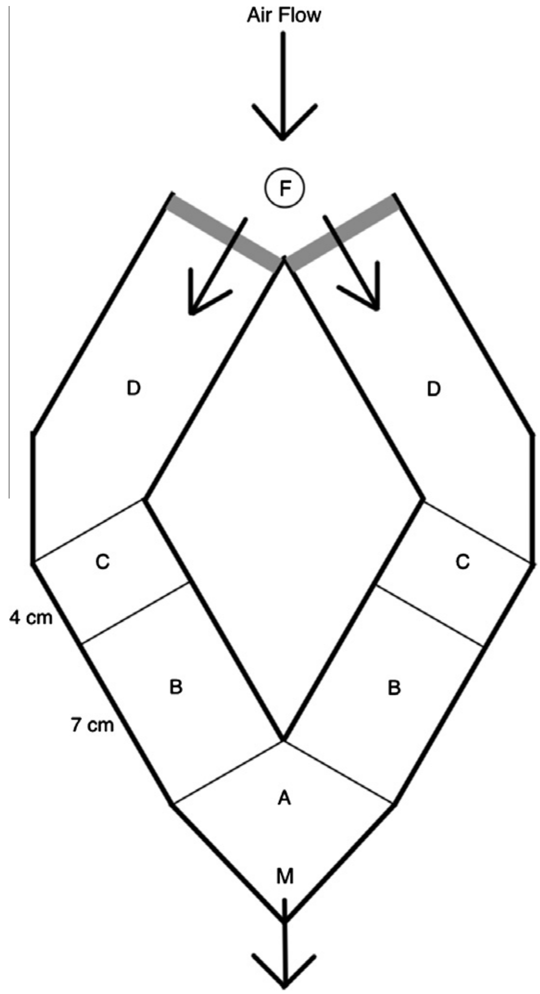

Fig. 1. Diagram (to scale) of two-choice olfactometer. Region A is the entry area, B the choice areas, C the treatment areas, and D the final sections. Females were sequestered at F and the males were introduced at M.

Approximately 4 cm beyond the ends of the corridors, we fixed a miniature fan to push air through the apparatus (Fig. 1). Scrubbing pads (Fig. 1, gray bars) were inserted in the ends of the corridors to disperse the airflow and prevent males from seeing females. We visually confirmed unidirectional airflow by observing smoke movement inside the corridors prior to any testing (Fig. 1, black arrows). Within each corridor we defined three regions (Fig. 1). The first 7 cm was the choice area, when the male entered this region it was clear that he had selected that arm of the olfactometer. The next 4 cm was a treatment area where a 4×5 cm piece of filter paper on the floor of the apparatus was treated with either herbicide or water. The final section consisted of all the space beyond the treatment area. To provide a surface with adequate traction, we covered the bottom of the olfactometer with filter paper that was replaced between each trial. In addition, we washed the olfactometer, wiped it down with ethanol, and allowed it to dry completely between each trial.

The olfactometer was located in an isolated booth in an environmentally controlled chamber. A camera mounted above the apparatus allowed us to record trials remotely from an adjacent room. Just before a trial, we used airbrushes (Hobbyist, W.R. Brown, North Chicago, IL, USA) with a very fine jet to spray two 5 cm by 4 cm filter paper rectangles so that their entire surface was damp. One piece was sprayed with 0.3 mL of 1.6% herbicide solution and the other with 0.3 mL distilled water. To prevent cross-contamination, one airbrush was used exclusively to dispense the distilled water and another for the herbicide. We placed the herbicide-treated paper in one corridor and the water treated paper in the other. The specific corridor into which treatments were placed (i.e. right or left) was randomized to account for any directional bias within the apparatus.

