OPTIMIZATION OF SEED PROPAGATION OF SEVEN NATIVE PLANT SPECIES

By

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Seven plants native to the moist habitats of the pine savannas, woodlands, and Hillside Bog natural area at the Crosby Arboretum, Picayune, MS, were evaluated under laboratory and nursery conditions to determine seed germination percentage, optimal germination temperature, and the effect of substrates on germination. These native plants include: titi (*Cyrilla racemiflora* L.), buckwheat tree (*Cliftonia monophylla* Britt.), flameflower (*Macranthera flammea* (Bartr.) Pennell), deertongue (*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*), pink coreopsis (*Coreopsis nudata* Nutt.), tall ironweed (*Vernonia angustifolia* Michx.), and swamp bay (*Persea palustris* (Raf.) Sarg.). Laboratory experimentation concluded with germination and determination of optimal temperature regimes. Tall ironweed had the highest rate of success in the nursery. Black Kow compost had suboptimal performance compared to Sunshine Mix 1 and pine bark / sand under nursery conditions. Several of the species tested had minimal germination and require further research to optimize germination and nursery growth.

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INTRODUCTION

There is an ever increasing need for ecological restoration due to many natural and man-made disasters such as hurricanes, toxic oil spills, and mining (Blanchard, 2007). At least 83 percent of the Earth's land surface has been altered with estimations of around 60 percent of our ecosystems being classified as degraded from such practices as overgrazing and logging (Groom et al., 2006). Many definitions have been given to define ecological restoration. For the purposes of this research ecological restoration is defined as "the returning of a system that has been altered, degraded, or destroyed to a state that closely mimics pre-disturbance conditions" (Anderson, 2007).

As land use intensifies from a more natural to a more artificial landscape, there is a great reduction in the populations of all species of flora and fauna. This reduction is directly associated with anthropogenic impacts, the presence of humans and their impacts on ecosystems, from practices such as unsustainable agriculture, leaching of toxic chemicals, urbanization, logging, and mining (Groom et al., 2006).

The Coastal Roots School Seedling Nursery Program (Coastal Roots) was developed by Louisiana State University (LSU) in 2000 to aid restoration efforts in the wake of anthropogenic impacts and natural disasters (Coleman and Bush, 2002). Coastal Roots has been adopted by the Coastal Research and Extension Center (CREC) of Mississippi State University (MSU) working with schools ranging from elementary to high schools (Coker et al., 2010). In constructing a small nursery on the schools' premises, science students are able to propagate, grow, and care for native plants to be used in restoration plantings within a predesignated restoration site. In the classroom, the students learn the issues and importance of ecological restoration, nursery maintenance, plant growth, wetlands, and other restoration and conservation issues.

To generate plant materials for restoration purposes, there is a great need for information on how to propagate many native species. Popular species are well known in the nursery industry and easily propagated, however, they represent a very small portion of the plant species which historically contribute to a given ecosystem's biodiversity. Many other species important to biodiversity have very little known about their propagation. Therefore, research was conducted to determine seed propagation requirements of seven native plant species. The objectives of this research were: 1) to identify native plants of significance for ecological restoration from various habitats located in southern Mississippi; specifically, plants with potential for production in a small, school-based nursery system; 2) to determine the optimal temperature regime producing the greatest germination percentage for each species; and 3) to determine the viability of seed propagation of these seven native plants in a small, school-based nursery system.

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CHAPTER II

LITERATURE REVIEW

2.1 Introduction

Nurseryman, ecological restorationists, and others alike have been propagating plant species from seed for many centuries. This method of propagation is one way we as humans mimic and alter natural processes to mass produce desirable plant species for certain uses such as the ornamental market and ecological restoration purposes. Seed carry genetic material that ultimately will result in seedlings of similar characteristics to that of the parent plant from each ecotype. This becomes extremely important with maintaining genetic diversity being a primary goal. Germinating seed from similar locations to which they will be planted is also important to the production of seedlings with the capability of thriving under a set of targeted circumstances (Dirr and Heuser, 2006; Luna and Wilkinson, 2009).

2.2 Seed collection and storage

To establish quality, native seedlings for a restoration project, one of the first steps should be to correctly identify the source plant from which the seed are taken with importance being placed on collecting from healthy, vigorous, local plant populations (Dirr and Heuser, 2006; Karrfalt, 2008; Lippitt et al., 1994). Being sure seed are able to be distinguished from trash and having a good knowledge of the seed and fruit structures are other important aspects (Karrfalt, 2008). To increase propagation success, collection should not occur until seed are completely ripened which can prove difficult to distinguish (Baskin and Baskin, 2001; Dirr and Heuser, 2006; Karrfalt, 2008). Seed from some plants require two to three years in order for maturity to be reached. At maturity, the seed are at their highest level of viability and vigor (Karrfalt, 2008). One good indication seed have ripened is when their fresh weight shows no further increase (Baskin and Baskin, 2001; Dirr and Heuser, 2006). Most often for collection purposes seed maturity is determined from the exogenous appearance of seed or fruits. Judging by appearance alone has proven to be a logical choice even though this may not always be the best indication due to variability in populations and weather (Karrfalt, 2008). Another method can be to harvest seed at the onset of natural dispersal (Baskin and Baskin, 2001). However, collecting the first group of seed or fruit that detach should be avoided due to the possibility of dispersal being caused from poor quality or death. Whichever method or combination of methods used, collection should take place before seed are lost to natural seedfall, predation, insect-feeding, or seed deterioration. Weather and location also play significant roles regulating the availability and maturity of seed. Therefore, paying attention to such events will greatly benefit seed collection efforts to acquire a high-quality seedlot (Karrfalt, 2008).

Methods to physically collect seed or fruit are directly dependent on the botanical nature of the mother plant. Some fruit may require sharp tools in order to remove them from the plant. Such fruit are termed persistent fruit due to their inability to detach readily. On the contrary, seed that disperse easily are gathered quickly by the bare hand. Use of rakes, hooks, shovels, or vacuums can aid with collecting seed from low plants or off the ground. Netting can also be used to collect seed falling from plants when allowing natural dispersal to take place. There are also a number of mechanical means ranging from seed harvesters to tree shakers which aid in mass collection efforts (Karrfalt, 2008).

Once seed are collected, proper identification should be maintained on seedlots by placing labels on the inside and outside of all containers (Karrfalt, 2008). Transportation of the seed should occur promptly and quickly to minimize potential loss of quality. It is best to use ventilated containers or bags for the transport of dry seed; whereas, fleshy fruit and recalcitrant seed can be placed in plastic bags and set inside a cooler to prevent damage from high temperatures and desiccation since maintaining hydration is crucial (Karrfalt, 2008; Luna and Wilkinson, 2009).

Once seed have been collected and transported back to the processing facilities, all necessary cleaning processes should be conducted. During any phase it is important to ensure all recalcitrant seed are kept moist throughout the process. This can be accomplished by placing seed under moistened burlap or within plastic bags of wet sand or peat at low temperatures. Orthodox seed can be spread over a mesh screen or placed in paper bags to allow them to dry. If placing in bags, be sure to keep the contents loose. Also, seed placed over mesh will need to be turned regularly throughout the day. It would be best to construct or purchase a drying rack that allows for good air flow when drying seed on screens. Temperatures should not exceed 27°C or fall below 18°C and a low relative humidity needs to be maintained to prevent mold from excess moisture (Luna and Wilkinson, 2009).

Seed need to be cleaned before being stored or planted. Cleaning procedures have the ability to increase germination percentages and the rate of germination of seed from certain plant species. However, depending on the quantity of seed to be cleaned, this can require expensive equipment and prove to be a labor intensive and time consuming task (Luna and Wilkinson, 2009).

Compared to orthodox seed, recalcitrant seed can usually be collected cleanly and stored or planted immediately so as not to dry out. This is not always the case though as some will need to be cleaned of debris and trash. A floatation test can be used to clean recalcitrant seed by filling buckets with water and then pouring seed into the buckets. Healthy, viable seed will normally sink to the bottom while trash, debris, and nonviable seed will float at the top of the water column. This method also helps to keep the seed hydrated, preventing desiccation. Some seed may need to soak longer than others, or overnight, to ensure all viable seed have sunk (Luna and Wilkinson, 2009).

Soaking fleshy fruit a few hours or a few days is often advantageous for the cleaning process. This softens the pulp and allows it to be separated more easily. The water needs to be changed every few hours or allowed to constantly flow to maintain a cool, fresh supply throughout the soaking process. Once properly soaked, seed from fleshy fruit are often extracted by a macerator. If adjusted correctly, the mechanism will de-pulp without causing damage to the seed itself. Bars rotate through the seed rubbing them together separating the pulp from the seed. While operating, water enters the hopper to flush the separated material away and the seed can be collected for further use. Small seedlots can be de-pulped using a modified food blender by coating the blades with a rubberized product (Karrfalt, 2008; Luna and Wilkinson, 2009).

Orthodox seed usually require a drying process before seed extraction. Once properly dried, many mechanisms or methods can be used to separate seed from capsules and trash (Karrfalt, 2008). Cleaning dehiscent fruit is normally easy due to the fruit opening at maturity. These seed can be extracted through simple procedures such as tumbling or shaking the fruit in paper bags and then separating (Luna and Wilkinson, 2009). Hulling is necessary for indehiscent fruit to remove fruit walls or wings. Mechanisms that accomplish this include: hammer mills, brush machines, de-wingers, and debearders; however, hammer mills can be too destructive for some seed. Once seed have been extracted, separation of trash and debris takes place. This cleaning process has been revolutionized by cleaning large quantities of seed using air-screen machines, screens, aspirators, and blowers (Karrfalt, 2008).

To prolong seed viability after collection, it is important to properly store any seed not immediately sown. Generally, viability can be enhanced and prolonged as seed moisture and temperature each decrease by 1% and 4°C, respectively (Lippitt et al., 1994). However, seed viability after long-term storage varies from species to species (Luna and Wilkinson, 2009). Seed should be dried to a maximum of 14% moisture and then stored under a low constant humidity between the range of 10 to 40%. Refrigerators, freezers, and room-temperature storage can be used to successfully store dry seed and maintain an environment with a low relative humidity (Lippitt et al., 1994; Luna and Wilkinson, 2009).

Recalcitrant seed need to be stored under cool, moist environments if not immediately sown and can only be stored for a limited time before viability is lost. To prolong viability of nondormant seed, seed moisture content needs to be maintained between 35 and 50%. Also, the seed should be stored in a way to allow for gas exchange. This is readily done by storing the seed in unsealed containers in peat moss under refrigeration (Luna and Wilkinson, 2009).

Storing dry seed in sealed containers helps to maintain low moisture content. Respiration from the seed within sealed containers decreases oxygen and increases carbon dioxide; thus, promoting prolonged viability. Maintaining low seed moisture content can also be accomplished by controlling humidity within storage environments. It is also necessary to use containers that will protect seed from predation by rodents and other pests (Lippitt et al., 1994).

2.3 Seed germination

Seed germination has been defined as "the beginning or resumption of growth" to include all processes that "commence with the water uptake by the seed (imbibition) and ends with the start of elongation by the embryonic axis, usually the radicle" (Bentsink and Koornneef, 2008; Bewley and Black, 1994; Raven et al., 2005). Water, oxygen, and temperature are three important factors affecting germination. The presence of light can also be important for germination of certain plant species. The process cannot commence unless imbibition takes place (Raven et al., 2005). Many of the processes involved during germination include protein hydration, subcellular structural changes, respiration, macromolecular syntheses, and cell elongation (Bewley and Black, 1994). In combination, these processes increase the rate of metabolism within the embryo in preparation for germination. Radicle penetration through the seed coat is a visual cue of the completion of germination; this occurrence is referred to as visible germination (Bewley and Black, 1994).

