A Chemical Analysis of Organic Herbicides

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Photo courtesy of: Henry's Fork Cooperative Weed Management Area

Introduction

Herbicides are a common way of eradicating unwanted plant growth. There are several herbicides on the market; however there is a perceived trade-off between effectiveness and sustainability.

One of the most widely commercialized herbicides in the world is glyphosate (Vivancos 2011). It is considered “virtually ideal” because of its broad spectrum and its supposedly low toxicity (Duke and Powles 2008). It kills plants by inhibiting the shikimic pathway (Vivancos 2011). This pathway is responsible for producing aromatic amino acids such as tryptophan and tyrosine. Glyphosate-induced alteration of amino acid metabolism allows the plant to accrue greater levels of antioxidant enzymes (Vivancos 2011). The plant photosynthesis pathway becomes severely reduced. It leads to oxidative stress for the plant, thus showing a strong correlation between amino acid metabolism and cellular redox state, which is dependent upon a balance between processes that require energy and processes that use energy (Vivancos 2011). Laboratory tests with human and animal cells suggest that glyphosate or glyphosate-containing herbicides cause genetic damage in both (Cox 2004). Several studies show that glyphosate alters the production of sex hormones. Glyphosate can mimic the estrogen hormone, which plays an important role in human reproduction. There have been several studies on the effects of glyphosate and practically every study shows that glyphosate causes genetic damage and even abnormal development in frogs (Cox 2004).

Juglone or 5-hydroxy-naphtalenedione is a compound that is derived from the leaves, roots and bark of species of plants in the Juglandaceae family. It is sometimes used as an herbicide but traditionally it is used as a natural dye for fabric and clothing.

It's initial form is hydrojuglone and becomes juglone when it comes into contact with air or soil (Shrestha 2009). Specifically, Black Walnut (*Juglans nigra*) is a plant that has allelopathic effects on other plants in that it is extremely toxic and negatively affects ATPase (which is imperative for cell metabolism) as well as decreases photosynthesis in leaf tissue (Shrestha 2009). It is thought to act as a respiration inhibitor by withholding the needed energy for metabolic activities (Black Walnut Toxicity). Similarly to glyphosate, it also affects aquatic plants (Shrestha 2009).

Figure 1. Leaves and roots of *Juglans nigra*

Photo courtesy of: Exploring the world of trees…a tree species blog; Hilton Pond Center for Piedmont Natural History

Juglone has been isolated and is commercially used in several products ranging from dietary supplements to skin care. The debate continues as to whether juglone is toxic to people. It is found to affect horses when used in their bedding material and causes some allergic reaction when pollen is shed (Funt & Martin). One of the known targets of juglone is the enzyme peptidyl-protyl isomerase or Pin1. P53 is a tumor suppressor in human and animal cells that is activated after DNA damage, while H2Ax is one of the several genes encoding the histone H2A. Peptidyl-protyl isomerase plays a role in the stabilization and activation of p53 following damage to DNA. The natural toxin juglone causes degradation of p53 and induces rapid H2Ax phosphorylation and cell death in human fibroblasts. If juglone inhibits transcription then it also induces p53 and inhibits Pin1, thus reducing the body's ability to fight tumor formation (Paulsen & Ljungman 2005). According to the German Commission E. Report, daily use of products that contain juglone are connected to increased risk of cancer of the tongue and leukoplakia of the lips (Paulsen & Ljungman 2005). Juglone also seems to inhibit the protein kinase C which phosphorylates other proteins such as serine (Paulsenn & Ljungman 2005). Juglone is found not to be safe because it can penetrate the plasma membrane and causes polarization by blocking potassium channels (Cox 2004).