Males were allowed to choose between water and herbicide areas in the presence ($n = 27$) or absence ($n = 27$) of females. For treatments with females, we sequestered a mature virgin female in a cylindrical container (1.5 cm in diam.) constructed of mesh screen between the fan and the end of the apparatus (Fig. 1); an empty container was used in trials without females. We then placed a mature virgin male under a 3 cm diam. glass vial in the entry area, activated the fan, and allowed him to acclimate for 5 min before lifting the vial. We recorded the male for 20 min or until his entire body crossed into the final section.

Upon review of the videotapes, we recorded whether the male selected the herbicide or water corridor. We also recorded the time when the male reached the choice area, when he reached the treatment area, and when he entered the final section. Three males were excluded because they either made multiple choices or laid down excessive silk in the vial before they were released. We included 26 trials with females and 25 without in our analyses.

We tested for a side bias within the olfactometer using Fisher's exact test to compare whether the male went left or right with the frequency with which herbicide or water was on the left arm of the olfactometer. We also used Fisher's exact test to determine if the frequency with which males selected water or herbicide corridors was altered in the presence of a female.

The total time it took for males to reach the final section was log-transformed to reach normality and compared in a two-way ANOVA with female presence/absence and the treatment selected as factors. Initial observations revealed that males either moved into one of the corridors very quickly (i.e., in a second or two) or took several minutes to make their selection. Because this led to a strongly bimodal distribution in decision time, we categorized males as fast deciders (those that moved into a corridor within one minute) or slow deciders (those that took longer than a minute to move into a corridor). We then used a three-way log-linear analysis to evaluate the effects of a female on the frequency with which fast or slow spiders selected herbicide or water and used contrasts to determine if the choices made by fast or slow spiders were independent of whether a female was present or not (Sokal and Rohlf, 1995).

3. Results

3.1. Pitfall experiment

Between one and four males were captured in 59 of the 125 traps we set (47.2%) in the 7 h time frame that traps were open. There was a three-way interaction between the presence/absence of herbicide, whether the treatment was located inside or outside of the trap and the number of males captured (Fig. 2) ($G^2 = 24.96$, $df = 12$, $p = 0.015$). Traps with herbicide on the filter paper inside with the female captured fewer males (0.6 ± 0.2 males/trap) than those where the filter paper was treated with distilled water (1.2 ± 0.1 males/trap) ($\mathrm{X}^2 = 5.69$, $df = 1$, $p = 0.017$; Fig. 2). This difference was due to the fact that 24% of the females in traps without herbicide attracted two or more males whilst only 12% of the females housed with herbicide drew in two males and none attracted more (Fig. 2). Traps with herbicide surrounding the opening also captured fewer males (0.5 ± 0.1 males/trap) when compared to traps with only water on the filter paper ring (1.0 ± 0.1 males/trap) ($\mathrm{X}^2 = 10.83$, $df = 1$, $p = 0.001$; Fig. 2). The dif-

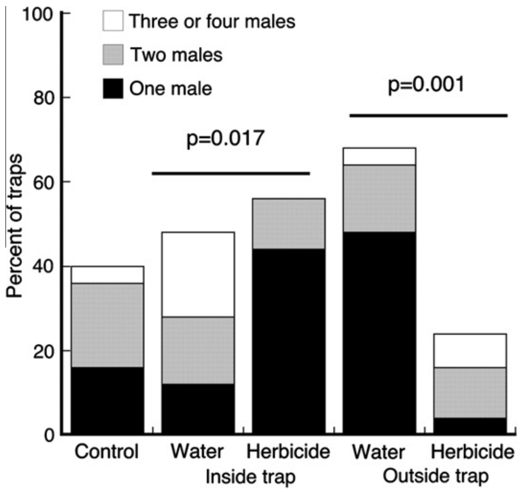

Fig. 2. Percent of pitfall traps that captured one, two, or more males across all treatments. Probabilities indicated were generated from planned contrasts comparing captures between traps with herbicide or water inside with the females, and between traps with herbicide or water surrounding them.