The germination process in a mature seed is initiated by the uptake of water and its ability to imbibe water is related to its water potential compared to the surrounding substrate. Water potential is an expression of the energy status of water. The diffusion of water occurs across the energy gradient from high to low water potential (Bewley and Black, 1994). Water uptake will occur when the seed's water potential is lower than its surroundings, provided some other force does not prevent the process. Three phases occur during water uptake: phase 1, imbibition; phase 2, lag phase; and phase 3, radicle extension during which water uptake increases again. At the onset, a rapid rate of imbibition, phase 1, occurs due to the low water potential of the seed. Almost immediately, minor metabolic activities begin. As water enters, the water potential of the seed is increased to that of the substrate. Therefore, a plateau in the rate of water uptake in phase 2 is reached as the water potentials of the seed and substrate approach equilibrium. It is during phase 2 the major metabolic processes become initiated for radicle emergence and germination (Bewley and Black, 1994). Swelling of the seed also occurs as it imbibes water (Bewley, 1997). The third phase commences as the radicle emerges from the seed indicating germination. This increase in water uptake results as structural changes take place within the cells of the radicle as it elongates (Bewley and Black, 1994). Metabolic processes resume almost immediately once imbibition has begun if the necessary enzymes and structures are still present within the imbibed seed after being desiccated (Bewley, 1997). Stored food reserves become digested and used by the embryo once enzymes are activated and synthesized from the absorption of water. As this occurs, cells begin to enlarge and divide. To have continuous growth, additional nutrients and water are required (Raven et al., 2005).

During germination, solutes such as sugars, organic acids, ions, amino acids, and proteins may rapidly leak from the seed as imbibition commences. Colonization of bacteria and fungi can result due to this leakage and ultimately result in death of the seed; however, some seed are known to leak protective agents such as proteinase inhibitors and lectins that combat invasions of microbes and insects (Bewley and Black, 1994).

Respiration is one of the immediate processes that will occur as a seed imbibes water; wherein, stored food reserves within the seed are broken down and converted into energy to be used during germination and growth (Bewley, 1997; Bewley and Black, 1994). Typically, the embryo consumes oxygen in three phases with the first resulting in a rate of rapid increase, followed by a lag phase, and then another increase in rate of oxygen consumption. Radicle penetration occurs during the transition between the second and third phase. The second burst of oxygen consumption in phase III is associated with radicle elongation and growth (Bewley and Black, 1994).

Penetration of the radicle through the testa, or seed coat, marks the completion of germination. Many have argued whether cell expansion alone or in combination with cell division is responsible for the initial penetration. Regardless, this cell expansion occurs in two phases with the first having a slow rate and the second a rapid rate of expansion also including an increased number of cells being present at later times. Several factors may contribute to the initiation of growth by the radicle. Reductions in osmotic potential allow for an increased amount of water to enter the cells of the radicle which in turn creates a higher turgor pressure leading to radicle elongation. This elongation can occur more freely as the cell walls become more relaxed or as the tissues around the tip of the radicle weaken. However, reductions in osmotic potential, relaxation, and tissues becoming weaker can lead to radicle growth individually or by combination (Bewley and Black, 1994).

Viable seed that fail to germinate after being exposed to favorable conditions are said to be dormant (Baskin and Baskin, 2001; Baskin and Baskin, 2004; Bewley and Black, 1994; Heather et al., 2010; Hilhorst, 2007). Primary dormancy is expressed when seed are dormant when released from the mother plant. Secondary or induced dormancy can occur once seed are imbibed and then subjected to unfavorable conditions for germination (Bewley and Black, 1994; Hilhorst, 2007).

Five types of dormancy exist: physiological, morphological, morphophysiological, physical, and combinational (physical plus physiological). Physiological dormancy is exhibited when the radicle is unable to penetrate the seed coat or other physical layers of the seed acting as barriers. This type of dormancy is the most common among all but one vegetation realm on earth, the matorral. Species dependent physiological dormancy can be broken through warm stratification or afterripening for subtropical and tropical seed, and cold stratification plus the previously two mentioned for temperate areas of the globe. If germination is delayed due to embryos being undifferentiated or underdeveloped, morphological dormancy has taken place. This type of dormancy requires the seed to have more time than nondormant seed to allow for morphological changes or embryo growth before germination. Physical dormancy is exhibited on occurrence of impermeability of water through the seed coat and can be broken through environmental cues (Baskin and Baskin, 2004). Also, combinations of these types can occur between morphological and physiological and physiological and physical. These combinations are known as morphophysiological and combinational dormancy; respectively (Heather et al., 2010).

Germinating temperatures vary widely between seed of different plant species. The quantity of seed that will germinate during a period of time and the germination rate are both highly influenced by temperature. Generally, minimum, maximum, and optimum temperature ranges exist specifically for each species (Bewley and Black, 1994; Raven et al., 2005). Seed may germinate throughout the temperatures found within the limits of the minimums and maximums. Even though germination can occur within this range, the germination rate may be slower when compared to the optimum temperature regime (Bewley and Black, 1994). Temperature has the ability to induce or break dormancy in seed. Low temperatures during the winter months are known to act as a stratification process and break dormancy in some warm-season species, while high temperatures brought forth by summer months can increase dormancy levels. However, some cool-season species are quite the opposite as higher temperatures reduce and lower temperatures increase dormancy levels (Allen et al., 2007). Alternating temperatures are normally more conducive to germination as opposed to constant ones. The fluctuating rhythm of temperature also mimics environmental conditions seed are naturally exposed to. Seed under such temperatures require 4.5 to 8 hours of either high or low temperatures per day in order to reach maximum germination percentages. Germination can be enhanced in the presence of a 1°C difference between high and low temperatures (Baskin and Baskin, 2001).

Light is another environmental factor affecting the germination potential of various seed after imbibition has taken place (Allen et al., 2007). As opposed to breaking dormancy, germination in seed exposed to continuous light may be inhibited due to the high-irradiance reaction (HIR) (Bewley and Black, 1994). As seed are exposed to high irradiances for prolonged periods of time the germination process can be halted (Baskin and Baskin, 2001; Bewley and Black, 1994). This photoinhibition occurs just before the radicle emergence which suggests this affects cell elongation (Bewley and Black, 1994). The highest level to which seed may still germinate will vary among species. In aid of reversing this effect on some species, germination is promoted by exposure to darkness of just one hour per day. Under incubation studies of seed germination, light should be available for 12 to 14 hours per day with high temperatures correlated with the light period (Baskin and Baskin, 2001). This mimics the natural environment more closely by simulating day and night with light and darkness and alternating temperatures.

2.4 School-based nurseries

The concept of school-based gardens has been successfully spreading for the past 20 years and more often than not reaches out to elementary students. School-based gardens have been in existence since 1918. They were originally initiated to address aesthetic purposes and then tailored for food production during the Second World War. School gardens may use potted plants, raised beds, vermicomposting, in-ground plantings, specific habitat and butterfly gardens, ponds, composting areas, and areas on the school grounds for learning. These gardens are often established for academic, behavioral, recreational, social, political, or environmental remediation purposes (Blair, 2009).

With the majority of the United States's population being raised in metropolitan areas, children have little knowledge of the world's ecosystems and their processes. These metropolitan areas usually do not allow children to explore and seek nature beyond the lawn and expanse of asphalt found outside their door, children therefore, are restricted to video games, television, and organized sports for recreation. Schoolyard gardening allows students, from rural or urban settings, to engage with nature and learn a wide variety of diverse biological aspects and cycles pertaining to all life (Blair, 2009).

Very similar to schoolyard gardens, nurseries are being used by schools throughout the U.S. to provide a learning base for school-aged children while growing native plants for ecological restoration purposes. These school-based nurseries have been adopted by many programs such as the Louisiana State University (LSU) Coastal Roots

(CR) School Seedling Nursery Program, which has now been adopted by the Coastal Research and Extension Center (CREC) of Mississippi State University (Coker et al., 2010). Working with native plants in a school-based nursery under the CR program, teachers are able to instruct students on the issues and importance of ecological restoration and allow them to participate in various aspects of ecological restoration and conservation (Caffey, 2001). In this program, students are able to gain knowledge and hands-on experience emphasizing native plants. The plants used are grown from seed in the school-based nurseries where students learn about nursery maintenance, plant growth, wetland issues, and other restoration and conservation issues (Coker et al., 2010). Once the seedlings are well established, students transplant them to the school's predetermined associated restoration site (Blanchard and Bush, 2008). Even though school-based nurseries vary slightly from school gardening, the basic premise of drawing kids outdoors to engage in hands-on learning is still at the core (Blair, 2009).

2.5 Use of native plants for conservation efforts

From thousands of years of use, at least 83 percent of the Earth's land surface has been altered with estimations around 60 percent of Earth's ecosystems classified as degraded. Past anthropogenic actions have even affected remote parts of the globe, such as the Amazon Basin, leaving the majority of ecosystems impacted (Groom et al., 2006; Hobbs et al., 2006). As land use has intensified, populations of native plant communities have become degraded, threatened, or extinct (Groom et al., 2006). The North American Continent has approximately 20,000 native plant species with estimations equaling 25 percent near extinction (Dorner, 2002; Harker et al., 1999). Some reports have indicated 58 percent of plants native to the United States have been lost (Groom et al., 2006). The definition of a native plant varies, but in the Southeast is generally defined as a species present in a given region preceding European settlement or presently occupying a site following the sequence of natural processes without human introduction (Booth and Jones, 2001; Dorner, 2002; Norcini, 2006). Estimations of 5,000 non-native plant species have accidentally entered U.S. ecosystems compared to the 17,000 native plants known to exist in the United States (Pimentel et al., 2000).

As of 2000, in the U.S., intentional and unintentional introductions have brought in nearly 50,000 exotic species (all species combined) for various reasons. While some have proven to be beneficial, many have inflicted significant negative consequences. Along with damaging ecosystems, major economic losses have been incurred on many sectors of industry including agriculture and forestry. From 1906 to 1991, almost $100 billion has been attributed to the damages of only 79 non-native species. Competition and predation from exotic species are also the primary factors behind 42% of the 958 native species threatened or endangered. Certain nonindigenous plant species have been known to outcompete or crowd out native plants within the U.S. and spread at a rate of 700,000 hectares per year. European purple loosestrife (*Lythrum salicaria*) is one such plant that invades wetlands and increases in coverage by 115,000 hectares per year. These plants also endanger wildlife associated with the native plant species becoming eradicated or reduced (Pimentel et al., 2000). Losing our native plants results in a loss of floral species diversity which indirectly reduces associated faunal diversity (Bischoff et al., 2010; Groom et al., 2006; Harker et al., 1999; Pimentel et al., 2000). Also, loss of ecosystem function is associated with the introduction of exotic plant species. For example, fire regimes can be greatly affected by the introduction of European cheatgrass

(*Bromus tectorum*) in the midwestern shrub-steppe habitat by reducing the fire interval and ultimately eliminating the shrub habitat (Pimentel et al., 2000).

As secondary aspects, native plants are chosen for many restoration projects due to their adaptation to the site, economic, and aesthetic benefits (Brzuszek et al., 2007; Dorner, 2002; Harker et al., 1999). Native plant communities within restoration sites depend less on human intervention for upkeep and therefore are more sustainable than non-natives. This is believed to be due to adaptive and survival traits specific to a given area (Dorner, 2002). From all this, it is understandable why restorationists use and emphasize native plants to accomplish the ecological goals of restoring ecosystem function and promoting biodiversity (Booth and Jones, 2001; Harker et al., 1999).

