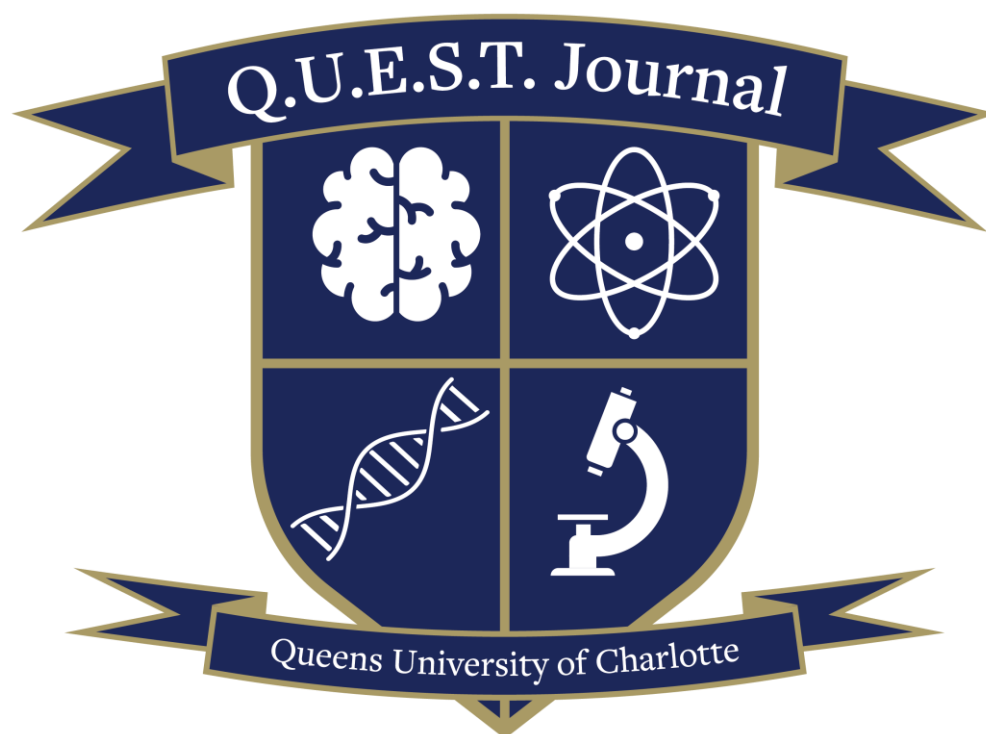


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Faculty Advisor Notes

Greetings to all those who quest for scientific knowledge! Much like 2020, 2021 continued to be a difficult time for students and faculty alike. However, much like last year, science inevitably continues, and students continue to produce important contributions to their scientific fields. Like many aspects of campus life, COVID 19 has greatly impacted student clubs and organizations at Queens University of Charlotte and the QUEST Journal finds itself without student leadership or members. Because we had papers in the process of publication, I did not feel it was appropriate to cancel the journal this year and punish those great students who had persevered in the scientific endeavor because QUEST Journal happens to find itself without student leadership at the moment. I have chosen to therefore take QUEST into receivership until it can be returned to its former glory.

I would like to thank the anonymous reviewers who have made this journal a truly peer-reviewed scientific journal. We could not create this authentic scientific publication without your time and efforts. This year has continued to be a difficult period where many of us are feeling overwhelmed. It gives me great hope whenever anyone takes time out of their day to help take part in the scientific process, especially when there is very little incentive to do so. Thank you again to our volunteer reviewers, your work is greatly appreciated. I also want to thank the authors of the paper contained within this volume: Makhaila, Josc, Irene, and Emma Rose. It would have been easier to coast through your final year at Queens without attempting to submit this paper and go through this process. Your perseverance is commendable, and you inspire me to maintain my own efforts. You were also incredibly patient with me as I went through the process of publishing your article. Thank you. Regardless of where COVID 19 will go in the future, science will continue and as long as students are willing to recognize the importance of disseminating their research, the QUEST Journal will continue to provide an outlet for communicating their research to the scientific community.

