

**EFFECTS OF TEMPERATURE AND STORAGE REGIMES ON THE  
GERMINATION RATES OF THREE NATIVE WARM-SEASON GRASSES**

A Thesis

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By

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**CERTIFICATE**

This is to certify that the thesis entitled “Effects of temperature and storage regimes on the germination rates of three native warm-season grasses” submitted for the award of Master of Science to the Nicholls State University is a record of authentic, original research conducted by Mr. Trevis Dale Olivier under our supervision and guidance and that no part of this thesis has been submitted for the award of any other degree diploma, fellowship, or other similar titles.

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## ABSTRACT

Historically, the coastal prairie of southwestern Louisiana, commonly referred to as “Cajun Prairie”, was estimated to cover approximately 2.5 million acres (Allain et al. 1999). Most of Louisiana’s coastal prairie is now used as cropland (sugarcane, rice and grain), or as pasture land for cattle grazing with a less than 1% of the historical coverage remaining (Allain et al. 1999). Big bluestem *Andropogon gerardii* Vitman, indiagrass *Sorghastrum nutans* (L.) Nash, and switchgrass *Panicum virgatum* L. are common species found throughout coastal prairies like those of southwestern Louisiana (Pitman 2000). A common characteristic of these grasses is seed dormancy. The goal of this project was to determine how different storage regimes affected the length of dormancy in a local seed lot as well as a commercial seed lot from a Texas supplier for each of these species. Tetrazolium tests were conducted on each species to determine the percentage of viable seed from both local and commercial seed lots. Each seed lot for each species was separated into a cold storage lot and an ambient temperature storage lot. Seed germination was quantified in incubators at 20°C, 25°C or 30°C with all other variables equal. For each lot, 4 replicate dishes of 100 seeds were randomly assigned to each of the 3 germination temperatures and allowed to germinate on a 12 hour light/dark cycle for 28 days. Germination counts were made every 7 days until the cycle was complete. This cycle was repeated every 60 days of storage for approximately 1 year (360 days). The mean percent viable seed was higher for commercial seeds than for local seeds in all three (3) species. When comparing germination rates for big bluestem and indiagrass, storage temperature had an effect when assessed over time. Results for big bluestem and indiagrass indicated that cold storage for more than 120 days of storage

may extend the life of viable seeds that perish after 120 days in ambient storage. When comparing germination rates for switchgrass, germination temperature had an effect on commercial seed. For most storage lengths, germination percentages were higher for commercial switchgrass seed germinated at 30°C than for commercial switchgrass seed germinated at 20°C, regardless of storage temperature. Results indicated that cold storage may not improve percent germination in switchgrass. This information may be useful when developing a germination protocol for use by commercial vendors, land managers, and conservationists.

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## GLOSSARY OF TERMS AND ABBREVIATIONS

ABA = Abscisic acid

ABI3 = ABA-INSENSITIVE3 gene

FUS3 = FUSCA3 gene

GA = Gibberellic acid

LEC1 = LEAFY COTYLEDON1 gene

LEC2 = LEAFY COTYLEDON2 gene

SMP = Solid matrix priming

C = Celsius

Chaffy = Seeds containing small, thin scales or bracts that are dry and membranous.

TTC = 2, 3, 5-triphenyl tetrazolium chloride

G1 = At very high risk of extinction due to extreme rarity (often 5 or fewer populations), very steep declines, or other factors.

G2 = At high risk of extinction or elimination due to very restricted range, very few populations, steep declines, or other factors.

Hardiness = The minimum temperature to which seeds can be exposed and still be able to resume growth.

Soundness = Seed is sound when it has an interior area that is filled from edge to edge with moist endosperm and/or embryo tissue.

Health = The condition of seeds based on the condition of the embryo tissue, the lack or presence of fungal infection, the age of the seed, and how likely the seed is to germinate and successfully grow when required environmental conditions are present.

Radicle = Embryonic root of a plant.

Caryopsis = A type of simple dry fruit that is monocarpelate (formed from a single carpel) and indehiscent (not opening at maturity) and resembles an achene, except that in a caryopsis the pericarp is fused with the thin seed coat.

Pericarp = The wall of a fruit, developed from the ovary wall.

## INTRODUCTION

### **The Coastal Prairie Ecosystem**

Until about 150 years ago, the southern United States coastal prairie system was comprised of approximately 3.64 million hectares (9.0 million acres) stretching across the Louisiana-Texas Gulf Coast (Allain et al. 1999). Coastal prairies once extended from Corpus Christi, Texas, to the pine savannas that ran north and south from Opelousas to Lafayette, Louisiana (Figure 1; Allain and Johnson 1998). The coastal prairie of southwestern Louisiana, commonly referred to as “Cajun Prairie”, was estimated to cover approximately 2.5 million acres (Allain et al. 1999). The habitat was termed “Cajun Prairie” because it covered much of the same lands that were settled by the exiled French Acadian settlers in the early nineteenth century that came to be known as Cajuns (Allain et al. 1999).

