# RESISTANCE TO THREE COMMON HERBICIDES IN CHAMELEON PLANT (*HOUTTUYNIA CORDATA* THUNB.), A HIGHLY INVASIVE EXOTIC SPECIES


by

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*This thesis is dedicated to my wife who supported me through it all.*

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# TABLE OF CONTENTS

# LIST OF FIGURES

# ABSTRACT

Chameleon plant (*Houttuynia cordata* Thunb.) is native to Southern and Southeastern Asia. It can reproduce sexually through seeds and asexually through rhizomes and is invasive in multiple countries including the U.S. There has been much research on *H. cordata* as a medicinal species, and its potential as an invasive species is well documented. However, its herbicidal resistance has not previously been quantified. The objective of this study is to assess *H. cordata's* resistance to herbicides. This study consisted of two rounds of tests to examine the resistance of *H. cordata* plants to three commonly used herbicides: SpeedZone, Weed-B-Gon, and Roundup. Two concentrations of each herbicide were used during each trial in the study: the recommended concentration and twice the recommended concentration. Herbicide treatments were applied outside the greenhouse. Herbicides were sprayed uniformly on the plants until the herbicide was dripping off the leaves. The growth of the treated plants was then monitored in the greenhouse. The herbicides generally reduced growth of the plants temporarily. However, plant extermination was not achieved. Plant samples from all herbicidal treatments regrew from rhizomes after all herbicide treatments. Results from the study showed that *H. cordata* could not be controlled by the recommended concentrations of herbicides commercially available for horticultural uses in the U.S. Doubling the recommended herbicide concentration was also ineffective in exterminating *H. cordata* plants. This research clearly showed that *H. cordata* has the potential to become a highly invasive species with the potential to negatively affect the ecological integrity of many communities in the U.S.

# CHAPTER 1. INTRODUCTION

Humans introduce non-native plants intentionally and unintentionally into almost every area in which they live or travel to (Gallardo & Aldridge, 2013). Studies have shown that invasive plant species' richness has a positive correlation with factors such as population density and tourism (Bhattarai et al., 2014). Currently, ornamental horticulture is the fastest growing segment of U.S. agriculture (Li et al., 2004). Much of this rising interest in horticulture can be attributed to the shift in overall focus from food growth to more personal horticulture. Personal gardens, however, often have a higher number of alien species than they do of native species (Smith et al., 2006). In fact, many invasive plants were originally ornamental plants that escaped the confines of a personal garden. It is estimated that more than 5,000 species of non-native ornamental plants have become naturalized in non-managed habitats in the U.S. due to improper supervision (LaRoe, 1995). Also, with the advent of buying plant species online, inexperienced horticulturists can purchase a plant based on how it looks without understanding the potential invasiveness of said plant if not properly contained. Due to these processes of globalization in regard to purchasing plants from all over the world, the number of invasive species is continually increasing (Lambertini et al., 2011, Sheppard et al., 2014). While some studies have observed native species adapt evolutionarily to compete with non-native competitors, many times the native plants will decline in abundance and dominance and, in extreme cases, localized extinction (Strauss et al., 2006, Sax et al., 2007). The spread of invasive plants is currently the second greatest threat to biodiversity loss in the United States, the first being loss of habitat (Pimental et al., 2000). Currently there are, according to the United States Park Services, approximately 1.4 million acres of United States land and water covered by exotic invasive plants (Perles, 2022). Invasive plants commonly cause urban and rural problems such as reduced water quality, which produces economic issues while people attempt to fix those problems (Potgieter et al., 2017). Ecologically, invasive plants can modify richness of both native and exotic species (plant and animal) (Fletcher et al., 2019). Usually, when exotic invasives are introduced to a non-native environment, the richness of native species is reduced and the abundance of exotic species increased (D'Antonio & Meyerson, 2002). Invasive species are also less likely to experience herbivory by native fauna, allowing them to spread uninhibited when native plants are preferably preyed upon. One study showed that non-native invasive species experience approximately 96% less herbivory than native species (Cappuccino & Carpenter, 2005). Non-native plants are also less susceptible to infection by viruses and fungi (Power, 2003). Moreover, invasive plants can also disrupt native plant-pollinator mutualisms by attracting pre-existing native pollinators away from the native plants, and therefore cause a negative impact upon the reproduction of the native plant species (Stout & Tiedeken, 2016).

One such invasive species, *Houttuynia cordata* Thunb., also known as the chameleon plant for its variety of colors, is a commonly used plant in personal gardening and is also a common herb used in certain cooking recipes. As such, it is easily obtained online as a plant and in grocery stores as freshly cut stems, both of which can be easily planted and grown in soil. This species possesses an aggressive system of rhizomes and has been anecdotally reported to be extremely difficult to eradicate and very resistant to herbicides. It is the purpose of this study to test these claims and, if found to be true, warn both the scientific community and the general populace about this potentially problematic plant.

# CHAPTER 2. LITERATURE REVIEW ON *HOUTTUYNIA CORDATA* THUNB.

*Houttuynia cordata* is a perennial ground cover plant native to Asia. Its heart shaped leaves can measure up to 7.5 cm in length and width (*Environment: Bay of Plenty Regional Council: Toi Moana*, 2003). Its leaves can display a variety of variegated colors including green, white, yellow, and red. The brighter colors, yellow and red, are more distinct when the plant grows in full sun. The leaves are simple with alternate arrangement along the stems and have been said to have a peppery scent when crushed. It is one of six species in the Saururaceae family which are all characterized by their unique flowers, aromatic properties, and creeping rhizomes. The rhizomes can easily break off and sprout new plants, and *H. cordata* produces clusters of tiny flowers on 2.5-to-3.8-cm spikes. At the base of each spike are four white petal-like structures (*Environment: Bay of Plenty Regional Council: Toi Moana*, 2003).

*Houttuynia cordata* has been used in traditional medicine for at least two millennia. In Southwest China, the consumption of *H. cordata* dates as far back as the Eastern Han dynasty, 25 C.E. to 220 C.E (Wang et al., 2021). It is currently used among diverse cultures across Asia for a variety of medicinal purposes. In Northeast India, *H. cordata* is eaten raw in salads for lowering blood sugar levels (Seal, 2011). A juice extract of the leaves has been used to treat cholera, dysentery, and blood deficiency (Hynniewta & Kumar, 2008). It has also been used as an astringent for drying out the skin (Seal, 2011). In Indochina, the plant is used for its cooling properties and is also used to stimulate menstrual flow (Lu et al., 2006). The leaves and stems can also be harvested during the growing season and made into decoctions. These are used internally to treat ailments such as cancer, coughing, dysentery, intestinal inflammation, and fever (Hynniewta & Kumar, 2008). These decoctions are also used externally to treat snake bites and skin disorders. Other traditional uses for *H. cordata* include treating measles, gonorrhea, eye troubles, swelling, pus drainage, and promoting urination (Lu et al., 2006).

*Houttuynia cordata* also has uses in modern medicine. *H. cordata* has been found to inhibit anaphylactic responses in mice (Li et al., 2005). It also has been found to have anti-mutagenic properties which may have use as cancer preventatives, including adjuvanticity (Chen et al., 2003, Kim et al., 2001, Wang et al., 2002). According to one study, *H. cordata* has been shown to have anti-inflammatory properties (Kumar et al., 2014). Other studies have shown the plant to have both anti-viral and anti-bacterial properties (Kumar et al., 2014, Hayashi et al., 1995). Another study found *H. cordata*. to have anti-obesity properties in mice (Miyata et al., 2010). Lastly, *H. cordata* has been shown to have direct anti-cancer properties by inducing apoptotic death in human cancer cells (Lai et al., 2010).

Although *H. cordata* may have many beneficial medicinal uses, it can pose a serious risk to non-native environments. As previously mentioned, *H. cordata* is a plant native to the Asian continent. It is specifically from the wetter areas of the Himalayas, allowing it to survive in damp and wet conditions where many other plants cannot. However, it is not an obligate aquatic plant. In order to thrive, *H. cordata* only needs enough rainfall or watering as other plant species that do not reside in damp environments (Wang, personal communication, 2022). Due to its variety of colors, it has become a sought-after addition to personal gardens in the U.S. Unfortunately, not all horticulturalists or gardeners who purchase this plant are aware of its potential for aggressive growth due to its rhizome system. This lack of consumer knowledge has facilitated the introduction and spread of this invasive species. Another reason this plant has spread so efficiently is because of its versatile medicinal purposes. Since 70–95% of the population of developing countries rely on traditional medicines and herbal remedies for primary care (Robinson & Zhang, 2011), purposeful growth of a medicinally beneficial plant such as *H. cordata* should be expected. The plant has also been suggested as a candidate for soil remediation at contamination sites due to its strong uptake of heavy metals including lead and mercury (Wu et al., 2009, Wang et al., 2021). With these positive uses for people, *H. cordata* has become a widely distributed plant globally.

According to the Global Invasive Species Database (GISD), *H. cordata* has established invasive alien ranges in the United States and New Zealand, and it has been reported as being naturalized in South America (GISD, 2006, Alves et al., 2013). In 2019 a naturalized population of *H. cordata* was found in Italy in the riparian zone of the river Stella with aggressive expansion of the population reported the next year (Liccari et al., 2021). *H. cordata* has been reported to survive in

USDA hardiness zones of 5 to 11 (average winter temperature range -28.9°C to 7.2°C) which would allow the plant to grow in most areas in the continental U.S., Europe, and Asia.

Most of the research conducted on *H. cordata* focuses on its medicinal uses. Some of the research mentions, but does not expand upon, *H. cordata*'s resistance to herbicides (Liccari et al., 2021). While it is important to explore the uses of plants, one must consider the potential issues of introducing exotic species into new communities and ecosystems. Since the impact of *H. cordata* has not yet been determined to pose a serious threat to any area, its removal has not been as thoroughly researched as some plants: kudzu (*Pueraria montana*) and Japanese honeysuckle (*Lonicera japonica*). Currently, the only known ways to completely remove *H. cordata* from an area is to manually remove the plant along with the surrounding soil and wait to see if the plant recovers and repeat the process. This can require multiple years if the plant is well established in the area. A recently proposed method of removal is the use of metasulfuron herbicide. However, this is a very strong herbicide that requires experience in herbicide application that most gardeners lack. Furthermore, since *H. cordata* is normally grown in personal gardens, gardeners usually loathe the use of a strong herbicide near other plants. The purpose of this study is to examine *H. cordata's* resistance to commonly available herbicides at different concentrations.

