



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.

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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) (Michaluková 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 non-lethal 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.

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