The construction of a railway system, which traversed the entire prairie district to connect southeastern Texas to New Orleans began soon after the arrival of the Cajun settlers and was completed in the 1880s (Fontenot and Freeland 1976). With the completion of the railroad an influx of speculators, farmers, and ranchers from the mid-western United States and neighboring Texas began purchasing Louisiana prairie lands along the railroad (Vidrine et al. 2001a). Towns soon developed along the railroad including present-day Eunice (St. Landry Parish), Iowa (Calcasieu Parish), Jennings, Welsh, Roanoke (Jefferson Davis Parish), Estherwood, Crowley, and Rayne (Acadia Parish; Fontenot and Freeland 1976). By the 1920s, overgrazing, large-scale rice production, land-clearing operations and alterations in hydrology had depleted much of the natural prairie landscape forcing the use of intensive farming practices among the

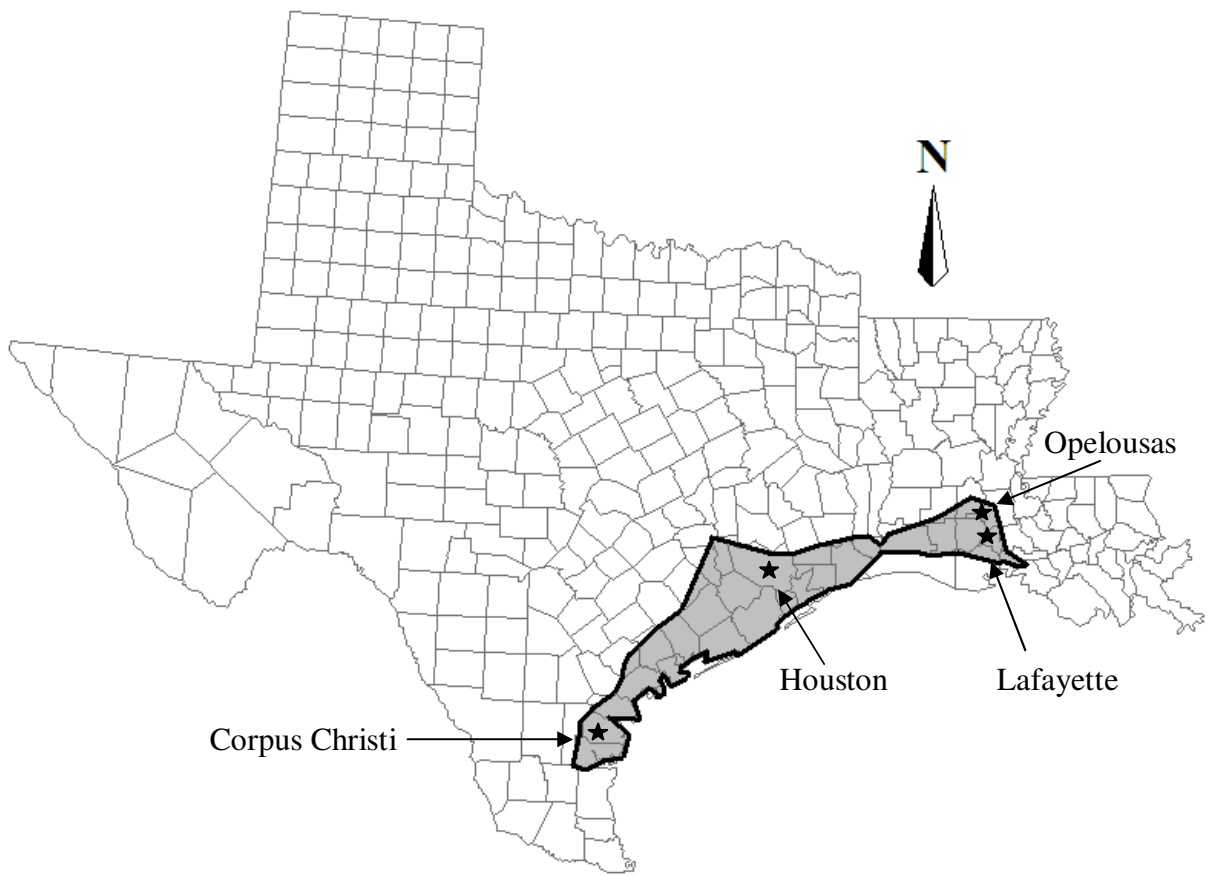


Figure 1. Coastal prairie habitat historically stretched from Corpus Christi, Texas to Lafayette, Louisiana.

settlers of the prairies (Vidrine et al. 2001b). Most of Louisiana's coastal prairie is now used as cropland (sugarcane, rice and grain), or as pasture land for cattle grazing. Louisiana's prairies are now remnant parcels totaling less than 1% of the historical coverage (Allain et al. 1999).

In the early 1980s, interest in the cultural and ecological aspects of the Cajun Prairie increased and eventually led to the opening of the Coastal Prairie exhibit at the Lafayette Natural History Museum in Lafayette, Louisiana in 1986. Also in 1986, biologists at Louisiana State University at Eunice rediscovered remnant strips of Cajun Prairie along the right-of-ways of the same railroads, which had initiated the demise of the Cajun Prairie a century earlier (Vidrine et al. 2001b). Since then continued interest in this habitat has led to other local, state, and federal projects to restore this habitat.

Currently, the coastal prairie is listed as "imperiled globally (G2)" by The Nature Conservancy (TNC) and the Texas Natural Heritage Program. The Louisiana Natural Heritage Program lists coastal prairie as "critically imperiled (G1)". Some of the organizations involved with coastal prairie restoration include the United States Department of Agriculture (USDA), The Nature Conservancy (TNC), Natural Resources Conservation Service (NRCS), United States Geological Survey (USGS), Louisiana Natural Heritage Program (LNHP), Louisiana Native Plant Initiative (LNPI), as well as many other organizations and universities. The Acadiana, Bayou Land, and Imperial Calcasieu Resource Conservation and Development (RC&D) Councils are also very involved in restoring this habitat. The RC&D's are non-profit councils funded in part by the USDA through the NRCS and through other sources like grants, workshop registration fees, private donations, in-kind donations, etc. Restoring this habitat has

become a primary goal for the USGS National Wetlands Research Center (NWRC) in Lafayette, Louisiana as well (Allain et al. 1999).