# CHAPTER 3. MATERIALS AND METHODS

This study consisted of two trials performed during the years 2021 and 2022. The trials will be referred to as the 2021 trial and the 2022 trial. The setup of these two trials was similar enough that they will be described together, noting where there were differences. This experiment was duplicated using two ecotypes of *H. cordata*. Both ecotypes were subject to the same methods. The study was conducted in the Department of Biology greenhouse at Indiana University Purdue University Indianapolis (IUPUI) Indianapolis, Indiana (39°46'24.0"N, 86°10'14.2"W).

## 3.1 **Propagation of Plants**

One ecotype of *H. cordata* was originally purchased from Saraga International Grocery in Indianapolis, Indiana as cut stems. These stems were allowed to root and become established plants in pots in the IUPUI greenhouse (referred to as “store-bought type”). In March 2021, the second ecotype was dug from an established colony in a private garden in Carmel, Indiana (referred to as “naturalized type”). These plants were allowed to become established plants in pots in the IUPUI greenhouse. The store-bought type was characterized by the typical appearance of *H. cordata* as previously described and had been rooted and in the greenhouse long enough for the rhizomes of both ecotypes to be equitable. The naturalized type was characterized by shorter stems and thicker, more leathery leaves. Leaves of both ecotypes had a solid green coloring and were not variegated. For the 2021 trial, 60 standard 14 cm height x 11 cm diameter pots by Dillen Products were filled with potting mix (Pro-mix bx mycorrhizae) and planted with three stems of the store-bought ecotype. A second set of 60 pots were filled and planted with three plants of the naturalized ecotype. All potted plants were allowed to grow and become established for eight weeks. After that time, the plants were compared visually, and the least healthy plants were removed until 48 pots of each ecotype remained. The 48 pots of each ecotype were divided into six groups of six experimental pots and 12 control pots for each ecotype. For the 2022 trial, survivors from the 2021 trial were allowed to recuperate for six months then were propagated by splitting rhizomes into 120 pots of the same brand of pots of the same dimensions. These plants were allowed to grow for another two months. The pots were then visually inspected to reduce the number to 112 pots per ecotype. The

112 pots were then randomly assigned to an herbicide treatment, resulting in six treatments with 16 replicates and a control that also had 16 replicates.

## 3.2 Selection, Preparation, and Application of Herbicides

Three herbicides were chosen for this study: SpeedZone (produced by pbi/ Gordon Corporation, Shawnee, Kansas), Weed-B-Gon (produced by Scotts, Marysville, Ohio), and Roundup (produced by Bayer, Leverkusen, German). These herbicides represent a range of treatment types. Roundup is a commonly used non-selective herbicide with isopropylamine salt as its active ingredient. Weed-B-Gon is a commonly used broadleaf specific herbicide with 2, 4-D, dimethylamine salt (8.66%), dicamba (0.37%), and MCPP-P, DMA salt (2.13%) as its active ingredients. SpeedZone is another broadleaf specific herbicide that uses slightly different active ingredients, including carfentrazone-ethyl (0.62%), 2, 4-D 2-ethylhexyl ester (28.57%), mecoprop-p acid (5.88%), and dicamba acid (1.71%). Herbicides were purchased as concentrates but diluted to two concentrations before use: recommended concentration and double the recommended concentration. All plants, the two ecotype groups and the control group, were moved outside the greenhouse to a low-wind area. Plastic spray bottles were used to apply the herbicide to the leaves and stems of the plants until the herbicide was dripping from the leaves, approximately three sprays each. The same procedure was used to spray the control group with tap water. The plants were returned to the greenhouse after the herbicide and water spray had dried. Pots were watered as needed for six weeks before data collection. This process was used for both the 2021 and 2022 trials.

## 3.3 Data Collection

Only the number of stems above the soil surface for each pot was counted in the 2021 trial. Data collection for the 2022 trial included number of stems at time of collection, stem height, leaf count, leaf area pre- and post-treatments, and rhizome dry weight. Stems were straightened as much as possible and stem height was measured with a ruler from the highest leaf node on the stem to the first root node below the surface of the soil. Leaves were counted for all plants. Leaf area, before treatment, was calculated from ruler measurements of height and width for the naturalized type. Leaves for the store-bought type were too numerous to be done fully by hand. Therefore before the treatment of the store-bought ecotype, leaf area was obtained by hand measuring the total leaf area of 10 stems randomly chosen from the sample group and taking the average of these stems. The number of plant stems in each store-bought pot was counted, then multiplied by the average leaf area. Average and total leaf area after treatment for both ecotypes was measured using a LI-COR LI-3100 Area Meter (LI-COR, Inc., Lincoln, Nebraska), which was calibrated every 30 minutes. Measurements were made over the course of two weeks, and samples were done in batches of five pots from each group per day to account for any growth that may have occurred during that time. After the removal of the stems, rhizomes were removed from the potted soil, washed, and manually scrubbed under a stream of tap water to remove any roots and root hairs. Rhizomes were then placed in paper bags and dried for 48 hours in an isotemp oven (Fischer Scientific, Pittsburg, Pennsylvania) at 80$^\circ$C. The dry rhizomes were then weighed using a Denver Instrument Company Model TL-104 scale but like.

### 3.4 Statistical Analysis

The effects of herbicidal treatments were examined using single factor analysis of variance (ANOVA). Comparisons between means of untreated and treated plant were made using least significant difference. Comparisons between means of plants of different concentrations of the same herbicide were also made using least significant difference. Regressions were also performed to compare the relationships between the number of stems in pot and total leaf area and of the number of leaves in a pot and total leaf area. Significance for the regression plots was determined by comparing the correlation coefficient to the positive critical value which was 0.426 at 95% confidence.

# CHAPTER 4. RESULTS

As the 2021 test was a proof-of -concept test, the only parameter tested was number of stems. With the data set being so small, its data is included in the number of stems section instead of having its own section.

## 4.1 Number of Leaves

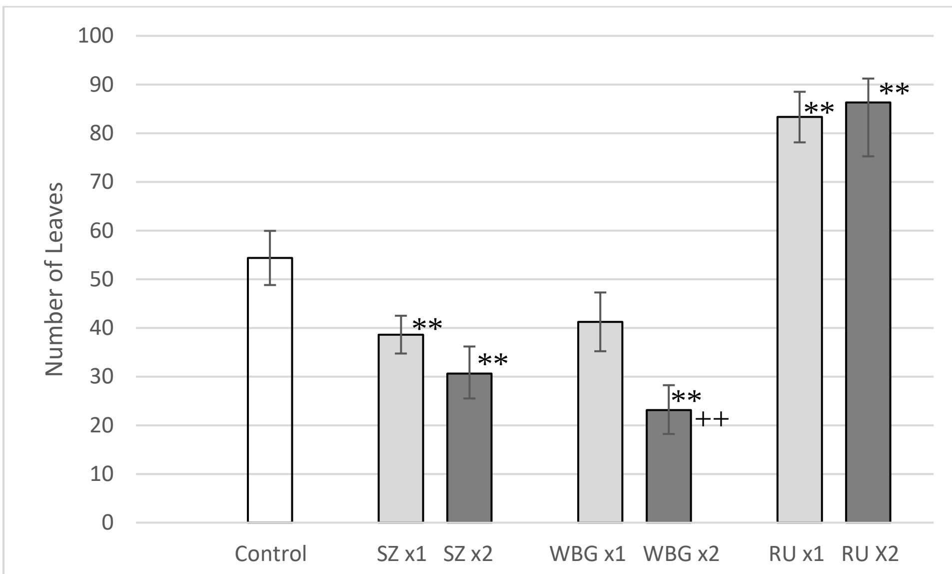


Figure 4.1 Average leaf number of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). Study was conducted in the Indiana University Purdue University Indianapolis (IUPUI) Department of Biology Greenhouse. n=16 for each treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. Plus signs (++) indicate significant difference between treatments of the same herbicide at $P < 0.01$.

In Figure 4.1 the *H. cordata* plants naturalized in the control group had 54.5 leaves on average. The plants treated with recommended and doubled concentrations of SpeedZone had 38.6 leaves and 30.6 leaves, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had 41.3 leaves and 23.1 leaves, respectively. The plants treated with recommended and doubled concentration of Roundup had 83.3 leaves and 86.3 leaves, respectively.

All treated plants, except those treated with the recommended concentration of Weed-B-Gon, had a significantly different leaf count from the control group. Plants that were given the broadleaf-specific treatment had a lower number of leaves, and those treated with Roundup had a higher number of leaves. Plants treated with Weed-B-Gon were also the only groups of plants that produced results with a significant difference between the recommended and doubled concentrations.

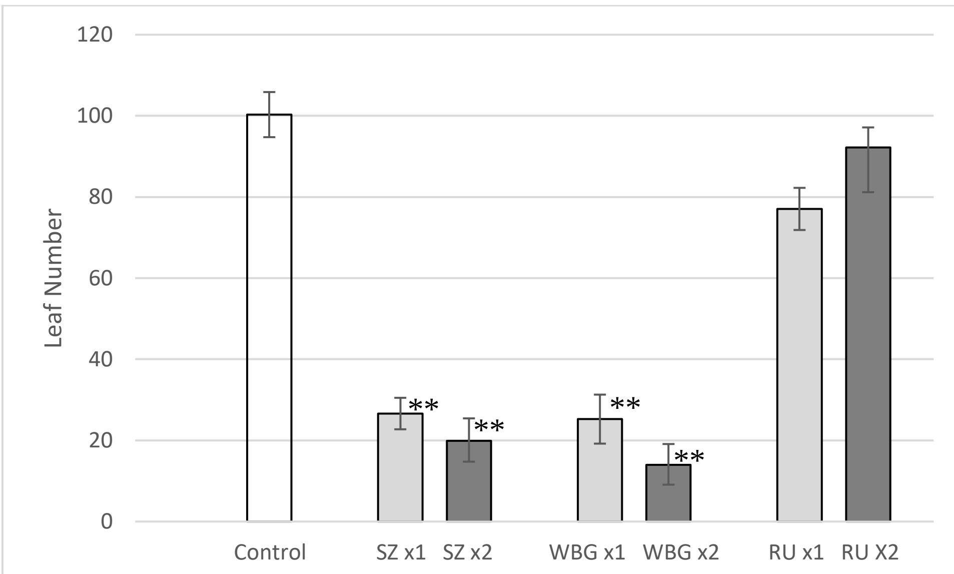


Figure 4.2. Average number of leaves of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. There were no significant differences between treatments of the same herbicide at $P < 0.01$.

