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AVOIDANCE OF THREE HERBICIDE FORMULATIONS BY EASTERN RED-BACKED SALAMANDERS (*PLETHODON CINEREUS*)

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***Abstract*.—The ability of terrestrial amphibians to avoid chemical pollutants, such as herbicides or fertilizers, is relatively unknown although such chemicals may occur in their habitats. We studied whether the Eastern Red-backed Salamander (*Plethodon cinereus*) would be able to behaviorally avoid three different herbicide formulations (Spectracide Brush Killer, Bayer Advanced Brushkiller Plus, and Roundup Weed and Grass Killer Concentrate Plus). Salamanders avoided all three formulations when encountered at their full recommended application concentration, and were able to detect and avoid the Bayer and Roundup formulations at 10% the full concentration. Salamanders did not avoid any formulation at 1% the full concentration. Our results suggest that salamanders are able to detect these herbicide formulations and behaviorally avoid them, perhaps reducing any negative effects from exposure to some field applications.**

Key Words.—amphibian; avoidance behavior; Eastern Red-backed Salamander; herbicide; *Plethodon cinereus*

INTRODUCTION

Herbicides are frequently used in forested habitats to help manage timber production (Shepard et al. 2004). In addition, herbicide drift into forests from neighboring agricultural fields can also occur (Gove et al. 2007). Such herbicide use and drift can occur over large areas. However, herbicides also can be used on a smaller scale in natural areas to control invasive plants (Carlson and Gorchov 2004; Simmons et al. 2007; Slaughter et al. 2007). The widespread use of these herbicides has raised questions about their potential effects on non-target organisms, including both plants and animals (Guynn et al. 2004). In particular, the effects on forest-dwelling amphibians, both aquatic and terrestrial, have been a subject of concern.

Unfortunately, we have relatively little information on such effects for terrestrial amphibians. Relyea (2005) found that overspraying of Roundup killed the majority of juvenile anurans tested, including *Lithobates sylvaticus*, *Anaxyrus fowleri*, and *Hyla versicolor*. Dinehart et al. (2009) also found increased mortality in juvenile anurans (*Anaxyrus cognatus* and *Spea multiplicata*) exposed to Roundup Weed and Grass Killer Ready-to-Use Plus, and Weed and Grass Killer Super Concentrated, but were not killed by Roundup Weather Max and Ignite 280SL. Agroxone 5 (formulation of 2,4-D) was lethal to adult *Triturus cristatus carnifex* (Zaffaroni et al. 1986). However, Cole et al. (1997) found that spraying with only glyphosate did not affect capture rates in several terrestrial salamanders, including *Plethodon vehiculum* and *P. dunni*. Glyphosate alone did not affect several species of terrestrial amphibians from the Oregon Coast Range (McComb et al. 2008), but commercial formulations using surfactants were not tested. Similarly, Jones et al. (2000) found little effect of herbicide treatment (tebuthimon) on amphibians and reptiles and Bernal et al. (2009) also found little effect of glyphosate formulations on Colombian frogs at normal application rates. Little is known about the ability of terrestrial amphibians to behaviorally avoid these herbicides as a means to potentially minimize the toxic effects. In one of the few published studies, Storrs Méndez et al. (2009) found that juvenile American Toads (*Anaxyrus americanus*) do not avoid soils treated with atrazine. However, adult amphibians may be able to detect and avoid herbicides, as evidenced by the ability of ovipositing anurans to avoid depositing eggs in contaminated pools or ponds (e.g., Takahashi 2007; Vonesh and Buck 2007; Vonesh and Kraus 2009).

We examined whether or not Eastern Red-backed Salamanders (*Plethodon cinereus*) would avoid substrates contaminated with three common household herbicide formulations (Spectracide Brush Killer Bayer Advanced Brushkiller Plus and Roundup Weed and Grass Killer Concentrate Plus), and thus whether or not they might be able to avoid exposure to lethal or sublethal concentrations of herbicides in nature if they are able to detect them. Because Eastern Red-backed Salamanders are frequently the most abundant terrestrial vertebrate in the forests of eastern North America (Casper 2005), understanding how they might behaviorally respond to herbicide use is very important, particularly given their important role in many temperate forest ecosystems (Welsh and Droege 2001; Davic and Welsh 2004). In addition, other less common species of *Plethodon* are also likely to experience such stressors and thus an understanding of the response of *P. cinereus* to herbicides will provide some understanding of how these other, more threatened, species may respond.

MATERIALS AND METHODS

We collected adult male and female Eastern Red Backed Salamanders (*Plethodon cinereus*; SVL about 45–55 mm) during mid-April 2010 and 2011 from under cover boards and natural cover objects on the Denison University Biological Reserve in Granville, Licking Co., Ohio, USA. The three studied herbicides were Spectracide Brush Killer (United Industries Corp., St. Louis, Missouri, USA; 2,4-D, 2-ethylexyl ester 9.74%, Dichlorprop-p, 2-ethylexyl ester 4.78%, Dicamba 1.65%; surfactant unkown), Bayer Advanced Brushkiller Plus (Bayer Crop Science LP, Research Triangle Park, North Carolina, USA; Triclopyr, triethylamine salt 8.8%; surfactant unknown), and Roundup Weed and Grass Killer Concentrate Plus (Monsanto Company, Marysville, Ohio, USA; Glyphosate, isopropylamine salt 18%, Diquat dibromide 0.73%; surfactant POEA).

