

# **Exploring the Effectiveness of Using Foliar Fertilizer Herbicide Treatments on Exotic Invasive Plant Species**

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## **Abstract**

As exotic invasive plant species out-compete native organisms and threaten natural ecosystems, the search for solutions often comes in the form of herbicides. Though there are many effective manual forms of controlling certain invasive species, as well as advancements in methods incorporating biological controls, herbicides are used to control and eradicate difficult intruders. This leads to the increased use of herbicides, which further threatens the sustainability of ecosystems. Herbicides have proven to be effective, though not in all scenarios. Effective solutions, therefore, must be explored in order to not only control and eradicate infestations of invasive species, but to lower the amount of herbicides required to produce effective treatments. As early as the 1950s, scientists began to explore the benefits of using foliar fertilizer to encourage the growth and well-being of crops; findings indicated that the addition of certain elements such as nitrogen and phosphorous increased both biomass and crop yield. Foliar fertilizer is seen to trick the plant into photosynthesis, increasing the movement of sugars, water and nutrients throughout its vascular system. The following discussion examines a series of experiments which explore the benefits of using fertilizers such as nitrogen and the potentials of magnesium, phosphorous, and potassium in aiding the effects of herbicides in the control and eradication of durable invasive species, by effectively encouraging the plant to utilize the herbicide more efficiently. The experiments also explore the use of adjuvants, such as Humboldt Sticky and ethylated oils, to further enhance treatment effectiveness, minimize impacts on non-target species, induce wake-up calls in root systems of invasive species, and lower the required concentration of herbicide.

## **1. Introduction**

### **1.1 History**

Exotic invasive species alter natural ecosystems, and threaten the native species dependent upon them. This makes the task of seeking solutions to combat intrusive progressions one of significant importance.

In 1953, Michigan State University received a grant from the United States DOE to conduct studies, using radioisotopes, to observe the effects of foliar absorption of fertilizer nutrients<sup>6</sup>. These studies would trace the movement of fertilizers throughout the vascular system of the plant. By using radioisotopes, the researchers found that foliar feeding a plant was eight to 10 times more effective than fertilizing the plant via soil application, in regard to the amount of nutrients required and the speed at which these nutrients were utilized by the plant<sup>6</sup>. Activity in the plant is increased when fertilizer is applied by the foliage; chlorophyll synthesis and photosynthesis increases as the foliage requires a greater amount of water, enabling the increased activity and movement of nutrients throughout the plant's vascular system<sup>6</sup>. In other words, direct application of fertilizers to leaves effectively induces the treated plant into performing higher rates of photosynthesis, leading to greater production by the plant and higher overall rates of functioning. For instance, researchers conducting experiments at Washington State University successfully engineered a variety of rice which yielded 35% more product than standard rice production yields<sup>6</sup>. These increased yield rates

were observed when a corn gene was inserted into rice DNA, enabling higher rates of photosynthesis within the organism<sup>6</sup>. Essentially, foliar solutions are another way in which to deliver nutrients throughout the plant, by accessing the organism's vascular system via stomata feeding<sup>21</sup>.

Foliar sprays are also widely used in the application of herbicides, from industrial agriculture to the control of exotic invasive species infestations; though, herbicides may not always prove to be effective in controlling persistent species<sup>2 8 10</sup>. Herbicides, such as glyphosate and triclopyr, fail to eradicate certain infestations, often calling for high concentrations of the chemical to conduct efficient treatments<sup>13</sup>. Nitrogen and other fertilizers may potentially increase the effectiveness and efficiency of applied foliar herbicides.

Recent studies suggest that increasing the uptake of nitrogen and phosphorous provided plants with an increased yield and quality<sup>6</sup>. Nitrogen is found to increase levels of aromatic amino acids and activate protein synthesis within plants<sup>1</sup>. Therefore, it could be advantageous to add nitrogen or similar fertilizers to glyphosate foliar sprays, as the chemical itself is a reducer of phenolic amino acids of the shikimate pathway, which effectively reduces the protein synthesis of the targeted organism<sup>3 4</sup>. This function of glyphosate, may keep the chemical from sufficiently infiltrating essential root systems, supporting the vitality of the targeted species<sup>7</sup>. Effectively, the chemical kills its entryway to more essential areas of plant biomass, which lie in the root systems of persistent species<sup>5</sup>. Aromatic amino acids are precursors for the synthesis of numerous biologically active compounds, essential for the regulation of biological processes within plants; as glyphosate is an inhibitor of these, an addition of nitrogen to foliar herbicide treatments may enhance its effects and the extent of the chemical's exposure within the plant<sup>7 9 24</sup>. As glyphosate works to kill the plant, the added nitrogen may effectively aid in increasing the activity and movement of the xylem and phloem throughout the vascular system. It is hypothesized that as nitrogen encourages the increase in levels of amino acids, countering glyphosate's amino acid inhibition, it could then aid the chemical's mobility throughout the plant and its ability to access key areas of energy within its biomass<sup>8</sup>.