Figure 2. Leukoplakia of the tongue. Photo courtesy of: dermRounds Dermatology Network

Figure 3. Aerial *Dioscorea bulbifera* Photo courtesy of: John

Air potato, *Dioscorea bulbifera*, is a non-native vine plant species that originally came from both Asia and Africa (Wheeler et al 2007). Although air potato's initial immigration path to Florida is unclear, its earliest documentation is 1905 (Air Potato Task Force 2008). Because air potato is an herbaceous, perennial twining vine it is very successful at climbing on native vegetation and destroying what lies beneath it. Air potato is easy to identify with its broad ovate-chordate leaves that have three main nerves that reach from the base of the leaf to the tip. The vine reproduces through bulblike growths; these aerial tubers called bulbils and are what give the vine its common name of air potato. The common name however, is misleading because the bulbils that are produced are not edible and can cause nausea if consumed due to the presence of cyanogens and dioscorine, a toxic alkaloid (Air Potato Task Force 2008). This plant was once considered an ornamental species but is now considered to be one of the most aggressive weeds (Tear 2004), and because air potato is able to

Figure 4. Tuber of *Dioscorea bulbifera* Courtesy of: John

f environments it has been found in several other states including Mississippi, Louisiana, Texas, and Hawaii (Wheeler et al 2007). Air potato is currently listed as a category I noxious weed by the Florida Department of Agriculture and Consumer Services (FLEPPC 2011) and it is vital that we begin to take back control from this species in order to protect our native plant communities.

Earpod Tree, *Enterolobium spp*., is native to Central Mexico and northern areas of South America but has now made its way over to Central Florida (Broschat 2007) and is newly considered to be an invasive species at UCF. The earpod tree is a fast growing, large, and drought tolerant, ornamental deciduous tree common in Costa Rica that is only considered invasive in certain conditions

Figure 5. Earpod transplant speimens in Toedter's truck. Photo courtesy of: John

(Rocha and Aguilar 2001). While it is no longer included on the Florida Exotic Pest Plant Council's invasive species list, the earpod tree is considered invasive according to UCF's

Landscape and Natural Resources Weed Management Plan (UCF LNR 2010). Currently at UCF the earpod tree population is quickly increasing and out competing natives and disrupting native plant communities in highly valued areas on campus (UCF LNR 2010). This species has become a high priority to control because of its rate of expansion and disruption to native plant species. These trees are also sometimes removed due to their low wind resistance, which is not conductive in Florida with our hurricane season. Earpod trees can be easily identified by their distinctive hard seed pods that are shaped like a human ear, thus where it gets the common name earpod tree. The leaves are doubly-compounded and the tree also produces many small white flowers (Culbert 2007). Although earpod tree may not be considered as invasive as air potato it is still very important to develop an effective way to reduce the population around the UCF campus in an attempt to preserve our many diverse ecosystems around the campus.

Figure 6. Earpod specimens in the field plot. Photo courtesy of: John

The reason we have chosen these two invasive species for this experiment is because of their difference in plant type. Air potato is a vine species while the earpod tree is a woody plant species. Using two different plant types allows us to see how each herbicide works on varying plant types. Glyphosate is labeled to work on grasses, broadleaf, and woody plants while juglone works well on many types of broad-leaved forbs but most grasses and some woody species can tolerate juglone.

Based on the information we have gathered we believe that the organic herbicide, juglone, will be just as effective at eradicating invasive species as the non organic herbicide, glyphosate.

Methods

The procedure will require air potato vines and earpod trees to be planted in a natural setting as well as a laboratory to ensure the validity of the data. The two plants will be treated with two different herbicides, varying in low, medium, and high concentration levels. These varying concentrations will allow us to view the potential efficiency of the herbicides; for example, while juglone might be effective, if it must be used at much higher concentrations than glyphosate, it may not be worth the trade-off.

Table 1. Concentration amounts for each herbicide

Herbicide	**Low Concentration**	**Medium Concentration**	**High Concentration**
Juglone (Organic)	1x: 0.00365g/liter (0.000365%)	20x: 0.075g/liter (0.0075%)	30x: 0.1095g/liter (0.01095%)
Glyphosate (Synthetic)	.64 oz/.5 gallon (1%)	1.92 oz/.5 gallon (3%)	3.2 oz/.5 gallon (5%)

There will be 48 specimens in the natural setting, with three control specimens for each variable in the field. There will be 32 specimens in the lab setting and two controls for the lab plot. Therefore there will be a total of 80 specimens. The synthetic herbicide chosen will be glyphosate, and the organic herbicide will be juglone.

