

The Effectiveness of Alternative Herbicides in Sustainable Weed Control Compared to

Roundup®

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Abstract

Monsanto's Roundup® is the most widespread pesticide in the world and it has possible deleterious impacts on humans and on the environment. Existing alternative eco-friendly herbicides have been shown to be inefficient and costly. For this reason, it is essential to explore alternative methods of weed management that have a minimal environmental impact. Throughout this study, the researcher compared the effectiveness of Roundup® as a weedkiller to that of 10% and 20% ascorbic acid by treating field plots in a backyard in residential Toms River and controlled monospecies trays vegetated with clover. Effectiveness was determined by measuring chlorophyll health in leaf samples from the plots and percent cover (or percent green) in each of the plots or trays. The field results, however, indicate that even though all of the herbicides had an impact on treated plants, Roundup® was consistently the most effective—having caused the greatest change in both chlorophyll health and percent cover in treated plots. This is because Roundup® is an herbicide that works from the roots up and ascorbic acid is a “burn off” herbicide which works from the top down. While this study was unsuccessful in developing an effective herbicide, it still provides some insight which can be used in future studies.

Introduction

Invented in 1970 by Monsanto chemist Dr. John Franz, glyphosate, the active ingredient in Roundup®, has rapidly become the world's most popular pesticide (Benbrook, 2016, para. 6). Roundup® is a nonselective, post-emergent herbicide used to manage vegetation that is considered harmful to agricultural crops or undesirable in ornamental gardens. A 2015 study published in *Environmental Science Europe* on the extent of usage of glyphosate (Roundup®) showed that “Two-thirds of the total volume of glyphosate applied in the U.S....has been sprayed in just the last 10 years” (Benbrook, 2016, para. 3). Glyphosate usage to this extent is causing researchers to question its impact on human and environmental safety.

Among the primary concerns is glyphosate's status as a carcinogen. This has been a major point of debate within the scientific community as there is a copious amount of research that supports both sides, some linking it to non-Hodgkin lymphoma and some recognizing no relation (Tarazona et al., 2017). The World Health Organization (WHO) has listed glyphosate as “probably a carcinogen”. However, as of December 12, 2017, the EPA recognizes glyphosate as a low toxicity substance and rejects its status as a carcinogen (Environmental Protection Agency, 2017).

Regardless of the product's main ingredient's status as a carcinogen, there is evidence that the inert ingredients are far more toxic than glyphosate itself. In 2014, French scientists studied a total of nine different pesticides to evaluate their respective toxicities to humans; among these was Roundup®, which “was among the most toxic herbicides and insecticides tested” (Mesnage, Defarge, de Vendômois, & Séralini, 2014, para. 1). Roundup® was tested separately from glyphosate in this study and was found to be 125 times higher than its active ingredient alone (para. 17, 12). Polyoxyethylene tallow amine (POEA), one of the inert ingredients still found in many Roundup® products, has been recognized as a toxin in aquatic

ecosystems by the U.S. Geological Survey (U.S. Geological Survey, 2017b, para. 1). As the usage of Roundup® and other glyphosate-based herbicides, scientists are also concerned with Roundup® running off into waterways and groundwaters. There is evidence that glyphosate runs off into surface waterways, though very little was found in groundwaters because glyphosate binds tightly to the soil (U.S. Geological Survey, 2017b). There is some debate as to whether or not glyphosate is a dangerous runoff chemical, but if glyphosate is running off, then it would also be likely that the inert ingredients are also producing runoff in waterways.

Over the years, scientists have been making an effort to develop alternatives that have less of an environmental impact than Roundup® and still are effective in managing weeds. Among the substances studied are acetic acid, clove oil, and limonene citric acid. Many of these are active ingredients in patented “environmentally-friendly” weed-killers. Much of the research to be found on alternatives is dated, but studies – including a study by the University of Massachusetts which spanned from 2004 to 2007 – show that many of these alternatives function best after multiple applications and at higher concentrations, up to 20% for acetic acid (Barker & Probst, 2008). These alternatives work from the top-down by interacting with the leaves and stems of the plant, often leaving the roots unharmed, which allows for rapid regrowth and makes these methods largely unsuitable and expensive for long-term management (Smith-Fiola & Gill, 2017, p. 2-3). In addition, there is some research which suggests that effectiveness of alternatives – specifically of acetic acid – is above 90% in the first few weeks, but dwindles over time (Chinery, 2001).

On January 9, 2018, a bill was introduced to the New Jersey Assembly which “[provides] a gross income tax credit for certain homeowners...for replacing all grass lawns...with stones” in order to limit the use of “chemical agents that could eventually flow into Barnegat Bay” (AB723,

2018, para. 1). The argument is that when people have grass lawns, they use fertilizers and pesticides to make the grass grow and get rid of undesirable plants that might not look aesthetically neat, thereby causing environmental issues in Barnegat Bay like eutrophication. This legislation could possibly encourage homeowners to decrease their usage of fertilizers. However, opportunist plants (i.e. weeds) still manage to grow through stone yards, looking even less aesthetically pleasing than in a grass lawn, which could result in homeowners using *more* herbicides, like Roundup®, in order to eliminate the leafy pests. Unlike with grass gardens, where homeowners must use pesticides relatively sparingly in order to prevent healthy grass from dying, there will be no “good” vegetation in the lawn to prevent them from overusing the herbicides. For this reason, it is useful to investigate alternative herbicides that kill off undesirable weeds, without the potential dangers that Roundup® and other herbicides pose.

