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POND SNAIL (*PHYSELLA HETEROSTROPHA*) MORTALITY IN RESPONSE TO GYPHOSATE AND ORGANIC BASED HERBICIDE CONTAMINATION

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ABSTRACT

Herbicides are commonly used around the world to prevent loss of crops but many have devastating effects on the surrounding terrestrial and aquatic ecosystems. One of the most commonly used active ingredients that is used in herbicides is glyphosate, which was applied on over 80 million acres in 2001, and has broad effects on many broadleaf plants (EPA 2001). Pesticides that are prone to runoff may contaminate aquatic ecosystems. Since glyphosate is potentially toxic to unintended targets, an organic alternative using citric acid is explored. Pond snail mortality over hour period in varying treatments of glyphosate or citric acid is compared.

Keywords: glyphosate (N-(*phosphonomethyl*)*glycine*)*, herbicides, organic herbicides, pond snail* (Physella heterostropha).

INTRODUCTION

Herbicides play an important role in society by aiding in the protection of crops against unwanted plants. However, the lethality and effects it has on terrestrial and aquatic ecosystems is overlooked by many. It is imperative to ask how the use of herbicides could be affecting daily life. The herbicides runoff into nearby water systems has been studied for many decades (Trichell, 1968); yet, some herbicides are rarely studied before being put into use by farmers around the globe.

In particular, Glyphosate (*N*-(phosphonomethyl)glycine) is a popular choice; it is the most widely used herbicide in the world, and one of the most commonly used herbicides to use glyphosate is RoundUp some would hail it as a miracle herbicide due to its ability to kill a broad range of broad-leaf plants (Gasnier, 2009; Duke and Powles, 2008). Others debate its toxicity on non-target species (Busse, 2001; Ayoola, 2008). Although the toxicity of glyphosate is a topic of debate, one proven merit of its use is that it has a relatively low half-live when compared to many other herbicides commonly used today. Glyphosate is one of the least persistent herbicides on the market, with a reported half-life ranging between 7 and 25 days (Duke et al., 2003; Battaglin, 2005). This greatly reduces the risk of an organism encountering glyphosate with such frequency as to impose a high possibility of chronic health problems. However, the risk of acute exposures during spraying seasons is still possible.

On the other side, the anti-GMO politics and yet uncertain toxicity of glyphosate has some pushing for more organic pesticides. In that case, acids and oils are popular choices for organic farmers (Franck et al., 2009). Citric acid lowers the pH of the environment, which facilitates aluminum uptake in plants. The acid itself is not especially toxic to the plants; it is the aluminum that kills plants near these water systems (Delhaize and Ryan, 1995). While comparatively expensive, clove oil is also an effective herbicide (Franck et al., 2009). The consumer herbicide Burn Out makes use of both of these ingredients and is a potential organic alternative to the use of glyphosate.

Snails are a common choice when looking at the health of an aquatic ecosystem, in particular the flora health. Snail shells are highly sensitive to pH changes and their tissues are known to store certain toxic heavy metals which makes them excellent bioindicators for aquatic ecosystem health (Dallinger et al., 1997). The pond snail (*Physella heterostropha*) was chosen for its dependence decaying organic matter (which typically lies on the sediment where herbicides are in their highest concentration), especially flora matter, its local abundance in an agricultural area, and its small body size (which means they have a lower body burden), making it particularly susceptible to acute exposures that may occur in the height of agricultural production each year. In this study, we compared the acute effects of two pesticides, KleenUp (a RoundUp knockoff that features the same main ingredient) and Burn Out on *Physella heterostropha* (an organic based herbicide) to determine if there is a significant difference in using a glyphosate based herbicide or an organic based herbicide mortality(commonly thought by many people in the general population to be safe). This study was a two pronged approach that looked at the lethal dosage at 50% mortality and dosage of each chemical and duration of exposure.