As interest, education, and demand have grown, native plants are marketed by the nursery industry now more than ever (Brzuszek et al., 2007). USDA Bureau of Plant Industry nurseries began the National Plant Materials Program in 1954 which ultimately gave way to a seed source for the native plant industry. Native seed were mass produced at these nurseries, established in 1935, to aid in reestablishing grass to the stripped native grasslands that resulted from the drastic events which lead up to the Dust Bowl. As of 1994, 71 percent of the plants released by the USDA were native instead of introduced or nonnative stock. This varied from the 1943-1970 era when introduced plants were released at a rate greater than natives. By the 1970s, attention to the negative effects brought forth from the introduction of nonnatives had grown. This concern lead restorationists to place an emphasis on using native plants for ecosystem function and structure overruling just meeting a conservation goal like soil stabilization with the use of introduced species (Booth and Jones, 2001; Richards et al., 1998).

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CHAPTER III

OPTIMIZING SEED GERMINATION TEMPERATURES FOR SELECT NATIVE SPECIES

3.1 Abstract

Ecological restoration has become a grave concern due to long-term, anthropogenic impacts. Determining suitable native plants for use in ecological restoration promotes biodiversity. The Coastal Roots Program, a Gulf States project, provides educational opportunities working with local schools to inform and instruct students and teachers regarding successful propagation of various native species for ecological restoration. Seven plant species native to the Crosby Arboretum, Picayune, MS, and surrounding natural areas were chosen due to the lack of commercial availability, propagation knowledge, wildlife significance, and threatened or endangered status. Seed of titi (*Cyrilla racemiflora* L.), buckwheat tree (*Cliftonia monophylla* Britt.), flameflower (*Macranthera flammea* (Bartr.) Pennell), deertongue (*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*), pink coreopsis (*Coreopsis nudata* Nutt.), tall ironweed (*Vernonia angustifolia* Michx.), and swamp bay (*Persea palustris* (Raf.) Sarg.) were collected, placed in paper coin envelopes, and stored at 5°C until germination tests were conducted. Seed were germinated on filter paper hydrated with 5 mL Captan® Fungicide 50WP (2.37 g a.i./ L H2O) solution in Petri dishes. To determine optimal germination temperature regimes, seed were germinated under 5 alternating temperature regimes set at 5˚C increments to simulate day and night temperatures: 10/5,

15/10, 20/15, 25/20, and 30/25˚C. To check for seed viability after germination, the remaining ungerminated seed were pricked and soaked overnight in 0.1% tetrazolium chloride (TZ). Germination tests indicated titi, pink coreopsis, and swamp bay had greatest germination under 30/25˚C day/night or 25/20°C day/night resulting in 1.1/1.7, 41.5/7.7, and 3.6/0.0% (2010/2011) overall germination, respectively. Flameflower and deertongue had highest germination percentages at 20/15˚C day/night or 15/10°C day/night resulting in 5.4/8.3 and 10.5/15.4% (2010/2011) germination, respectively. Tall ironweed germinated best under 20/15°C day/night resulting in 36.2% germination. TZ tests on buckwheat tree seed proved none were viable.

3.2 Introduction

Increasing awareness and concern for the restoration of degraded or disturbed ecosystems have resulted in individuals and organizations undertaking conservation activities. Programs have been initiated to engage adults and children. LSU established the Coastal Roots (CR) program which was subsequently adopted by Mississippi State University's Coastal Research and Experiment Center (CREC) creating an opportunity for precollege school systems to participate in conservation. By emphasizing and using native plants through the CR program, teachers are able to provide students with hands-on experience and knowledge on the issues and importance of ecological restoration and conservation (Caffey, 2001). Students learn about nursery maintenance, seed germination and plant growth, wetland issues, and other restoration and conservation issues as seedlings are being grown in the school-based nurseries for transplanting into the landscape (Coker et al., 2010).

The Coastal Roots Program in Mississippi is in its first stages being adopted in 2008 by the CREC. Woolmarket Elementary School in Biloxi, MS was chosen as the first school for the nursery program. A nursery was installed at the site in 2009 with three other schools scheduled over the next 3 years. With the onset of school-based nursery production, native plants will be allocated to the chosen schools based on a specific site in need of reparations. The initial site correlation with the first school will be a location at the Crosby Arboretum, Picayune, MS (Coker et al., 2010). This converted arboretum was once an old strawberry farm and later a pine plantation. Crosby Arboretum was donated by the L.O. Crosby, Jr. family to the Crosby Arboretum Foundation who later partnered with Mississippi State University. The purpose of this donation was the preservation, protection, and exhibition of plants native to the Pearl River Drainage Basin in memory of the late L.O. Crosby, Jr. The native status of plants was based from acquired nearby tracts of land showcasing natural plant communities of the area (Brzuszek and Clark, 2009). The arboretum acquired seven natural areas within close proximity: Hillside Bog, Steep Hollow, Mill Creek, Red Bluff, Talowah, Dead Tiger Creek Savanna, and Dead Tiger Creek Hammock. These areas host a great level of plant diversity and showcase many of the native plant communities present within the southern region of Mississippi (Drackett, 2009).

Site adaptations, economic gain, ecological function, genetic biodiversity, and aesthetic benefits are major reasons native plants are emphasized for restoration projects (Brzuszek et al., 2007; Dorner, 2002). There are varying definitions of a native plant, but in the Southeast a native plant is generally defined as a species present in a given region preceding European settlement or presently occupying a site following the sequence of natural processes without human introduction (Dorner, 2002; Norcini, 2006). For purposes of this study, the definition of a native plant entails a species which once inhabited and is adapted to Crosby Arboretum and its surrounding natural areas. Native plants have adaptive and survival characteristics associated to a given site that allow for less maintenance by humans (Dorner, 2002).

Successful propagation and production protocols for native species allow commercial growers to add a niche to their market by providing the seed or vegetatively propagated plants to conservationists (Kaye, 2001). There has been an increase in demand for native plants in recent years especially by landscape architects and landscape contractors. As demand increases, supplies have not met the need (Brzuszek and Harkess, 2009). More research is needed to provide propagation and production guidelines for additional species to increase the diversity and availability of native species. Lack of availability is a major reason more consumers do not use native plant species (Brzuszek et al., 2007).

Seven plant species native to Crosby Arboretum that hold potential for the commercial industry and ecological restoration are: titi (*Cyrilla racemiflora* L.), buckwheat tree (*Cliftonia monophylla* Britt.), swamp bay (*Persea palustris* (Raf.) Sarg.), flameflower (*Macranthera flammea* (Bartr.) Pennell), tall ironweed (*Vernonia angustifolia* Michx.), deertongue (*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*), and pink coreopsis (*Coreopsis nudata* Nutt.). Titi and buckwheat tree belong to the Cyrillaceae which is made up of only three genera. Both have been reported as monophyletic (Weaver, 1996; Zhang and Anderberg, 2002) and an important source of nectar for honey in Mississippi (Lieux, 1981); however, in large stands of titi and years of abundant nectar, death among bee broods has occurred and is known as "purple brood" (Weaver, 1996). "Purple brood" is a nutritional deficiency as larvae, pupae, and young feed on the nectar, turn purple, and then die (Sanford, 1987). Their native ranges are found in the southeastern United States with titi also growing well in Central America, the Greater and Lesser Antilles, and parts of South America (Weaver, 1996). Typically in the United States, titi is a tardily deciduous or evergreen, dioecious, small tree or large shrub reaching an average height of 10 to 15 feet with potential to grow 25 feet (Dirr, 1998; Dute et al., 2004; Kirkman et al., 2007), but grows extensively larger in the tropics. Although titi is highly polymorphic across its global distribution, a spreading habit is usually observed growing as wide as it is tall with multiple crooked stems located in shrub-dominated wetlands of the U.S. Titi grows best in acidic sand-based soils with high organic matter and the occurrence of temporary inundation and periodic burning. Its leaves turn shades of orange and scarlet during late fall and small clusters of white flowers born on spike-like racemes are produced in abundance beginning in June or July. Flowering gives rise to small, 0.3 cm diameter, drupe type fruit shaped like an egg (Weaver, 1996). The flowers host over 25 families of insects with halictid bees being the primary pollinator (Dute et al., 2004). Fruit set occurs around August maturing from October through December (Weaver, 1996). Dispersal of the dry indehiscent capsules occurs by animals and water, and the plant also spreads by root-sprouting. Each nonsplitting capsule may host two to three seed that lack a seed coat (Miller and Miller, 2005; Zhang and Anderberg, 2002). Weaver (1996) and Dute et al., (2004) reported seed to be rarely fertile and stated self-pollinated stands of titi to be parthenocarpic; as well as, cross-pollination is needed to actually fertilize the ovule (Dute et al., 2004; Weaver, 1996). Dirr and Heuser (2006) suggest to harvest the seed in the yellow-brown transition, allow a drying period, and place in airtight containers under refrigeration. Four cultivars exist within the nursery industry: *Cyrilla racemiflora*

‘Argyle,’ *C. racemiflora* ‘Graniteville,’ *C. racemiflora* ‘Kristi,’ and *C. racemiflora* ‘Spring Cloud’ (Hatch, 2007).

Occurring in the same family, buckwheat tree closely relates to titi. They share characteristics of possessing two to three seed lacking a seed coat within a winged, dry indehiscent drupe-type fruit (Kirkman et al., 2007; Zhang and Anderberg, 2002). Buckwheat tree is a better source of nectar than titi for bee pollinators since “purple brood” is not associated with the species. Buckwheat tree is a dioecious, evergreen, large shrub to small tree reaching maximum heights and widths of 15 to 20 feet by 8 feet and usually forms thickets (Kirkman et al., 2007; Odenwald and Turner, 2006). It can be found growing in wet areas that are temporarily inundated such as floodplains, bayheads, and swampland edges. Flowers are produced on racemes from March to April, and fruit mature in late summer. As winter approaches, the fruit turn brown and then persist into the winter (Dirr, 1998; Kirkman et al., 2007). Dispersal of the winged capsules occurs primarily by water. Root-sprouting also increases colonization (Kirkman et al., 2007; Miller and Miller, 2005). Three cultivars exist within the nursery industry: *Cliftonia monophylla* ‘Berry Pink,’ *C. monophylla* ‘Chipola Pink,’ and *C. monophylla* ‘Van Cleve’ (Hatch, 2007).

A member of the Lauraceae, swamp bay (*Persea palustris* (Raf.) Sarg.) is listed as endangered by the state of Arkansas (U.S. Department of Agriculture, 2009b.). This plant is an important component in restoration projects along the Atlantic Coast with its native range occurring in the southeastern U.S. (Colodney, 2005). Commercial availability has been extremely limited due to infestations of wasp larvae within the seed (Dehgan and Sheehan, 1991). Swamp bay closely resembles its counterpart, and is sometimes argued to be a variety of redbay (*Persea borbonia*). Differences between the two species are the dense pubescence that occurs on the young twigs and petioles and the rust-colored midvein of swamp bay. Swamp bay is a monoecious, small, evergreen tree that can reach a maximum height of 25 to 30 feet but, this rarely occurs. It grows best under wet conditions such as swamps, wetland hardwood hammocks, marshland edges, and wet pine flatwoods where is persists as an understory tree (Dehgan and Sheehan, 1991; Kirkman et al., 2007). Bay leaves are very aromatic and have been used for spice and tea (Odenwald and Turner, 2006). Swamp bay flowers appear in June with fruit maturation occurring in October. Fruit take the form of a subglobose drupe with a purple to black coloration of the thin fleshy skin that covers the seed (Colodney, 2005; Dirr, 1998). Colodney (2005) stated the fleshy exocarp needs to be removed due to inhibition of germination occurring from its presence. Due to its recalcitrant nature, swamp bay seed must be keep moist and germination benefits from a 2 month cold, moist stratification (Colodney, 2005).