In Figure 4.2 the *H. cordata* plants in the store-bought control group had 100.3 leaves on average. The plants treated with recommended and doubled concentrations of SpeedZone had 26.6 leaves and 19.9 leaves, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had 25.3 leaves and 14.0 leaves, respectively. The plants treated with recommended and doubled concentrations of Roundup had 77.1 leaves and 92.3 leaves, respectively. All plants treated with a broadleaf-specific herbicide produced numbers of leaves significantly lower than the control group. There were no plants that had a significant difference in the number of leaves between the recommended concentration and the doubled concentration of the same herbicide.

## 4.2 Total Leaf Area Before Treatment

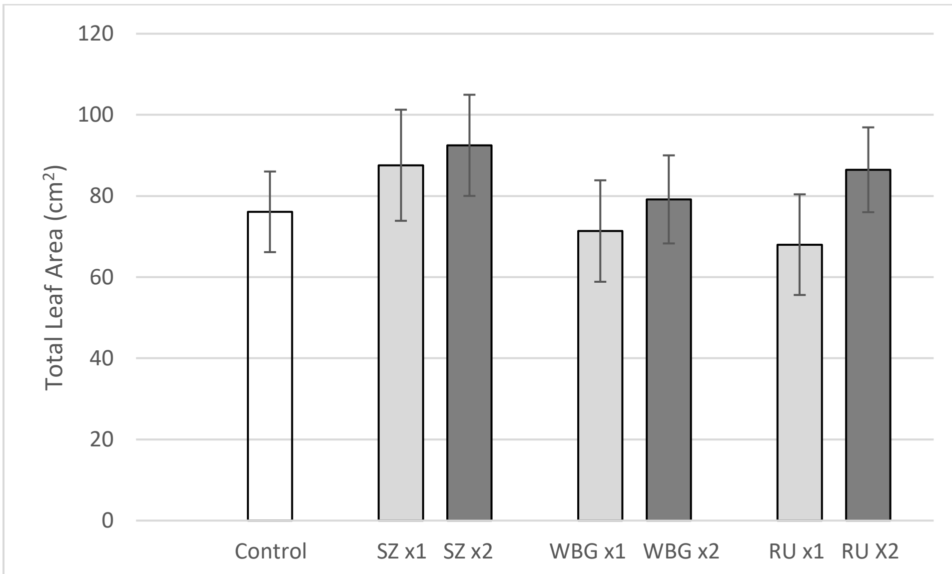


Figure 4.3. Total leaf area of naturalized *Houttuynia cordata* before 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. There was no statistical difference between any of the test groups before the herbicides were applied.

As shown in Figure 4.3 prior to herbicidal treatment, the *H. cordata* plants in the naturalized control group had an average total leaf area of 76.1 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had total leaf areas of 87.9 $cm^2$ and 92.5 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had total leaf areas of 71.4 $cm^2$ and 79.2 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had total leaf areas of 68.0 $cm^2$ and 86.5 $cm^2$, respectively. There were no statistical differences between the plant groups' lead areas prior to herbicidal treatment.

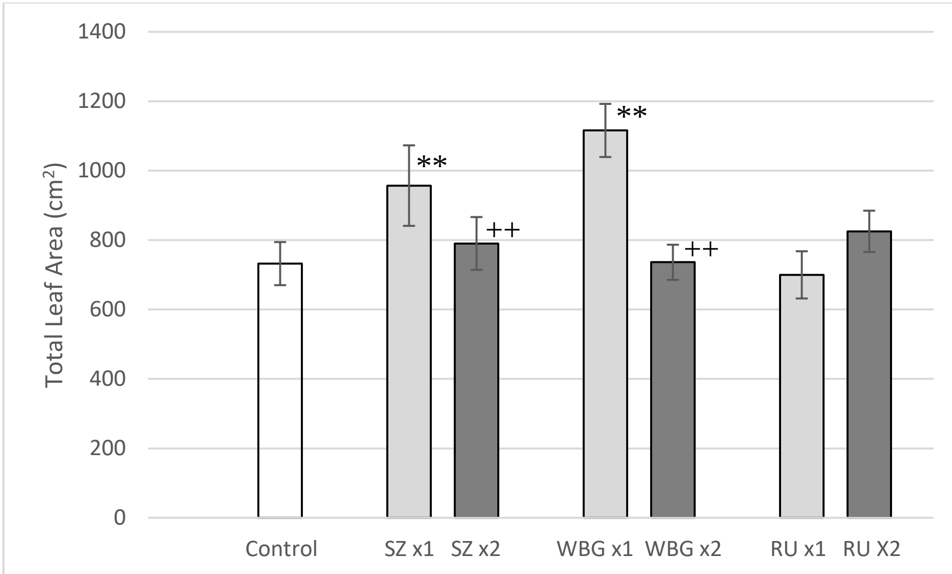


Figure 4.4. Total leaf area of store-bought *Houttuynia cordata* before 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. Plus signs (++) indicate significant difference between treatments of the same herbicide at $P < 0.01$.

Figure 4.4 shows that prior to herbicidal treatment, the *H. cordata* plants in the store-bought control group had an average total leaf area of 732.2 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had total leaf areas of 956.9 $cm^2$ and 790.3 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had total leaf areas of 1115.8 $cm^2$ and 736.1 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had total leaf areas of 699.8 $cm^2$ and 825.2 $cm^2$, respectively. Prior to treatment, plants to be treated with single concentrations of SpeedZone and Weed-B-Gon were significantly higher than the control group. Plants in these same groups also had significantly larger leaf areas than plants to be treated with the doubled concentrations of the same herbicide.

## 4.3 Total Leaf Area After Treatment

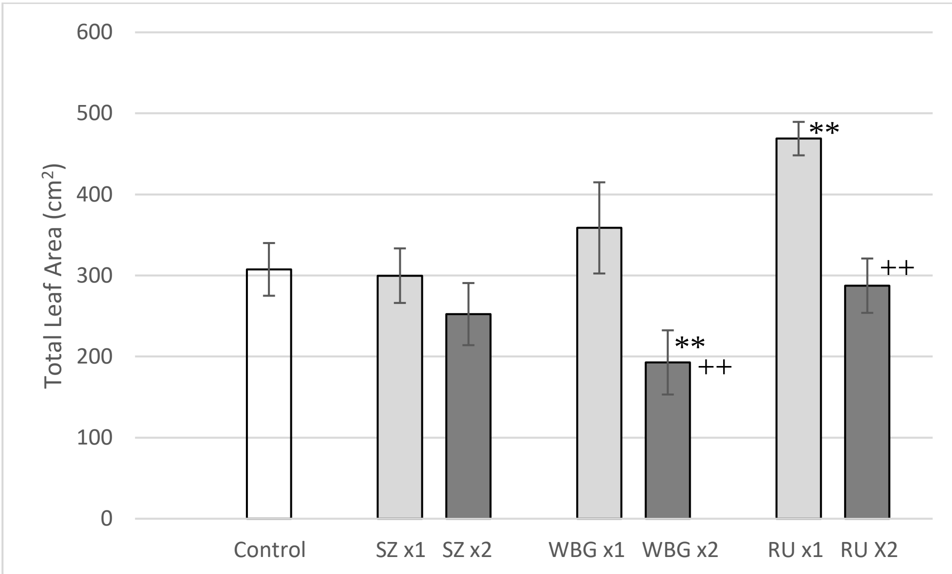


Figure 4.5. Total leaf area of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. Plus signs (++) indicate significant difference between treatments of the same herbicide at $P < 0.01$.

In Figure 4.5 the *H. cordata* plants in the naturalized control group had an average total leaf area of 307.5 $cm^2$ after treatment. The plants treated with recommended and doubled concentrations of SpeedZone had total leaf areas of 299.8 $cm^2$ and 252.4 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had total leaf areas of 358.8 $cm^2$ and 192.8 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had total leaf areas of 468.9 $cm^2$ and 287.5 $cm^2$, respectively. The plants treated with Weed-B-Gon doubled concentration and the Roundup recommended concentration were the only ones that had significant differences from the control group. Plants treated with Weed-B-Gon and Roundup had significant differences in total leaf area between the recommended concentration treatment and double the recommended concentration treatment.

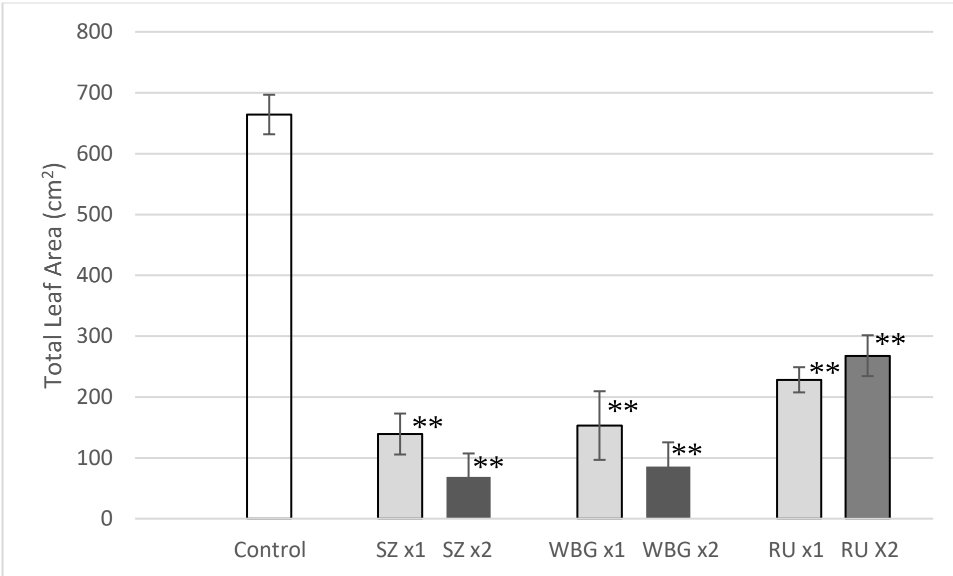


Figure 4.6. Total leaf area of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. There were no significant differences between treatments of the same herbicide at $P < 0.01$.

