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The Effects of Herbicide on the Endangered Herb Baptisia Arachnifera and Preliminary Nutrient Survey of Leaf Tissue and Soil

Ruth Ann C. Steinbrecher

THE EFFECTS OF HERBICIDE ON THE ENDANGERED HERB *BAPTISIA ARACHNIFERA* AND PRELIMINARY NUTRIENT SURVEY OF LEAF TISSUE AND SOIL

by

RUTH ANN CONSTANCE STEINBRECHER

(Under the Direction of Subhrajit Saha)

ABSTRACT

Baptisia arachnifera (Hairy Rattleweed) is an endangered herbaceous legume that only occurs in Wayne and Brantley Counties of Georgia, United States. Many of the remaining populations exist in areas now managed for timber. This study investigated the effects of Imazapyr, an herbicide commonly used in timber management, on growth and survivorship of *B. arachnifera* under both field and greenhouse conditions. This study also analyzed leaf and soil samples from six populations of *B. arachnifera* to determine the nutrient content of the leaves and soil. A recensus of a *B. arachnifera* population was also conducted in a site without commercial timber management. In the greenhouse, all *B. arachnifera* that were treated with herbicide died, regardless of herbicide application level within ten weeks. Control treatments survived and grew throughout the study. Field results showed that plants treated with low and high levels of herbicide died 2-4 weeks before the control plants, which also died due to heat stress. The concentrations of micronutrients (Al, B, Cu, Fe, Mn, Na and Zn) in leaf tissue differed across sites. Calcium concentration in the soil was found to be higher in where *B. arachnifera* are present when compared to sites that did not contain *B. arachnifera*. A higher percentage of sub-adult *B. arachnifera* were found in the 2013 census than the 2010 census of the site without commercial timber management. However, a higher percentage of seedlings, juveniles and reproductive *B. arachnifera* were found in the 2010 census. The finding of this study do not support the use of herbicide Imazapyr on sites with *B. arachnifera*. Future directions for research should include a closer look at how other competition controls such as burning and thinning affect each life stage of *B. arachnifera*, as well as studies on the overall health of each individual population of this endangered species on both managed and unmanaged timber land.

INDEX WORDS: Hairy rattleweed, Imazapyr, Greenhouse study, Field study, Micronutrients, Georgia, Endangered species

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RUTH ANN CONSTANCE STEINBRECHER

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RUTH ANN CONSTANCE STEINBRECHER

Major Professor: Subhrajit Saha
Committee: Lissa M. Leege
Ray Chandler

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CHAPTER 1
LITERATURE REVIEW

Approximately one third of the 17,000 native vascular plant species in the United States are considered endangered or threatened (Negrón-Ortiz 2014). In 1973, the Endangered Species Act was passed in the United States and this act states the importance of conservation: "…other species of fish, wildlife and plants have been so depleted in numbers that they are in danger of or threatened with extinction;" and that "these species of fish, wildlife and plants are of esthetic, ecological, educational, historical, recreational, and scientific value to the Nation and its people" (Endangered Species Act, 1973). A study by Zavaleta et al., 2010 showed that ecosystems with high plant biodiversity are more productive and are better able to withstand and recover from climate extremes, pests and disease over long periods.

Conservation efforts should focus primarily on species that are rare, as these species have a greater chance of extinction than those that are common (Davies et al. 2004, Johnson 1998, Levin et al. 1996, Pimm et al. 1988). Species rarity is often measured by species abundance and range size (Kunin, 1997; Murray et al., 2002). It has been found that rare species are at a greater risk of genetic simplification that can ultimately reduce a species' ability to adapt to changing environmental conditions, and lead to higher rates of inbreeding (Lande, 1998).

When trying to guide recovery efforts of endangered species, it is important to study and understand the patterns of the decline of the species (Leidner and Neel, 2011). Some examples of these patterns may include reductions in geographic range, number of populations, or overall abundance (Leidner and Neel, 2011). Discovering what causes these patterns is equally as important to the conservation efforts of this endangered species.

For the scientific management of an endangered species, it is important to study the biology of growth and survival requirements of the subject of interest. Species biology (more specifically, natural history) has been identified as "the key to plant preservation" because of its ability to reveal factors that limit long-term persistence (Massey and Whitson, 1980). In order to gain optimum knowledge of how to stabilize and promote recovery of a species, work must be done both with wild populations as well as organisms that are kept in a controlled environment. Havens et al. stated that effective plant conservation includes addressing information about species distribution and rarity, as well as providing public education to mitigate threats facing endangered species (Havens et al., 2014).

Human activities influence species rarity when they result in habitat destruction and/or degradation on plant populations (Fiedler and Ahouse, 1992). One example of this is commercially exploiting a forest that is home to an endangered species (Ben and Lassere, 2008). The logging of forests endangers approximately 109 plant and animal species in the United States alone (Czech and Devers, 2000). Brockway and Lewis (2003) found that plant species richness in the understory of thinned forests in a longleaf pine bluestem ecosystem decreased 44% from 50 to 28 species, while clear cut areas dropped 39% from 49 to 30 species on average. Plant species diversity also followed a declining trend through time. Timber management was found to have both positive and negative effects on ten different species of rare plants (including *Astragalus agnicidus*, *Campanula californica*, *Erythronium revolutum*, *Horkelia tenuiloba*, *Lycopodiu clavatum*, *Mitella caulescens*, *Pleuropogon hooverianus*, *Senecio bolander*, *Sidalcea bolanderi*, *Sidalecea malachroides* and *Usnea longissima*) found in a coast redwood forest habitat (Scholars and Golec, 2007).

The southeastern plain region of the United States is an ecoregion that is forested predominantly with species of oak, hickory and pine. Forest cover accounts for approximately 52% of the ecoregion, with the cycle of forest cutting and subsequent regrowth dominating changes to the forest land (Napton et al., 2010). The longleaf pine ecosystem resides in this ecoregion, but has greatly dwindled in its initial coverage at the time of European settlement of 30 million ha to a current coverage of only 1.2 million ha that mostly exist in isolated fragments (Outcalt and Sheffield 1996, USF 2003). Disruption of fire regimes, urbanization and land conversion are the primary reasons for the decline of this ecosystem (Brockway et al. 2006, Outcalt 2000, Van Lear et al., 2005). The longleaf ecosystem is home to approximately 187 species of rare plant species, most of which have narrow habitat requirements (Walker 1993, Walker 1998).

One such species is *Baptisia arachnifera* (also known as hairy rattleweed) a federally endangered species that is endemic to only Wayne and Brantley Counties of southeast Georgia. Most of its populations exist of land that is currently managed for timber (U.S. Fish and Wildlife Service, 1984). *Baptisia arachnifera* is classified with a rarity rank of G1, S1 (globally and state critically imperiled with 5 or fewer occurrences or fewer than 1,000 individuals) (Fish and Wildlife Service, 1996).This federally endangered perennial legume derives its name from the dense tomentose hairs that cover the leaves and stem (Kral, 1983), giving it a “cobwebby” appearance. It prefers the open pinewoods and mixed pine-hardwoods with sandy soil, common in the Coastal Plain of SE Georgia (U.S.Fish and Wildlife Service, 1984).

Baptisia arachnifera has been listed as endangered since 1978 due to loss of habitat and low numbers of individuals (U.S. Fish and Wildlife Service, 1984). Studies have linked the decline of the species with pine tree bedding practices and fire suppression in timber management sites (U.S. Fish and Wildlife Service, 1978). The species has lost nearly 90% of individuals in monitoring populations in sites managed for timber over the past 23 years (Leege, 2009). *B. arachnifera* is also susceptible to seed mortality caused by weevils (Petersen et al., 1998) and fungus (Green and Palmbal, 1975). All of the remaining populations are within 16 km of each other. Ceska et al. (1997) showed that the close proximity of the populations and the reduction in population sizes suggests that the now separate populations may be fragments of a once more continuous gene pool. Studies have shown that without aggressive management and protection effort the species could go extinct (U.S. Fish and Wildlife Service, 1984).

Estep (2012) observed that seeds that escaped pre-dispersal mortality collected from the natural range of *Baptisia arachnifera* can be used to obtain numerous seedlings within a greenhouse, which can then be used to transplant back into the wild populations. Conducting surveys of the species and monitoring the population and acquiring biological knowledge of *B. arachnifera* are all components of the recovery plan for this species (U.S. Fish and Wildlife Service, 1984).

Timber management and *Baptisia arachnifera*

Timber management is thought to have strong impacts on population of *Baptisia arachnifera.* The species population has declined 89% over the past 20 years in sites managed for timber (Leege, 2007). Events in the timber cycle include cutting of mature trees, (either by clear cutting or by thinning) bedding of plantation rows, planting of new tress and spraying of

herbicide (Burrows n.d.). Suppression of fire in these managed areas leads to growth of other shrub species such as gallberry (*Ilex coriacea*), saw palmetto (*Serenoa repens*) and fetterbrush (*Lyonia lucida*). These plants have the potential to shade out Baptisia arachnifera (U.S. Fish and Wildlife Service, 1978).

Herbicide is also used in the timber cycle to control competing vegetation (Addington et al., 2012). This herbicide application may benefit *Baptisia arachnifera* by eliminating competition much like it does for the pine tree seedlings, but here have not been any studies conducted on the effects of how this herbicide might be affecting *Baptisia arachnifera*.

The Nature Conservancy property

A population on land that is not managed for timber is owned by The Nature Conservancy of Georgia (Figure 1.1). This property is located in Brantley County of Georgia, United States, This protected piece of land offers a chance to further study the species undergoing management practices, and critically evaluate restoration progress. A survey conducted in 2010 identified every *Baptisia arachnifera* individual in a portion of the Nature Conservancy property (Leege and Estep, 2011). Various management practices have been conducted since that time, including thinning and burning, yet the consequences of these treatments have not been studied. A recensus of the *Baptisia arachnifera* population on the Lewis tract is required to evaluate the effects of burning and thinning on population growth and reproduction.

Nutrients in the plant and soil

Another aspect of *Baptisia arachnifera's* ecology that is important to consider is the nutrient (especially micronutrient) content of the soil. Nutrients are critical for plant health, and

deficiency may cause several disorders and poor plant growth. In legume crop farming it has become common practice to add nutrients such as zinc, boron, copper, molybdenum and nickel to increase crop yield and increase drought tolerance (Ashraf et al., 2012). Iron has been recognized as an especially important micronutrient for nitrogen-fixing plants (Brear et al., 2013). It is possible that one or more nutrient(s) may play critical role(s) in growth & survival of the species. On the other hand, soils are the reservoirs & sources of the nutrients and that may determines the presence and distribution of *Baptisia arachnifera*. No studies of nutrients in the soil and leaf tissue of Baptisia arachnifera have previously been, leaving questions about if they impact distribution and health of this species.

This study addressed the following questions:

1) What are the effects of Imazapyr (an herbicide used in timber management) on *Baptisia arachnifera* in field and greenhouse conditions? And
2) What is the micronutrient content of *Baptisia arachnifera* leaves and how does this vary across sites?
3) Do soil micronutrient levels differ in sites with and without *Baptisia arachnifera*?

The objectives of this study were:

- To determine the effects of the herbicide Imazapyr on the health and survivorship of *Baptisia arachnifera* and two commonly-occurring plant species in a climate controlled greenhouse setting.
- To determine the effects of the herbicide Imazapyr on the health and survivorship of *Baptisia arachnifera* in a natural environment (field) setting.
- To determine the leaf and soil nutrient status of *Baptisia arachnifera*.
- To conduct a recensus of a *Baptisia arachnifera* population in a natural, unmanaged tract of land.

CHAPTER 2
EFFECTS OF HERBICIDE ON *BAPTISIA ARACHNFIERA* UNDER GREENHOUSE AND FIELD CONDITIONS

Introduction

Herbicides are used in land management to control undesirable plant growth, whether the plant growth is an invasive species that threatens native populations (Rice et al. 1997, Sheley et al. 2000, Crone et al. 2009) or a species that competes with a crop on a farm (Newton et al. 1996, Gressel 1999, Kabambe et al 2008). Many herbicides that are used are non-selective and broad spectrum, meaning that they are used to kill many types of plants and are not going to target one plant in particular (Cox 1996). Several studies have found that these non-selective broad range herbicides have the potential to negatively affect non-target species of both animals and plants (Schuster and Schroeder, 1990, Faust et al. 1993, Obrigawitch et al. 1998, Power et al. 2013).

In the timber cycle herbicide is applied just after planting of new pine seedlings to eliminate the pioneer species that act as competitors (Addington et al., 2012). In timber management, Imazapyr ((4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-pyridinecarboxylic acid) is the herbicide used to control weeds such as Gallberry (*Ilex coriacea*) and other native grasses (Nelson and Centrell, 2002).

Imazapyr is absorbed quickly through plant tissue and can be taken up by roots. It is translocated in the xylem and phloem to the meristematic tissues, where it inhibits the enzyme acetohydroxy acid synthase (AHAS), also known as acetolactate synthase (ALS). ALS catalyzes the production of three branched-chain aliphatic amino acids, valine, leucine, and isoleucine,

required for protein synthesis and cell growth (Tu et al., 2001). Plants usually die slowly, usually aftera month of being sprayed (Shaner, 1991).

In a timber stand the Imazapyr applied is absorbed by both *Baptisia arachnifera* and other plants, therefore the toxic impact are distributed among all plants present. The ecosystem in which *Baptisia arachnifera* exists hosts many other understory plant species, such as broomsedge blustem (*Andropogon viriginicus*), gallberry (*Ilex coriacea*), and rusty lyonia (*Lyonia ferruginea*) (Chafin et al., 2007). These understory plants contribute to making the longleaf pine ecosystem one of the most species rich plant communities outside of the tropics (Peet and Allard, 1993).

This herbicide application may benefit *Baptisia arachnifera* by eliminating competition, but there have been no studies conducted to confirm this. Conversely, a 1998 study by Sawicka and Selwet found that herbicide application to legumes can decrease the activity of root-nodule bacteria. A greenhouse study was conducted to monitor effects of herbicide in a climate controlled environment, and an herbicide field study was conducted to imitate the natural growing conditions of *Baptisia arachnifera* and include the variables that were either absent or controlled in the greenhouse study (temperature, precipitation, predation, etc.). The natural growing conditions that *Baptisia arachnifera* prefers include open pine woods and mixed pine-hardwoods with sandy soil in the coastal plain area (USFWL, 1984).