Nitrogen and other fertilizers may also encourage the functions of herbicides which act as synthetic auxins, such as triclopyr (Garlon 3A)<sup>7 26</sup>. Triclopyr mimics the phytohormonal activity of plants, essentially inducing the plant into rapid and uncontrollable growth until the plant can not support its functions any longer<sup>7 26</sup>. It is speculated that nitrogen may help to facilitate this effect, by increasing vascular system activity, protein synthesis, and photosynthesis<sup>7</sup>.

Furthermore, studies show the delivery and allocation of elements into root systems can be increased by applying sugars, light and phytohormones<sup>7</sup>. For instance, stem injection experiments to control Japanese knotweed, used ethephon, a precursor of ethylene via ethylated seed oil, as an adjuvant to attempt to break rhizome-bud dormancy of the organism<sup>7 12 25</sup>. The study concluded that not only did this catalyze activity of dormant membranes, but it helped to stimulate growth of both lateral and basal rhizome buds<sup>7 25</sup>. This increased the accumulation of triclopyr within the basal buds and proximal regions of the plant's rhizome system<sup>7</sup>. In addition, wetting agents, such as Humboldt Sticky, create a microscopic bond between foliar sprays and foliage<sup>21</sup>. This bond gives the foliar spray increased access to the vascular system of the plant and enhances the organism's utilization of the treatment<sup>21</sup>.

It is the purpose of the following experiments to explore and assess a variety of herbicide, nitrogen fertilizer, and Humboldt Sticky mixtures, as foliar treatments to control exotic invasive species.

## 1.2. Exotic Invasives - Test Species - Background information

### 1.2.1. *japanese stiltgrass (microstegium vimineum)*

In 1919, Japanese stiltgrass, also known as Nepalese browntop, arrived from Asia in Tennessee as porcelain packaging material<sup>30</sup>. This invasive is persistent in both full sun and deep shade, producing 100 to 1000 seeds per plant<sup>30</sup>. These seeds are viable in the soil for at least three years<sup>30</sup>. This species grows thick and tall, up to two-to-three feet and produces many viable seeds, therefore, imposing a particular threat to the emergence and survival of native ground vegetation<sup>29</sup>.

### 1.2.2. *english ivy (hedera helix)*

Introduced from eastern Asia and northern Africa, English ivy is a woody vine threatening native vegetation at all forest levels<sup>15</sup>. The vine effectively blocks out sunlight from reaching its host with thick-waxy-green leaves<sup>15</sup>. It is found throughout most of the United States and serves as an aggressive predator within native ecosystems<sup>15</sup>.

### 1.2.3. *japanese wisteria (wisteria floribunda)*

Japanese wisteria is a woody vine which is able to climb and engulf canopies leaving a lack of adequate sunlight for the host in order for it to efficiently carry out photosynthesis<sup>16</sup>. Introduced in 1816 as an ornamental, the vine is now widely present throughout the eastern part of the country<sup>18</sup>. As the vine has an extensive root system and may grow up to seventy feet long, mechanical control may cause infestations to worsen due to root dispersal and self-propagation properties<sup>16 17</sup>. Many studies, therefore, suggest the use of chemical methods to control established infestations of this invasive vine.

### 1.2.4. *japanese knotweed (fallopia japonica)*

Japanese knotweed is an herbaceous perennial, introduced to North America in the 1800s as an ornamental<sup>19</sup>. Native to Asia, this tuberous invasive may grow up to 10-feet in height. Its broad-leaf cover inhibits the development of native ecosystems once the plant is well established. Furthermore, the weed is easily self-propagated, needing no more than one-cubic centimeter of its organic matter to produce a new plant<sup>7</sup>. Growing in dense patches, Japanese knotweed has both an extensive above and below ground presence, which can reach up to several meters below the earth's surface<sup>7</sup>. It also has a vast rhizome network, which may branch out horizontally for several meters in any direction. As Japanese knotweed has allelochemical properties, native species in the presence of this invasive receive a shortage of nutrients, effectively reducing their chances of sustaining life<sup>7</sup>.

### 1.2.5. *kudzu (pueraria montana)*

Kudzu was first introduced to the United States around 1876<sup>20</sup>. In the early 1900s it was sold as an affordable feed for livestock, and by 1933 the Soil Erosion Service (now the Natural Resource Conservation Service) claimed it to be an effective vegetative agent in ameliorating and alleviating soil erosion<sup>20</sup>. By the 1950s, kudzu populated much of the South, and in 1997, the United States Congress labelled Kudzu a “noxious weed”<sup>20</sup>. Today, it covers about seven-million acres in the Southeast United States<sup>28</sup>. Kudzu can grow up to 60-feet in one season<sup>27</sup>. It is a perennial vine which climbs and has tuberous roots which may reach a depth of 12-feet<sup>27</sup>. These roots are generated from a crown root which is situated within its vines; one crown root may generate up to 30 vines<sup>27</sup>. The seeds are limited, but may be viable for several years<sup>20</sup>. Kudzu is also a nitrogen fixing plant<sup>28</sup>.