Members of the herbaceous Asteraceae (composites) contribute greatly to the level of species diversity in many ecosystems. Composites are not only useful for their aesthetics but for their utilitarian aspect as well. Many of these species provide forage, nectar, and serve as host plants for various native wildlife and insects (Coffey and Kirkman, 2006). Three species categorized in this family include: tall ironweed (*Vernonia angustifolia* Michx.), deertongue (*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*), and pink coreopsis (*Coreopsis nudata* Nutt.). These plants are native to the southeastern U.S. Tall ironweed and deertongue can be found growing in Mississippi, Alabama, Georgia, the Carolinas, and Florida with the addition of Louisiana for deertongue. Pink coreopsis is found in the previously mentioned states with the exclusion of the Carolinas. Tall ironweed is a perennial plant that can reach heights of four feet and showcases purple, disk flowers that bloom from June until September. It grows best in sand-based soils and on dry roadsides and readily occurs in longleaf pine ecosystems (Glitzenstein et al., 2007; Nelson, 2005). Seed germination research conducted in a nursery and laboratory setting indicated two and three percent germination; respectively, after the seed had been cleaned and stratified. A temperature regime of 30°C during an eight hour light period and 20°C in darkness for 16 hours was used in the laboratory experiment (Barbour, 2007). Barbour and Glitzenstein (2007) reported higher germination percentages of cleaned seed and an indication of the need to stratify tall ironweed seed. However, detailed information of their research remains unknown.

Deertongue ranges in height from two to five feet and has a purple inflorescence that blooms from August until November (Nelson, 2005). Its aromatic leaves smell like vanilla after being dried and crushed due to the presence of coumarin. They also have been used to flavor tobacco and have been found to contain several non-volatile pharmacological components (Wahlberg et al., 1972). Deertongue is an herbaceous perennial that grows in moist to dry sites including bogs, pond margins, roadsides, flatwoods, and sandhills (Nelson, 2005). Deyrup et al. (2002) report deertongue to be a host plant of seven different bee species in Florida. It is also reported to be a host plant of butterflies such as the little metalmark (*Calephelis virginiensis*) (Hall et al., 2007).

Pink coreopsis ranges in heights of two to five feet and showcases pink, yellow centered disk flowers that bloom from March until June. It is commonly found growing in wet areas such as roadside ditches, flatwoods, bogs, wetland edges, and wet pinelands where it resembles a rush (Nelson, 2005). Nelson (2005) reports pink coreopsis being critically imperiled in Alabama and Mississippi and imperiled in Louisiana. This categorization in Alabama and Mississippi refers to counts of five or fewer occurrences throughout each state. In Louisiana alone, pink coreopsis populations have been reduced to only St. Tammany Parish. Habitat loss, hydrological changes, and the suppression of fire regimes have been considered the primary causes behind reductions in pink coreopsis populations (Nelson, 2005).

Flameflower (*Macranthera flammea* (Bartr.) Pennell) is reported to be a member of the Orobanchaceae by some and the Scrophulariaceae by others (Nelson, 2005; U.S. Department of Agriculture, 2009a.). Its native range is in the southeastern U.S. where it can be found growing in Louisiana, Mississippi, Alabama, Georgia, and Florida. It occurs in the transition zone between wet and dry habitats such as wet thicket and bay edges, wetland margins, and pitcher plant bogs. This biennial reaches heights of four to nine feet and takes the shape of a shrub. Flameflower has 2.5 - 4.5 cm orange flowers that form a candelabra-like inflorescence from August until October. The flowers are pollinated by hummingbirds and provide a source of essential energy for them before their migration southward. After flowers wilt, a 1.25 cm dehiscent capsule remains containing small, dark colored seed that disperse upon splitting. Reports indicate flameflower acts as a hemiparasitic plant attaching itself to roots of other plants (Nelson, 2005). Flameflower is considered to be a rare plant in Louisiana, threatened in Georgia, and endangered in Florida (Chafin, 2008; Louisiana Dept. of Wildlife and Fisheries Natural Heritage Program, 2009; U.S. Department of Agriculture., 2009a.).

It is important to evaluate native species not commonly found in commercial production to determine viability of germination. Research on seed germination is necessary to increase the diversity of native plant species used for ecological restoration. Providing beneficial information to organizations, programs, and the commercial industry is important for the increased use of native plants. To correlate with the Coastal Roots Program, this study focused on determining the optimal germination temperature regime of seven native species to achieve the highest germination percentage. The Coastal Roots Program, coordinating school systems to provide ecological restoration and increase student and teacher awareness and knowledge, will benefit from this research. Not only the Coastal Roots Program, but the commercial nursery industry, ecological restorationists, and interested individuals will benefit from increased knowledge of seed germination from additional native species.

3.3 Materials and Methods

Working in association with Crosby Arboretum, seven species native to their properties were chosen for seed germination research due to their threatened and endangered status, lack of commercial availability, ornamental value, and significance to wildlife populations.

3.3.1 Seed collection and storage

Seed germination studies were conducted for two consecutive years. Seed were collected from titi, buckwheat tree, flameflower, and deertongue in November 2009, and October 2010 at Crosby Arboretum. Swamp bay seed was also collected from Crosby Arboretum in November, 2009, but seed numbers were lacking and infested by an insect in October 2010 and thus purchased from Louisiana Forest Seed Company (Lecompte, LA) in January 2011. Due to the lack of local seed source, tall ironweed and pink coreopsis were acquired elsewhere. Tall ironweed, Florida ecotype, was purchased from Ernst Conservation Seeds (Meadville, PA) in January 2010, and from the Florida Wildflowers Growers Cooperative (Crescent City, FL) in January 2011 due to lack of availability from Ernst Conservation Seeds. Tall ironweed acquired from Ernst Conservation Seeds was collected from their site in Live Oak, FL. Pink coreopsis seed was collected from the Apalachicola National Forest, Liberty County, FL, in May 2010 and May 2011. Once collected, the seed were cleaned and prepared as follows: deertongue and tall ironweed were debearded and aspirated to remove trash by airflow; titi, buckwheat tree, flameflower, and pink coreopsis were sieved to remove as much trash and debris as possible; the fleshy exocarp found on the seed of swamp bay was removed and the seed was placed in sealed plastic bags with moisture under refrigeration (5°C). Seed from the other species were counted by an electronic seed counter and placed in coin envelopes. Each year, individual coin envelopes contained 50 seed and were stored under refrigeration (5°C) until May 2010, and January 2011, except for swamp bay which received a two month cold, moist stratification at 5°C (Colodney, 2005).

3.3.2 Germination procedure

Petri dishes (100 x 15 mm) were lined with Whatman© filter paper (#1, Whatman International Ltd., Maidstone, England) and hydrated with a 5 mL Captan® Fungicide 50WP (2.37 g a.i./L H2O) (Southern Agricultural Insecticides, Boone, NC) solution. Seed from each plant species were placed within the Petri dishes and then placed inside five germination incubators. The incubators were set at varying temperature regimes under a long-day photoperiod (16 hr). Temperatures were alternated to simulate environmental conditions set at 5°C increments to distinguish day and night ranges: 10/5, 15/10, 20/15, 25/20, or 30/25°C. Eight replications of 50 seed per species per temperature regime were used for all species except deertongue and swamp bay in 2010.

Due to the lack of seed in 2010, deertongue had six replications of 19 seed exposed to only three temperature regimes while swamp bay had six replications of 23 seed exposed to three temperature regimes. Lighting, 50 to 60 μmol/m^2/s, was correlated to day temperatures.

Seed were placed under alternating temperature regimes for 28 days with germination counts conducted every two days. Determination of the optimal germination temperature was then made for each species based on the regime with the highest germination percentage within the 28-day period. Any remaining ungerminated seed from each species were placed within the determined optimal temperature for an additional 10 days with germination counts conducted every two days to determine quiescence. After completion of the 10-day germination period, viability tests were conducted on the remaining ungerminated seed. Seed were pricked with a hypodermic needle within close proximity to their embryos and then soaked in a 0.1% 2,3,5-triphenyl-2H-tetrazolium chloride (TZ) solution for 12 hours. The remaining viable seed were determined from the presence of a pink to red stained embryo which classified them as being dormant (Baskin and Baskin, 2001; Peters, 2000). Total viability was calculated by combining the germinated and remaining viable or dormant seed.

3.3.3 Statistical analysis

The data collected included: germination counts conducted every two days, overall percentage of germination, and an overall percentage of seed viability. The data were analyzed as a randomized complete design, using GLM procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC) with mean separation according to the least significant difference test, $P_\alpha = 0.05$.

3.4 Results and discussion

3.4.1 2010 germination study

Seed germination percentages varied across all plant species. In 2010, pink coreopsis and tall ironweed had the highest overall germination percentages of 41.5 and 36.2%, respectively. Deertongue, flameflower, swamp bay, and titi had germination percentages below 11%: 10.5, 5.4, 3.6, and 1.1%; respectively (Table 3.1). Significant differences were found in deertongue, pink coreopsis, and titi in relation to germination under separate temperature regimes for the 28-day period (Table 3.3). The 28-day germination period indicated titi, pink coreopsis, and swamp bay germinated best under warmer temperature regimes (30/25°C); whereas, flameflower, tall ironweed, and deertongue had highest germination counts at moderate temperatures (20/15°C) (Tables 3.1 to 3.3). At the completion of the overall 38 day experiment, TZ tests indicated viable, but dormant seed by red to pink staining of the embryos. Dormant seed counts were added to the number of germinated seed to compute total viability of the seedlot from each species. Viability percentages for buckwheat tree, deertongue, pink coreopsis, titi, swamp bay, flameflower, and tall ironweed were as follows: 0, 12.3, 60.2, 3.1, 35.5, 9.9, and 66.0%; respectively (Table 3.1).

3.4.2 2011 germination study

Germination percentages varied between consecutive years among species. In 2011, pink coreopsis and tall ironweed resulted in 7.7 and 9.4% germination, respectively, which was noticeably lower than the year prior. Deertongue, flameflower, swamp bay, and titi resulted in 2011 germination percentages of 15.4, 8.3, 0.0, and 1.7%; respectively (Table 3.2). Titi had its highest germination occurring in the warmer temperature regime (30/25°C) for both years (Tables 3.2 to 3.4). Flameflower, tall ironweed, pink coreopsis, and deertongue varied from the year prior and had higher germination counts under 15/10, 25/20, 25/20, and 15/10°C; respectively (Tables 3.3 to 3.4; Figs. 3.1 to 3.4). Since swamp bay concluded with no germination in 2011, the optimal temperature regime of 30/25°C, determined in 2010, was used during the remainder of the experiment in 2011 (Table 3.2). No germination was observed for the buckwheat tree during the germination periods from either year. Viability percentages for buckwheat tree, deertongue, pink coreopsis, titi, swamp bay, flameflower, and tall ironweed differed from 2010 and 2011 (Table 3.1 to 3.2).

3.4.3 Discussion

In both consecutive years, germination counts across temperature regimes indicated warmer temperatures (30/25°C) greatly reduced the rate of flameflower germination (Fig. 3.1). Reduced numbers of viable seed remained after flameflower was exposed to higher temperatures. In 2010 and 2011, no germination was observed under the temperature regime of 30/25°C and only a minimal amount of seed remained viable, 2 and 3%; respectively. This appears to indicate the seeds' inability to survive higher temperature ranges; however, no documentation has been published to support this claim.

An indication of a cold, moist stratification (10/5°C) was found to enhance the germination percentage of tall ironweed and is supported by a report from Barbour and Glitzenstein (2007). This was especially observed from the results of 2010, whereas this was not as evident the following year (Fig. 3.2). The seed source differed in 2011 and initial observations upon arrival lead to the belief that much of the seed had desiccated and were not viable before undergoing experimentation.

In 2010, germination counts across the temperature regimes indicated warmer temperatures (30/25°C) increased the germination of pink coreopsis. However, in 2011, pink coreopsis had higher germination only after seed were first exposed to cooler temperatures of 10/5 and 15/10°C and then placed under the optimal temperature determined to be 25/20°C (Fig. 3.3). This suggests pink coreopsis may enter a form of dormancy and benefits from a cool, moist stratification process similar to *Coreopsis floridana* and *C. leavenworthii* (Rukuni, 2008). However, it has been reported that an after-ripening period is beneficial to the germination of some Coreopsis species (Kabat, 2004; Norcini and Aldrich, 2007; Rukuni, 2008). Further research needs to be conducted to address after-ripening effects on pink coreopsis seed.