Table 2. Number of replicates per concentration of each herbicide for both Air Potato and Ear Pod, respectively.

Field plants	**None**	**Low**	**Medium**	**High**
Juglone (Org)	3x	3x	3x	3x
Glyphosate (Syn)	3x	3x	3x	3x
Lab plants	**None**	**Low**	**Medium**	**High**
Juglone (Org)	2x	2x	2x	2x
Glyphosate (Syn)	2x	2x	2x	2x

The herbicides will be applied to the plants once and repeated as necessary. We will observe vegetation changes over a five-week span by our research team in order to obtain results. Pictures will be taken weekly to document the mortality rate of the invasive plants. The same will be done within the lab to analyze the difference in a controlled setting against a natural one. In the field we will require a set of materials including:

Figure 7. Collecting Air Potato specimen for the lab plots

1. Juglone
2. Glyphosate
3. Herbimax Oil Surfactant and Adjuvant
4. Tracker (admiral liquid)
5. Invasive species of plant
    - ❖ Air potato
    - ❖ Earpod Tree

6. Solo Accupower 416 5gal Backpack Sprayer
7. Chemical hand sprayer 32oz
8. Snake chaps
9. Camera (documentation of plant reciprocation)
10. Designated plots
11. Chart (representing plant growth or stunt)
12. ArcGIS mapping

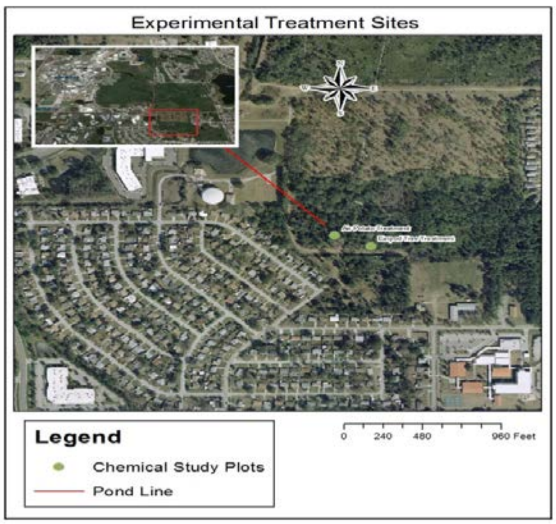


Figure 8. Plot areas for treatment

Courtesy of: Jason Toedter

The lab in located on UCF campus in the biology greenhouse. The plots will be designed to mimic the plots and setup in the field. The specimens in the lab will be treated with the same herbicides but in a sterile environment, therefore will require

1. Gloves
2. Goggles
3. Clean plots (to ensure no cross contamination to ensure results)
4. Shovels (for transplanting lab plots)

Once the air potato and earpod tree were transplanted and moved to the lab plots the experiment was ready for the application of herbicides. Two days passed and when we arrived at the plot to continue the experiment we realized that most the air potato and some earpod were dying before the application of herbicides. To combat this situation we decided to reduce the amount of plant replicates from three to two and make sure the plants we transplanted were healthy enough to survive the transition.

Data Analysis

We will begin to analyze the data by conducting observations twice a week. When we arrive to our designated plots we will first capture a photo and document any observable changes. Qualitative changes will be recorded according to:

- Plant Height (We will measure the plant height to determine if the species is growing, shrinking, or stable).
- Coloration (We will observe the coloration of the species and compare it to control to help determine the effects of the herbicide).
- Species per plot (We will count the species within the plot to determine if they are reproducing, stable, or dying off).

All of the observations made will help us categorize the species on a scale of 0-3 with the use of decimals if needed. This scale will help us formulate graphs to show the life cycle of the species within the plot.

0: Death, species no longer shows any signs of life.

1: Poor, Species are still alive but show signs of death. Plant is brown, withered, limp, and is approaching end of life.