## 2. Preliminary Experiments: Exploring combinations of foliar nitrogen fertilizer, glyphosate and Humboldt Sticky.

The following preliminary experiments were applied on June 27th, 2019 in order to observe the effects of foliar applied nitrogen fertilizer (Miracle-Gro Brand) and Humboldt Sticky in aiding glyphosate in the control of invasive species. Tests were undertaken on four exotic invasive species: Japanese stiltgrass, English ivy, Japanese wisteria, and Japanese knotweed.

### 2.1. Experimental Method

#### 2.1.1. *japanese stiltgrass*

It is suggested that stiltgrass may be effectively treated with 0.5%-2.0% glyphosate at 41% through late summer<sup>14</sup>. For this experiment eight 10x10 foot plots of well-established Japanese stiltgrass were chosen. Situated under a forest canopy on a slight slope. Plots receiving Treatment #1 were solely exposed to 10% glyphosate. Plots designated for Treatment 2 were given one ounce of Humboldt Sticky per gallon, mixed with 10% glyphosate. Treatment 3 consisted of 10-tablespoons of nitrogen fertilizer per gallon and 10% glyphosate. Finally, Treatment 4 included the combination of all three components.

Two 2 1/2 gallon sprayers were used in carrying out Treatments 1-4 and their replicates, making eight total plots. The stiltgrass was sprayed until fully covered, but not dripping. Plots were selected and managed in order to decrease the potential influences of treatments applied to neighboring plots; a minimum of two feet was left between plots.

Table 1. Japanese stiltgrass - Plot layout

<b>Treatment 3</b> (gly., N)	<b>Treatment 2</b> (gly., HS)		<b>Treatment 1</b> (gly.)	<b>Treatment 2</b> (gly., HS)	<b>Treatment 4</b> (gly., HS, N)
		<b>Treatment 4</b> (gly., HS, N)	<b>Treatment 3</b> (gly., N)	<b>Treatment 1</b> (gly.)	

\*(gly. = 10.0% glyphosate; N = 10-tablespoons of nitrogen fertilizer per gallon; HS = 1-ounce of Humboldt Sticky per gallon)

Treatments 1 and 2 were delivered from sprayer 1, while sprayer 2 performed Treatments 3 and 4. Application rates varied due to mechanical differences in the two sprayers; though, initially calibrated to similar droplet size, sprayer 2 eventually developed a larger droplet size than sprayer 1. Such development resulted from a clog formulation in the nozzle of sprayer 2 <sup>12</sup>. These changes were especially apparent in Test 4. On another note, sprayer 1 dispensed more rapidly than sprayer 2, leading to about 50% more usage of treatment mixtures than in Treatments 3 and 4.

### 2.1.2. english ivy

The same four treatments used in the Japanese stiltgrass experiment described previously, were used to treat four, five-by-five foot plots of English ivy. These plots were selected on a nearby slope under a full-forest canopy. The plots were separated by at least two feet.

Table 2. English ivy - Plot layout

<b>Treatment 1</b> (gly.)	<b>Treatment 2</b> (gly., HS)	<b>Treatment 3</b> (gly., N)	<b>Treatment 4</b> (gly., HS, N)
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\*(gly. = 10.0% glyphosate; N = 10-tablespoons of nitrogen fertilizer per gallon; HS = 1-ounce of Humboldt Sticky per gallon)

The treatments were carried out with the same sprayers used previously; sprayer 1 serving for Treatments 1 and 2, and sprayer 2 used to apply Treatments 3 and 4. The sprayers persisted with the same variability described in the stiltgrass treatment, with regards to droplet size and flocculation issues in sprayer 2.

### 2.1.3. Japanese wisteria

Only one preliminary test was conducted on a flat plot of Japanese wisteria, about three-by-five feet in area. Treatment 4, previously mentioned, was used to complete this test. This plot, like the previous plots, rests under full shade.

### 2.1.4. Japanese knotweed

One preliminary test was conducted on a young and scattered population of Japanese knotweed. This site was also situated under full-forest coverage. Treatment 4 was prepared in sprayer 1 to complete the test.

## 2.2. Preliminary Experimental Observations & Results

### 2.2.1. *japanese stiltgrass*

All plots were successfully killed, though variations in results were difficult to identify. Further observations should be taken in the spring to explore any significant plot differences with regards to the emergence of native ground cover and reemerging stiltgrass. It is also suggested that treatments be conducted with lower levels of glyphosate to observe the different effects of applied mixtures.

### 2.2.2. *english ivy*

Observable results show that Plots 1, 2, and 4 received the most effective treatments having the quickest die-off of the vine, and the least amount of regrowth.