According to the USGS Coastal Prairie Restoration Information System there are at least 650 plant species associated with the Cajun Prairie habitat (Allain 2007).

Elevation, soil organic content, cations, and environmental factors associated with topography are the primary factors that influence species composition throughout this habitat (Grace et al. 2000). A natural topographic feature of the prairie that affected species composition was the presence of pimple mounds (Grace et al. 2000). How these 0.5 to 1 meter high raised areas, which stretched up to 10 meters across, originated is unknown; however, indicator species analysis revealed that predominately upland and colonizing species were largely restricted to these mounds (Grace et al. 2000).

Throughout the Great Plains and across much of the southern United States, big bluestem *Andropogon gerardii* Vitman, indiagrass *Sorghastrum nutans* (L.) Nash, and switchgrass *Panicum virgatum* L. are recognized as valuable forage and conservation species (Pitman 2000). These are three of the most prominent grasses in Coastal Prairie remnants. A Coastal Prairie restoration project that began in 1988, for example, evaluated the density of big bluestem, indiagrass and switchgrass in 1995 and again in 2000, and determined that all three grasses successfully survived early succession (Vidrine et al. 2001a). These are common grass species that are important to the health of the coastal prairie ecosystem.

Warm-season grasses such as these, however, are typically slow to establish because of chaffy, hairy seeds that are difficult to plant using conventional grain drills, seedlings that have low vigor and do not compete well with weeds, and exhibit seed

dormancy (Henning 1993). Association of Official Seed Analysts (AOSA) defined vigor as those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions (AOSA 2000). Vigor can be associated with seed age, hardiness, soundness, and health (AOSA 2000). Seed dormancy is a major challenge associated with the use of local ecotypes in restoration plantings (USDA 2007).

Commercial seed is developed and selectively bred for high seed production and reduced dormancy to allow for more rapid establishment (Gustafson et al. 2004). Because of these grow out practices genetic diversity is reduced in commercial seed (Gustafson et al. 2005). Restoration experts agree that plant materials native to an area should be used in conservation projects when possible to achieve long term sustainability (USDA 2007). Local seed, however, is not selectively bred for reduced dormancy and often requires a longer time period to establish than commercially produced seed. Gaining a better understanding of seed dormancy in locally collected seeds and practical, efficient dormancy reducing treatments that can be applied on a commercial scale is a critical step in developing commercial production of local ecotypes for these three grasses.

### **Seed Dormancy**

Seed dormancy is the failure of an intact, viable seed to complete germination under favorable conditions (Bewley 1997). Baskin and Baskin (2004) suggested another definition for dormancy, which is any seed that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favorable for its germination. A seed is considered germinated

when radicle protrusion occurs; however, one or several factors may prevent radicle protrusion (Adkins et al. 2002). These factors may be attributed to two basic mechanisms of seed dormancy. The first mechanism involves factors which occur within the tissues surrounding the embryo typically known as the seed coat (Figure 2). The second mechanism involves those processes that occur within the embryo (Figure 2).

Although the seeds of warm-season grasses maintain the ability to germinate, dormancy often occurs due to one or more of the tissues surrounding the embryo. Seed coats can prevent germination by preventing water uptake and/or gas exchange by acting as an impermeable barrier, acting as a mechanical barrier preventing embryo expansion, or by production of germination inhibitors (Adkins et al. 2002). Dormancy due to an impermeable seed coat can be overcome by weakening the seed coat and pericarp tissues that surround the caryopsis. In natural systems this occurs by the action of saprophytic fungi, fire, or chemical action when the seed passes through the digestive tract of an animal (Adkins et al. 2002). Seed coats may sometimes contain toughened tissues as well. In the Australian channel millet *Echinochloa turnerana*, for example, these tissues do not allow for the expansion of the embryo and the emergence of the radicle (Conover and Geiger 1984). These tissues are usually mechanically weakened