FIELD SITE AND SNAIL COLLECTION

Pond snails were collected in Petersburg Spring (Figs 1-3), a cold spring (~ $10^{\circ}C$) with an abundance of macroinvertebrates including *P. heterostropha*. This site was chosen for this study because it does not neighbor agricultural land thus allowing for a population not currently being affected by pesticides. Snails were primarily found in the lentic portion of the spring near the end of the brook (*Fig.* 1).

Figure 1. Picture of the field site, Petersburg Spring in Petersburg, Pennsylvania.

Figure 2 and 3. Photos of two different habitats (rocks and watercress) in Petersburg Spring where pond snails were collected. Spring located in Petersburg, Pennsylvania.

Figure 4. Lab setup of the pond snail bins as stored in Juniata College Ecology Department's laboratory fridge near native temperatures (~$10^{\circ}C$). The bins in the left tray contained different concentrations of Kleen Up weed and grass killer and the bins in the right tray were different concentrations of Burn Out weed and grass killer.

MATERIALS AND METHODS

Study Methods

The pond snail *P. heterostropha* was collected from Petersburg Spring in Petersburg, PA because of it high population abundance which allowed for high collection volume thus

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allowing for a greater range in body sizes (N ≈ 450). Spring pH was measured within 5 meters of the source with a Markson digital pH Meter Model 88 (Markson LabSales, Honolulu, HI). Snail were collected with dip nets and placed in 1 L plastic containers with native spring water with no more than 50 per container to allow for normal dissolved oxygen concentrations.

One glyphosate herbicide, Kleen up (40% w/v glyphosate),and one organic based herbicide, Burn Out (8.9% and 2.6% w/v citric acid and clove oil, respectively), were mixed into 0.25 L of natural spring water using a static model of exposure in the following doses: 0.010, 0.027, 0.030, 0.055, 0.070, 0.109, 0.150, 1.75, 3.5, 5.2, 6.9 and 8.9 mL The pH of each exposure treatment (see *Table 1*) was then measured using a Markson digital pH Meter Model 88 (Markson LabSales, Honolulu, HI). A control sample, using only native spring water, was used for each dose. Snails were randomly placed into each exposure dose and controls (N = 15). Mortality was recorded after a 24 hour period for each sample stored in a Chase refrigeration unit (Chase Industries, Cincinnati, OH) at 10°C (*Fig.* 4).

Statistical Analysis

Linear analysis was conducted between the mortality vs time for the initial concentrations of Kleen Up and Burn Out. LSRs were graphed using MiniTab version 17 and mortality rates were compared using an ANOVA analysis. Dose response curves were generated using Sigma Plot version 9. The LD_{50} of each dose response curve was graphically calculated using Rstudio dose.p function for a general linear model (GLM) under the MASS library package. Standard errors were converted to standard deviations to determine the significant differences or similarity for the LD_{50}'s of Kleen Up and Burn Out.

RESULTS

Dosage and Time

Significant relationships were found between dosage and time groups ($P < 0.05$) of each LSR and there was significant differences among the slopes (*ANOVA:* N = 4, $P = 0.006$, $F = 21.28$). However, though there were also significant relationships among each LSR ($P < 0.05$), when compared to time and herbicide type no significant difference was found ($N = 4$, $P = 0.229$, $F = 1.88$).

LD_{50} Analysis

LD_{50}'s were calculated on Rstudio using a GLM (see *Fig. 6 A and B*) and error was represented by the calculated one standard deviation for Kleen Up (DF = 13, $LD_{50} = 1.91 \pm 0.71$ mL) and Burn Out (DF = 13, $LD_{50} = 1.81 \pm 0.75$ mL). The LD_{50}'s showed no significant difference at one standard deviation over a 24 hour exposure period.

