Herbicide Tolerance and Weed Control of Longleaf Pine Native Understory Species

by

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Abstract

The restoration of longleaf pine (*Pinus palustris*) is an important environmental issue due to the shrinking size of this once dominant forest type. The restoration of this ecosystem requires the presence of native understory species such as wiregrass (*Aristida beyrichiana*), muhly grass (*Muhlenbergia capillaris*), yellow Indian grass (*Sorghastrum nutans*), Florida ticktrefoil (*Desmodium floridanum*), narrow-leaf sunflower (*Helianthus angustifolius*) and many others. The commercial propagation of these understory species is a relatively new aspect of nursery management, which subsequently means that nursery managers have little knowledge on how to grow and propagate these plants, and also how to control unwanted weeds amongst them as well. In addition, the use of herbicides as part of ecological restoration of longleaf has been reported to decrease seedling survival. These trials will examine the effects of several herbicides applied at varying rates to further evaluate their effect upon the selected understory plants. These herbicides include: atrazine (AAtrex®), imazapyr (Chopper®), imazamox (Clearcast®), lactofen (Cobra®), s-metolachlor (Dual Magnum®), oxyflurofen (Goal 2XL® and Goaltender®), pendimethalin (Prowl H_2O®), imazapic (Plateau®), imazethapyr (Pursuit®), halosulfuron-methyl (Sedgehammer®), sulfentrazone (Spartan Charge®), dicamba + 2,4-D (Weedmaster®) and butyric acid (2,4-DB®). For both the seed production study and the grass seedling study, successful herbicide treatments were identified that are tolerated by the native plant species and effective on target weed species. The successful herbicide treatments for the native plant seed production include atrazine, dicamba + 2,4-D, imazapic, and sulfentrazone. The successful treatments for the native grass seedlings include halosulfuron-methyl, lactofen, pendimethalin and a low rate of

oxyfluorfen. These treatments will provide nursery managers and plant growers with proven tools which can be used to grow native plants and aid longleaf pine restoration initiatives. Additionally, an indicator species, sorghum, was identified as a potential imazapyr bioassay. This indicator species will allow land managers and foresters to easily and efficiently determine if a site that has been treated with imazapyr is safe to plant longleaf pine seedlings.

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Chapter 1

Introduction and Literature Review

1.1 INTRODUCTION

1.1.1 *Longleaf Pine History*

The southeastern forests of the U.S. have changed significantly over the past 500 years. The southeast, which was previously dominated by the longleaf pine (*Pinus palustris*) grassland ecosystem and estimated to have covered more than 74 million acres in the southeast from Virginia to Texas, is now dominated by other southern pine species such as loblolly pine (*Pinus taeda)* and slash pine (*Pinus elliottii)* (Frost, 1993). Longleaf pine was and still is regarded as the most diverse ecosystem in the United States, only second behind the tropics (Brockway et al, 1998). Prior to European settlement, these forests were maintained by Native Americans and natural fire disturbances. Longleaf pine thrive when there are understory fires to control competitive species such as oaks (*Quercus spp.)* and other woody stems such as yaupon (*Ilex vomitoria*) and sweetgum (*Liquidambar styraciflua*). Fire not only controls competitive vegetation but it helps to facilitate longleaf pine regeneration allowing longleaf pine seeds to settle onto bare mineral soil rather than into the litter layer. The natural understory of longleaf pines consists partly of wiregrass (*Aristida beyrichiana)* and little bluestem (*Schizachyrium scoparium*) which are important in providing a fuel source for understory fires (Landers et al, 1995).

Native Americans would often practice understory burning because this would make hunting easier by reducing vegetation, the number of biting insects, and aid them from other tribes and predators by increasing visibility (Van Lear et al, 2005). Although the Native

Americans performed understory fires, there were also natural occurrences of wildfires. The Southeast experienced a high frequency of storms due to the proximity to the Gulf of Mexico. These storms would often bring lightening which would cause wildfires. These wildfires accomplished the same objectives as the anthropogenic fires and what is still accomplished through the use of prescribed fires today (Landers et al, 1995; Van Lear et al, 2005; Croker, 1987).

As the Europeans settled North America and began colonization, they harvested much of the southern pines for ship building and naval supplies (such as turpentine and tar for ships), farming, and the expansion of their communities (Frost, 1993). Once harvested, it was noticed that longleaf pine did not regenerate as well as the other southern pine species such as loblolly and slash pine (Harrington et al, 2003). There were three contributing factors to this. First, longleaf pine is traditionally a poor seed producer, and its large seeds have a limited dispersal range (Landers et al, 1995). The seed would often get caught in the grasses and litter on the ground if the site has not been burned to reduce the vegetation cover (Burns and Honkala, 1990). Additionally, some longleaf pine forest harvests were so thorough that there wasn’t a seed source available for regeneration (Van Lear et al, 2005). Second, longleaf pine seedlings may remain in a stemless grass stage for 1-3 years before they begin vertical growth (Landers et al, 1995). Grass stage growth habit deterred many from planting longleaf pine because it appeared that the species grew more slowly than either loblolly or slash pine, which then slowly replaced longleaf pine across the landscape. However, the slower growth of longleaf pine has since been proven to be incorrect. Researchers have shown that longleaf pine is able to catch up to loblolly pine trees by age 12-15 on poor sites and by age 25-30 on average sites (Johnson, 1999). A third reason contributing to poor longleaf pine regeneration is that longleaf pine seedlings that did establish were often eaten by wild hogs and cattle that roamed the woods. Potential impacts of grazing are increased when the seedlings stay in a grass stage for several years, which is more vulnerable to grazing (Croker, 1987).

During a period of declining Native American populations, the forests were not burned as regularly as they once were. This lack of fire negatively impacted the longleaf pine stands by allowing competitive vegetation to suppress and out-compete the longleaf pine (Van Lear et al, 2005; Dale et al, 2002). Fire suppression continued throughout the 1900s as the United States adopted a fire exclusion policy that was detrimental to the remaining longleaf pine stands and to the natural forest succession of stands that had been harvested. In addition, due to the management practices of industrial and private landowners in place at the time much of the harvested longleaf pine was not regenerated. The culmination of these factors led to the reduction of longleaf pine in the southeast from an estimated 74 million acres to about 3 million acres by the 1990s (Landers et al, 1995; Frost, 1993). Once this reduction in acreage was recognized by the forestry community, numerous restoration efforts began to restore longleaf ecosystems in southeastern forest lands.

1.1.2 *Longleaf Pine Ecosystems*

Longleaf pine is adapted to grow in the warm climates (both moist and dry) common to the southeast (Burns and Honkala, 1990). The longleaf pine ecosystem was maintained by frequent low- to mid-intensity burning, resulting from lightening and Native American land management practices. Native understory species provided the fuel source required to carry a fire (Outcalt, 1992). Understory species include warm season grasses such as wiregrass (*Aristida beyrichiana*), muhly grass (*Muhlenbergia capillaris*), little bluestem (*Schizachyrium scoparium*),big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans*)

(Brockway and Lewis, 1997). As the density of wiregrass and other grass species increase they tend to suspend pine litter off the ground which decreases moisture content, providing an optimum fuel source for fire. (Mulligan et al, 2002). The frequent fires suppressed other competitive species and woody stems, benefiting wiregrass seed production and growth, if the fires occurred during the growing season (Mulligan and Kirkman, 2002; Aschenbach et al, 2010). Without the native understory plant species and dried pine straw, there would be limited fuel for fires and the longleaf pine ecosystem would eventually be overcome by other species which would suppress the natural regeneration of the longleaf pine and ultimately change the overall forest type (Kush et al, 1999).

Longleaf pine ecosystems are extremely diverse in regards to both vegetation and wildlife. The understory is comprised of the aforementioned native grasses, legumes (e.g. *Tephrosia virginiana*, *Desmodium floridanum*), and composites (e.g. *Helianthus angustifolius*). The legumes and composites are important for the survival and habitat for many wildlife species e.g. gopher tortoises (*Gopherus polyphemus*), indigo snakes (*Drymarchon couperi*), quail (*Colinus virginianus*), turkey (*Meleagris gallopavo*), and red cockaded woodpecker (*Picoides borealis*) (Val Lear et al, 2005). The open, grassland understory created by these native understory species provides cover and nutrition for wildlife, adding to the value that longleaf pine ecosystems can bring to the environment and emphasizing the importance that understory species have on the ecology and habitat of these systems.

Until the latter half of the 20th century, artificially-regenerated longleaf pine stands often lacked the understory plant species that contribute to the important environmental processes of the ecosystem. These early restoration efforts occurred through the USDA’s Conservation Reserve Program (CRP). However, as participation in this program and knowledge of the longleaf pine ecosystem increased, the importance of these understory species was realized. As a result, the newest version of the CRP as well as other restoration initiatives requires establishment of native plants in the understory of planted longleaf pine, particularly native warm-season grasses (Kaeser and Kirkman, 2010). Additionally, the CRP offers subsidies to landowners who reforest their land with longleaf pine (Farm Service Agency, 2011). Although, there are qualifications and restrictions as to who can receive the subsidies, this is a beneficial movement for longleaf pine ecosystem restoration.

1.1.3 *Longleaf Pine Restoration*

The restoration of the longleaf pine ecosystem is becoming increasingly popular with non-industrial landowners throughout the southeast. Due to the improvements of forest management, the commercial production of seedlings, and increased knowledge of longleaf pine, restoration of this ecosystem has become significantly more successful.

With the CRP's requirement of re-establishing native ground cover species, commercial production of native understory plants in nurseries became more common. Today, as these native ground covers have become recognized as an integral part of longleaf pine restoration they are being mass-produced across the southeast. The increase in production has made it easier to incorporate native understory species into the restored ecosystems and increases the probability of success. While restored ecosystems may not match the appearance of natural stands, the primary focus in restoration is to use available tools and species to mimic the plant composition and ecological disturbances, both natural and anthropogenic, to recreate those environments.

A prominent movement for longleaf pine ecosystem restoration is being led by America's Longleaf. In 2009, the organization created a 15 year goal of increasing longleaf pine forest types in the southeast from 3.4 million acres to 8 million acres. Their conservation plan included three primary objectives: 1) to maintain existing longleaf pine stands, 2) to improve land that is characterized as having a longleaf pine cover type but lacks understory species, and 3) to convert land that is not currently characterized as a longleaf pine ecosystem into one (America's Longleaf, 2009).

America's Longleaf also identified research needs for proper longleaf pine ecosystem restoration. One key research need concerned understory species and plant community composition, including guidelines and standards for the commercial production of understory plant species, and increased knowledge of the community composition and the species that comprise them (America's Longleaf, 2009). While the restoration program has made progress, there are some production issues that have impeded the process. These research needs stem from both inside and outside of the commercial production of the understory native plant species. Many forest nurseries have developed production systems to commercially grow these plants in seed production areas and in nursery container systems similar to forest-tree production. However, in both the seed production areas and the native plant production areas, weeds can be a significant. A large concern is that there are no herbicide guidelines for effective control of weeds without also adversely affecting the understory native plant species.

In order to commercially grow understory native plant species, a constant source of seed is required. The process begins with growing plants for the production of seed which is then harvested and cleaned to remove undesirable weed seeds that might have been collected during the harvest. It is during both the seed and seedling production that the managers are struggling to control weeds. Commercial production of understory species is a new aspect of nursery management and few herbicides are available to control troublesome weeds that do not also harm the understory crop species (grasses, legumes and forbs). Often, herbicides are labeled to control both the weeds and the desired understory species which poses a problem when nursery managers spray herbicide and adversely affect their native understory plant crop.

In order to cultivate native understory seedlings in containers, there must be a seed production area or orchard from which to gather seed. Seed production areas establish plants in bed rows which are managed to ensure that an adequate amount of seed is produced for the production of native plant seedlings. Weeds are an issue within the seed production areas. Native plants in seed production areas tend to be spaced widely apart to allow for maximum seed production. Herbicide weed control methods used in these areas can be over the top, directed, or spot-spray applications where only the weeds are treated. Spot spraying ensures that the established plants are not harmed by the herbicide and are released from competition from unwanted weeds.

Container-grown seedlings are another form of commercial production of these plants that are typically grown in a peat moss substrate. During the growing season, it is inevitable that weeds will become established within the containers. When weeds become severe enough, steps must be taken to control them using herbicides. Depending on the amount of active ingredient in the herbicide there may be damaging effects to the crop species in addition to the weeds

1.1.4 *Herbicides*

Herbicides are a common forest management tool for the control of unwanted vegetation. They allow land managers to control plants that either inhibit or compete with the crop plants. Herbicides vary greatly in their selectivity with respect to species controlled. For example, hexazinone and triclopyr control broadleaf weeds and have relatively little effect on grasses whereas imazapyr is not selective and is active against many plant types. There are two primary pathways that herbicides can enter a plant: 1) through the roots and 2) through the foliage. Some herbicides can only do one or the other while others have been engineered to do both (Pike and Hager, 1998). Other compounds such as crop oil or surfactants can be added to increases their effectiveness (Dayan et al, 1996). Herbicides have several modes of action that disrupt plant function and growth. Herbicides that enter the plant through the roots have numerous methods of altering plant function including 1) root mitotic inhibitors block cell division, 2) pigment inhibitors, 3) shoot inhibitors affect cell growth and division, and 4) photosynthetic inhibitors block electron transfer. Herbicides that enter the plant through the foliage inhibit plants in three ways: 1) meristematic (lipid) inhibitors, 2) membrane disruptors, and 3) photosynthetic inhibitors. Herbicides that are capable of entering the plant through both soil and foliage applications can 1) affect protein synthesis and cell division which in turn impacts the new growth of the plant, and 2) they can block the acetolactate synthase enzyme which inhibits the plant's metabolism (Pike and Hager, 1998). Herbicides are a versatile and customizable tool that can be tailored to specific weeds. The different modes of action allow herbicides to be used independently or combined with each other create a specifically designed treatment.

1.1.5 *Herbicides and Native Plant Interactions*

Many troublesome weeds are encountered in the commercial production of the native plants found in longleaf pine ecosystems, especially in the seed production areas. They include: *Conyza canadensis* (horseweed), *Solidago canadensis* (goldenrod), *Croton glandulosus* (Vente conmigo), *Gamochaeta purpurea* (purple cudweed), *Oxalis stricta* (common yellow woodsorrel) and many others. Weed control is necessary as they are detrimental to the growth of the desired species. Herbicides provide effective and economical control of troublesome weeds. However, this often means controlling a grass within a grass or a broadleaf weed within a desired broadleaf plant. This can place severe limitations on the availability of the herbicide and combination of herbicides used.

1.1.6 *Herbicides and Longleaf Pine Interactions*

Several studies have been conducted in longleaf pine stands to determine how the understory plant community is affected by various herbicides currently used in forest settings. Freeman and Jose (2009) used four herbicide treatments, hexazinone, imazapyr, sulfometuron methyl, and sulfometuron methyl + hexazinone tank mix, to 1) identify differences in shrub control, 2) assess effects on wiregrass, total herbaceous cover, and community composition, 3) compare longleaf pine seedling growth and survival rates among treatments, and 4) compare fire temperatures and vegetation responses to prescribed fire among treatments. They reported all four treatments resulted in an increase in wiregrass cover over the control, with the sulfometuron methyl + hexazinone treatment resulting in the highest wiregrass cover. Kaeser and Kirkman (2010) evaluated nine pre- and post-emergence herbicides on non-target native plant species of the longleaf pine ecosystem. Their results showed that wiregrass is sensitive to hexazinone at the higher rate (3.30 kg/ha), whereas herbicides such as aminopyralid and triclopyr controlled pre-emergence grasses more than anticipated and that all pre-emergence legumes were sensitive to butyric acid. Jose et al (2010) examined how the four herbicides used in Freeman and Jose (2009), affect both the growth of the seedlings and the understory plant composition when applied over the top of 1-year old longleaf pine seedlings. They reported that none of the herbicides reduced the percent cover of wiregrass when sprayed at various rates. Brockway (2000) reported that hexazinone increased the foliar cover of wiregrass while decreasing the amount of turkey oak (*Quercus laevis*) and other oak species (*Quercus chapmanii*, *Quercus geminate*, *Quercus myrtifolia*) that had begun to invade the longleaf pine ecosystem. The majority of these field studies found that herbicide applications and fire are beneficial in controlling competitive species while benefiting the growth of both the longleaf and native understory species.

Although the conservation of established longleaf stands is an important aspect of the longleaf restoration plan, another component that needs research is the successful establishment of longleaf pine after harvesting. Common silvicultural practices in the forest industry involve several steps to prepare the site for replanting. In fact, prior to reforesting any land type, whether it be cropland, pastures, or forest land, the area is often sprayed with the herbicide imazapyr. Oftentimes, once the vegetation has died, prescribed fire is used to clean up the remaining plant debris. Then longleaf seedlings are planted, along with understory plants such as wiregrass, little bluestem, Indian grass, muhly grass, goat's rue, and Florida ticktrefoil. However, longleaf pine is particularly sensitive to imazapyr. Imazapyr is both a foliar and soil active herbicide (Dickens et al, 2012) and pine seedlings should not be planted within 60 days of a 48 oz ai/ac or greater treatment. Once the recommended wait time has passed it is theoretically safe to plant longleaf pine seedlings without seedling survival concerns. However, a rapid test to determine soil herbicide levels would prevent planting too soon, but currently none are available.

1.1.7 *Herbicide Bioassay*

An herbicide bioassay is a vegetative method used to detect amounts of herbicide in the soil that may impact the plant health (Pfeiffer, 2004). The use of a bioassay technique is an easy and inexpensive way to test soil for herbicide toxicity (Rashid et al, 2001). The presence or absence of herbicides is determined by whether indicator plants grown in treated soil show symptoms of herbicide injury (Pfeiffer, 2004). A bioassay for imazapyr could be a useful tool to use in the restoration process of longleaf pine ecosystems. Currently, foresters and land managers do not have a method for determining soil toxicity. They simply wait the recommended amount of time according to the herbicide label and hope that the longleaf seedlings won't be affected.

1.1.8 *Conclusion*

Through this project, herbicide regimes and growth guidelines will be developed to provide nursery managers with the means to successfully grow native understory species. This research project has three primary studies: 1) herbicide tolerance of understory grasses, 2) herbicide tolerance and weed control in seed production areas of understory species, and 3) the development of an imazapyr bioassay study for outplanting longleaf pine. This research will benefit the longleaf pine re-establishment efforts by providing information on how to commercially produce native plant seedlings more efficiently as well as providing a quick bioassay for use before replanting longleaf pine and native plants.

Chapter 2

Herbicide Weed Control and Tolerance of Mature Native Understory Plant Species

2.1 ABSTRACT

Successful restoration of longleaf pine ecosystems requires establishment of both longleaf pine and key understory species. For commercial production of native understory species in forest nurseries, a dependable source of viable seed is required. Collecting seed in the wild may prove to be unreliable, difficult to collect, or not viable. Some companies have recently begun growing native plants for the purpose of seed production. To date there are no herbicide protocols to help control weeds in these species that are often similar to the weed species growing amongst them. The purpose of this study was to identify herbicide treatments for successful control of weeds without detrimental effects to the understory species. Fourteen herbicide treatments were applied to six native plant species grown for seed production to determine 1) how well the plants tolerate the herbicides and 2) how well the treatments control weeds. Plant injury was evaluated using an injury rating scale of 1-9 (1=no injury and 9=mortality) and weed control was evaluated by collecting a weed sample at the conclusion of the study. Successful herbicide treatments that are tolerated by the native plants and provide weed control were identified for each understory species. Atrazine and dicamba + 2,4-D were the two optimal herbicide treatments for use on native grasses, whereas sulfentrazone offered the best results on narrow-leaf sunflower (*Helianthus angustifolius*). Imazapic provided the best results when applied to Florida ticktrefoil (*Desmodium floridanum*) and goat's rue (*Tephrosia virginiana*). Native plant producers now have additional tools that they can use in their production system to increase the availability of these important understory plants.

2.2 INTRODUCTION

The amount of land supporting longleaf pine ecosystems has been decreasing for several hundred years. The restoration of this ecosystem is becoming increasingly popular throughout the southeastern United States. Through improvements in forest management, commercial production of longleaf pine seedlings, and increased knowledge of longleaf pine reforestation practices, restoration efforts have become significantly more successful than before. In 2006, the Conservation Reserve Program (CRP) added a requirement that native ground cover species be re-established along with longleaf pine on croplands. Prior to this, native understory plants were not being grown for commercial purposes in nurseries. Today, these native ground covers are recognized as an integral part of longleaf pine restoration and they are being produced across the southeast. The increase in production has made it easier to incorporate native understory species into the ecosystem and increases the probability of restoration success. While restored ecosystems may not equal the appearance of natural ecosystems, the primary focus of restoration efforts is to use the tools available to mimic the plant composition and natural and anthropogenic disturbances.

One prominent movement for longleaf pine ecosystem restoration is being led by America's Longleaf. In 2009, America's Longleaf designed a 15-year goal of increasing longleaf pine forest types in the southeast from 3.4 million acres to 8 million acres. Their conservation plan contained three primary objectives: 1) to maintain existing longleaf pine stands, 2) to improve land that is characterized as having a longleaf pine cover type but lacks understory species, and 3) to convert land that is not currently characterized as a longleaf pine ecosystem into one (America's Longleaf, 2009).

In addition, America's Longleaf identified research areas that are needed in order to properly restore longleaf pine. One key research need concerned longleaf pine understory species and community composition, including guidelines and standards for the commercial production of longleaf pine understory plant species, and increased knowledge of the community composition and the species that comprise them (America's Longleaf, 2009). While progress has been made on these research questions, there are some production issues that have yet to be addressed. Many forest nurseries have recently developed production systems to commercially grow these native understory plants in seed production areas and in nursery container systems similar to forest-tree production. However, in both the seed production areas and the native plant seedling production areas, weeds can pose significant issues (e.g. out-competing, stunting) to production. A major concern is that herbicide guidelines are lacking that effectively control weeds, but not also adversely affect the understory native plant species.

For commercial production of understory native plants, a constant and reliable source of seed is necessary. The process begins with the production of plants of these understory species from which seed is harvested. Seeds are cleaned to remove undesirable weed seeds that might have been collected during the harvest. Reducing weeds will make the whole process more efficient by preventing weeds from getting tangled on the combine during harvest and it will make the seed cleaning process easier by removing undesirable plant seeds. Reduced competition of the native plants may also improve seed production. As weeds control in native plants is a new market, few herbicides are available to control weeds while not harming the understory crop species. Understory crop species include warm season grasses (e.g. *Aristida beyrichiana, Schizachyrium scoparium, Sorghastrum nutans*) and legumes (e.g. *Tephrosia virginiana, Desmodium floridanum*). Often, herbicides are labeled to control both the weeds and the desired understory species which poses an obvious problem if nursery managers spray herbicide which can adversely affect the crop.

In order to produce native understory seedlings, there must be a reliable seed production area from which to gather seed. Seed production areas are comprised of established native plants in bed rows which must be properly managed to ensure that an adequate amount of seed is produced. Weed control in these areas can use herbicides either over-the-top, directed, or spot-spray application method where only the weeds are treated. Spot spraying ensures that the established plants are not harmed by the herbicide and can be released from competition from the weeds; however this is a time consuming method and is focused on post-emergence control.

This project will identify herbicide treatments that can be used to control competitive weeds growing amongst the established native plants and make the seed harvest and cleaning process more efficient.

2.3 MATERIALS AND METHODS

A study investigating the herbicides for the control of weeds in native understory plant seed production areas was conducted at the Lolly Creek Farm (Worth County, GA). Six desirable understory plant species were used in this study: 1) wiregrass (*Aristida beyrichiana*), 2) Indian grass (*Sorghastrum nutans*), 3) little bluestem (*Schizachyrium scoparium*), 4) goat's rue (*Tephrosia virginiana*), 5) Florida ticktrefoil (*Desmodium floridanum*), and 6) narrow-leaf sunflower (*Helianthus angustifolius*). The plant species were grouped by plant type and each group received their own set of herbicide treatments. The grasses (wiregrass, Indian grass, and little bluestem) received applications of seven herbicides: 1) atrazine, 2) lactofen, 3) s-metolachlor, 4) oxyfluorfen (Goal 2XL®), 5) oxyfluorfen (GoalTender®), 6) pendimethalin, and 7) dicamba + 2,4-D (Table 2.1). The legumes (goat's rue and Florida ticktrefoil) received six different herbicide applications: 1) imazamox, 2) pendimethalin, 3) imazapic, 4) imazethapyr, 5) sulfentrazone, and 6) butyric acid (Table 2.2). The composite forb (narrow-leaf sunflower) received applications of five herbicides: 1) pendimethalin, 2) s-metolachlor, 3) sulfentrazone, 4) sulfentrazone + s-metolachlor, and 5) sulfentrazone + pendimethalin (Table 2.3). The herbicide rates were chosen based on the herbicide labels.

Prior to herbicide treatments, plots (6 ft wide x 20 ft long) were randomly laid out in the production beds with colored pin flags that corresponded to the various treatments. There was no space between plots, but there was about a foot between production beds. As some weeds require multiple applications of herbicide during the growing season (such as *Ambrosia artemisifolia* in the legume plots), and weed species may change over the season, a comparison of one and two applications of herbicides was made. All plants received an application at time A and half received applications at time A and B. The first application (A) occurred on March 27 and 31, 2014 and the second application (B) occurred on May 5 and 6, 2014. The herbicides were applied using a hand-held spray wand that was CO_2 powered with four nozzles calibrated to spray 187 l/ha at 172 kPa when moving 10 meters per 10 seconds (20 gallons/acre at 25 psi at 30 ft per 10 seconds). The spray wand was held approximately 1 ft above the native plants as the herbicides were applied.

At weeks 2, 4, 6, 8, and 10 post treatment, the native plants were evaluated for injury using a scale from 1-9 (1=no injury and 9=mortality). Plots were also visually evaluated to determine how much of the treated area was occupied by weeds (anything other than the target plant). Ten weeks after the second herbicide application (July 10 and 11) a 1 ft x 5 ft frame was placed in the center of every plot and weeds that fell within the counting frame were collected and returned to Auburn University. The weeds within each plot were then identified and enumerated, dried at 70° C for 48hours and weighed to determine weed biomass (g) by treatment.

The data (injury, weed coverage, and weed biomass) was analyzed using SAS 9.3. A Duncan's and Dunnett's test was used to determine how the treatments compared to each other (Duncan's) as well as how they compared to the control group (Dunnett's).

2.4 RESULTS

The effectiveness of an herbicide to control weeds within the seed production area was dependent on the tolerance of the native plant crop species as well as the susceptibility of weeds within that crop. If an herbicide failed to meet either of those qualifications, then the treatment is not useful. A summary of each native plant treated and the efficacy of each herbicide is discussed below.