The goal of this experiment is to develop an alternative organic herbicide and experimentally compare its effectiveness to that of Roundup®. Because Roundup® translocates from the roots up through the stem of treated plants and most alternative herbicides attack the cell walls of the plant, I hypothesized that the developed alternative herbicide will react similarly to other alternatives like vinegar, resulting in Roundup® being the more effective herbicide. Given this hypothesis, I predicted that Roundup® would reduce the chlorophyll health and percent cover of treated plants more than the developed alternative herbicide. In addition, I predict that Roundup-treated plants will have significantly less regrowth than the alternative herbicide.

Methods

Alternative Herbicide Mixture

Active ingredient. I selected ascorbic acid as the active ingredient in the alternative herbicide for this experiment. Ascorbic acid ($\text{HC}_6\text{H}_7\text{O}_6$), commonly known as vitamin C, is a water-soluble white powder commonly used as a dietary supplement and antioxidant found in many fruits and vegetables (National Center for Biotechnology Information, n.d.-a). It is also commonly used as a household cleaner, particularly for stubborn stain removal and rust removal in swimming pools. In addition, it is considered similar in chemical properties with citric acid, and is therefore frequently used as a citric acid substitute (Source from ascorbic acid package). Although there is evidence that ascorbic acid can promote plant health (as it does in humans), its effectiveness in cleaning and similarity to citric acid – which is commonly used as a cleaner and an ingredient in some organic herbicides – makes it a viable and inexpensive candidate herbicide (Bauernfiend, 1982).

Inert ingredients. Although it is ineffective in killing a weed, a surfactant is an essential ingredient in every herbicide. The surfactant reduces the surface tension of a liquid (like water, which has a very high surface tension) and allows the herbicide to spread over and permeate into the cell wall, rather than beading over the cell wall and rolling off of the plant (Curran, n.d.). I chose yucca (*Yucca angustifolia*) extract as a surfactant in the alternative extract. Yucca extract is known to have detergent or soap-like properties, a strong indicator of a potential surfactant. More further solidify its status as a potential surfactant, it is also sold in stores as a soil additive which allows nutrients to be more quickly absorbed into the soil and thereby more easily accessible to the plant's roots.

Mixture contents. I mixed two ascorbic acid treatments: a 10% ascorbic acid solution and a 20% ascorbic acid solution. Both concentrations consisted of 5mL of yucca extract.

Field Experimental Design

The first component of the experiment took place in a standard stone yard in order to compare the effectiveness of the alternative herbicide to that of Roundup® under the same conditions that they would typically be used by the average homeowner.

Study site. The study site was located in a stony yard behind a residence near Main St. in Toms River, New Jersey (Figure 1). The yard had roughly 13-20 cm of rocks above a weed barrier and was overgrown with diverse vegetation (Figure 2).

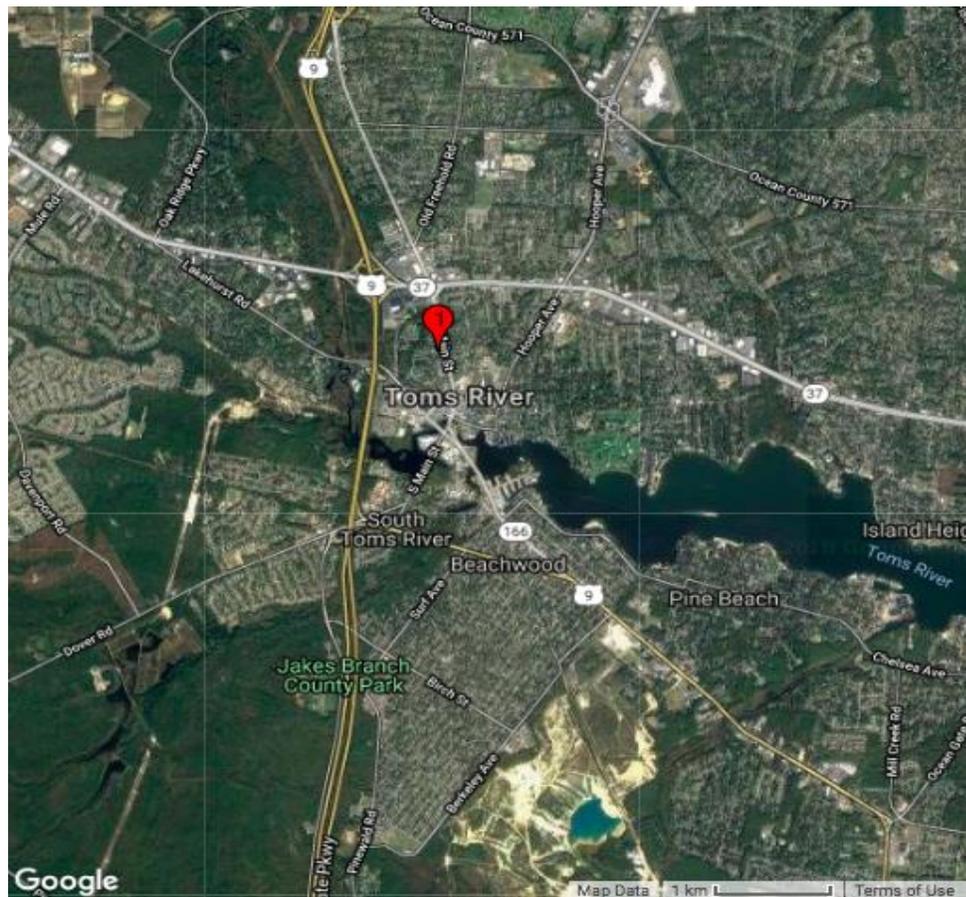


Figure 1: Satellite image of site location.