Even though germination occurred across all temperature regimes, a reduction in viable seed was observed after deertongue seed was exposed to higher temperatures and could be the result of seed death from high temperatures. A greater number of seed germinated after exposure to lower temperatures; thus, a cool, moist stratification may be beneficial for this species (Fig. 3.4).

In both years, only minimal germination percentages of 1.1/1.7% and 3.6/0.0% (2010/2011) occurred among titi and swamp bay; respectively. It is logical to observe the highest germination of titi and swamp bay seed under higher temperature regimes since many woody, temperate species germinate best while subjected to 30°C days and 20°C nights (Bonner, 2008). These results coincide with previous reports of titi seed being rarely fertile (Dute et al., 2004; Weaver, 1996), however, to my knowledge, there are no published germination percentages available. Minimal germination percentages from this study could have been due to collection from an isolated population. The entire stand may have vegetatively propagated itself from one mother plant through root-sprouting rendering its flowers to be only self-pollinated. Dute et al. (2004) and Weaver (1996) suggest cross-pollination is required for successful fertilization of the species. If so, it is logical to believe cross-pollination did not occur since the stand of titi where the seed were collected was distant to other populations on the property resulting in low fertilization percentages.

Fungi established on the swamp bay seed early into the study. Findings in the literature indicate the occurrence of fungi can influence and inhibit seed germination in some species such as soybeans and barley by competing and limiting the amount of oxygen that enters the seed (Harper and Lynch, 1981; Nik, 1980). The colonization of fungi on the swamp bay seed in this experiment may have had adverse effects resulting in the low germination percentages observed for the species. Thereto, a problem may have arisen from the stratification process. Colodney (2005) reported a two month stratification process under refrigeration was used to achieve good results in swamp bay seed, but no germination percentages were given. However, findings by Bonner (2008) indicated redbay (*Persea borbonia* (L.) Spreng.) resulted in no germination after being stratified under 3°C for 28 days. Further research needs to be conducted to determine the effects of a stratification process.

As buckwheat tree resulted in no germination from both consecutive years, further research is needed to determine the cause. It may be necessary to collect seed earlier in the year before they begin to dry and turn brown on the plant. Personal observations indicated the seed appeared desiccated upon collection. Also, being in the same family as titi, problems may have arisen in the pollination and fertilization of the flowers. No reports were found indicating buckwheat tree needs cross-pollination in order to fertilize the ovule, but this may be the case and requires additional research.

3.5 Conclusion

For ecological restoration to be most beneficial, biodiversity needs to be promoted (Groom et al., 2006). With programs arising to help aid against harmful impacts on the environment through restoration efforts, additional research needs to be conducted on native plants. Germination of most of the selected native species from this research was influenced by temperature; therefore, optimal temperature regimes and germination percentages were able to be determined for all species, except buckwheat tree. This determination greatly enhances the knowledge of seed propagation for these native plant species and gives some indication of the applicability of using these species within restoration efforts and the commercial native plant industry. Based on the results from 2010, additions to the Coastal Roots Program's plant palette could include pink coreopsis and tall ironweed which would benefit restoration efforts by promoting biodiversity. However, the source of the seed may prove to be difficult and limiting. The results from 2011 for both species vary in germination and viability percentages. Further, research needs to be continued to enhance germination percentages for each additional species since all but pink coreopsis and tall ironweed exhibited germination percentages below 15 percent with small quantities of seed remaining viable.

Table 3.1 Seed germination (after 28 days across varying temperature regimes), quiescent, dormant, dead seed, total germination (combines germination and quiescent percentages), and viability percentages of seven native southeastern United States species under controlled environments in 2010. Seed were exposed to varying temperature regimes (10/5, 15/10, 20/15, 25/20, and 30/25°C) in growth incubators for 28 days and then placed under the optimal regime for an additional 10 days to determine quiescence. Seed counts equaled 2000 seed. Viability percentages were calculated by adding the numbers of germinated seed and dormant seed, dividing the sum by the total number of seed in the study, and multiplying by 100.

		2010							
Common Name	**Latin Name**	***n***	**Germination (%)**	**Quiescent (%)**	**Dormant (%)**	**Dead (%)**	**Total Germination (%)**	**Total Viability (%)**	**Optimal Temperature (°C)**
Buckwheat tree	*Cliftonia monophylla* Britt.	2000	0.0	0.0	0.0	100.0	0.0	0.0	N/A
Deertongue	*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*	342	9.4	1.1	1.8	87.7	10.5	12.3	20/15
Pink coreopsis	*Coreopsis nudata* Nutt.	2000	22.1	19.3	18.8	39.8	41.4	60.2	30/25
Titi	*Cyrilla racemiflora* L.	2000	0.1	1.0	2.0	97.0	1.1	3.1	30/25
Swamp bay	*Persea palustris* (Raf.) Sarg.	414	1.5	2.1	31.9	64.5	3.6	35.5	30/25
Flameflower	*Macranthera flammea* (Bartr.) Pennell	2000	1.0	4.4	4.5	90.1	5.4	9.9	20/15
Tall ironweed	*Vernonia angustifolia* Michx.	2000	16.4	19.8	29.8	34.1	36.2	66.0	20/15

Table 3.2 Seed germination (after 28 days across varying temperature regimes), quiescent, dormant, dead seed, total germination (combines germination and quiescent percentages), and viability percentages of seven native southeastern United States species under controlled environments in 2011. Seed were exposed to varying temperature regimes (10/5, 15/10, 20/15, 25/20, and 30/25°C) in growth incubators for 28 days and then placed under the optimal regime for an additional 10 days to determine quiescence. Seed counts equaled 2000 seed. Viability percentages were calculated by adding the numbers of germinated seed and dormant seed, dividing the sum by the total number of seed in the study, and multiplying by 100.

		2011							
Common Name	**Latin Name**	***n***	**Germination (%)**	**Quiescent (%)**	**Dormant (%)**	**Dead (%)**	**Total Germination (%)**	**Total Viability (%)**	**Optimal Temperature (°C)**
Buckwheat tree	*Cliftonia monophylla* Britt.	2000	0.0	0.0	0.4	99.6	0.0	0.4	N/A
Deertongue	*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*	2000	13.0	2.4	2.0	82.6	15.4	17.4	15/10
Pink coreopsis	*Coreopsis nudata* Nutt.	2000	1.4	6.3	24.3	68.0	7.7	32.0	25/20
Titi	*Cyrilla racemiflora* L.	2000	0.9	0.8	0.5	97.8	1.7	2.2	30/25
Swamp bay	*Persea palustris* (Raf.) Sarg.	2000	0.0	0.0	0.6	99.4	0.0	0.6	N/A
Flameflower	*Macranthera flammea* (Bartr.) Pennell	2000	4.6	3.7	11.8	79.9	8.3	20.1	15/10
Tall ironweed	*Vernonia angustifolia* Michx.	2000	5.7	3.7	0.2	90.4	9.4	9.6	25/20

Table 3.3 Mean percentage germination of seven native southeastern United States species under controlled environments in 2010 and 2011. Eight reps of 50 seed were placed under individual temperature regimes, from left to right, 10/5, 15/10, 20/15, 25/20, and 30/25°C for 28 days to allow for germination to determine optimal temperature regimes.

	2010 Temperature Regimes (°C)					2011 Temperature Regimes (°C)				
Common Name	**10/5**	**15/10**	**20/15**	**25/20**	**30/25**	**10/5**	**15/10**	**20/15**	**25/20**	**30/25**
Buckwheat tree	0.00 a[z]	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Deertongue	N/A[y]	N/A	2.63 a	0.88 b	1.14 b	1.50 ab	2.10 a	1.98 a	1.48 ab	1.10 b
Pink coreopsis	1.75 c	1.13 c	1.48 c	3.70 b	5.78 a	0.23 ab	0.08 b	0.00 b	0.35 a	0.23 ab
Titi	0.00 b	0.00 b	0.00 b	0.00 b	0.08 a	0.00 b	0.00 b	0.08 ab	0.25 a	0.25 a
Swamp bay	N/A	N/A	0.00 a	0.22 a	0.51 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Flameflower	0.00 b	0.15 ab	0.25 a	0.25 a	0.00 b	0.00 b	1.48 a	1.28 a	0.10 b	0.00 b
Tall ironweed	0.23 b	0.78 b	3.28 a	2.85 a	3.13 a	0.00 c	0.35 c	0.83 b	1.40 a	0.98 ab

[z] Letter following mean seed germination indicates significant difference across the row within year (temperature regimes) according to Fisher's Protected LSD $P_\alpha = 0.05$.

[y] N/A = Not applicable.

Table 3.4 Seed germination percentages over a 28 day period of seven native southeastern United States species under controlled environments in 2010 and 2011. Percentages based on the number of seed which germinated per dish containing 50 seed. Seed were placed under alternating temperature regimes, from left to right, 10/5, 15/10, 20/15, 25/20, and 30/25°C to allow for germination to determine optimal temperature regimes.

	2010 Temperature Regimes (°C)					**2011 Temperature Regimes (°C)**				
Common Name	**10/5**	**15/10**	**20/15**	**25/20**	**30/25**	**10/5**	**15/10**	**20/15**	**25/20**	**30/25**
Buckwheat tree	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Deertongue	N/A	N/A	5.26	1.75	2.34	2.40	3.35	3.15	2.35	1.75
Pink coreopsis	2.80	1.80	2.35	5.90	9.25	0.35	0.10	0.00	0.55	0.35
Titi	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.10	0.40	0.40
Swamp bay	N/A	N/A	0.00	0.48	0.97	0.00	0.00	0.00	0.00	0.00
Flameflower	0.00	0.25	0.40	0.40	0.00	0.00	2.35	2.05	0.15	0.00
Tall ironweed	0.35	1.25	5.25	4.55	5.00	0.00	0.55	1.30	2.25	1.55