Unlike conditions in a natural environment, plants were not subject to a range of temperatures and were not limited with access to water, and there were also no natural pests or predators in the greenhouse experiment. In a natural environment, *Baptisia arachnifera* is subject to predation by seed-eating insects such as *Apion rostrum* (USFWL, 1984), as well as fungal

infections in their seed pods (Estep, 2012). These factors are all causes of stress that may affect plant growth. Also, the combinations of these stresses that were not present in the greenhouse may also produce unique plant responses in a natural environment (Mittler, 2006). Factors such as drought and heat have been shown to cause stress responses in legumes (Nayyar et al., 2014). It has also been found in studies that include both a greenhouse and field experiment that greenhouse data has a potential to be skewed due to superior growth conditions in the greenhouse (Limpens et al., 2012). This study will attempt to answer the question of what the effects are of herbicide on *Baptisia arachnifera* is in both a climate-controlled greenhouse study and a field study.

Materials and Methods

Greenhouse Experiment

The greenhouse herbicide study was conducted at research greenhouse attached to the natural sciences building located on Georgia Southern University's campus in Statesboro, Georgia, United States. The greenhouse temperature averaged approximately 27.7 degrees Celsius in the duration of the study.

I gathered *Baptisia arachnifera* seeds the previous year from wild plants in populations known to have high numbers of reproductive plants (Figure 2.1). Based on seed availability of each plant, seed pods were pulled off of the plant and placed in a paper bag with the site written on the outside of the bag. No more than 5% of the total seed pods were taken from each plant. In the lab, I removed the seeds from the pods and stored in petri dishes at room temperature until it was time to plant them. Seeds were randomly selected from each site and planted in seed trays until they were all used in the greenhouse on September 30, 2013.

Over half of the 812 seeds planted germinated after about 12 days and were allowed to grow for 9 months before being randomly chosen to be transplanted in 35.56 cm diameter pots with common competitor plants, including grasses of the genus *Andropogon* and Gallberry (*Ilex coriacea*). *Ilex coriacea* was chosen as a representative woody competitor, and *Andropogon* species of grass were gathered from field sites containing *Baptisia arachnifera* to represent grass competitors. At the time they were gathered, I was unable to key out grasses to species because of the lack of reproductive parts of the grass. Plants were then allowed to establish for several weeks.

The competitor plant species and plant percent cover was determined by consulting existing community data collected in 2009 in sites containing *Baptisia arachnifera* (Leege-personal communication). This was collected in one by one meter plots within sites and included species composition, percent cover and density of plant species. Two of the most commonly occurring species in plots with *Baptisia arachnifera* were *Andropogon* sp. and *Ilex.* Data on percent cover of competitor plants in each 1 meter by 1 meter plot were used to determine how to fill each 35.56 cm diameter pot. *Ilex* composed 58% area, *Andropogon* composed 35% area and *Baptisia arachnifera* composed 7% area (Figure 2.2).

Herbicide application levels were researched and taken from the literature as well as the chemical fact sheet for Imazapyr of recommended doses of Imazapyr to apply to a pine tree stand (Addington et al. 2012, Havens et al. 2014). Low (946.4 ml per 0.405 hectares, treatment 1), medium (1419.5 ml per 0.405 hectares, treatment 2) and high (1892.7 ml per 0.405 hectares treatment 3) dosage levels were chosen to evaluate different responses of *Baptisia arachnifera* and other plants to a range of doses of herbicide. A control of water was also used (treatment 4).

Each level was scaled to be appropriate for the size of the greenhouse study. The recommended doses taken from literature are the amount of Imazapyr that should be added to a minimum of 18.93 liters of water. Each pot in the greenhouse study was receiving 4 ml of liquid in one spray. The spray bottles of each herbicide treatment were filled to hold 96 ml of liquid to ensure that there would be more than enough of each treatment for all of the pots.

The amount of Imazapyr in each treatment bottle was determined by figuring out how many ml of Imazapyr it would take in 4 ml of water to be equivalent to each level of Imazapyr in 18.9 liters of water (Table 2.1). Treatment levels were assigned to each pot using a randomized complete blocks design. Herbicide was applied using a hand sprayer spraying one foot above the pot with the sprayer centered to ensure even application to all plant species.

There were 12 replications of each of the 4 treatments, with a total of 48 pots (Figure 2.3). I estimated growth and vigor of *Baptisia arachnifera* and its competitor species before application of the herbicide. Plant height was measured from the soil surface to the tip of the plant. The number of leaves that were green (no yellowing) of each plant was recorded. Plants were also measured with a SPAD 502 P chlorophyll meter (Spectrum Technologies, Inc.) and NDVI (Normalized Difference Vegetation Index). The SPAD 502 P chlorophyll meter works by emitting two frequencies of light, one at a wavelength of 660 nm (red) and one at 940 nm(infrared). Leaf chlorophyll absorbs red light but not infrared. The difference in absorption is measured by the meter and termed "optical density difference" which is a ratio of reflection vs. absorption (SPAD 502 P chlorophyll meter product manual). The Field Scout CM 1000 NDVI meter works in a similar fashion by calculating the plant's reflectance measurements in the red and near infrared portion of the spectrum (Govaerts and Verhulst, 2010). After application

plants were left to grow for one week. Height, leaf number, chlorophyll and NDVI data was then taken twice a week on the same days of the week for 10 weeks (until plant death). Survivorship curves were created for each treatment and each plant species.

I compared results using a repeated measure ANOVA with parameters being treatment, time, treatment crossed with time and treatment nested within pot. Differences in means were tested using a Tukey's HSD Test. All statistics were done using JMP Pro 10 ® (2012).

Field Experiment

A field site in Brantley County, Georgia, United States (31°20'08.51" N, 81°54'27.23" W, Figure 2.4) was chosen based on its close proximity to a population of *Baptisia arachnifera* that is not currently under management from a timber company. Brantley County has a humid subtropical climate, with an average of 132.08 cm of rain per year, a July high of 33.3 degrees and January low of 3.33 degrees Celsius.

Researchers did not want the existing population to be sprayed with herbicide so a site was chosen under a Georgia Power powerline cut for the experiment. This way greenhouse-grown *Baptisia arachnifera* could be planted in an area that they naturally occur, and be treated with chemicals without harming *Baptisia* that already grew there. The field site was located in a Georgia Power power-line cut next to a *Baptisia arachnifera* population. The land where the population occurs is currently owned by the Nature Conservancy.

Baptisia arachnifera seeds were gathered from the Nature Conservancy property (Figure 1.1) and five sites that spray herbicide (Figure 2.1). The five sites were chosen based on which sites were known to have a high number of reproductive plants. Fruiting plants were haphazardly

chosen. Pods were harvested based on the availability of the seeds for each plant and stored in brown paper bags.

Back in the lab seeds were removed from seeds pods and stored inside until plating time in the greenhouse. 812 seeds were planted randomly in flat seeds trays filled with Miracle-Gro Moisture Control Potting Mix. Plants were watered every day and started to germinate after about 12 days. Over half of the seeds planted germinated. Plants were watered and grown for 9 months before being transplanted out in the field. The plants were roughly 17.78-25.4 cm in height when they were transplanted.

60 plants were arranged in in a grid in a 6.4 x6.4 meter area (Figure 2.5) to make laying down irrigation pipes easier. Plants were placed 0.9 m apart from each other so that different treatments would bleed over into each other. A solar-powered automatic irrigation system was constructed (Figure 2.6) The irrigation system consisted of a 454.2 liter tank that was placed out into the field and a 12 V pump that was connected to a battery and solar panel for power. A timer was rigged so that the plants would be watered for 10 min at 4 AM in the morning on the days that I wanted them to be watered. A hose line ran from the pump and tank to a main PVC line that ran parallel to the rows of plants. Drip irrigation hosing was laid out along each row of plants, and a drip head (3.8 liters per hour release) was inserted along the hose wherever a plant occurred. Another 454.2 liter tank was purchased and used as a refill tank to transport water out to the field tank. Plants were watered every day for two weeks to help them establish. Water was weaned back on the third week to plants only receiving water every three days. On the fifth week of the plants living in the field, *Baptisia arachnifera* data parameters were taken on height from

the surface of the soil to the tip of the plant and number of green leaves. Chlorophyll measurements were taken using a SPAD 502 P meter (Spectrum Technologies, Inc.).

Five leaves were measured from each plant and the value was averaged. NDVI measurements were also taken on each *Baptisia arachnifera* using a Field Scout CM 1000 (Spectrum Technologies, Inc.).

Plants were then randomly assigned one of four different treatments: low: 946.4 ml of Imazapyr per 0.405 hectares (treatment 1), medium: 1419.5 ml Imazapyr per 0.405 hectares (treatment 2), high: 1892 ml of Imazapyr per 0.405 hectares (treatment 3) and water control (treatment 4). Each treatment was scaled in a similar fashion to the greenhouse experiment to be appropriate for the 0.82 m^2 area around each *Baptisia arachnifera* plant (Table 2.2). Two sprays from the hand sprayer were needed to cover the area completely, so each area received 8 ml of each solution. The solution was sprayed over each area containing the plant, using a 0.9 m by 0.9 m square made out of PVC pipe as a guide. The PVC square was positioned so that the *Baptisia* was in the middle of the square, and the spray was distributed evenly over the entire area inside of the square. Plant height, green leaf number, chlorophyll and NDVI data were taken again on each plant one week after the application of the treatments, and were then measured twice a week for the next eight weeks. Survivorship curves for each treatment were calculated and graphed.

For the field herbicide experiment, results were analyzed with a repeated measures ANOVA with parameters being treatment, time, and treatment crossed with time. Means were compared using a Tukey's HSD test. All statistics were done using JMP Pro 10 ® (2012).

Results

Greenhouse Experiment

Survivorship curves for *Baptisia arachnifera* showed that the death of all plants treated with herbicide within 8-10 weeks while the control plants did not die. The high treatment showed total population death at week eight making it the fastest of all treatments to die, followed by medium treatment at week nine, and low treatment at week ten. (Figure 2.7).

Heights of *Baptisia arachnifera* did not differ significantly among herbicide treatments and control (P=0.3780). However, a significant interaction was found between treatment and time (P=0.002), as well as time alone (P=<0.0001, Table 2.3) Average height of the low, medium and high treatments was reduced by 14%, 5.4% and 12.8% respectively while average height of the control treatment *Baptisia* were found to have grown 3.6% on average (Figure 2.8).

Herbicide treatments reduced *Baptisia arachnifera* leaf number relative to control with respect to treatment (P=0.0351, Table 2.4) as well as time (P=<0.0001, Table 2.5). Plants treated with herbicide treatment 1, 2 and 3 showed a reduction in leaf number averages by 27.2%, 26.4%, and 18.8% respectively (Figure 2.9). The control plants lost 6.7% of their leaves on average.

Baptisia arachnifera chlorophyll content was not affected by treatment type (P=0.2376) but differed with respect to both time (P=<0.0001). A treatment x time interaction was found (P=0.0080, Table 2.6) as well. Some treatments (including control) dipped in number on week six, but data varied widely and did not seem to have a definite trend.

NDVI values for *Baptisia arachnifera* were not affected by treatment (P=0.9515), or time (0.1741). There was also not an affect from treatment crossed with time (P=0.7120, Table 2.7 and Figure 2.11).

Survivorship curves for *Andropogon* sp. of grass showed that all three treatments of herbicide showed death of plants treated. All herbicide treated plants were all dead at week eight (Figure 2.12). No plants died in the control treatment across the entire study. Andropogon sp. height did not show any effects from treatment (P=0.1553), time (P=0.1079) or treatment crossed with time (P=0.2541, Table 2.8 and Figure 2.13). Leaf number of *Andropogon* species was not affected by treatment (P=0.3914) but did show an affect by time (P=<0.0001). All treatments, including the water control showed leaf loss over time (Figure 2.14). There was not a significant interaction from treatment crossed with time (P=0.8366, Table 2.9).

Chlorophyll content of *Andropogon* sp. leaves was affected over time (P=<0.0001) and by treatment (P=0.0160) and did not show a significant treatment/time interaction (P=0.5191, Table 2.10). A similar spike in chlorophyll was found in all treatments at the week eight measurement. None of the treatments followed a steady pattern of decline, with each treatment having many fluctuations over time (Figure 2.15). *Andropogon* NDVI values were not affected by time (P= 0.6938) or treatment (P=0.3812) and did not show a significant treatment/time interaction (P=0.4790, Table 2.12 and Figure 2.16).

The survivorship curves for *Ilex coriacea* in the greenhouse study were similar to the other two species, with the plants in three herbicide treatments were all dead at the end of the study while all control plants survived. Medium and high treatments both had all of their plants dead at week eight and all of the plants in low treatment were dead at week nine (Figure 2.17). Ilex height changed over time (P=<0.0001), with all three herbicide treatments showing a loss in height while the control treatment actually showed growth in height from the beginning to the

end of the study (Figure 2.18). However, height was not statistically affected by treatment (P=0.7413) or treatment crossed with time (P=0.0672, Table 2.13).

Ilex leaf number showed an effect from treatment (P=0.0017), time (P=<0.0001) and treatment/time interaction (P=0.0166, Table 2.14). Low treatment averages showed a 45% loss in leaves, medium treatment showed a 63% loss in leaves, and high treatment showed a 54% loss of leaves. Even the control treatment 4 lost 29% of their leaves on average (Figure 2.19). Average *Ilex* chlorophyll readings showed a change over time (P=<0.0001, Figure 2.20) but were not affected by treatment (P=0.5643) and did not show a significant treatment/time interaction (P=0.9899, Table 2.15). *Ilex* NDVI was not effected by treatment (P=0.7917) or time (0.5337) and did not show a significant treatment/time interaction (P=0.3872, Table 2.16, Figure 2.21).

Field Study

Survivorship curves for *Baptisia arachnifera* showed that all treatments including control died in the field. Plants mortality occurred most rapidly in low treatment, showing total plant death around week six, followed by high treatment showing total plant death around week eight. Medium treatment and control treatment 4 both showed total plant death at week nine (Figure 2.22). *Baptisia* height changed over time (P=<0.0001), an average height loss among treatments of 1.4 % (Figure 2.23), but did not show a response to treatment (P=0.4175) although there was a response due to a treatment/time interaction (P=<0.0001, Table 2.17). *Baptisia* leaves followed the same pattern, with decrease across all treatments that averaged to 3.5% leaf loss (Figure 2.24) among all four treatments (P=<0.0001). There was a treatment crossed with time interaction (P=<0.0001), but no difference between treatments alone (P=.01721, Table 2.18).