Plot selection. A total of 12 1m^2 plots were created in parts of the yard with the most plant diversity. Within each plot, I laid out a meter-square quadrat split into twenty-five 20cm sample squares. I randomly selected five squares and identified and counted all the species within them in order to determine species diversity and density within each of the plots.



Figure 2. Image of the site prior to treatment.

Treatment. Of the 12 plots, three plots were treated with 1kg of 10% ascorbic acid, three plots were treated with 1kg of 20% ascorbic acid, and three plots were sprayed with Roundup® for about 2.25 minutes. The final three were left as a control. The plots were given two treatments with their respective herbicide through the course of this experiment.

Evaluation of effectiveness. I used two methods to measure the effectiveness of the herbicides: chlorophyll health and percent cover.

Chlorophyll health. Before each treatment and for two days afterwards, I collected three leaf samples of each species available within each plot and used a Hantech Handheld Chlorophyll Fluorometer to attain an Fv/Fm value.

Percent cover. Percent cover describes the amount of green within a plot and is used to estimate plant size and the health of plants within the plot. Before and for two days following each treatment, I took an overhead picture of each plot from a height of about 1.5 meters. Each picture I installed into ImageJ, a scientific image analysis program (Figure 3). Then, I selected all of the green within the plots and attained a count of green

pixels. I divided the number of green pixels by the total number of pixels to get a percent cover value.

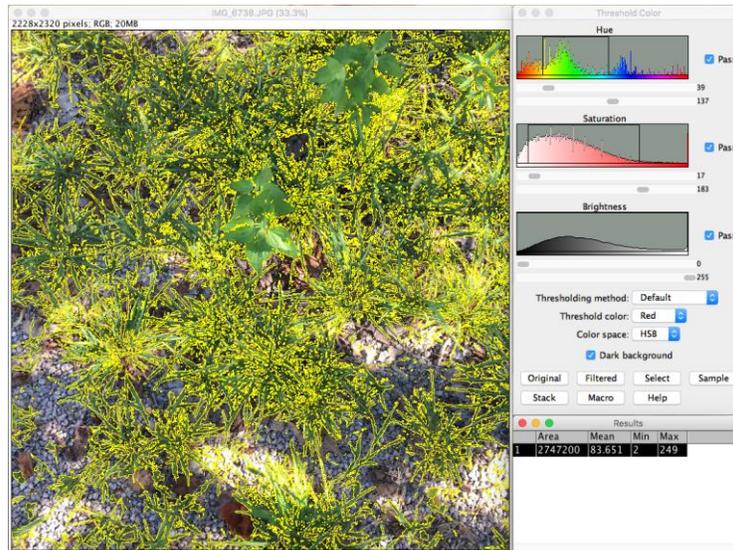


Figure 3. I used hue, saturation, and brightness sliders in Image J to select the green pixels in each of the plot images.

Regrowth in treated plots. After the final treatment, I left the plots for two weeks before returning to collect images of each individual plot to be processed for percent cover.

Data analysis. To analyze chlorophyll data, I calculated the average chlorophyll values for each of the plots, resulting in three averages per type of treatment. Next, I ran an ANOVA test between the averages of the different treatments to compare the impact of each treatment on chlorophyll health. For each type of treatment, I also grouped all chlorophyll values and ran a t-test assuming unequal variances between initial and final chlorophyll values to assess the effect of the herbicide on treated plots. I ran an ANOVA test between the percent cover values of each herbicide and a t-test between initial and final percent cover for each herbicide. An alpha of 0.05 or less was used to determine significance for all tests.

Monospecies Tray Experimental Design

Sample subjects. I chose white clover (*Trifolium repens*) as my sample subject in the monospecies trays. White clover is a common perennial weed in many New Jersey weeds and is known to have a germination period of about three days, making it convenient to grow in a lab setting (Hall, n.d.).

Tray setup and monitoring. A total of 16 trays were filled with organic MiracleGro® topsoil and vegetated with 5.0 grams of clover seeds and placed on tables outdoors in an area with minimal tree cover (Figure 4). All trays were watered equally on a regular basis depending on rainfall. I monitored and recorded rainfall data using the Weather Underground Station located in the Village of Toms River (KNJTOMSR29), which is about a mile and a half away from the tray site. Additionally, I set out two data loggers amid the trays to measure light intensity and temperature.



Figure 4. Sixteen trays were set on tables outdoors at the Save Barnegat Bay EcoCenter in an area with minimum tree cover.