2: Fair, Species is alive but is beginning to show signs of deterioration. Leaves are beginning to brown; plant growth is slowing or has already stopped.

3: Excellent, Species is thriving, showing growth, lush, green, and shows no sign of deteriorating.

Field Data Results:

Glyphosate application to the Air potato, *Dioscore abulbifera*:Following the application of glyphosate on November 16, 2012, the air potato resulted in a 100% death rate with all concentrations. The control specimen with no herbicide application remained in good health. (Table 3)

Juglone application to the Air potato, *Dioscorea bulbifera:* After the application of Juglone, the species resulted in a 100% death rate at the low and medium concentrations. The high concentration yielded a death rate at 67 percent. (Table 4)

Glyphosate application to The Earpod Tree, *Enterolobium spp:* Glyphosate resulted in 0% death with all concentrations of herbicide. All concentrations had an effect on the species but no concentration ultimately lead to death.

Juglone application to The Earpod Tree, *Enterolobium spp:*,The application of Juglone, resulted in 0% death at all concentrations. The low concentration showed the greatest signs of death followed by the medium and high concentrations.

Table 3: Life cycle of Air potato, *Dioscorea bulbifera*in a natural setting following the application of Glyphosate on November 16, 2012.

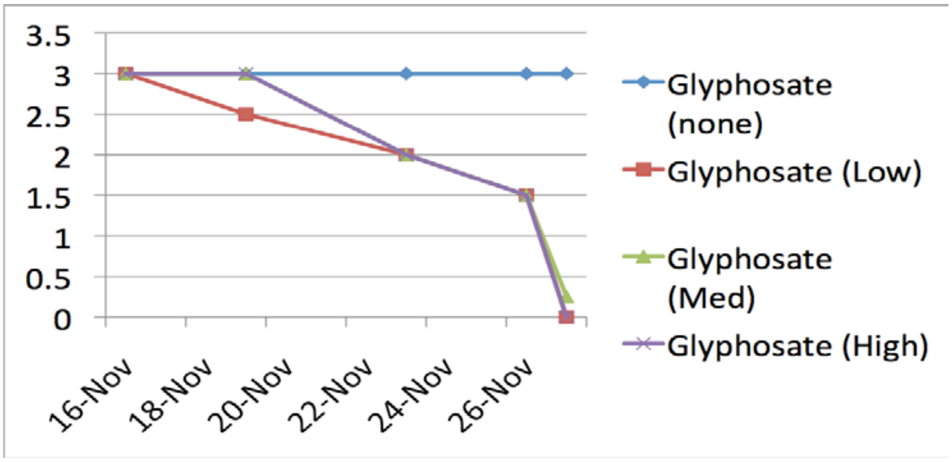
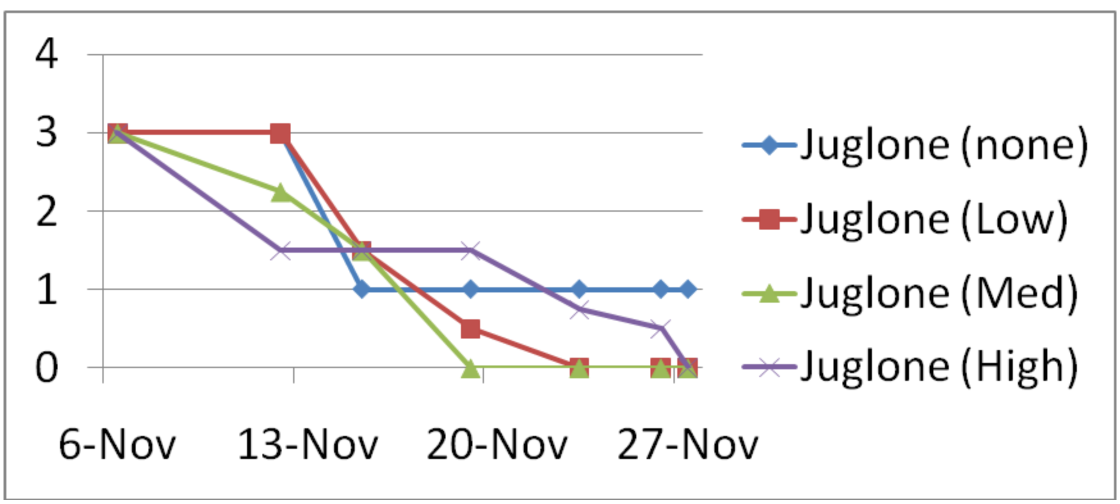