As shown in Figure 4.6, after herbicidal treatment, the *H. cordata* plants in the store-bought control group had a total leaf area of 664.2 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had total leaf areas of 139.1 $cm^2$ and 68.8 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had total leaf areas of 453.0 $cm^2$ and 85.7 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had total leaf areas of 228.1 $cm^2$ and 267.7 $cm^2$, respectively. All plants treated with herbicide had significantly lower total leaf area than the control group. There were no plant groups that had a significant difference between the recommended concentration and the doubled concentration.

## 4.4 Total Leaf Area Difference Before/After Treatment

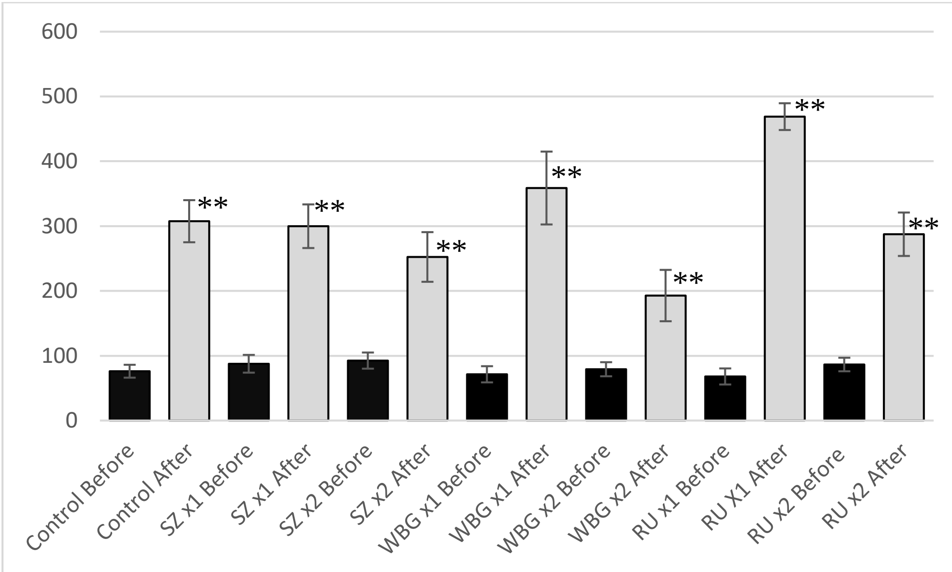


Figure 4.7. Starting and ending leaf area values of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference between start and end values at $P < 0.01$.

Figure 4.7 shows the beginning and ending values of each naturalized treatment group. The difference between the starting leaf area and the ending leaf area for the *H. cordata* plants in the naturalized control group was 231.4 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had differences of 212.3 $cm^2$ and 159.9 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had differences of 287.4 $cm^2$ and 113.7 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had differences of 400.9 $cm^2$ and 201.1 $cm^2$, respectively. All plant groups had a significant increase in leaf area between the start and end of the study.

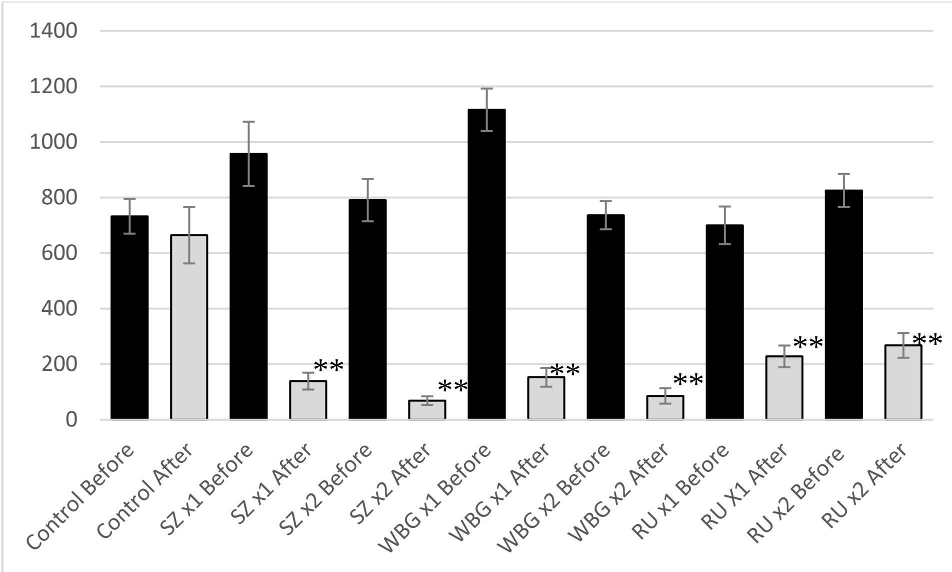


Figure 4.8. Starting and ending leaf area values of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference between start and end values at $P < 0.01$.

In Figure 4.8 the difference between the starting leaf area and the ending leaf area for the *H. cordata* plants in the store-bought control group was 68.0 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had differences of 817.9 $cm^2$ and 721.6 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had differences of 962.8 $cm^2$ and 650.4 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had differences of 471.7 $cm^2$ and 557.5 $cm^2$, respectively. All plants produced an ending total leaf area significantly lower than the starting total leaf area. The control group's beginning and end values did not have a significant difference.

## 4.5 Average Leaf Area

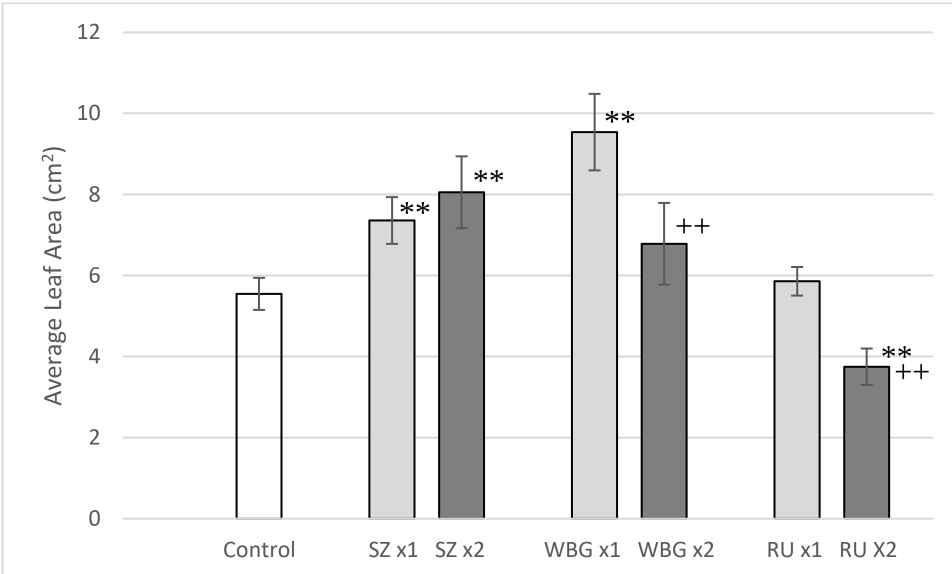


Figure 4.9. Average leaf area of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. Plus signs (++) indicate significant difference between treatments of the same herbicide at $P < 0.01$.

In Figure 4.9 the *H. cordata* plants in the naturalized control group had an average leaf area of 5.5 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had leaf areas of 7.4 $cm^2$ and 8.1 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had leaf areas of 9.5 $cm^2$ and 6.8 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had leaf areas of 5.9 $cm^2$ and 3.7 $cm^2$, respectively. Plants treated with SpeedZone recommended and doubled concentration and the Weed-B-Gon recommended concentration treatments had a significantly higher average leaf area than the control. Plants treated with a double concentration of Roundup had leaf area that was significantly lower than the control group. Plants treated with Weed-B-Gon, and Roundup produced a significant difference between the recommended and twice the recommended concentrations of the herbicide.

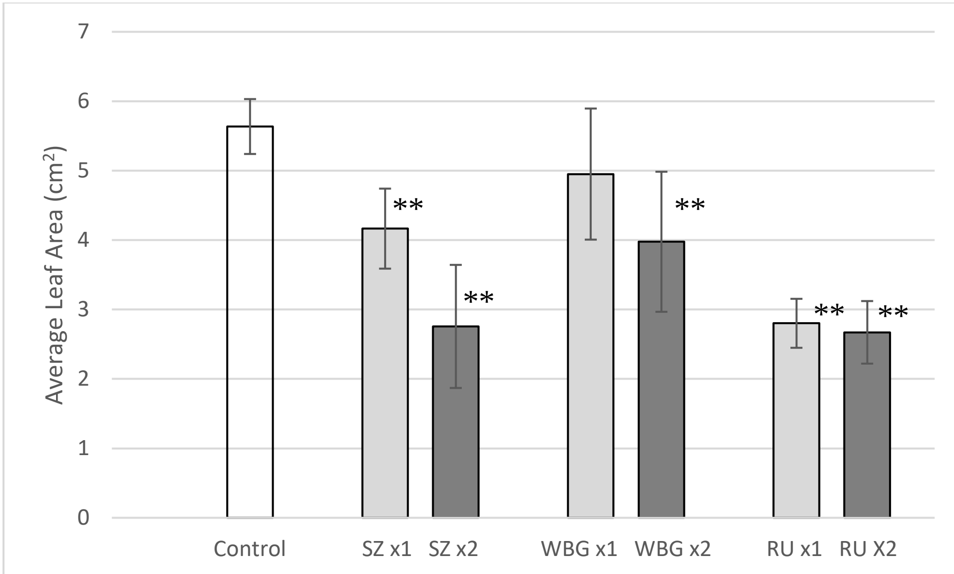


Figure 4.10. Average leaf area of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. There was no significant difference between treatments of the same herbicide at $P < 0.01$.

