

The effect of glyphosate on the wolf spider *Rabidosa punctulata* communication

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ABSTRACT

Communication between wolf spiders is an important component for reproduction. Spiders have the ability to communicate through visual cues, acoustic/vibratory signals, and pheromones. Glyphosate is known to be used in farming as an herbicide. This study explored whether the presences of glyphosate interfered with the detection of female wolf spider pheromones in her silk by male wolf spiders. I ran a repeated measures experiment on to see if the glyphosate that was applied to the soil had any effect on the wolf spider communication across different timeframes. I found that there is a significant difference in courtship latency between the control (no glyphosate) and some of the glyphosate conditions. Interestingly, the effects of glyphosate were more pronounced the longer it had been on the substrate. This discovery means that the glyphosate effected the detection by the male and the more it was broken down in the soil the more effect observed.

Keywords: *wolf spiders, roundup, glyphosate, pheromones*

INTRODUCTION

Glyphosate (commonly known as Roundup) is a commonly used herbicide in commercial and residential areas. Glyphosate is a nonselective herbicides that farmers use because it is a simple and cost-effective way to control annual, perennial and aquatic weeds (Roberts, 2005). Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which blocks aromatic amino acid biosynthesis resulting in the death of the plant (Mesnage, 2017). Farmers apply Roundup in three different techniques. The first technique happens two weeks before planting in early spring. Thirty-three percent of farmers use this technique because the soil is not disturbed often (National, 2019). The second technique is applying Roundup during planting, called crop-destruct method due to the crops not being harvestable, performed during the summer (National, 2019). The third technique is after planting, when the Roundup is applied once the crops have ceased in production, used during the months of November and December (National, 2019). In 2016, there was an average of 300 million pound of glyphosate applied (Mesnage, 2017).

The target plants are often not the only organism affected by this herbicide. Other non-weeds have also been shown to be affected by the amount and the length of exposure of the glyphosate (J, 2018). Beyond the effects on plants, other important members of the prairie ecosystem are affected as well.

Spiders are arthropod predators found in nearly every terrestrial ecosystem. Arachnids are a natural pest control, so pesticides in some ecosystems are not needed. These secondary consumers allow the ecosystem to stay within homeostasis, since they are controlling the number of primary consumers present within the ecosystem. Grass sites are abundant in

Lycosidae and *Gnaphosidae*, two common ground-dwelling arachnids. Lycosids are particular in their habitat choice, as they will leave if conditions are poor (Weeks, 2000). If secondary consumers are not residing in areas the potential for that ecosystem to get out of homeostasis grows significantly, due to primary consumers numbers increasing rapidly. With the increasing number of primary consumers, farmers have applied more pesticides into the ecosystem as a way of controlling the primary consumers (Farinós, 2018).

Exposure to different pesticides have shown mixed effects on spider populations. Researchers found an increase in ground dwelling predators in cotton fields treated with the glyphosates (Farinós, 2018). However, exposure to sublethal pesticides has been seen to effect predation, competition between organisms, density-dependent relationships, and the development of the population (Farina, 2018). Virginie Party and colleagues wrote an article that looked at the affects that odor backgrounds on the pheromones of moths. They found that the volatile compounds effected the male's pheromones trigged walking response (Party, 2013), delaying the response of the male moths.

One aspect of spider biology that has not been investigated in relation to the application of pesticides is its effect on spider communication systems. Spiders have the capability to communicate in various forms, including one of their more important modalities, chemical (Kronestedt, 1996). Using a variety of pheromones, wolf spiders can gather a variety of information on the spider that was there, including sex, species, and body condition (Roberts, 2005). The detection of pheromones is important for the initiation of male courtship displays to females (Bell, 2015).

Upon detection of female silk, male *Rabidosa punctulata* court by raising their front legs and vibrating them quickly in the air. Courtship allows for the male to gain the attention of the female he wishes to mate with, while also getting an arousal or stimulus from the female (Robinson, 1982). So if the glyphosate masks the pheromone, the male possibly will miss the opportunity to mate.