Baptisia chlorophyll differed among treatments (P=0.0412) with low treatment having a statistically lower chlorophyll content than control treatment (Figure 2.25), but not medium or high treatments (Table 2.19). An effect over time and from an interaction of treatment and time was not found (P=0.1002, P=0.6210, Table 2.20). *Baptisia* NDVI did not show an effect from time (P=0.6790), treatment (P=0.3413), or a treatment/time interaction (P=0.3881, Table 2.21, Figure 2.26).

Discussion

In the greenhouse, plants that were treated with herbicide eventually all died within 8-10 weeks of being treated, while control plants did not. *Baptisia's* death from the treatment of herbicide is supported by the findings of a 2010 study by Kaeser and Kirkman in which native legumes were found to be more sensitive and at risk of damage and death from herbicide application regardless of herbicide application rate or concentration. In this study, plants were considered dead when they had no green pigment left and showed no new growth as well as loss of leaves. This was true for all species. This indicates that when it comes to overall survivorship, the low, medium and high levels did not matter as they all resulted in plant death. In both the greenhouse and the field, the parameters of height, leaf number, chlorophyll ad NDVI did not necessarily follow the same patterns as the survivorship data did, indicating that the use of just one of these parameters along would not be a good assessment of the plant's true response to the treatment of herbicide.

Andropogon did not show response to treatment for height and leaf number but did lose leaves over time. This is interesting that there would not be a difference in leaf number of *Andropogon* between treatments and control, as other studies have shown that one of the first

responses of *Andropogon* to herbicide treatment is loss in number of leaves (Twain et al. 2002, Baer and Groninger 2004). Both *Baptisia* height and leaf number response showed a treatment time interaction, although they did not show an overall effect from treatment. This is because *Baptisia's* response to the treatment changed over time, with heights of the control growing a few centimeters while herbicide treatments shrunk and dropped leaves.

Chlorophyll and NDVI did not follow any sort of linear pattern, unlike the parameters of height and leaf number which when treated with herbicide generally decreased over time when treated with herbicide for all species. In the greenhouse, there was a varying level of sunlight and shade among all of the plants and this may have contributed to the vacillations of chlorophyll data going up and down over time. Chlorophyll values have been shown to been effected by the inconsistent sun and shade among plants in both a greenhouse and field setting (Jifon et al., 2005). The NDVI meter does best in measuring large, continuous plants like turf grass (Govaerts and Verhulst, 2010) and was probably unable to distinguish values in the greenhouse or the field because of the plant's close proximity to each other.

In the field, no treatment effect from any parameter that was measured was shown. This is probably because of the heat and water stress that the young plants faced when they were transplanted in the middle of summer caused stress that may have compounded with the stress of herbicide application.

Management Recommendations

Due to the death of all *Baptisia arachnifera* plants that were treated with herbicide, it is not recommended that Imazapyr be used on sites that contain this species. Further research

should investigate the effects of burning on different life stages of *Baptisia arachnifera* to see if burning could be used as an effective control on competitor species. Future field studies with *Baptisia arachnifera* should be conducted on existing populations, or plant at a cooler time of year and allow more time for plants to establish.

CHAPTER 3
NUTRIENT SURVEY OF *BAPTISIA ARACHNIFERA* LEAF TISSUE AND SOIL

Introduction

Macro and micronutrients are required in plants for both developmental and physiological processes. Deficiency of a nutrient may result in disorder, sickness and even death of plants. Timber management practices and consequences of those practices could possibly affect the availability of nutrients to plants (Blake and Golding, 2002).

Application of fertilizer may affect nutrient levels available to plants. Dickens et al. (2003) recognized five common fertilizer types in the southeastern United States and lists their N-P-K values. These include triple superphosphate (TSP, 0-46-0), diammonium phosphate (DAP, 18-46-0) for phosphorous fertilization, ammonium nitrate (34-0-0) and urea (46-0-0_ for nitrogen, and muriate of potash (MOP, 0-0-60) for potassium. The amount of fertilizer applied varies and is usually based off of a previous foliar analysis (Akers et al., 2013).

Soil nutrients can be lost from timber sites through the removal of biomass as well as through the increased nutrient mobilization and leaching that can occur when during soil disturbance (Pritchett and Wells 1978, Jurgensen et al. 1979). Pritchett and Wells also found that both harvesting and mechanical site preparation have the potential to accelerate the mineralization of nutrients. Additionally, it has been found that the total harvesting of trees on sites may lead to increased erosion rates and/or percolation losses of nutrients in the soil (Mroz et al. 1985). A 1972 study by Boyle and Ek found that short cutting rotations and clear cutting of sites leads to increased loss of nutrients and reduction of site quality.

The purpose of this study was to determine if there were differences in soil nutrients in sites that did and did not have *Baptisia arachnifera* present, and also find out if there were differences in leaf nutrients in *Baptisia arachnifera* foliage among sites with *Baptisia arachnifera*. This data is preliminary data that is a potentially important first step in finding out which nutrients are most important to *Baptisia arachnifera*. These data could also be used in the future to help find out what levels of nutrients *Baptisia arachnifera* does well in.

Materials and Methods

Leaves of *Baptisia arachnifera* were collected from five sites owned by a timber company and a sixth site owned by the Nature Conservancy in Brantley County, GA, United States. *Baptisia arachnifera* leaves were collected in Spring 2014 from six different sites that have a history and presence of *Baptisia arachnifera* populations (Figure 1.1 and 2.1). Five of these sites were on timber company property (notated in results as sites 1-5, treatments including bedding, thinning, spraying of herbicide, tree harvesting with no burning taking place) and the sixth is owned by the Nature Conservancy (notated in results as site 6, treatments including sporadic burning and thinning, with no bedding and spraying of herbicide). At each site every *Baptisia* plant was found and assigned a number. Ten mature *Baptisia arachnifera plants* were randomly selected from each site and 10-15 recently mature leaves were collected from each plant, totaling 60 sets of samples.

Soil was collected from the same six sites that leaves were collected from, and was also collected from six sites on that were owned by the same timber company but did not have any presence or historical record of *Baptisia arachnifera* existing on them (notated in results as sites 7-12). Field sites had a mixture of soil profiles (Table 3.1) with most being loamy sand and some

being fine sane profiles (United States Department of Agriculture, 2014). At each of the 12 sites, soil samples were collected from two sampling points at a depth of 0 – 10 cm, the average rooting depth of *Baptisia arachnifera*. Soil sampling location was chosen by randomly selecting two plants for adjacent sampling, or by randomly selecting two locations in the sites that did not have plants. Total number of samples was 24.

Soil analysis was done by the Soil Water and Plant Lab at the University of Georgia, Athens, United States. They tested the soil for the following nutrients: Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, and Zn. The methodology involved HNO3 Microwave Digestion (EPA 3051) - HNO3 matrix and ICP – Inductively coupled plasma spectrography (EPA 200.7).

Soil C and N were analyzed at Georgia Southern University. A measurement of 100 milligrams of soil was measured using an XSE Analytical Balance (105 DU model, Mettler Toldeo) from each sample after being dried in an Isotemp drying oven (Fischer Scientific) and ground using a ball grinder (8000M Mixer/Mill, SPEX Sample Prep). The 100 milligrams was carefully put inside of an aluminum cup that was folded closed and run through a Flash 2000 Combustion NC Soil Analyzer (CE Elantech, Inc., NJ, USA).

The plant tissue was also tested at the Soil, Water and Plant Lab at the University of Georgia. They tested for the following nutrients: Al, B, Cd, Cr, Cu, Fe, Mn, Mo, Na, Ni, Pb and Zn and the methodology involved the Microwave - Acid (HNO_3) Digestion, ICP Method using a CEM Mars5 microwave digestion system, Thermo Jarrell-Ash model 61E ICP.

Tests were run to see if there were differences between nutrients in leaf tissue between sites, and differences in soil nutrients that did and did not contain *Baptisia arachnifera*. Analysis was done by using a nonparametric Wilcoxon test and Mann-Whitney U test when appropriate.

Means were compared using a Steel-Dwass test. All statistics were done using JMP Pro 10 ® (2012).

Results

Leaf tissue

Aluminum, boron, copper, iron, manganese, sodium and zinc concentrations were found to be different among sites (Table 3.2). Aluminum concentrations for sites 1, 3, 4 and 6 had a 50% lower average ppm concentration than sites 2 and 5 (P=<0.0001, Table 3.3). Boron concentrations were 50.7% higher in sites 1, 2 and 4 than sites 3, 5 and 6 (P=<0.0001, Table 3.4). For copper, concentrations in sites 1, 2, 3 and 4 were found to be 66.4% lower than in sites 5 and 6 (P=<0.0001, Table 3.5). Iron was 50.8% higher in site 2 than all of the other sites (P=0.0005, Table 3.6) and manganese was 81.2% higher in site 6 than site 5. Sites 1, 2, 3 and 4 were 64.6% lower in manganese concentration than site 5 (P=0.0001, Table 3.7). Sodium was 63.8% lower in sites 6 and 5 than sites 2 and 1, and sites 1 and 2 were 13% higher in sodium concentration than sites 3 and 4 (P=0.0001, Table 3.8). Finally, zinc was 87.7% higher in site 6 than the sites with the lowest concentration of zinc, sites 1 and 3 (P=0.0015, Table 3.9).

Soil

Calcium, carbon and nitrogen were found to be differing in concentrations between sites that did and did not have *Baptisia arachnifera* (Table 3.10). Calcium concentration decreased by 83.9% on sites that did not have *Baptisia* (P=0.0222). Nitrogen decreased by 45.2% on sites without *Baptisia*, and carbon decreased by 76.8% on sites that did not have *Baptisia* (P=0.0039, Table 3.11 and 3.12).

Discussion

The data from this study suggests that different sites that are managed for timber differ significantly in select nutrients in both soil and plant leaves. The managing of land for timber has the potential to change the nutrient content of the soil through practices like tilling and draining as well as the addition of fertilizer (Cucci et al., 1994; Lian and Lee, 1997; Oskarsen et al., 1996; Randall and Schmitt 1990). These are all management treatments that could have been done on these lands, and so could explain the differences in nutrients between sites. The mobility of nutrients determines how long they will retain in the soil and how fast they will leach from the system (Gul et al., 2013).

Baptisia arachnifera leaf micronutrient levels average across all of the sites samples compared to soybean (*Glycine max*) leaf levels showed that micronutrient levels in *Baptisia* had 91.7% less zinc, 24.2% less boron and 70.7% less iron but 62% more copper and 51% more manganese than soybean levels (Yasari 2012, Vasconcelos et al. 2014).Young (2007) found that a congener (same genus) species *B. lanceolata* occurred on soil with higher levels of manganese than *B. arachnifera*. Young postulated that this might mean that *B. arachnifera* has a lower tolerance for manganese than *B. lanceolata*. High levels of aluminum were found on sites 5 and 6. Aluminum has been found to be toxic to plants that grow in soils with a pH of 5.5 or lower even in very small amounts (Rout et al., 2001) with soybean being a prime example of a plant that is stressed by aluminum (Wagastuma and Ezoe 1985, XueLin et al. 2009, Duressa et al. 2010,) but has also developed some tolerance to it (Bianchi-Hill et al. 2000, Silva et al. 2001,

Villagarcia et al. 2001). It could be possible that *Baptisia* is tolerant to aluminum toxicity, but more research would be needed to discover that answer.

This study found that there was a significant difference in manganese between all timber managed sites and site 5 and 6, site 5 and 6 having almost triple the amount of manganese in the leaves. There may be significance in the data that was found that site six, which is owned by the Nature Conservancy and not managed for timber and site 5 which is managed for timber site are more similar to each other in nutrient content than other sites.

Calcium was 6.2 times higher in the soil in sites with *Baptisia arachnifera* than sites that did not have *Baptisia.* One study found that that calcium in the soil helped reduce damage caused by the toxic effects of aluminum on root growth (Brady et al, 1993) and this raises the question of it calcium is having the same effect on aluminum concentrations in *Baptisia*.

Management Implications

The data that were gathered in this study will hopefully serve as a stepping-stone for answering further questions about nutrient requirements of *Baptisia arachnifera*. A suggestion for further research would be for the overall health and population number of the different *Baptisia* sites to be analyzed and compared with the nutrient levels found for each site. This could then be developed into suggestions for fertilizer application on greenhouse-grown *Baptisia* as well as what soil profile/nutrient profile transplanted *Baptisia* would be most successful in.

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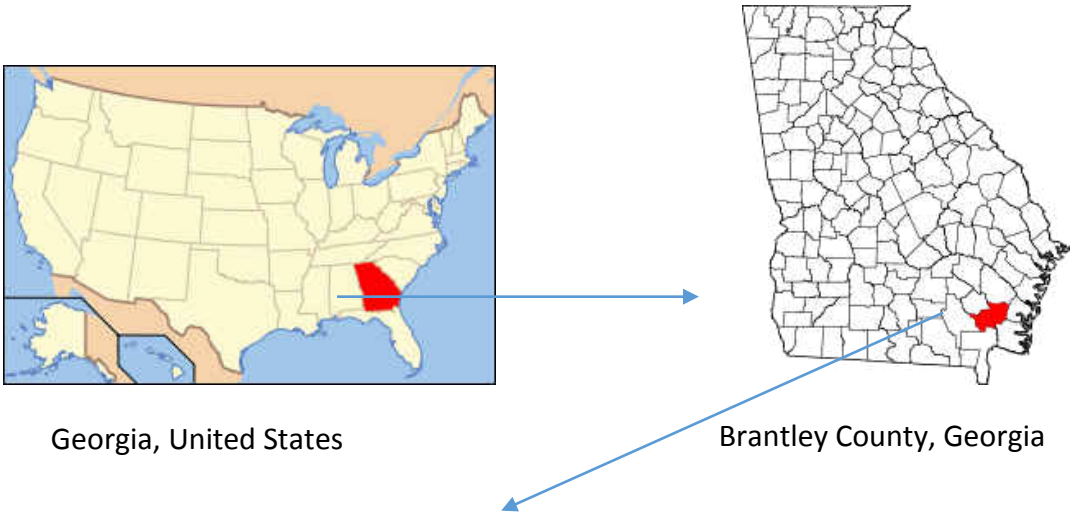


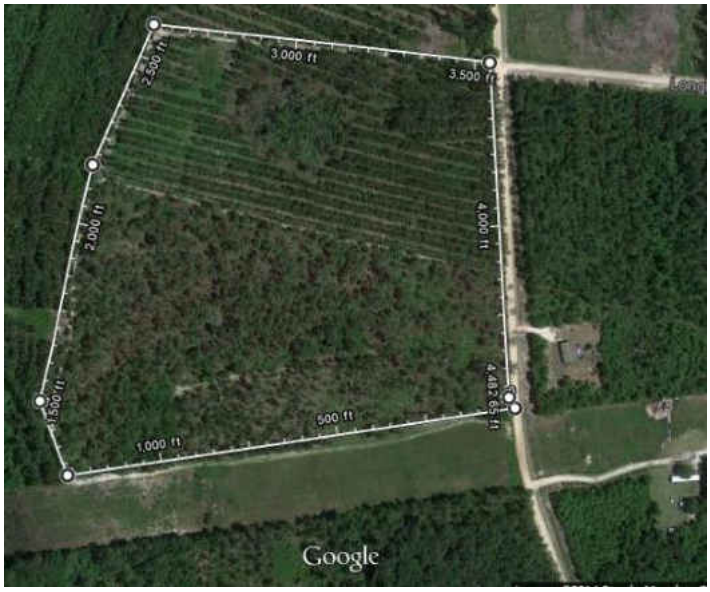


Figure 1.1: The Nature Conservancy property, outlined by a ruler.