2.4.1 *Little Bluestem*

A total of fourteen different herbicide treatments were applied to little bluestem. After ten weeks, four treatments were detrimental to the growth of little bluestem plants. These were the single and sequential applications of oxyfluorfen (Goal 2XL® and GoalTender®). The other herbicide treatments caused only minor damage to the little bluestem with herbicide injury ratings of 2 or less (Table 2.4). Eight herbicide treatments resulted in weed coverage of less than 10%: single and sequential applications of lactofen, dicamba + 2,4-D, atrazine and sequential applications of s-metolachlor, and pendimethalin. At the end of the study period, the dry biomass of weeds was greatest in the single and sequential application of oxyfluorfen (GoalTender®) (> 10.0 grams). Single and sequential applications of atrazine and dicamba + 2,4-D (Table 2.4) offered effective control of weeds with no damage to little bluestem.

2.4.2 *Indian Grass*

Fourteen different herbicide treatments were applied to plots growing Indian grass. Ten weeks following treatment application, the sequential application of oxyfluorfen (GoalTender®) (5.8) was the only treatment detrimental to the health of the target plant (Table 2.5). The remaining herbicide applications had injury ratings of 3.0 or less. Over the course of the study, all of the herbicide treatments resulted in a substantial amount of weeds within the plots when compared to the other native plant species tested in the study. A sequential application of dicamba + 2,4-D resulted in an average of 17.9% weed coverage of the plots. All other treatments had 20% or more average weed coverage. Single and sequential applications of atrazine and dicamba + 2,4-D (Table 2.5) performed better than all other treatments when weeds were collected for dry weight biomass.

2.4.3 *Wiregrass*

Like Indian grass, the sequential applications of oxyfluorfen (Goal 2XL® (4.9) and GoalTender® (3.9)) were detrimental to wiregrass (Table 2.6). The other treatments resulted in injury ratings on wiregrass of 2.0 or less with several herbicide treatments resulting in no injury. The single and sequential applications of dicamba + 2,4-D resulted in the lowest weed coverage in the wiregrass plots over a ten-week period, 6.9 and 9.4% coverage respectively (Table 2.6). As part of management of the production system, the wiregrass plots were burned before the weeds could be collected, and thus, weed biomass in these plots was not collected.

2.4.4 *Florida Ticktrefoil*

None of the twelve different herbicide treatments resulted in significant injury to Florida ticktrefoil. Sequential applications of imazapic and imazethapyr resulted in an injury rating of 2.6 and 2.4, respectively (Table 2.7). In addition to acceptable injury, imazethapyr applied sequentially reduced weed coverage (14.6%) ten weeks after treatment application (Table 2.7). Other herbicide treatments that had low weed biomass were the single (6.94 g) and sequential application (3.26 g) of imazapic (Table 2.7).

2.4.5 *Goat's Rue*

None of the twelve herbicide treatments sprayed over-the-top of goat's rue resulted in serious injury to the plant. The most injury occurred in the plots that received sequential applications of sulfentrazone (2.4) and butyric acid (2.1) (Table 2.8). Weed control with the single and sequential applications of butyric acid resulted in weed coverage of more than 20% (Table 2.8). However, the sequential applications of imazamox, and imazapic provided acceptable control of weeds based on the dry weight biomass (2.0 g and 1.9 g) (Table 2.8).

2.4.6 *Narrow-leaf Sunflower*

Ten different herbicide treatments were applied to narrow-leaf sunflower. Sequential applications of s-metolachlor + sulfentrazone (average injury rating of 6.3) and pendimethalin + sulfentrazone (average injury rating of 4.3) were detrimental to the health of narrowleaf sunflower. Other herbicide treatments tested on narrow-leaf sunflower had an injury rating of 3.0 or less (Table 2.9). Sequential applications of sulfentrazone and pendimethalin resulted in 10% or less average weed coverage (Table 2.9). The sequential application of sulfentrazone had the lowest dry weight biomass (3.74 g) of the plots that received an herbicide treatment (Table 2.9). The remaining treatments had >10 grams of weed biomass at the end of the study. Weed control varied among the treatments and weed coverage and biomass was lowest in the control plots. .

2.5 DISCUSSION

This study was able to identify herbicides that will significantly aid in the production, growth and cultivation of established native plants that are used for seed production. Controlling weedy competition for desirable native plant species grown for seed production will increase seed and seedling production. There was a wide range of both weed control and herbicide tolerance of the native plant species amongst the herbicides tested. The herbicides that were the most successful include dicamba + 2,4-D, atrazine, imazamox, butyric acid, sulfentrazone, imazapic and imazethapyr. Some herbicide treatments were acceptable at the single application but became injurious to native plants when applied twice. In contrast, some sequential applications positively enhanced the performance of the herbicide. This was especially true with sulfentrazone and imazapic when applied to goat's rue, as well as sulfentrazone and pendimethalin when applied to narrow-leaf sunflower (Table 2.9).

Herbicides have been an important cultural practice in forestry for decades. Herbicides can be used for site preparation that aid in the establishment and release of southern pines. Removing less desired and competitive understory species is important to maintain the survival of the desired native understory plant species when restoring longleaf ecosystems. These desirable plant species include those tested including wiregrass, bluestem grasses as well as various composites, forbs and legumes. It should be noted that herbicides are not meant to replace fire within longleaf ecosystems but be used in addition to fire for the removal of competitive non-native species. Multiple herbicides that have been reported in past research to be beneficial for the cultivation of native plants found in longleaf pine ecosystems.

Some of the herbicides and plant species used in this study performed as in previous studies. Kaiser and Kirkman in (2010) evaluated the effects of nine herbicides at two rates each on ten native understory species grown in a greenhouse environment. They found atrazine

(AAtrex®) to be detrimental to grass species, whereas, in this study atrazine did not adversely affect the native grass species and it effectively controlled undesirable species. The difference in results could be attributed to the age and sensitivity of the plants. The young seedlings appear to be more susceptible to herbicide damage than mature established plants. Both studies tested butyric acid and imazapic on native legume species and achieved similar results in respect to injury level.

Another study by Freeman and Jose (2009) evaluated the effects of four herbicide treatments on native grasses and understory species within a study site that was being converted from slash pine to longleaf pine. The four treatments were imazapyr, hexazinone, sulfometuron, and sulfometuron + hexazinone tank mixture. Four years post- application, wiregrass cover had increased in all treatments except for the control group. The treatment that resulted in the largest increase of wiregrass was the sulfometuron + hexazinone tank mixture. Although our study at Lolly Creek did not analyze the size or biomass of the wiregrass, most of the herbicides used were tolerated by the established wiregrass plants (with the exception of oxyfluorfen) and weed control was observed when dicamba + 2,4-D and atrazine were used compared to control plots.

As the native plant market expands to meet the demand for longleaf pine ecosystem restoration and plants become more widely grown throughout the southeast, new obstacles and situations may arise which will require the use of herbicides. These obstacles could include growing other native plant species commercially, weeds becoming tolerant of herbicides, or the removal or addition of usable herbicides. It is important to know which herbicides to use in specific situations so that optimal and efficient land management can take place and aid in the restoration and maintenance of longleaf pine ecosystems.

Dicamba + 2,4-D and atrazine were the best herbicides for weed control in little bluestem and Indian grass. Injury was minimal or nonexistent with both single and sequential applications of the herbicides and provided successful weed control. Since the single application of both atrazine and dicamba + 2,4-D provided similar weed control to the sequential application, the single application of either herbicide would be most efficient in weed control with respect to cost. A few of the more problematic weeds in little bluestem and Indian grass were *Digitaria sanguinalis* (crabgrass) and *Solidago canadensis* (goldenrod), both of which were controlled with these herbicide treatments (Figures 2.1 and 2.2). As part of the management of the production system, the land manager of Lolly Creek uses prescribed winter burns on the little bluestem and Indian grass fields which remove most of the undesirable plant species and the dead plant tissue from the previous growing season. Atrazine and dicamba + 2,4-D treatments also worked well in wiregrass, resulting in low injury and successful weed control (Table 2.6). However, since the wiregrass field was burned before weed biomass could be collected, a complete weed biomass analysis could not be provided. The prescribed burn controlled the undesirable plant species and allowed the wiregrass to grow uninhibited. Additionally, growing season burns have been shown repeatedly to improve wiregrass seed production which is the ultimate goal of the landowner (Mulligan and Kirkman, 2002). Growing season burns may be the best treatment for wiregrass, not only for seed production but also for weed control. However, in some cases herbicides may also be required to control weeds left behind by fire.

Weed control and seed production in the Florida ticktrefoil and goat's rue was successfully obtained with imazapic (Table 2.7). The single and sequential applications of imazapic resulted in comparable weed control and injury ratings when used over Florida ticktrefoil. Thus, a single application would be more economical. In contrast, goat's rue required a sequential application of imazapic to yield successful control of undesirable plant species. Neither the single nor sequential applications of imazapic caused significant damage to either species. Although the sequential applications of imazapic was the best treatment at removing undesirable plant species within goat's rue, sequential applications of imazamox and butyric acid also significantly reduced the amount of weeds .

Of all the herbicide treatments applied to narrow-leaf sunflower, the control group had the least amount of weed biomass. This occurred because the narrow-leaf sunflower was injured by the herbicide applications reducing crop size and coverage, allowing the weeds to grow with less competition. The herbicide treatment that resulted in the least amount of weed biomass was the sequential application of sulfentrazone although it did cause minor damage to the non-target plants (3.1) (Table 2.9). Since a higher level of weed control can be achieved by not applying any herbicides, no treatment is recommended for narrow-leaf sunflower.

At least one beneficial herbicide treatment was found for every species that was included in the study. Even though a successful treatment was discovered to control weeds without causing excessive damage to the target plant, the treatment is not always necessary. Areas should be evaluated carefully and if it is found to have an excessive amount of weeds, then herbicides may be used.

Chapter 3

Herbicide Tolerance of Native Plant Seedlings

3.1 ABSTRACT

America's Longleaf, an organization with an initiative to increase the acreage of longleaf pine ecosystems throughout the southeast, has specified three primary restoration practices: 1) to maintain existing longleaf stands, 2) to improve land that is characterized as having a longleaf cover type but lack understory species, and 3) to convert land that is not currently characterized as a longleaf ecosystem into one. As a result of increased restoration efforts, forest-tree nurseries have begun to produce container-grown native grass seedlings on a commercial scale. This study evaluated the response of container-grown native grass seedlings (wiregrass (*Aristida beyrichiana*), little bluestem (*Schizachyrium scoparium*), Indian grass (*Sorghastrum nutans*) and muhly grass (*Muhlenbergia capillaris*)) to several herbicides, to aid in their commercial production in order for use in longleaf ecosystem restoration projects. Herbicides were applied to the grass seedlings using a CO_2 powered spray wand. Seedlings were evaluated to determine herbicide effects. Lactofen, halosulfuron-methyl, pendimethalin and oxyfluorfen (depending on the rate) were all tolerated by the grasses and could be used in the commercial production of these plants to control weeds.

3.2 INTRODUCTION

Restoration of longleaf pine ecosystems is becoming increasingly popular with non-industrial landowners throughout the southeast. One prominent movement for longleaf ecosystem restoration is being led by America's Longleaf. In 2009, the organization created a 15-year-goal of increasing longleaf pine forest types in the southeast from 3.4 million acres to 8 million acres. Their conservation plan developed three primary objectives: 1) to maintain existing longleaf pine stands, 2) to improve land that is characterized as having a longleaf pine cover type but lacks understory species, and 3) to convert land that is not currently characterized as a longleaf pine ecosystem into one (America's Longleaf, 2009). In addition, the America's Longleaf identified research areas that are needed in order to properly restore longleaf pine. One key research need concerned longleaf pine understory species and community composition, including guidelines and standards for the commercial production of longleaf pine understory plant species, and increased knowledge of the community composition and the species that comprise them (America's Longleaf, 2009). Due to the improvements of forest management, the commercial production of longleaf pine seedlings, financial incentives and increased knowledge of longleaf pine, restoration of this ecosystem has become more successful. Prior to the Conservation Reserve Program's (CRP) additional requirement of reestablishing native ground cover species, understory plants were not regularly grown for commercial purposes in nurseries. Today, since these understory ground covers have become recognized as an integral part of longleaf pine restoration they are being widely produced across the southeast. The increase in understory plant production has made it easier to incorporate native understory species into the ecosystems and increased the probability of successful ecosystem restoration. These native plant species are important because of the value that they bring to the ecosystem: ground cover and food for numerous wildlife species and a fuel source for prescribed fire (Van Lear et al, 2005; Outcalt et al, 1999). While the restoration program has made progress, there are some production issues that have impeded the process. Many forest nurseries have developed production systems to commercially grow native understory species in container systems similar to forest-tree production. However, in the native plant production areas, unwanted weeds can be significant issue in the production of these understory species. One of the larger concerns is that there are no herbicide guidelines to effectively control weeds that do not also adversely affect the understory native plant species. These trials were undertaken to determine the efficacy of currently available broad spectrum herbicides for the control of weed species commonly associated with the native plant production systems and their herbicide tolerance.

3.3 MATERIALS AND METHODS

3.3.1 *Understory Plant Seedling Treatment – Goldsboro, NC*

To evaluate herbicide efficacy and native seedling tolerance, five herbicides labeled for use in forest-tree nurseries were tested on the understory container seedlings. These were: 1) halosulfuron-methyl, 2) lactofen, 3) oxyfluorfen (Goal 2XL®), 4) oxyfluorfen (GoalTender®) and 5) pendimethalin (Table 3.1). The herbicide treatments were applied over-the-top of germinated wiregrass (*Aristida beyrichiana*), muhly grass (*Muhlenbergia capillaris*) and Indian grass (*Sorghastrum nutans*) using a CO_2 powered applicator calibrated to deliver 234 L/ha (25 gallons of water per acre) when moving 10 meters per 10 seconds (30 ft per 10 seconds). The rates used were based on previous native plant trials and application information obtained from each herbicide label (Jackson et al, 2015). The herbicide treatments were applied as a post-emergence to the seedlings and both pre- and post-emergence to some weeds at two rates on July 3, 2013 (Table 3.1). The seedlings were sown eleven weeks prior to the herbicide application. Each treatment was applied to five container trays of each of the native plants, with five non-treated container trays of each plant species used as a control.

At 2, 3, 4, 6 and 8 weeks post treatment, the native plants in each tray were evaluated for herbicide injury using a rating scale of 1-9, where 1=no injury and 9=mortality (Kaiser and Kirkman, 2010). Data was analyzed using the PROC GLM in SAS (SAS version 9.3, SAS Institute Inc.) and treatment means were separated using both the Duncan and Dunnett's test with an alpha < 0.05.

3.3.2 *Understory Plant Seedling Treatment – Moultrie, GA*

This study was a replication of what was conducted in North Carolina the previous summer. To evaluate herbicide efficacy and native plant tolerance, four different herbicides labeled for use in nurseries were tested on the understory container seedlings at a second nursery that included 1) halosulfuron-methyl, 2) lactofen, 3) oxyfluorfen (Goal 2XL®), 4) pendimethalin (Table 3.2). The herbicide treatments were applied over-the-top of the plants using a CO_2 powered applicator calibrated at 234 L/ha (25 gallons per acre) when moving 10 meters per 10 seconds (30 ft per 10 seconds). The herbicides were applied as a post-emergence to the seedlings and both a pre- and post-emergence to some weeds on June 26, 2014 on, Indian grass and little bluestem, and wiregrass was sprayed on August 6, 2014. The Indian grass and little bluestem seedlings were sown six weeks prior to the herbicide application, and the wiregrass seedlings were sown eight weeks prior to the herbicide application. The difference in age at the time of application was due to scheduling and size of the seedlings. The herbicide rates were determined based on results from previous herbicide trials and from the herbicide labels. Each herbicide treatment was applied to five container trays of native understory plant seedlings with a total of 25 container trays of each native understory plant species.

At 1, 2, 3 and 6 weeks post treatment, Indian grass and little bluestem and 2, 4 and 6 weeks post application for wiregrass plants were examined for herbicide injury and mortality using the following scale: 1-9, where 1=no injury and 9=mortality (Kaiser and Kirkman, 2010). The data from Indian grass and little bluestem was sampled at different time than the wiregrass due to application dates and observed herbicide effects. Data was analyzed using the PROC GLM in SAS (SAS version 9.3, SAS Institute Inc.) and the treatment means were separated using both the Duncan and Dunnett's test with an alpha < 0.05.

3.4 RESULTS

3.4.1 *Indian grass, muhly grass and wiregrass – Goldsboro, NC*

Of the five herbicides (Table 3.1) that were used over the top of wiregrass seedlings, only oxyfluorfen (Goal 2XL® and GoalTender®) were detrimental to the understory plant seedlings. The high rates of oxyfluorfen (Goal 2XL® and GoalTender®) resulted in injury ratings of 5.6 and 5.4, respectively (Table 3.2). The other herbicide treatments did little to no damage to the wiregrass seedlings (injury ratings from 1.0 – 1.4) (Table 3.2). None of the herbicides applied to the muhly grass or Indian grass seedlings caused damage (injury ratings of 1.0) (Table 3.3 and 3.4).

3.4.2 *Indian grass, little bluestem, and wiregrass – International Forest Company*

Oxyfluorfen (Goal 2XL®) was the only herbicide that caused injury to the wiregrass seedlings (1.4) (Table 3.8), however, the injury was minimal and did not hinder the growth and viability of the seedlings. The other herbicide treatments were tolerated by the wiregrass seedlings (injury ratings of 1.0) (Table 3.7). All four herbicides tested at IFCO caused minor damage to the Indian grass seedlings (injury ratings of 1.4 – 2.1 respectively) (Table 3.5). Although the treated seedlings were damaged when compared to the non-treated group, the injury was minimal and did not cause any long term problems with the growth and viability of the seedlings later in the growing season. Only pendimethalin caused minor injury to the little bluestem seedlings (1.3) (Table 3.6). The other herbicides were tolerated by little bluestem. None of the herbicides, including pendimethalin, were detrimental or hindered the growth or viability of the native plant seedlings.

3.5 DISCUSSION

These studies identified several herbicides that can be applied over the top of native grass seedlings in order to control weeds. The ability to minimize competition will benefit nurseries by providing another management tool to grow native plant seedlings more efficiently. Furthermore, the production of quality seedlings will benefit longleaf pine ecosystem restoration as outlined in America's Longleaf Restoration Initiative (America's Longleaf, 2009).

The ability of the native grass seedlings to tolerate herbicides is critical in identifying viable and successful herbicide treatments for control of weeds. The intent of using herbicides is to limit competition. However, if the herbicides are more detrimental than beneficial then one problem is being replaced by another. The need to limit competition without negatively affecting the native plant emphasizes the importance of finding the right herbicide(s) and the correct usage rate. Brockway et al (1998) conducted a plant cover, diversity and biomass study in which they reported that using a low rate of hexazinone can control woody species while benefiting the growth of wiregrass, a key longleaf ecosystem species. The site, which had become dominated by turkey oak (*Quercus laevis*), was treated with a low rate of hexazinone which reduced turkey oak in both the overstory and understory. The reduction of turkey oak provided available space and nutrients for wiregrass that was seen to increase for two growing seasons following the hexazinone treatment (Brockway et al, 1998)

Nursery trials conducted in North Carolina on native plants revealed that wiregrass does not tolerate compounds containing oxyflurofen (Goal 2XL® and GoalTender®) (Jackson et al, 2015). Consequently, oxyfluorfen (Goal 2XL®) was examined a second time at IFCO the following year at half of the lowest rate that had been previously used in North Carolina (6 oz/ac). When used at this rate oxyfluorfen did minimal damage (1.4) to the seedlings, allowing its use and providing nursery managers with another herbicide treatment option to control weeds. These trials at North Carolina and IFCO revealed that wiregrass seedlings can tolerate a number of other herbicides which are already used in forest-tree nurseries. This is beneficial because the nurseries will be able to utilize herbicides that they already use on tree seedlings.

Weeds were not an issue in the nursery in 2014 and thus, a weed-control component to the study was not incorporated. Once the herbicides are known to be tolerated by the grass species it is important to know how well they will control weeds. Norcini et al (1997) evaluated the tolerance of wiregrass seedlings to six herbicides as well as the herbicides' ability to control bittercress (*Cardamine hirsuta*) competing with the wiregrass seedlings. They found that imazaquin and imazapic were moderately tolerated by the wiregrass seedlings, while the other four herbicides (isoxaben, oryzalin, oxadiazon, and isoxaben + trifluralin) were too detrimental to the wiregrass to be useful, regardless of their ability to control bittercress.

Due to the limited amount of native plant seedlings available at North Carolina, only two herbicides were evaluated on Indian grass and muhly grass; lactofen and halosulfuron-methyl. Both herbicides, at both rates, were tolerated by the Indian grass and muhly grass seedlings, with injury ratings of 1.0. When the trials were replicated the following year, the rate of halosulfuron-methyl and lactofen were increased, and the high rate of pendimethalin was used again, whereas the rate of oxyfluorfen (Goal 2XL) was decreased. Minor damage was caused by all four herbicide treatments at IFCO which was different from the complete lack of injury that was seen at North Carolina. This difference in results between the two study sites could be attributed to the change in herbicide rates as well as the age of the seedlings when the herbicide was applied. The grass seedlings in North Carolina were sprayed about 11 weeks after the seeds were sown whereas the grass seedlings at IFCO were sprayed at a younger age. The Indian grass and little bluestem grasses were sprayed about 6 weeks after sowing and the wiregrass seedlings were sprayed about 8 weeks after sowing. The wiregrass was sprayed at a later time than the Indian grass and little bluestem at IFCO because the seeds were not sown at the same time. Some of the herbicide rates were also adjusted based on the results seen in North Carolina (Table 3.1 and 3.2). All four herbicide treatments at IFCO were tolerated by the Indian grass and could be viable treatments for weed control. This is important because it provides the forest nursery with additional tools to use in order to limit competition to produce a better seedling, which also benefits the overall restoration effort.

Trials at IFCO indicated that little bluestem was even more tolerant than Indian grass seedlings to the herbicides and rates tested. Pendimethalin did minor injury to the little bluestem seedlings but the other herbicides were well tolerated. The ability of little bluestem seedlings to tolerate these commonly used herbicides suggests that they could be viable treatments in both forest-nursery and seed production areas.

The limited availability of muhly grass seedlings limited evaluation of this particular native plant species, making it difficult to provide recommendations. However, from what was tested, the muhly grass seedlings were tolerant of both rates of lactofen and halosulfuron-methyl. The next step in this herbicide evaluation would be to increase the rates of the herbicides to adequately evaluate the tolerance of muhly grass to these herbicides. Also, the incorporation of more herbicides would be ideal to find more viable treatments to cover a broader spectrum of weeds.

Nurseries which grow these native grass seedlings for use in longleaf pine ecosystem restoration programs now have information and tools to use to produce the native plant material more efficiently. Knowing which herbicides are tolerated by the native grass species is critical, to allow effective control of weeds. All four species proved to be tolerant of the majority of the herbicide treatments that were evaluated. Oxyflurofen (Goal 2XL® or GoalTender®) should be used with caution. It can be tolerated by wiregrass when used at 6 oz ai/ac.

Chapter 4

Imazapyr Bioassay to Aid in the Restoration of Longleaf Pine Seedlings

4.1 ABSTRACT

Chemical site preparation is a common practice prior to planting longleaf pine seedlings. Arsenal® and Chopper® are two herbicides that are commonly used in this process and contain the active ingredient imazapyr. Longleaf pine is particularly sensitive to imazapyr when compared to other southern pine species (e.g. loblolly, slash, and shortleaf pine). If longleaf pine seedlings are planted too soon after the herbicide application, residual amounts of herbicide may still be present and may affect seedling survival. Currently, there is no simple field test that can be used to determine if residual amounts of imazapyr are present in the soil. The purpose of this study was to develop a simple soil bioassay test to determine if the site is safe to plant longleaf pine seedlings. The study took place in a greenhouse at Auburn University. Four rates of imazapyr (Chopper®) were applied to two soil types and six indicator plant species were evaluated for their response to soil containing imazapyr over time. In addition to the six possible indicator species, longleaf pine seed was sown as well. Seeds of all seven species were sown weekly, beginning the day that the herbicide was applied and continuing for 14 weeks. Based on the survival, discoloration and the biomass of the plants, sorghum could be used as an indicator species that, when exposed to imazapyr, will turn purple indicating that imazapyr is still present in the soil. If purple coloration does not appear on the plants, then the site is safe to plant longleaf pine seedlings.

4.2 INTRODUCTION

The longleaf pine ecosystem has been decreasing in acreage over the past several hundred years. A number of factors have contributed to this decline: change in fire regimes, European settlement, slower regeneration rates than other southern pines, and the need for the proper native understory community. Longleaf pine ecosystems are unique and are regarded as the most diverse ecosystems in North America (Brockway et al, 1998). As a result, longleaf pine ecosystem restoration is becoming increasingly popular with non-industrial landowners throughout the southeast. One of the first steps in longleaf ecosystem restoration is site preparation to aid in establishment of longleaf pine seedlings. Site preparation methods can include chemical, mechanical and prescribed fire treatments. A common site preparation chemical used in forestry is imazapyr which is the active ingredient in Chopper® and Arsenal® herbicides. A survey in 2004 revealed that imazapyr is the most commonly used forestry herbicide in the Southeast U.S. (Freeman and Jose, 2009). Imazapyr is a non-selective herbicide that is used to kill competing hardwood vegetation for the release of pine seedlings. Imazapyr can be absorbed by: roots, stem, and foliage and interferes with the production of three amino acids which are critical for the production of proteins within the plant. Imazapyr can move within a plant via the xylem and phloem reaching areas of new plant growth where the herbicide accumulates. The formation and growth of new roots can be severely impacted by imazapyr, eventually resulting in stunted plants. Because imazapyr is soil active, it is recommended that after imazapyr applications, longleaf seedlings not be planted for 60 days. However, longleaf pine seedlings are particularly sensitive to imazapyr, and if planted before imazapyr has dissipated, seedlings can exhibit herbicide injury symptoms including stunted growth or seedling mortality. Since imazapyr is typically applied during site preparation before longleaf pine seedlings have been planted, imazapyr impacts the production of new roots and damages previously existing roots. Without new roots, longleaf pine seedlings are less efficient at nutrient and water up-take. Stunting can be exacerbated in longleaf pine seedlings because they naturally remain in a "grass stage" for 1 to 3 years. The grass stage is a period of growth where the seedlings store up nutrients and put on little vertical growth. Throughout this time the apical meristem is protected from low-intensity fires by the long bushy pine needles surrounding it. The grass stage of longleaf pine is one of the reasons why other southern pine species were favored in reforestation instead of longleaf pine in the past (Johnson, 1999).