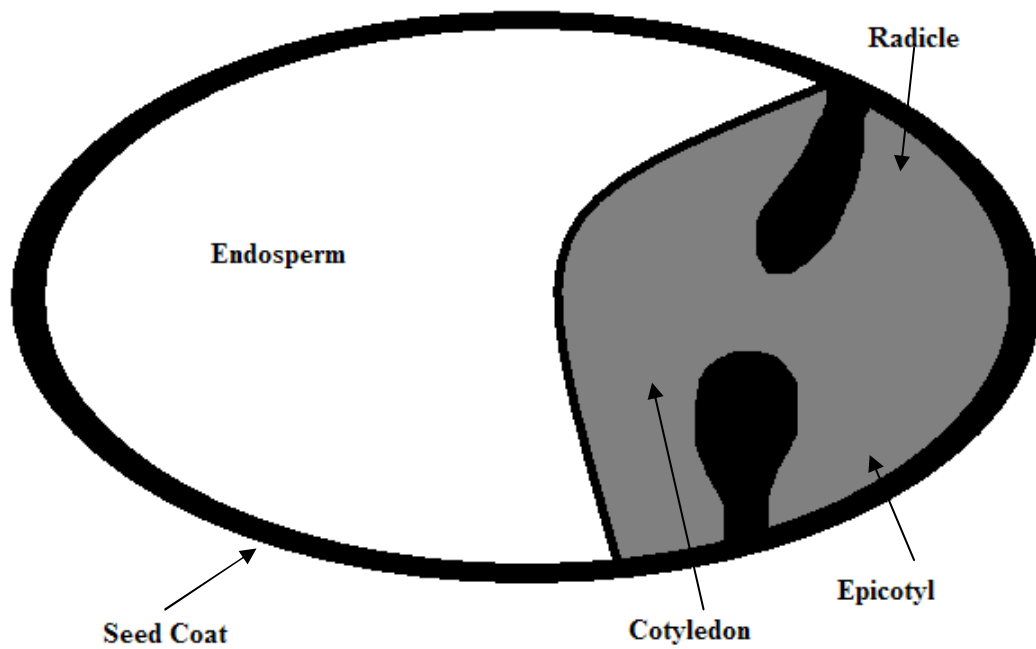


Figure 2. Basic seed anatomy of grasses including big bluestem, indiagrass, and switchgrass. Some dormancy processes occur within the embryo while others occur within the seed coat. All of these processes prevent radicle protrusion. The embryo is the light grey shaded area including the cotyledon, epicotyl, and radicle.

over time by either the production of embryo induced enzymes or by physical factors such as temperature fluctuation (Conover and Geiger 1984).

Embryo dormancy is believed to serve as a survival mechanism for many grass species that may experience unfavorable seasonal or periodic germination conditions (Simpson 1990). Genes responsible for germination are often suppressed by environmental conditions like drought, low temperatures, and other climatic factors (fire, frost, flooding, etc.) resulting in dormancy (Finch-Savage and Leubner-Metzger 2006). Dormancy in embryos may sometimes result from lack of an essential enzyme or growth process associated with a suppressed gene (Dyer 1993). Plant hormones such as abscisic acid (ABA), gibberellic acid (GA), and ethylene are also associated with seed dormancy and germination. Abscisic acid inhibits endosperm rupture thus causing seed dormancy (Adkins et al. 2002). Conversely, GA induces endosperm rupture meaning embryo germination is dependant on GA concentration (Adkins et al. 2002). In warm season grasses, the balance between germination promoters and inhibitors is not completely understood but a number of grasses have been reported to overcome dormancy following the application of GA (Zarnstorff et al. 1994; Adkins et al. 2002).

As stated earlier, environmental factors (drought, fire, temperature change, etc.) can alter the expression of genes and may cause mutations that become apparent when genes are expressed. Two groups of seed dormancy/germination mutants are recognized. The first group collectively functions in the overall control of seed maturation and mutations within these genes reduce dormancy (Parcy et al. 1997). The four gene loci ABA-INSENSITIVE3 (ABI3), FUSCA3 (FUS3) and LEAFY COTYLEDON (LEC1 and LEC2) play key roles in controlling mid- and late seed development (Meinke et al. 1994).

ABI3 positively controls sensitivity to ABA and the breakdown of chlorophyll and FUS3 and LEC1 and 2 control the suppression of leafy traits in cotyledons and anthocyanin accumulation (Meinke et al. 1994; Parcy et al. 1997). All of these processes are needed for germination and maturation to occur (Bentsink and Koornneef 2008). Mutations in any of these loci affect multiple processes including the accumulation of storage proteins, acquisition of dormancy and desiccation tolerance (Bentsink and Koornneef 2008).

The second group of mutations is associated with ABA biosynthesis and its mode of action. ABA regulates various aspects of plant growth and development, including seed dormancy (Bentsink and Koornneef 2008). Mutations associated with ABA are more directly related to seed dormancy rather than seed development like the first type of mutations discussed (Bentsink and Koornneef 2008). Abscisic acid deficient (*aba1*) mutants, for example, typically exhibit the absence of ABA-induced dormancy and marked reductions in seed dormancy (Bentsink and Koornneef 2008).

Big bluestem, switchgrass, and indiagrass exhibit seed dormancy after harvest, which is defined by an increase in the percentage of germinated seeds after some form of treatment. Seed dormancy is a major obstacle to successful establishment of these species. Therefore, much research and testing has focused on stratification techniques to rapidly break seed dormancy. Unstratified seed of these species obtain the highest percentage of germinated seeds after two years of storage (Emal and Conard 1973; Shen et al. 2001; Owsley n.d.). Many studies have compared the effects of various stratification techniques on the germination rates of these seeds (Coukos 1944; Hallett and Bewley 2002; Hanson and Johnson 2005). A few common seed stratification methods including pre-chilling, hull removal, caryopsis scarification, priming, storage

(after-ripening), leaching, light, alternating temperatures, chemical soaking, and different combinations of these treatments have been examined in these grasses (Beckman et al. 1993; Fulbright 1988; Haynes et al. 1997).

For example, two solid matrix seed priming (SMP) treatments (moistened and wet-chill) were applied to big bluestem and switchgrass seeds and their effects on germination rates were determined. The moistened and wet-chill (4°C) treatments increased seedling emergence of switchgrass 35 and 150%, respectively and SMP treatments increased big bluestem emergence by 18% (Beckman et al. 1993). According to Karssen et al. (1990), priming seems to occur when irreversible products are formed that are tolerant of desiccation and stimulate dormant seeds to germinate.