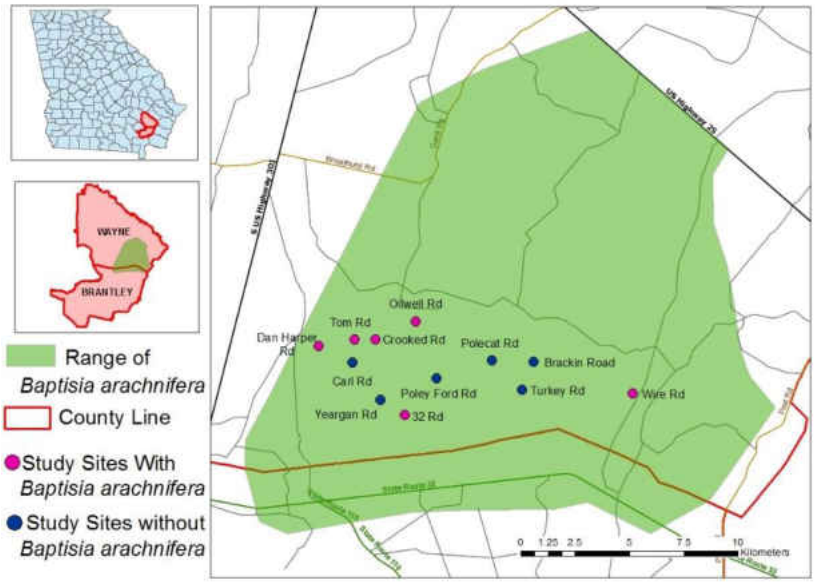


Figure 2.1: Field Sites with and without *Baptisia arachnifera*.

Figure 2.2: All three plants in the greenhouse experiment pot shortly after planting. From top and going clockwise, *Ilex coriacea* (gallberry), *Baptisia arachnifera* (Hairy Rattleweed), and *Andropogon* sp. of grass.

B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
4	4	3	2	2	4	2	2	1	3	1	4
3	1	2	4	3	2	3	3	3	4	3	3
2	2	1	1	1	3	4	1	4	2	4	2
1	3	4	3	4	1	1	4	2	1	2	1

Figure 2.3: Experimental design of the 48 pots in the greenhouse. Each letter in each cell represents a pot. Blocks are labeled 1-12 across the top and include the four cells beneath them. 4=control treatment, 3=high treatment, 2=medium treatment and 1=low treatment of Imazapyr.

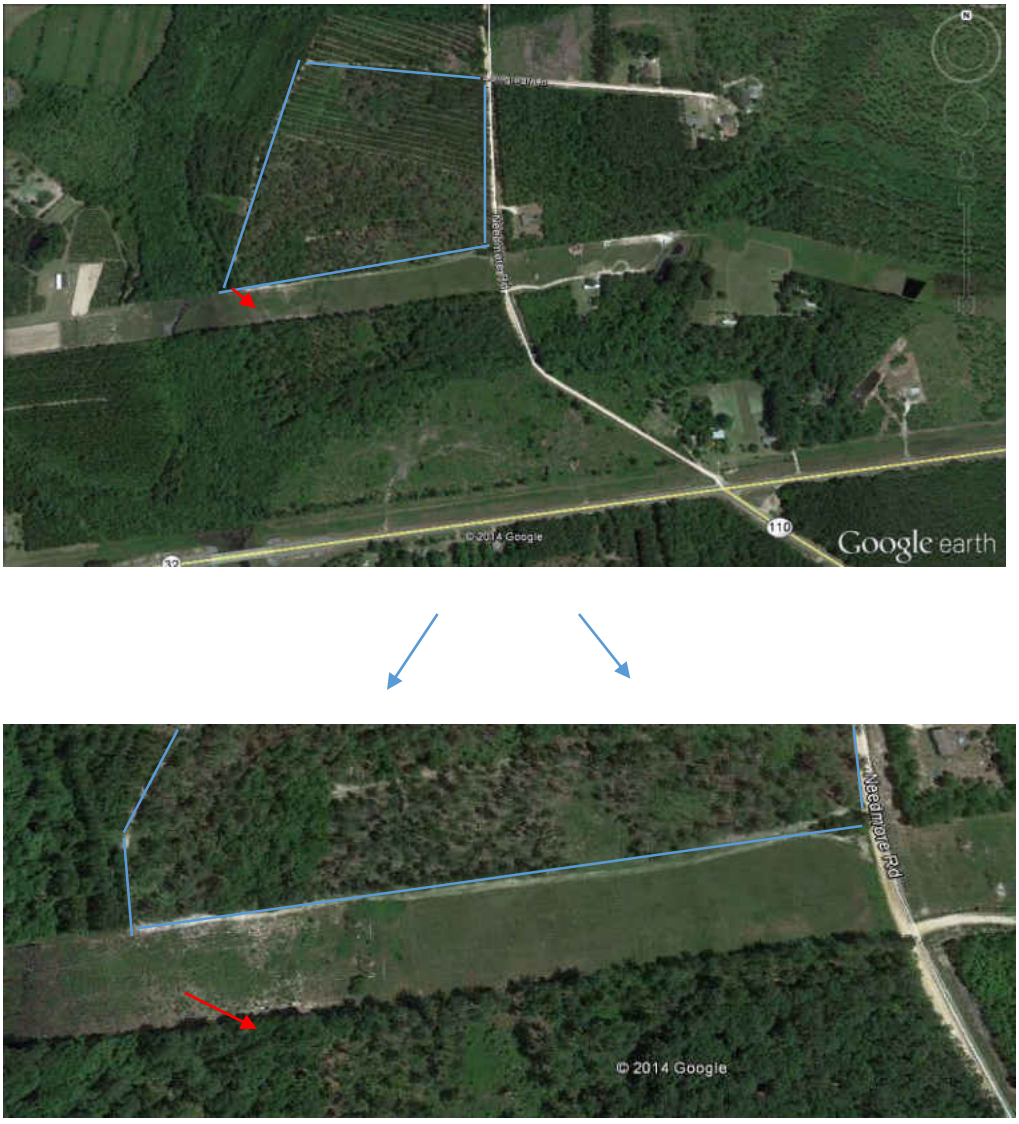


Figure 2.4: Location of field experiment site (red arrow) with respect to Nature Conservancy owned property (blue outline).

Figure 2.5: *Baptisia arachnifera* in the field herbicide study. Plants were laid out in a grid so as to easily run irrigation lines. Each flag represents a location where a *Baptisia* is planted.

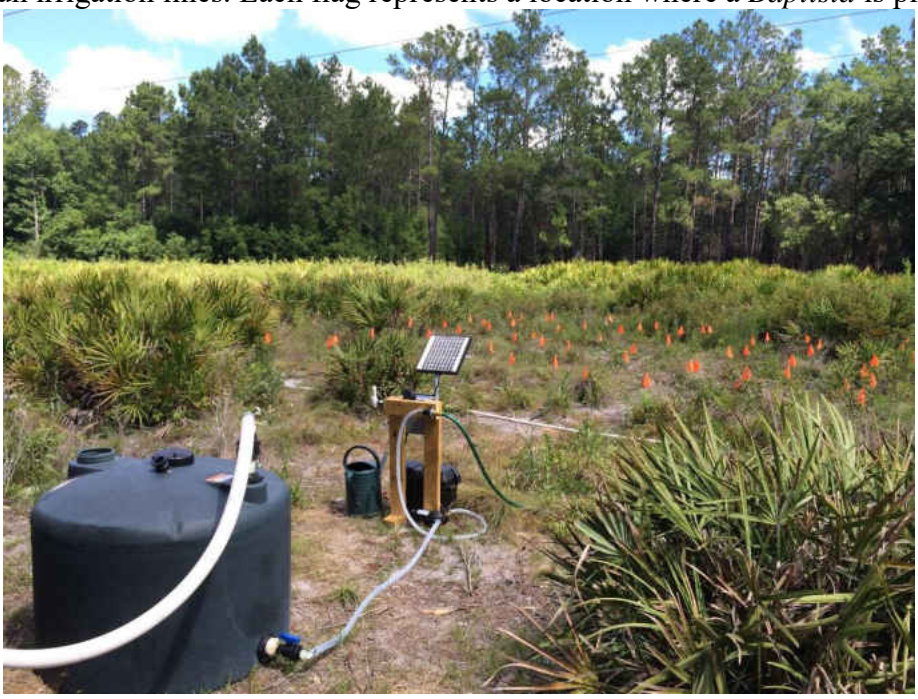


Figure 2.6: Field herbicide experiment irrigation setup.

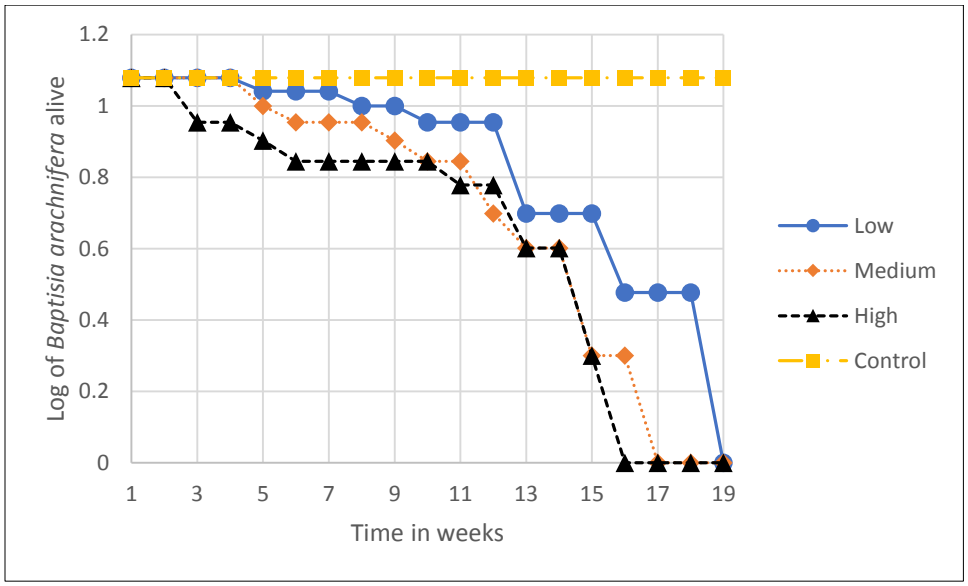


Figure 2.7: Survivorship curves of *Baptisia arachnifera* in the greenhouse study in weeks.

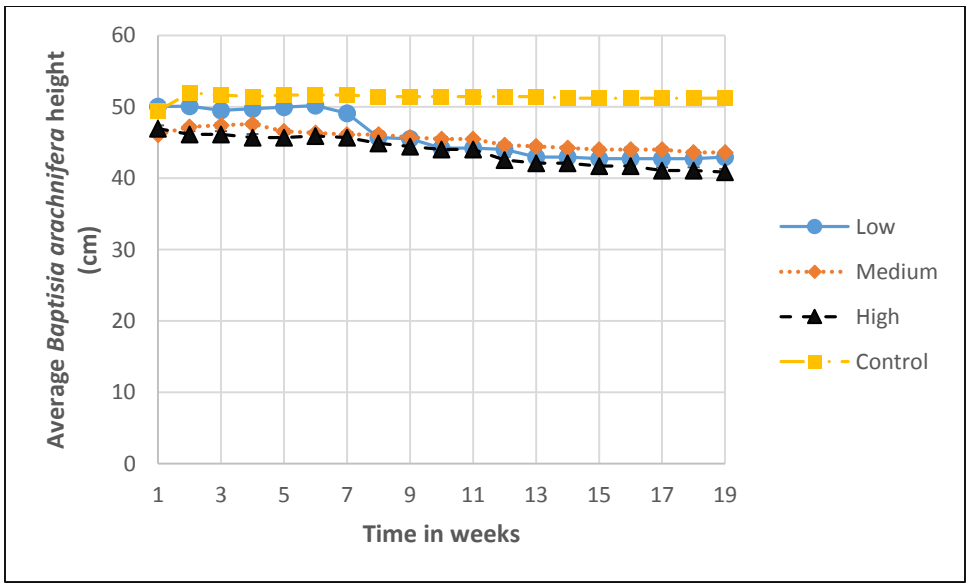


Figure 2.8: Average *Baptisia arachnifera* height of treatments 1-4 across time.