For successful re-establishment of longleaf pine in the southeastern United States, steps to avoid stunting or seedling mortality due to residual imazapyr in the soil should be taken. The time period to wait between an application of imazapyr and planting longleaf pine seedlings is dependent upon soil type and environmental factors (Hagar and Nordby, 2007). Tests to determine persistence within the soil are time consuming and expensive. However, a novel inexpensive and quick solution to this problem would be a bioassay test that can be used to determine if it is safe to plant longleaf pine seedlings.

A bioassay uses vegetation to detect if there are significant amounts of herbicide in the soil to impact the plant health (Pfeiffer, 2004). Bioassays are an easy and inexpensive way to test soil for herbicide toxicity (Rashid et al, 2001). "The presence or absence of herbicides is based on whether indicator plants grown in soil show symptoms of herbicide injury. Bioassays are designed to detect bio-available amounts of herbicides in soil; that amount which is readily available for plant uptake" (Pfeiffer, 2004).

Bioassays have been used in numerous applications: to determine contamination in compost (Fauci et al, 2013), to determine endomycorrhizal levels in soil (Moorman and Reeves, 1979), and assessing the contamination in freshwater through plant uptake (Folsom and Price, 1991). A bioassay for imazapyr may be a useful tool in the restoration process of longleaf pine ecosystems, for landowners and managers regarding site preparation and safe planting time for longleaf pine seedlings. Experimental greenhouse trials were conducted to identify a rapid plant bioassay for the detection of residual imazapyr in soils to allow verification that a site sprayed with imazapyr is safe for planting.

4.3 MATERIALS AND METHODS

Six fast-growing plant species were evaluated for use in the bioassay: 1) tomato (*Lycopersicon lycopersicum*), 2) sorghum (*Sorghum bicolor*), 3) cucumber (*Cucumis sativus)*, 4) lettuce (*Lactuca sativa v. buttercrunch)*, 5) radish (*Raphanus sativus*), and 6) cabbage (*Brassica oleracea)*. Longleaf pine seeds were also sown to evaluate how imazapyr affected them over time. Ray-leach SC7 Stubby cells (6.5 cubic inches, 98 cells per tray) were filled with either a coarse soil (sandy clay loam) or fine soil (loamy sand) collected from nursery beds of two forest-tree nurseries (Shellman, GA and Camden, AL) in the southern United States. Soil was analyzed for soil texrture by the ALFA Research and Extension Center at Auburn University. The Ray-leach containers were then sprayed with imazapyr (Chopper®) at three different rates 2.17 L/ha, 3.28 L/ha, and 4.37 L/ha (30, 45 and 60 oz ai/acre) using a hand-held CO_2 powered boom sprayer calibrated to spray 234 l/ha (25 gallons water /acre). After spraying, a subset of cells were sown with the six vegetable plants as well as longleaf seeds, to determine the effects of imazapyr on germination and seedling development.

Beginning on the same day of the herbicide application and every week for 14 weeks, seeds of each vegetable species and longleaf pine was sown into five Ray-leach tubes per herbicide treatment and soil type. Each week's sowing replication was grown for 15 weeks during which time information on plant growth data was recorded, which included survival, chlorosis, and plant injury (e.g. stunting, wilting). An equal amount of water was distributed to all seedlings being grown in coarse textured soil, and an equal amount of water was distributed to all the seedlings being grown in the fine textured soil. Fifteen weeks post sowing, the plants (shoots and roots) were carefully removed from the soil in the tubes. Pictures were then collected to document plant size and root structure by treatment. The plants were then dried in an oven at 70^{o} C for 48 hours to determine the dry weight by soil type and time since herbicide treatment.

Data was analyzed using the PROC GLM in SAS (SAS version 9.3, SAS Institute Inc.) and the treatment means were separated using the Duncan test to determine differences among the variable and the Dunnett's test to compare the injury and biomass to the non-treated controls. The data were separately analyzed by soil type and vegetable species.

4.4 RESULTS

Most of the plant species sown to the treated soil had higher survival rates when grown in the coarser textured sand soils rather than the heavier clay-textured soils. The plants grown in clay exhibited symptoms for a longer period than those grown in sand because the herbicide is able to leach throughout the soil profile of the sand faster than the clay due to the larger particle size (Whiting et al, 2014).

4.4.1 *Tomato*

Tomato plants initially had greater survival at all three herbicide rates in sand (76-95%) compared to seeds sown in clay textured soil (18-62%) (Table 4.1 a). Tomato seed sown in clay textured soil had low survival rates until seeds were sown four weeks after herbicide treatment (38-78%). Overall, tomato plants grown in the clay textured soil experienced more chlorosis and injury than the tomato grown in sand (Figure 4.1); (1-23% chlorosis and 1-33% injury) (Table 4.1 a, b, c). Tomato plants grown in sand, had greater dry weights than those plants sown in the heavier textured soil (Table 4.1 c). Although the above ground growth was not always indicative of the herbicide effects, the below ground growth revealed the negative effects it had on the roots and overall growth of the plants (Figures 4.9 and 4.10).

4.4.2 *Sorghum*

Sorghum sown to all three herbicide rates had lower survival than sorghum sown to the non-treated control containers in either soil (Table 4.2 a, b, c). When comparing survival across the three imazapyr rates, sorghum plants in both fine and coarse soil textures had similar survival rates (Table 4.2 a, b, c). Four weeks after sowing sorghum, plants developed a purple coloration if sown in imazapyr treated soil regardless of the rate (Table 4.2a; Figure 4.2 and 4.3). The purple coloration remained throughout the entire 14 week experiment. Injury to sorghum (wilting, stunting, and necrosis) plants in the imazapyr treated soils was negligible, regardless of rate. At the end of the 14-week experiment, sorghum plants grown in the clay soil had considerably more biomass than those sorghum plants grown in the sand-textured soil (Table 4.2 a, b, c). Although the above ground growth was not always indicative of the herbicide effects, the below ground growth revealed the negative effects it had on the roots and overall growth and biomass of the plants (Figures 4.11 and 4.12).

4.4.3 *Cucumber*

Cucumber plant survival was similar between the two soil types. Cucumber survival was 70% four weeks after the application of herbicide. Cucumber plants became chlorotic three weeks after sowing in the coarse and fine textured imazapyr treated soil (Figure 4.4). Of the two soil types and symptom expression, cucumber plants sown in the sand were more chlorotic throughout the study than the cucumber plants grown in the heavier clay soils (Table 4.3 a, b, c). Injury (wilting, damage, stunting, and necrosis) to cucumber plants were comparable between the two soil types. Cucumber plants in both soil types had similar biomass results through six weeks post-sowing after the initial application. After 6 weeks, cucumber plants growing in the clay-treated soil had more biomass than cucumber plants sown in the sand-treated soil (Table 4.3 b). At the end of the experiment, cucumber plants grown in the non-treated clay soil were larger than the grown in non-treated sand soil (Table 4.3 b, c). Although the above ground growth was not always indicative of the herbicide effects, the below ground growth revealed the negative effects that imazapyr had on the roots and overall growth of the plants (Figures 4.13 and 4.14).

4.4.4 *Lettuce*

The lettuce plants grown in sand had higher survival rates than those plants grown in clay; however, lettuce plants that were sown in clay four weeks after herbicide application had an increase in survival over those sown earlier (Table 4.4 a, b, c). Survival rates of lettuce sown in clay soils never equaled those grown in sand, but they did increase with time after herbicide application. Lettuce grown in both soil types had substantial chlorosis throughout the study period (up to 46%). Although lettuce grown in both soil types had similar chlorosis rankings, the lettuce plants grown in sand tended to exhibit more injury than those grown in clay (up to 29%) (Figure 4.5). Among the different herbicide rates and time since sowing, lettuce plants grown in sand had greater dry weight than those grown in clay (Table 4.4 a, b, c). Although the above ground growth was not always indicative of the herbicide effects, the below ground growth revealed the negative effects imazapyr had on the overall plant growth (Figures 4.15 and 4.16).

4.4.5 *Radish*

The radish plants sown to herbicide-treated soils had equal survival among both soil textures (15-30%). However, the radish grown in the non-treated sand had higher survival rates than those grown in non-treated clay. Plants in the herbicide-treated soils, regardless of soil type, had chlorosis that ranged from 3 to 25% over the experimental period (Table 4.5 a, b, c). For plants grown in the sand-textured soils, chlorosis became more evident on plants that were sown two weeks after herbicide application (Table 4.5 a). Radish plants grown in clay displayed chlorosis three weeks after herbicide application (Table 4.5 a). There was evidence of plant injury to radish throughout all herbicide treatments across the entire 15-week study. Radish plants grown in the sand tended to have more dry weight for the first portion of the study. However, plants sown nine weeks after the herbicide application, biomass was similar between both soil textures (Table 4.5 a, b, c) (Figure 4.6). Although the above ground growth was not always indicative of the herbicide effects, the below ground growth revealed the negative effects imazapyr had on the roots and overall growth of the plants (Figures 4.17 and 4.18).

4.4.6 *Cabbage*

The cabbage plants sown in sand had better overall survival for the first three weeks across all treatments when compared to the cabbage seed sown to the heavier textured clay soil (Table 4.6 a). The survival of cabbage plants when compared between the sand and clay textured soil treatments were similar on plants that were sown four weeks after the herbicide application (Table 4.6 a). Generally, cabbage plants grown in sand were more chlorotic than the plants grown in clay for the first four weeks (3-45%) (Table 4.6 a). The amount of chlorosis was similar among the two soil types and three herbicide treatments at five weeks after the herbicide application. Herbicidal injury was negligible until week ten. Prior to week ten, less than 6.7% of the plants were injured among all treatments. After week ten, the percentage of injured plants increased across all treatments and peaked at 16% (Table 4.6 a, b, c). The plant biomass grown in treated soil tended to be greater in sand than in clay throughout the study 0.0658 g vs. 0.0468 g, respectively (Table 4.6 a, b, c) (Figure 4.7). Although the above ground growth was not always indicative of the herbicide effects, the below ground growth revealed the negative effects imazapyr had on the overall plant growth with significant root reduction (Figures 4.19 and 4.20).

4.4.7 *Longleaf Pine*

Longleaf pine sown in the lighter textured sand had higher survival rates than the longleaf sown to the heavier textured clay soils during first three weeks after the herbicide application (90.6% compared to 79.3%) (Table 4.7 a). Three weeks post-application, seedling survival was similar between soil types. Longleaf pine grown in both soil textures had similar needle chlorosis that could not be correlated to herbicide applications at any time of sowing. With respect to aboveground symptoms, longleaf pine grown in clay exhibited more foliage injury than longleaf seedlings grown in sand. At the end of the 15-week experiment, longleaf pine grown in sand had more biomass than those seedlings grown in the clay-textured soils, 0.2255 g vs. 0.1662 g, respectively (Table 4.7 a, b, c) (Figure 4.8). While the above ground growth and seedling appearance did not always indicate of the herbicide effects, the below ground root growth revealed the negative effects imazapyr had on the roots and overall growth of the longleaf seedling (Figures 4.21 and 4.22).

4.5 DISCUSSION

Identifying an indicator plant that could be used to detect the presence of imazapyr in soils treated for replanting would be a great tool for use as a rapid bioassay tool. These greenhouse trials were one of the first attempts to develop a protocol that would aid in the successful planting of longleaf pine seedlings as part of the numerous re-establishment programs in the southern United States. Of the six species tested, there was a wide range of symptoms and plant growth observed. Tomato and lettuce had a better tolerance to imazapyr, whereas cucumber was susceptible. Those plants that need some tweaking to use as a bioassay include radish and cabbage as these species exhibited symptoms but the cause could not be clearly identified. Of the six plants tested, the one that clearly resulted in an imazapyr soil detection system was sorghum. When exposed to soil containing imazapyr the sorghum will turn purple indicating the presence of the chemical in the soil as soon as three weeks post-sowing.

Based on these trials, the greatest potential for a bioassay for the detection of imazapyr in forest soils would be sorghum. Sorghum was susceptible to both imazapyr-treated soils and exhibited symptoms shortly after sowing, compared to non-treated soils. Sorghum survival in treated soil was significantly less (20%) compared to the control (100%). Sorghum in treated soils gave a strong indication of the presence of imazapyr with plants developing a dark red coloration. In the early weeks of exposure to imazapyr (0-7 weeks) sorghum plants mortality occurred shortly after they began to experience the reddening of the foliage. However, sorghum plants experienced less discoloration and survival and biomass increased with decreasing amounts of dark-red purple coloration with more time after herbicide application (8-14 weeks).

Tomato and cucumber would not work as they did not express strong visual symptoms of imazapyr. Tomato plants did show a difference in growth when compared to the tomato sown to the non-treated soils, but this reduction was not significant. The higher levels of chlorosis and injury to tomato grown in clay soils was most likely a result of the soil characteristics compared to sand-textured soils. Cucumber was not a good option as a bioassay plant either as this plant had poor germination, was sensitive to water stress, and like the tomato, there was not a strong indication that imazapyr was present in the soil. Cucumber has a shallow root system which is why their survival percentage peaked after plants had been sown four weeks after the herbicide application. It took four weeks for the imazapyr to leach through the soil profile and out of contact of the cucumber roots.

Lettuce was clearly impacted by the imazapyr treated soils, but occasionally throughout the study the plant would appear to be dead yet would recover (Figure 4.5). The ephemeral nature of the plant made it difficult to determine how much the imazapyr was impacting the lettuce growth. Lettuce grown in sand resulted in more biomass at the end of the experiment and exhibited more injury during the data collection period. Like cucumber, radish was too sensitive to imazapyr, which may be why the survival rates were similar in both soil types.

Longleaf pine was also sown to imazapyr treated and non-treated soils to compare the above- and below ground growth habits alongside the vegetables. Similar to the vegetables, the above ground symptoms of imazapyr exposure on longleaf pine foliage was not as evident as the below ground effects. The longleaf pine grown in sand had more biomass because of the effective drainage of the herbicide that leached out of the root zone. Although, the above-ground foliage of the longleaf pine grown in the non-treated soil were better than the control, it was difficult to tell how much the longleaf grown in the treated soil were being affected by imazapyr.

There were no above ground symptoms indicating that longleaf seedlings were being affected by the imazapyr; however, when the plants were removed from the soil there was considerably more root growth for plants grown in the non-treated soil than those grown in the treated soil (Table 4.7 a, b, c; Figures 4.21 and 4.22). This supports the fact that imazapyr impacts new root growth and can have devastating effects on longleaf pine seedlings that are planted in soil containing imazapyr (Dickens et al. 2012).

Bioassays can be used to determine tolerance of herbicides, phytotoxity, degradation of the chemical over time, and selectivity among others (Espana et al, 2011). The bioassay performed in this study examined how different plant species are able to tolerate imazapyr and if they display a reaction that can be explicitly identified and attributed to the presence of imazapyr. Another bioassay study that is common is the examination of weed resistance to certain chemicals. This has become especially important as weeds become resistant to chemicals due to repeated use. However, a common theme among all bioassays is the importance of speed. The tests are designed to provide the researcher with fast results which could allow for more tests and adaptations as necessary.

One bioassay study in Washington State was developed to quickly determine if compost contained a herbicide contaminate. A sample of compost was found to contain clopyralid (trade name Stinger®), an active hormone disrupting chemical that is labeled for use on turfgrass which is why it was found in yard debris and compost. The Washington State Department of Agriculture removed the use of clopyralid on lawns and turf with an exception given to golf courses where the use of clopyralid was a necessity for the upkeep and integrity of the course. A method was developed which incorporated the planting of peas into any substance suspected of containing clopyralid and observing the results. A list of symptoms that are known to be caused by exposure to picolinic acids, the herbicide group that contains clopyralid, was provided for homeowners, gardeners, and compost sites. The goal of that study was to provide a method for homeowners and researchers to determine if a given substance contained clopyralid based on how the pea plants responded ("leaf cupping and distortion or curling of stems") (Anonymous, 2002).

The study from Washington State is similar to the bioassay outlined in this paper. A method and indicator plant was identified to determine the presence of a chemical based on the indicator plant's response. The same results are being sought within this study to provide landowners and land managers with the means to quickly and effectively assess the chemical contamination of soils. Overall, tomato was not a viable indicator species because it did not provide a definitive sign that could be used to determine if imazapyr was still in the soil. It was stunted and impacted by imazapyr, but that alone is not sufficient. The cucumber plants did not have adequate survival rate during the experimental time frame with non-treated soils similar to the herbicide treated soils. In contrast, lettuce was too hardy to be a viable indicator. It would be stunted and would appear to be dead but would recover and begin growth again. Radish and cabbage were both too sensitive. They were chlorotic in both treated and non-treated soil which makes it difficult to determine if the chlorosis was due to the herbicide or some other reason.

The sorghum was the best indicator species. The difference between the sorghum grown in the non-treated groups versus the treated soil was extreme. When the sorghum grew in the treated soil it would turn a burgundy/red which indicated that there was imazapyr in the soil. The sorghum grown in the non-treated soil would sometimes have slight reddening of the foliage but it was not similar to results seen in the treated soil. The sorghum also responded differently with time exposed to imazapyr. As sorghum was exposed earlier to the herbicide, it became smaller and more discolored. As time went on, the sorghum increased in size and took longer for discoloration to occur because it took time for the roots to grow and extend into the soil zone that still had imazapyr. Of the six possible indicator species tested, sorghum has the best chance of informing land managers and foresters if soil has residual imazapyr from site preparation practices.

Chapter 5

Summary and Conclusions

5.1 HERBICIDE WEED CONTROL AND TOLERANCE EVALUATION OF ESTABLISHED NATIVE PLANT SPECIES

This study was able to identify herbicides that will significantly aid in the production, growth and cultivation of established native plants that are used for seed production. By controlling weedy competition with for a number of highly desired native plant species, their production will increase. Of the herbicide treatments that were evaluated there was a wide range of both weed control and tolerance of the herbicide on the native plant species. The herbicides that were the most successful include dicamba + 2,4-D, atrazine, imazamox, butyric acid, sulfentrazone, imazapic and imazethapyr. Some herbicide treatments were acceptable at the single application but became injurious to native plants when applied twice these include both tank mixes applied to narrow-leaf sunflower. In contrast, some sequential applications positively enhanced the performance of the herbicide this was especially true with sulfentrazone and imazapic when applied to goat's rue as well as sulfentrazone and pendimethalin when applied to narrow-leaf sunflower (Table 2.9).

5.2 HERBICIDE TOLERANCE OF NATIVE PLANT SEEDLINGS IN NURSERIES

These studies identified several herbicides that can be applied over the top of native grass seedlings in order to control unwanted weeds. All of the treatments, with the exception of the oxyfluorfen (Goal 2XL® and GoalTender®) applications in North Carolina, are tolerated by the native grass seedlings and are acceptable applications for use in forest-tree nurseries. The ability to minimize competition will benefit nurseries by providing them with more management tools to grow native plant seedlings more efficiently. Furthermore, the production of quality seedlings will benefit longleaf ecosystem restoration such as what was outlined in America's Longleaf Restoration Initiative (America's Longleaf, 2009).

5.3 IMAZAPYR BIOASSAY

These greenhouse trials were one of the first attempts to develop a protocol that would aid in the planting of longleaf pine seedlings as part of the numerous re-establishment programs in the southern United States. The identification of an indicator plant that could be used to detect the presence of imazapyr in soils treated for replanting would be a great tool for use as a rapid bioassay tool. Of the six species tested, there was a wide range of symptomology and plant growth. Those that were too tolerant of the herbicide or too susceptible to herbicide levels in the soil included tomato, cucumber and lettuce. Those plants that need some tweaking to use as a bioassay include radish and cabbage as these species exhibited symptoms but the cause could not be clearly identified. Of the six plants tested, the one that clearly resulted in an imazapyr soil detection system was sorghum. When exposed to soil containing imazapyr the sorghum will turn purple indicating the presence of the chemical in the soil.

This bioassay test can be used to determine when it is safe to plant longleaf pine seedlings as well as to determine why planted seedlings are being stunted or dying. When using the test to determine if soil is safe for longleaf pine, it is recommended to begin using sorghum between 50-60 days after the application and to continue using sorghum every other week until the soil is deemed safe based on the sorghum. This may delay planting dates and these steps should be factored into the process.

5.4 FINAL RESEARCH SUMMARY AND POTENTIAL RESEARCH

The studies outlined above identified herbicide treatments and indicator species that are both successful and unsuccessful. These treatments can be used to grow and cultivate native plants more efficiently which will benefit longleaf pine restoration. However, although the research accomplished in these studies is beneficial, more research needs to be done to improve upon this. To better understand how the herbicides affect the established native plants, the seed production of the plants should be analyzed to determine if the herbicides are limiting the amount of seeds that the plants are able to produce. Furthermore, the native plant seedlings could be out-planted to determine if there is an herbicide effect once the plants leave the nursery. Lastly, the use of sorghum as an imazapyr bioassay indicator species needs to be taken into the field and tested. Perhaps the use of a sorghum seedling may be better than sowing sorghum seed. All of that and more can be beneficial to the research that has already been done and can benefit forestry and restoration efforts throughout the southeastern U.S.

Tables

Table 2.1. 2014 Lolly Creek herbicide trial applied to little bluestem (*Schizachyrium scoparium*), Indian grass (*Sorghastrum nutans*), and wiregrass (*Aristida beyrichiana*).

Herbicides	Active Ingredient	Rates (L/ha)
AAtrex®	Atrazine	2.34
Cobra®	Lactofen	1.17
Dual Magnum®	S-metolachlor	1.53
Goal 2XL®	Oxyfluorfen	1.75
GoalTender®	Oxyfluorfen	2.63
Prowl H_2O®	Pendimethalin	1.75
Weedmaster®	Dicamba + 2,4-D	2.34
Control	N/A	N/A

Table 2.2. 2014 Lolly Creek herbicide trial applied to Florida ticktrefoil (*Desmodium floridanum*) and goat's rue (*Tephrosia virginiana*).

Herbicides	Active Ingredient	Rates (L/ha)
Clearcast®	Imazamox	0.44
Prowl H_2O®	Pendimethalin	1.75
Plateau®	Imazapic	0.58
Pursuit®	Imazethapyr	0.44
Spartan Charge®	Sulfentrazone	0.27
2,4 DB®	Butyric Acid	2.34
Control	N/A	N/A

Table 2.3. 2014 Lolly Creek herbicide trial applied to narrow-leaf sunflower (*Helianthus angustifolius*).

Herbicides	Active Ingredient	Rates (L/ha)
Prowl H_2O®	Pendimethalin	1.75
Dual Magnum®	S-metolachlor	1.53
Spartan Charge®	Sulfentrazone	0.27
Dual Magnum®+ Spartan®	S-metolachlor + Sulfentrazone	1.53 + 0.27
Prowl H_2O® + Spartan®	Pendimethalin + Sulfentrazone	1.75 + 0.27
Control	N/A	N/A

Table 2.4. 2014 Lolly Creek herbicide trial results of little bluestem (*Schizachyrium scoparium*).

Herbicide	Application	Average Injury[a]	Weed Coverage[b]	Weed Biomass[c]
GoalTender®	Single	5.0 b**	12.1 bc	19.939 a
GoalTender®	Sequential	7.9 a	20.4 a	11.055 ab
Goal 2XL®	Single	3.1 c	11.4 bcd	7.709 bc
Goal 2XL®	Sequential	5.1 b	11.7 bc	7.745 bc
Dual Magnum®	Single	1.1 e	18.9 a	4.498 bc
Dual Magnum®	Sequential	1.3 de	9.2 cd	1.638 bc
Prowl H_2O®	Single	1.1 e	16.8 ab	2.920 bc
Prowl H_2O®	Sequential	2.0 d	7.5 cd	3.245 bc
Cobra®	Single	1.2 de	9.5 cd	1.398 bc
Cobra®	Sequential	1.7 de	7.5 cd	2.265 bc
Weedmaster®	Single	1.0 e	8.2 cd	0.170 c
Weedmaster®	Sequential	1.5 de	5.0 d	0.255 c
AAtrex®	Single	1.0 e	7.7 cd	0.218 c
AAtrex®	Sequential	1.2 e	6.3 cd	0.000 c
Control	N/A	1.0 e	20.0 a	5.035 bc

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).
[b] = Weed coverage is a percentage representing how much of the research plot was comprised of weeds.
[c] = Weed biomass is the dry weight of the weed sample in grams.
**Means with different letters are significantly different ($p < 0.05$).

Table 2.5. 2014 Lolly Creek herbicide trial results of Indian Grass (*Sorghastrum nutans*).

Herbicide	Application	Average Injury[a]	Weed Coverage[b]	Weed Biomass[c]
GoalTender®	Single	2.9 b**	35.7 ab	21.01 abc
GoalTender®	Sequential	5.8 a	41.3 a	21.15 abc
Goal 2XL®	Single	1.6 de	32.9 abc	16.58 abc
Goal 2XL®	Sequential	2.9 b	29.6 abc	28.06 ab
Dual Magnum®	Single	1.1 de	36.3 ab	17.22 abc
Dual Magnum®	Sequential	2.0 cd	21.3 bc	25.50 abc
Prowl H_2O®	Single	1.0 e	27.7 abc	6.31 bc
Prowl H_2O®	Sequential	1.5 de	27.9 abc	8.57 bc
Cobra®	Single	1.3 de	30.9 abc	35.75 a
Cobra®	Sequential	1.4 de	27.5 abc	23.93 abc
Weedmaster®	Single	1.1 de	22.7 bc	0.13 c
Weedmaster®	Sequential	2.7 bc	17.9 c	0.72 c
AAtrex®	Single	1.0 e	23.6 bc	0.00 c
AAtrex®	Sequential	1.5 de	25.0 bc	0.42 c
Control	N/A	1.0 e	26.3 abc	18.96 abc

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

[b] = Weed coverage is a percentage representing how much of the research plot was comprised of weeds.

[c] = Weed biomass is the dry weight of the weed sample in grams.

**Means with different letters are significantly different ($p < 0.05$).

Table 2.6. 2014 Lolly Creek herbicide trial results of wiregrass (*Aristida beyrichiana*).

Herbicide	Application	Average Injury[a]	Weed Coverage[b]	Weed Biomass[c]
GoalTender®	Single	2.1 c**	20.8 a	N/A
GoalTender®	Sequential	4.9 a	13.8 abc	N/A
Goal 2XL®	Single	1.4 d	14.4 abc	N/A
Goal2XL®	Sequential	3.9 b	16.3 abc	N/A
Dual Magnum®	Single	1.0 d	16.0 abc	N/A
Dual Magnum®	Sequential	1.0 d	13.8 abc	N/A
Prowl H_2O®	Single	1.0 d	17.7 ab	N/A
Prowl H_2O®	Sequential	1.0 d	16.9 abc	N/A
Cobra®	Single	1.0 d	17.1 abc	N/A
Cobra®	Sequential	1.3 d	13.8 abc	N/A
Weedmaster®	Single	1.0 d	6.9 c	N/A
Weedmaster®	Sequential	1.0 d	9.4 bc	N/A
AAtrex®	Single	1.0 d	11.3 abc	N/A
AAtrex®	Sequential	1.0 d	13.1 abc	N/A
Control	N/A	1.0 d	15.3 abc	N/A

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).
[b] = Weed coverage is a percentage representing how much of the research plot was comprised of weeds.
[c] = Weed biomass is the dry weight of the weed sample in grams.
**Means with different letters are significantly different ($p < 0.05$).