Treatments. Four of the trays were treated with 100g of 10% ascorbic acid. Four trays were treated with 100g of 20% ascorbic acid. The next four were sprayed with Roundup® for 30 seconds. The final four were left untreated as a control.

Data collection. Similar to the field plots, I used percent cover to evaluate the effectiveness of the herbicides within the trays. Before and for two days after treatment, I took an overhead picture of each tray from a height of about 0.3 meters and analyzed them in ImageJ.

Results

Field Experiment

Chlorophyll health. Plots treated with Roundup® showed a significantly higher change in chlorophyll health than either the 10% or the 20% ascorbic acid ($F = 12.765$, $P = 0.0020$).

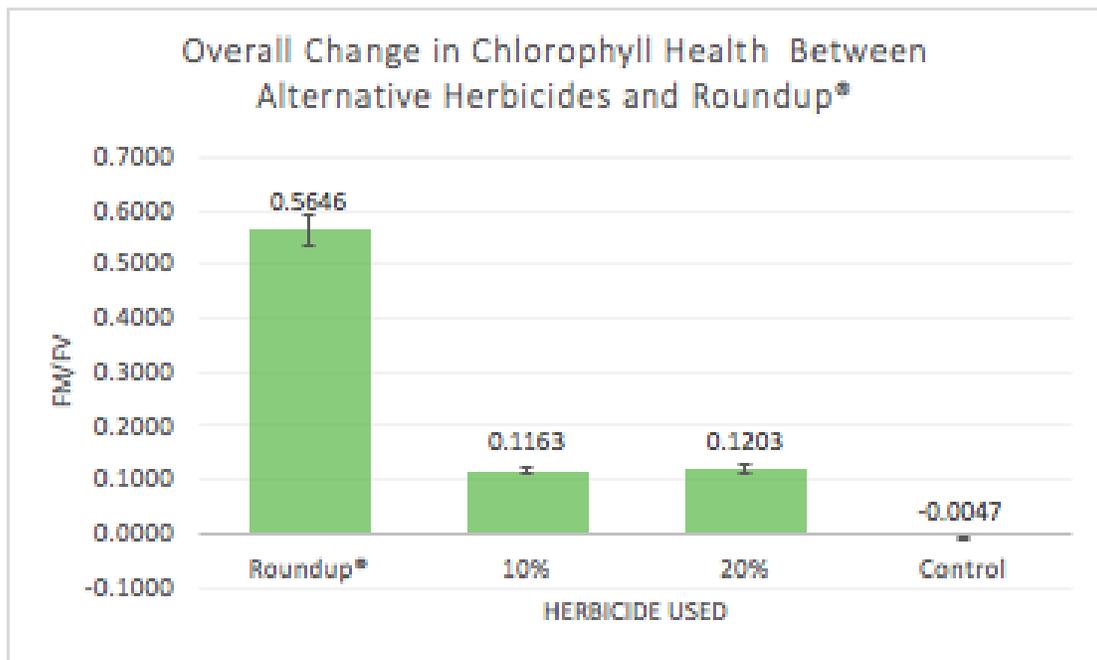


Figure 5. Comparison between change in chlorophyll health in control plots and plots treated with 10% and 20% ascorbic acid and Roundup®.

10% ascorbic acid. The 10% ascorbic acid significantly decreased the chlorophyll health of treated plots ($n = 81$, $t = 2.023$, $P = 0.0005$).