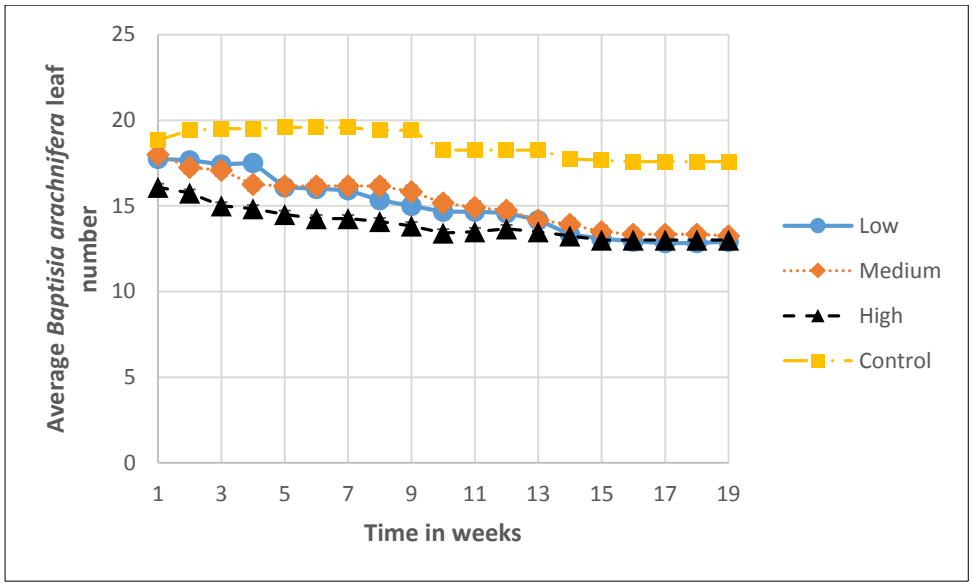


Figure 2.9: Average *Baptisia arachnifera* leaf number across time.

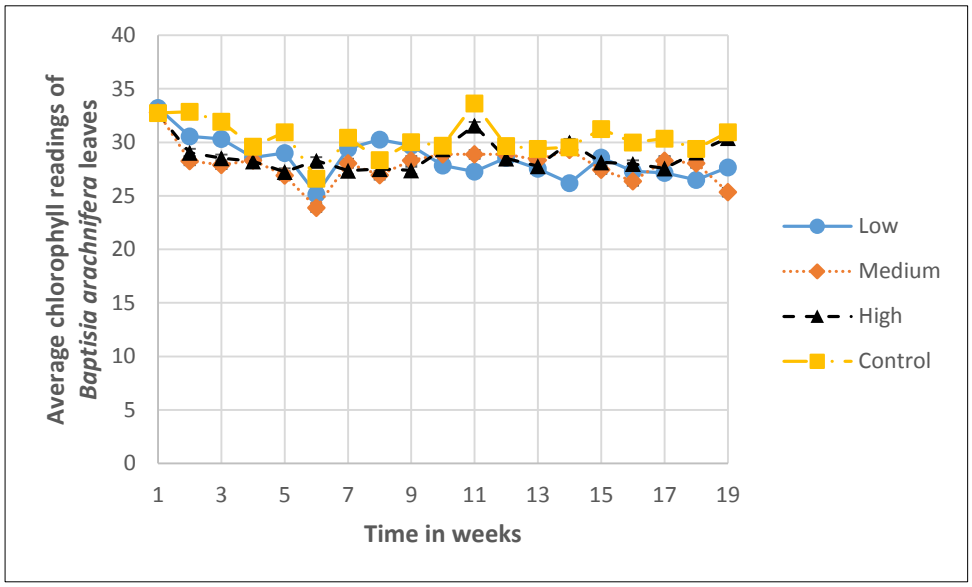


Figure 2.10: Average *Baptisia arachnifera* chlorphyll content across time.

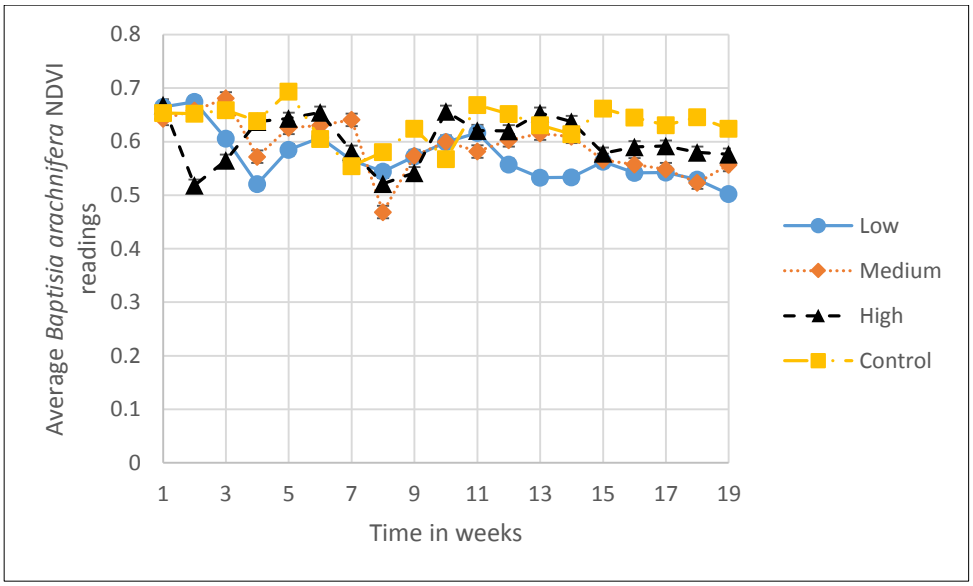


Figure 2.11: Average *Baptisia arachnifera* NDVI readings across time.

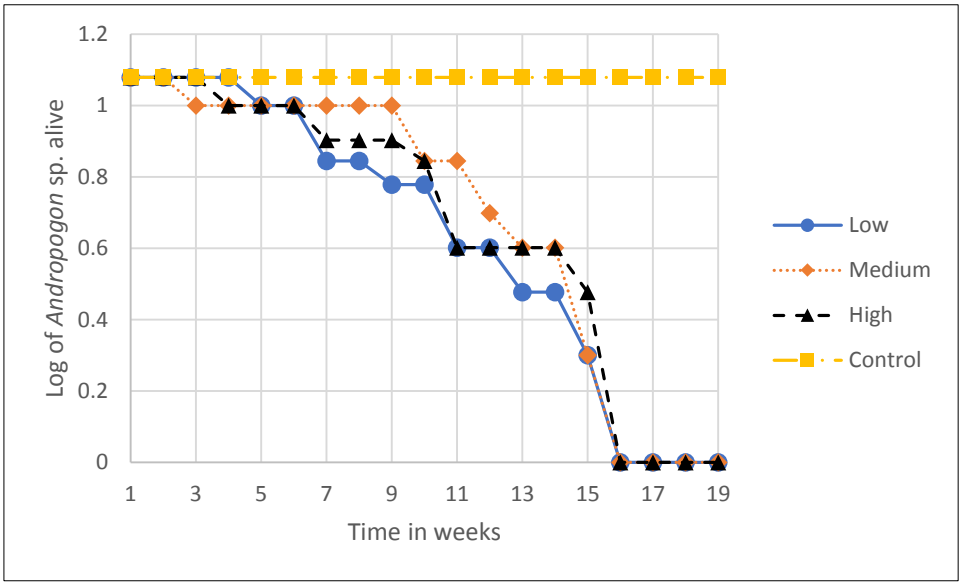


Figure 2.12: Survivorship curves for *Andropogon sp.* in the greenhouse study.

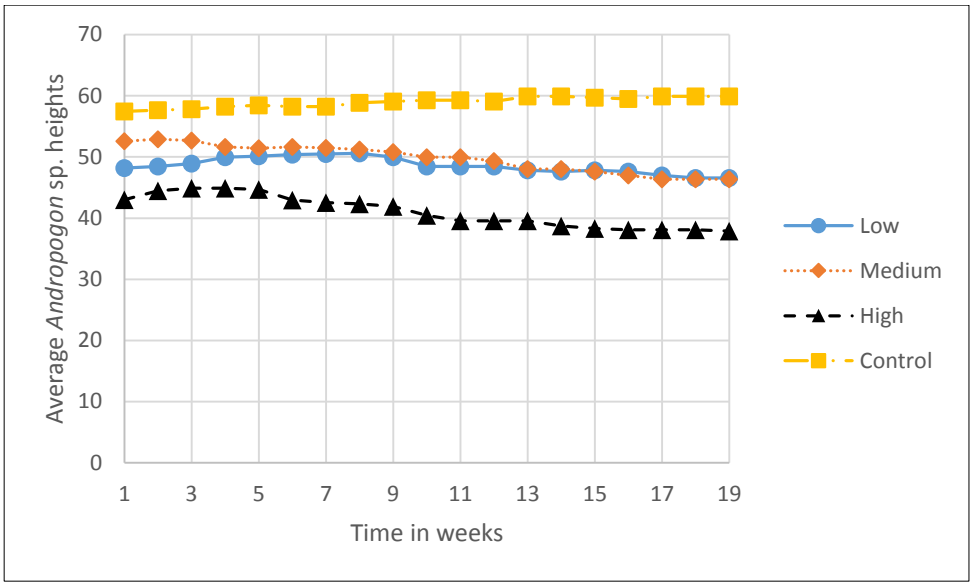


Figure 2.13: Average *Andropogon* sp. height across time.

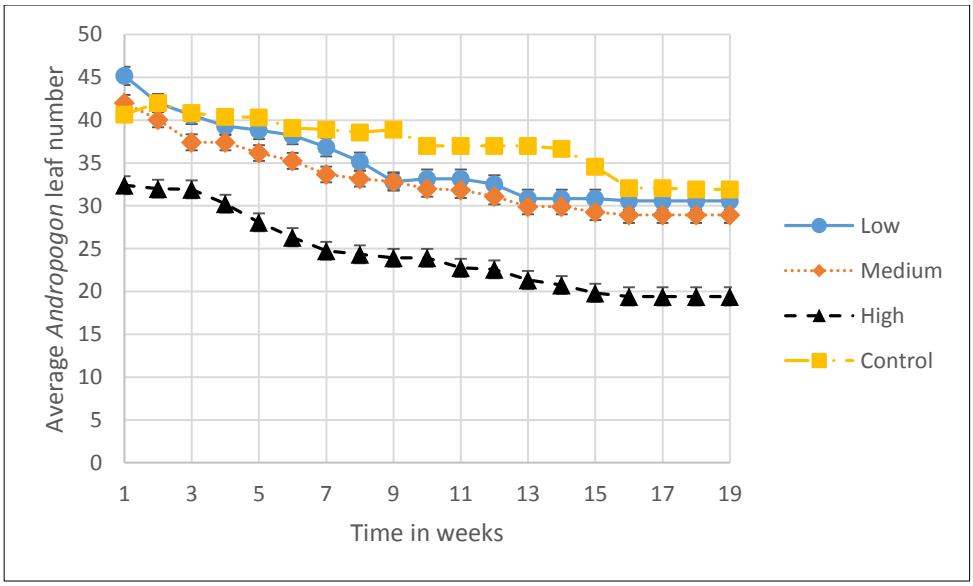


Figure 2.14: Average *Andropogon* sp. leaf number across time.

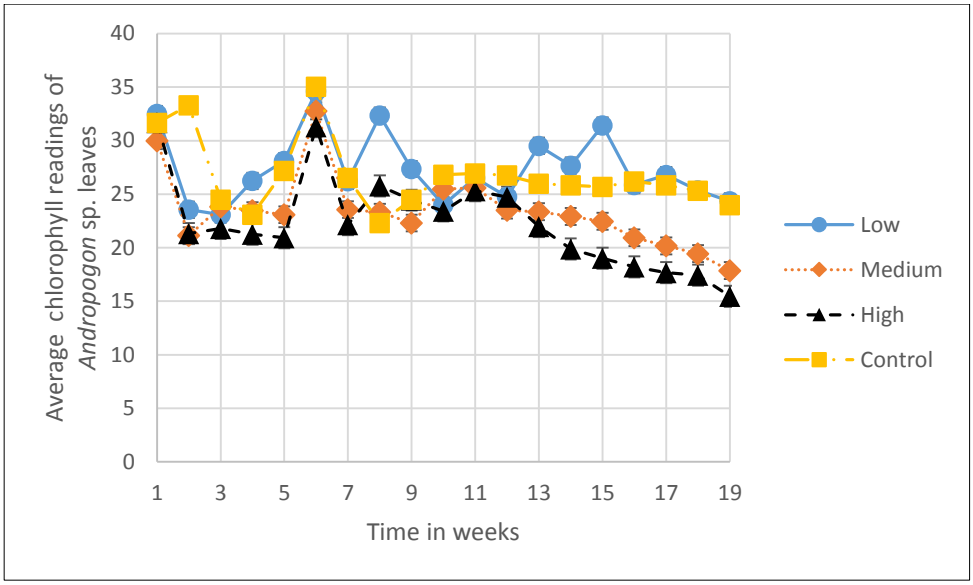


Figure 2.15: Average *Andropogon* chlorophyll readings over time.

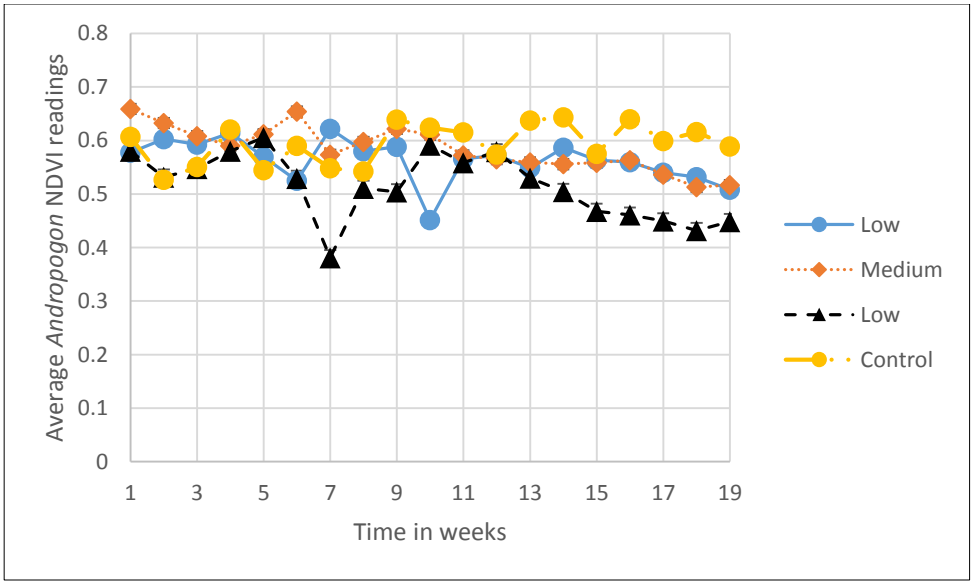


Figure 2.16: Average *Andropogon* NDVI readings over time.

