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EFFECTS OF THE HERBICIDE ATRAZINE ON *RANA SYLVATICA* DEVELOPMENT

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ABSTRACT

Newly hatched wood frog (*Rana sylvatica*) tadpoles were treated with sublethal concentrations of the commonly used herbicide atrazine. Tadpoles remained in dilutions of 75 ug/L and 250 ug/L for 10 d after which measurement of total length, torso length, and weight were measured. Significant differences were found in the torso length and overall length with the control organisms being smaller than those in the treatments. No conclusions can be drawn from these data without further research.

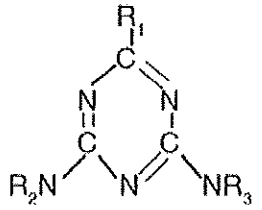
Keywords: Atrazine, body size, development, Rana sylvatica, *wood frog.*

INTRODUCTION

Agriculture is Pennsylvania's number one industry. There are approximately 45,000 farms in the state and Pennsylvania is second in the nation in the number of acres of farmland preserved for agricultural use (PA Farm Bureau, 2000). With this large quantity of farmland, herbicide acts as an important tool in order to boost crop production. One of the mostly widely used herbicides in the US is atrazine, which exceeds a usage of 30 kilotons each year (Raven, 1983).

Atrazine is a selective triazine herbicide (Fig. 1) that is used to control broadleaf and grassy weeds in agriculture and conifer reforestation plantings. Yet it can persist in the environment for a relatively long period of time, with a half-life of 224 days at 25°C and pH of 4-7 (Miller, 1999). Although the use of this chemical is intended to remove only unwanted vegetation, its presence in the environment can have affects on other life-forms, especially those in aquatic environments. In a 1992 survey of water quality at streamflow-gauging stations throughout the corn and soybean belt of the United States, Thurman et al. (1992) detected atrazine in 98% of post-planting water samples. Of these samples, 55% were over the Maximum Contaminant Level (MCL) set by the Environmental Protection Agency.

Figure 1 - *Structure of diamino-s-triazine.*



Despite the fact that atrazine is not the most lethal herbicide, it has been shown to have many sublethal affects in organisms. Atrazine may disrupt endocrine functions in amphibians, which are generally considered to be more sensitive to aquatic contaminants than other aquatic vertebrates, partly because of their highly permeable skin (Larson *et al.*, 1998). The combination of atrazine's widespread usage, relatively long period of persistence, and sublethal effects in animals indicates that atrazine may have detrimental effects on animal life located near agricultural areas.

Larson et al. (1998) have shown that, at sublethal concentrations, atrazine can have significant effects on larval size and hormone concentration in tiger salamanders (*Ambystoma tigrinum*). Larson *et al.* (1998) suspected that interactions between stress hormones (corticosterone) and thyroid hormones (thyroxine) promoted metamorphosis. Corticosterone acts to convert thyroxine into triiodothyronine in larvae, which in turn promotes differentiation, but may also slow growth during the time of differentiation. However, stress-hormone concentrations rise in response to stressors in the environment, as does thyroxine in stressed amphibians (Norris, 1997). Therefore, increased amounts of stressor hormone and thyroid hormones should produce increased differentiation or metamorphosis, yet the organism should appear smaller than normal due to the thyroid's ability to hinder growth. These developmental patterns was earlier recognized by Rose and Armentrout (1976), who found that amphibian larvae often have the ability to accelerate metamorphosis in response to deteriorating conditions in larval habitat. Also, their study showed that amphibians, which went through metamorphosis quicker, had a tendency to be smaller adults.

Thus, in an attempt to test the generality of these findings, we investigated the effects of atrazine on the wood frog, *Rana sylvatica*. Wood frogs are found throughout the Northeastern US, as well as Canada and Alaska. These frogs, like many others, use vernal ponds to breed, which occurs one night a year. Vernal pools contain limited amounts of stagnant water, and thus are vulnerable to high concentration effects if exposed to herbicide runoff. Therefore, we believe that vernal ponds in agricultural areas are critical systems to study for potential effects of herbicides on aquatic species. Based on the studies of Rose and Armentrout (1976) and Larson *et al.* (1998), we hypothesized that over a 10-day study, wood-frog tadpoles would experience a decrease in size and weight after exposure to sublethal concentrations of atrazine.