Table 2.7. 2014 Lolly Creek herbicide trial results of Florida Ticktrefoil (*Desmodium floridanum*).

Herbicide	Application	Average Injury[a]	Weed Coverage[b]	Weed Biomass[c]
Prowl H_2O®	Single	1.0 b**	34.5 bc	15.28 b
Prowl H_2O®	Sequential	1.1 b	47.5 a	129.53 a
Spartan Charge®	Single	1.0 b	28.6 bcde	45.08 b
Spartan Charge®	Sequential	1.3 b	56.1 a	61.85 b
Plateau®	Single	1.0 b	17.0 fg	6.94 b
Plateau®	Sequential	2.6 a	25.4 cdefg	3.26 b
2,4 DB®	Single	1.0 b	32.1 bcd	35.83 b
2,4 DB®	Sequential	1.0 b	36.7 b	32.51 b
Pursuit®	Single	1.0 b	20.2 efg	23.04 b
Pursuit®	Sequential	2.4 a	14.6 g	13.69 b
Clearcast®	Single	1.0 b	22.9 defg	31.08 b
Clearcast®	Sequential	1.0 b	26.7 bcdef	28.18 b
Control	N/A	1.0 b	35.5 bc	52.58 b

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).
[b] = Weed coverage is a percentage representing how much of the research plot was comprised of weeds.
[c] = Weed biomass is the dry weight of the weed sample in grams.
**Means with different letters are significantly different ($p < 0.05$).

Table 2.8. 2014 Lolly Creek herbicide trial results of goat's Rue (*Tephrosia virginiana*).

Herbicide	Application	Average Injury[a]	Weed Coverage[b]	Weed Biomass[c]
Prowl H_2O®	Single	1.0 b**	17.7 bcd	15.126 ab
Prowl H_2O®	Sequential	1.1 b	17.1 bcd	13.617 ab
Spartan Charge®	Single	1.4 b	15.4 bcd	13.190 ab
Spartan Charge®	Sequential	2.4 a	13.8 cd	7.174 ab
Plateau®	Single	1.2 b	14.4 bcd	12.189 ab
Plateau®	Sequential	1.3 b	15.0 bcd	1.898 b
2,4 DB®	Single	1.0 b	21.0 abc	11.575 ab
2,4 DB®	Sequential	2.1 a	25.6 a	2.555 b
Pursuit®	Single	1.1 b	15.7 bcd	8.576 ab
Pursuit®	Sequential	1.1 b	12.9 d	8.363 ab
Clearcast®	Single	1.1 b	12.1 d	6.800 ab
Clearcast®	Sequential	1.0 b	11.7 d	2.055 b
Control	N/A	1.0 b	21.5 ab	23.060 a

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).
[b] = Weed coverage is a percentage representing how much of the research plot was comprised of weeds.
[c] = Weed biomass is the dry weight of the weed sample in grams.
**Means with different letters are significantly different ($p < 0.05$).

Table 2.9. 2014 Lolly Creek herbicide trial results of narrow-leaf sunflower (*Helianthus angustifolius*).

Herbicide	Application	Average Injury[a]	Weed Coverage[b]	Weed Biomass[c]
Prowl H_2O®	Single	1.3 fg**	20.5 bc	25.85 ab
Prowl H_2O®	Sequential	1.3 fg	9.2 d	10.32 b
Dual Magnum®	Single	1.4 fg	15.2 cd	38.32 ab
Dual Magnum®	Sequential	2.8 cd	25.8 ab	20.75 ab
Spartan Charge®	Single	1.9 ef	22.5 abc	29.95 ab
Spartan Charge®	Sequential	3.1 c	10.0 d	3.74 b
Dual Magnum®+ Spartan®	Single	3.1 c	13.8 cd	18.65 ab
Dual Magnum®+ Spartan®	Sequential	6.3 a	14.6 cd	38.32 ab
Prowl H_2O® + Spartan	Single	2.1 de	30.7 a	29.87 ab
Prowl H_2O® + Spartan	Sequential	4.3 b	13.3 cd	26.58 ab
Control	N/A	1.0 g	13.8 cd	2.38 b

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).
[b] = Weed coverage is a percentage representing how much of the research plot was comprised of weeds.
[c] = Weed biomass is the dry weight of the weed sample in grams.
**Means with different letters are significantly different ($p < 0.05$).

Table 3.1. 2013 North Carolina herbicide treatments applied to wiregrass (*Aristida beyrichiana*), Indian grass (*Sorghastrum nutans*) and muhly grass (*Muhlenbergia capillaris*).

Herbicide	Active Ingredient	Low Rate (L/ha)	High Rate (L/ha)
Cobra®	Lactofen	0.29	0.58
Goal 2XL®	Oxyflurofen	0.58	1.16
GoalTender®	Oxyflurofen	0.88	1.75
Pendulum Aquacap®	Pendimethalin	1.24	2.48
Sedgehammer®	Halosulfuron-methyl	35.01 g/ha	70.03 g/ha
Control		N/A	N/A

Table 3.2. 2014 IFCO herbicide treatments applied to wiregrass (*Aristida beyrichiana*), Indian grass (*Sorghastrum nutans*) and little bluestem (*Schizachyrium scoparium*).

Herbicide	Active Ingredient	Rate (L/ha)
Cobra®	Lactofen	0.58
Goal®	Oxyflurofen	0.44
Pendulum Aquacap®	Pendimethalin	2.48
Sedgehammer®	Halosulfuron-methyl	105.08 g/ha
Control	N/A	N/A

Table 3.3. 2013 North Carolina herbicide trial results of wiregrass (*Aristida beyrichiana*).

Herbicide	Application Rate (L/ha)	Average Injury[a]
Goal 2XL®	1.16	5.6 a**
GoalTender®	1.75	5.4 a
GoalTender®	0.88	3.9 b
Goal 2XL®	0.58	3.8 b
Cobra®	0.58	1.4 c
Cobra®	0.29	1.2 c
Sedgehammer®	2.47 g/ha	1.1 c
Pendulum Aquacap®	2.48	1.0 c
Pendulum Aquacap®	1.24	1.0 c
Sedgehammer®	1.27 g/ha	1.0 c
Control	N/A	1.0 c

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

** Means with different letters are significantly different ($p < 0.05$).

Table 3.4. 2013 North Carolina herbicide trial results of muhly grass (*Muhlenbergia capillaris*).

Herbicide	Application Rate (L/ha)	Average Injury[a]
Cobra®	0.58	1.0**
Cobra®	0.29	1.0
Sedgehammer®	70.03g/ha	1.0
Sedgehammer®	35.01g/ha	1.0
Control	N/A	1.0

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

** Means with different letters are significantly different ($p < 0.05$).

Table 3.5. 2013 North Carolina herbicide trial results of Indian grass (*Sorghastrum nutans*).

Herbicide	Application Rate (L/ha)	Average Injury[a]
Cobra®	0.58	1.0**
Cobra®	0.29	1.0
Sedgehammer®	70.03 g/ha	1.0
Sedgehammer®	35.01 g/ha	1.0
Control	N/A	1.0

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

** Means with different letters are significantly different ($p < 0.05$).

Table 3.6. 2014 IFCO herbicide trial results on Indian grass (*Sorghastrum nutans*).

Herbicide	Rate (L/ha)	Injury Rating[a]
Sedgehammer®	105.08 g/ha	2.1 a**
Goal 2XL®	0.44	2.0 a
Cobra®	0.58	1.5 b
Pendulum Aquacap®	2.48	1.4 b
Control	N/A	1.0 c

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

** Means with different letters are significantly different ($p < 0.05$).

Table 3.7. 2014 IFCO herbicide trial results on little bluestem (*Schizachyrium scoparium*).

Herbicide	Rate (L/ha)	Injury Rating[a]
Pendulum Aquacap®	2.48	1.3 a**
Goal 2XL®	0.44	1.0 b
Cobra®	0.58	1.0 b
Sedgehammer®	105.08 g/ha	1.0 b
Control	N/A	1.0 b

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

** Means with different letters are significantly different ($p < 0.05$).

Table 3.8. 2014 IFCO herbicide trial results on wiregrass (*Aristida beyrichiana*).

Herbicide	Rate (L/ha)	Injury Rating[a]
Goal 2XL®	0.44	1.4 a**
Cobra®	0.58	1.0 b
Pendulum Aquacap®	2.48	1.0 b
Sedgehammer®	105.08 g/ha	1.0 b
Control	N/A	1.0 b

[a] = Injury rating scale of 1-9 (1=no injury and 9=mortality) which was a measure of phytotoxicity due to herbicide exposure (Kaiser and Kirkman 2010).

** Means with different letters are significantly different ($p < 0.05$).

Table 4.1a. Survival, chlorosis, injury percentages and average biomass per plant of tomato (*Lycopersicon spp*) sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Tomato									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	76.3 b**	18.8 c	3.8 b	0.0 b	2.5	0.0	0.0169	0.0023
	45	95.0 a	62.5 b	12.5 a	21.3 a	0.0	0.0	0.0394	0.0029
	60	77.5 b	21.3 c	1.3 b	3.8 b	0.0	0.0	0.0440	0.0032
	C	70.0 b	87.5 a	0.0 b	0.0 b	0.0	0.0	0.1000	0.3422
p-value		<.0001	<.0001	.0569	.0103	.4774			
1	30	61.3 b	20.0 b	0.0	0.0 b	1.3	0.0	0.0222	0.0059
	45	84.0 a	73.3 a	0.0	17.3 a	0.0	2.7	0.0724	0.0024
	60	18.7 c	4.0 b	0.0	0.0 b	1.3	0.0	0.0376	0.0000
	C	93.3 a	74.7 a	0.0	0.0 b	0.0	0.0	0.3169	0.2628
p-value		<.0001	<.0001		.0690	.6182	.3032		
2	30	81.3 b	16.0 b	6.7	0.0 b	2.7	1.3	0.0443	0.0024
	45	76.0 b	85.3 a	0.0	9.3 a	0.0	2.7	0.0531	0.0095
	60	58.7 c	16.0 b	4.0	4.0 ab	1.3	0.0	0.0465	0.0018
	C	100.0 a	96.0 a	0.0	0.0 b	0.0	0.0	0.1959	0.1568
p-value		<.0001	<.0001	.1955	.2179	.5405	.5897		
3	30	86.7 b	49.3 c	4.0 ab	4.0 ab	0.0	0.0	0.0542	0.0027
	45	96.0 a	78.7 b	0.0 b	14.7 a	0.0	4.0	0.0133	0.0028
	60	72.0 c	38.7 c	9.3 a	8.0 ab	0.0	1.3	0.0924	0.0054
	C	97.3 a	100.0 a	0.0 b	0.0 b	0.0	0.0	0.2434	0.1047
p-value		<.0001	<.0001	.1635	.1758		.4924		
4	30	97.3 a	45.3 c	0.0 b	2.7	0.0	0.0	0.0879	0.0041
	45	89.3 b	74.7 b	0.0 b	4.0	2.7	1.3	0.0719	0.0035
	60	98.7 a	18.7 d	4.0 a	1.3	8.0	1.3	0.0690	0.0000
	C	100.0 a	100.0 a	0.0 b	0.0	0.0	0.0	0.1495	0.0714
p-value		.0023	<.0001	.1661	.0079	.4344	.6182		

Table 4.1b. Survival, chlorosis, injury percentages and average biomass per plant of tomato (*Lycopersicon spp*) sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Tomato									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	96.0 a**	66.7 b	0.0	2.7 ab	0.0 b	0.0	0.0495	0.0064
	45	80.0 b	77.3 b	0.0	5.3 a	0.0 b	2.7	0.0426	0.0273
	60	72.0 c	46.7 c	0.0	0.0 b	6.7 a	0.0	0.0497	0.0490
	C	94.7 a	94.7 a	0.0	0.0 b	0.0 b	0.0	0.0573	0.0475
p-value		<.0001	<.0001		.0590	.1925	.3032		
6	30	100.0 a	72.0 b	1.3	6.7	5.3 a	0.0	0.0453	0.0030
	45	88.0 b	60.0 b	1.3	1.3	0.0 b	0.0	0.0116	0.0028
	60	97.3 a	76.0 b	0.0	0.0	6.7 a	2.7	0.0715	0.0268
	C	98.7 a	100.0 a	0.0	0.0	0.0 b	0.0	0.0659	0.1303
p-value		.0244	<.0001	.6182	.1480	.0013	.3032		
7	30	68.0 c	64.0 b	1.3	2.7	0.0 b	1.3 ab	0.0661	0.0029
	45	76.0 bc	72.0 b	2.7	2.7	1.3 b	1.3 ab	0.0837	0.0095
	60	92.0 a	44.0 c	0.0	0.0	14.7 a	5.3 a	0.0283	0.0020
	C	82.7 ab	97.3 a	0.0	0.0	0.0 b	0.0 b	0.0789	0.0973
p-value		<.0001	<.0001	.5897	.6182	.0430	.5264		
8	30	88.0 b	65.3 a	0.0	1.3	2.7	6.7	0.0961	0.0327
	45	97.3 a	45.3 b	0.0	0.0	2.7	4.0	0.1019	0.0031
	60	98.7 a	69.3 a	0.0	0.0	1.3	9.3	0.0269	0.0311
	C	86.7 b	76.0 a	0.0	0.0	1.3	2.7	0.0480	0.1361
p-value		.0021	<.0001		.4770	.5561	.1379		
9	30	69.3 b	53.3	6.7	0.0	8.0	2.7 ab	0.0683	0.0032
	45	74.7 b	61.3	8.0	0.0	12.0	8.0 a	0.0127	0.0110
	60	100.0 a	56.0	1.3	1.3	9.3	4.0 ab	0.0647	0.0404
	C	70.7 b	53.3	1.3	0.0	2.7	0.0 b	0.0499	0.0461
p-value		<.0001	<.0001	.0004	.4770	.2315	.0379		

Table 4.1c. Survival, chlorosis, injury percentages and average biomass per plant of tomato (*Lycopersicon spp*) sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Tomato									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	69.3 c**	61.3 ab	0.0	4.0 a	12.0 ab	12.0 a	0.1347	0.0136
	45	89.3 b	65.3 a	0.0	0.0 b	9.3 ab	13.3 a	0.0307	0.0129
	60	100.0 a	34.7 c	0.0	0.0 b	24.0 a	0.0 b	0.0323	0.0022
	C	56.0 d	50.7 b	0.0	0.0 b	2.7 b	2.7 b	0.0571	0.0411
p-value		<.0001	<.0001		.1661	.0913	.0001		
11	30	84.0 c	50.7 c	0.0 b	0.0	10.7 ab	10.7 b	0.0913	0.0027
	45	89.3 bc	78.7 a	0.0 b	0.0	13.3 a	21.3	0.0576	0.0206
	60	100.0 a	66.7 ab	5.3 a	1.3	17.3 a	24.0 a	0.0866	0.0090
	C	93.3 ab	58.7 bc	1.3 b	1.3	4.0 b	1.3 b	0.0544	0.1383
p-value		.0003	<.0001	.0347	.6182	.0001	.0003		
12	30	41.3 b	58.7 a	0.0	5.3 a	20.0 a	17.3 a	0.0142	0.0156
	45	96.0 a	33.3 c	0.0	0.0 b	18.7 a	6.7 bc	0.1483	0.0022
	60	90.7 a	46.7 b	0.0	0.0 b	10.7 ab	12.0 ab	0.0931	0.0267
	C	93.3 a	32.0 c	0.0	0.0 b	2.7 b	0.0 c	0.0625	0.0135
p-value		<.0001	<.0001		.2066	.0167	.0064		
13	30	86.7 a	77.3 a	0.0	1.3	8.0 ab	33.3 a	0.1001	0.0073
	45	100.0 a	34.7 b	0.0	0.0	20.0 a	4.0 b	0.0887	0.0034
	60	66.7 c	26.7 b	0.0	0.0	18.7 a	0.0 b	0.0531	0.0020
	C	100.0 a	26.7 b	0.0	0.0	0.0 b	0.0 b	0.0763	0.0150
p-value		<.0001	<.0001		.4770	.0261	.0028		

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2]Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3]Average biomass per plant was determined from data collected 15 weeks after sowing.

Table 4.2a. Survival, chlorosis, injury percentages and average biomass per plant of sorghum *(Sorghum bicolor)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Sorghum									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	22.5 b**	22.5 b	0.0 b	0.0 b	0.0	0.0	0.0152	0.0173
	45	25.0 b	16.3 b	0.0 b	0.0 b	0.0	0.0	0.0251	0.0056
	60	22.5 b	18.8 b	0.0 b	0.0 b	0.0	0.0	0.0197	0.0225
	C	48.8 a	87.5 a	36.3 a	47.5 a	0.0	0.0	0.3470	0.6736
p-value		.0001	<.0001	<.0001	.0003				
1	30	20.0 b	20.0 b	0.0 b	0.0 b	0.0	0.0	0.0196	0.0113
	45	21.3 b	21.3 b	0.0 b	0.0 b	0.0	0.0	0.0287	0.0177
	60	22.7 b	12.0 b	0.0 b	0.0 b	1.3	1.3	0.0264	0.0210
	C	93.3 a	93.3 a	66.7 a	40.0 a	0.0	0.0	0.4788	1.1286
p-value		<.0001	<.0001	<.0001	.0088	.4770	.4770		
2	30	21.3 b	20.0 b	0.0 b	0.0 b	0.0	0.0	0.0220	0.0361
	45	20.0 b	24.0 b	0.0 b	1.3 b	0.0	0.0	0.0255	0.0156
	60	14.7 b	10.7 b	0.0 b	0.0 b	0.0	0.0	0.0177	0.0176
	C	100.0 a	97.3 a	57.3 a	50.7 a	0.0	0.0	0.2720	0.2399
p-value		<.0001	<.0001	<.0001	.0005				
3	30	16.0 b	26.7 b	5.3 b	6.7 b	0.0	0.0	0.0338	0.0189
	45	20.0 b	14.7	6.7 b	1.3 b	0.0	0.0	0.0267	0.0181
	60	20.0 b	16.0 b	6.7 b	5.3 b	0.0	0.0	0.0292	0.0170
	C	80.0 a	100.0 a	48.0 a	41.3 a	0.0	0.0	0.2892	0.3372
p-value		<.0001	<.0001	.0011	.0242				
4	30	13.3 b	13.3 b	5.3 b	2.7 b	0.0	0.0	0.0339	0.0257
	45	13.3 b	13.3 b	5.3 b	4.0 b	0.0	0.0	0.0221	0.0145
	60	20.0 b	16.0 b	6.7 b	5.3 b	0.0	0.0	0.0255	0.0226
	C	100.0 a	100.0 a	58.7 a	34.7 a	0.0	0.0	0.3155	0.2844
p-value		<.0001	<.0001	.0005	.0737				

Table 4.2b. Survival, chlorosis, injury percentages and average biomass per plant of sorghum *(Sorghum bicolor)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Sorghum									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	14.7 b**	17.3 b	6.7 b	9.3 b	0.0	0.0	0.0470	0.0178
	45	20.0 b	13.3 b	12.0 b	5.3 b	0.0	0.0	0.0636	0.0246
	60	12.0 b	21.3 b	5.3 b	4.0 b	0.0	0.0	0.0221	0.0200
	C	100.0 a	97.3 a	61.3 a	40.0 a	0.0	0.0	0.2126	0.3329
p-value		<.0001	<.0001	<.0001	.1074				
6	30	16.0 b	13.3 b	9.3 b	6.7 b	0.0	0.0 b	0.0200	0.0231
	45	14.7 b	13.3 b	8.0 b	6.7 b	0.0	0.0 b	0.0269	0.0258
	60	14.7 b	17.3 b	8.0 b	4.0 b	0.0	0.0 b	0.0287	0.0250
	C	100.0 a	100.0 a	41.3 a	44.0 a	0.0	5.3 a	0.2821	0.4587
p-value		<.0001	<.0001	.0093	.0022		.0768		
7	30	9.3 b	17.3 b	4.0 b	9.3	0.0	0.0	0.0273	0.0231
	45	13.3 b	17.3 b	6.7 b	9.3	0.0	0.0	0.0221	0.0269
	60	13.3 b	13.3 b	5.3 b	6.7	0.0	0.0	0.0214	0.0210
	C	100.0 a	100.0 a	64.0 a	26.7	5.3	0.0	0.1977	0.4400
p-value		<.0001	<.0001	.0003	.0645	.3696			
8	30	26.7 c	16.0 b	20.0 bc	8.0	0.0 b	0.0	0.1949	0.0311
	45	14.7 c	17.3 b	8.0 c	10.7	0.0 b	0.0	0.0238	0.0281
	60	42.7 b	17.3 b	32.0 b	10.7	0.0 b	0.0	0.1192	0.0272
	C	92.0 a	100.0 a	65.3 a	29.3 a	8.0 a	0.0	0.1790	0.5747
p-value		<.0001	<.0001	.0009	.0210	.2192			
9	30	22.7 b	18.7 b	16.0 b	12.0	4.0 ab	0.0	0.1769	0.0408
	45	13.3 b	13.3 b	6.7 b	6.7	0.0 b	0.0	0.0233	0.0381
	60	16.0 b	13.3 b	9.3 b	6.7	0.0 b	0.0	0.0246	0.0382
	C	92.0 a	73.3 a	42.7 a	20.0	13.3 a	2.7	0.1812	0.9163
p-value		<.0001	<.0001	.0003	.0171	.1639	.4770		

Table 4.2c. Survival, chlorosis, injury percentages and average biomass per plant of sorghum *(Sorghum bicolor)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Sorghum									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	48.0 b**	13.3 b	41.3 a	6.7 b	2.7 b	0.0	0.2132	0.0290
	45	16.0 c	13.3 b	9.3 b	6.7 b	0.0 b	0.0	0.0250	0.0262
	60	13.3 c	13.3 b	6.7 b	6.7 b	0.0 b	0.0	0.0228	0.0256
	C	85.3 a	100.0 a	36.0 a	33.3 a	17.3 a	2.7	0.1868	0.4118
p-value		<.0001	<.0001	.0062	.0218	.0909	.4770		
11	30	49.3 b	13.3 b	38.7 a	6.7	1.3 b	0.0	0.2839	0.0353
	45	25.3 c	14.7 b	16.0 b	8.0	2.7 b	0.0	0.1497	0.0420
	60	13.3 d	13.3 b	6.7 b	6.7	0.0 b	0.0	0.0613	0.0404
	C	82.7 a	74.7 a	42.7 a	22.7	13.3 a	0.0	0.1708	0.3960
p-value		<.0001	<.0001	.0033	.0012	.0312			
12	30	16.0 c	29.3 b	6.7 b	20.0 ab	0.0 b	9.3 a	0.0573	0.1245
	45	30.7 b	13.3 c	24.0 b	6.7 b	1.3 b	0.0 b	0.3881	0.0380
	60	13.3 c	13.3 c	6.7 b	6.7 b	0.0 b	0.0 b	0.0482	0.0226
	C	100.0 a	73.3 a	53.3 a	24.0 a	14.7 a	0.0 b	0.1948	0.8737
p-value		<.0001	<.0001	.0070	.0020	.0992	.0020		
13	30	42.7 b	13.3 b	30.7 b	6.7 b	6.7 b	0.0	0.2866	0.0367
	45	9.3 c	8.0 b	2.7 c	1.3 b	0.0 b	0.0	0.0847	0.0318
	60	8.0 c	6.7 b	1.3 c	0.0 b	0.0 b	0.0	0.0474	0.0277
	C	100.0 a	84.0 a	57.3 a	26.7 a	26.7 a	4.0	0.1324	0.5855
p-value		<.0001	<.0001	.0015	.1319	.0104	.4770		

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2]Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3]Average biomass per plant was determined from data collected 15 weeks after sowing.