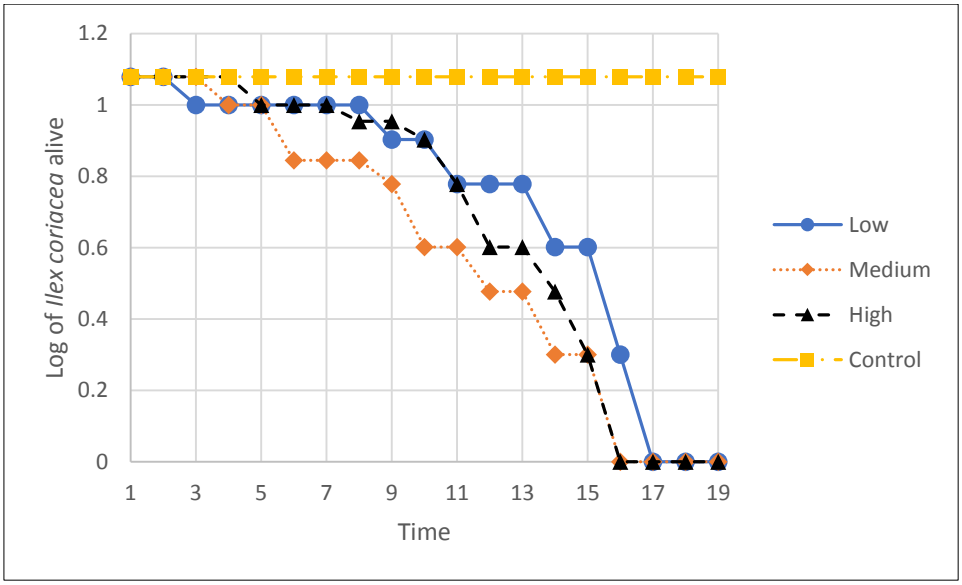


Figure 2.17: Survivorship curves of *Ilex coriacea* over time in a greenhouse study.

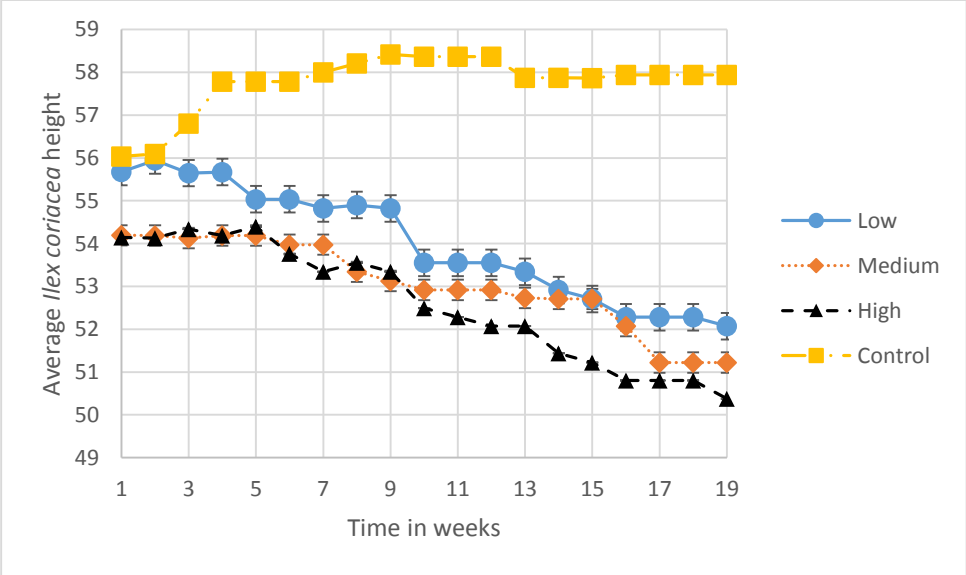


Figure 2.18: Average *Ilex coricea* height over time.

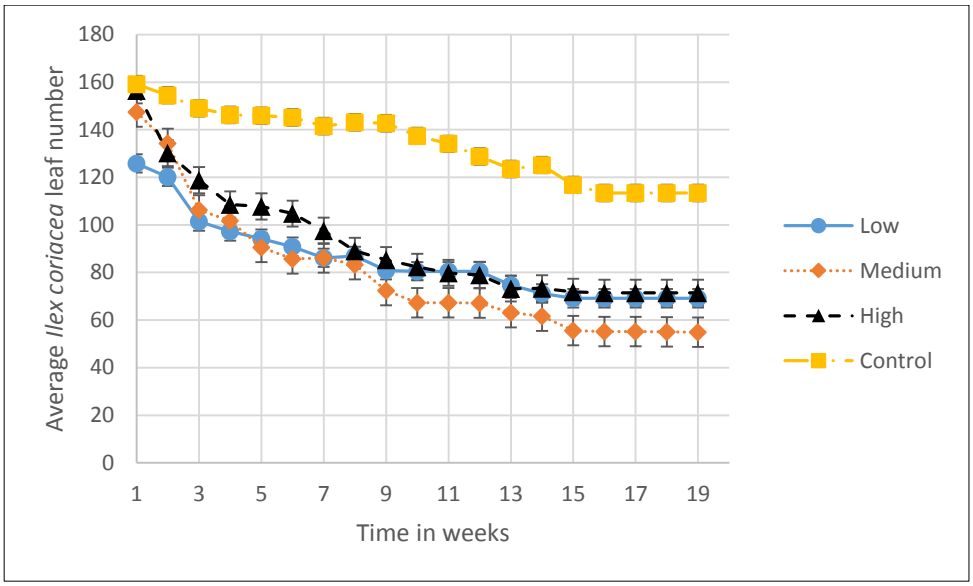


Figure 2.19: Average *Ilex coriacea* leaf number over time.

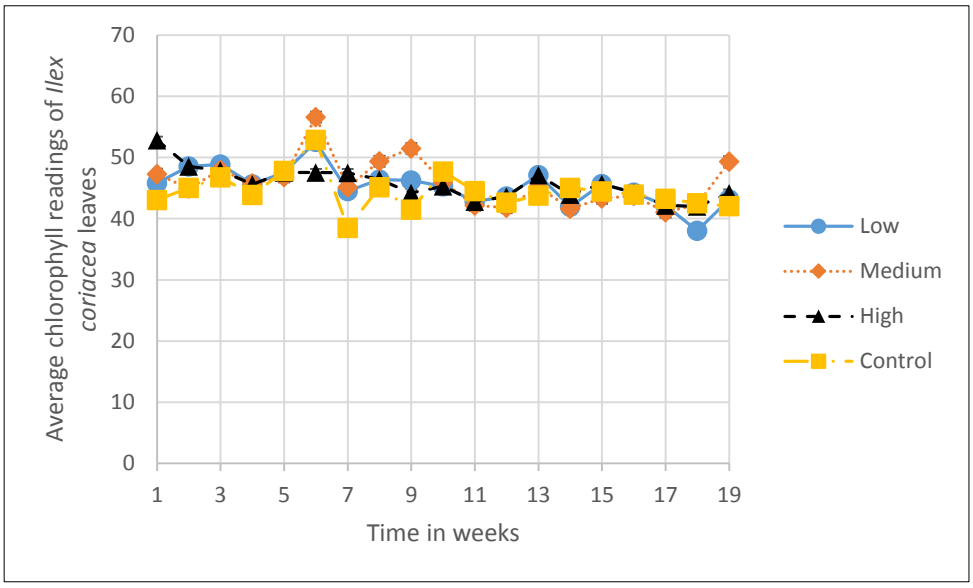


Figure 2.20: Average *Ilex coriacea* chlorophyll readings over time.

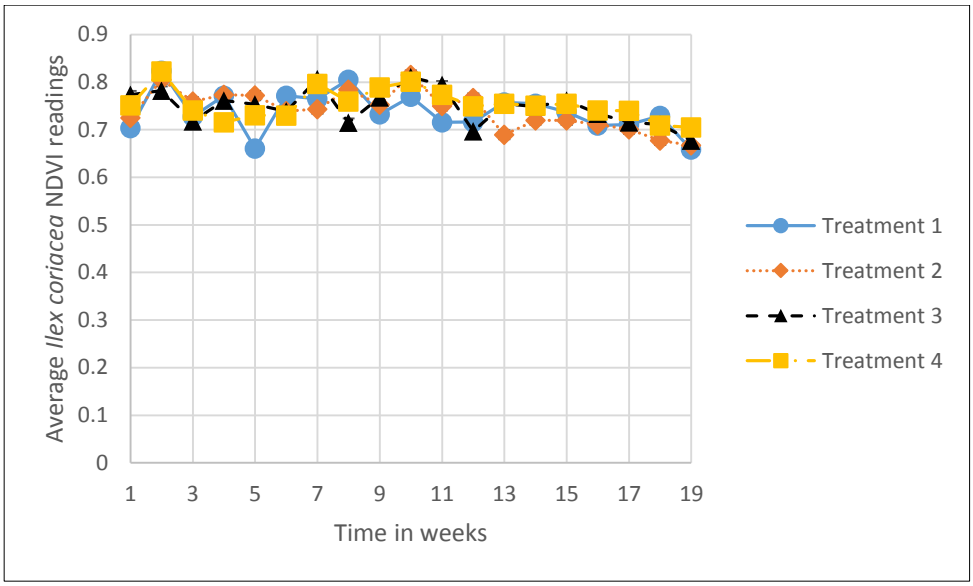


Figure 2.21: Average *Ilex coriacea* NDVI readings over time.

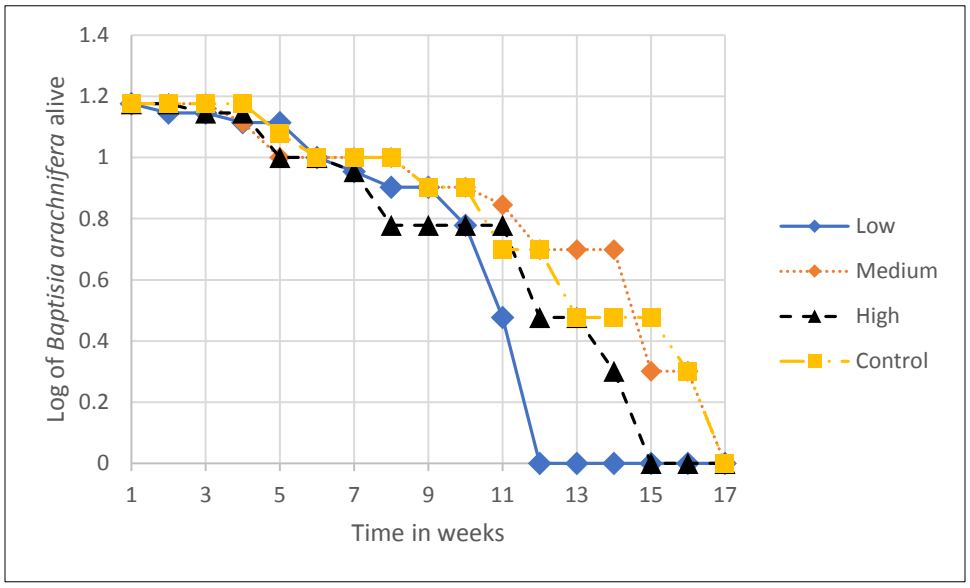


Figure 2.22: Survivorship of *Baptisia arachnifera* in a field experiment over time.

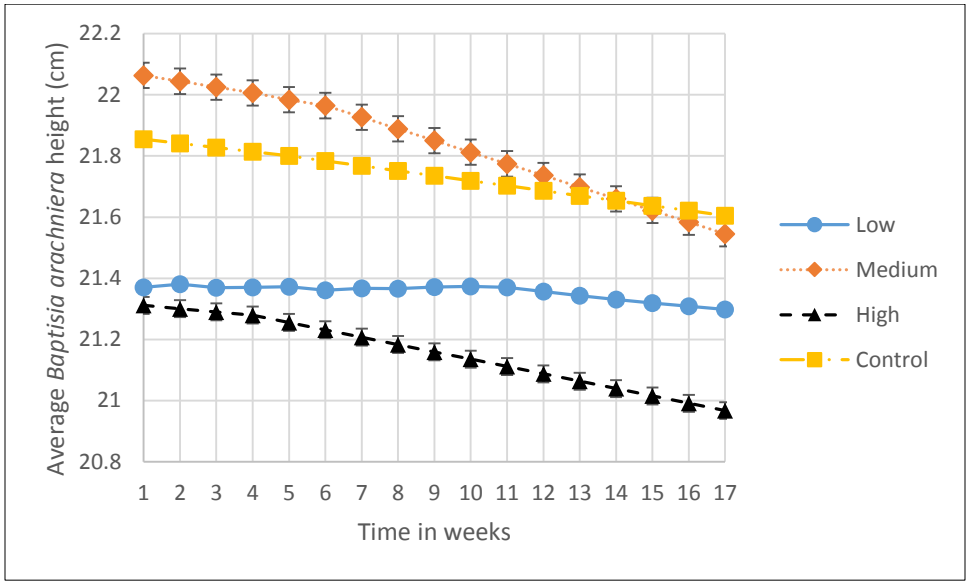


Figure 2.23: Average *Baptisia arachnifera* height over time in a field experiment.

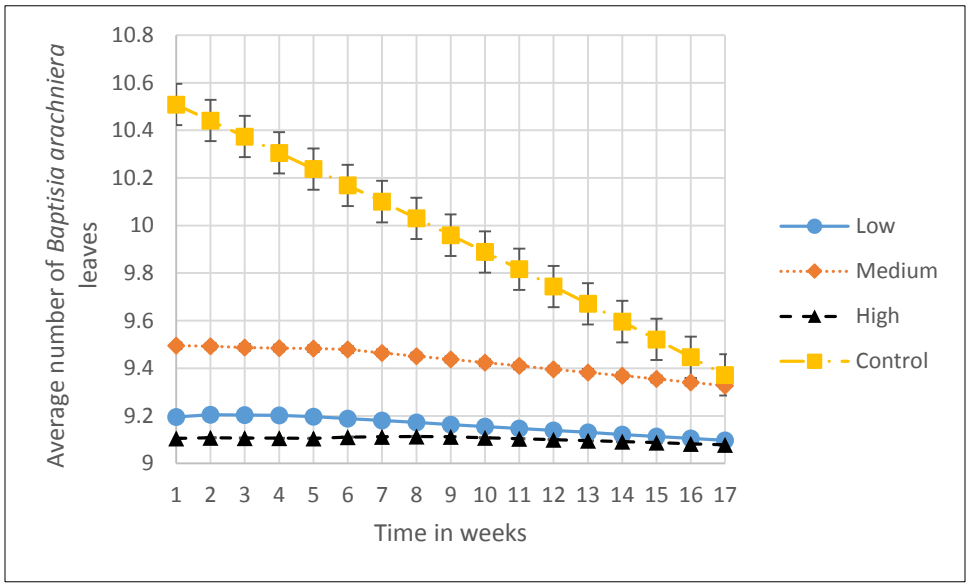


Figure 2.24: Average number of *Baptisia arachnifera* leaves over time in a field experiment.