As Plots 1 and 2 were treated with sprayer 1, emitting about 50% more than sprayer 2, this may indicate that higher concentrations of herbicide may control the vine more effectively than lower concentrations. Furthermore, this may suggest that a combination of glyphosate and nitrogen, at the same application rate as Plots 1 and 2, may be as equally as effective as the first two treatments. Plot 3, receiving a lower application rate than Plots 1 and 2, supported the most regrowth out of all the test areas. Plot 4, treated with sprayer 2 at an application rate 50% lower than that observed in Plots 1 and 2, showed regrowth similar to that observed in Plots 1 and 2. These results suggest that the combination of nitrogen fertilizer and Humboldt Sticky, in a lower concentration of glyphosate, is equally effective in controlling the invasive as are higher concentrations of the herbicide as a solo chemical or in combination with Humboldt Sticky as a regulator.

Further research should be conducted in this area, to explore the effects of Humboldt Sticky and nitrogen as chemical regulators and enhancers in combination with lower concentrations of herbicide. This should be done to assess each individual component's abilities and interactions with the selected herbicide.

### 2.2.3. *japanese wisteria*

Treatment 4 proved to be effective in killing the vine. No regrowth is currently present. The original live-vine structure, now remains brittle and lifeless. Wisteria often requires multiple herbicide applications, therefore further observations should be taken to assess the possible regeneration of the vine in the spring.

### 2.2.4. *japanese knotweed*

By day eight, the effects of Treatment 4 were physically apparent throughout the treated population. Stems and foliage were already of yellow and brown pigment. On day 13, the majority of the knotweed was without foliage, and stems and stalks were completely brown in pigment, though still exhibiting fleshiness. By day 20, the plants were brittle and lifeless. There is currently no regrowth or sprouting in the proximity of effectively treated plants.

## 3. Secondary Experiments: Continued Exploration of the Effects of Foliar Fertilizer and Herbicide Combinations on Japanese Knotweed and Kudzu

A series of secondary experiments were carried out to further explore the implications of preliminary results and observations. Glyphosate and triclopyr are used in the following experiments on kudzu and Japanese knotweed. Most importantly, the concentration of glyphosate was adjusted to lower levels from pilot experiments involving Japanese knotweed, to better observe the differentiation between the effects of varying treatments. The spraying of these three sites took place over a two-day period, on September 2nd and 3rd, 2019. Observations and monitoring of the sites was completed weekly.

### 3.1. Experimental Method

#### 3.1.2. kudzu

In this experiment, four treatments were carried out. Treatment 1 consisted of 10% triclopyr (Garlon 3A). Treatment 2 was a mixture of 10% triclopyr and one-ounce of Humboldt Sticky per gallon. Treatment 3 was made of 10% triclopyr with 10-tablespoons of nitrogen per gallon. Treatment 4 was a combination of all three ingredients. Each plot was about 20x20 feet in sum. Plots were neighboring without separation on a soft slope at the front of the site, which increased to a steep slope by the back of the site.

Table 5. Kudzu - Plot layout

		<b>Treatment 4</b> <i>(tri., HS, N)</i>
<b>Treatment 3</b> <i>(tri., N)</i>	<b>Treatment 2</b> <i>(tri., HS)</i>	<b>Treatment 1</b> <i>(tri.)</i>

\*(*tri.* = 10% triclopyr; *N* = 10-tablespoons of nitrogen fertilizer per gallon; *HS* = 1-ounce of Humboldt Sticky per gallon)

Treatment was completed under fair weather conditions with a slight breeze present. Two sprayers were used, with one sprayer experiencing flocculation issues in the applications of Treatments 3 and 4. The foliar treatment was applied until full-foliage coverage, except for treatment occurring after flocculation arrived in the tank. Despite attempts to relieve the tank of resulting solids, the problem persisted and treatment was carried out anyway. Plots were monitored weekly after treatment was executed.

#### 3.1.3. japanese knotweed

As seen in the preliminary results of initial tests with Japanese knotweed, a combination of nitrogen fertilizer, Humboldt Sticky, and 10% glyphosate were effective in killing the above ground portions of the treated population. Therefore, the purpose of these secondary experiments is to further explore preliminary results and the effects of different treatment combinations, including combinations which incorporate the use of triclopyr, on the invasive species as a mature and well-established specimen. The plants differed in these experiments than in preliminary rounds as the plots were denser, more mature and well-established infestations. The factor of sun exposure was also significantly different in this phase, as one site is exposed to full sun, while the other is exposed to partial shade.

Before implementing treatments, steps were taken to clear other vegetative species present in the plots, ranging from mulberry vines, to privet and pokeweed. Overhanging tree branches were partially cut back in order to avoid damage to nearby trees, while dead stalks still connected to the knotweed crown were removed. Finally, plots were marked with flags.

##### 3.1.3.1. hill plots

Eight plots were selected for this site. Plots 5 through 8 were treated with a 2.0% glyphosate combination. Plot 8 received a treatment of 2.0% glyphosate in combination with 10-tablespoons of nitrogen fertilizer per gallon. Plot 7's treatment consisted of 2.0% glyphosate. Plot 6 was given a treatment of 2.0% glyphosate and one-ounce of Humboldt Sticky per gallon, while Plot 5 received a combination of all ingredients: Humboldt Sticky, nitrogen fertilizer and 2.0% glyphosate. Plots 1 through 4 were designated to receive treatments of these same combinations, but with a concentration of 2.1% glyphosate. Only plots 2 and 4 received their designated treatments; Plot 4 received a treatment of 2.1% glyphosate, while Plot 2 received a combination of 2.1% glyphosate and nitrogen fertilizer. Plot 3 was left untreated. Lastly, Plot 1 was alternatively treated with triclopyr at 10%, nitrogen fertilizer and Humboldt Sticky.