According to Haynes et al. (1997), 24 months of storage under cool, dry conditions (approximately 13°C) increased germination rate in switchgrass. This study also indicated an increase of 60% in germination rate of scarified seeds, an increase of 61% in germination rate of seeds treated with sodium hypochlorite (NaOCl), and a 62% increase in germination rate of seeds that were pre-chilled (Haynes et al. 1997). A similar study conducted with indiangrass seed produced similar results (Emal and Conard 1973). Seeds stored at room temperature remained dormant for 7 months before dormancy began to decline and moist chilling for 4 weeks at 4-6°C prior to germination resulted in near maximum germination (Emal and Conard 1973).

Switchgrass, big bluestem, and indiangrass are members of the family Poaceae the “true grasses,” which contains approximately 338 genera. All three species are warm-season perennial grasses that are adapted to warm-day climates and require that soil temperatures remain above 10° C for germination and seedling survival following

germination. For each of these species there are many different cultivars. According to the International Code of Nomenclature for Cultivated Plants, a cultivar is a group of individual plants which is collectively distinct for a particular combination of attributes, is uniform in its overall appearance and when propagated by appropriate means will retain those attributes (Trehane 2004).

### ***Big Bluestem***

Big bluestem is native to the United States ranging throughout most of the country excluding the west coast, Nevada, and Idaho (USDA 2009). Big bluestem and other bluestem species belong to the genus *Andropogon* which contains 20 species. Big bluestem is a common grass of the tallgrass prairies of North America and uses C4 photosynthesis, which results in rapid growth throughout the summer months (Gould and Shaw 1983). Big bluestem is a tall warm-season grass that grows to a height of 1.8 to 2.4 meters (6 to 8 feet) and can be distinguished from other warm-season grasses by blue coloration at the base of the culm and purplish, 3-part flower clusters that resemble a turkey's foot. Big bluestem is valued in prairie conservation and restoration projects because its extensive root system makes it an efficient user of water and its thick clumps firmly hold soil (Porter 1970).

There are nine cultivars of big bluestem which include: 'Bison', 'Bonilla', 'Niagara', 'Rountree', 'Earl', 'Pawnee', 'Champ', 'Sunnyview', and 'Kaw' (Everett 1991). 'Kaw' big bluestem was the cultivar used in this study because it is typically the most successful cultivar throughout the southeastern region of the U.S based on adaptability (USDA 2009). The 'Kaw' cultivar was released by the Manhattan Plant Materials Center in Manhattan, Kansas in the 1940's (USDA 2009).

For big bluestem, flowering time is dependant on photoperiod. Flowering is triggered by a peak in day length followed by days of decreasing day length (Owsley n.d.). Southern ecotypes of big bluestem commonly exhibit higher levels of seed dormancy than northern ecotypes (Owsley n.d.). ‘Kaw’ and other more northerly cultivars are often planted in the southeast because these cultivars tend to germinate earlier than southern cultivars resulting in faster growth and establishment of stands. Although dormancy is prevalent in big bluestem, particularly more in southern cultivars, there are many dormancy breaking techniques that effectively reduce dormancy.

Solid matrix priming (SMP), for example, was shown to increase germination rates of big bluestem by 18% (Beckman et al. 1993). SMP is a process in which seeds are mixed with a solid matrix material and provided aeration and the minimum amount of water to ensure osmoconditioning but not germination (Beckman et al. 1993). Another study by Hsu et al. (1985) showed that chilling also increased germination rate in big bluestem. Chilled seeds had the highest germination rates between 20 and 30°C compared with 12 to 20°C for unchilled seeds because lower temperatures probably partially met the chilling requirement (Hsu et al. 1985).

### ***Indiangrass***

Indiangrass inhabits the same range as big bluestem and is also a C4 warm season grass (USDA 2009). Indiangrass grows to a height of about 0.9 to 1.5 m (3 to 5 ft). The seed heads of indiangrass are single, golden brown, plume-like panicles. Indiangrass has small, fluffy seeds with small, hair-like structures called awns. Indiangrass is often used in mixed grass cultures to reduce wind erosion, for roadside cover, for hay and forage pasture, and for wildlife habitat (USDA 2009).

There are eleven cultivars of indiangrass which are ‘Cheyenne’, ‘Holt’, ‘Llano’, ‘Minnesota Common’, ‘Nebraska 54’, ‘Osage’, ‘Oto’, ‘Pawnee’, ‘Rumsey’, ‘Tomahawk’, and ‘Lometa’ (USDA 2009). ‘Lometa’ was used in this study, and was released by the Knox City Plant Materials Center in Knox City, Texas in cooperation with the USDA Soil Conservation Service in 1981. ‘Lometa’ was developed as an alternative to the ‘Cheyenne’ cultivar because ‘Cheyenne’ is not well adapted to the moist soils of southern Texas (Texas Agricultural Experiment Station and USDA 1983). ‘Lometa’ indiangrass was selected for this study because ‘Lometa’ tolerates moist clay soils well and because this cultivar originated from a similar latitude as that of stands in Louisiana. Pitman (2000) showed that ‘Lometa’ indiangrass had greater field establishment in a Louisiana field planting than did cultivars from more northern latitudes.