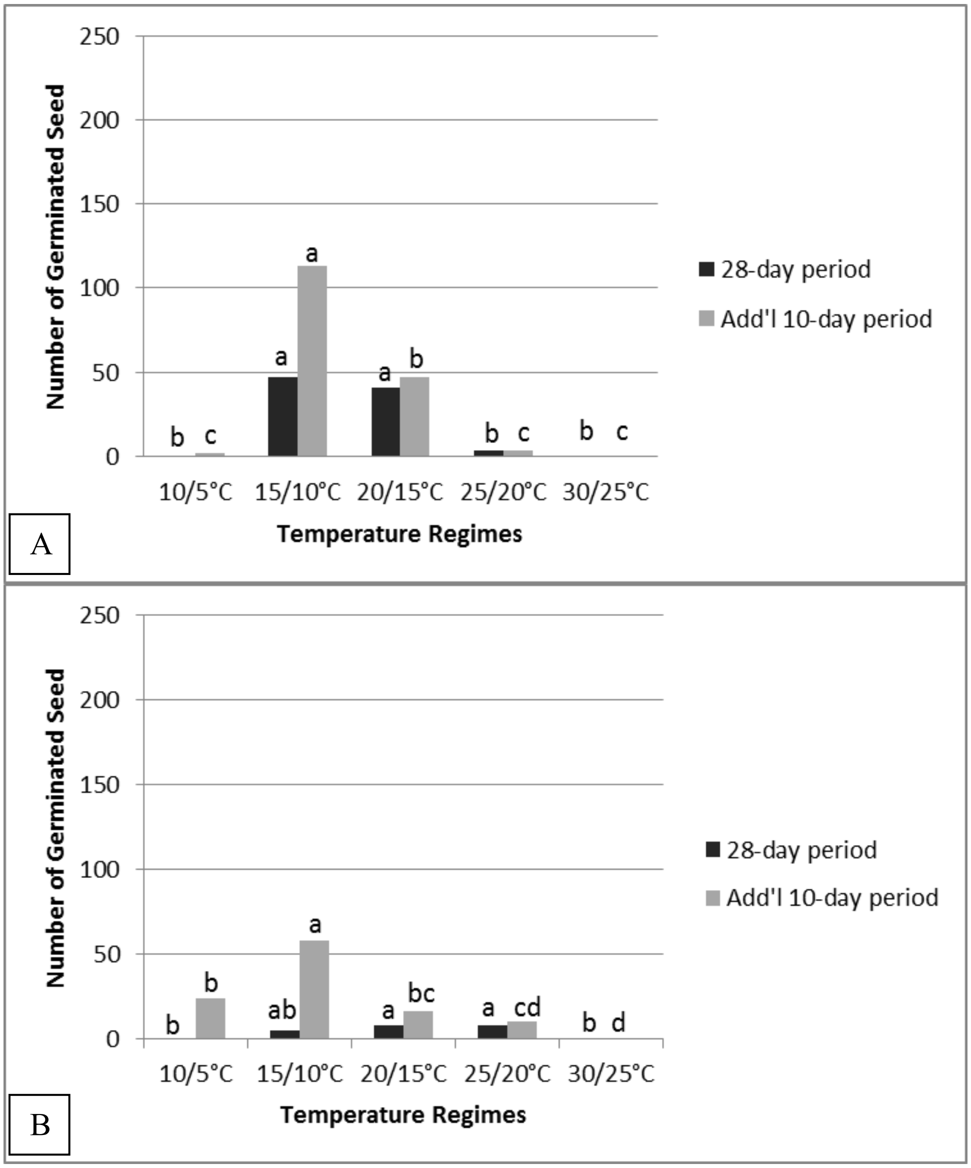

Figure 3.1 Seed germination counts across temperature regimes to determine optimal temperature (20°C light / 15°C dark) in 2010 (A) and (15°C light / 10°C dark) in 2011 (B) combined with the additional ten day period to determine quiescence under the optimal regime for flameflower (*Macranthera flammea* (Bartr.) Pennell). Germinated seed counts were out of a total of 2000 seed. Treatments followed by the same letter within time period are not significantly different according to Fisher's Protected LSD $P_\alpha = 0.05$.

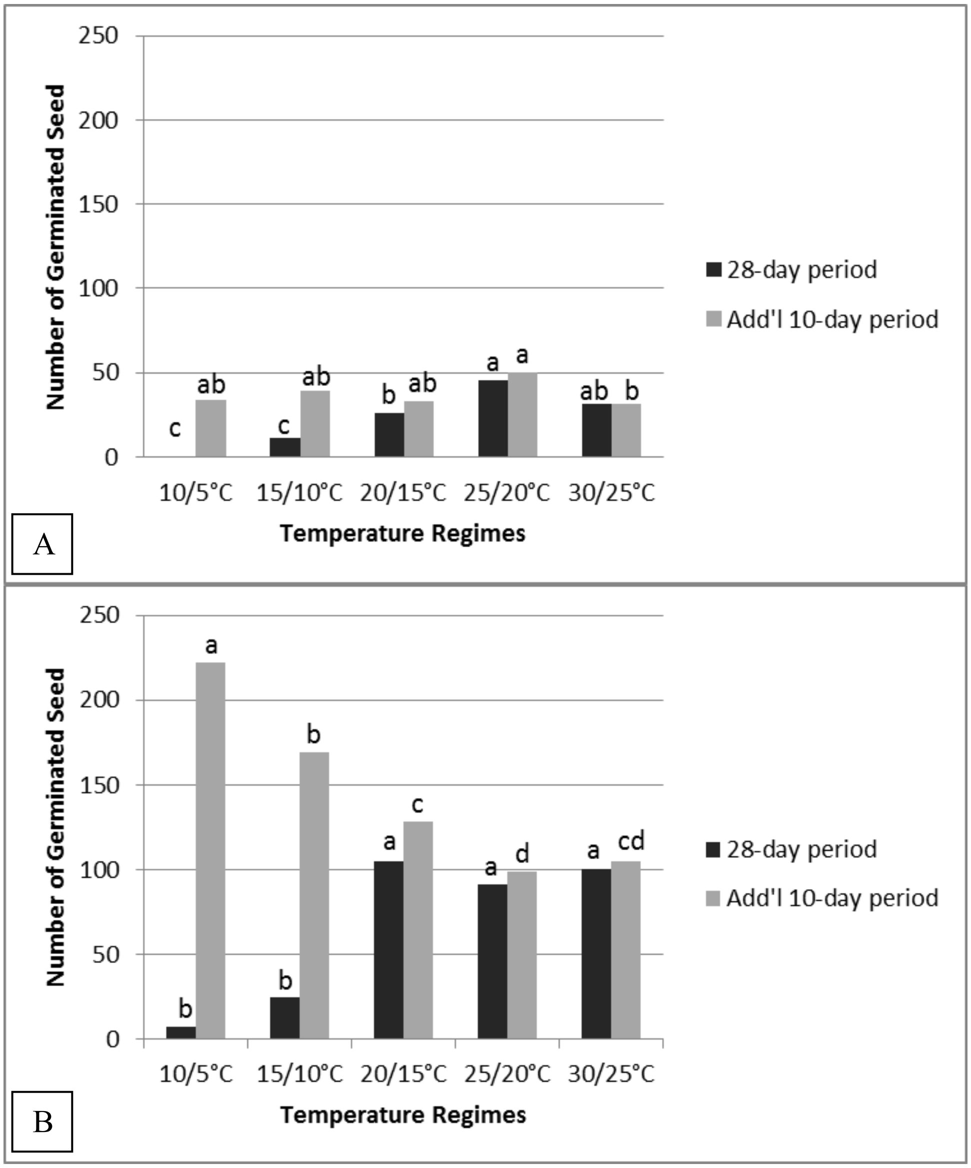

Figure 3.2 Seed germination counts across temperature regimes to determine optimal temperature (20°C light / 15°C dark) in 2010 (A) and (25°C light / 20°C dark) in 2011 (B) combined with the additional ten day period to determine quiescence under the optimal regime for tall ironweed (*Vernonia angustifolia* Michx.). Germinated seed counts were out of a total of 2000 seed. Treatments followed by the same letter within time period are not significantly different according to Fisher's Protected LSD $P_\alpha = 0.05$.

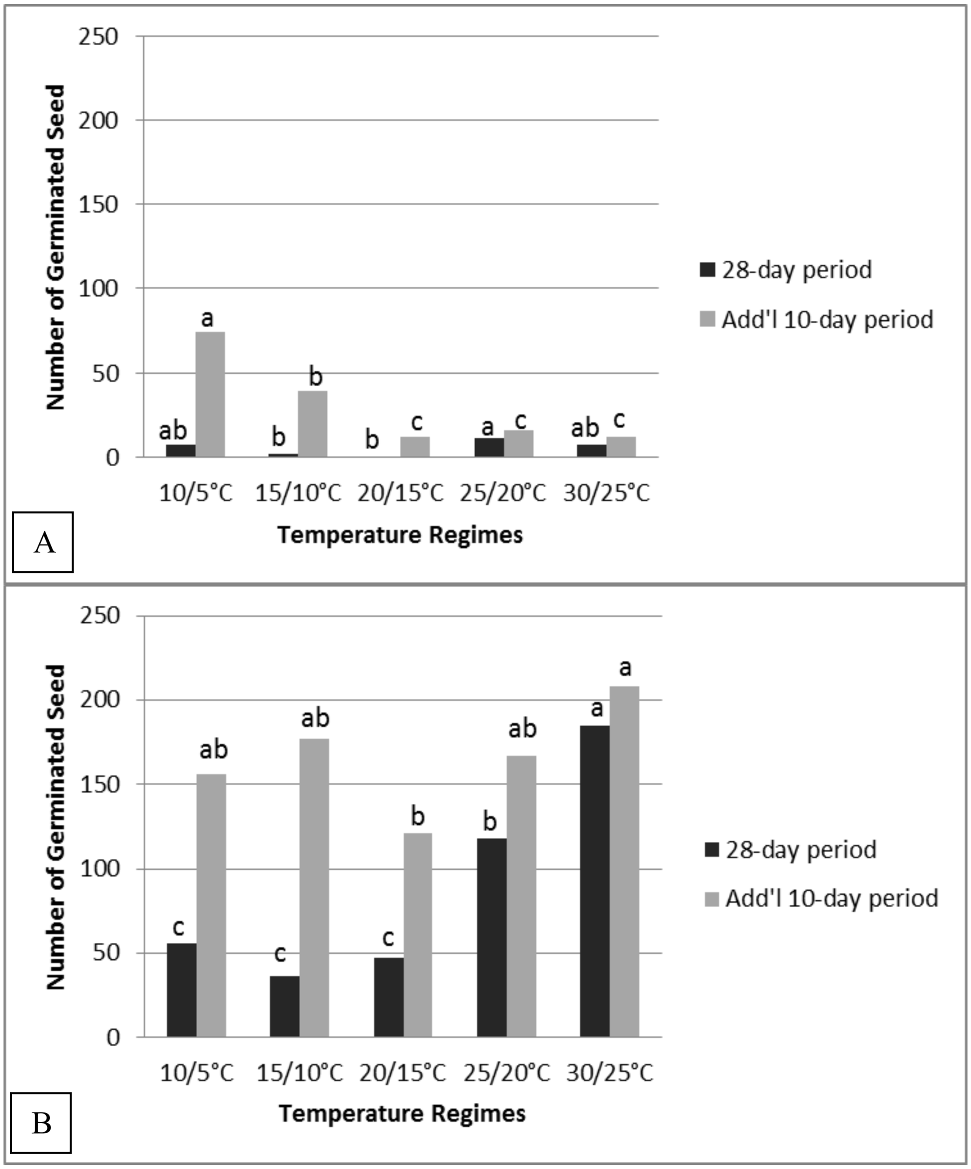

Figure 3.3 Seed germination counts across temperature regimes to determine optimal temperature (30°C light / 25°C dark) in 2010 (A) and (25°C light / 20°C dark) in 2011 (B) combined with the additional ten day period to determine quiescence under the optimal regime for pink coreopsis (*Coreopsis nudata* Nutt.). Germinated seed counts were out of a total of 2000 seed. Treatments followed by the same letter within time period are not significantly different according to Fisher's Protected LSD $P_\alpha = 0.05$.