Table 4: Life cycle of Air potato, *Dioscorea bulbifera*in a natural setting following the application of Juglone on November 6, 2012.



<u>Lab Data Results</u>:

Glyphosate application to the Air potato, *Dioscorea bulbifera:* Following the application of glyphosate the air potato resulted in a 100% death rate at all concentrations. The control with no herbicide application showed a 0% death .

Juglone application to the Air potato, *Dioscorea bulbifera:* Juglone resulted in a 100% death at all concentrations. The control specimen showed no signs of death.

Glyphosate application to The Earpod Tree, *Enterolobium spp:* Glyphosate resulted in 0% death at all concentrations. The high concentration of glyphosate produced the highest signs of death between all concentrations. The control specimen showed no signs of death.

Juglone application to The Earpod Tree, *Enterolobium spp:,Juglone resulted in 50% death for the low concentration, 0% death for medium and high concentrations. The low concentration of herbicide resulted in the highest rate of death. The control species had 0% death.*

Table 5 : Life cycle of Air potato, *Dioscorea bulbifera* in a natural setting following the application of Juglone on November 6, 2012.

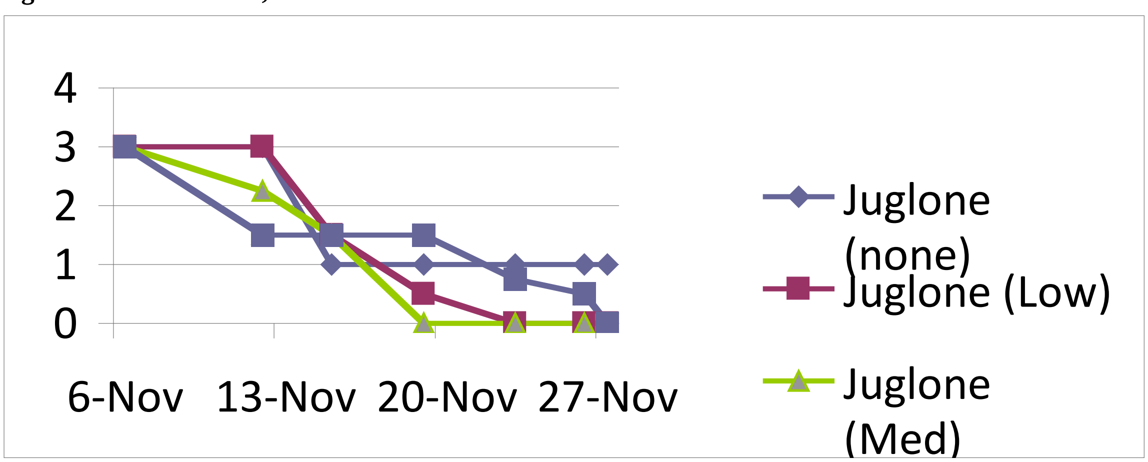


Table 6: Life cycle of The Earpod Tree, *Enterolobium spp* in a natural setting following the application of Glyphosate on November 16, 2012