Indiangrass exhibits high seed dormancy based on germination rate and field establishment studies (Emal and Conard 1973; Rafii and Barnett 1970; Fulbright 1988). A variety of treatments have been effective in increasing germination rates in indiangrass stands. Pre-chilling, for example, breaks dormancy resulting in higher germination percentages (Emal and Conard 1973). Emal and Conard (1973) exposed indiangrass seeds to 4 weeks of moist chilling at 4-6° C prior to germination testing and found that for the first 7 months chilled seeds obtained near maximum germination and those seeds of the same source that were not chilled maintained near 100% dormancy. Chemical treatments have also been effective in increasing germination rates (Watkinson 1998). Sodium hypochlorite (NaOCl) soaking for 60 minutes prior to germination and

germination in gibberellic acid (GA) solution was found to increase germination rate from less than 10% to 65% (Watkinson 1998).

### ***Switchgrass***

Switchgrass *Panicum virgatum* ranges from Mexico to Canada and across the majority of the U.S. excluding the Pacific coast states (USDA 2009). Switchgrass grows to a height of 0.9 to 1.5 meters (3 to 5 feet) and can be distinguished from other warm-season grasses by white patches of hair at the point where leaves attach to stems.

Switchgrass stems are round and typically have a reddish tint and switchgrass seed heads have open, spreading panicles of tiny hairless seeds (USDA 2009). Switchgrass is highly valued as a perennial ornamental because it stabilizes soil and produces attractive flowers and foliage (Haynes et al. 1997). It is also commonly used for livestock feed, soil and wildlife conservation, and biofuel production.

Interest in the commercial production of switchgrass is expected to increase as more research focuses on the use of switchgrass as a renewable energy source (Haynes et al. 1997; Adler et al. 2006). Switchgrass has one of the highest potentials for use as a biofuel crop in the United States, because it grows well under a wide range of conditions (Comis 2006). Cultivar trials centered in Virginia, Alabama, and Texas have identified three (3) high-yielding switchgrass cultivars (McLaughlin et al. 1999). These include Alamo in the deep south, Kanlow at intermediate latitudes, and Cave-in-Rock for the upper Midwest (McLaughlin et al. 1999).

Switchgrass ecotypes are adapted to a variety of latitudes in the U.S. (Casler et al. 2004; Casler et al. 2007). Increasingly later maturity and more rapid stem elongation rate were evident in more southern ecotypes resulting in high biomass yield and longer

retention of photosynthetically active tissue when compared to more northern ecotypes (Casler et al. 2004). More northern ecotypes, however, exhibited an increase in cold tolerance resulting in higher survival, stand longevity, and sustained biomass yields at colder temperatures (Casler et al. 2004). Biomass production, conservation, or restoration projects should use populations from as close to the native range as possible to ensure maximum growth and survival of stands and to maintain the ecological integrity of the site.

The cultivars of switchgrass are ‘Blackwell’, ‘Cave-in-Rock’, ‘Dacotah’, ‘Forestburg’, ‘Grenville’, ‘Kanlow’, ‘Nebraska 28’, ‘Pathfinder’, ‘Shelter’, ‘Summer’, ‘Trailblazer’, and ‘Alamo’ (USDA 2009). ‘Alamo’ was the cultivar used in this study. ‘Alamo’ was released by the Knox City Plant Materials Center in Knox City, Texas in cooperation with the Soil Conservation Service and the Texas Agricultural Experiment Station in 1978. ‘Alamo’ was developed for southern areas with rainfall > 63.5 centimeters (25 inches) per year and grows in a range of soil types from clays to fine sands (Texas Agricultural Experiment Station 1979). ‘Alamo’ was used in this study because this cultivar has the closest latitudinal origin to that of Louisiana prairie habitat and because ‘Alamo’ is adapted to grow in moist clay soils typical in Louisiana.

Seed dormancy is a major obstacle in switchgrass establishment, particularly in seeds harvested in fall and planted the following spring (Zarnstorff et al. 1994). Many seed treatments, however, have been effective in breaking seed dormancy and increasing percent germination in switchgrass (Haynes et al. 1997; Jensen and Boe 1991; Zarnstorff et al. 1994). Jensen and Boe (1991) found scarification to increase switchgrass germination by 73%. Their study also showed increases in germination percentage after

six months of storage at room temperature. Zarnstorff et al. (1994) also found increased storage time resulted in increased germination percentage. Seeds stored at 5° C had an increase in final germination over a 4 year period.

Seed priming (SMP) increased germination percentage (Beckman et al. 1993). According to (Beckman et al. 1993) SMP significantly increased switchgrass germination by up to 50%, and this increase was greater (up to 150%) when combined with wet-chill treatment. Wet-chill treatment increased germination percentage in switchgrass when combined with other dormancy breaking methods and when used alone (Beckman et al. 1993; Haynes et al. 1997; Zarnstorff et al. 1994).

This study focused on the effects of storage length, storage temperature, germination temperature, and seed source on percent germination. The primary objective of this study was to quantify and compare germination rates of commercially obtained seed of big bluestem, indiangrass, and switchgrass to the germination rates of locally obtained seed under different storage and germination regimes.

### **Project Goal and Objectives**

Big bluestem, indiangrass, and switchgrass are dominate perennial warm-season grasses throughout the coastal prairies of Texas and Louisiana. In Louisiana, native stocks of these species are in high demand for revegetation projects, conservation programs, roadside enhancement, biofuel production, and many other uses (Gutormson and Patin 2002). Although obtaining large quantities of non-local and cultivar grass seeds may be relatively affordable, available, and desirable given the quantity of seeds required for prairie restoration, research indicates genetic and plant performance differences between local and non-local seed sources in all three of these species

(Gustafson et al. 2005). The lack of commercially available plant materials that are adapted to Louisiana is a primary factor contributing to stand failure (USDA 2007). In addition, genetically adapted plants are more likely to ensure the ecological integrity of conservation plantings.