Table 3. Japanese knotweed - Hill-plot layout

<b>Plot 1</b> ( <i>tri.</i> 10%, <i>HS</i> , <i>N</i> )	<b>Green Plot</b> ( <i>No</i> <i>treatment</i> )	<b>Plot 2</b> ( <i>gly.</i> 2.1%, <i>N</i> )	<b>Plot 3</b> ( <i>No</i> <i>treatment</i> )	<b>Plot 4</b> ( <i>gly.</i> 2.1%)	<b>Plot 5</b> ( <i>gly.</i> 2.0%, <i>HS</i> , <i>N</i> )	<b>Plot 6</b> ( <i>gly.</i> 2.0%, <i>HS</i> )	<b>Plot 7</b> ( <i>gly.</i> 2.0%) <hr/> <b>Plot 8</b> ( <i>gly.</i> 2.0%, <i>N</i> )
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\*(*tri.* = *triclopyr*; *gly.* = *glyphosate*; *N* = 10-tablespoons of nitrogen fertilizer per gallon; *HS* = 1-ounce of *Humboldt Sticky* per gallon)

Primarily, spraying was carried out from under the knotweed canopy to access the underleaf stomata. Though, as some foliage was difficult to reach, certain plants were treated from the top. Before spraying commenced, neighboring plots were artificially separated, using large pieces of cardboard and ropes to construct a spray barrier. These boards were strategically placed in order to separate the foliage and high reaching stems that are often intermingled amongst densely populated stands of Japanese knotweed. Despite this effort, some overspray occurred, affecting adjacent plots, due to miss fire from under-canopy spraying. These effects will be further discussed in the results section.

### 3.1.3.2. stream plots

Four tests were completed at this site using 2% triclopyr. These four plots dress the slightly sloped bank of a stream. triclopyr was the chemical preference, as it poses no threat around water and amphibious species<sup>26</sup>. Plot 1 received a treatment of 2% triclopyr. Plot 2 received a combination of 10-tablespoons of nitrogen fertilizer per gallon and 2% triclopyr. Plot 3 was given a combination of one-ounce of *Humboldt Sticky* per gallon and 2% triclopyr. Lastly, Plot 4 received a mixture of 10-tablespoons of nitrogen fertilizer, one-ounce of *Humboldt Sticky* with 2% triclopyr. These plots were sprayed and separated in the same fashion as expressed in the previous Hill Plot method. Over-spray from adjacent plots may also play a factor at this site.

Table 4. Japanese knotweed - Stream-plot layout

<b>Treatment 1</b> ( <i>tri.</i> )	<b>Treatment 2</b> ( <i>tri.</i> , <i>N</i> )	<b>Treatment 3</b> ( <i>tri.</i> , <i>HS</i> )	<b>Treatment 4</b> ( <i>tri.</i> , <i>HS</i> , <i>N</i> )
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\*(*tri.* = 2.0% *triclopyr*; *N* = 10-tablespoons of nitrogen fertilizer per gallon; *HS* = 1-ounce of *Humboldt Sticky* per gallon)

## 3.2. Secondary Experimental Observations & Results

### 3.2.1. kudzu

Variations were not apparent in the effects of different treatments. Signs of applied treatment effects were evident, throughout all plots, as soon as three days preceding treatment applications. On day three, browning and yellowing of the vine and foliage was already present. By day 13, the effects had expanded throughout the plots, leaving scarce traces of vine life. On day 23, the vine appeared brown, brittle and lifeless across all plots. These tests indicate that high percentages of herbicide are effective along with fertilizer and adjuvant combinations in controlling kudzu.

Further research should be carried out in this area to assess the effects of lower concentrations of herbicide with the various combinations of fertilizer and adjuvant presented above. Also, potential benefits of incorporating phosphorous, magnesium and potassium fertilizers instead of nitrogen should be explored, as kudzu presents itself as a nitrogen fixing plant<sup>28</sup>. If nitrogen, other mentioned fertilizers and *Humboldt Sticky* prove to be effective, this could reduce the herbicide required to treat and eradicate kudzu infestations, as well as to potentially decrease the quantity of treatments needed to achieve infestation eradication. Finally, such implications may be positive for the emergence and nourishment of native ground cover, as such fertilizers could expedite native growth and enhance its nourishment once kudzu does not hold such a dominant presence in the area.