Table 1.Shows the pH of each individual bin tested. All measurements recorded on April 5th, 2016: seven days after setup of the Kleen Up and Burn Out bins with 1.75 and 3.50 mL of herbicide, five days after setup of the Kleen Up and Burn Out bins with 0.880, 0.440 and 0.200 mL of herbicide, same day as the setup of the Kleen Up and Burn Out bins with 0.109, 0.055 and 0.027 mL of herbicide. pH measured using Markson Digital pH Meter Model 88

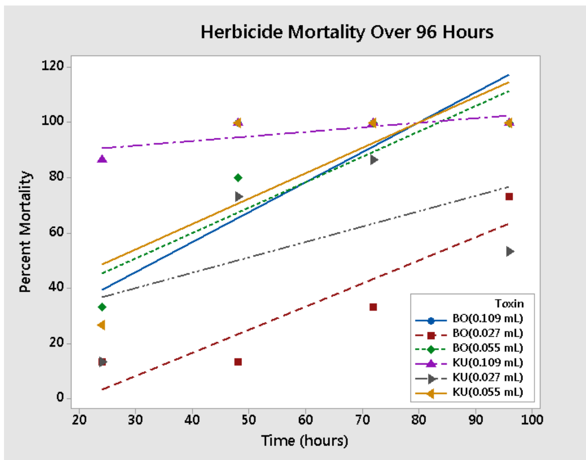

Figure 5. Slopes were calculated using a LSR method in MiniTab 17 (BO = Burn Out, KU = Kleen Up). Slopes indicate the rate of mortality over a 96 hour period and were compared using an ANOVA with simultaneous Tukey test which shows that only dosage and not herbicide type affects mortality.

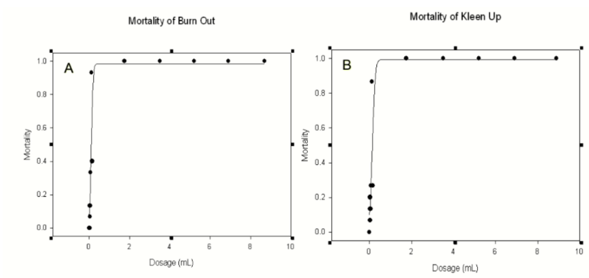

Figure 6. Dose response curves of Burn Out (A) and Kleen Up (B) experimental trials. Data displayed were collected after a 24 hour exposure period using a static exposure model. No significant difference between the LD_{50} of each herbicide was found when using Rstudio dose.p function from the MASS library for a GLM model.

DISCUSSION

ANOVA testing shows no statistical difference in mortality over time when comparing the mortality rates over time between Kleen Up and Burn out. However, there was a significant difference when comparing the dosages, which has been widely documented among toxicological studies. However, we found no significant difference when comparing the LD_{50} of each herbicide. Due to the generality previous studies have shown in glyphosate herbicides, and there relatively high mortality on other test species, we thought that the Kleen Up would have higher mortality rates at lower dose levels. Though this was not shown in the results, we think that pH (see Table 1) could have been a factor in having similar mortality.

We hypothesize that the lowered pH could have been a confounding factor that could have altered the biochemical detoxification pathways; especially for the organic pesticide, that is intentional as part of the pesticide mechanism of action. Since the snails generally prefer alkaline conditions (though they were collected from a slightly acidic spring,) the severe decrease in pH alone may have been enough to cause mortality (NYSDEC). The heavy decomposition of snail shells was also observed and may be related to the pH decline, rather than to the toxicity of the pesticides. We mention pH decline because it is possible that calcium may be leached from calcium containing shells to stabilize hydronium atoms, and free radicals like H^{-1}, causing less calcium to be present for gastropods and other macroinvertebrates.

Further studies into the potential benefits of alternative pesticides is recommended. Pesticides are not likely to go away any time soon, and in that case, research into their effects are imperative to the health of not only snails, but to all species. When safer alternatives to any pesticide, even already relatively safe ones, are found, a concerted effort must be put forth to make the switch for the sake of our future.

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