FIELD SITE

On March 24, 2000, *R. sylvatica* egg samples were obtained from a ditch along Route 26 heading south toward Huntingdon, PA. The water in the ditch was slow moving with a trickling drainage spout approximately 5 feet away. There was an abundance of grass, pine needles, and cattails in the water and the surrounding area. Multiple ditches similar to this were in the area, but only this one had a sufficient amount of water to contain eggs.

METHODS AND MATERIALS

The egg masses were transported back to Juniata College in plastic buckets containing the water in which they were laid. The egg masses were divided into 3 smaller groups of approximately 30 eggs, which were placed into 3 equally sized large, glass dishes and allowed to hatch at room temperature. Glass plates were placed partially over the dishes to minimize evaporation, yet still allow gas exchange.

On March 28, 2000, approximately three days after hatching, we set up our treatment solutions of atrazine. A small amount (~49 ml) of atrazine was obtained from a nearby farm in a premixed solution containing one pound (453 g) atrazine per quart of water. To facilitate accurate measurements of the treatment concentrations, we used a 10 µl syringe to dilute the original 49 mL of atrazine in one liter of water. The treatment concentrations were 75 µg/l (3.5 µl of the above base solution in one liter of sample water) and 250 µg/l (10.8 µl of the base solution in one liter of sample water). The sample water collected from our field site was used in the control group.

The tadpoles were cultured in their respective atrazine concentrations for ten days, during which they were fed fish food daily. On the tenth day, the length and mass of the tadpoles were measured. A small amount of water was placed into a clear petri dish, along with one tadpole. A ruler was placed under the petri dish, and measurements of total length and torso length were measured. The same tadpole *was*

then weighed in a small weighing boat using an analytical balance. The tadpoles were weighed and measured on the same day to maximize data consistency. Analysis of variance and Tukey tests (Minitab) were used to compare tadpole sizes among the treatment groups.

RESULTS

Various size measures of the tadpoles in the three treatment groups are given in Table1. Among the treatment groups there was a significant difference in torso size ($P = 0.001$) and the overall size ($P < 0.001$) of the tadpoles, but no significant difference in the weight ($P = 0.151$) or torso length/total length ratio ($P = 0.374$). There was also no difference in the weight to length ratio ($P = 0.887$).

Tukey tests showed that torso length was significantly greater in the 250 µg/l atrazine group than in the control group ($P < 0.001$) and marginally significantly greater in the 75 µg/l atrazine group than in the control group ($P = 0.051$). However, torso length did not differ significantly between the 75 µg/L treatment and the 250 µg/l treatment ($P = 0.161$).

Similarly, total length was significantly greater in the 75 µg/l treatment than in the control ($P = 0.026$), and also for the 250 µg/l treatment compared to the control ($P < 0.001$). However, again no difference was observed between the two atrazine-treatment groups ($P = 0.076$).

Table 1. Body size measures of Rana sylvatica *tadpoles at different concentrations of atrazine.*

Control					
	Torso length	**Total length**	**Weight**	**Torso/Total**	**Length/Weight**
Mean	6.21	18.34	0.10	0.34	191.90
Std. Dev.	0.76	1.43	0.023	0.039	47.21

75 µg/l					
	Torso length	**Total length**	**Weight**	**Torso/Total**	**Length/Weight**
Mean	6.69	19.28	0.11	0.35	181.28
Std. Dev.	1.44	2.3	0.026	0.059	28.78

250µg/l					
	Torso length	**Total length**	**Weight**	**Torso/Total**	**Length/Weight**
Mean	7.15	20.19	0.11	0.35	204.32
Std. Dev	0.88	1.23	0.015	0.03	76.31

DISCUSSION

We hypothesized that *R. sylvatica* tadpoles in the treatment groups would be smaller than those in the control group, but instead we found just the opposite to be true. Since there was essentially no difference in tadpole weights among the treatments, perhaps the larger tadpoles treated with atrazine had less body reserves than their control counterparts. If this were the case, then atrazine may have indeed had some impact on tadpole size. However, conrtary to this hypothesis, weight/length ratios were not significantly smaller in the atrazine treatment groups. This result was surprizing, but should be viewed with caution because of the large standard deviations associated with our weight and length/weight means.