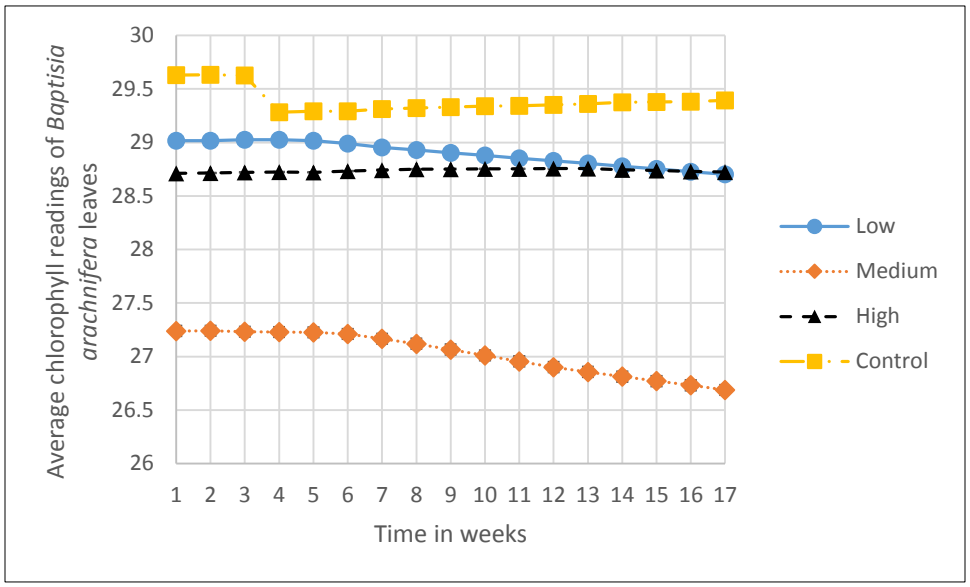


Figure 2.25: Average chlorophyll readings of *Baptisia arachnifera* over time in a field experiment.

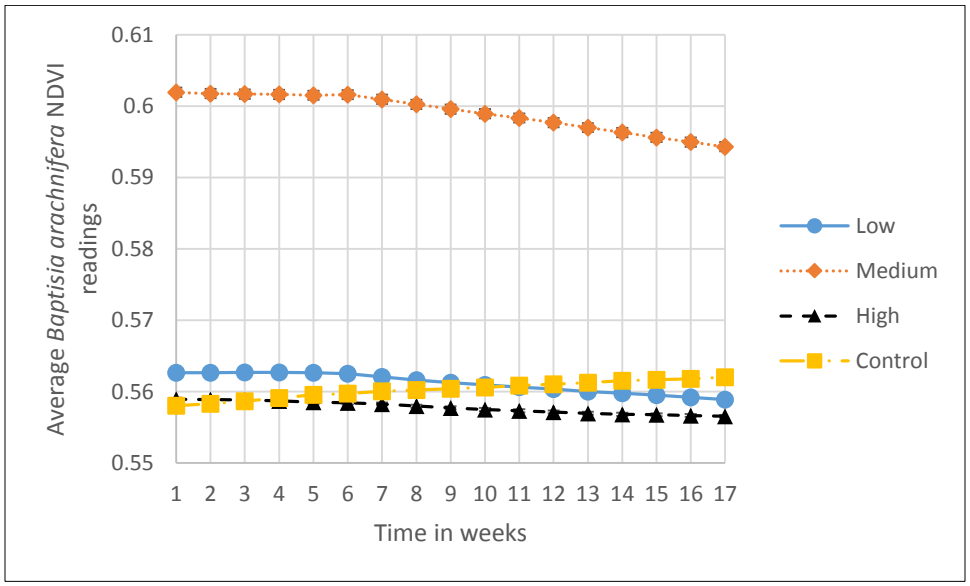


Figure 2.26: Average *Baptisia arachnifera* NDVI readings over time.

Table 2.1: Greenhouse experiment herbicide treatments. All units are in ml.

Treatment		Literature Imazapyr	Literature Water Volume	Experiment Imazapyr per bottle	Experiment Water per bottle	Experiment Imazapyr per pot	Experiment Water per pot
1	Low	946.4	18927.1	4.8	96	0.2	4
2	Medium	1419.5	18927.1	7.2	96	0.3	4
3	High	1892.7	18927.1	9.6	96	0.4	4

Table 2.2: Field experiment herbicide treatments. All units are in ml.

Treatment		Literature Imazapyr	Literature Water Volume	Experiment Imazapyr per bottle	Experiment Water per bottle	Experiment Imazapyr per area	Experiment Water per area
1	Low	946.4	18927.1	9.6	192	0.4	8
2	Medium	1419.5	18927.1	14.4	192	0.6	8
3	High	1892.7	18927.1	19.2	192	0.8	8

Table 2.3: Effect tests of height differences in *Baptisia arachnifera* tested in a greenhouse study with respect to treatments, time and treatment crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	9.9727	<0.0001*
Treatment	3	3	1.0548	0.3780
Treatment*Time	54	54	1.8806	0.0002*

* Indicates values of significance

Table 2.4: Differences in leaf number among herbicide treatments applied to *Baptisia arachnifera* in a greenhouse study. Levels not connected by the same letter are significantly different.

Treatment	P-Value	Significance Level	Least Squared Mean
1 (low)	0.0351	AB	14.9
2 (medium)		AB	15.2
3 (high)		B	13.9
4 (control)		A	18.6

Table 2.5: Effect tests of leaf number differences in *Baptisia arachnifera* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	21.7877	<0.0001*
Treatment	3	3	1.0548	0.0351*
Treatment*Time	54	54	1.1842	0.1767

* Indicates values of significance

Table 2.6: Effect tests of chlorophyll content differences in *Baptisia arachnifera* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	7.0346	<0.0001*
Treatment	3	3	1.4632	0.2376
Treatment*Time	54	54	1.5507	0.0080*

* Indicates values of significance

Table 2.7: Effect tests of NDVI differences in *Baptisia arachnifera* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	1.3086	0.1741
Treatment	3	3	0.1140	0.9515
Treatment*Time	54	54	0.8824	0.7120

* Indicates values of significance

Table 2.8: Effect tests of height differences in *Andropogon sp.* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	1.4340	0.1079
Treatment	3	3	1.8318	0.1553
Treatment*Time	54	54	1.1255	0.2541

* Indicates values of significance

Table 2.9: Effect tests of leaf number differences in *Andropogon* sp. tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	1.4340	0.1079
Treatment	3	3	1.8318	0.1553
Treatment*Time	54	54	1.1255	0.2541

* Indicates values of significance

Table 2.10: Effect tests of chlorophyll content differences in *Andropogon* sp. tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	4.0589	<0.0001*
Treatment	3	3	3.8303	0.0160*
Treatment*Time	54	54	0.9791	0.5191

* Indicates values of significance

Table 2.11: Differences in chlorophyll readings among herbicide treatments applied to *Andropogon* sp. in a greenhouse study. Levels not connected by the same letter are significantly different.

Treatment	P-Value	Significance Level	Least Squared Mean
1 (low)	0.0160	A	27.38
2 (medium)		AB	23.43
3 (high)		B	2.31
4 (control)		AB	27.23

Table 2.12: Effect tests of differences in NDVI readings for *Andropogon* sp. tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	0.8066	0.6938
Treatment	3	3	1.0473	0.3812
Treatment*Time	54	54	0.4790	0.4790

* Indicates values of significance

Table 2.13: Effect tests of differences in height measurements for *Ilex coriacea* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	7.1536	<0.0001*
Treatment	3	3	0.4175	0.7413
Treatment*Time	54	54	1.3169	0.0672

* Indicates values of significance

Table 2.14: Effect tests of differences in leaf number for *Ilex coriacea* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	44.4143	<0.0001*
Treatment	3	3	5.9322	0.0017*
Treatment*Time	54	54	1.4762	0.0166*

* Indicates values of significance

Table 2.15: Effect tests of differences in chlorophyll readings for *Ilex coriacea* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	4.2967	<0.0001*
Treatment	3	3	0.6878	0.5643
Treatment*Time	54	54	0.6004	0.9899

* Indicates values of significance

Table 2.16: Effect tests of differences in NDVI readings for *Ilex coriacea* tested in a greenhouse setting with respect to treatment, time and treatement crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	18	18	0.9364	0.5337
Treatment	3	3	0.3467	0.7917
Treatment*Time	54	54	0.3872	0.3872

* Indicates values of significance

Table 2.17: Effect tests of height differences in *Baptisia arachnifera* tested in a field study with respect to treatments, time and treatment crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	16	16	35.5074	<0.0001*
Treatment	3	3	0.4175	0.4175
Treatment*Time	48	48	4.2300	<0.001*

* Indicates values of significance

Table 2.18: Effect tests of leaf number differences in *Baptisia arachnifera* tested in a field study with respect to treatments, time and treatment crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	16	16	1.7258	<0.0001*
Treatment	3	3	75.6118	0.1721
Treatment*Time	48	48	4.7893	<0.001*

* Indicates values of significance

Table 2.19: Differences in chlorophyll readings among herbicide treatments applied to *Baptisia arachnifera* in a field experiment. Levels not connected by the same letter are significantly different.

Treatment	P-Value	Significance Level	Least Squared Mean
1 (low)	0.0412	B	42.3
2 (medium)		AB	29.0
3 (high)		AB	22.7
4 (control)		A	21.1

Table 2.20: Effect tests of chlorophyll content differences in *Baptisia arachnifera* tested in a field study with respect to treatments, time and treatment crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	16	16	1.48	0.1002
Treatment	3	3	2.93	0.0412*
Treatment*Time	48	48	0.92	0.6210

* Indicates values of significance

Table 2.21: Effect tests of NDVI differences in *Baptisia arachnifera* tested in a field study with respect to treatments, time and treatment crossed with time.

Parameters	Nparm	DF	F Ratio	Prob>F
Time	16	16	0.6790	0.6790
Treatment	3	3	0.8066	0.3413
Treatment*Time	48	48	0.3881	0.3881

* Indicates values of significance

Table 3.1: Soil profiles of field sites. Site 6 is owned by the Nature Conservancy. B=sites with *Baptisia arachnifera*. NB=sites that did not have *Baptisia arachnifera*.

Site	GPS	Soil Type
1B	31°20’36.46” N, 81°54’00.89” W	Leefield loamy sand, Albany-Leefield complex, Mascotte find sand
2B	31°20’36.21” N, 81°54’01.71” W	Albany-Leefield complex, Leefield loamy sand, Rigdon-Olustee complex
3B	31°20’45.29” N, 81°53’48.43” W	Surrency mucky fine sand, Mascotte find sand
4B	31°20’36.55” N, 81°53’56.60” W	Surrency mucky fine sand, Mascotte find sand
5B	31°20’49.78” N, 81°46’57.96” W	Rigdon-Olustee complex, Mandarin fine sand, Leon find sand
6B	31°20’19.70” N, 81°54’20.27” W	Bonifay loamy sand, Fuquay loamy sand, Leefield loamy sand, Olustee loamy fine sand, Mascotte find sand
7NB	31°20’33.28” N, 81°53’52.83” W	Surrency mucky fine sand, Mascotte find sand
8NB	31°20’31.92” N, 81°53’40.66” W	Mascotte find sand, Rigdon-Olustee complex
9NB	31°20’32.54” N, 81°54’02.27” W	Olustee loamy fine sand, Mascotte find sand
10NB	31°21’09.77” N, 81°47’18.07” W	Rigdon-Olustee complex, Mandarin fine sand
11NB	31°21’12.11” N, 81°47’29.07” W	Rigdon-Olustee complex, Mascotte find sand
12NB	31°21’12.89” N, 81°47’24.98” W	Rigdon-Olustee complex, Mandarin fine sand

Table 3.2: Plant Leaf Micronutrient Averages by Site (ppm). Each site had ten plants sampled from it. The numbers here are averages of those values.

Site	Al*	B*	Cd	Cr	Cu*	Fe*	Mn*	Mo	Na*	Ni	Pb	Zn*
CR	56.1	33.1	<0.8	<1	5.6	56.4	44	<1	5076.9	1.4	<2.0	11.6
DH	93.3	31.9	<0.8	<1	5.6	79.9	38.2	<1	5964.1	1.2	<2.0	12.2
WR	96.6	18	<0.8	<1	17	55.7	105.5	<1	1491.1	1.4	5.3	11.6
LT	47.2	21.2	<0.8	<1	11.6	52.1	191.2	<1	2663.1	1.1	<2.0	19.9
TR	42.5	32.2	<0.8	<1	3.6	51.9	39.3	<1	5515.9	1.3	<2.0	10.9
OW	43.3	25.5	<0.8	<1	4.4	48.8	27.8	<1	5076.6	1.4	<2.0	10.6

*Indicates that there were significant differences of this micronutrient between sites.

Table 3.3: Average leaf concentration of aluminum (ppm) by site. Levels not connected with the same letter are significantly different.

Site	5	2	4	6	3	1
Al (ppm)	96.6	93.3	56.1	47.2	43.3	42.5
Level of Significance P=<0.0001	A	A	B	B	B	B

Table 3.4: Average leaf concentration of boron (ppm) by site. Levels not connected with the same letter are significantly different.

Site	4	1	2	3	6	5
B (ppm)	33.1	32.2	31.9	25.5	21.2	18
Level of Significance P=<0.0001	A	A	A	B	B	B

Table 3.5: Average leaf concentration of copper (ppm) by site. Levels not connected with the same letter are significantly different.