May your QUEST bring you success,

Dr. Scott M. Weir

Faculty Advisor of the QUEST Journal 2017-2021

The Effects of Hi-Yield Super Concentrate Killzall™ II on *Hexastylis* Leaf Growth, Color, and Wilting

Makhaila Anderson, Josc Fink, Irene Kuriakose, Emma Rose Stockton

Affiliations: Biology department, Queens University of Charlotte

Contact Author: Irene Kuriakose, kuriakosei@queens.edu

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ABSTRACT: *Hexastylis naniflora* (heartleaf ginger) is a small, heart-shaped plant native to Southeastern North America and is currently federally threatened. In this study, we partnered with the Catawba Land Conservancy (CLC), a non-profit organization that aims to conserve the lands of the Southern Piedmont region of North Carolina, to research how the pesticide formulation Killzall can be used to eliminate invasive plant species like kudzu (*Pueraria montana*) while conserving the non-target species *Hexastylis naniflora*. The active ingredient in Killzall, glyphosate, is used in weed control but has been found to produce negative effects on non-target plants due to residue left in the environment after application. Over the course of 5 weeks, 23 *Hexastylis minor* plants were treated and observed in a lab. The control group consisted of 4 plants and the remaining 19 were divided into four groups, receiving varying doses of the glyphosate mixture, ranging from the highest application rate on the Killzall label to 0.25x the highest application rate. Each week the plant length, leaf length, and leaf color were recorded using predetermined scales. Even after a second dose was added, little change occurred in color and growth. Since the *Hexastylis* in our study showed little to no physical changes in response to the herbicide, we concluded the plant has a high resistance to glyphosate and that the herbicide poses little risk of nontarget contamination to the plant. Past literature suggests that this unexpected resistance could be due to a hormesis effect or reduced translocation of the herbicide.

Introduction

The genus *Hexastylis* of the family *Aristolochiaceae* consists of nine species and thirteen taxa and is found across the Southeastern United States (Niedenberger 2010). Before being categorized as its own genus, *Hexastylis* was paired with the *Asarum* genus found primarily in the East, but the two genera can be distinguished by their leaf morphology. According to Davis et al. (2014), *Hexastylis* has thicker, evergreen, leaves that form a heart or triangular shape. Currently, some species in the genus are federally threatened and conservation efforts, such as creating markers to identify the genus and examining genetic diversity, are being made to protect these plants (Hamstead et al. 2015).

A threat certain *Hexastylis* species are facing is the invasion of kudzu (*Pueraria montana*), an invasive vine native to Asia. Kudzu can overtake and ultimately destroy seedlings and

trees of native plants, primarily those in the southeastern U.S. One common method of regulating this invasive species is the use of herbicides, though total elimination of kudzu requires retreatment (Berisford et al. 2006). However, an unexpected consequence of this method is the contamination of nontarget plants. Barbosa et al. (2020) reported that when glyphosate was used on weeds, the seeds dispersed droplets of the herbicide to nontarget coffee plants, leading to impaired growth.

The active ingredient in High-yield Super Concentrate Killzall™ II, which will be referred to as Killzall, is glyphosate, the most widely used systemic herbicide in the world (Christoffoleti et al. 2008; Lacerda et al. 2019). It is used for weed control and is known for showing broad-spectrum control and low toxicity in non-target plants (Lacerda et al 2019). Under acute toxicity conditions, the U.S. Environmental Protection Agency

defines glyphosate as “practically non-toxic and not an irritant”, but glyphosate has potential for harmful toxic effects, as residue remains in the environment (Bai and Ogbourne 2016). Glyphosate, having an extended half-life and a slow degradation process, increases the risk of persistence, especially in the event of reapplication (Bai and Ogbourne 2016). It has been indicated that medium to coarse sized droplets negatively affect retention in plants compared to fine droplets and that plant absorption and translocation increase with droplet size (Feng et al. 2003).