### 3.2.2. japanese knotweed - hill plots

Table 6. Emerging Sprouts - Hill plots

<b>Plots:</b> (x) ——— <b>Time:</b> (y)	<b>Plot 8</b> ( <i>glyphosate</i> 2.0%, N)	<b>Plot 2</b> ( <i>glyphosate</i> 2.1%, N)	<b>Plot 7</b> ( <i>glyphosate</i> 2.0%)	<b>Plot 4</b> ( <i>glyphosate</i> 2.1%)	<b>Plot 6</b> ( <i>glyphosate</i> 2.0%, HS)	<b>Plot 5</b> ( <i>glyphosate</i> 2.0%, HS, N)	<b>Plot 3</b> (No treatment )	<b>Plot 1</b> ( <i>triclopyr</i> 10%, HS, N)
<b>Day 33</b>	0	0	1	0	0	0	0	0
<b>Day 36</b>	0	0	6	0	0	0	0	0
<b>Day 45</b>	0	0	10	0	1	1	0	0
<b>Day 52</b>	1	0	11	0	2	1	0	0
<b>Day 66</b>	0	0	7	0	3	0	0	0

\*(N = 10-tablespoons of nitrogen fertilizer per gallon; HS = 1-ounce of Humboldt Sticky per gallon)

Browning of foliage was evident in all plots within the first week following treatment. Further browning of the above ground plant mass was seen by day 33. The first sign of knotweed sprouts was observed in Plot 7, 33 days following treatment. This may be an early indicator that glyphosate, alone, may be more effective applied in higher concentrations during the late summer; as Plot 4 showed no signs of regrowth at this time, though, it did support a green stalk that seemingly went untouched during treatment.

On day 36, 6 sprouts were present in Plot 7, while no other plots showed signs of sprouting. By day 45, one sprout was present in Plots 5 and 6 indicating live rhizome activity; though, it is to be noted that Plot 5's sprout stemmed from a broken stalk which was left untreated during application. A total of 10 sprouts were in Plot 7 on this day. Plot 1 showed no signs of regrowth, though, eight to 10 sprouts were present around its perimeter. This may suggest enhanced lateral bud action or potential rhizome connections between Plot 1 and the untreated-neighboring plot.

By day 52, the sprout population in Plot 7 increased to eleven; while Plot 8 hosted a single sprout. It may be possible that this sprout belonged to the underground rhizome system predominantly present in Plot 7 as it sat close to both Plot 7 and 8, though resulting in the designated area for Plot 8. Further research in how the knotweed rhizome moves and develops may help in assessing these results. Plot 6 increased to two sprouts; and Plot 5's sprout showed signs of deterioration. This occurrence in Plot 5 may hint at the treatment combination's ability to translocate efficiently within the rhizome and furthermore, amongst dormant rhizome buds.

On day 66, Plot 7 had only seven sprouts, with many showing signs of deterioration. Plot 6 supported three sprouts, while Plot 5's untreated sprout died out. The sprout originally noted to be in Plot 8 also failed to thrive. If it is correct to assume that this sprout's underground rhizome did in fact belong to Plot 8, this may suggest that nitrogen presents benefits in aiding in the translocation of glyphosate within the organism's underground rhizome system.

The previously mentioned green stalk from Plot 4, showed signs of diminution on this day. This may suggest that translocation is less effective in treatments that only use glyphosate. Finally, the untreated plot neighboring Plot 1 showed signs of advanced diminution on this day. This could be due to seasonal changes, which may also suggest that some of the sprout die-out may be linked to these changes. Otherwise, this might indicate effective translocation of treatment to the rhizome buds from the neighboring-treated plot.

### 3.2.3. *japanese knotweed* - stream plots

Table 7. Emerging Sprouts - Stream plots

<b>Plots (x):</b> Time (y):	<b>Plot 1</b> ( <i>tri.</i> )	<b>Plot 2</b> ( <i>tri.</i> , <i>N</i> )	<b>Plot 3</b> ( <i>tri.</i> , <i>HS</i> )	<b>Plot 4</b> ( <i>tri.</i> , <i>HS</i> , <i>N</i> )
<b>Day 33</b>	5	1	1	0
<b>Day 36</b>	6	1	2	0
<b>Day 45</b>	9	1	2	0
<b>Day 52</b>	8	1	3	0
<b>Day 66</b>	7	1	3	0

\*(*tri.* = 10% triclopyr; *N* = 10-tablespoons of nitrogen fertilizer per gallon; *HS* = 1-ounce of Humboldt Sticky per gallon)

By day 33, stalks began to show signs of browning and sprout life in Plots 1-3. There were five sprouts present in Plot 1, while only one sprout was found to be present in Plots 2 and 3. As Plot 1 was treated with only triclopyr at 2%, this suggests that fertilizer and regulators, such as Humboldt Sticky, enhance the effects of triclopyr when applied as a combination treatment.

On day 36, Plot 1 had six sprouts; Plot 2 had one, and Plot 3 supported an additional sprout in its population. Plot 4 remained sproutless. On day 45, Plot 1 had nine sprouts, while Plots 2-4 remained unchanged. By day 52, one sprout had died off from the nine previously recorded sprouts present in Plot 1. Plot 3 had an additional sprout, while Plots 2 and 4 remained the same as the prior recording.