Figure 4.10 shows the control group store-bought *H. cordata* plants in the control group had an average leaf area of 5.6 $cm^2$. The plants treated with recommended and doubled concentrations of SpeedZone had leaf areas of 4.2 $cm^2$ and 2.8 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had leaf areas of 4.9 $cm^2$ and 4.0 $cm^2$, respectively. The plants treated with recommended and doubled concentrations of Roundup had leaf areas of 2.8 $cm^2$ and 2.7 $cm^2$, respectively. Plants treated with all herbicide treatments, except the Weed-B-Gon recommended concentration, produced results significantly less than the control.

## 4.6 Average Number of Stems at Time of Data Collection

### 4.6.1 2021 Study

During the 2021 study, the sample size differed from the 2022 study. The controls had a sample size of 12 pots of plants and the six herbicide treatments had a sample size of six pots of plants each, instead of 16.

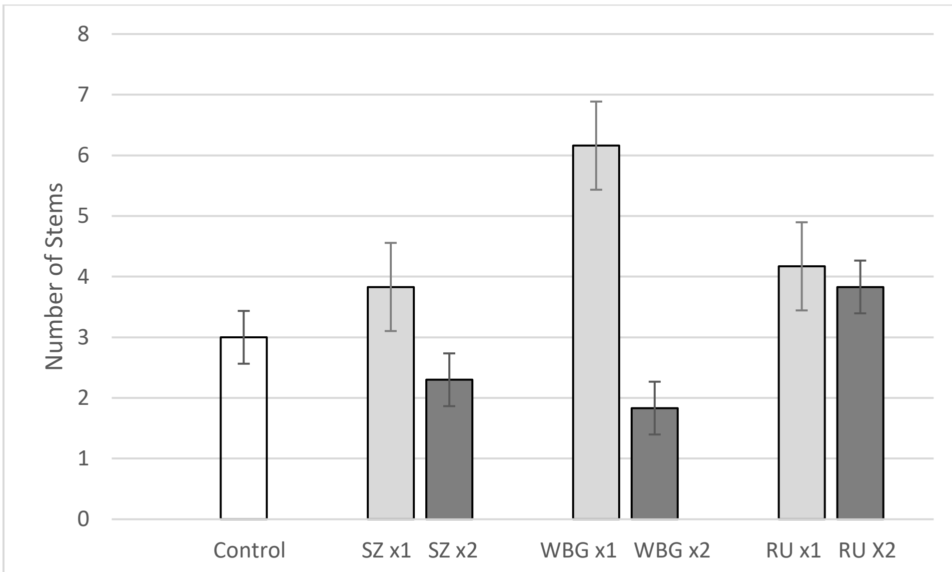


Figure 4.11. Average number of stems of naturalized *Houttuynia cordata* after 2021 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=12 for the control. n=6 for all other treatments. Standard error bars were used for this graph.

In Figure 4.11 the control group naturalized *H. cordata* plants in the control group had an average number of 3 stems. The plants treated with recommended and doubled concentrations of SpeedZone had 3.8 stems and 2.3 stems, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had leaf areas of 6.16 stems and 1.8 stems, respectively. The plants treated with recommended and doubled concentrations of Roundup had leaf areas of 4.17 stems and 3.8 stems, respectively. As stems were counted *en mass* by treatment group, no statistical data is available for the 2021 test. Error bars indicate one standard error in Figure 4.11.

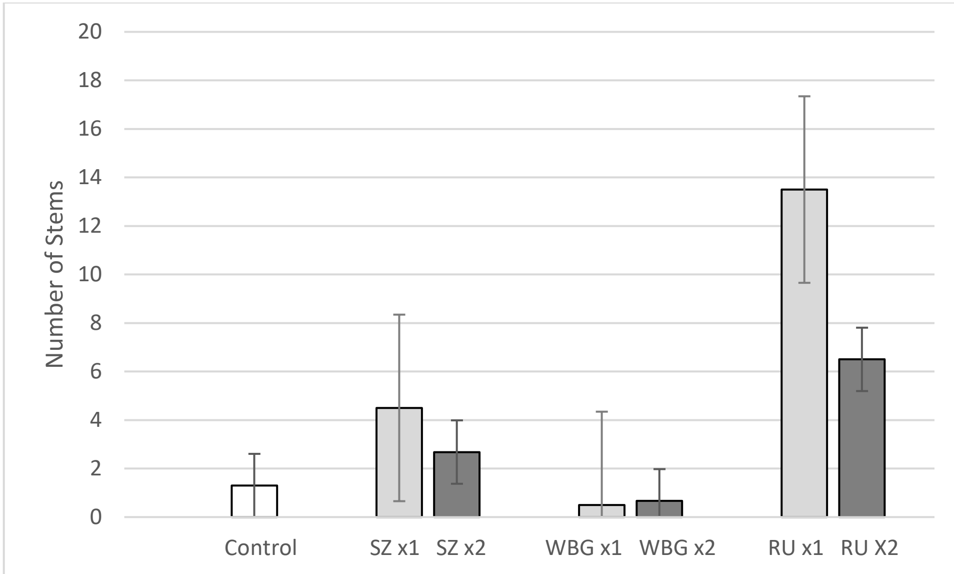


Figure 4.12. Average number of stems of store-bought *Houttuynia cordata* after 2021 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=12 for the control. n=6 for all other treatments. Standard error bars were used.

The control group store-bought *H. cordata* plants in the control group had an average number of 1.3 stems in Figure 4.12. The plants treated with recommended and doubled concentrations of SpeedZone had 4.5 stems and 2.7 stems, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had leaf areas of 0.5 stems and 0.67 stems, respectively. The plants treated with recommended and doubled concentrations of Roundup had leaf areas of 13.5 stems and 6.5 stems, respectively. Qualitative results gathered by visual observation showed that the naturalized type regrowth produced thick, almost leathery leaves that continued to persist to the end of the 2022 study. The stems of these plants were also stunted when compared with the control group before treatment.

### 4.6.2 2022 Study

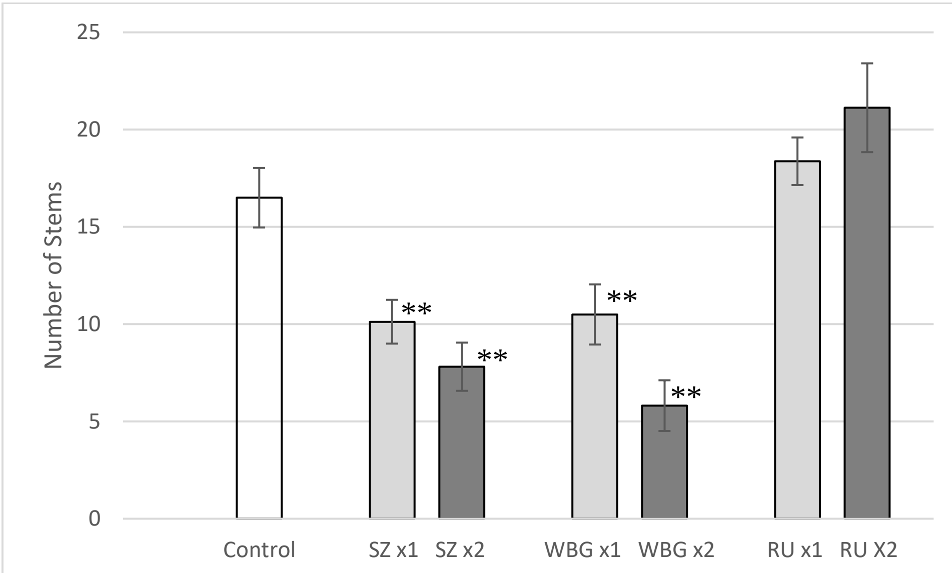


Figure 4.13. Average number of stems of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. There was no significant difference between treatments of the same herbicide at $P < 0.01$.

As shown in Figure 4.13 the control group naturalized *H. cordata* plants in the control group had an average number of 16.5 stems. The plants treated with recommended and doubled concentrations of SpeedZone had 10.1 stems and 7.8 stems, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had leaf areas of 10.5 stems and 5.8 stems, respectively. The plants treated with recommended and doubled concentrations of Roundup had leaf areas of 18.4 stems and 21.1 stems, respectively. All plants treated with broadleaf-specific herbicides produced results that were significantly lower than the control group for this parameter. There were no treated plants that produced significant differences between the recommended concentration and the doubled concentration of herbicide.

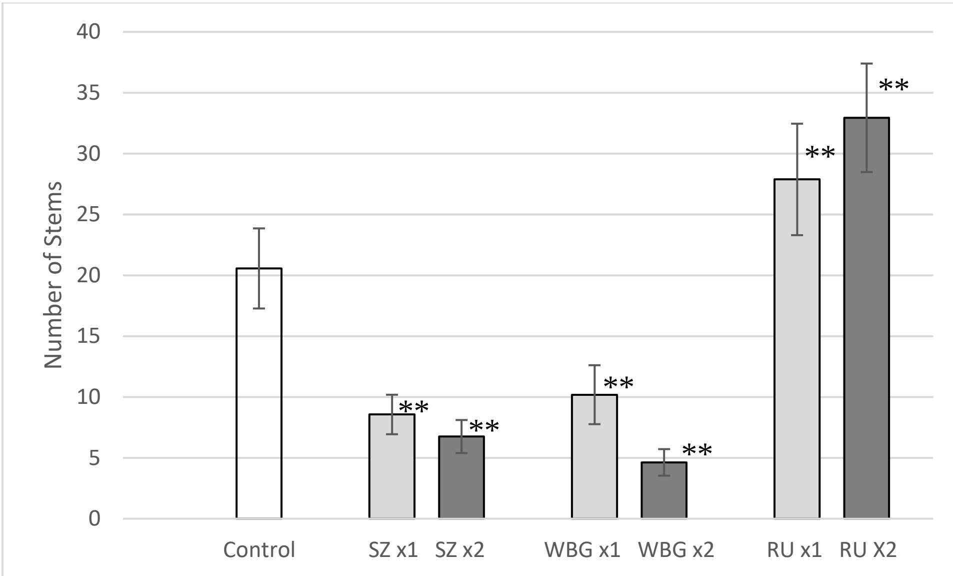


Figure 4.14. Average number of stems of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. There were no significant differences between treatments of the same herbicide at $P < 0.01$.