The goal of this project is to determine whether there is a difference in the germination rates of locally versus non-locally obtained ecotypes of big bluestem, indiagrass, and switchgrass under various stratification scenarios and lengths of storage. This information will be used for the production of commercially available plant material from local ecotypes. This project will help to determine if native south Louisiana obtained seeds of big bluestem, indiagrass, and switchgrass will produce germination rates comparable to the currently used non-local, commercially available seeds. This project will quantify conditions that yield the highest germination rate from dormant seed thus increasing the efficiency of seed use.

The primary objective is to compare germination rates of commercially obtained seed of indiagrass, switchgrass, and big bluestem to the germination rates of locally obtained seed under various storage methods. Another objective of this project is to determine seed viability of each seed lot used for this study. Seed viability will be determined using tetrazolium testing as described in the Tetrazolium Testing Handbook prepared by the Tetrazolium Testing Committee of the Association of Official Seed Analysts (AOSA 2000). This test will provide rapid estimates of seed viability (< 24 hours), which will be useful in testing dormant seed lots. Assessment of the percentage of viable seed prior to germination is critical for estimating germination rates by providing a baseline of percent dead seed.

## METHODS

For each of the three species for both seed lots (commercial and local), cold storage at 4°C was compared to storage at ambient outdoor temperature. Germination rates were assessed after: 0, 60, 120, 180, 240, 300, and 360 days of storage beginning 4 months after harvest of local seed. Local seed was stored in paper bags at room temperature (~21°C) in a temperature controlled laboratory until germination testing began. Germination percentages were quantified at three temperatures to determine the temperature at which the highest germination rate occurred. The lowest germination temperature averaged 20°C as published in the AOSA manual for big bluestem and the highest germination temperature averaged 30°C, which is the average high soil temperature for the germination period of the locally adapted ecotype. The middle temperature averaged 25°C which is the median temperature between the AOSA temperature and the local temperature. Storage and germination temperature treatments were studied primarily because of the practicality of using these methods on a large-scale in local seed production. These are the most feasible and efficient dormancy breaking methods.

### **Seed Lots**

Commercial seeds of big bluestem ('Kaw' cultivar), indiagrass ('Lometa' cultivar), and switchgrass ('Alamo' cultivar) were purchased from Browning Seed Company located in Plainview, Texas, (34° 11' 28" N, 101° 43' 8" W) and delivered to Nicholls State University in ready to germinate condition. The commercial big bluestem seed was grown in Missouri, the commercial indiagrass was grown in Texas, and the commercial switchgrass was grown in Oklahoma. The local seed lot was hand-harvested

from four (4) indigenous populations located along Highway 14 in Kaplan, Louisiana (Vermillion Parish; 30°06'26"N, 92°07'26"W) in October of 2007, and stored in paper bags located in a temperature controlled laboratory at Nicholls State University at room temperature (~21°C) until processing to remove all debris (Figure 3). For switchgrass and big bluestem, whole seed heads were clipped and placed in paper bags to be processed. For indianguass, seed was hand-stripped from the stalks into paper bags to be processed. Local seed was cleaned at the NRCS Plant Materials Center in Golden Meadow, Louisiana, using a bundle seed thrasher, fluffy seed debearder, and an air screen seed cleaner on 6 November 2007. After cleaning, seed was stored in paper bags in a temperature controlled laboratory (~21°C) at Nicholls State University for four (4) months until commercial seed was received. Once commercial seed was received in February 2008, germination test were conducted beginning with day 0 germination trial starting on 14 February 2008.

### **Storage and Treatment of Seed**

Ecotypes (local and commercial) were exposed to similar storage lengths and treatments. Seeds were stored at two temperatures with uncontrolled humidity, controlled refrigerated at 4° C ( $4.48 \pm 0.75^{\circ}\text{C}$ ) and ambient local temperature ( $21.3 \pm 6.3^{\circ}\text{C}$ ) at the Nicholls State University Farm (Figure 4). Germination rates were assessed after 0, 60, 120, 180, 240, 300, and 360 days of storage with cold storage beginning on 14 February 2008 (Figure 4).