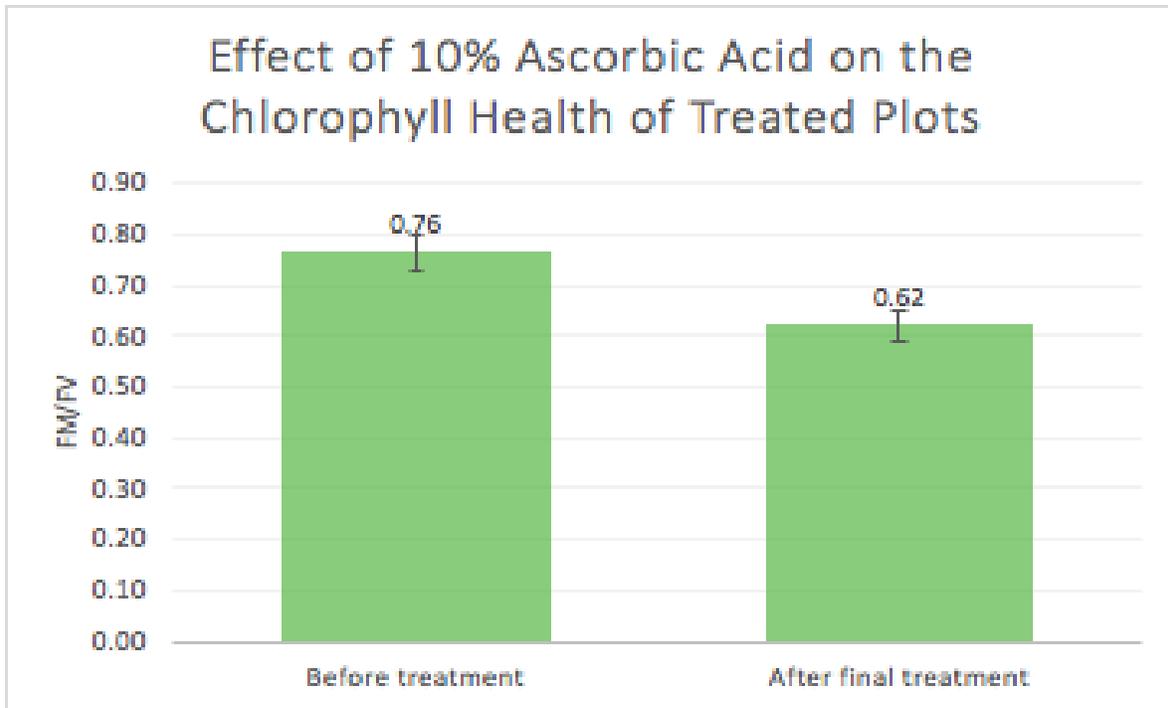


Figure 6. Mean chlorophyll health of plots before and after being treated with 10% ascorbic acid using 5% error bars.

20% ascorbic acid. The 10% ascorbic acid significantly decreased the chlorophyll health of treated plots ($n = 62$, $t = 2.010$, $P = 0.0004$).

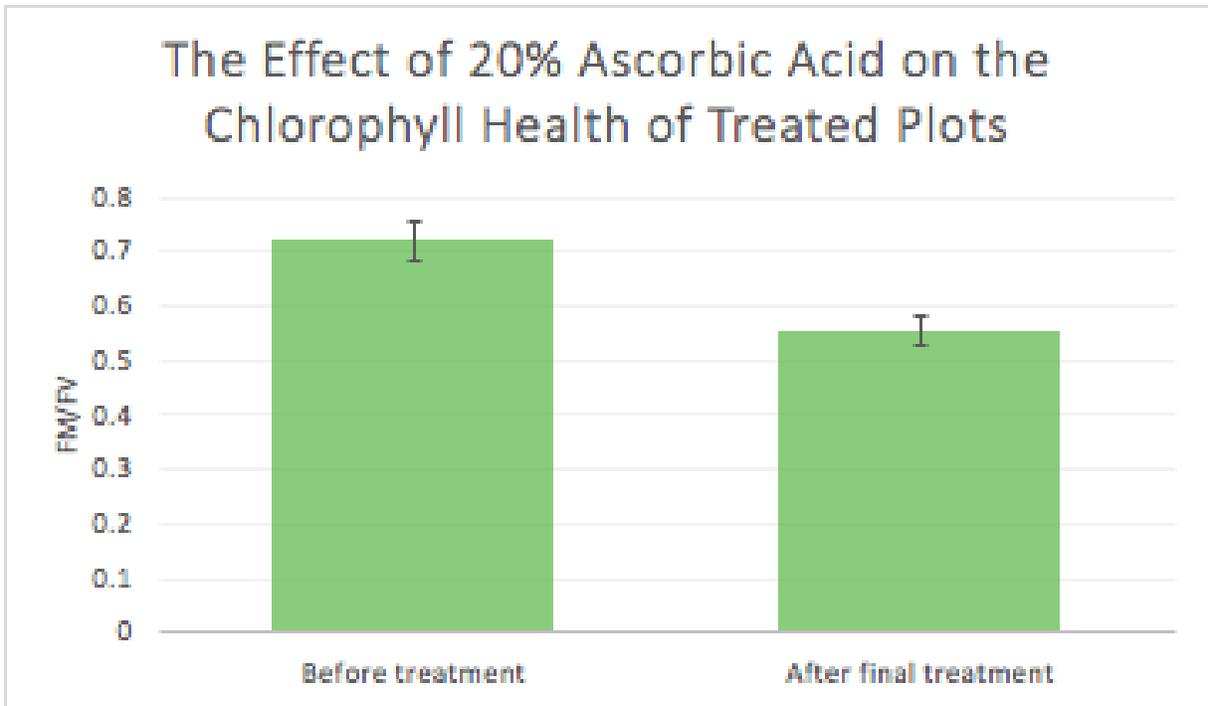


Figure 7. Mean chlorophyll health of plots before and after being treated with 20% ascorbic acid, using 5% error bars.

Roundup®. The Roundup significantly decreased the chlorophyll health of treated plots ($n = 76, t = 2.028, P > 0.0001$).