Figure 4.14 shows the *H. cordata* plants in the store-bought control group had an average number of 20.6 stems. The plants treated with recommended and doubled concentrations of SpeedZone had 8.6 stems and 6.8 stems, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had leaf areas of 10.2 stems and 4.6 stems, respectively. The plants treated with recommended and doubled concentrations of Roundup had leaf areas of 27.9 stems and 32.9 stems, respectively. All plants treated with herbicide produced results significantly different from the control group with the broadleaf-specific herbicides having lower stem count and the Roundup having higher stem count. There were no treated plants that produced results with a significant difference between the recommended and doubled concentrations of the same herbicide.

## 4.7 Stem Height

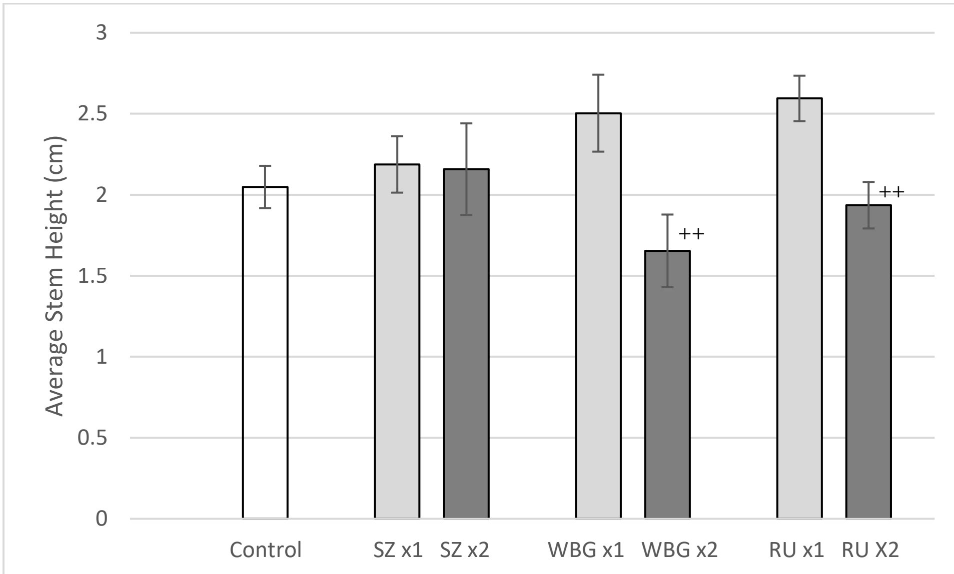


Figure 4.15. Average stem height of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. No herbicide treatments showed significant difference from the control at $P < 0.01$. Plus signs (++) indicate significant difference between treatments of the same herbicide at $P < 0.01$.

In Figure 4.15 the *H. cordata* plants in the naturalized control group had an average stem height of 2.1 cm. The plants treated with recommended and doubled concentrations of SpeedZone had 2.2 cm and 2.2 cm, respectively. The plants treated with recommended and doubled concentration of Weed-B-Gon had stem heights of 2.5 cm and 1.7 cm, respectively. The plants treated with recommended and doubled concentrations of Roundup had stem heights of 2.6 cm and 1.9 cm, respectively. No treated plants produced results significantly different from the control group. Plants treated with Weed-B-Gon, and Roundup showed a significant difference between their single and doubled concentration treatments.

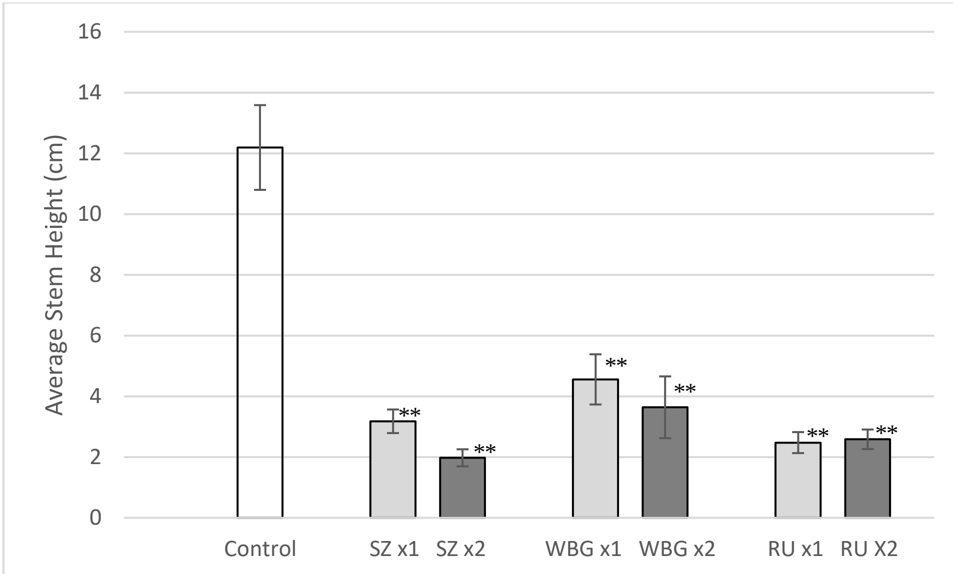


Figure 4.16. Average stem height of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Asterisks (**) indicate significant difference from the control at $P < 0.01$. There were no significant differences between treatments of the same herbicide at $P < 0.01$.

In Figure 4.16 the *H. cordata* plants in the store-bought control group had an average stem height of 12.2 cm. The plants treated with recommended and doubled concentrations of SpeedZone had stem heights of 3.2 cm and 2.0 cm, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had stem heights of 4.6 cm and 3.6 cm, respectively. The plants treated with recommended and doubled concentrations of Roundup had stem heights of 2.5 cm and 2.6 cm, respectively. All treated plants produced results significantly lower than the control group.

## 4.8 Rhizome Dry Weight

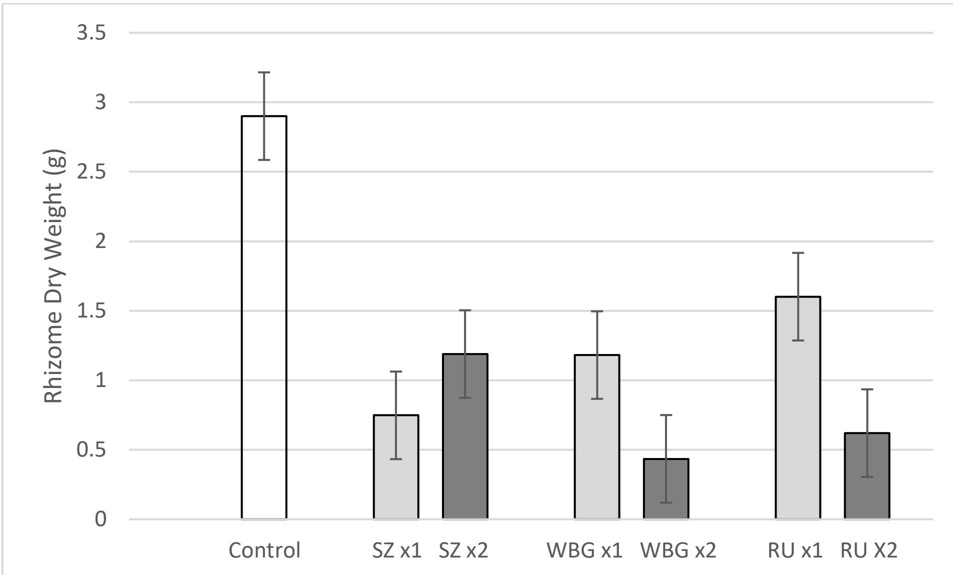


Figure 4.17. Average rhizome dry weight of naturalized *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. No statistical data available. Error bars are one standard error.

As shown in Figure 4.17 the *H. cordata* plants in the naturalized control group had an average rhizome dry weight of 2.9 g. The plants treated with recommended and doubled concentrations of SpeedZone had weights of 0.8 g and 1.2 g, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had weights of 1.2 g and 0.4 g, respectively. The plants treated with recommended and doubled concentrations of Roundup had weights of 1.6 g and 0.6 g, respectively. Rhizomes were weighed together, therefore no statistical data is available.

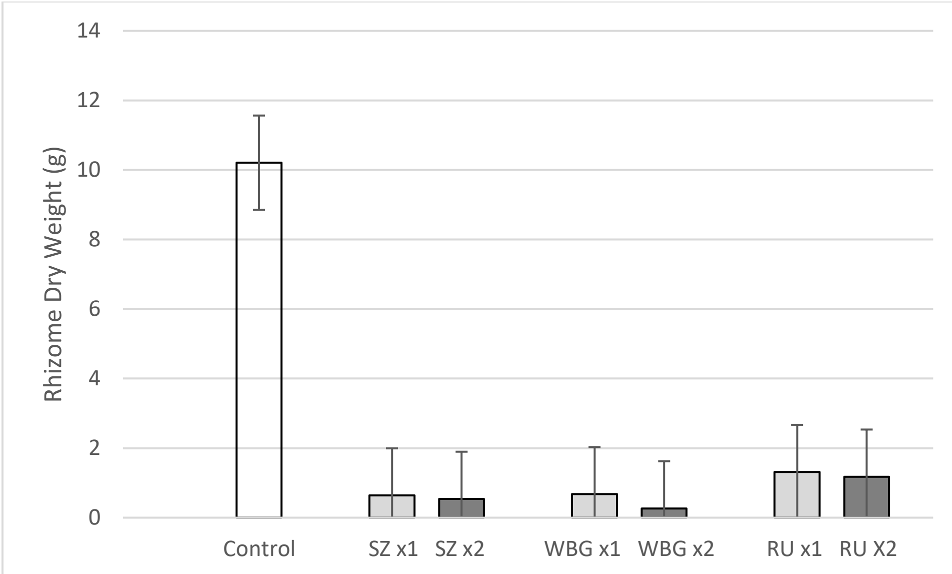


Figure 4.18. Average rhizome dry weight of store-bought *Houttuynia cordata* after 2022 study. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. No statistical data available.