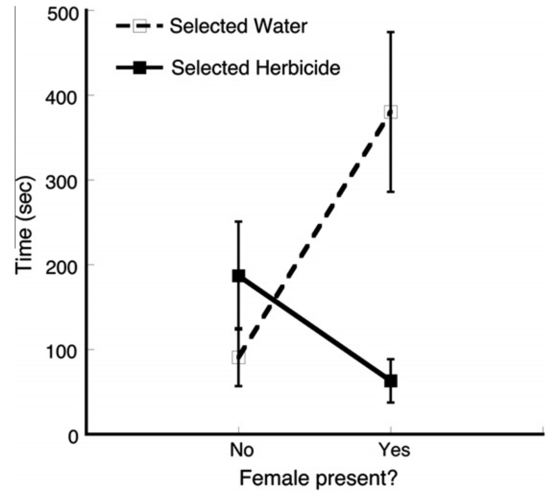

Fig. 3. Total time (s) (mean ± SE) that it took males to move from entry area to the final section of the olfactometer (from M to area D in Fig. 1) categorized by whether a female was present or not and the treatment selected.

ference between these latter two treatments was due to the number of traps that captured males; only six of the 25 traps surrounded by herbicide captured a male whereas 18 of the 25 surrounded by water were found with at least one male (Fig. 2).

3.2. Olfactometer experiment

A total of 26 males chose the water-treated corridor and 25 chose the herbicide-treated corridor. The choice of a corridor with herbicide or water was not affected by side (Fisher exact $p = 0.785$) or the presence or absence of a female (Fisher exact $p = 0.786$).

All males moved completely through the olfactometer past the treatments and into the final section. These times were normal after log transformation and the ANOVA revealed significant differences among the groups (Table 1). There was a significant interaction between the presence of female and the treatment selected on the time it took males to reach the final section (Table 1); when females were present, males that selected water took significantly longer than males that selected the herbicide corridor (Fig. 3).

Approximately half of the spiders were categorized as slow (26) and the other half as fast (25). There was a significant three-way interaction in the log linear analysis suggesting that the decision speed, the presence of females, and the selection of herbicide or water were related ($G^2 = 9.54$, $df = 4$, $p = 0.049$). The presence of females had an effect on whether or not slow males selected corridors treated with herbicide or water ($X^2 = 5.33$, $df = 1$, $p = 0.021$) but corridor selection for fast males was independent of the presence of a female ($X^2 = 0.18$, $df = 1$, $p = 0.677$) (Fig. 4). Specifically, slow males were more likely to select the water treatment when females were present (Fig. 4).

Table 1
Results of two-way ANOVA conducted on the total time (log-transformed) for males to move from the entry area to the final section of the olfactometer. Untransformed data are presented in Fig. 3.

Factor	*df*	Sums of squares	*F*-ratio	*P*
Whole model	3	808 612	5.04	0.004
Female presence/absence	1	64 444	1.21	0.278
Herbicide or water	1	146 242	2.74	0.105
Interaction	3	559 032	10.46	0.002

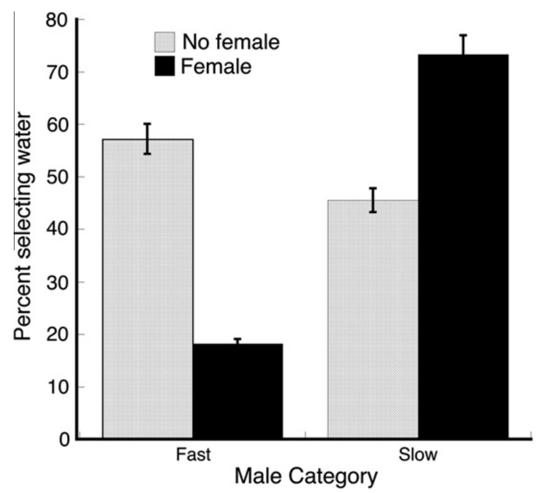

Fig. 4. Percent of males selecting the water (i.e. avoiding herbicide) categorized by their level of activity in the first 60 s in the olfactometer (fast vs. slow) and whether or not the treatment included a female.