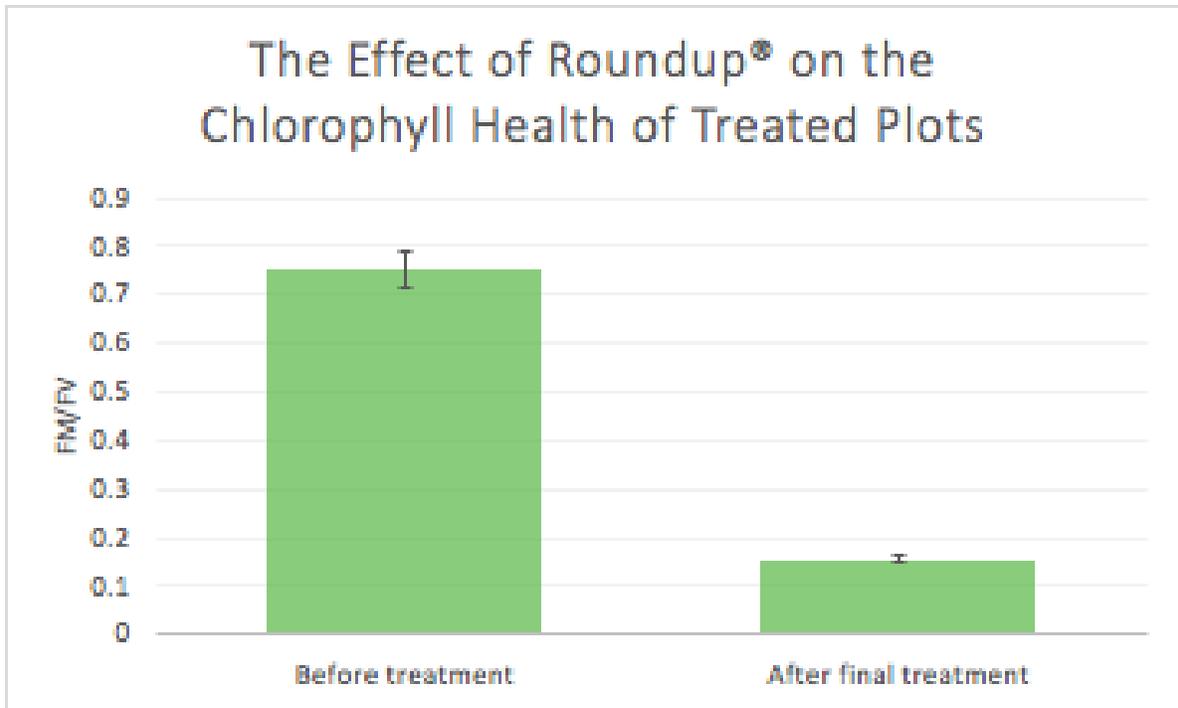


Figure 8. Chlorophyll health of plots before and after being treated with Roundup®, using 5% error bars.

Percent cover. Roundup-treated plots showed a significantly higher change in percent cover than either the 10% or the 20% ascorbic acid ($n = 24$, $F = 5.140$, $P = 0.0285$). The percent cover only increased in control plots.

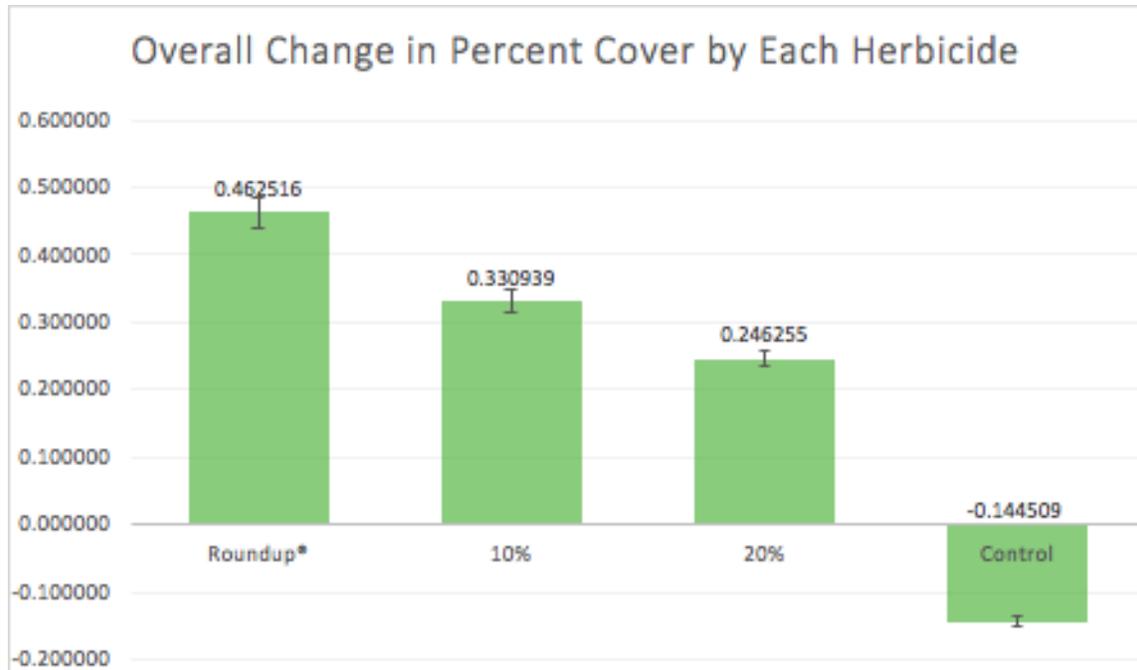


Figure 9. Comparison of percent cover between each herbicide after final application using 5% error bars .

10% ascorbic acid. There was no significant difference between initial and final percent cover in plots treated with 10% ascorbic acid ($t = 2.216$, $P = 0.1570$).

20% ascorbic acid. There was no significant difference between initial and final percent cover in plots treated with 20% ascorbic acid ($t = 0.875$, $P = 0.2370$).