Figure 4.18 shows the *H. cordata* plants in the store-bought control group had an average rhizome dry weight of 10.2 g. The plants treated with recommended and doubled concentrations of SpeedZone had weights of 0.6 g and 0.5 g, respectively. The plants treated with recommended and doubled concentrations of Weed-B-Gon had weights of 0.7 g and 0.3 g, respectively. The plants treated with recommended and doubled concentrations of Roundup had weights of 1.3 g and 1.2 g, respectively. The rhizomes were dry and weighed *en masse* ant therefore no statistical information was available.

## 4.9 Number of stems/Leaf Area Comparison

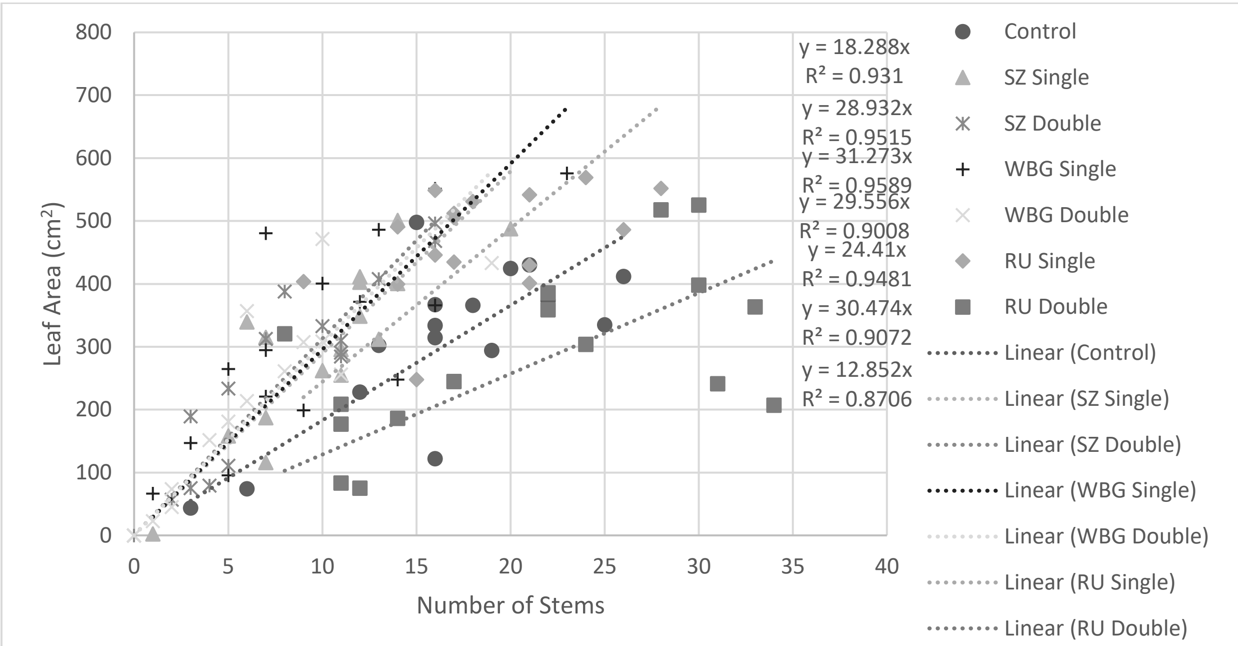


Figure 4.19 Regression of leaf area with reference to number of stems for the naturalized ecotype in the 2022 test. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Trendline intercepts set at 0.0. Equations are listed in key order.

The regression in Figure 4.19 shows that there was a positive correlation between stem count and leaf area for the naturalized ecotype. Plants in the control group showed a slope of 18.3 $cm^2$/stem and an $R^2$ value of 0.93. Plants that were treated with SpeedZone recommended concentration and recommended concentration doubled had slopes of 28.9 $cm^2$/stem and 31.3 $cm^2$/stem, respectively and $R^2$ values of 0.95 and 0.96, respectively. Plants treated with Weed-B-Gon recommended and doubled concentrations had slopes of 29.6 $cm^2$/stem and 24.4 $cm^2$/stem and $R^2$ values of 0.90 and 0.95, respectively. Plants treated with Roundup recommended and doubled concentrations had slopes of 30.5 $cm^2$/stem and 12.9 $cm^2$/stem and $R^2$ values of 0.91 and 0.87, respectively. All values from this figure were significant.

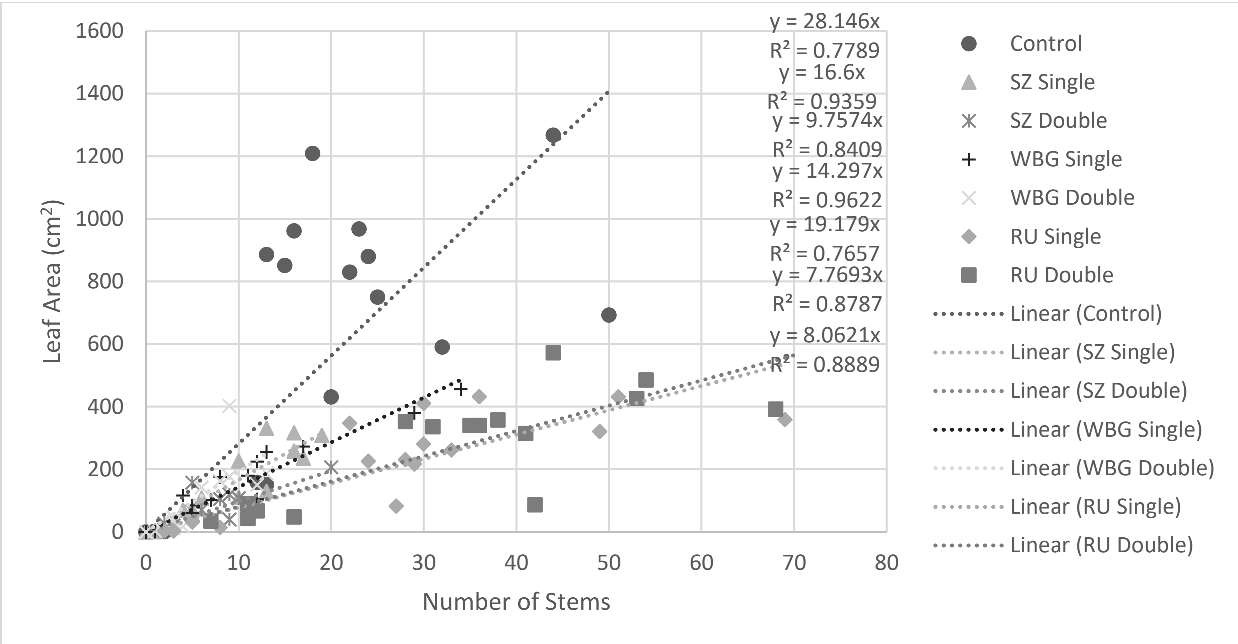


Figure 4.20 Regression of leaf area with reference to number of stems for the store-bought ecotype in the 2022 test. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Trendline intercepts set at 0.0. Equations are listed in key order.

The regression in Figure 4.20 shows that there was a positive correlation between stem count and leaf area for the store-bought ecotype. Plants in the control group showed a slope of 28.1 $cm^2$/stem and an $R^2$ value of 0.78. Plants that were treated with SpeedZone recommended concentration and recommended concentration doubled had slopes of 16.6 $cm^2$/stem and 9.8 $cm^2$/stem, respectively and $R^2$ values of 0.94 and 0.84, respectively. Plants treated with Weed-B-Gon recommended and doubled concentrations had slopes of 14.3 $cm^2$/stem and 19.2 $cm^2$/stem and $R^2$ values of 0.96 and 0.77, respectively. Plants treated with Roundup recommended and doubled concentrations had slopes of 7.8 $cm^2$/stem and 8.1 $cm^2$/stem and $R^2$ values of 0.88 and 0.89, respectively. All values from this figure were significant.

## 4.10 Number of Leaves/Leaf Area Comparison

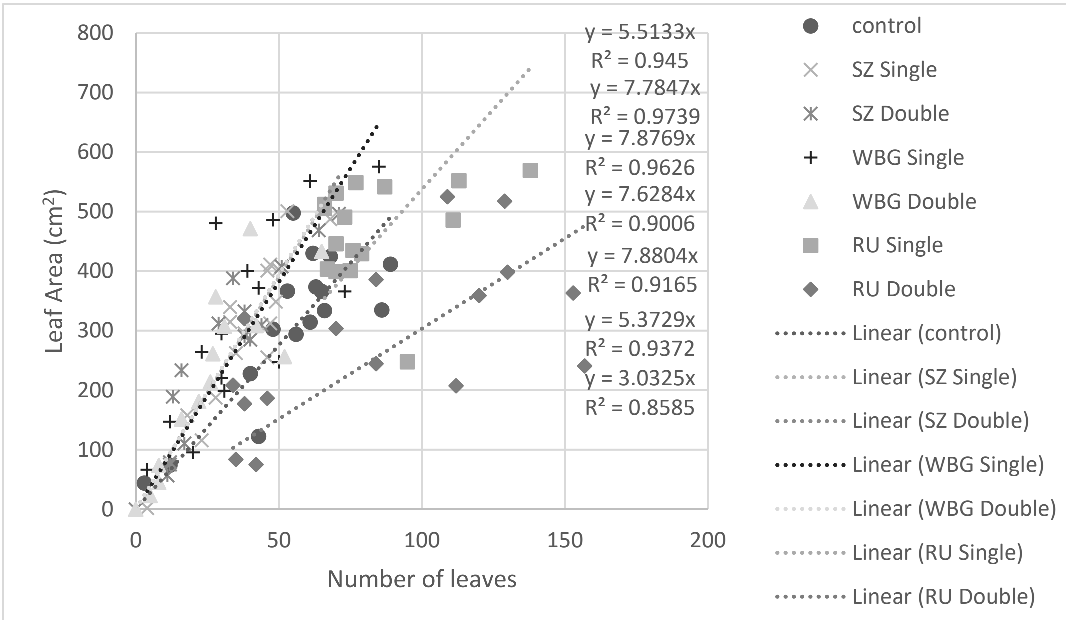


Figure 4.21. Regression of leaf area with reference to the number of leaves for the store-bought ecotype in the 2022 test. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Trendline intercepts set at 0.0. Equations are listed in key order.