Table 4.3a. Survival, chlorosis, injury percentages and average biomass per plant of cucumber *(Cucumis spp)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Cucumber									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	37.5 a**	31.3 b	8.8 a	7.5 b	10.0	5.0	0.0252	0.0106
	45	41.3 a	21.3 b	3.8 bc	6.3 b	11.3	3.8	0.0156	0.0118
	60	18.8 b	20.0 b	0.0 c	0.0 b	3.8	2.5	0.0118	0.0127
	C	32.5 ab	87.5 a	7.5 ab	27.5 a	2.5	1.3	0.2517	0.3617
p-value		.0051	<.0001	<.0001	.0022	.0632	.2948		
1	30	25.3 c	14.7 c	0.0 b	0.0 b	0.0	0.0	0.0167	0.0000
	45	37.3 bc	57.3 b	4.0 b	6.7 b	6.7	10.7	0.0153	0.0422
	60	46.7 b	8.0 c	4.0 b	0.0 b	9.3	1.3	0.0123	0.0000
	C	93.3 a	78.7 a	34.7 a	26.7 a	13.3	4.0	0.2643	0.7702
p-value		<.0001	<.0001	.0022	.0467	.5723	.3118		
2	30	46.7 bc	22.7 c	12.0 b	0.0 b	5.3	0.0 b	0.0240	0.0126
	45	56.0 ab	62.7 b	26.7 a	17.3 a	9.3	10.7 a	0.0214	0.0283
	60	32.0 c	10.7 c	4.0 b	1.3 b	8.0	2.7 b	0.0176	0.0057
	C	70.7 a	82.7 a	26.7 a	20.0 a	2.7	0.0 b	0.1623	0.0876
p-value		<.0001	<.0001	<.0001	.0031	<.0001	.0775		
3	30	46.7 b	58.7 b	28.0	6.7 b	0.0 b	13.3	0.0200	0.0131
	45	46.7 b	70.7 ab	24.0	33.3 a	5.3 b	21.3	0.0255	0.0356
	60	54.7 b	58.7 b	33.3	10.7 b	14.7 a	5.3	0.1100	0.0144
	C	89.3 a	78.7 a	28.0	9.3 b	4.0 b	6.7	0.1906	0.1911
p-value		<.0001	<.0001	<.0001	.0022	.2183	.0375		
4	30	52.0 b	58.7 a	20.0 ab	12.0	13.3	13.3 a	0.0296	0.0156
	45	49.3 b	53.3 ab	28.0 a	10.7	9.3	12.0 ab	0.0273	0.0272
	60	45.3 b	38.7 b	16.0 ab	10.7	10.7	9.3 ab	0.0183	0.0148
	C	82.7 a	53.3 ab	6.7 b	2.7	2.7	1.3 b	0.0596	0.1584
p-value		<.0001	<.0001	<.0001	.0696	<.0001	.0002		

Table 4.3b. Survival, chlorosis, injury percentages and average biomass per plant of cucumber *(Cucumis spp)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Cucumber									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	42.7 c**	57.3 b	14.7 ab	14.7 a	8.0 b	13.3 a	0.0261	0.0329
	45	40.0 c	41.3 b	16.0 ab	2.7 b	13.3 ab	13.3 a	0.0203	0.0286
	60	61.3 b	34.7	21.3 a	2.7 b	25.3 a	12.0 a	0.1248	0.0138
	C	89.3 a	81.3 a	5.3 b	0.0 b	9.3 b	0.0 b	0.0895	0.1868
p-value		<.0001	<.0001	<.0001	.0028	.0009	<.0001		
6	30	40.0 b	61.3 b	6.7 b	16.0	9.3	22.7 a	0.0116	0.0327
	45	45.3 b	52.0 b	16.0 ab	14.7	14.7	13.3 ab	0.0212	0.0333
	60	42.7 b	41.3 b	25.3 a	9.3	14.7	6.7 b	0.0219	0.0123
	C	78.7 a	100.0 a	9.3 b	17.3	5.3	6.7 b	0.1118	0.2526
p-value		<.0001	<.0001	<.0001	.7586	.0040	.0211		
7	30	38.7 b	60.0 b	12.0 ab	21.3	12.0 ab	24.0	0.0211	0.0365
	45	44.0 b	50.7 bc	21.3 a	16.0	17.3 a	18.7	0.0222	0.0202
	60	44.0 b	36.0 c	18.7 ab	6.7	10.7	13.3	0.0110	0.0110
	C	65.3 a	81.3 a	6.7 b	4.0	4.0 b	16.0	0.0391	0.1328
p-value		<.0001	<.0001	<.0001	.0477	.0003	.0313		
8	30	58.7 ab	50.7 ab	14.7 ab	20.0 a	13.3	22.7 a	0.1383	0.0334
	45	49.3 bc	49.3 ab	22.7 a	13.3 a	24.0	28.0 a	0.0342	0.0256
	60	45.3 c	41.3 b	17.3 a	8.0 ab	21.3	16.0 ab	0.0194	0.0303
	C	68.0 a	60.0 a	1.3 b	0.0 b	20.0	4.0 b	0.0466	0.0641
p-value		<.0001	<.0001	<.0001	.0018	<.0001	.0001		
9	30	37.3 b	42.7	12.0 ab	16.0 a	26.7 a	9.3 ab	0.0156	0.0260
	45	36.0 b	45.3	21.3 a	12.0 ab	17.3 ab	14.7 ab	0.0168	0.0229
	60	37.3 b	48.0	18.7 a	20.0 a	10.7 b	22.7 a	0.0216	0.0132
	C	50.7 a	53.3	4.0 b	0.0 b	25.3 ab	6.7 b	0.0446	0.0643
p-value		<.0001	<.0001	<.0001	<.0001	<.0001	.0029		

Table 4.3c. Survival, chlorosis, injury percentages and average biomass per plant of cucumber *(Cucumis spp)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Cucumber									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	36.0 b**	49.3 ab	14.7 ab	14.7	17.3 b	18.7 a	0.0166	0.0427
	45	42.7 ab	40.0 b	25.3 a	14.7	29.3 a	14.7 ab	0.0243	0.0260
	60	37.3 b	41.3 b	22.7 a	16.0	13.3 b	16.0 ab	0.0165	0.0133
	C	50.7 a	57.3 a	6.7 b	2.7	32.0 a	2.7 b	0.0692	0.1570
p-value		<.0001	<.0001	<.0001	.0123	<.0001	<.0001		
11	30	42.7 c	40.0 b	13.3	9.3	16.0 b	17.3	0.0148	0.0171
	45	46.7 bc	41.3 b	21.3	12.0	26.7 ab	18.7	0.0135	0.0299
	60	65.3 a	41.3 b	18.7	9.3	33.3 a	16.0	0.2241	0.0473
	C	54.7 b	66.7 a	9.3	5.3	38.7 a	12.0	0.0399	0.1476
p-value		<.0001	<.0001	<.0001	.0061	<.0001	.0023		
12	30	29.3	48.0 a	12.0	8.0	18.7	20.0	0.0202	0.0475
	45	38.7	34.7 b	9.3	2.7	26.7	17.3	0.0198	0.0120
	60	32.0	33.3 b	14.7	8.0	20.0	17.3	0.0113	0.0166
	C	36.0	33.3 b	6.7	0.0	26.7	2.7	0.0268	0.0421
p-value		<.0001	<.0001	<.0001	.0074	<.0001	.0035		
13	30	36.0 b	52.0 a	10.7	16.0 a	22.7	29.3 a	0.0158	0.0336
	45	36.0 b	26.7 b	21.3	0.0 b	25.3	24.0 a	0.0257	0.0135
	60	30.7 b	26.7 b	12.0	4.0 b	25.3	20.0 ab	0.0211	0.0108
	C	50.7 a	28.0 b	12.0	1.3 b	30.7	1.3 b	0.1008	0.0387
p-value		<.0001	<.0001	<.0001	.0313	<.0001	.0067		

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2]Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3]Average biomass per plant was determined from data collected 15 weeks after sowing.

Table 4.4a. Survival, chlorosis, injury percentages and average biomass per plant of lettuce *(Lactuca sativa)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Lettuce									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	28.8 c**	23.8 a	2.5 b	2.5	7.5 a	0.0	0.0072	0.0000
	45	30.0 c	3.8 b	5.0 b	0.0	0.0 b	0.0	0.0172	0.0000
	60	62.5 b	20.0 ab	20.0 a	2.5	1.3 b	0.0	0.0510	0.0000
	C	93.8 a	2.5 b	10.0 ab	0.0	5.0 ab	0.0	0.1727	0.0000
p-value		<.0001	.0076	.0375	.6007	.0112			
1	30	66.7 b	21.3 b	28.0 ab	2.7 b	1.3	2.7 ab	0.0053	0.0000
	45	29.3 c	78.7 a	9.3 bc	44.0 a	5.3	9.3 a	0.0146	0.0165
	60	53.3 b	12.0 b	37.3 a	2.7 b	9.3	0.0 b	0.0172	0.0000
	C	93.3 a	93.3 a	6.7 c	9.3 b	5.3	0.0 b	0.1560	0.2518
p-value		<.0001	<.0001	.0286	.0079	.0338	.3143		
2	30	88.0 a	28.0 b	34.7 a	8.0	13.3	0.0	0.0237	0.0000
	45	100.0 a	88.0 a	29.3 a	20.0	17.3	4.0	0.0272	0.0508
	60	52.0 b	14.7 c	18.7 ab	6.7	5.3	1.3	0.0464	0.0000
	C	100.0 a	94.7 a	9.3 b	9.3	6.7	2.7	0.5321	0.0965
p-value		<.0001	<.0001	.1232	.1076	.0006	.0439		
3	30	73.3 b	53.3 b	18.7	20.0	13.3	1.3 b	0.0106	0.0031
	45	84.0 b	85.3 a	16.0	26.7	16.0	13.3 a	0.0513	0.0069
	60	85.3 b	62.7 b	30.7	16.0	10.7	2.7 ab	0.0624	0.0239
	C	97.3 a	94.7 a	17.3	12.0	10.7	4.0 ab	0.1180	0.0841
p-value		<.0001	<.0001	.0984	.0005	.0004	.3993		
4	30	70.7 b	80.0 a	5.3	22.7	5.3 b	13.3	0.0096	0.0037
	45	80.0 b	69.3 ab	17.3	22.7	14.7 ab	10.7	0.0126	0.0030
	60	92.0 a	56.0 b	21.3	17.3	17.3 a	5.3	0.0576	0.0000
	C	93.3 a	74.7 a	14.7	8.0	10.7 ab	4.0	0.0669	0.0886
p-value		<.0001	<.0001	.2395	.0020	<.0001	.0037		

Table 4.4b. Survival, chlorosis, injury percentages and average biomass per plant of lettuce *(Lactuca sativa)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Lettuce									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	90.7 a**	76.0 ab	12.0	22.7 a	14.7 a	13.3 a	0.0143	0.0087
	45	90.7 a	73.3 b	13.3	20.0 a	8.0 ab	2.7 b	0.0430	0.0333
	60	80.0 b	44.0 c	20.0	9.3 ab	1.3 b	9.3 ab	0.0672	0.0014
	C	89.3 a	86.7 a	9.3	4.0 b	4.0 b	5.3 ab	0.0767	0.0728
p-value		<.0001	<.0001	<.0001	<.0001	.0012	.0105		
6	30	56.0 b	72.0 b	8.0	22.7	5.3 ab	14.7 ab	0.0114	0.0093
	45	69.3 b	73.3 b	14.7	33.3	17.3 a	24.0 a	0.0127	0.0094
	60	96.0 a	62.7 b	24.0	10.7	2.7 b	6.7 b	0.0609	0.0212
	C	88.0 a	98.7 a	20.0	24.0	6.7 ab	5.3 b	0.1170	0.0773
p-value		<.0001	<.0001	.0394	.0594	.1127	.0057		
7	30	52.0 c	65.3 b	10.7 b	13.3	5.3 b	9.3 a	0.0623	0.0066
	45	84.0 a	62.7 b	28.0 a	12.0	28.0 a	6.7 ab	0.0165	0.0057
	60	66.7 b	33.3 b	12.0 b	8.0	8.0 b	0.0 b	0.0258	0.0000
	C	85.3 a	100.0 a	21.3 ab	25.3	6.7 b	4.0 ab	0.0503	0.1034
p-value		<.0001	<.0001	<.0001	.0563	.0010	.0871		
8	30	100.0 a	65.3 ab	33.3 ab	18.7 ab	10.7 ab	14.7 a	0.0503	0.0221
	45	89.3 b	54.7 b	34.7 ab	21.3 a	14.7 a	6.7 ab	0.0284	0.0085
	60	80.0 c	62.7 ab	24.0 b	8.7 ab	2.7 b	8.0 ab	0.1188	0.1711
	C	98.7 a	68.0 a	38.7 a	6.7 b	4.0 b	2.7 b	0.0729	0.0734
p-value		<.0001	<.0001	<.0001	.0068	.0027	.0034		
9	30	58.7 b	46.7 c	18.7	18.7	17.3	6.7	0.0088	0.0029
	45	45.3 b	46.7 c	18.7	24.0	13.3	13.3	0.0086	0.0022
	60	96.0 a	60.0 b	18.7	16.0	24.0	12.0	0.0406	0.0723
	C	46.7 b	70.7 a	17.3	18.7	5.3	4.0	0.0405	0.0784
p-value		<.0001	<.0001	.8598	.0044	.3068	.0005		

Table 4.4c. Survival, chlorosis, injury percentages and average biomass per plant of lettuce *(Lactuca sativa)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Lettuce									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	61.3 a**	65.3 b	14.7	16.0 b	5.3	9.3	0.0437	0.0127
	45	46.7 b	38.7 c	10.7	12.0 b	12.0	12.0	0.0044	0.0000
	60	68.0 a	37.3 c	22.7	9.3 b	17.3	5.3	0.0996	0.0066
	C	49.3 b	100.0 a	22.7	46.7 a	9.3	6.7	0.0321	0.0717
p-value		<.0001	<.0001	.0002	.0964	.1849	.4346		
11	30	89.3 a	54.7 c	44.0	18.7	6.7	22.7 a	0.0494	0.0038
	45	77.3 b	69.3 b	34.7	25.3	9.3	16.0 ab	0.0497	0.0830
	60	89.3 a	74.7 ab	28.0	24.0	6.7	5.3 c	0.0700	0.0972
	C	76.0 b	82.7 a	46.7	20.0	8.0	12.0 bc	0.0325	0.0315
p-value		<.0001	<.0001	<.0001	.0024	.4481	.0002		
12	30	17.3 d	53.3 b	4.0 b	16.0 ab	8.0 b	22.7 a	0.0000	0.0234
	45	64.0 b	9.3 c	29.3 a	0.0 b	18.7 ab	0.0 c	0.0435	0.0000
	60	85.3 a	52.0 b	29.3 a	20.0 a	29.3 a	13.3 b	0.0538	0.0483
	C	37.3 c	92.0 a	14.7 ab	25.3 a	5.3 b	6.7 bc	0.0307	0.0410
p-value		<.0001	<.0001	.0138	.0004	.0320	.0010		
13	30	96.0 a	46.7 b	37.3 ab	14.7	16.0 ab	16.0 a	0.0732	0.0057
	45	88.0 b	26.7 c	20.0 b	13.3	22.7 a	0.0 b	0.0668	0.0000
	60	81.3 c	32.0 c	32.0 ab	12.0	26.7 a	9.3 ab	0.0672	0.0000
	C	98.7 a	81.3 a	45.3 a	13.3	9.3 b	13.3 a	0.0300	0.0914
p-value		<.0001	<.0001	.0235	<.0001	.0281	.1405		

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2] Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3] Average biomass per plant was determined from data collected 15 weeks after sowing.

Table 4.5a. Survival, chlorosis, injury percentages and average biomass per plant of radish *(Raphanus sativus)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Radish									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	26.3 bc**	18.8	5.0	6.3	2.5	3.8	0.0050	0.0123
	45	17.5 c	10.0	2.5	0.0	7.5	0.0	0.0092	0.0000
	60	31.3 b	22.5	5.0	3.8	2.5	5.0	0.0016	0.0000
	C	86.3 a	22.5	15.0	1.3	0.0	0.0	0.3050	0.3919
p-value		<.0001	.0163	.0184	.6577	.4991	.0050		
1	30	38.7 b	18.7 b	18.7	0.0	0.0	5.3	0.0169	0.0029
	45	34.7 b	22.7 b	17.3	13.3	6.7	1.3	0.0100	0.0035
	60	28.0 b	17.3 b	13.3	2.7	4.0	0.0	0.0018	0.0092
	C	100.0 a	49.3 a	22.7	8.0	0.0	8.0	0.3113	0.4739
p-value		<.0001	<.0001	.0006	.2201	.5934	.0073		
2	30	26.7 b	25.3 b	17.3	12.0	0.0	5.3	0.0076	0.0037
	45	26.7 b	26.7 b	18.7	20.0	5.3	6.7	0.0096	0.0049
	60	25.3 b	20.0 b	17.3	5.3	0.0	4.0	0.0095	0.0125
	C	100.0 a	68.0 a	25.3	10.7	0.0	4.0	0.2976	0.0974
p-value		<.0001	<.0001	<.0001	.0007	.4770	.0046		
3	30	21.3 b	21.3 b	10.7	12.0	0.0	1.3 ab	0.0076	0.0061
	45	20.0 b	22.7 b	13.3	16.0	0.0	0.0 b	0.0106	0.0034
	60	21.3 b	24.0 b	14.7	9.3	0.0	2.7 ab	0.0037	0.0051
	C	100.0 a	89.3 a	8.0	30.7	0.0	9.3 a	0.3082	0.0689
p-value		<.0001	<.0001	<.0001	.0303		.3848		
4	30	20.0 b	21.3 b	13.3	21.3	0.0	0.0	0.0089	0.0014
	45	21.3 b	21.3 b	14.7	10.7	0.0	0.0	0.0062	0.0035
	60	21.3 b	18.7 b	14.7	9.3	0.0	0.0	0.0056	0.0018
	C	90.7 a	84.0 a	22.7	9.3	2.7	2.7	0.2290	0.0836
p-value		<.0001	<.0001	<.0001	.0006	.3032	.4770		

Table 4.5b. Survival, chlorosis, injury percentages and average biomass per plant of radish *(Raphanus sativus)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Radish									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	21.3 b**	21.3 b	14.7	13.3	1.3	0.0 b	0.0072	0.0055
	45	26.7 b	22.7 b	14.7	16.0	2.7	0.0 b	0.0061	0.0029
	60	24.0 b	24.0 b	14.7	12.0	1.3	0.0 b	0.0024	0.0048
	C	86.7 a	70.7 a	21.3	20.0	4.0	12.0 a	0.1195	0.0235
p-value		<.0001	<.0001	<.0001	.0001	.6047	.0373		
6	30	22.7 b	22.7 b	20.0	21.3	0.0	0.0 b	0.0074	0.0058
	45	16.0 b	24.0 b	10.7	21.3	0.0	0.0 b	0.0082	0.0047
	60	24.0 b	21.3 b	18.7	13.3	0.0	0.0 b	0.0027	0.0057
	C	81.3 a	76.0 a	17.3	25.3	0.0	5.3 a	0.0704	0.0492
p-value		<.0001	<.0001	<.0001	<.0001		.2066		
7	30	16.0 b	21.3 b	10.7	21.3	0.0	0.0 b	0.0069	0.0070
	45	18.7 b	24.0 b	13.3	21.3	0.0	0.0 b	0.0073	0.0049
	60	20.0 b	20.0 b	20.0	20.0	0.0	0.0 b	0.0055	0.0044
	C	68.0 a	58.7 a	10.7	12.0	1.3	12.0 a	0.0124	0.0090
p-value		<.0001	<.0001	<.0001	.0002	.4770	.0867		
8	30	36.0 b	20.0 b	17.3	12.0	9.3 a	0.0 b	0.0807	0.0045
	45	21.3 b	22.7 b	17.3	14.7	0.0 b	0.0 b	0.0107	0.0046
	60	25.3 b	20.0 b	17.3	13.3	2.7 b	0.0 b	0.0049	0.0032
	C	65.3 a	56.0 a	6.7	6.7	5.3 ab	6.7 a	0.0153	0.0377
p-value		<.0001	<.0001	<.0001	<.0001	.0236	.0292		
9	30	20.0 b	26.7 b	14.7 ab	24.0 b	0.0	1.3	0.0086	0.0076
	45	20.0 b	18.7 b	17.3 a	18.7 b	0.0	1.3	0.0066	0.0025
	60	16.0 b	20.0 b	14.7 ab	13.3 ab	0.0	0.0	0.0055	0.0056
	C	53.3 a	53.3 a	1.3 b	0.0 b	1.3	0.0	0.0143	0.0321
p-value		<.0001	<.0001	<.0001	<.0001	.4770	.6182		

Table 4.5c. Survival, chlorosis, injury percentages and average biomass per plant of radish *(Raphanus sativus)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Radish									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	20.0 b**	22.7 b	17.3 a	22.7 a	1.3	0.0	0.0064	0.0067
	45	13.3 b	24.0 b	10.7 ab	21.3 a	0.0	1.3	0.0048	0.0052
	60	18.7 b	17.3 b	18.7 a	17.3 a	0.0	0.0	0.0057	0.0065
	C	49.3 a	56.0 a	4.0 b	0.0 b	2.7	0.0	0.0216	0.1250
p-value		<.0001	<.0001	<.0001	<.0001	.5897	.4770		
11	30	16.0 c	16.0 b	14.7	14.7	0.0 b	0.0 b	0.0068	0.0053
	45	20.0 c	16.0 b	17.3	13.3	2.7 b	0.0 b	0.0062	0.0045
	60	33.3 b	17.3 b	21.3	16.0	13.3 a	0.0 b	0.0082	0.0476
	C	60.0 a	50.7 a	12.0	8.0	2.7 b	4.0 a	0.0186	0.0518
p-value		<.0001	<.0001	<.0001	<.0001	.0890	.1661		
12	30	12.0 b	17.3 b	6.7	17.3	0.0	0.0	0.0048	0.0045
	45	13.3 b	20.0 b	13.3	13.3	0.0	0.0	0.0063	0.0050
	60	13.3 b	17.3 b	12.0	17.3	0.0	0.0	0.0033	0.0038
	C	37.3 a	36.0 a	5.3	6.7	1.3	0.0	0.0167	0.0151
p-value		<.0001	<.0001	.0008	<.0001	.4770			
13	30	14.7 b	16.0 ab	14.7	16.0 a	0.0	0.0	0.0051	0.0050
	45	20.0 b	14.7 ab	18.7	14.7 a	2.7	0.0	0.0081	0.0046
	60	13.3 b	13.3 b	13.3	13.3 a	0.0	0.0	0.0045	0.0039
	C	48.0 a	26.7 a	6.7	0.0 b	1.3	0.0	0.0073	0.0133
p-value		<.0001	<.0001	<.0001	<.0001	.0323			

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2] Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3] Average biomass per plant was determined from data collected 15 weeks after sowing.

Table 4.6a. Survival, chlorosis, injury percentages and average biomass per plant of cabbage *(Brassica oleracea)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Cabbage									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	35.0 b**	15.0 ab	15.0 a	0.0	0.0	5.0	0.0037	0.0046
	45	16.3 c	3.8 b	3.8 bc	0.0	3.8	0.0	0.0091	0.0000
	60	40.0 b	27.5 a	8.8 ab	2.5	0.0	0.0	0.0021	0.0000
	C	58.8 a	20.0 a	0.0 c	0.0	0.0	0.0	0.1815	0.2816
p-value		<.0001	<.0001	.0001	.4774	.4774	.4774		
1	30	65.3 b	22.7 c	36.0 a	2.7	0.0	6.7	0.0155	0.0030
	45	52.0 b	45.3 b	33.3 a	12.0	0.0	0.0	0.0078	0.0045
	60	54.7 b	16.0 c	28.0 a	0.0	0.0	0.0	0.0206	0.0000
	C	97.3 a	84.0 a	2.7 b	0.0	0.0	1.3	0.2533	0.3245
p-value		<.0001	<.0001	<.0001	.3720		.5306		
2	30	57.3 b	45.3 b	32.0 a	13.3 b	0.0	5.3	0.0133	0.0103
	45	68.0 b	60.0 b	30.7 a	28.0 a	0.0	0.0	0.0192	0.0053
	60	62.7 b	13.3 c	41.3 a	2.7 b	0.0	2.7	0.0063	0.0034
	C	100.0 a	97.3 a	2.7 b	0.0 b	0.0	0.0	0.4563	0.2658
p-value		<.0001	<.0001	<.0001	.0261		.1216		
3	30	61.3 b	60.0 b	45.3 a	21.3 ab	0.0	0.0	0.0078	0.0063
	45	60.0 b	48.0 b	45.3 a	34.7 a	0.0	0.0	0.0098	0.0056
	60	53.3 b	53.3 b	36.0 a	16.0 ab	1.3	2.7	0.0884	0.0047
	C	100.0 a	100.0 a	0.0 b	0.0 b	0.0	0.0	0.3630	0.1870
p-value		<.0001	<.0001	<.0001	.0912	.4770	.3032		
4	30	40.0 b	50.7 b	33.3 a	28.0 a	0.0	0.0	0.0090	0.0054
	45	52.0 b	44.0 b	29.3 a	32.0 a	0.0	0.0	0.0188	0.0041
	60	38.7 b	56.0 b	30.7 a	24.0 a	0.0	0.0	0.0039	0.0033
	C	100.0 a	81.3 a	1.3 b	0.0 b	0.0	0.0	0.1538	0.0929
p-value		<.0001	<.0001	<.0001	.0070				

Table 4.6b. Survival, chlorosis, injury percentages and average biomass per plant of cabbage *(Brassica oleracea)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Cabbage									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	62.7 b**	41.3 b	36.0 a	21.3 a	1.3	0.0 b	0.0554	0.0033
	45	62.7 b	41.3 b	42.7 a	22.7 a	0.0	0.0 b	0.0350	0.0103
	60	61.3 b	42.7 b	37.3 a	21.3 a	0.0	0.0 b	0.0582	0.0044
	C	100.0 a	85.3 a	0.0 b	0.0 b	0.0	5.3 a	0.1283	0.1389
p-value		<.0001	<.0001	<.0001	<.0001	.4770	.2066		
6	30	44.0 c	70.7 a	24.0 a	53.3 a	0.0	0.0	0.0064	0.0075
	45	40.0 c	48.0 b	32.0 a	28.0 b	0.0	0.0	0.0060	0.0036
	60	69.3 b	48.0 b	38.7 a	28.0 b	0.0	0.0	0.0225	0.0060
	C	100.0 a	80.0 a	1.3 b	1.3 c	0.0	0.0	0.1150	0.1012
p-value		<.0001	<.0001	<.0001	<.0001				
7	30	85.3 b	65.3 b	40.0 a	48.0 a	0.0	0.0	0.0430	0.0095
	45	54.7 c	38.7 c	36.0 a	25.3 b	0.0	0.0	0.0750	0.0055
	60	46.7 c	37.3 c	40.0 a	28.0 b	1.3	0.0	0.0163	0.0018
	C	100.0 a	88.0 a	0.0 b	0.0 c	1.3	2.7	0.1705	0.1440
p-value		<.0001	<.0001	<.0001	<.0001	.6182	.4770		
8	30	86.7 b	54.7 b	32.0 a	33.3 a	4.0	2.7	0.1158	0.0855
	45	60.0 c	33.3 c	38.7 a	28.0 a	1.3	1.3	0.1009	0.0038
	60	66.7 c	53.3 b	24.0 a	28.0 a	2.7	0.0	0.0288	0.0285
	C	100.0 a	76.0 a	1.3 b	4.0 b	0.0	4.0	0.0908	0.2217
p-value		<.0001	<.0001	<.0001	<.0001	.7294	.3300		
9	30	24.0 c	44.0 b	18.7 b	37.3 a	0.0 b	0.0	0.0068	0.0056
	45	20.0 c	33.3 c	12.0 bc	29.3 a	0.0 b	1.3	0.0045	0.0034
	60	68.0 b	45.3 b	36.0 a	41.3 a	6.7 a	0.0	0.0518	0.0061
	C	98.7 a	60.0 a	2.7 c	1.3 b	0.0 b	1.3	0.0897	0.0672
p-value		<.0001	<.0001	<.0001	<.0001	.1925	.6182		

Table 4.6c. Survival, chlorosis, injury percentages and average biomass per plant of cabbage *(Brassica oleracea)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Cabbage									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	98.7 a**	36.0 b	29.3 b	33.3 a	14.7	0.0	0.0475	0.0050
	45	29.3 c	25.3 b	26.7 b	25.3 a	0.0	0.0	0.0041	0.0028
	60	49.3 b	33.3 b	48.0 a	33.3 a	8.0	0.0	0.0278	0.0043
	C	88.0 a	86.7 a	20.0 b	0.0 b	10.7	0.0	0.0659	0.1192
p-value		<.0001	<.0001	<.0001	<.0001	.0829			
11	30	54.7 c	40.0 c	45.3 a	34.7 a	10.7 a	0.0 b	0.0174	0.0062
	45	82.7 b	46.7 bc	48.0 a	33.3 a	5.3 ab	6.7 ab	0.1093	0.0078
	60	89.3 ab	68.0 a	41.3 a	30.7 a	10.7 a	9.3 a	0.1556	0.0687
	C	98.7 a	52.0 b	4.0 b	4.0 b	0.0 b	6.7 ab	0.1028	0.1151
p-value		<.0001	<.0001	<.0001	<.0001	<.0001	.0155		
12	30	20.0 b	37.3 b	18.7 ab	32.0 a	0.0 b	0.0	0.0060	0.0046
	45	26.7 b	21.3 c	24.0 a	14.7 ab	0.0 b	6.7	0.0090	0.0034
	60	42.7 a	26.7 c	22.7 ab	25.3 a	8.0 a	0.0	0.0741	0.0030
	C	34.7 ab	70.7 a	6.7 b	5.3 b	4.0 ab	5.3	0.0566	0.1890
p-value		<.0001	<.0001	<.0001	<.0001	.2809	.5460		
13	30	68.0 b	28.0	48.0 a	26.7 a	16.0 a	0.0	0.0449	0.0035
	45	34.7 d	24.0	33.3 b	24.0 a	2.7 b	0.0	0.0041	0.0028
	60	50.7 c	24.0	38.7 ab	24.0 a	13.3 ab	0.0	0.0331	0.0039
	C	94.7 a	26.7	25.3 b	9.3 b	5.3 ab	2.7	0.0636	0.0086
p-value		<.0001	<.0001	<.0001	<.0001	.0167	.4770		

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2]Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3]Average biomass per plant was determined from data collected 15 weeks after sowing.