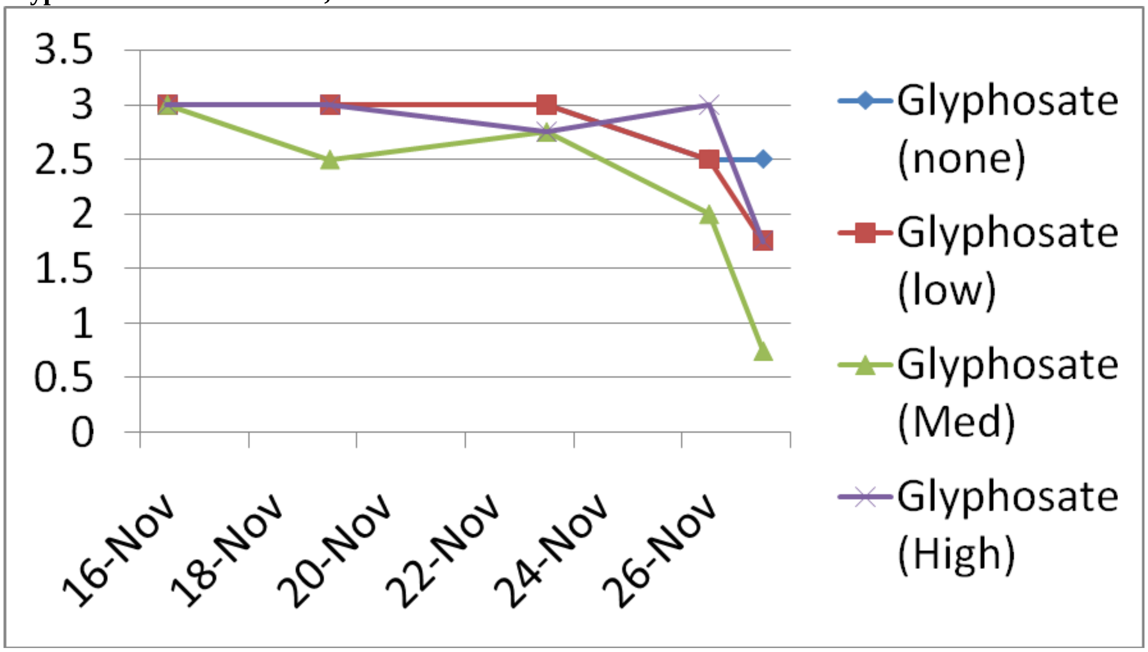


Table 7: Life cycle of Air potato, *Dioscorea bulbifera*in a lab setting following the application of Juglone on November 6, 2012.