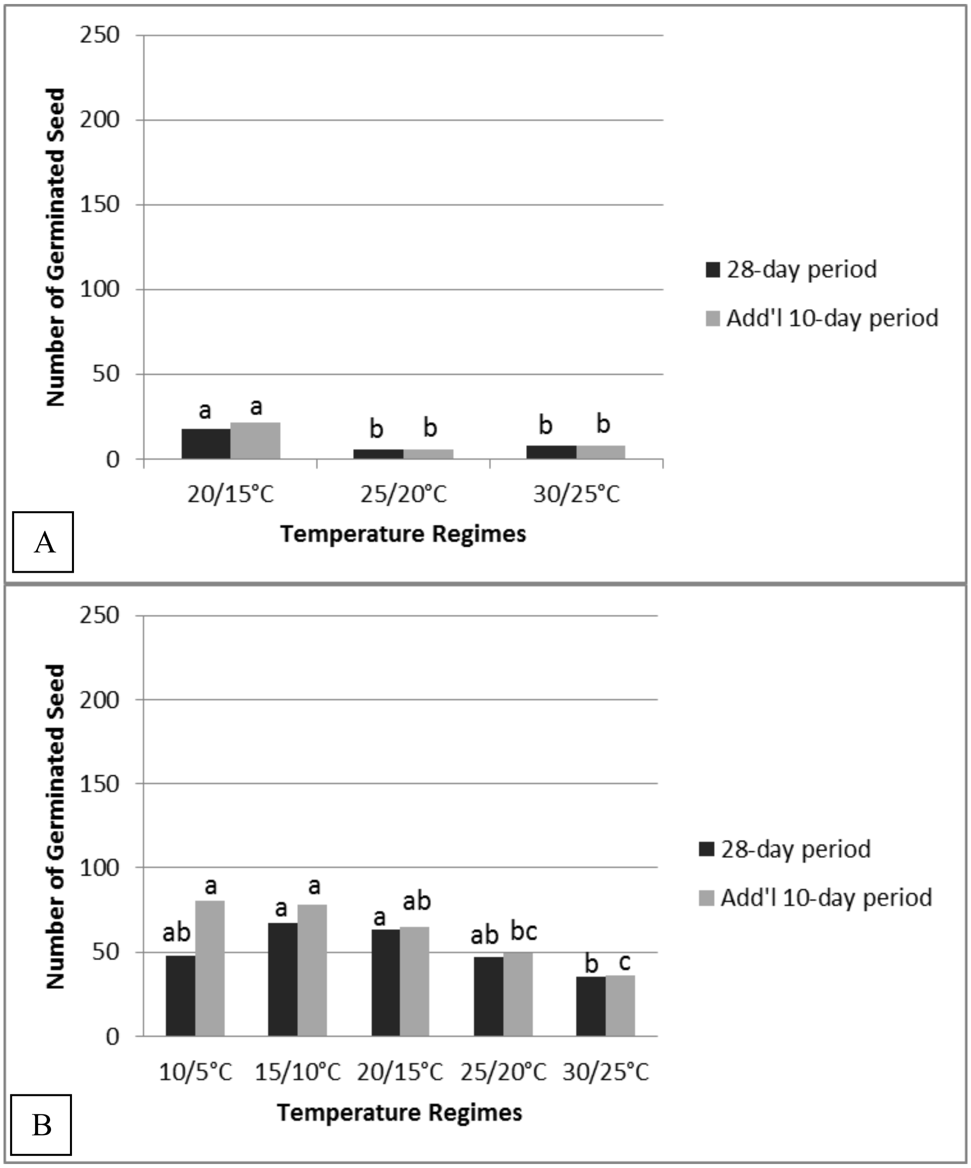

Figure 3.4 Seed germination counts across temperature regimes to determine optimal temperature (20°C light / 15°C dark) in 2010 (A) and (15°C light / 10°C dark) in 2011 (B) combined with the additional ten day period to determine quiescence under the optimal regime for deertongue (*Carphephorus odoratissimus* (Gmel.) Herb. var. *odoratissimus*). Germinated seed counts were out of a total of 342 seed in 2010 and 2000 in 2011. Treatments followed by the same letter within time period are not significantly different according to Fisher's Protected LSD $P_\alpha = 0.05$.

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CHAPTER IV

SELECT NATIVE SPECIES GROWN IN A SCHOOL NURSERY

4.1 Abstract

School-based nurseries are becoming a widely used tool to aid in and inform students and teachers about ecological restoration. Composite species (pertaining to the Asteraceae) greatly contribute to the level of plant diversity found within an ecosystem. Reintroducing composites to degraded sites promotes flora diversity and can indirectly promote fauna diversity. This research tested the potential of adding two composites to the plant palette of Coastal Roots, a Gulf States project partnering with precollege schools to teach ecological restoration using on-site school-based nurseries. In 2010 and 2011, Tall ironweed (*Vernonia angustifolia* Michx.) and pink coreopsis (*Coreopsis nudata* Nutt.) seed were sown in a nursery setting to determine germination percentages and the effect of different substrates. In 2010, seed were sown in Ray Leach "Cone-tainers"™ (3.8 x 21 cm) filled with Sunshine Mix 1 or Black Kow®. In 2011, seed were sown in plastic 288 plug trays (1.75 x 2.54 cm^3 per cell) filled with Sunshine Mix 1, Black Kow®, or pine bark / sand in a 75:25 ratio by volume and then transplanted to 606 Ball Cell-Paks (5.1 x 5.7 cm^3 per cell). Tall ironweed seed sown in Sunshine Mix 1 and pine bark / sand resulted in the highest germination percentages, 38.54 and 35.41%; respectively. Seedlings grown in pine bark / sand, Sunshine Mix 1, and Black Kow® resulted in growth indices of 284.56, 192.76, and 64.54 cm^3; respectively, after 17 days of growing in a greenhouse on a capillary mat.

4.2 Introduction

School-based nurseries are being used by several programs throughout the world to engage and inform school-aged children while establishing native plants to aid with restoring ecosystem functions to degraded sites. The Coastal Roots (CR) program introduced by Louisiana State University (LSU) in January 2000 is one such program using school-based nurseries to provide precollege students an opportunity to participate and learn about conservation and ecological restoration. In 2008, Mississippi State University's Coastal Research and Extension Center (CREC) adopted the CR program. Nursery maintenance, plant growth, wetland issues, as well as other restoration and conservation issues are all learned while students gain hands-on experience by emphasizing and growing native plant seedlings in their school-based nursery (Coker et al., 2010). Each school has an associated site in need of reparations to which the seedlings will be transplanted after they have become well established within the nursery (Blanchard and Bush, 2008). Students begin to see and understand the need and issues of ecological restoration by being provided an opportunity to work with native plants as they participate in restoration and conservation firsthand (Coker et al., 2010).

Coastal Roots is in the preliminary stages in Mississippi with Woolmarket Elementary School in Biloxi, MS the first school to partner with the program. Once partnered, the schools are assigned a specific site in need of reparations. A location within Crosby Arboretum, Picayune, MS was the designated site paired with Woolmarket Elementary (Coker et al., 2010). The land which now makes up Crosby Arboretum was once used to farm strawberries and pine trees. Intentions for the donation of the property by the L.O. Crosby, Jr. family were to preserve, protect, and exhibit plants originally found within the Pearl River Drainage Basin. This donation was made to the Crosby

Arboretum Foundation who later joined with Mississippi State University. A native plant palette was identified from acquired nearby tracts of land where great levels of diversity within natural plant communities of that specific area still existed (Brzuszek and Clark, 2009). Many plant communities on these properties are native to South Mississippi (Drackett, 2009).

Restorationists use native plants to promote genetic diversity, aesthetics, and ecological function, but also because they are naturally adapted to the specific site conditions and generally require less maintenance compared to non-native plants (Brzuszek et al., 2007; Dorner, 2002). Native plants have been defined in many ways. A prominent definition in the Southeast is: a species present in a given region preceding European settlement or presently occupying a site following the sequence of natural processes without human introduction (Dorner, 2002; Norcini, 2006). For this study, a native plant is defined as a species which once inhabited and is adapted to Crosby Arboretum and its surrounding natural areas. Due to adaptive and survival traits, native plants tend to be more sustainable compared to non-natives since they exhibit less dependence from humans for upkeep and maintenance after planting in restoration sites (Dorner, 2002). Commercial growers are able to market native plants to conservationists once successful propagation and production protocols have been established (Kaye, 2001). Also, the use of native plants by landscape architects and landscape contractors has increased over the past few years, however, supplies are not meeting the need (Brzuszek and Harkess, 2009). Additional research would benefit the industry by providing greater knowledge of propagation and production methodology which in turn would increase the diversity and availability of native species in the Southeast (Brzuszek et al., 2007).

The level of species diversity found in many ecosystems can be increased by increasing the number of composites (members of the Asteraceae) present. Composites offer several benefits ranging from beautification to utilitarian aspects. These species provide forage, nectar, and serve as host plants for various native wildlife and insects (Coffey and Kirkman, 2006). Tall ironweed and pink coreopsis are two composite species native to the southeastern United States, particularly the Crosby Arboretum. Tall ironweed persists in sand-based soils typical of dry roadsides and longleaf pine ecosystems. This perennial plant blooms with purple disk flowers during the months of June through September and reaches a height of four feet (Glitzenstein et al., 2007; Nelson, 2005). Previous nursery and laboratory germination studies of cleaned and stratified seed reported two to three percent germination. In the laboratory, a temperature regime of 30/20°C light/dark was used where light persisted for eight hours and darkness for 16 hours (Barbour, 2007). Hard plastic propagation flats were used to plant seed for the nursery study and approximately 44 days later, a germination count was conducted (Barbour, 2007). Higher germination percentages and a need for a stratification process were reported for tall ironweed (Barbour and Glitzenstein, 2007). However, detailed information of their research is unclear. Coffey and Kirkman (2006) conducted a germination phenology study from December 2001 until April 2004 on tall ironweed. They reported a germination percentage of 15 ± 1.28 percent after seeding standard flats and placing on benches exposed to outdoor conditions under 63% shade cloth (Coffey and Kirkman, 2006).

Pink coreopsis is a warm-season perennial that resembles a rush. The plant reaches two to five feet in height as it blooms pink with yellow disk flowers from March until June. Roadsides ditches, flatwoods, bogs, wetland edges, and wet pinelands are examples of the wet to moist areas where pink coreopsis grows best. Reports indicate pink coreopsis is endangered in Alabama and Mississippi and threatened in Louisiana (Nelson, 2005). Accounts of five or fewer occurrences throughout each state of Alabama and Mississippi have been made. Pink coreopsis in Louisiana is only known to be remaining in St. Tammany Parish. Habitat loss, hydrological changes, and the suppression of fire regimes take the primary blame for the reduction in pink coreopsis populations (Nelson, 2005).

As many native plant species have little production information known in the commercial industry, research concerning proper nursery propagation methodology becomes important. Research on seed propagation is needed in order to promote genetic diversity among the native plant species available for ecological restoration. Organizations, programs, and the commercial industry will become more adept at using additional native plants once needed research has been conducted and reported. Many will benefit from research conducted on additional native plant species as it particularly pertains to seed propagation methodology within a nursery environment. The objective of this study was to determine the viability and successful germination of tall ironweed and pink coreopsis in a school-based nursery.

4.3 Materials and Methods

Coordinating with Crosby Arboretum, two plants native to their properties, tall ironweed and pink coreopsis, were chosen for seed germination research due to their threatened and endangered status, lack of commercial availability, ornamental value, and significance to wildlife populations.

4.3.1 Seed collection and storage

Independent germination studies were conducted under nursery settings for two consecutive years. Since there was an insufficient quantity of available seed at Crosby Arboretum, tall ironweed and pink coreopsis were purchased or collected from other sources. In 2010, tall ironweed, Florida ecotype, was purchased from Ernst Conservation Seeds, Meadville, PA, but collected from their satellite site in Live Oak, FL; and in 2011, tall ironweed was purchased from Florida Wildflowers Growers Cooperative, Crescent City, FL. In 2010, pink coreopsis was collected from populations located in Apalachicola National Forest, Liberty County, FL, however, there was an insufficient amount collected in May 2011 to repeat the nursery study. Once the seed were in possession, tall ironweed was debearded and aspirated whereas pink coreopsis was sieved to remove trash and debris. Tall ironweed and pink coreopsis seed were then counted and placed in paper coin envelopes. Fifty seed were placed in individual coin envelopes before being stored under refrigeration (5°C) until August 2010 and June 2011.

4.3.2 Nursery study

Ray Leach "Cone-tainers"™ (3.8 x 21 cm) (SC10 Super, Stuewe and Sons, Inc., Tangent, OR) were filled with either Sunshine Mix 1 (SunGro Horticulture, Bellevue, WA) or Black Kow® (Black Gold Compost Co., Oxford, FL) for the nursery study in August 2010. Each rep consisted of 49 cone-tainers of each substrate placed in RL98 trays (Stuewe and Sons, Inc., Tangent, OR). One seed per cone-tainer was sown within each of the five reps for the germination study. Once prepared, five reps of each species were placed within a simulated school-based nursery exposed to outdoor conditions with an overhead mist irrigation according to the Coastal Roots standard (Coleman and Bush, 2002). Mist irrigation was programmed to operate twice a day, 7:30 a.m. and 7:30 p.m., for five minutes per cycle. Irrigation volume was not measured. Emergence of the cotyledons marked germination. The germination experiment was terminated 61 days later.