This study examined whether the presence of glyphosate affected the male wolf spider ability to sense the female wolf spider silk in the area.

The effect that roundup has on spider communication is important due to the effect that comes on a secondary consumer. If the reproduction of a secondary level organism is affected, the potential for the homeostasis of the ecosystem to become unbalanced increases greatly.

MATERIALS AND METHODS

In order to obtain wolf spiders for the study, we went to a pasture in McPherson County, KS. We collected 120 *Rabidosa punctulata* wolf spiders and then, housed them in individual plastic containers that are 8.4 x 8.4 x 11.0 cm (Chai, 2016). Spiders containers were checked for molts every two to three days and fed three crickets once a week. Water was provided through a wick in the bottom of the cage (Chai, 2016). Only immature females were collected, however, some of the males were already mature in the field.

Ten to fifteen days following maturation of females, trials began. Twenty-five males were ran through a repeated trials. Females ran through only two conditions, but I made sure that males were not paired with the same female during different conditions. The experimental conditions were the control group (no glyphosate), immediate exposure after the application of roundup (0 days), seven days after application of roundup, and fourteen days after application of roundup. Each trial was examined for a total time of 15 minutes. All trials, for every condition, took place in plastic circular arenas that have a diameter of 20.2 cm, and a height of 7.3 cm (Wilgers, 2016), that had the walls darkened to help remove visual distractions (Wilgers, 2016). Peat moss soil was placed inside the arena about a centimeter thick that may or may not contain roundup depending on condition, and the silk the female has laid down for that trail. Depending on the condition, the peat moss was sprayed 25 times with standard Roundup prior to the female laying her silk. The peat moss was placed inside a container, where the soil was sprayed and then dated for records. The amount of sprays was calculated from the standard amount of Roundup applied to Kansas farmlands. After the female has laid her silk, she will be removed from the arena, whereupon the male was introduced and I watched for his display of courtship for a maximum of 15 minutes. In each trial, I observed

the latency to begin courtship and the number of courtship bouts expressed by the male wolf spider. All male spiders were ran through conditions in the same format (7 day, 14 day, control, then 0 days). For the immediate exposure, I waited 10 minutes before introducing the male so the fumes of the roundup would not have an influence. I used a non-parametric repeated measures ANOVA followed by pairwise comparisons (Durbin-Conover) to analyze my results.

RESULTS

Male courtship was affected by the presence of glyphosate. The presence of glyphosate resulted in greater latency to court ($\chi^2=21.8$ df=3 p<.001; Figure 1). The effect was more pronounced the longer the glyphosate had been on the substrate. Male spiders had a greater latency as the glyphosate broke down over the days (Table 1; Figure 1).

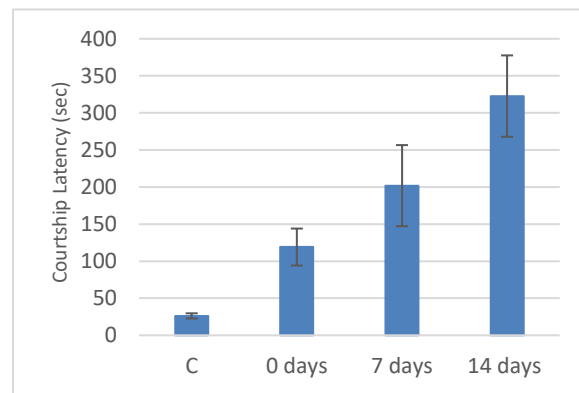


Figure 1. Courtship latency for *Rabidosa punctulata* across different treatments with exposure to Glyphosate (C = Control, 0 days = 0 days after application, 7 days = 7 days after application, 14 days = 14 days after application)