Finally, on day 66, observations showed diminution of seven sprouts present in Plot 1. Plot 2's sprout still showed signs of good health with broad foliage and dark-green biomass. Plot 3 and 4 remained unchanged from the previous recording.

These results suggest that not only is glyphosate more effective in combination with nitrogen and Humboldt Sticky as separate additive agents in treatment combinations, but they also show great benefits in compatibility with one another in aiding the effectiveness of glyphosate within Japanese knotweed.

### 3.2.3. *comparisons between Japanese knotweed sites*

Results from both sites suggest, that up to this point in observations, nitrogen and Humboldt Sticky could serve as beneficial adjuvants in enhancing the ability of glyphosate and triclopyr in controlling the invasive and its sprouting rhizome buds. Furthermore, it is shown that the two adjuvants may work together to surpass the efficiency of the two as separate combinations in works with herbicide. Further observations are being taken to explore these results in greater detail.

## 4. Conclusions

The purpose of these experiments was to examine the effects of mixing herbicide applications with nitrogen fertilizer and Humboldt Sticky to control exotic invasive species. Though some plots showed no significant results due to high levels of herbicide application, sufficient evidence from preliminary and secondary experiments suggest that the addition of a wetting agent, Humboldt Sticky, or nitrogen fertilizer to foliar herbicide applications may increase the

effectiveness and efficacy of the chemical in killing the targeted invasive species. These two agents in combination together may further increase these effects of the herbicide in treatment.

Though these preliminary results implicate successful treatments with foliar nitrogen fertilizer, herbicide and Humboldt Sticky mixtures, further experiments with rigorous replication should be conducted in order to produce supportable statistical analyses that can be broadly applied in the field. Research into other foliar fertilizers, such as phosphorus, magnesium and potassium, should be explored as these fertilizers may be more effective in aiding the herbicide, depending on the target species. These fertilizers may also provide greater benefits for native species than nitrogen<sup>11</sup>.

Another area of relevance would be to research the root systems of targeted exotic invasive species. For instance, as seen with Japanese knotweed, dormant rhizome buds are difficult for herbicides to affect, leaving vital parts of the plant without treatment<sup>22 23</sup>. Ethylated oils may serve as an activator of these dormant areas and further enable the herbicide's activity within the specimen<sup>7 12 25</sup>. By exploring the complexities of different exotic invasive species' structures, mixtures can then be specified as to what fertilizers, adjuvants and herbicide will be most effective.

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## 6. References

1. Williams, L.E., Miller, A.J. (2001). Transporters responsible for the uptake and partitioning of nitrogenous solutes. *Annual Review Plant Physiology Plant Molecular Biology*. 52: 659-88.
2. *Journal of Agricultural and Food Chemistry*. (March 1, 1954). Pesticide-Fertilizer Combinations; Herbicides with Nitrogen Solutions Biggest News in Fertilizer-Pesticide Mixtures.2:6(292-93).
3. Jacob, K.D. (September 15, 1954). Status and Problems of Fertilizer-Pesticide Mixtures. *Journal of Agricultural and Food Chemistry*.2:19(270-276).
4. Maeda, H., Dudareva, N. (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annual Review of Plant Biology*. 63:73-105.
5. Herrmann, K.M., Weaver, L.M. (1999). The Shikimate Pathway. *Annual Review of Plant Physiology and Molecular Biology*. 50:473-503.
6. Foliar Fertiliser Ltd. (2004). How does foliar fertilizer work?/Photosynthesis & Agriculture. Retrieved from: [http://www.foliarfert.com/pages/how\\_does\\_foliar\\_fertiliser\\_work.htm](http://www.foliarfert.com/pages/how_does_foliar_fertiliser_work.htm)
7. Bashtanova, U.B., Beckett, K.P., Flowers, T.J. (2009). Review: Physiological Approaches to the Improvement of Chemical Control of Japanese Knotweed (*Fallopia japonica*). *Weed Science*. 57(584-592).
8. Tahir, M., Nadeem, M.A., Tanveer, A., Ayub, M., Hussain, A., Naeem, M., Javeed, H.M.R. (2011). The effect of urea as adjuvant on herbicide effectiveness in maize with full and reduced doses of herbicide. *Pakistan Journal of Life Social Sciences*. 9:1(45-51).
9. Felton, D.E., Ederer, M., Steffens, T., Hartzell, P.L., Waynant, K.V. (2017.) UV-Vis Spectrophotometric Analysis and Quantification of Glyphosate for an Interdisciplinary Undergraduate Laboratory. *Journal of Chemical Education*. 95(136-140).
10. Klosterboer, A.D., Bardsley, C.E. (1968). Assessing Compatibility of Herbicides and Nitrogen Solutions. *Weed*

Science. 16:4(468-470).