4. Discussion

Our experiments suggest that a commercial formulation of a glyphosate-based herbicide affects mate location in a wolf spider that is common in agroecosystems where these chemicals are routinely applied. When virgin females were sequestered in pitfall traps with herbicide they attracted fewer males, while fewer traps containing females captured any males when there was an herbicide-laden surface surrounding the entrance (Fig. 2). Both of these results demonstrate that herbicide interfered in some way with the ability of males to find females and both of these results could be explained by a volatile component of the herbicide interfering with the release, reception, or perception of the airborne pheromones that females use to attract males. Our laboratory olfactometer results provided stronger support for the hypothesis of volatile info-disruption (Figs. 3,4). Thus far, our experiments do not reveal whether the herbicide might alter pheromone output, mask or dilute the pheromones, and/or alter the male's ability to detect and respond appropriately to the pheromones. Nevertheless these results and those of Evans et al. (2010) confirm that both males and females react to this common herbicide and that the timing of spraying has implications for the population success of *P. milvina*.

Male *P. milvina* clearly react to this herbicide formulation and our data suggest that an airborne component of the herbicide affected their approach to females. In pitfall treatments where the herbicide surrounded the trap, males would have received a combination of female pheromones and volatile herbicide components, but then would have had direct contact with the herbicide as they moved closer to the traps. In the olfactometer, however, males had to make a decision before contacting the herbicide and the slow males, i.e. those that took longer than one minute to move into a corridor, were more likely to select the side with no herbicide (Fig. 4). Interestingly, in treatments without females, even slow males did not discriminate between herbicide and water corridors of the olfactometer. Thus, we have no evidence that males are repulsed by the volatile herbicide components alone; rather, the herbicide seemed only to affect the males' ability to detect and/or respond to female pheromones.

The interaction between herbicide and female airborne signals can also explain the fact that females in contact with herbicide drew fewer males to their traps than the females housed with water (Fig. 2). Although this species normally slows down on moist or wet surfaces (Wrinn, 2010), here, males actively moving about the fields would encounter only dry filter paper as they came close to a cup and, presumably as a result, most of these traps captured males. However, there was a lower per trap capture, which was likely due to the same signal interference phenomenon we observed in the olfactometer experiment. On the other hand, we know that female *P. milvina* detect this herbicide formulation because they spend less time walking and cover less distance on surfaces treated with the same concentration of the same herbicide (Evans et al., 2010). Hence, it is entirely possible that females altered the production or composition of their pheromones when in contact with herbicide in a manner that influenced male captures.

The relatively high captures of males in control traps with no females to attract them and the number of fast males in our olfactometer studies are consistent with the biology of *Pardosa* species, which have been characterized as "sit and move" predators (Samu et al., 2003). Both sexes of this species are inherently very active, opportunistic colonizers (Walker et al., 1999; Marshall et al., 2000) but males in particular tend to forage less and cover larger distances than females (Walker and Rypstra, 2002, 2003). In the field, males moving widely across fields encountered no information that would cause them to be attracted to or repelled by empty control traps, whereas information emanating from all other traps would affect captures based on the males' receptivity and their ability to assess the status of females. Similarly, we suspect that the difference between fast and slow males observed in the olfactometer study was due to how they perceived the cues in the apparatus as well as their receptivity at that moment. Once receptive males detect the chemical cues of desirable females, they slow down and engage in a visual search punctuated by periodic courtship activity (Schlosser, 2005; Rypstra et al., 2009). This characteristic also explains why it took the males that selected the water corridor the longest to reach the approach areas when females were present in the olfactometer (Fig. 3).