Table 1. Courtship Latency pairwise comparison

Comparison	Statistic	p-value
C – 0 days	4.376	<.001*
C – 7 days	3.615	<.001*
C – 14 days	6.089	<.001*
0 – 7 days	0.761	0.451
0 – 14 days	1.713	0.094
7 – 14 days	2.474	0.018

*Significant after Bonferroni correction

My results suggest that courtship rate was not affected by glyphosate ($\chi^2=3.08$ df=3 p=0.38) Once males began courting a female, they responded with relatively the same bouts per minute across all treatments (Figure 2)

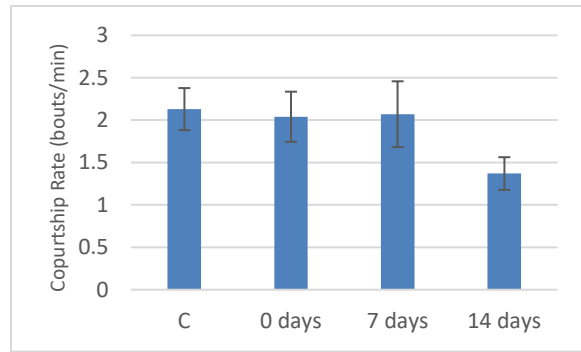


Figure 2. Courtship rate for *Rabidosa punctulata* across different treatments with exposure to Roundup (C = Control, 0 days = 0 days after application, 7 days = 7 days after application, 14 days = 14 days after application)

DISCUSSION

There was significant evidence that glyphosate does affect the communication between wolf spiders. Spiders delayed their courtship when in the presence of roundup applied up to 14 days prior.

Interestingly, the greatest latency to court was when males were exposed to roundup that was applied 14 days ago. This may have occurred due to the breakdown of the glyphosate. X Yang and colleagues wrote an article analyzing the characteristics of glyphosate as it decays. Yang and his colleagues found that the residues of the glyphosate were detectable in 0-30 cm of topsoil after 748 days of application, this lets me know the male spider having a hard time detecting the pheromones of the female makes sense in the 14 days after application since the residues of the glyphosate had the ability to influence the pheromones the male detected. Yang also discovered that there was also 57% concentration of glyphosate in 0-10 cm of the topsoil 7 days after application, if this experiment was performed again after having this knowing I would make sure the soil that the glyphosate was applied to was less than 10cm, so to ensure the glyphosate was solely influencing the male spider and not the residues of the glyphosate.

An article titled Herbicides and their Lethal and Sublethal Effects on the Chemical Communication System of *Xenopus levis* focused on herbicides affecting tadpoles. K. A. Yuill Proctor analyzed the effects of herbicides present at sublethal levels on tadpoles. Proctor found that atrazine – an herbicide that inhibits photosynthesis- affected amphibians directly and indirectly by the spray's runoff into the water sources, females were affected by being drawn to female-female chemical cue interactions. When amphibians were introduced to glyphosate, they found that depending on if the glyphosate was organic or

synthetically made had different effects on the amphibians (Proctor, 2004). Synthetic glyphosate had less of an effect on the amphibians unlike the organic glyphosate (Proctor, 2004). Another discovery about the synthetic glyphosate was if the glyphosate pesticides had polyethoxylated tallowamine surfactant present in the solution, the herbicide was toxic rather than herbicides synthetically made that had glyphosate solely present (Proctor, 2004). I did not analyze rather the glyphosate in the roundup was paired with polyethoxylated tallowamine, but if the glyphosate was paired that would also make sense for the males having difficulty detecting the female pheromones since the toxicity would be increased.

The male spiders were ran through the same conditions in the same order, 7 day then 14 day then control (no application) then 0 day. This method could have affected my results by the males because males become accustomed to the experiment and may consistently respond differently in early versus late trials. This would have been a concern if males continuously shortened their courtship latencies across the different treatments, but this pattern was not evident.

In conclusion, the glyphosate affected the latency of the male due to the residues the glyphosate was down into in the 14 day after application trials. The residues of the glyphosate may have been blocking the pheromones of the female on the silk.

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