Table 4.7a. Survival, chlorosis, injury percentages and average biomass per plant of longleaf pine *(Pinus palustris)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Longleaf									
Week[1]	Treatment (oz/ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3](g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
0	30	92.5**	47.5 b	7.5 ab	2.5 b	3.8	2.5	0.0950	0.0715
	45	92.5	78.8 a	1.3 b	11.3 a	0.0	3.8	0.1107	0.0977
	60	87.5	81.25	13.8 a	0.0 b	6.3	2.5	0.1088	0.1208
	C	81.3	86.3 a	12.5 ab	2.5 b	3.8	0.0	0.4526	0.3490
p-value		<.0001	<.0001	.0076	.0847	.2735	.0170		
1	30	92.0 a	73.3 bc	4.0	1.3	2.7 b	9.3 ab	0.0950	0.0728
	45	93.3 a	86.7 ab	0.0	9.3	0.0 b	14.7 a	0.1329	0.0915
	60	85.3 b	68.0 c	14.7	8.0	10.7 a	4.0 ab	0.0801	0.0852
	C	93.3 a	89.3 a	14.7	2.7	0.0 b	0.0 b	0.5385	0.3084
p-value		<.0001	<.0001	.1214	.0968	.0165	.0759		
2	30	93.3 a	88.0 a	4.0 b	1.3 b	1.3	12.0 a	0.1247	0.1064
	45	93.3 a	93.3 a	8.0 ab	6.7 ab	4.0	1.3 b	0.1681	0.1085
	60	89.3 b	70.7 b	8.0 ab	9.3 a	4.0	17.3 a	0.1117	0.0920
	C	93.3 a	88.0 a	13.3 a	0.0 b	0.0	0.0 b	0.3120	0.2905
p-value		<.0001	<.0001	<.0001	<.0412	.0020	.0389		
3	30	93.3 a	93.3	2.7 b	2.7 b	0.0 b	0.0 b	0.1283	0.1746
	45	80.0 b	93.3	10.7 ab	26.7 a	1.3 b	5.3 a	0.1815	0.1327
	60	90.7 a	93.3	6.7 b	4.0 b	9.3 a	4.0 ab	0.1867	0.1185
	C	93.3 a	93.3	21.3 a	5.3 b	0.0 b	0.0 b	0.4607	0.3130
p-value		<.0001	<.0001	.0021	<.0001	.0172	.0241		
4	30	94.7	98.7	9.3	12.0 a	0.0 b	4.0	0.1618	0.1427
	45	92.0	92.0	14.7	8.0 ab	2.7 b	6.7	0.1425	0.0123
	60	96.0	93.3	17.3	1.3 b	20.0 a	1.3	0.0892	0.1023
	C	94.7	94.7	8.0	0.0 b	2.7 b	0.0	0.4148	0.2844
p-value		<.0001	<.0001	.0002	.0156	.0022	.0174		

Table 4.7b. Survival, chlorosis, injury percentages and average biomass per plant of longleaf pine *(Pinus palustris)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Longleaf									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
5	30	93.3 a**	89.3 a	14.7	6.7	6.7	8.0	0.1544	0.1434
	45	69.3 b	93.3 a	8.0	4.0	2.7	0.0	0.2201	0.1834
	60	66.7 b	58.7 b	10.7	8.0	6.7	8.0	0.1333	0.1376
	C	93.3 a	90.7 a	12.0	4.0	4.0	8.0	0.5011	0.3000
p-value		<.0001	<.0001	.2049	.0132	.0596	.5043		
6	30	97.3	93.3 a	9.3	4.0	0.0 b	0.0	0.1571	0.1372
	45	94.7	93.3 a	2.7	9.3	1.3 b	0.0	0.1463	0.2090
	60	93.3	93.3 a	6.7	4.0	0.0 b	2.7	0.1479	0.1362
	C	93.3	62.7 b	6.7	5.3	10.7 a	2.7	0.3683	0.3224
p-value		<.0001	<.0001	.0588	.0473	.0070	.5741		
7	30	84.0 b	90.7 ab	5.3 bc	12.0	5.3	5.3	0.2090	0.1441
	45	93.3 a	93.3 a	10.7 ab	13.3	0.0	13.3	0.2181	0.1340
	60	93.3 a	93.3 a	17.3 a	18.7	4.0	6.7	0.1157	0.0977
	C	92.0 a	89.3 b	0.0 c	17.3	5.3	13.3	0.3148	0.4079
p-value		<.0001	<.0001	<.0001	<.0001	.3590	<.0001		
8	30	92.0	86.7 a	8.0 ab	6.7	0.0 b	4.0 b	0.2224	0.1895
	45	93.3	57.3 b	16.0 a	4.0	14.7 a	8.0 ab	0.2358	0.1080
	60	93.3	93.3 a	8.0 ab	0.0	2.7 b	1.3 b	0.2511	0.1327
	C	93.3	85.3 a	4.0 b	9.3	0.0 b	16.0 a	0.4174	0.4718
p-value		<.0001	<.0001	.0709	.4755	.0208	.0600		
9	30	93.3 ab	85.3 b	1.3 b	6.7 ab	5.3	2.7 b	0.1841	0.2126
	45	89.3 b	86.7 ab	4.0 b	8.0 ab	2.7	4.0 b	0.1016	0.1301
	60	93.3 ab	94.7 a	12.0 a	2.7 b	8.0	2.7 b	0.1839	0.1626
	C	96.0 a	78.7 b	1.3 b	20.0 a	2.7	17.3 a	0.3566	0.2973
p-value		<.0001	<.0001	.0261	.1445	.4363	.2842		

Table 4.7c. Survival, chlorosis, injury percentages and average biomass per plant of longleaf pine *(Pinus palustris)* sown in soils treated with imazapyr and grown in the greenhouse, 2014.

Longleaf									
Week[1]	Treatment (oz.ac)	Survival[2] (%)		Chlorosis[2] (%)		Injury[2] (%)		Average Biomass Per Plant[3] (g)	
		Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay
10	30	96.0 a**	65.3 b	5.3 ab	18.7 ab	0.0	10.7	0.2093	0.1339
	45	76.0 b	92.0 a	6.7 ab	16.0 ab	1.3	4.0	0.1576	0.1262
	60	93.3 a	74.7 b	9.3 a	24.0 a	2.7	10.7	0.2100	0.1296
	C	97.3 a	64.0 b	0.0 b	8.0 b	2.7	14.7	0.4388	0.4023
p-value		<.0001	<.0001	.0882	<.0001	.7458	.0476		
11	30	94.7	84.0 a	14.7 b	12.0	4.0 b	9.3	0.2543	0.1400
	45	93.3	85.3 a	30.7 a	16.0	16.0 a	4.0	0.2087	0.1469
	60	96.0	94.7 a	5.3 bc	8.0	5.3 b	8.0	0.2700	0.1736
	C	93.3	52.0 b	0.0 c	5.3	5.3 b	13.3	0.4050	0.0808
p-value		<.0001	<.0001	.0001	.0018	.0164	.2252		
12	30	86.7	89.3 a	1.3 b	18.7 a	2.7	6.7	0.1296	0.1090
	45	96.0	77.3 a	16.0 a	1.3 b	4.0	2.7	0.2050	0.0987
	60	94.7	90.7 a	18.7 a	17.3 a	0.0	14.7	0.1793	0.1426
	C	93.3	52.0 b	0.0 b	9.3 ab	0.0	17.3	0.2069	0.0568
p-value		<.0001	<.0001	.0005	.0643	.1161	.3854		
13	30	98.7 a	92.0 a	1.3 b	8.0 ab	0.0 b	5.3	0.2692	0.0938
	45	97.3 a	73.3 b	12.0 a	16.0 a	0.0 b	1.3	0.2026	0.1306
	60	89.3 b	60.0 b	0.0 b	1.3 b	6.7 a	1.3	0.1912	0.1459
	C	98.7 a	33.3 c	0.0 b	2.7 b	0.0 b	13.3	0.3589	0.0653
p-value		<.0001	<.0001	.1215	.0292	.0292	.5510		

** Means with different letters are significantly different ($p < 0.05$).

[1] Corresponds to how long after the herbicide application the seeds were sown. Each week was grown for a 15 week period.

[2]Survival, chlorosis, and injury percentages determined from data collected over a 15 week period.

[3]Average biomass per plant was determined from data collected 15 weeks after sowing.

Figure 2.1. 2014 Lolly Creek herbicide trial weed count of little bluestem (*Schizachyrium scoparium*).

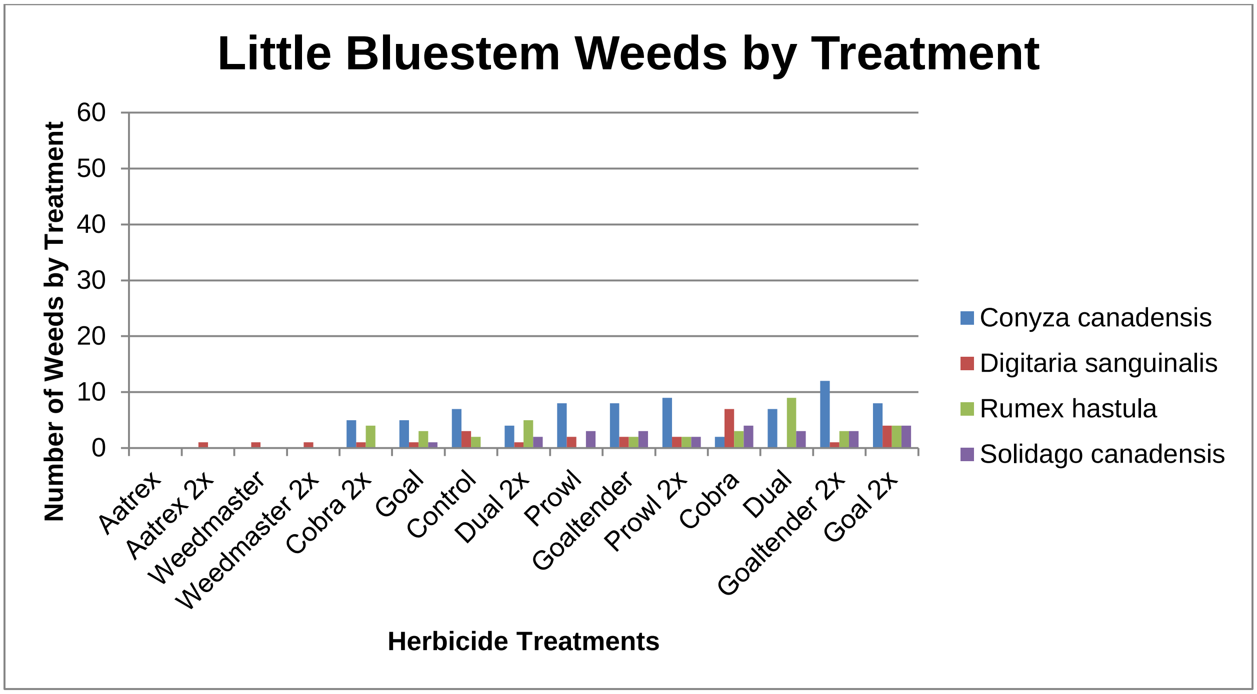


Figure 2.2. 2014 Lolly Creek herbicide trial weed count of Indian Grass (*Sorghastrum nutans*).

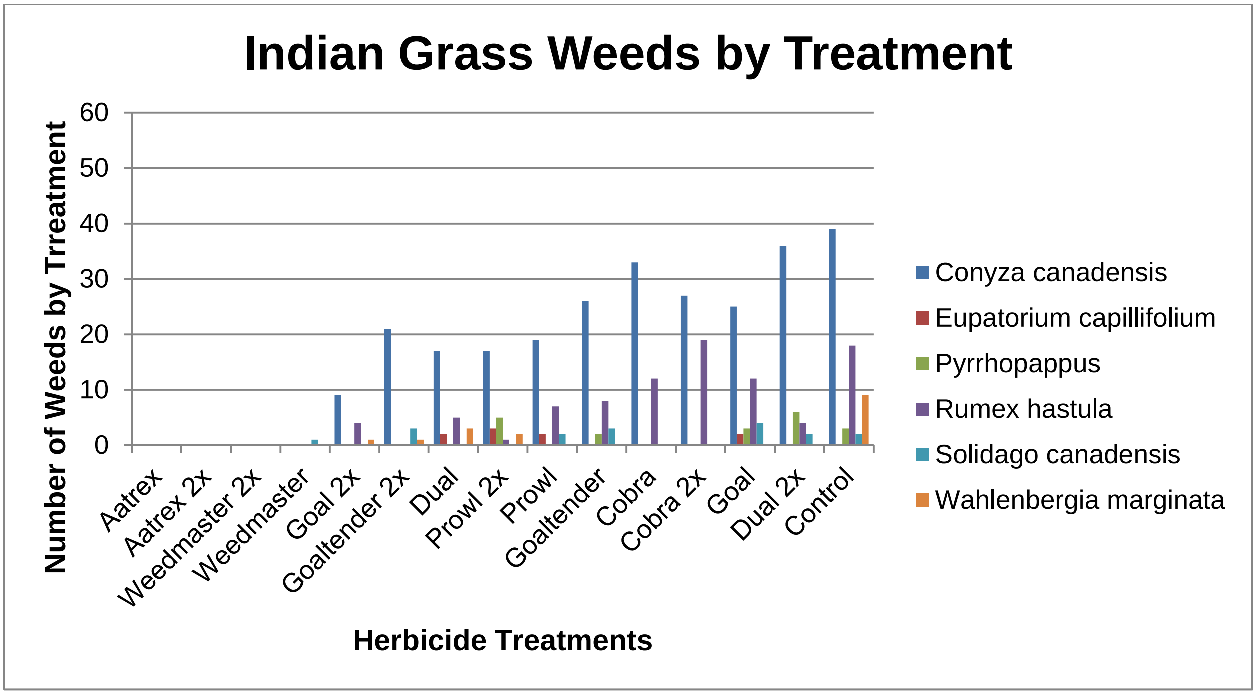


Figure 2.3. 2014 Lolly Creek herbicide trial weed count of Florida Ticktrefoil (*Desmodium floridanum*).

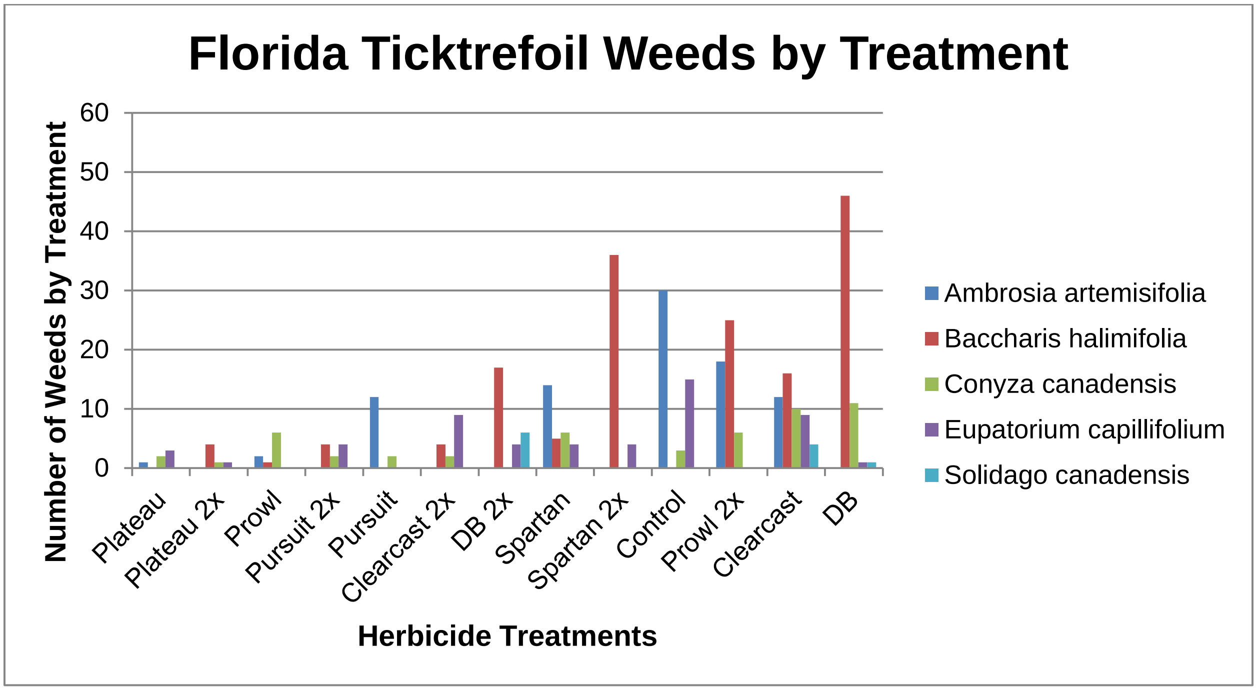


Figure 2.4. 2014 Lolly Creek herbicide trial results of goat's Rue (*Tephrosia virginiana*).

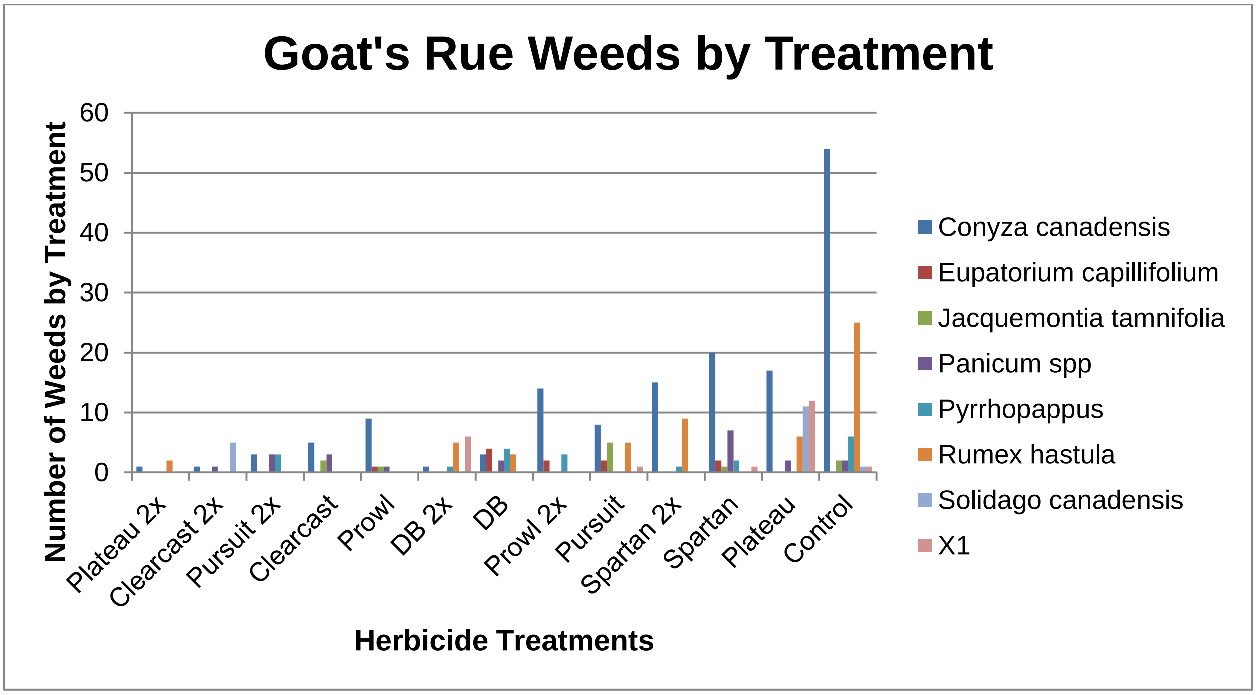


Figure 2.5. 2014 Lolly Creek herbicide trial results of narrow-leaf sunflower (*Helianthus angustifolius*).

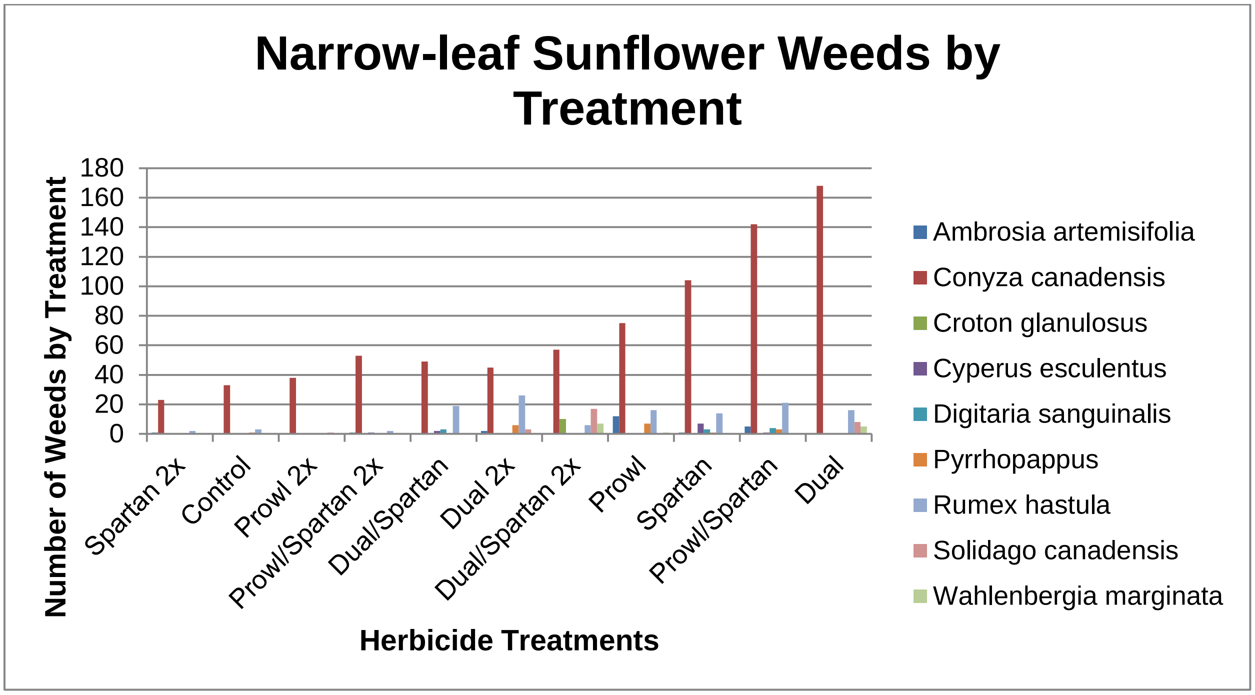


Figure 3.1. 2013 Indian grass seedlings grown in North Carolina.

Figure 3.2. 2013 muhly grass seedlings grown in North Carolina.

Figure 3.3. 2013 wiregrass seedlings grown in North Carolina.

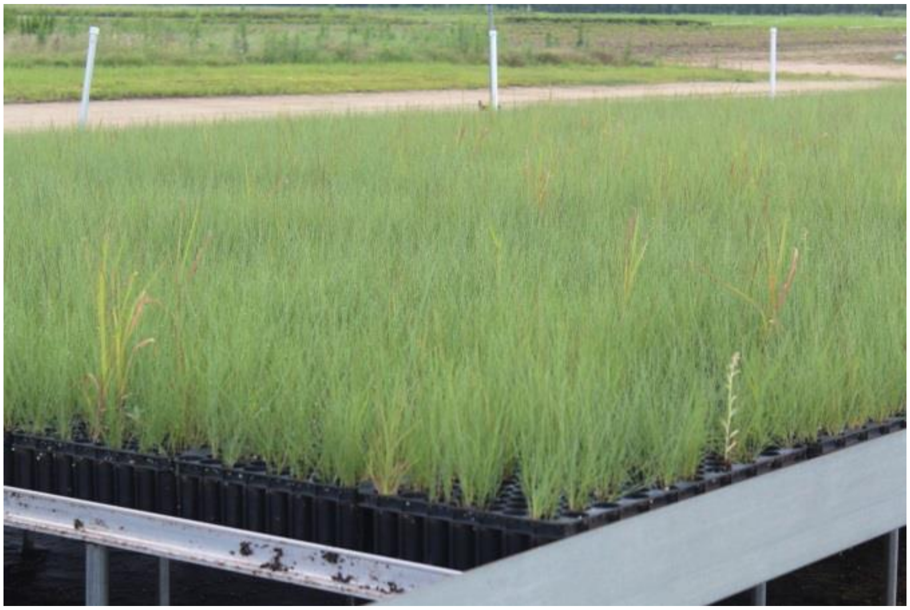


Figure 3.4. 2014 Indian grass grown at IFCO.

Figure 3.5. 2014 little bluestem grown at IFCO.