The regression in Figure 4.21 shows that there was also a positive correlation between number of leaves and leaf area for the naturalized ecotype. Plants in the control group showed an slope of 5.5 $cm^2$/leaf and an $R^2$ value of 0.95. Plants that were treated with SpeedZone recommended concentration and recommended concentration doubled had slopes of 7.8 $cm^2$/leaf and 7.9 $cm^2$/leaf, respectively and $R^2$ values of 0.97 and 0.96, respectively. Plants treated with Weed-B-Gon recommended and doubled concentrations had slopes of 7.6 $cm^2$/leaf and 7.9 $cm^2$/leaf and $R^2$ values of 0.90 and 0.92, respectively. Plants treated with Roundup recommended and doubled concentrations had slopes of 5.4 $cm^2$/leaf and 3.0 $cm^2$/leaf and $R^2$ values of 0.94 and 0.86, respectively. All values from this figure were significant.

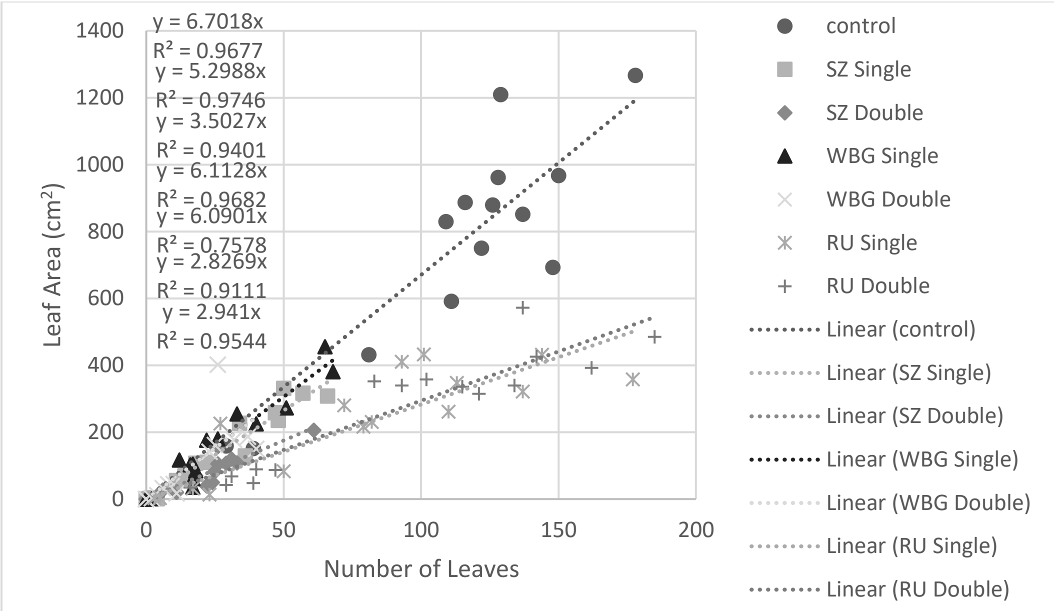


Figure 4.22. Regression of leaf area with reference to number of leaves for the store-bought ecotype in the 2022 test. Herbicides used were SpeedZone (SZ), Weed-B-Gon (WBG) or Roundup (RU) at the recommended concentration (x1) or twice the recommended concentration (x2). n=16 per treatment. Trendline intercepts set at 0.0. Equations are listed in key order.

The regression in Figure 4.22 shows that there was also a positive correlation between the number of leaves and leaf area for the store-bought ecotype. Plants in the control group showed a slope of 6.7 $cm^2$/leaf and an $R^2$ value of 0.97. Plants that were treated with SpeedZone recommended concentration and recommended concentration doubled had slopes of 5.3 $cm^2$/leaf and 3.5 $cm^2$/leaf, respectively and $R^2$ values of 0.97 and 0.94, respectively. Plants treated with Weed-B-Gon recommended and doubled concentrations had slopes of 6.1 $cm^2$/leaf and 6.1 $cm^2$/leaf and $R^2$ values of 0.97 and 0.76, respectively. Plants treated with Roundup recommended and doubled concentrations had slopes of 2.8 $cm^2$/leaf and 2.9 $cm^2$/leaf and $R^2$ values of 0.91 and 0.95, respectively. All values from this figure were significant.

# CHAPTER 5. DISCUSSION

The data produced in this study supports the hypothesis that current commercially available herbicides cannot control *H. cordata* as there were no plants that were successfully eradicated by the herbicide they were treated with. Generally, plants treated with broadleaf-specific herbicides, SpeedZone and Weed-B-Gon, had less growth than untreated plants for all parameters except the naturalized leaf area (Figures 4.3 and 4.5). Plants treated with the non-selective herbicide, Roundup, were generally unaffected and even exceeded the control groups in the stem height, number of stems, leaf area, and number of leaves parameters. The store-bought plants were more affected by herbicide application than the naturalized plants. Finally, increasing the herbicide concentration did not cause a significant difference in the regrowth of parameters for any of the herbicides used.

It is not surprising that the plants treated with the broadleaf-specific herbicides struggled to regrow after application as the active ingredient for both herbicides, dicamba, is known to persist and be active in soils up to 12 weeks after application (Friesen, 1965). This also explains why plants treated with SpeedZone showed more inhibition to regrowth, as it contains a higher percentage of dicamba in its active ingredients. The active ingredient in Roundup, glyphosate, is known to sorb closely to soils and become immobile (Vereecken, 2005). Therefore, after initial application to the plant leaves, there was no herbicidal influence during the regrowth. It may also be that given the strength of the herbicide used, the stems were killed without the herbicide reaching the rhizomes and roots.

As previously mentioned, in all parameters tested, the naturalized ecotype was less affected by the herbicides than the storebought ecotype. This may be that the naturalized plants have adapted to imperfect environments whereas the storebought ecotype has only ever been grown in near-perfect conditions. This will be an important factor to consider in future studies since using a storebought type may not yield results applicable to the field. Also, though doubling the concentrations of the herbicides did not produce a significant difference in this study, there was a general reduction in growth. This indicates that increasing the herbicide concentration further may produce a significant difference from the recommended concentrations. However, the possibility of soil toxicity, specifically when using herbicides such as glyphosate, must be considered before using such extreme concentrations in field testing (Rose et al., 2016).

In Figure 4.20 every treated group had a lower ratio of leaf area to stem count than the control group. This means that after treatment the plants increased their production of new stems over growth of preexisting leaves. This suggests that store-bought *H. cordata* may increase the number of stems and it produces in response to chemical or manual pruning. This is corroborated by Figure 4.22 as the ratios of number of leaves to leaf area again favored number of leaves. However, in Figures 4.19 and 4.21 the only treated group that had a regression favoring the number of stems or number of leaves was the group treated with a doubled concentration of Roundup. This again shows a difference between store-bought and naturalized *H. cordata.*

As there is very little information on the herbicidal resistance of *H. cordata,* this paper will compare the information gained during this study to other species of plants that spread through rhizomes, the first of which will be Hooker's evening primrose (*Oenothera elata)*. This plant is another broadleaf plant that has aggressive rhizomes and is difficult to eradicate via herbicides. When treated with herbicides, *O. elata* generally has a negative response to both glyphosate and dicamba preparations in both above ground mass and below ground mass (Bates, 2013). As *H. cordata* aboveground mass only had a negative response to dicamba preparations, a stronger herbicide may be needed to kill its rhizomes. Another type of plant that shows this sort of resistance to herbicides is the invasive Japanese knotweed (*Polygonum cuspidatum*). *Polygonum cuspidatum* is another herbaceous plant that spreads aggressively through its rhizomes and has been shown to be immune to eradication by glyphosate, imazapyr, and synthetic auxins (Bashtanova et al., 2009). Comparing the results of this study to the results from other similar studies shows a common trend of failure of herbicides to control herbaceous plants that spread by rhizomes.

Individuals looking to replicate this study will want to consider the limitations of this study. First, this study was developed and implemented in two years. As this was plant research, two years is not enough time to produce a thorough study as there is only one growing season each year and long-term effects past a two-year period could not be determined. The sample size of this study was also small with only 16 pots per different herbicide treatment. A larger sample size would be expected to reduce error in the data, allowing for more precise statistical analysis. Also, only unvariegated *H. cordata* was used in this study. Studies using variegated varieties of the plant may have different results. The results of the study are nevertheless still valid in identifying the ineffectiveness of commercially available herbicides on the eradication of *H. cordata*.

Further studies in this field may include a repeat of this study where the stems of the untreated plants are manually removed at the time of treatment to determine how much of the loss of rhizome mass in the treated plants was due to herbicidal influence or simply using stored energy to regrow above ground biomass. It may also be beneficial to study the depth to which current herbicides penetrate rhizome systems such as the kind *H. cordata* possesses, and to study the impact herbicides that stay active in the soil, such as dicamba have on rhizomes over time.

# CHAPTER 6. CONCLUSION

The goal of this study was to determine if current commercially available herbicides could eradicate the plant *Houttuynia cordata*. To achieve this, two different ecotypes of *H. cordata* were treated with three herbicides at recommended and double concentrations: SpeedZone, Weed-B-Gon, and Roundup. The physiological responses of the plants to these herbicides were then measured and compared to an untreated control group. The results of the treatments showed that plants treated with non-selective herbicides showed little to no lasting response after initial stem death, whereas plants treated with broadleaf-specific herbicides showed a significant reduction in growth. However, there were no groups of plants that were completely eradicated.

The fact that no groups of plants were eradicated by the treatment they received supports the hypothesis that *H. cordata* cannot be eradicated by current commercially available herbicides. The species also does not, in most circumstances, show a significant difference in response to increases in the herbicide concentration used, which also supports the hypothesis. However, the fact that rhizome weight did drastically decrease may indicate that eradication is a possibility with future commercial herbicides.

This research has shown that current herbicides are not enough to exterminate *H. cordata.* This is a common result when studying other research that experimented on herbicidal resistance in plants that spread through rhizomes. *H. cordata* is an especially resistant plant according to this study, therefore, further research is required to determine how best to eradicate this invasive species.

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