Procedural problems could possibly have influenced our results. First, separating frog eggs is an extremely difficult task due to the sticky jelly surrounding them. When trying to equally partition eggs into dishes, it was practically impossible to allocate an even number to each dish. As a result, our control group

ended up with almost twenty more tadpoles than the treatment groups. Thus increased crowding in the control group could have inhibited tadpole growth and thus their body size.

Another problem that we encountered was that our solution of atrazine was too concentrated to accurately treat our tadpoles. On a farm, a typical solution of atrazine is formulated by mixing one pound of atrazine for every quart of water, a very concentrated solution from which to obtain 75 and 250 μg/L. The farm provided us with approximately 49 ml, which was diluted to one liter. However, even after this dilution, we still needed only 3 and 10 μl to make our treatments. The only equipment that Juniata College has to measure such small quantities is a 10 μl syringe, not a very accurate tool in comparison to an automatic pipette, for example. Therefore, it was difficult to see when there was actually a particular quantity of solution in the syringe, and more importantly, if it came out at all.

Our group also encountered a problem with weighing the tadpoles on the analytical balances. As can be seen in Table 1, tadpole weights varied greatly. This was probably due to the varying amounts of water in the weighing boats and the fact that the tadpoles themselves were wet. One tiny drop of water on a tadpole when placing it in the weight boat dramatically changed the readings that we obtained. Absorbing some of the water with tissue paper seemed to alleviate some of the problem, but it did not eliminate it.

A final problem with this study was the very limited amount of time in which to perform it. Because of the time issue, we were only able to treat our tadpoles for ten days, a very short period of time. We feel that more reliable data could have been obtained if the tadpoles were given longer exposure times. Also, the rates of metamorphosis of the tadpoles should also have been examined. It was very difficult to determine if there were any changes in the rates of metamorphosis among our groups partly because our specimens were so young.

There are many options for further research on this subject. First, this experiment could be repeated making the corrections that we suggested above to see if the results are different. In addition, these same methods could be repeated using different species such as spring peepers, spotted salamanders, fish, or other aquatic animals. Furthermore, hormone samples could be obtained from the subjects to see if there is any correlation between hormone concentration, body size, and rate of development. Future research along these same lines could provide valuable information regarding the impacts herbicides have on the environment, and perhaps on humans, as well.

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LITERATURE CITED

Larson, D.L., S. McDonald, A. Fivizzani, W. Newton, and S. Hamilton. 1998. Effects of herbicide atrazine on *Ambystoma tigrinum* metamorphosis: Duration, larval growth, and hormonal response. Physiological Zoology 71: 671-679.

Miller, T. L. 1999. The extension toxicology network. http://ace.orst.edu/info/extoxnet/

Norris, D.O. 1997. Vertebrate endocrinology. Academic Press, San Diego, USA.

Pennsylvania Farm Bureau. 2000. Pennsylvania Agriculture Information. http://www.pfb.com/news/aginfo.html.

Raven W.S. 1983. The pesticide dilemma. Pages 493-517 in *Environment.*

Rose, F.L. and D. Armentrout 1976. Adaptive strategies of *Ambystoma tigrinum* Green inhabiting the Llano Estacado of West Texas. Journal of Animal Ecology **45**: 713-729.

Thurman E.M., D.A. Goolsby, M.T. Meyer, M.S. Mills, M.L. Pomes, and D.W. Kolpin. 1992. A reconnaissance study of herbicides and their metabolites in surface water of the midwestern United States using immunoassay and gas chromatography/ mass spectrometry. Environmental Science and Technology **26**:2440-2447.