The steps that go into creating pesticide guidelines and the toxicity tests prior to consumer use is a crucial part of how we can best protect our environments from over toxification. It is law to abide by the pesticide’s label, but some pesticide formulations can be more toxic than the label states. This could be due partially to non-active ingredients added after toxicity testing is done on the active ingredient. However, it is most likely due to the main active ingredients in the pesticide which in this study was glyphosate. This difference can have a great impact on the target plant, but especially is of concern for the non-target plant (White and Boutin 2007).

Glyphosate is currently being used as an herbicide by the Catawba Lands Conservancy (CLC), a non-profit organization created to permanently conserve and manage the land of the Southern Piedmont of North Carolina for community benefit (<https://catawbalands.org/who-we-are/>). For this experiment, we will be partnering with the CLC to research how the pesticide Killzall can be used to eliminate the invasive plant species kudzu and conserve the non-target species *Hexastylis naniflora*. The CLC will then be able to apply these data to their pesticide use in protected areas of the Southern Piedmont of NC.

As glyphosate is applied to *Hexastylis*, physiological changes may be indicated by changes in leaf color. The control should remain green as there is no treatment of the glyphosate. The treatment plants, starting with the lowest dose, should show growing signs of wilting and an increase in the coverage of yellow on the leaf as the dose concentration increases. Plants that

turn brown, have extreme yellowing, and wrinkles should be considered unrevivable. For our experiment, we expect that the application of glyphosate to the *Hexastylis* plants will lead to changes in color and increased wilting, indicating that the herbicide will display non-target toxicity to the threatened species.

Materials and Methods

Plants Species and Experimental setup:

Wild ginger (*Hexastylis minor*, collected from CLC Preserves by Sean Bloom) plants were received in the lab with initial leaf lengths, color, and sizes recorded. *Hexastylis minor* will act as a surrogate species for the endangered *Hexastylis naniflora*. Once collected, the plants remained in the Queens University of Charlotte greenhouse for 2 days and in a lab closet for 7 days until the start of treatment.

The plants continued to grow in 12.7 cm² plastic pots with soil and remained uncovered in the lab. Part of the soil provided from the CLC Preserves was mixed with soil from the Queens University of Charlotte greenhouse and remained uncovered in the lab. The soil provided was Daddy Pete’s Plant Pleaser Potting Mix. The main ingredients in the potting mix were aged pine bark fines, cow manure, perlite, dolomitic lime, and gypsum. These ingredients add nutrients, minerals and help prevent the soil from compacting to provide the plants with an optimal condition for growth. The lab temperature was measured using a temperature logger and recorded once a week. The temperature was recorded to be consistently between 22°C and 23°C. Once the doses were given, the plants were moved and placed in a storage closet with four 40 watt CFL light bulbs pointed at the plants that gave off 450 lumens, as the plants needed to be kept out of direct sunlight. The plants stayed in the same shaded location night and day for the remainder of the experiment.

For this study the CLC provided us with 23 plants. Four of the 23 plants were not treated and were considered the control group. There were 4 groups of plants dosed with glyphosate. The control and lowest dose group each had four

plants in their groups. The remaining three treatment groups had 5 plants in each group that received different levels of pesticide solutions.

Preparing and Applying the Pesticide:

Our highest dose was the highest Killzall label application rate of 4 quarts/acre (935.4 uL/m²). We scaled this application rate down to the area of the plant containers, which are 161.29 cm². For example, for 935.4uL/m², we added 15.1 uL to each replicate plant container. Our other treatments were lower applications of 701.6, 467.7, and 233.9 uL/m² (Table 1). For each application rate, we scaled the volume applied down to 161.29 cm² containers.