The herbicide effects revealed by these data suggest that, at the time of spraying, there would be a reduction in mating opportunities for both sexes due to an increased difficulty in mate location, a finding that has implications for the intensity of sexual selection in *P. milvina*. The mating system of this species conforms to stereotypical sex roles of choosy females and competitive males (Emlen and Oring, 1977). Females produce eggsacs that weigh more than 30% of their body mass that they attach to their abdomen and carry; after hatching, the spiderlings ride on the female's abdomen before dispersing (Colancecco et al., 2007). Presumably because of this large parental investment, virgin females reject 30–40% of the males that court them in laboratory trials (Rypstra et al., 2003; Wilder and Rypstra, 2008). The air- and substrate-borne chemical signals from females are sufficient to attract several males that commence intense courtship activity and engage in high levels of male–male aggression as they vie for the female's attention (Rypstra et al., 2009). Since herbicide exposure seemed to reduce the ability of female signals to draw in males, it could potentially break down this choosy and competitive mating system. Specifically, if females are less likely to attract males and/or they attract fewer males, it will have the effect of shifting the operational sex ratio in such a way that some choosy individuals do not have the proper signals to attract a mate they deem suitable and may remain unmated. This situation could give females that were comparatively indiscriminant in mate choice a reproductive advantage, regardless of their quality. Thus, herbicide, if sprayed at a critical time in the *P. milvina* mating period, may be a selective agent that reduces the importance of male signaling and female choice in the mating system of *P. milvina* and ultimately relaxes the intensity of sexual selection for populations living in agroecosystems (Kokko and Jennions, 2008).

The effects of glyphosate-based herbicides on the behavior of predatory arthropods reported thus far are small and likely to be short-lived. By itself, the info-disruption uncovered here probably has a minor impact on population and communities of spiders in the fields, especially when compared to the changes caused by the dramatic shifts in the plant communities that result from herbicide application (Haughton et al., 1999, 2001b). However, we know little about the full range of impacts imposed by a pulse of herbicide on the life history and behavior and what their collective influence may be on population density or agricultural production. Here we have evidence that reproduction may be compromised when either males or females of *P. milvina* are exposed to herbicide due to interference with chemical signaling. Additionally, merely walking on an herbicide-treated surface reduced survival of *P. milvina* females over a long (60 d) period (Evans et al., 2010) and reduced the viability of *P. milvina* eggsacs (Wrinn, 2010). If we start to add these effects together and consider that genetically modified crops enable farmers to spray the same herbicide on their fields multiple times in a season, glyphosate-based herbicides may be a much more important selective factor on *P. milvina* populations than is typically assumed. In addition, there is strong evidence to suggest that generalist predators can exert significant top-down control and increase plant production, especially in agricultural ecosystems (Halaj and Wise, 2003). Even small epigeal spiders, including *P. milvina*, can have significant effects on herbivory experienced by economically important plants (Hlivko and Rypstra, 2003; Birkhofer et al., 2008). Thus, the application of glyphosate-based herbicides, previously considered innocuous to animals, may be having some complex and unintended effects on the food web by shifting predator population success and community structure.

These studies demonstrate that glyphosate-based herbicides are "info-disruptors" (sensu Lurling and Scheffer, 2007) and reduce the efficacy of natural infochemicals important to mate location in *Pardosa milvina*. They also suggest that an airborne component of this herbicide plays a role in such disruption. To our knowledge, this is the first study to explore the degree to which any formulation of a glyphosate-based herbicide influences chemical communication among common terrestrial arthropods in agroecosystems. Recent reviews stress the need to address the potential impact of anthropogenic chemicals on animal communication pathways (Lurling and Scheffer, 2007; Klaschka, 2008, 2009). These data suggest a subtle but significant effect of herbicide on the signaling that allows males and females to co-locate, which can alter the landscape for sexual selection and reproductive success. The nature of the effects, especially because they occur in a common predatory species that can play an important role in the agricultural food web, is potentially problematic; however, the circumstances under which these effects influence population viability, community structure, and/or the food web remain to be explored.

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