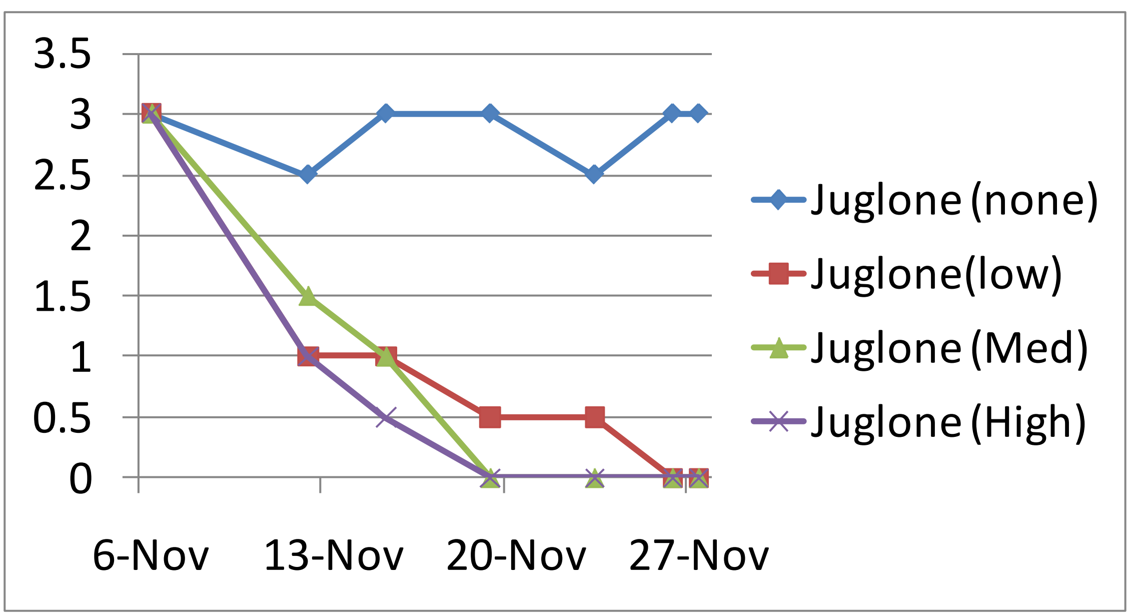
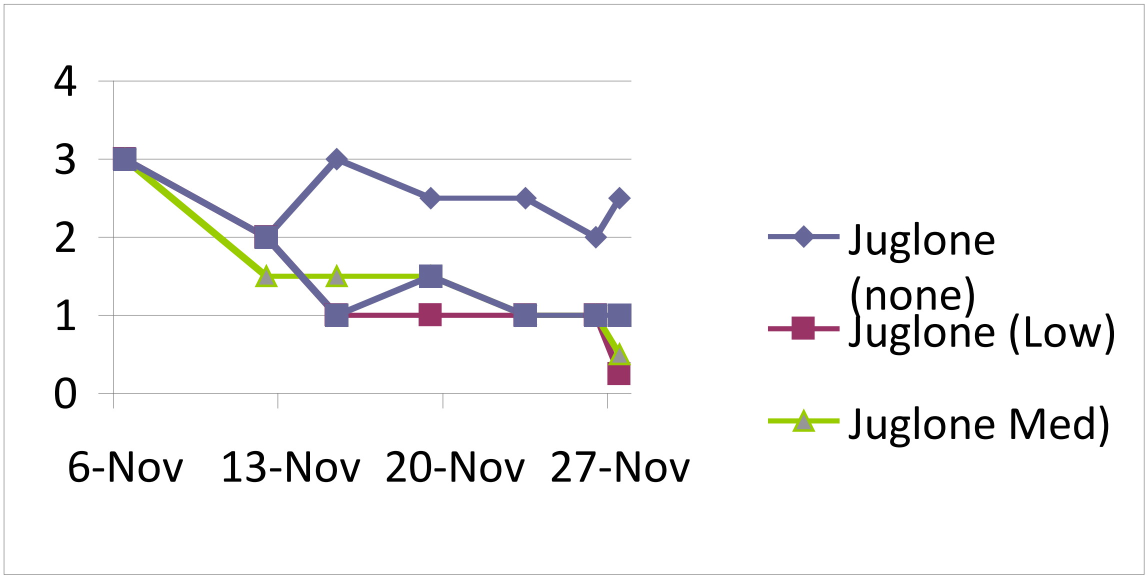


Table 8: Life cycle of The Earpod Tree, *Enterolobiumspp* in a lab setting following the application of Juglone on November 6, 2012.



<u>Discussion</u>

The least expensive herbicide for the air potato plants in the field was juglone at a low concentration (Table 9). It was just as effective as the medium concentration with a 100% death rate, however the medium concentration killed the plants at least 4 days earlier (Figure 9). This is only significant in effectiveness if time is a major constraint.

Figure 9. Juglone at medium concentration applied to *Dioscorea bulbifera*

The most effective herbicide for ear pod trees in the field plots was glyphosate at a medium concentration (Table 9). However, a greater decline in health was seen in the low and high concentrations of juglone. The cost of the low concentration of juglone was less than half of the medium concentration of glyphosate. It is interesting to note that of the ear pods in the field plots, the controls for the glyphosate group had the greatest overall decline in health (Figure 10). This suggests either too close of a proximity to the ear pods that were sprayed, or other climatic factors.

The least expensive herbicide for the air potato plants in the lab was juglone at a low concentration (Figure 11). The organic high concentration killed the plants at least 4 days earlier than the low concentration (Table 3), but as mentioned earlier, this is only significant in effectiveness if time is a major constraint.

Figure 11. Air potato shortly after juglone application

Figure 10. Ear pod control for glyphosate

The most cost effective herbicide for the ear pods in the lab was the juglone at a low concentration (Table 4). It had the same death rate as the synthetic herbicide at a medium concentration, but with a greater decline in health.