Each of the 4 dilutions contained a mixture of the calculated pesticide dosage with 10 mL of water. From each mixture, 10mL was pipetted into a spray bottle and applied evenly across the surface of the plant leaves and container. Application of pesticide occurred twice. Once at the beginning of the experiment in week one and a second time at the beginning of week three. A second dose was applied because no changes were observed in the first doses given to the plants. For the second dose the concentrations were the same for each group. The plants were also watered the day prior to applying the second concentrations.

Prior to the initial dosing, wilting/leaf size, and color of leaves were observed. Measurements were then repeated weekly on Friday. In week three, another dose was given to all *Hexastylis* plants, as no changes in measurements or physical characteristics were found in previous weeks. In week 4, final plant data were recorded and compared to week 1 of our study.

Data Collection:

Plant length was measured in centimeters using a ruler. The color of the leaves was recorded using a leaf color chart (<https://cguru.usda.gov/carya/Manual/lfcolor.html>). Leaf wilting was monitored using a similar wilting scale (Wong et al. 2005). The endpoint to determine death of the plant was 8-10 on the color scale and over 5 on the leaf wilting scale.

Table 1. Volume of Killzall dosage calculated for each treatment group for the surface area of the containers (161.29 cm²) used to hold Hexastylis minor plants. Treatment group doses are based on label specified application rates.

Treatment Group	n	Volume of Hi-Yield Super Concentrate Killzall™ II added to each replicate (uL)
Control	4	0
233.9 uL/m ²	4	3.70
467.7 uL/m ²	5	7.50
701.6 uL/m ²	5	11.3
935.4 uL/m ²	5	15.1

Results

Overall, there were no noticeable changes between plant groups regarding wilting and leaf color, there was not enough visible evidence of change in the plants so we did not conduct statistical analysis of the plants features. There was also no noticeable change in leaf size for each of the plants. All plants had a leaf color score of between 1 and 2, and a wilting score of 0. At the end of the study leaf wilting continued to remain minimal.

Discussion

This study was used to assess the effect of the formulation Hi-Yield Super Concentrate Killzall™ II on *Hexastylis* leaf growth, color, and wilting. We predicted that Killzall would produce negative effects on each physical characteristic recorded in this study. In our four-week study, we did not observe changes in *Hexastylis* leaf growth, color, or wilting. Since there were no noticeable physical characteristic changes found after applying the first dose of Killzall to our *Hexastylis* groups, a second round of Killzall dosing was applied at the same concentration as the first treatment. This was used to determine if time was a factor that affected the outcome of our study, or if there was potential that our first treatment was not potent enough to produce an effect. The second round of dosing was conducted as a

continuation of our first experiment, where the only change in our methods was including the second dose. There were no visible changes in leaf growth, color or wilting found after the second application of Killzall on *Hexastylis* leaf growth, color, and wilting.

Oftentimes plants that are sprayed with an herbicide containing glyphosate will present with negative effects. However, in our experiment no plants died and there were no noticeable signs of wilting and/or color change which lead us to speculate the plants may have some form of resistance to glyphosate. Populations of *Lolium rigidum*, *Lolium multiflorum*, and *Conyza canadensis* have shown resistance to glyphosate which may be due to reduced translocation because glyphosate accumulated in leaf tips rather than the roots of the plants (Preston and Wakelin 2008). In one study, glyphosate-resistant glyphosate-treated soybeans benefited from the aminomethylphosphonic acid (AMPA) part of an herbicide formulation 28 days after the treatment was administered (Ding et al. 2010). Through an effect known as hormesis, where low doses of a usually toxic agent help with plant growth, one study showed that glyphosate was able to assist in increasing the dry weight of sugarcane and eucalyptus plants by means of photosynthesis and an effect on CO₂ (Renan et al. 2017). Another study presented similar results reporting that even some target plants receive low enough doses of glyphosate to help with growth by any combination of the following: the action of photosynthesis, having a shorter life cycle, inducing shikimic acid accumulation, and increasing seed production (Brito et al. 2017). Hormesis and reduced translocation could be possible explanations as to why we did not see any negative changes to the ginger plants.