We used 15 cm diameter petri dishes as experimental arenas. We lined the bottom of each petri dish with two halves of a 15 cm diameter filter paper. One half was dipped into aged tapwater and the other half into the herbicide formulation. We determined full herbicide concentrations using the recommended dilutions provided by the manufacturer on the label: Bayer (31.25 mL/L), Spectracide (39.1 mL/L), and Round up (46.9 mL/L). We ran separate choice tests on the full, 10%, and 1% concentrations of each herbicide formulation. In addition, we conducted a double control set of trials in which both sides of the filter paper were treated with aged tapwater. We did not determine actual concentrations of the herbicides. For the full concentration and control trials, we used 35 salamanders per formulation (total number of salamanders used = 140). For the 10% and 1% concentration trials, we used 20 salamanders per formulation and concentration (total number of salamanders used = 120). No salamanders were used more than once.

For each trial, we placed a salamander in the middle of the petri dish. When each of the salamanders was in their respective dishes, we covered the dishes and started timers. We recorded the location of the salamander on either the control or herbicide side of the petri dish every 15 min for 1 h. We determined the location of the salamander by where the majority of the front portion of their body lay. For each individual, we calculated the proportion of observations the salamander was on the water side of the petri dish. We used a Wilcoxon signed-rank test to compare the mean proportion of observations on the water side to the expected mean of 0.5 for random use of the sides of the petri dish. We ran separate tests for each herbicide and concentration. Means are given ± 1 S.E.

RESULTS

For our double control trials, we found that there was no preference for either side of the Petri dish (mean proportion on left side = 0.54 ± 0.08; W = 24.0, df = 34, $P = 0.60$).

At the full concentration of Spectracide, we observed salamanders on the water side of the petri dish significantly more than would be expected by chance (mean = 0.76 ± 0.057; W = 186, df = 34, $P < 0.001$). Salamanders did not show a significant preference for water or Spectracide sides of the petri dish at either the 10% (mean = 0.39 ± 0.08; W = -34.5, df = 19, $P = 0.13$) or the 1% concentration (mean = 0.46 ± 0.06; W = -6.0, df = 19, $P = 0.75$).

In the full concentration trial for the Bayer formulation, salamanders significantly avoided the Bayer-treated side of the petri dish (mean = 0.81 ± 0.057; W = 225.5, df = 34, $P < 0.001$). The salamanders also significantly avoided the Bayer-treated side of the petri dish at 10% concentration (mean = 0.70 ± 0.08; W = 52.5, df = 19, $P = 0.049$). Salamanders did not show any preference at the 1% concentration (mean = 0.48 ± 0.09; W = -4.5, df = 19, $P = 0.92$).

Salamanders significantly avoided the side of the petri dish treated with the full concentration of the Round-up formulation (mean = 0.69 ± 0.07; W = 127.5, df = 34, $P = 0.009$). Salamanders also avoided the Round-up treated side of the petri dish at the 10% concentration (mean = 0.76 ± 0.07; W = 69.5, df = 19, $P = 0.003$). No preference was observed at the 1% concentration (mean = 0.59 ± 0.07; W = 11.5, df = 19, $P = 0.29$). All salamanders survived the experiment.

DISCUSSION

Our results suggest that Eastern Red-backed Salamanders are able to detect and avoid all three herbicide formulations at their full label application rates, and to avoid both the Bayer and Round-up formulations at 10% the label concentration. The actual avoidance response in the field may take the form of dispersal from the area, as has been observed in response to clear-cutting (Semlitsch et al. 2008), or by taking refuge belowground. The ability to avoid these herbicide formulations would potentially allow Red-backed Salamanders to minimize exposure to the potentially toxic effects of these herbicides which have been shown to be lethal to some species of terrestrial amphibians (e.g., Zaffaroni et al. 1986; Relyea 2005;

Dinehart et al. 2009). However, the inability of these salamanders to respond to more dilute concentrations may limit their ability to avoid exposures to these herbicides. For example, our results suggest Eastern Red-backed Salamanders could avoid exposure to herbicides from small-scale spot treatment of the understory which would be expected to result in higher concentrations at ground level, but might not be able to avoid exposure to larger scale applications, such as aerial treatments, which would be expected to result in lower concentrations at ground level.

We used commercially-available formulations of the herbicides that contained both active ingredients and surfactants. The response we observed could therefore be driven by either the active ingredients or the surfactants. We suspect that the surfactants are likely the major factor in the salamanders' responses since in several other contexts the surfactants of herbicides have been shown to be more toxic to amphibians than the active ingredients (e.g., Mann and Bidwell 2001; Mann et al. 2003; Howe et al. 2004). Unfortunately, the specific surfactant was known only for one of the formulations we used (Roundup), and thus we cannot generalize about the relative effects of different surfactants and their formulations.

In addition to the results of our study, *P. cinereus* have also been shown to avoid soils contaminated with high levels of lead (Bazar et al. 2010) and urea (Gaglione et al., unpubl. data) and to choose substrates based on pH (Mushinsky and Brodie 1975). Thus, Eastern Red-backed Salamanders appear to have the ability to detect and avoid a wide range of contaminants, which might be expected given the ability of these salamanders to detect and respond to chemical cues from conspecifics and predators (Maerz et al. 2001; Sullivan et al. 2002; Martin et al. 2005; Dantzer and Jaeger 2007).

From a management perspective, our results suggest that Eastern Red-backed Salamanders will be able to behaviorally respond to herbicide treatments that may be used in controlling invasive species or other weeds in forest habitats, especially if such use is targeted and used on relatively small areas. Unfortunately, we know too little about the toxicity of field applications of herbicides to know if temporary avoidance of herbicides would be effective, or even if avoidance behaviors and the salamander's mobility would allow salamanders to escape the toxic herbicides when used on a larger scale. In addition, it is unclear if there may be any negative consequences for the avoidance behavior, such as reduced foraging or increased risk of predation due to greater movement.

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