Site	5	6	4	2	3	1
Cu (ppm)	17	11.6	5.6	5.6	4.4	3.6
Level of Significance P=<0.0001	A	A	B	B	B	B

Table 3.6: Average leaf concentration of iron (ppm) by site. Levels not connected with the same letter are significantly different.

Site	2	4	5	6	1	3
Fe (ppm)	79.9	56.4	55.7	52.1	51.9	48.8
Level of Significance P=<0.0005	A	B	B	B	B	B

Table 3.7: Average leaf concentration of manganese (ppm) by site. Levels not connected with the same letter are significantly different.

Site	6	5	4	1	2	3
Mn (ppm)	191.2	105.5	44	39.3	38.2	27.8
Level of Significance P=<0.0001	A	B	C	C	C	C

Table 3.8: Average leaf concentration of sodium (ppm) by site. Levels not connected with the same letter are significantly different.

Site	2	1	4	3	6	5
Na (ppm)	5964.1	5515.9	5076.9	5076.6	2663.1	1491.1
Level of Significance P=<0.0001	A	A	B	B	C	C

Table 3.9: Average leaf concentration of zinc (ppm) by site. Levels not connected with the same letter are significantly different.

Site	6	2	4	5	1	3
Zn (ppm)	19.9	12.2	11.6	11.6	10.9	10.6
Level of Significance P=<0.0115	A	AB	AB	AB	B	B

Table 3.10: Soil nutrient results by site (ppm). Underlined sites contain *Baptisia arachnifera* plants. Calcium, nitrogen and carbon were found to have a significant difference between sites with and without *Baptisia arachnifera*. *Indicates significant difference in sites with and without *Baptisia arachnifera.*

Site	Al	B	Cd	Cr	Cu	Fe	Mn	Mo	Na	Ni	Pb	Zn	C*	S	Mg	Ca*	K	N*	P
LT	1287	<1	<0.5	<0.5	<0.5	578	<0.5	<0.5	<5	<1	<2.5	<0.5	4441.2	<8	<1.5	25.73	<20	503.7	<6
DH	2329	<1	<0.5	<0.5	<0.5	1387	<0.5	<0.5	<5	<1	<2.5	<0.5	9820.4	19.87	<1.5	<5	<20	737.7	<6
WR	3423	<1	<0.5	<0.5	<0.5	1157	<0.5	<0.5	<5	<1	<2.5	<0.5	12932	31.86	<1.5	39.83	<20	795.6	<6
CR	926	<1	<0.5	<0.5	<0.5	235	<0.5	<0.5	<5	<1	<2.5	<0.5	11779.2	<8	<1.5	11.51	<20	693.3	<6
OW	2121	<1	<0.5	<0.5	<0.5	633	<0.5	<0.5	<5	<1	<2.5	<0.5	10493.1	73.58	<1.5	98.93	<20	674.3	<6
TR	2320	<1	<0.5	<0.5	<0.5	891	<0.5	<0.5	<5	<1	<2.5	<0.5	12969.5	10.77	<1.5	<5	<20	870.3	<6
BR	2108	<1	<0.5	<0.5	<0.5	880	<0.5	<0.5	<5	<1	<2.5	<0.5	3193.7	<8	<1.5	<5	<20	447.5	<6
YR	3116	<1	<0.5	<0.5	<0.5	880	<0.5	<0.5	<5	<1	<2.5	<0.5	482.9	<8	<1.5	<5	<20	348.4	<6
PF	869	<1	<0.5	<0.5	<0.5	347	<0.5	<0.5	<5	<1	<2.5	<0.5	2906.8	<8	<1.5	<5	<20	378.3	<6
CL	1137	<1	<0.5	<0.5	<0.5	394	<0.5	<0.5	<5	<1	<2.5	<0.5	2720.7	<8	<1.5	<5	<20	411.5	<6
DT	989	<1	<0.5	<0.5	<0.5	166	<0.5	<0.5	<5	<1	<2.5	<0.5	3230.9	<8	<1.5	<5	<20	379.3	<6
PC	563	<1	<0.5	<0.5	<0.5	197	<0.5	<0.5	<5	<1	<2.5	<0.5	1959.2	<8	<1.5	<5	<20	378.6	<6

Table 3.11: Carbon (ppm) analysis results from soils collected from sites with and without *Baptisia arachnifera*.

Baptisia arachnifera	**Mean**	**Standard Deviation**	**P-value**
Present	10405.94	3186.466	0.0039*
Absent	2415	1053.4	

* Indicates values of significance.

Table 3.12: Nitrogen (ppm) analysis results from soils collected from sites with and without *Baptisia arachnifera*.

Baptisia arachnifera	**Mean**	**Standard Deviation**	**P-value**
Present	712.4902	124.7673	0.0039*
Absent	390.6093	34.29749	

* Indicates values of significance.

APPENDIX

RECENSUS OF A NATURAL POPULATION

Introduction

The last known population of *Baptisia arachnifera* under natural area exists on the property of The Nature Conservancy of Georgia. This land, known as the Lewis Tract, is not commercially harvested for timber and undergoes minimal management that are limited to a few burn treatments/occasional tree thinning every few years This site offers use as a control population to areas that are managed for timber. In the summer of 2010 a census was taken of every existing *Baptisia arachnifera* in the site Georgia Southern University graduate student Timothy Estep of every existing *Baptisia arachnifera* on the area in hopes of starting a population census. The information gathered about from the population trends after treatments such as burning and thinning can provide valuable knowledge on the what the best management practices for land that contain *Baptisia arachnifera* can be. Addressing information about species distribution has the potential to be an effective plant conservation tool (Havens et al., 2014).

Materials and Methods

A census was taken over an area of land totally approximately 12.1 hectares (Figure 1). As was done in the previous census, every *Baptisia arachnifera* was located by walking up and down belt transects throughout the area. When a plant was located, GPS coordinates were taken and life stage was also determined for each plant (Figure 1.2). The different life stages were as follows: seedling (under 15.2 cm), juvenile (over 15.2 cm, unbranched), sub-adult (branched, but no reproductive structures), and adult (reproductive structures i.e. flowers and seed pods). Height of each plant and leaf number of each plant was measured for each plant, and for adult plants the

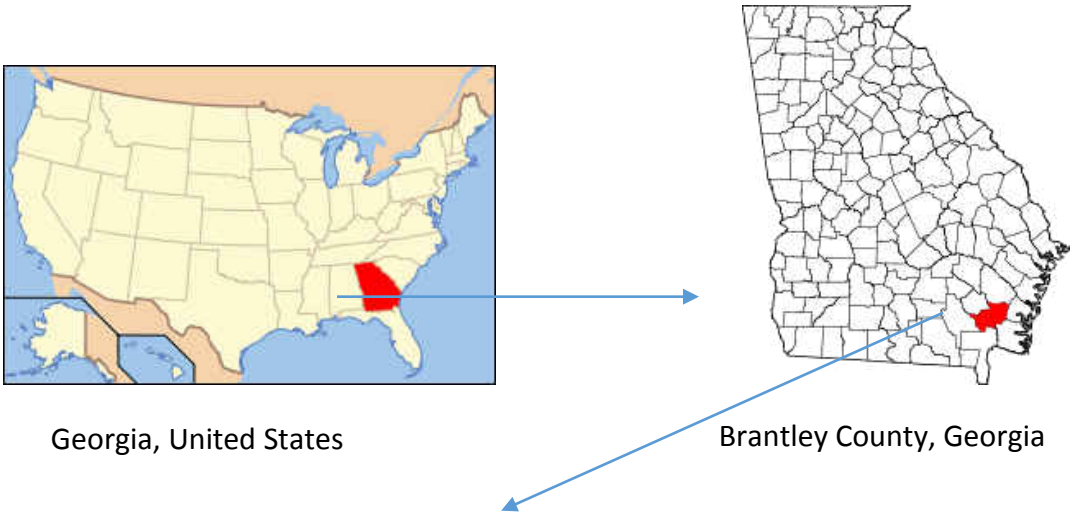
number of seed pods and flowers was also recorded. Burning and thinning treatments took place between January 2010 and March 2012 (Table 1.1).

Results

355 *Baptisia arachnifera* were found in 2013 as compared to the 444 that were found in 2010. A higher percentage of sub-adults were found in the 2013 census compared to the 2010 census. However, the 2010 census had a higher percentage of seedlings, juveniles, and reproductive plants (Table 1.2)

Management Implications

Burning is an effective way to control and clear understory and has been shown to directly influence plant community composition and vegetation structure (Morrison et al. 1995; Moreira 2000). Wall et al. (2012) reported that the demographic response of species to fire in fire-dependent ecosystems is variable. The results of this study found different number of life stages, and one of the reasons for this may be *Baptisia arachnifera's* response to fire treatments. More research needs to be done to see if there is any effect on fire on different life stages of *Baptisia arachnifera.*



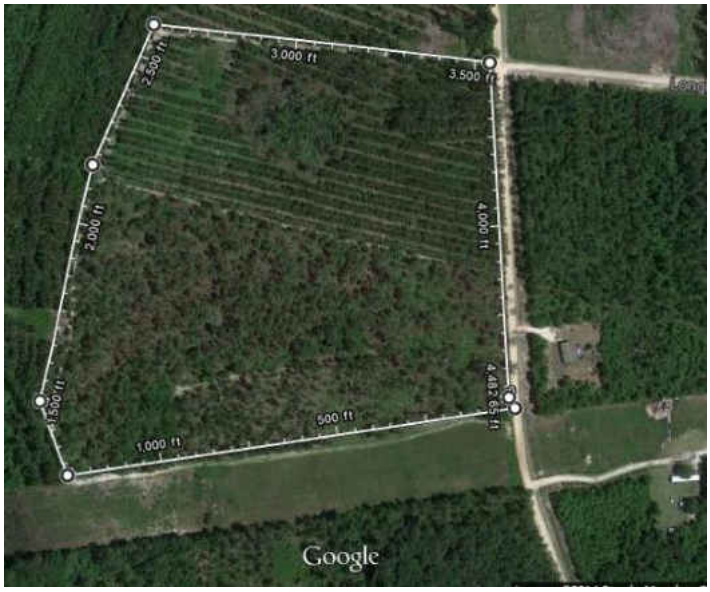


Figure 1.1: The Nature Conservancy property, outlined by a ruler.

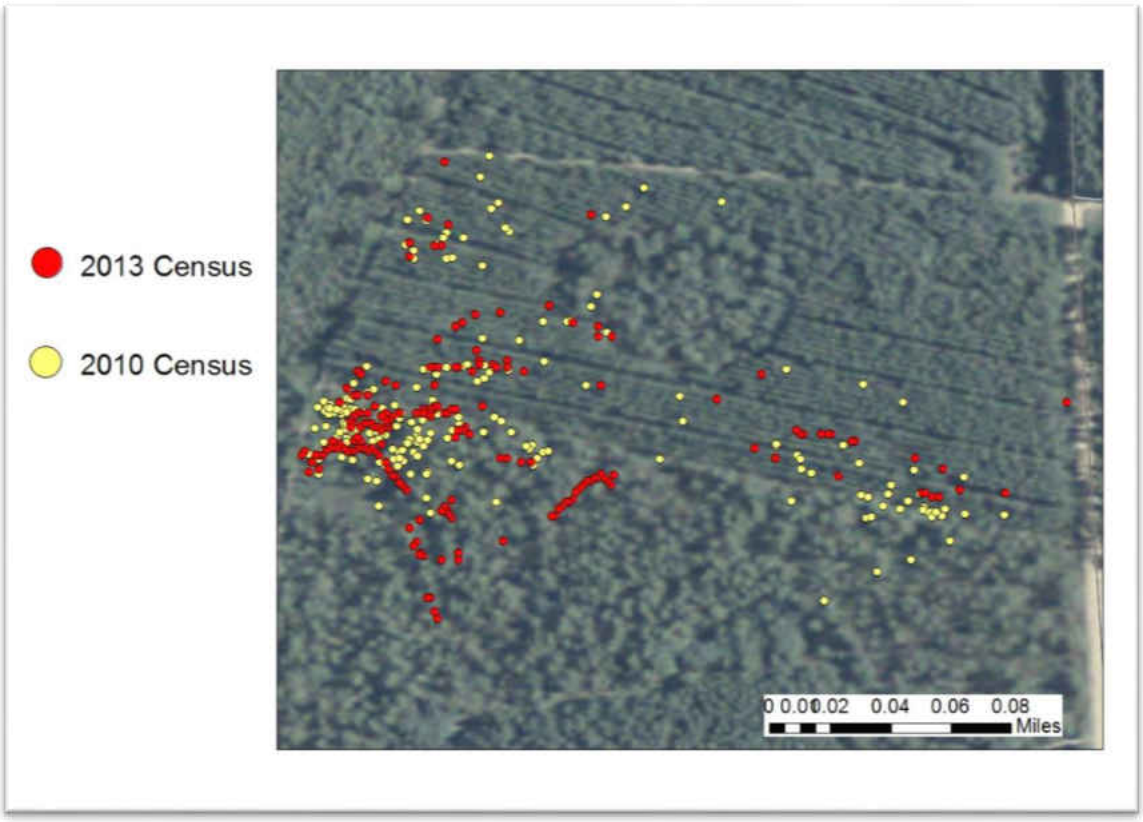


Figure 1.2: GIS map of Lewis tract area with all *Baptisia arachnifera* from the 2010 and 2014 census marked.

Table 1.1: Treatments/Census done on Lewis Tract property between 2010 and 2013. The area of land was burned in January 14, 2010 and the first census was conducted by Timothy Estep in Summer 2010. The area was burned again in fall of 2010, and thinned in Summer 2011. Another burn was performed on March 21, 2012, with the second census taking place in summer 2013.

Date	Burning	Thinning
January 14, 2010	X	
Summer 2010	**Census-Estep**	
Fall 2010	X	
Summer 2011		X
March 21, 2012	X	
Summer 2013	**Census-Steinbrecher**	

Table 1.2: Findings of the 2010 and 2013 census of *Baptisia arachnifera* on Lewis Tract property.

	2010		2013	
Plant Stage	**Count**	**Percent**	**Count**	**Percent**
Seedling	22	4.95	1	0.28
Juvenile	78	17.57	55	15.49
Sub adult	175	39.41	196	55.21
Reproductive	169	38.06	103	29.01
Total	444		355	