For future experiments, factors such as temperature, *Hexastylis* growth stage, droplet size and application time could be altered and examined for effects on glyphosate efficacy. In Ganie et al. (2017), glyphosate efficacy on ragweed was analyzed at both high temperatures (29° C) and low temperatures (20°C). Results found that there was improved efficacy and less resistance to glyphosate at high temperatures, due to better absorption and translocation of the herbicide at

these temperatures. The study recommended applying glyphosate at warmer days, approximately at 29°C, which could be a change to consider for increased efficacy as our herbicide was applied at 22-23°C. Similarly, Singh and Singh (2004) concluded that glyphosate is more effective at earlier growth stages in citrus groves weed species, possibly due to the larger surface area of growing leaves allowing for better absorption of the herbicide. Also, as previously mentioned by Feng et al. (2003), droplet size can impact the retention and absorption of glyphosate in plants, so droplet size could be modified for future studies. A fourth factor to consider altering is time as Mohr et al. (2007) found that applying glyphosate to broadleaf weeds at 6:00PM was more effective in comparison to application at 6:00AM. However, for barnyard grass and velvet leaves, applications at 2:00PM were more effective than 8:00PM and soybean was found to be unaffected by differing time applications (Mohr et al. 2007). These changes in glyphosate efficacy at different times could be due to the diurnal changes of leaf movements. It has also been found that different soil sorption characteristics have the ability to affect mobility and leachability of a compound, where glyphosate is strongly absorbed by soil minerals due to competition between glyphosate and phosphate for surface sites (Broggaard and Gimsing 2008). According to de Jonge et al. (2001), glyphosate sorption capacity will be reduced with increases in phosphorus status in the soil. Both soil sorption and application times could be investigated with *Hexastylis* to find conditions that improve herbicide efficacy.

Besides altering environmental factors, adding organosilicon adjuvants could improve the effectiveness of glyphosate as well. Organosilicon surfactants are added to glyphosate to maintain its efficacy in rainfall, which often dilutes the herbicide (Reddy and Singh 1992). Silicone adjuvants are able to help glyphosate be more effective by increasing how well it is absorbed by the plant and its uptake time. When the adjuvant Kinetic was added to glyphosate and applied to six different weed species in Reddy and Singh (1992), the herbicide showed improved efficacy in rainfall for five of the species. While our experiment was conducted indoors

and rain was not a factor, applying a silicone adjuvant to glyphosate applications of *Hexastylis* to prevent dilution of the herbicide could be a consideration for future experiments.

Based on the results we generated, we concluded that the glyphosate at the 4 different doses we calculated may not have severe effects on non-target plants, however we cannot support this statement solely based on our study. Other research we have found describes the toxicity that plants and organisms get that comes from exposure to glyphosate and that research would support the statement that non-target terrestrial and aquatic plants and organisms are at risk (Gill et al. 2018; Schweizer et al. 2019; de Saraiva et al. 2016), including fish, microorganisms, plants, and small animals (Murussi et al. 2016). Future studies could include the use of different plants and different pesticide combinations to show different hardiness of plants as well as a variety of plant responses. Studies such as these are influential to the way we use pesticides and can help us determine if we should change the way we use pesticides based on risk assessment and experimentation. Risk of glyphosate on target organisms can be shown by herbicide resistance of organisms. In a New Zealand study assessing the risk of herbicides on various wheat and barley crops, species were classified as low risk if they showed high herbicide resistance (Ngow et al. 2020).

Since the *Hexastylis* in our study showed little to no physical changes in response to the herbicide, we concluded that the plant has high resistance to glyphosate and that the herbicide poses little risk of nontarget contamination to the plant. Therefore, based on the low risk of the herbicide in this study, the CLC can be confident in the safety of *Hexastylis* even with exposure to Killzall.

Acknowledgements

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