Table 9. Most effective herbicide per plot variable; least expensive herbicide between the most effective concentrations per herbicide

	Most effective	Least Expensive
Ear Pod Field	Glyphosate- Medium concentration: %33 death %56 decline	Juglone- Low concentration: $2.01
Ear Pod Lab	Juglone- Low concentration: %50 Death %92 decline	Juglone- Low concentration: $1.34
Air Potato Field	Juglone- Low concentration: %100 death %100 decline	Juglone- Low concentration: $2.01
Air Potato Lab	Juglone- Low concentration: % 100 death %100 decline	Juglone- Low concentration: $1.34

In every variable, the organic herbicide has proven cheaper; the most expensive concentration of organic herbicide is less than half the cost for the cheapest concentration of the synthetic herbicide. For most variables, the organic herbicide was proven more effective in killing plants, and when the synthetic herbicide had a higher death rate, plants sprayed with the organic herbicide still had a greater overall decline in health.

Table 10. Cost of each herbicide according to concentration level

	Low concentration	Medium concentration	High concentration
Organic all plants	$6.68	$6.94	$7.06
Synthetic all plants	$15.12	$15.92	$16.72
Organic per plant	$0.67	$0.69	$0.71
Synthetic per plant	$1.51	$1.59	$1.67

Some of the barriers that may have affected the outcome include plant locations, recent weather patterns, and cross contamination of herbicide concentration. The lab plants were first placed in an area of lower elevation than its surroundings. Precipitation would create small pools, decreasing the health of the air potatoes. The plants were then moved to a higher elevation where water could not accumulate. Hurricane Sandy caused a greater than normal increase in precipitation, further decreasing the health of the lab plants. The hurricane reached Florida October 29th, 2012; the large amount of wind also affected the plants and even turned some lab specimens over. Luckily, there was no flooding in either the lab or field plots.

Both herbicides inhibit the photosynthetic pathway of the plants (Vivancos 2011; Shrestha 2009). As plants decreased in 'health', the first signs were yellowing and wilting of leaves. The ear pods that were sprayed with glyphosate decreased only somewhat in health, with a total of 2 plants dying that were sprayed. Glyphosate's stated effectiveness is on grass, sedge and broad-leaved species; this may account for its ineffectiveness as compared to air potato (Cox 2004).

This experiment has shown that the organic herbicide juglone is just as effective at eradicating invasive plants as its synthetic counterpart, glyphosate. There is no need to use a synthetic substitute when a naturally occurring phytochemical fulfils the same utility.

Juglone is still a hazardous herbicide that should be used with extreme caution. However, it is an option that allows for the most social equity. Monsanto is the producer of Roundup, which is an herbicide whose main active ingredient is glyphosate. They also alter their genetically modified crops to be resistant to glyphosate (Norsworthy 2012). This creates both a hindrance in the economic development of farmers that use their seeds which produce infertile offspring as well a disparity in social justice, since the farmers becomes reliant on the corporation who originally sold them the seeds for another crop. With an option like juglone, farmers are no longer dependent on these corporations.

The patent on glyphosate has expired, allowing for companies such as Bayer, Syngenta and Nufarm to sell herbicide products that contain glyphosate (Brandli & Reinacher 2012). China produces 400,000 tons of glyphosate annually, about half of total global production (Brandli & Reinacher 2012). A study in Germany tested the urine of a population in Berlin that did not previously come into any contact with the herbicide, and found extremely high levels of glyphosate in their urine, about 5 to 20 times more than the limit for drinking water (Brandli & Reinacher 2012). It is likely to be from the produce consumed that was sprayed with glyphosate.

Humans have irrevocably altered native ecosystems all around the world. Invasive species infiltrate healthy ecosystems from several venues, such as ballast water from ships (Deacutis and Ribb 2002). Preventative measures are extremely helpful, however mitigation methods have become a necessity. This research reinforces the potential for effective, sustainable eradication of plants that threaten the integrity of UCF's local biodiversity.

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