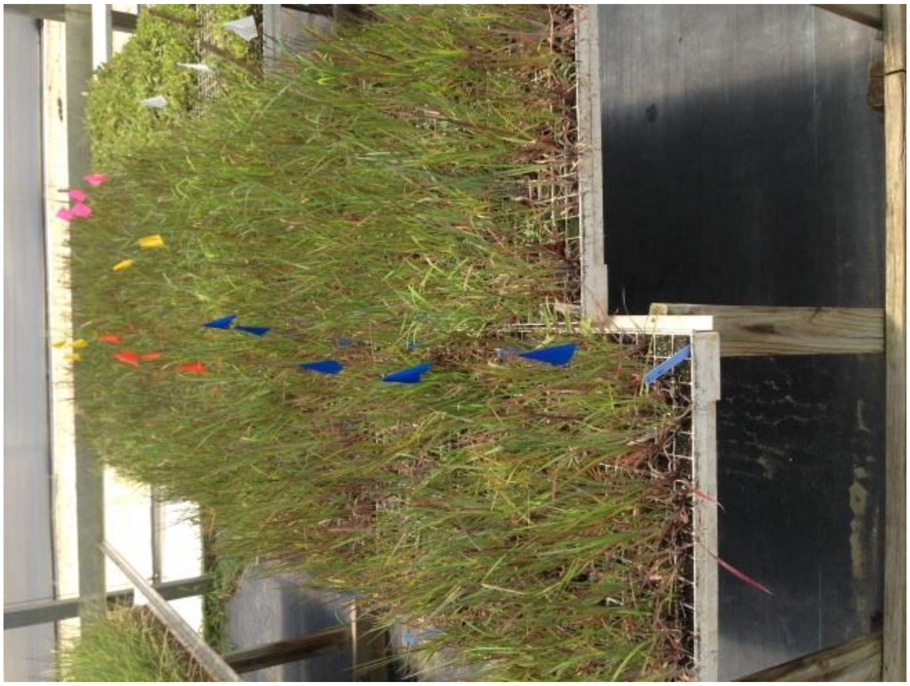


Figure 3.6. 2014 wiregrass seedlings grown at IFCO.

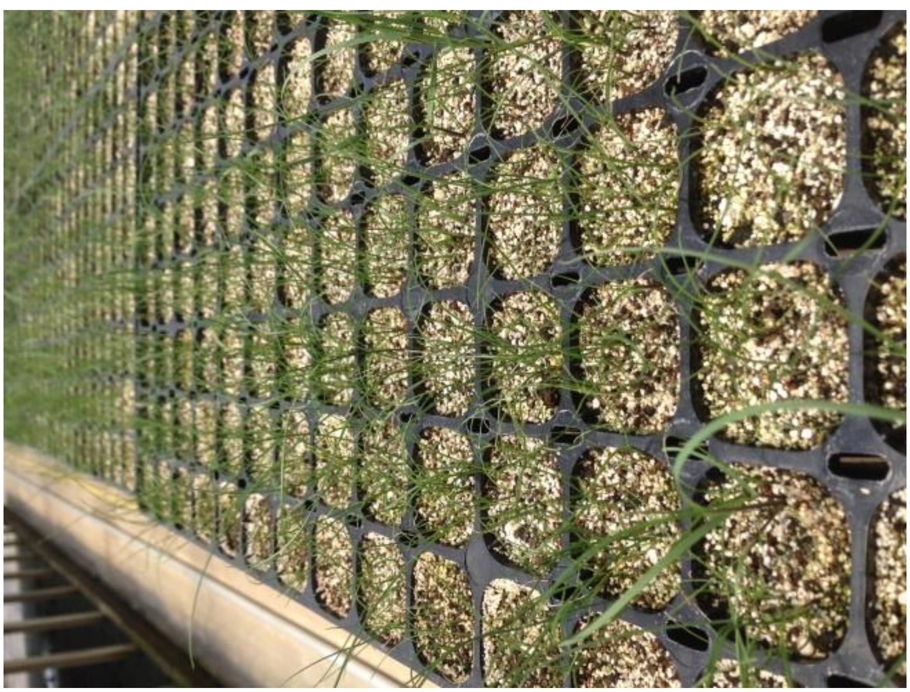


Figure 4.1. Tomato plants grown in coarse textured soil treated with 60 oz/ac of imazapyr. Image taken 16 weeks after treatment and 12 weeks after sowing.

Figure 4.2. Sorghum plants grown in fine textured soil treated with 60 oz/ac of imazapyr. Image taken 9 weeks after treatment and 4 weeks after sowing. Note the purple coloration of the plants.

Figure 4.3. Sorghum plants grown in coarse textured non-treated soil. Image taken 4 weeks after treatment and sowing.

Figure 4.4. Cucumber plants grown in fine textured soil treated with 45 oz/ac of imazapyr. Image taken 6 weeks after treatments and 5 weeks after sowing. Note the chlorosis of the plants.

Table 4.5 Lettuce grown in fine textured soil treated with 60 oz/ac of imazapyr. Image taken 18 weeks after treatment and 6 weeks after sowing.

Figure 4.6. Radish plants grown in coarse textured non-treated soil. Image taken 6 weeks after treatment and 4 weeks after sowing.

Figure 4.7. Cabbage plants grown in coarse textured non-treated soil. Image taken 9 weeks after treatment and 7 weeks after sowing.

Figure 4.8. Longleaf pine seedlings grown in coarse textured non-treated soil. Image taken 9 weeks after both treatment and sowing.

Figure 4.9. Tomato plants grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.10. Tomato plants grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.11. Sorghum plants grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.12. Sorghum plants grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.13. Cucumber plants grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.14. Cucumber plants grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.15. Lettuce plants grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.16. Lettuce plants grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.17. Radish plants grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

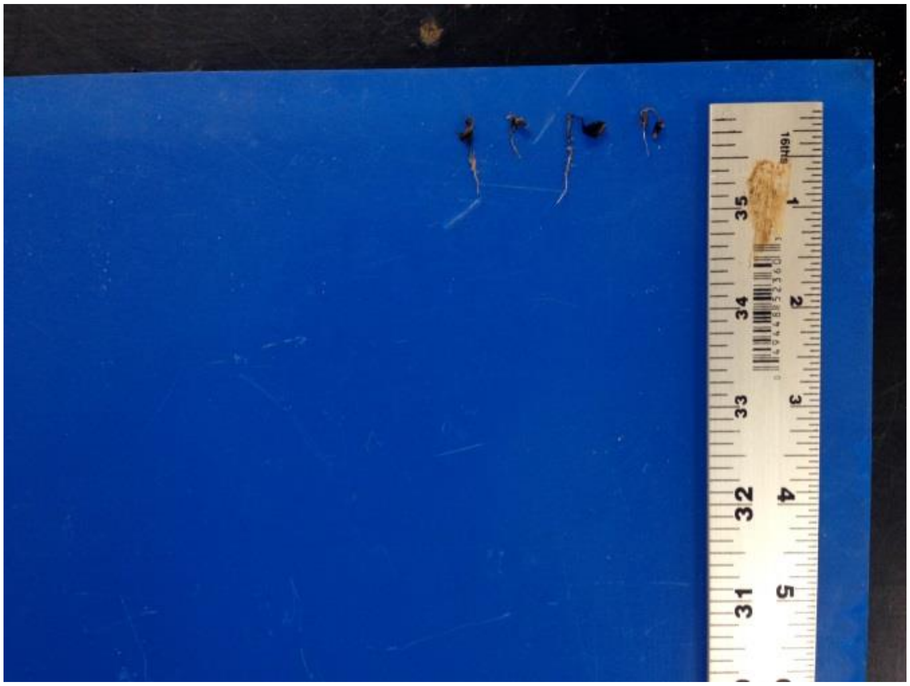


Figure 4.18. Radish plants grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.19. Cabbage plants grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.20. Cabbage plants grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.21. Longleaf pine grown in coarse textured soil treated with 30 oz/ac of imazapyr. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

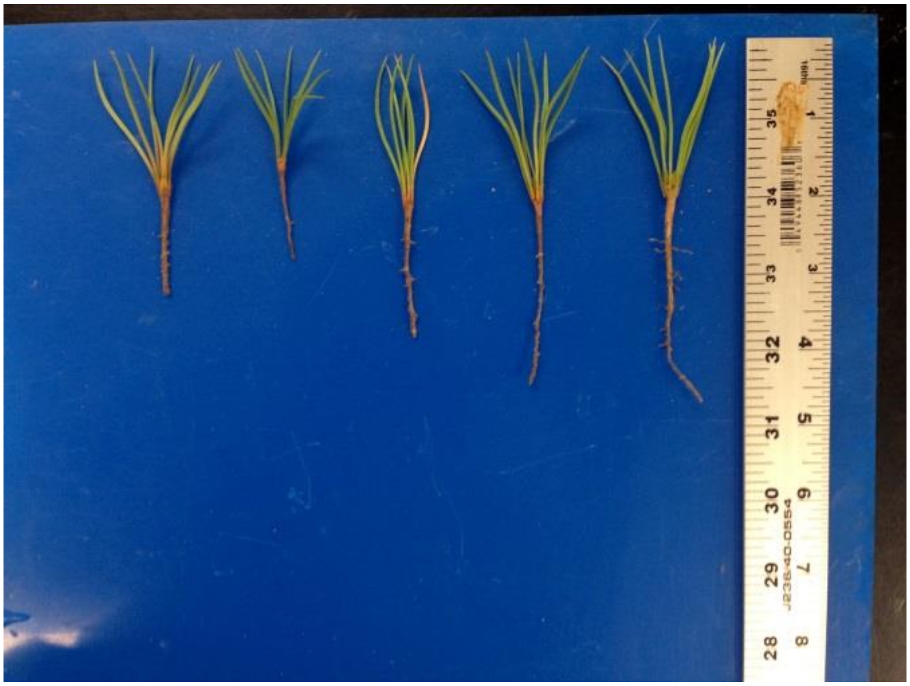


Figure 4.22. Longleaf pine grown in coarse textured non-treated soil. Image taken 19 weeks after the treatment was applied to the soil and 15 weeks after sowing.

Figure 4.23 Dry weight of tomato (*Lycopersicon spp*) grown in sand across all three herbicide rates.

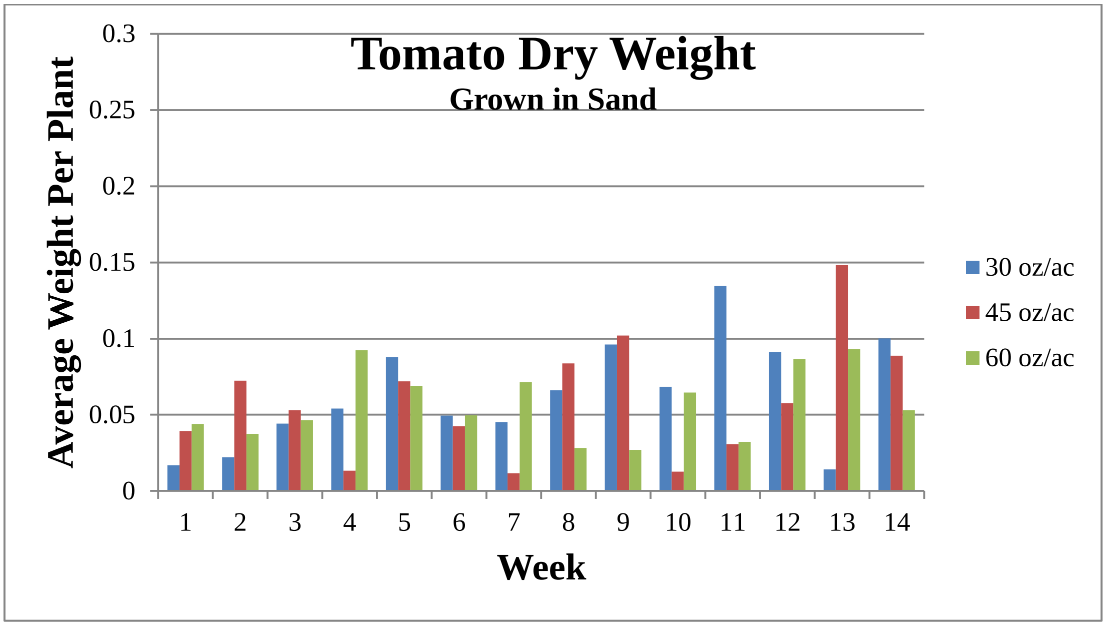


Figure 4.24 Dry weight of tomato (*Lycopersicon spp*) grown in clay across all three herbicide rates.

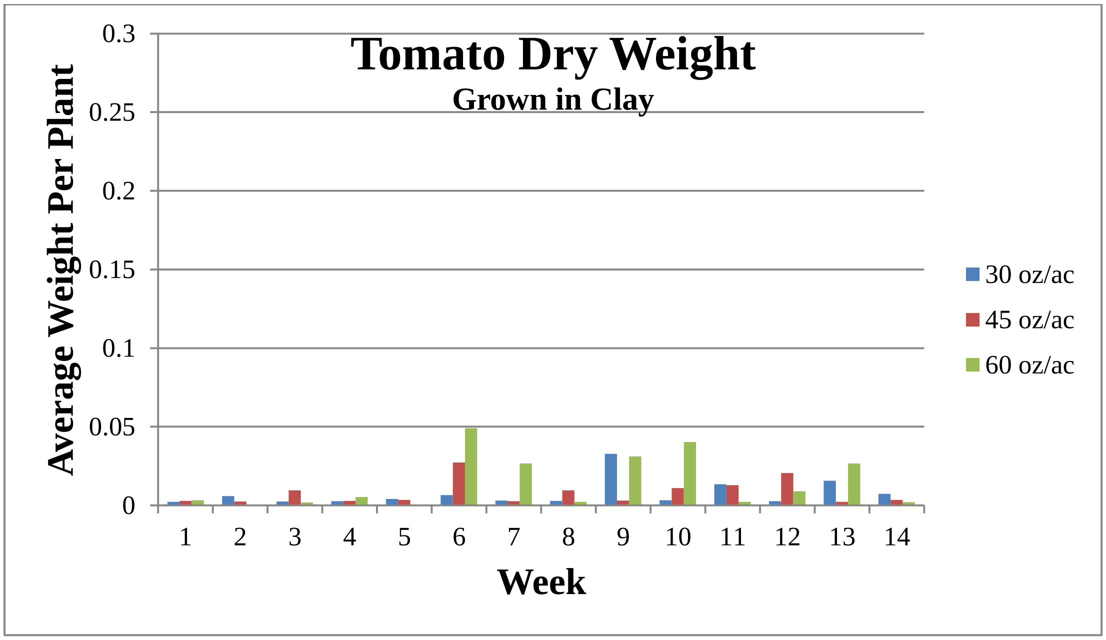


Figure 4.25 Dry weight of sorghum *(Sorghum bicolor)* grown in sand across all three herbicide rates.

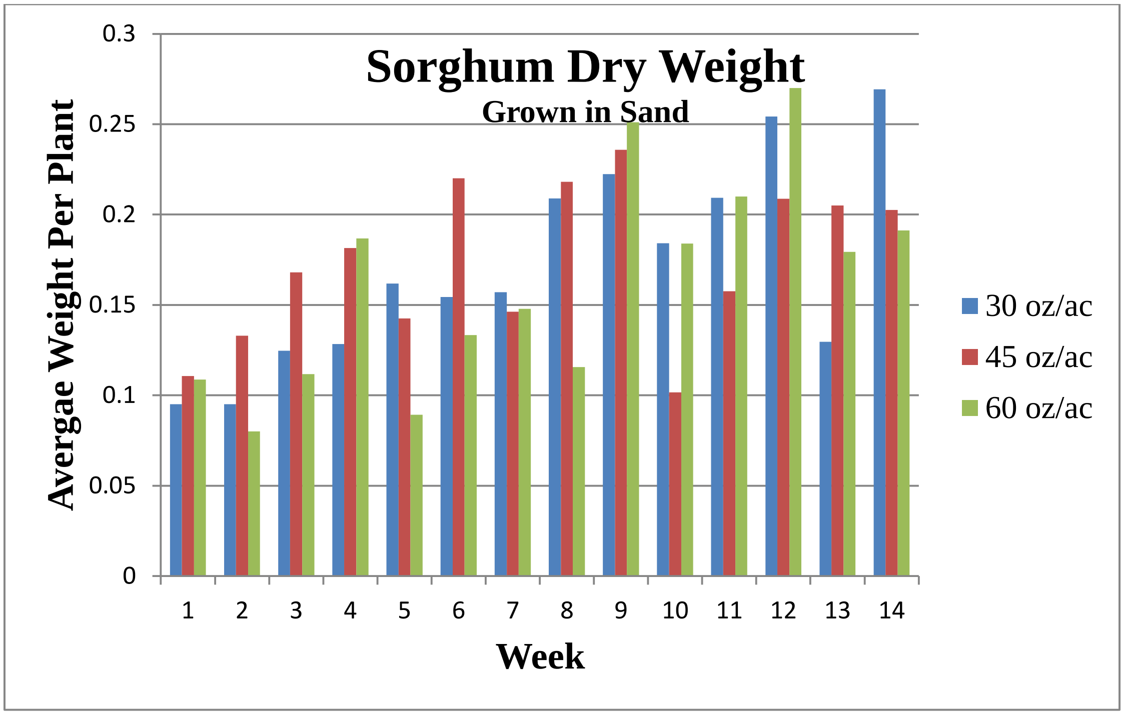


Figure 4.26 Dry weight of sorghum *(Sorghum bicolor)* grown in clay across all three herbicide rates.

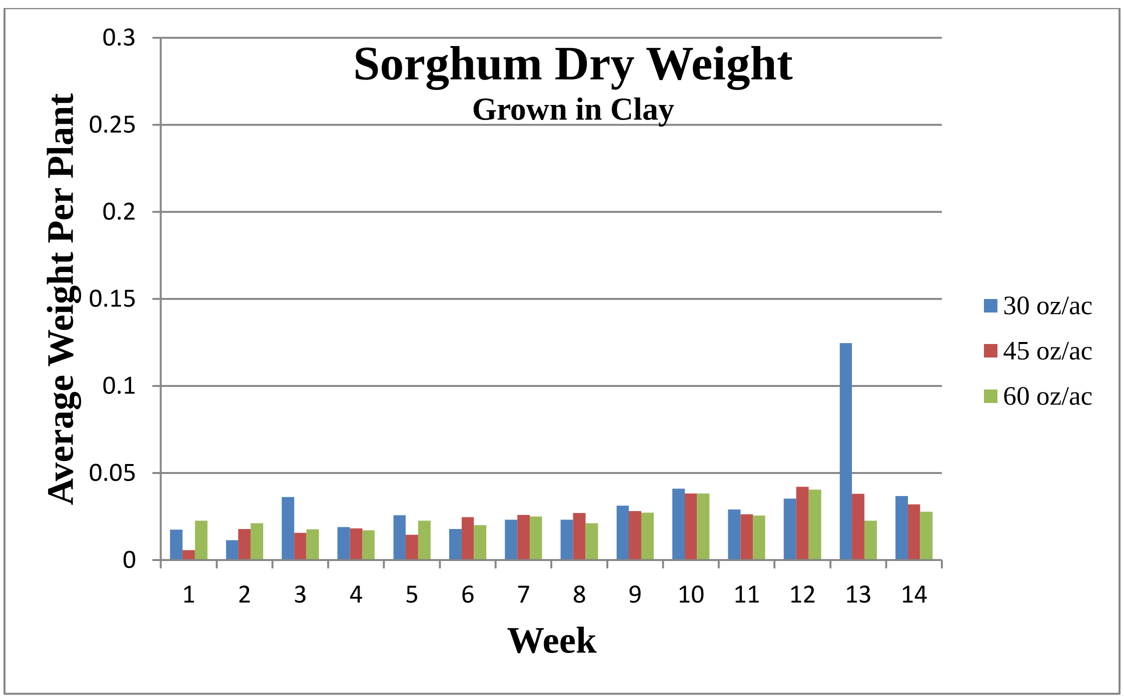


Figure 4.27 Dry weight of cucumber *(Cucumis spp)* grown in sand across all three herbicide rates.

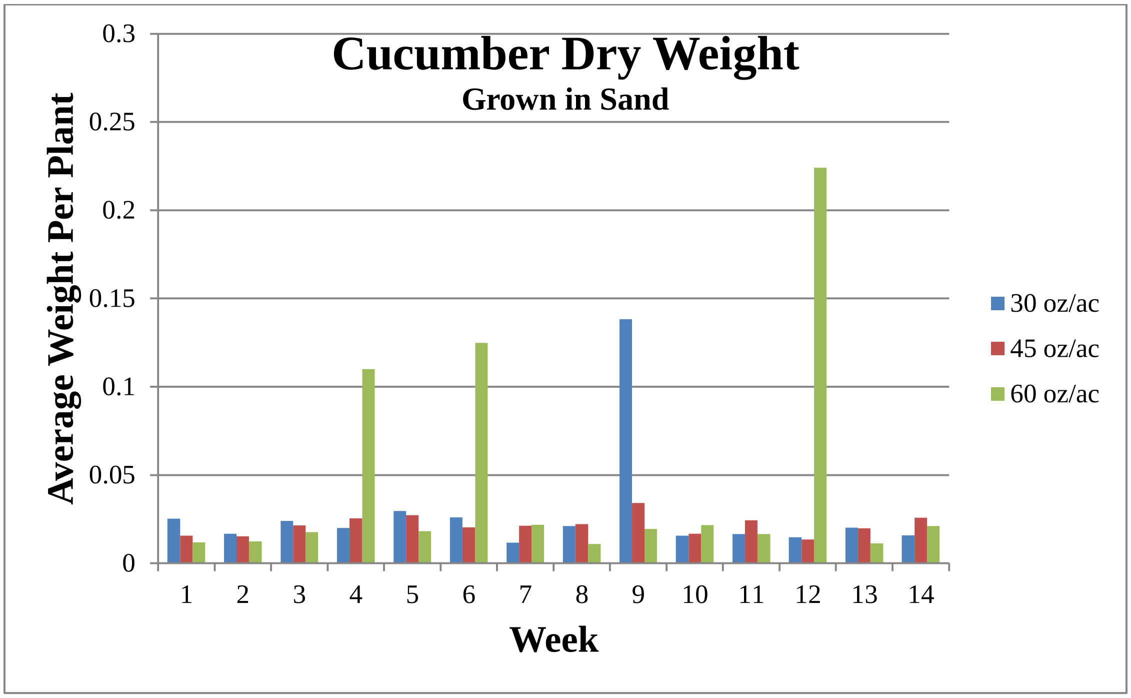


Figure 4.28 Dry weight of cucumber *(Cucumis spp)* grown in clay across all three herbicide rates.

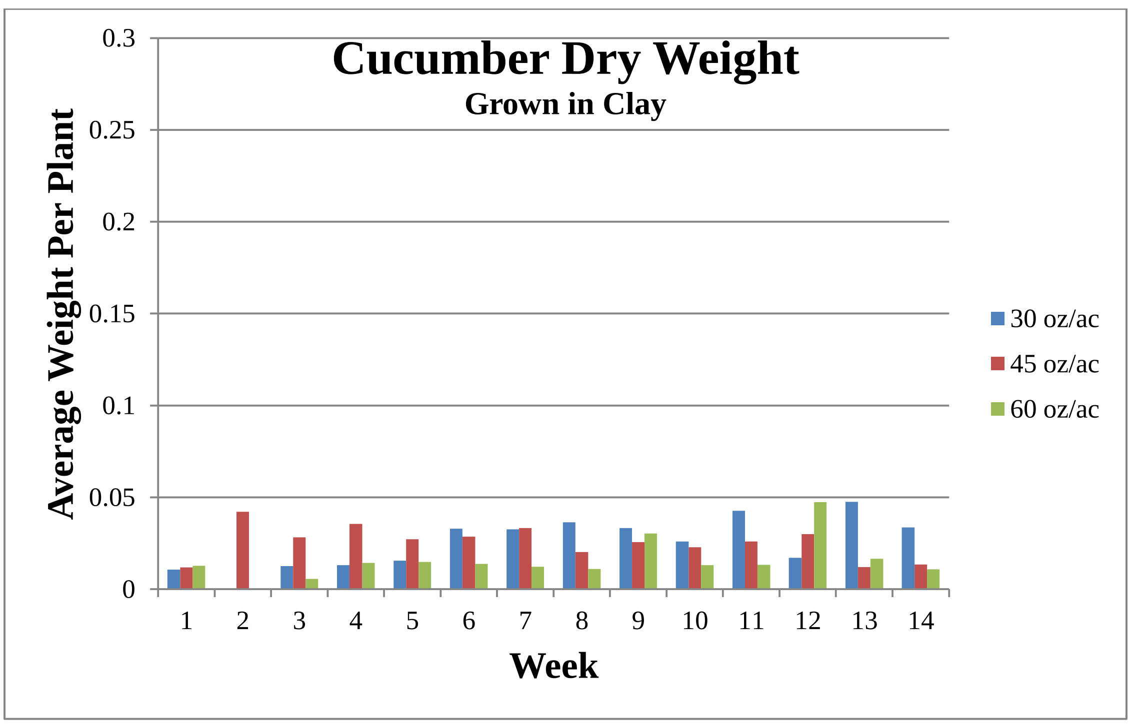


Figure 4.29 Dry weight of lettuce *(Lactuca sativa)* grown in sand across all three herbicide rates.

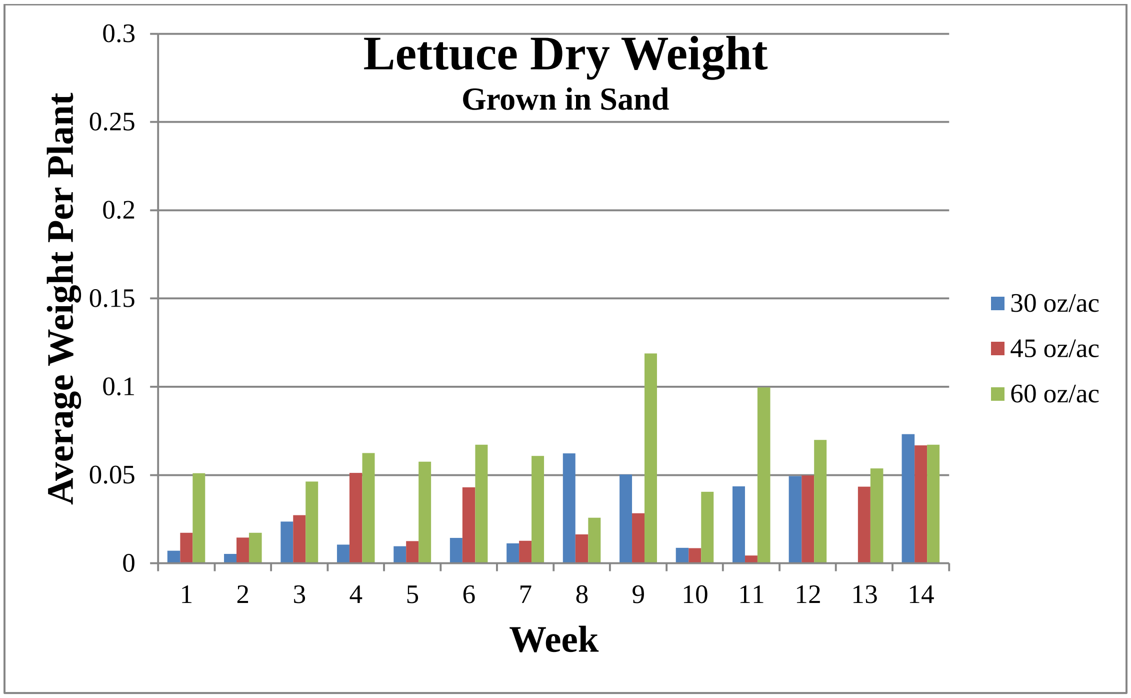


Figure 4.30 Dry weight of lettuce *(Lactuca sativa)* grown in clay across all three herbicide rates.