Roundup®. Roundup® significantly decreased the percent cover of treated plots ($t = 9.141$, $P = 0.0028$)

Regrowth in Treated Plots. Percent cover increased in plots treated with 10% and 20% ascorbic acid and in control plots, but decreased in plots treated with Roundup® ($n = 24$, $F = 7.454$, $P = 0.0124$)

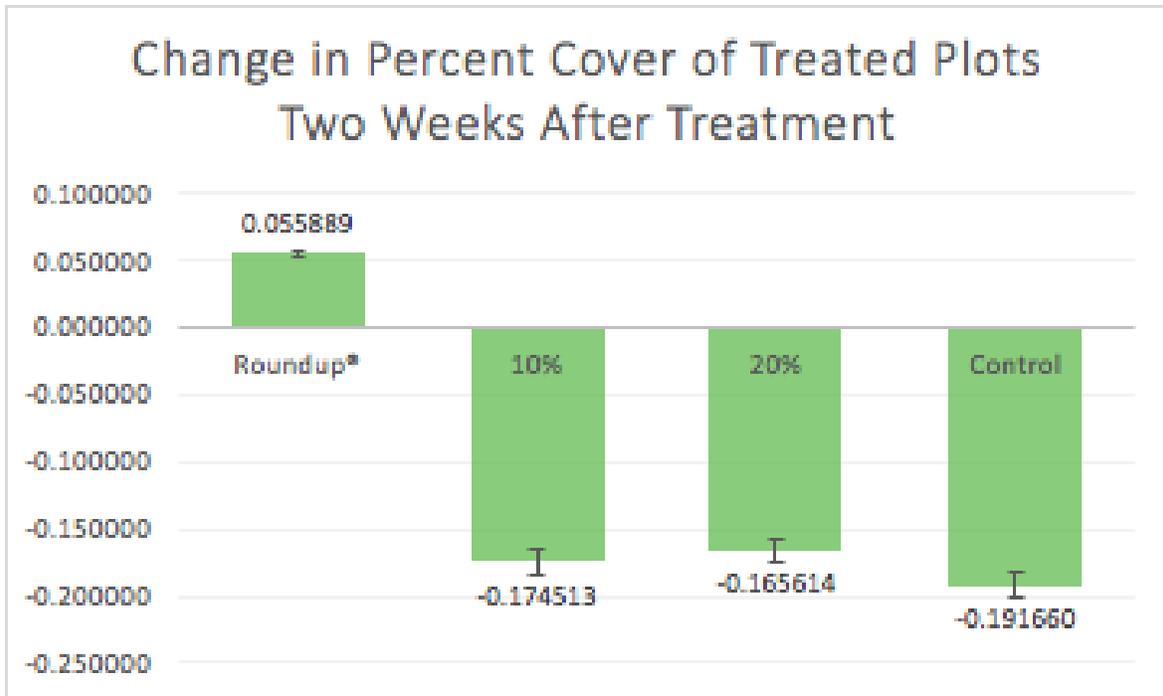


Figure 10. Comparison of percent cover between each herbicide two weeks after the final application using 5% error bars.

Monospecies Tray Experiment

Due to heavy rainfall prior to treatment, all plants in half of the trays died. Therefore, there were only two replicates per treatment remaining. The percent cover decreased in all trays, but most significantly in those treated with Roundup®.

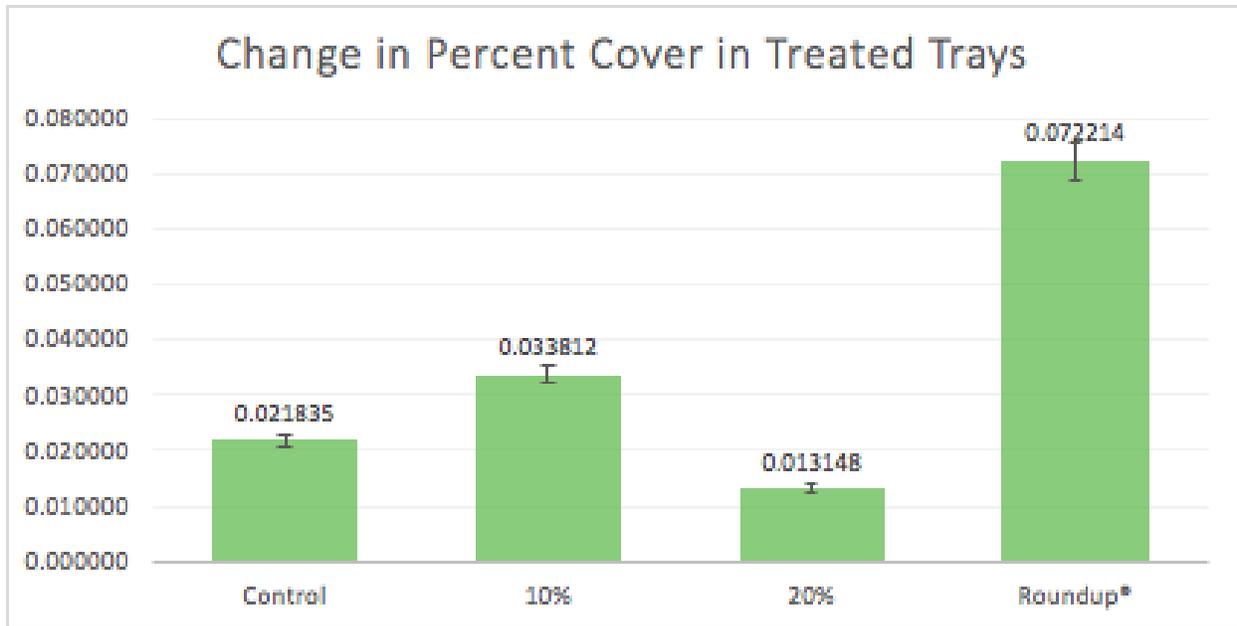


Figure 9. Comparison of percent cover between each herbicide in the monospecies trays using 5% error bars. Each treatment only had two replicates and none of the trays had a consistent amount of living plants in them prior to treatment, making them unsuitable for data analysis.