In June 2011, three media (substrates), Sunshine Mix 1, Black Kow®, and pine bark / sand in a 3:1 ratio by volume were used. Soil physical properties were determined using the method by Hidalgo (2001) (Table 4.1). A hard plastic 288 plug tray (1.75 x 1.75 x 2.54 cm per cell) was filled with the substrates and then subdivided by cutting the tray into 24-cell sections. Each substrate consisted of four 24-cell experimental units. After sowing one seed into each individual cell, reps were placed on a capillary mat within a greenhouse under 65% shade. Additional seed were subsequently sown into the experimental substrates in 606 Ball Cell-Paks (5.1 x 5.1 x 5.7 cm per cell) (George J. Ball Inc., Chicago, IL) and placed on a capillary mat to germinate and to observe seedling growth. The mat was hand watered as needed with a fertigation system dispensing water soluble fertilizer (200 mg N · L-1, Peter's Professional® 20-10-20, Scotts Sierra Horticultural Products Co., Marysville, OH). The germination study was terminated 37 days later as no further germination was observed. Seedlings from the germination study, after developing true leaves, were transplanted into 606 Ball Cell-Paks and left on the capillary mat to observe further growth. Growth measurements were taken approximately 17 days afterwards. Height was measured from the surface of the substrate to the highest part of the plant. Two width measurements were taken, one across the plant and the other 90° from the first. A growth index was then calculated as a volume using the formula: Growth Index = $\pi \cdot \{[(w1 + w2)/2]/2\}2 \cdot h$ (Hidalgo, 2001).

4.3.3 Data collection and analysis

The effects of substrate on germination and growth were tested. Germination counts were conducted every four days and growth indices were measured upon the completion of the study. Data were analyzed as a randomized complete design, using GLM procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC) with mean separation according to the least significant difference test, $\alpha = 0.05$.

4.4 Results and Discussion

4.4.1 2010 nursery study

No difference, due to substrate, was observed in the mean number of germinated seed for tall ironweed in 2010. Tall ironweed seed sown in Sunshine Mix 1 and Black Kow® for approximately 60 days germinated at 39.2 and 31.4%; respectively. Tall ironweed cotyledons emerged and the seedlings ceased growth once the first true leaves began to develop. Pink coreopsis had no germination by completion of the study.

4.4.2 2011 nursery study

The mean numbers of seed that germinated in Sunshine Mix 1 and pink bark / sand were not greater than in Black Kow® (Fig. 4.1). Tall ironweed seed germination was 38.5% in Sunshine Mix 1, 21.9% in Black Kow®, and 35.4% in pine bark / sand after 37 days. At the completion of the study, growth indices indicated differences in growth of seedlings between the substrates. Seedlings grown in pine bark / sand and Sunshine Mix 1 resulted in a greater mean growth by measuring plant volume than those in Black Kow® (Figs. 4.2 and 4.3). Visual inspection also noted a reduction in growth exhibited by the seedlings grown in Black Kow® (Fig. 4.3). Pink coreopsis was not repeated due to the lack of germination in the nursery study of the previous year and an inadequate amount of fresh seed in 2011.

4.4.3 Discussion

There was no difference in germination of tall ironweed in the substrates tested in 2010 or 2011. There was a difference found between growth of the seedlings with the least growth in Black Kow®. Black Kow® has a lower percentage of air space and appeared to remain wetter and more compacted for a longer period of time compared to the other two substrates. These conditions are not conducive to efficient plant growth and could have resulted in reduced growth. The germination percentages of tall ironweed observed in the two consecutive nursery studies were greater than those reported by Barbour (2005) and Coffey and Kirkman (2006).

The pink coreopsis seed may have entered into dormancy after being exposed to refrigeration, therefore, requiring a stratification process to break dormancy. Coreopsis species have been reported to require either after-ripening or stratification to enhance germination (Kabat, 2004; Norcini and Aldrich, 2007; Rukuni, 2008). Further nursery research is needed to determine the cause for the lack of germination in pink coreopsis seed in 2010.

4.5 Conclusion

As the use of school-based nurseries has become more recognized and widely distributed, additional plants native to specific regions need to be researched to promote the greatest breadth of biodiversity for the restoration of ecosystems. Coastal Roots is one such program in the Gulf states that takes advantage of these nurseries to engage school-aged children and their teachers in ecological restoration. To enhance the Coastal

Roots plant palette, tall ironweed and pink coreopsis were tested under nursery settings to determine seed germination percentages using various substrates under conditions similar to the Coastal Roots nursery design.

Upon completion of the study, substrate was found to affect seed germination and growth of tall ironweed. Seed sown in Sunshine Mix 1 and pine bark / sand grew better than those sown in Black Kow®. Sunshine Mix 1 and pine bark / sand are recommended for growing tall ironweed in a nursery setting. Tall ironweed holds potential to be incorporated into the Coastal Roots Program but needs further work to improve germination. Pink coreopsis failed to meet any desirable goal; therefore, further research is needed before it can be recommended for use in the Coastal Roots Program.

Table 4.1 Soil physical properties for Sunshine Mix 1, pine bark / sand 3:1 v/v, and Black Kow®, from left to right, pore space, air space, water holding capacity (percentage), and bulk density (g/cc).

Substrate	Pore Space (%)	Air Space (%)	Water Holding Capacity (%)	Bulk Density (g/cc)
Sunshine Mix 1	90.9	28.3	62.6	0.1
Pine bark / sand 3:1 v/v	74.7	33.6	41.1	0.5
Black Kow®	81.4	27.7	53.7	0.4

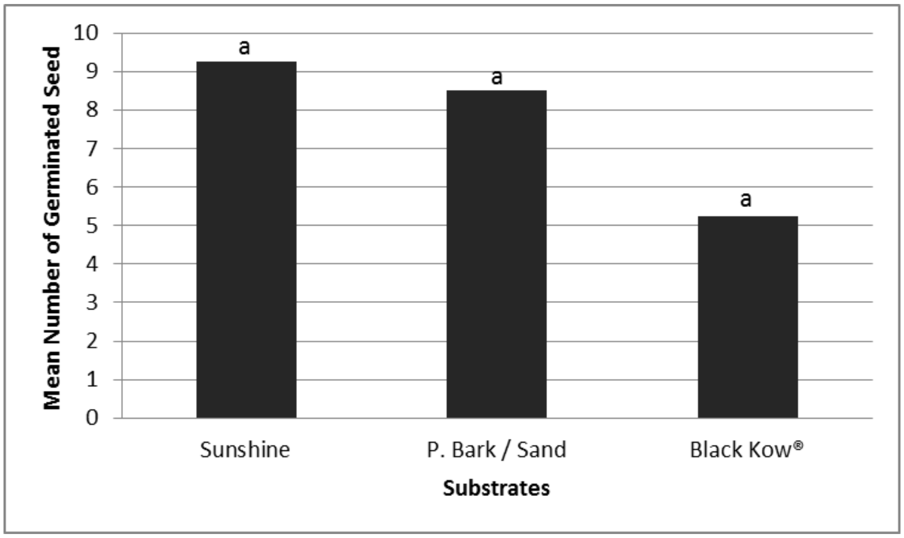

Figure 4.1 Nursery germination of tall ironweed (*Vernonia angustifolia* Michx.) seed in 2011. Mean seed germination out of 96 seed sown in Sunshine Mix 1, pine bark / sand 3:1 v/v, or Black Kow® compost after 37 days from June until July. Means separation using Fisher's Protected LSD $P_\alpha = 0.05$.

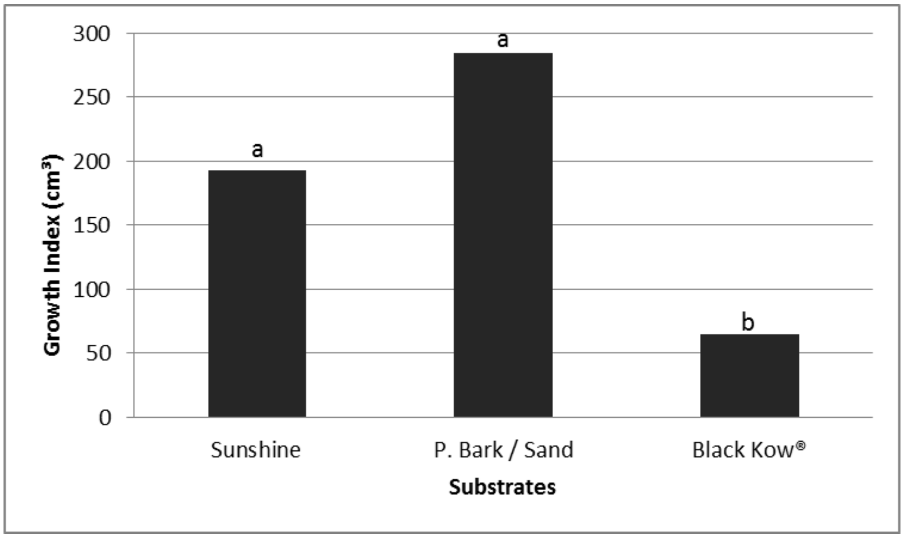

Figure 4.2 Growth indices (cm³) of tall ironweed (*Vernonia angustifolia* Michx.) grown in Sunshine Mix 1, pine bark / sand 3:1 v/v, or Black Kow® compost in July 2011. Growth index = π·r2·h. Mean separation using Fisher's Protected LSD $P_\alpha = 0.05$.

Figure 4.3 Vertical (A) and overhead (B) photographs depicting growth variations among tall ironweed (*Vernonia angustifolia* Michx.) seedlings grown in, from left to right, Sunshine Mix 1, Pine bark / sand 3:1 v/v, and Black Kow® for 17 days. Photographs were taken on 12 August 2011.

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CHAPTER V

CONCLUSION

The promotion of biodiversity is essential to achieve success in restoring ecosystem function during ecological restoration attempts. The basis of this study was to evaluate native species to increase the Coastal Roots plant palette to promote a greater range of plant diversity. The objective was to determine the viability of select plant species indigenous to Crosby Arboretum for the Coastal Roots Program by conducting seed germination experiments in laboratory and nursery settings.

Germination percentages were low and inadequate to provide a sound basis to determine the optimal temperature regime for some of the plant species in the study. However, temperature effects on seed germination for many of the species were observed, and provided beneficial knowledge about the propagation of those species. Optimal temperature regimes for germination ranged from 15/10°C (day/night temperatures) to 30/25°C. The resulting germination percentages and temperature effects greatly contribute to the determination of tall ironweed being a successful addition to the Coastal Roots plant palette.

Tall ironweed germination percentages observed in the nursery study remained consistent with those found under laboratory experimentation. Substrate did not affect germination of tall ironweed, however, it did affect the growth of tall ironweed seedlings.

Of the three substrates tested, Sunshine Mix 1, pine bark / sand, and Black Kow®, Black Kow® was not found to be suitable for the germination or growth of plants in a school nursery system.

In conclusion in this study, tall ironweed holds the greatest potential for induction into the Coastal Roots plant palette. Sunshine Mix 1 or pine bark / sand are recommended for the successful growth of this species. This herbaceous plant species addition would provide benefits to ecological restoration and the commercial industry by promoting biodiversity and broadening the propagation knowledge of students, teachers, and nursery propagators. Further research is needed to understand and enhance seed germination among other native plant species before dismissing their use in the Coastal Roots Program and nursery industry.