11. Ehrenfeld, J.G. (2005). Effects of Nitrogen Supply on the Dynamics and Control of Japanese Barberry and Japanese Stiltgrass. *United States Department of Agriculture*. Retrieved from: <https://reeis.usda.gov/web/crisprojectpages/0192809-effects-of-nitrogen-supply-on-the-dynamics-and-control-of-japanese-barberry-and-japanese-stiltgrass.html>
12. Tu, M., Randall, J.M. (2003). Weed Control Methods Handbook: Chapter 8 - Adjuvants. *The Nature Conservancy*. Retrieved from: <https://www.invasive.org/gist/products/handbook/21.Adjuvants.pdf>
13. Jones, D., Bruce, G., Fowler, M.S., Law-Cooper, R., Graham, I., Abel, A., Street-Perrot, A., Eastwood, D. (2018). Optimising physiochemical control of invasive Japanese knotweed. *Biological Invasions*. 20:8(2091-2105).
14. US EPA. (2017). Pesticide Product Label: Glyphosate 41% Superconcentrate Herbicide. Retrieved from: [https://www3.epa.gov/pesticides/chem\\_search/ppls/084009-00027-20170310.pdf](https://www3.epa.gov/pesticides/chem_search/ppls/084009-00027-20170310.pdf).
15. US Forest Service. (n.d.) English Ivy. Retrieved from: [https://www.fs.usda.gov/naspf/sites/default/files/naspf/pdf/english\\_ivy.pdf](https://www.fs.usda.gov/naspf/sites/default/files/naspf/pdf/english_ivy.pdf).
16. Dickens, E.D. (2015). Wisteria (Wisteria sp.) Control herbicide options. *Georgia Forest Productivity: Publication Series*. 8(1-4).
17. Wisteria Control Plan. (2016). Retrieved from: <http://www.wachng.org/Documents/Wisteria2016.pdf>.
18. Martin, T. (n.d.). Weed Notes. *Wildland Invasive Species Team/The Nature Conservancy*. Retrieved from: <https://www.invasive.org/gist/moredocs/wisspp01.pdf>.
19. Gover, A., Johnson, J., Sellmer, J. (2007). Managing Japanese Knotweed. *US Department of Agriculture & Penn State University*. Retrieved from: [https://www.nrcs.usda.gov/Internet/FSE\\_DOCUMENTS/nrcs142p2\\_017951.pdf](https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_017951.pdf).
20. Everest, J.W., J.H. Miller, D.M. Ball, and M. Patterson. (1999). Kudzu in Alabama: History, Uses, and Control. *Alabama Cooperative Extension System*. ANR-65.
21. Humboldt Nutrients. (n.d.) Humboldt Sticky: Product Information. Retrieved from: <http://www.humboldtnutrients.com/products/high-powered-additives/humboldt-sticky/>.
22. Phlorum. (2014, March 5th). Novel Ash Die Back Treatment to Combat Japanese knotweed. Retrieved from: <https://www.phlorum.com/blog/2014/03/05/ash-die-back-japanese-knotweed-mitochondrial-inhibitor/>.
23. Phlorum. (2014, April 9th). *How to Control Japanese knotweed*. Retrieved from: <https://www.phlorum.com/blog/2014/04/09/control-japanese-knotweed/>.
24. *Creative Proteomics*. (n.d.). Aromatic Amino Acids Analysis Service. Retrieved from: <https://www.creative-proteomics.com/services/aromatic-amino-acids-analysis-service.htm>.
25. Soll, J. (2007). Control of Invasive Knotweed on a Landscape Scale: Lessons learned from seven years of experiments and field trials. *The Nature Conservancy*. Retrieved from: <https://www.wsweedscience.org/wp-content/uploads/slide-presentations/15%20Soll.pdf>.
26. US EPA. (2008). Pesticide Product Label: Triclopyr 4. Retrieved from: [https://www3.epa.gov/pesticides/chem\\_search/ppls/066222-00153-20080429.pdf](https://www3.epa.gov/pesticides/chem_search/ppls/066222-00153-20080429.pdf).
27. Missouri Department of Conservation. (n.d.) Kudzu: Background, Life History. Retrieved from: <https://mdc.mo.gov/trees-plants/problem-plant-control/invasive-plants/kudzu-control>.
28. Hickman, J.E., Wu, S., Mickley, L., Lerdau, M. (2010). Kudzu (*Pueraria montana*) invasion doubles emissions

of nitric oxide and increases ozone pollution. *Proceedings of the National Academy of Sciences of the United States of America*. 107(22):10115-10119.

29. Gover, A., Johnson, J., Lloyd, K., Sellmer, J. (2008). Invasive Plant Species Management: Japanese stiltgrass (*Microstegium vimineum*). *Penn State, Department of Horticulture*. Retrieved from: <https://plantscience.psu.edu/research/projects/vegetative-management/publications/state-parks-invasive-species-management-quick sheets/4.-japanese-stiltgrass-microstegium-vimineum>.

30. Apsley, D., Smith, A. (2011). Controlling Non-Native Invasive Plants in Ohio Forests: Japanese stiltgrass. *Ohio State University, the College of Food, Agricultural, and Environmental Sciences*. Retrieved from: <https://ohioline.osu.edu/factsheet/F-70-11>.