Discussion

As indicated in the results, all herbicides had an impact on their respective plots, but Roundup® was without compare the most effective of the three, thus supporting my original hypothesis. All of the herbicides showed a statistically significant decrease in chlorophyll health, but Roundup® decreased the chlorophyll health of treated plots the most and was the only herbicide to have a statistically significant change in percent cover. In addition, it was the only herbicide to show a decrease in percent cover two weeks after the final treatment. This suggests that whereas Roundup® was capable of completely killing the plants, the ascorbic acid only decreased the health of the plants within treated plots, not kill them.

I used percent cover (or percent green) in ImageJ to evaluate the macro-effect of the herbicides. This method gives me an estimate of how quickly the herbicide works and how great an impact it has on a plant—whether it kills it immediately or slowly starts deteriorating its

health. ImageJ proved to be a useful tool for doing so, as it selects individual pixels within a determined hue, saturation, and brightness level. That being said, it is flawed in that it is subjective to the researcher. For example, ImageJ cannot take into consideration shadows and reflection from the sun, leaving the researcher to decide what is green and what is not. In addition, since color is a gradient, it is more difficult to determine what level of green should be considered in percent cover. ImageJ did provide more accurate percent estimates and may be useful in future studies when analyzing vegetative cover.

Contrary to percent cover, chlorophyll health was used to evaluate the micro-effect of the herbicides—whether the plant was being affected at all by the herbicide. As previously mentioned, I used a Hantech handheld chlorophyll fluorometer to measure the chlorophyll health of the plants. To do so, I used special clips with a hole in it and clipped my sample leaves and let them sit for a few minutes. This activates the chlorophyll in the leaves and allows them to absorb a certain wavelength from the fluorometer. A very healthy plant should have an Fv/Fm value of about 0.80 or higher. If the Fv/Fm value decreased subsequent to treatment, that would indicate that the herbicide affected the health of that plant. Like ImageJ, the chlorophyll fluorometer also had some flaws, especially when it came to larger leaf samples. The leaves were not usually uniformly impacted and sometimes only the edges would be withered, but the rest of the sample was still healthy. This made it difficult to determine where the hole on the clips should go and could have had an effect on the final results.

Ascorbic acid is considered a burn-off herbicide like vinegar or limonene, meaning that it attacks the cell walls of the plants, rather than the roots. Studies have shown that such herbicides are more effective on young plants and in higher concentrations (Smith-Fiola & Gill, 2017). Also, as with any herbicide, some plants are more easily impacted by them than others.

For example, annuals are much more vulnerable and easy to impact than perennials (“A guide to weed”, 2011). The plants within plots were diverse and mostly older, consisting of up to nine different species and a maximum plant height of about 35cm. Ascorbic acid (in both concentrations) likely affected the leaves of the plants, resulting lower chlorophyll values, but overall did not reach the roots to have any major impact on the overall size and health of the plant, which is the reason for the minimal change in percent cover. Time constraints and poor weather prevented me from applying a third and fourth application to the plots, but perhaps with more applications the ascorbic acid would be more effective.

The monospecies trays were destroyed by an extreme rainfall event, leaving only eight trays and two replicates per treatment. This does not allow for credible data analysis, but could give some insight as to how to approach this experiment in the future, should someone wish to replicate or improve it. The percent cover in all of the trays decreased due to heavy rainfall, but trays treated with Roundup® showed the greatest decrease. This could be because there was an unequal amount of surviving plants in each tray. This means that even if the herbicides were potentially equal in effectiveness, the change would be lower in trays with fewer plants. In the future, this experiment should be executed in a greenhouse, where the trays are protected from extreme weather events.

The role of glyphosate in the United States continues to be a relevant and ongoing debate in the media, within the scientific community, and branching out in the judicial system. On August 10, 2018, a California jury ruled against the EPA’s conclusion that glyphosate is not a carcinogen when a terminally-ill patient with non-Hodgkins Lymphoma won a lawsuit against Monsanto for not labelling the cancer risk on Roundup® products (Gillam, 2018). The debate and uncertainty of Roundup® and other glyphosate-based products’ effect on humans and the

environment are the reason why it is essential to continue to investigate new alternatives that can compete with glyphosate in effectiveness, but without the uncertainty of future impacts.

Conclusion

The use of ImageJ or other visual graphics programs may be useful in assessing percent cover, especially using the color aspect ratios that I chose in this study. Overuse of glyphosate-based herbicides is an ever-growing problem with long-term effects that have yet to be completely understood. While ascorbic acid may not be a viable alternative herbicide for homeowners to use, we can use that knowledge to further research new alternatives.

Acknowledgements

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