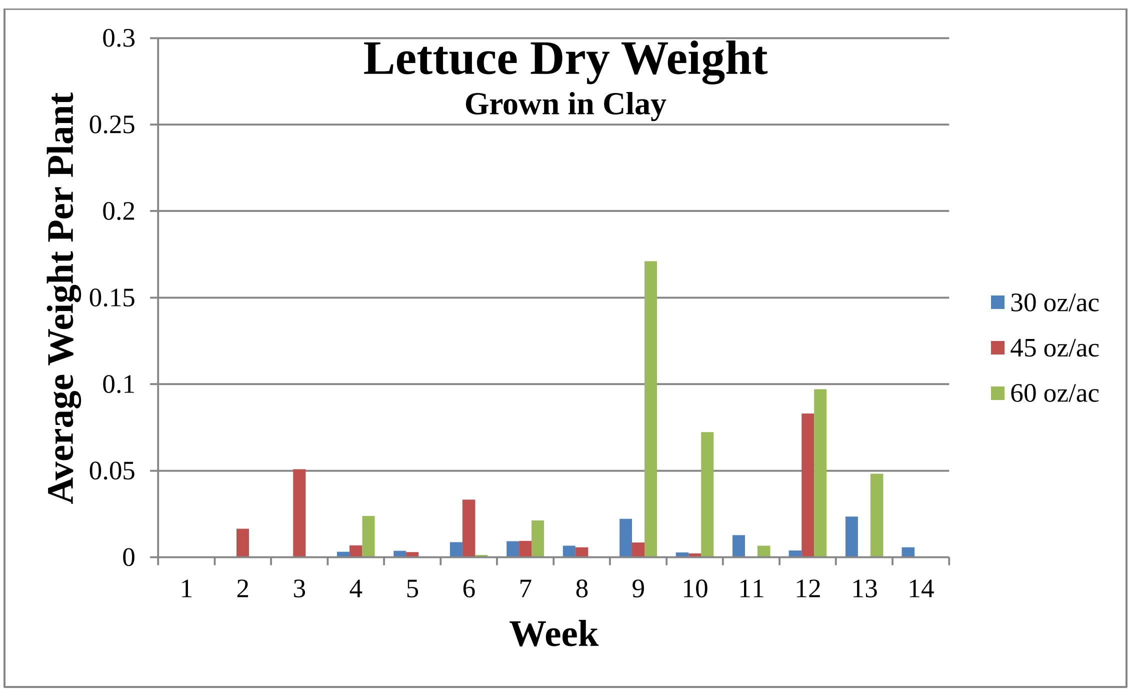


Figure 4.31 Dry weight of radish *(Raphanus sativus)* grown in sand across all three herbicide rates.

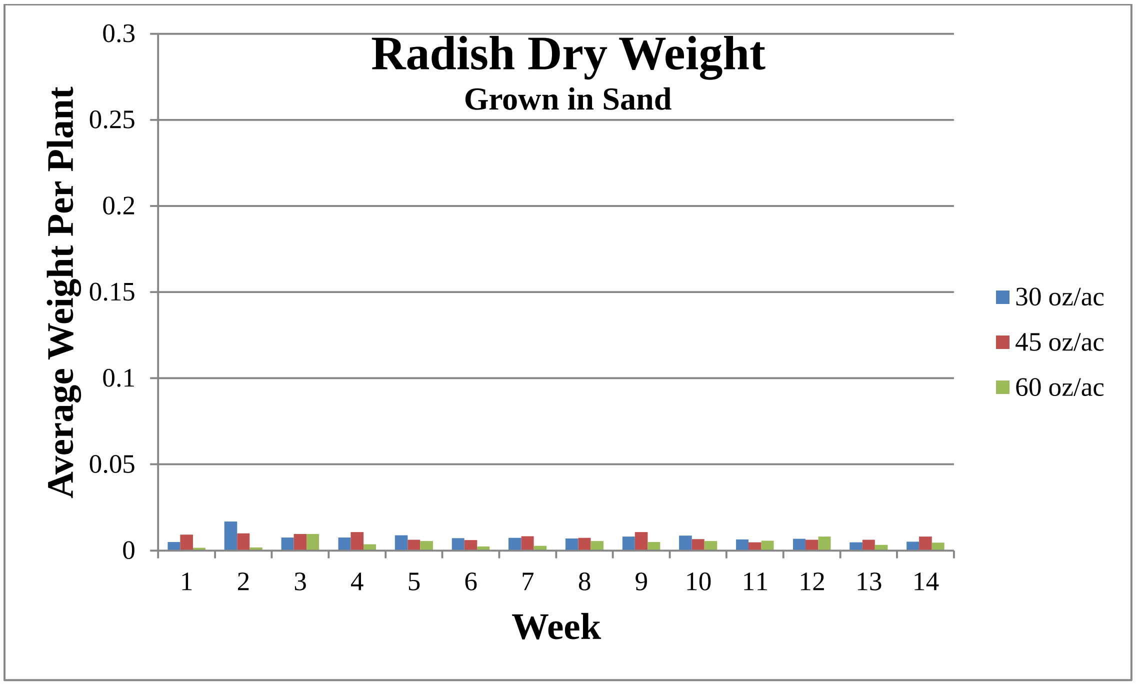


Figure 4.32 Dry weight of radish *(Raphanus sativus)* grown in clay across all three herbicide rates.

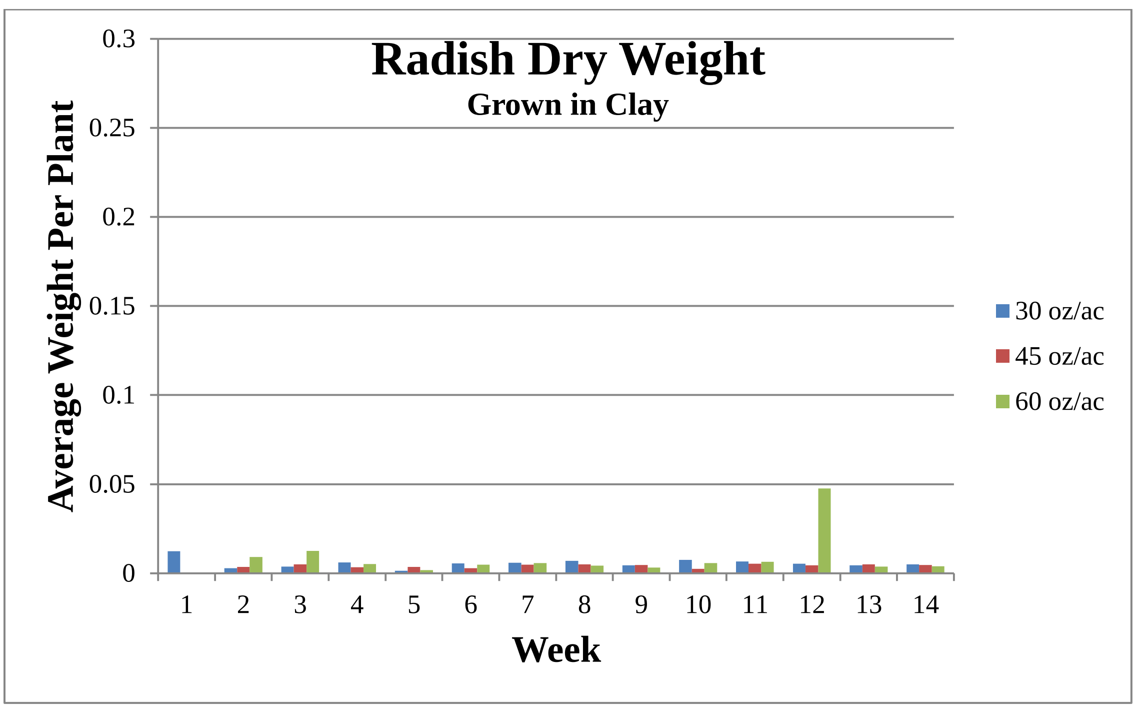


Figure 4.33 Dry weight of cabbage *(Brassica oleracea)* grown in sand across all three herbicide rates.

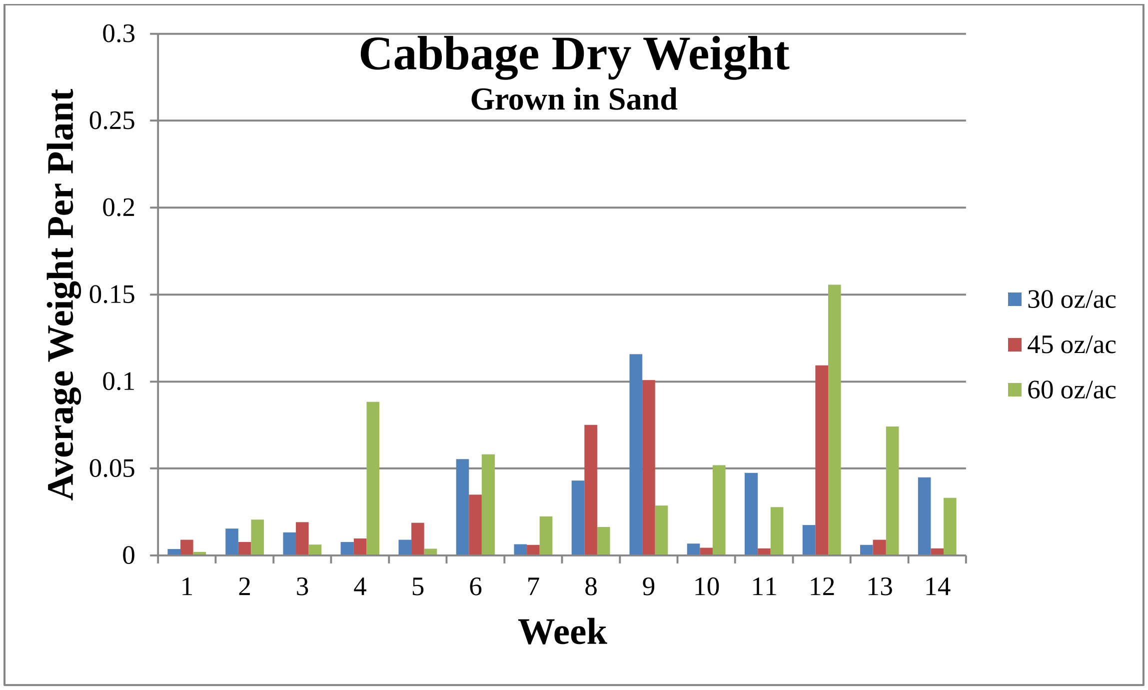


Figure 4.34 Dry weight of cabbage *(Brassica oleracea)* grown in clay across all three herbicide rates.

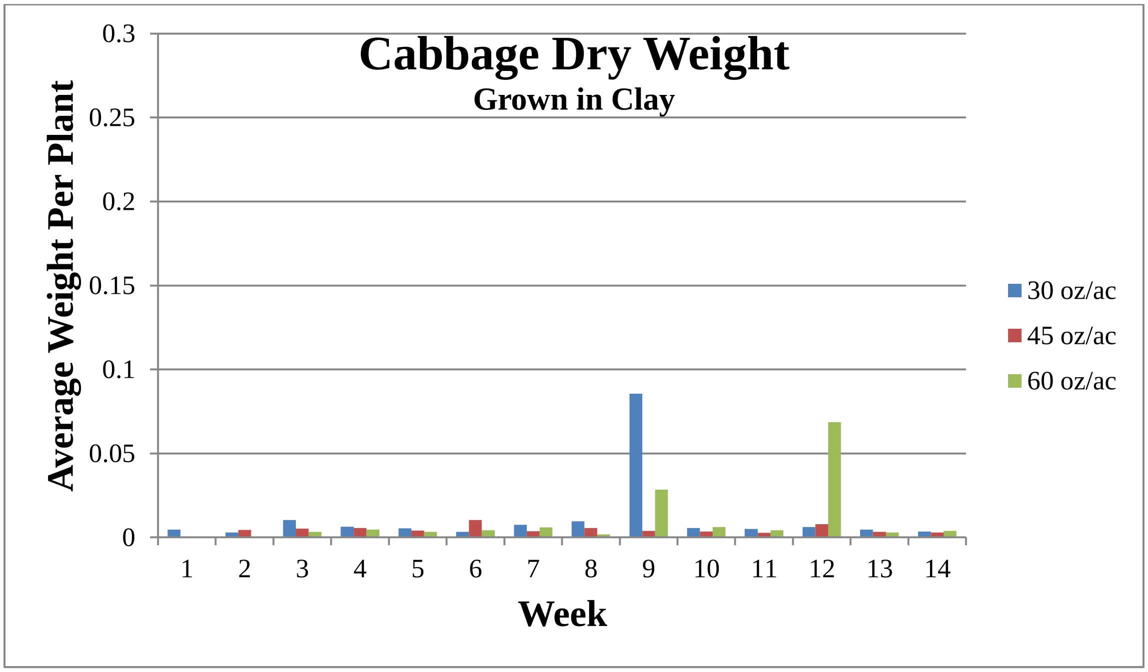


Figure 4.35 Dry weight of longleaf pine *(Pinus palustris)* grown in sand across all three herbicide rates.

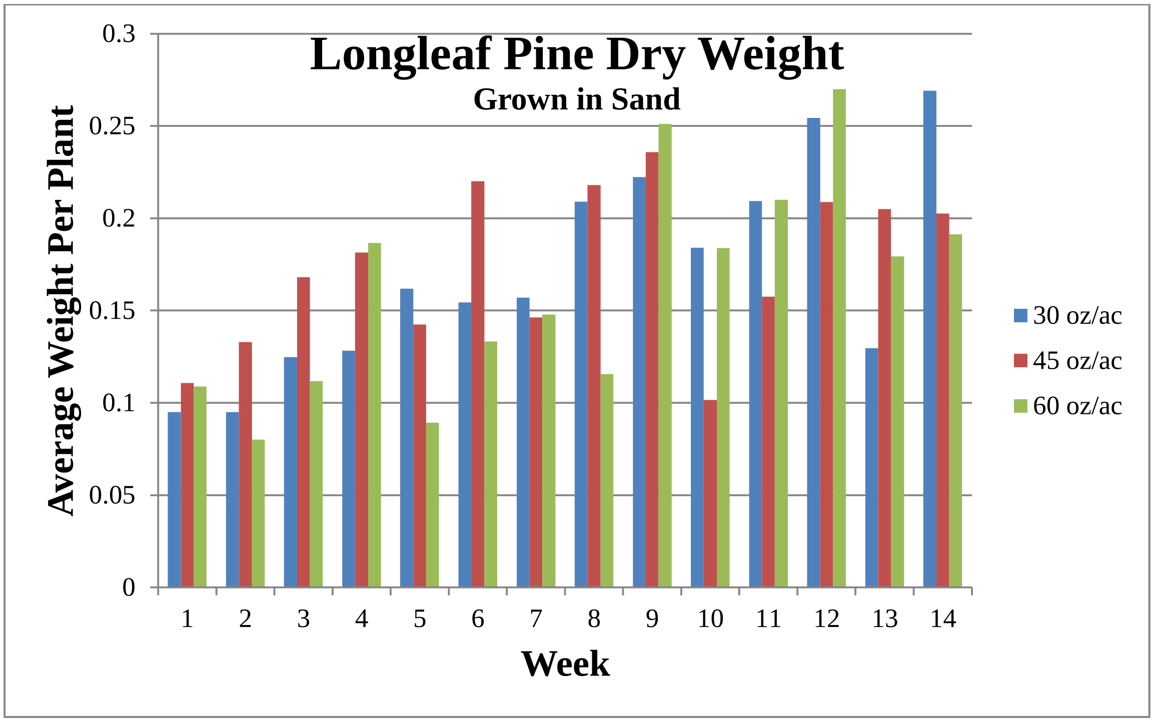
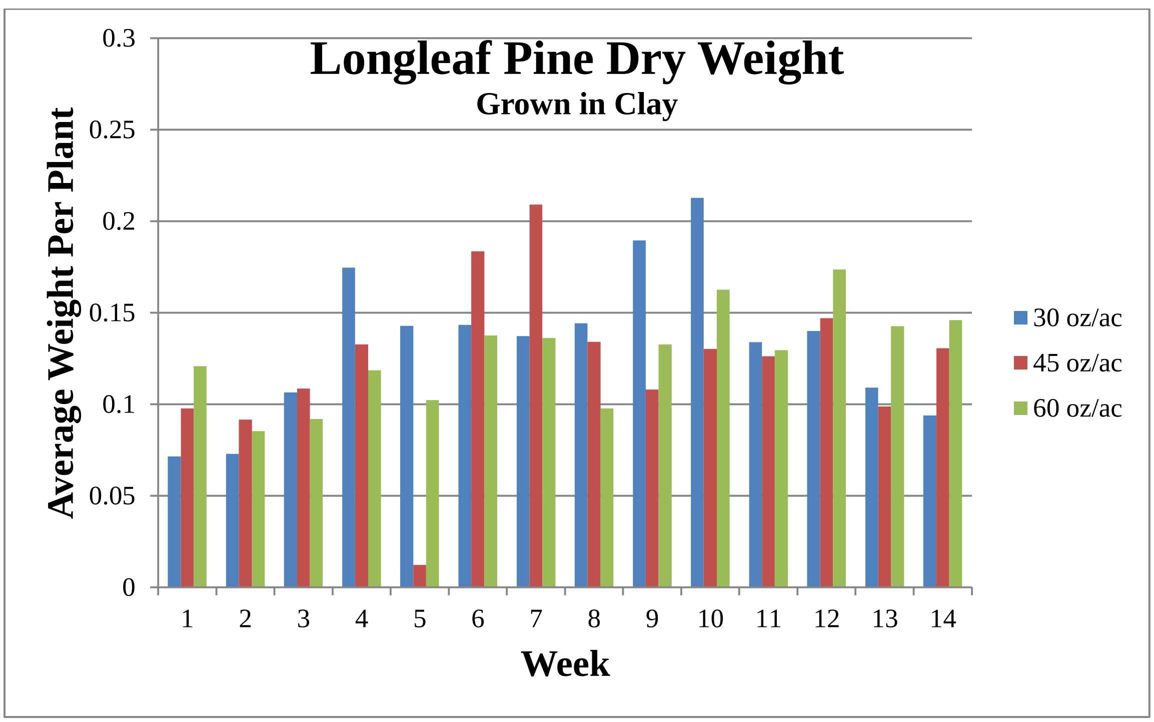


Figure 4.36 Dry weight of longleaf pine *(Pinus palustris)* grown in clay across all three herbicide rates.



References

America's Longleaf. 2009. Range-wide conservation plan for longleaf pine. Report prepared for the Steering Committee of the Regional Working Group for America's Longleaf.

Aschenbach, T.A., Foster, B.L., Imm, D.W. 2010. The Initial Phase of a Longleaf Pine Wiregrass Savanna Restoration: Species Establishment and Community Responses. Restoration Ecology. 18:762-771.

Anonymous. Bioassay Test for Herbicide Residues in Compost: Protocol for Gardeners and Researchers in Washington State. 2002. Washington State University. Washington State Department of Ecology.

Brockway, D.G., Outcalt, K.W. 2000. Restoring Longleaf Pine Wiregrass Ecosystems: Hexazinone Application Enhances Effects of Prescribed Fire. Forest Ecology and Management. 137:121-138.

Brockway, D.G., Outcalt, K.W., Wilkins, R.N. 1998. Restoring Longleaf Pine Wiregrass Ecosystems: Plant Cover, Diversity and Biomass Following Low-Rate Hexazinone Application on Florida Sandhills. Forest Ecology and Management. 103:159-175.

Brockway, D.G., Lewis, C.E. 1996. Long-term Effects of Dormant-Season Prescribed Fire on Plant Community Diversity, Structure and Productivity in a Longleaf Pine Wiregrass System. Forest Ecology and Management. 96:167-183.

Burns, R.M., and Honkala, B.H. 1990. Silvics of North America: 1. Conifers; 2. Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC. vol.2, 877 p.

Croker, T.C.Jr. 1987. Longleaf Pine: A History of Man and a Forest. USDA Forest Service. Forestry Report R8-FR7.

Dale, V.H., Beyeler, S.C., Jackson, B. 2002. Understory Vegetation Indicators of Anthropogenic Disturbance in Longleaf Pine Forests at Fort Benning, Georgia, USA. Ecological Indicators. 1:155-170.

Dayan, F.E., Green, H.M., Weete, J.D., Hancock, G. 1996. Postemergence Activity of Sulfentrazone: Effects of Surfactants and Leaf Surfaces. Weed Science. 44:797-803.

Dickens, D., Minogue, P., Moorhead, D. 2012. A Guide to Using Imazapyr for Chemical Site Preparation in Southern Pine Plantation Establishment. The University of Georgia, Cooperative Extension Service. 5 pp.

Espana, P.S., Loureiro, I., Escorial, C., Chueca, C., Montanya, I.S. 2011. The Bioassay Technique in the Study of Herbicide Effects. ISBN. 978-953-307-975-2.

Farm Service Agency. 2011. Conservation Reserve Program: Longleaf Pine Initiative. United States Department of Agriculture. Fact Sheet.

Fauci, M., Bezdicek, D.F., Caldwell, D., Finch, R. 2013. Development of Plant Bioassay to Detect Herbicide Contamination of Compost at or Below Practical Analytical Detection Limits. Bulletin of Environmental Contamination and Toxicology. 68(1): 79-85.

Folsom, B. L. Jr., Price, R. A. 1991. A Plant Bioassay for Assessing Plant Uptake of Contaminants from Freshwater Soils or Dredged Material. American Society for Testing and Materials. 2:172-177.

Freeman, J.E., Jose, S. 2009. The Role of Herbicide in Savanna Restoration: Effects of Shrub Reduction Treatments on the Understory and Overstory of a Longleaf Pine Flatwoods. Forest Ecology and Management. 257:978-986.

Frost, C. 1993. Four Centuries of Changing Landscape Patterns in the Longleaf Pine Ecosystem. North Carolina Plant Conservation Program.

Futch, S. H., Singh, M. 1999. Herbicide Mobility Using Soil Leaching Columns. Bulletin of Environmental Contamination and Toxicology. 62: 520-529.

Hager, A.G., Nordby, D. 2007. Herbicide Persistence and How to Test for Residues in Soils. Illinois Agricultural Pest Management Handbook. 15:343-350.

Harrington, T.B., Dagley, C.M., Edwards, M.B. 2003. Above- and Belowground Competition from Longleaf Pine Plantations Limits Performance of Reintroduced Herbaceous Species. Forest Science. 49:681-695.

Jackson, P.D., Enebak, S.A., West, J., Hinnant, D. 2015. Assessing Tolerance of Longleaf Pine Understory Herbaceous Plants to Herbicide Applications in a Container Nursery. BSSRC. Forest Service. 265-271.

Johnson, R. 1999. Does Longleaf Make Dollars and Sense? Alabama’s Treasured Forests. Vol. 18. 4:26-27.

Jose, S., Ranasinghe, S., Ramsey, C.L. 2010. Longleaf Pine (Pinus palustris P. Mill.) Restoration Using Herbicides: Overstory and Understory Vegetation Responses on a Coastal Plain Flatwoods Site in Florida, U.S.A. Restoration Ecology. 18:244-251.

Kaeser, M.J., Kirkman, K.L.. 2010. The Effects of Pre- and Post-emergent Herbicides on Non-target Native Plant Species of the Longleaf Pine Ecosystem. Journal of the Torrey Botanical Society. 137:420-430.

Kush, J.S., Meldahl, R.S., Boyer, W.D. 1999. Understory Plant Community Response after 23 Years of Hardwood Control Treatments in Natural Longleaf Pine (*Pinus palustris*) Forests. Canadian Journal of Forest Research. 29:1047-1054.

Landers, L.J., Van Lear, D.H., Boyer, W.D. 1995. The Longleaf Pine Forests of the Southeast: Requiem or Renaissance? Journal of Forestry. Vol. 93. 11:39-44.

Moorman, T., Reeves, B.F. 1979. The Role of Endomycorrhizae in Revegetation Practices in the Semi-arid West. II. A Bioassay to Determine the Effect of Land Disturbance on Endomycorrhizal Populations. American Journal of Botany. 66(1): 14-18.

Mulligan, M.K., Kirkman, K.L. 2002. Burning Influences on Wiregrass (*Aristida stricta*) Restoration Plantings: Natural Seedlings Recruitment and Survival. Restoration Ecology. Vol. 10. 2:334-339.

Mulligan, M.K., Kirkman, K.L., Mitchell, R.J. 2002. *Aristida beyrichiana* (Wiregrass) Establishment and Recruitment: Implications for Restoration. Restoration Ecology. Vol. 10. 1:68-76.

Norcini, J.G., Aldrich, J.H. 1997. Bittercress Control in Wiregrass Tubeling Production. Proceedings-Florida State Horticultural Society. 110:133-135.

Outcalt, K.W. 1992. Factors Affecting Wiregrass (Aristida stricta Michx.) Cover on Uncut and Site Prepared Sandhills Areas in Central Florida. Ecological Engineering. 1:245-251.

Outcalt, K.W., Williams, M.E., Onokpise, O. 1999. Restoring *Aristida stricta* to *Pinus palustris* Ecosystems on the Atlantic Coastal Plain, U.S.A. Restoration Ecology. Vol. 7. 3:262-270.

Pfeiffer, M.. 2004. Herbicide Detection in Soils via Bioassays. Pesticide Training Resources.

Pike, D.R., Hager, A. 1998. How Herbicides Work. University of Illinois: Department of Crop Sciences.

Rashid, A., Sharma, P., Evans, I. 2001. Plant Bioassay Techniques for Detecting and Identifying Herbicide Residues in Soil. Alberta Agriculture, Food and Rural Development. Agdex 609-1.

Schmidtling, R.C., Hipkins, V. 1998. Genetic Diversity in Longleaf Pine (*Pinus palustris*): Influence of Historical and Prehistorical Events. Canadian Journal of Forest Research. 28:1135-1145

Tchan, Y. T., Roseby, Joan E., Funnell, G. R. 1975. A New Rapid Specific Bioassay Method for Photosynthesis Inhibiting Herbicides. Soil Biology Biochemistry. 7: 39-44.

Van Lear, D.H., Carroll, W.D., Kapeluck, P.R., Johnson, R. 2005. History and Restoration of the Longleaf Pine-Grassland Ecosystem: Implications for Species at Risk. Forest Ecology and Management. 211:150-165.

Whiting, D., Wilson, C., Moravec, C., Reeder, J.. 2014. Managing Soil Tilth: Texture, Structure, and Pore Space. Colorado State University Extension. Colorado Master Gardener Program. CGM GardenNotes #213.