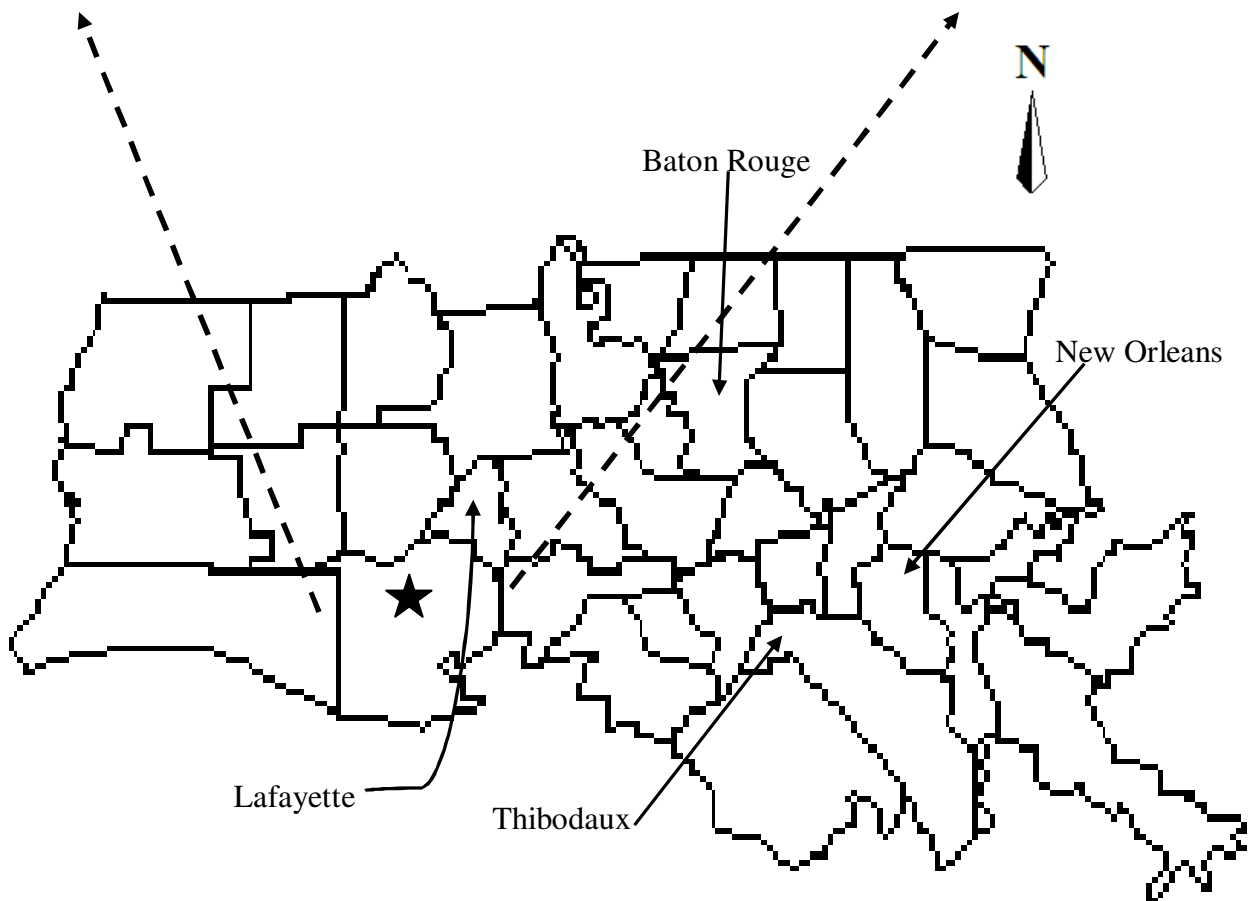
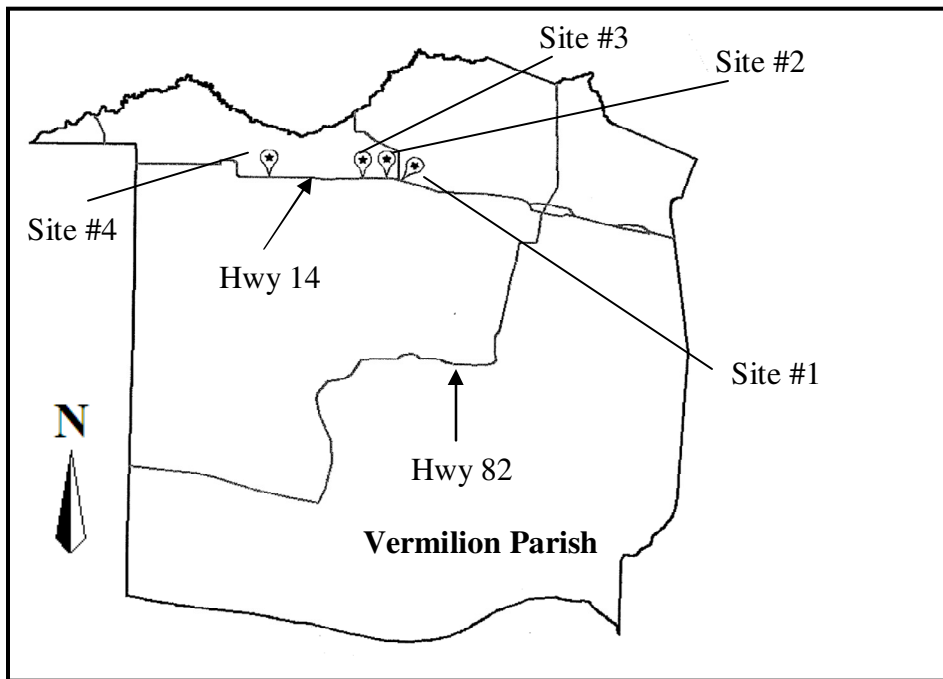


Figure 3. A star depicts the approximate local collection site in Kaplan, Louisiana ( $30^{\circ}06'26''\text{N}$ ,  $92^{\circ}07'26''\text{W}$ ). Seed was collected from four (4) sites along Highway 14. Site #1 ( $30^{\circ}0'20.82''\text{N}$ ;  $92^{\circ}18'44.22''\text{W}$ ), site #2 ( $30^{\circ}0'24.72''\text{N}$ ;  $92^{\circ}19'26.34''\text{W}$ ), site #3 ( $30^{\circ}0'22.00''\text{N}$ ;  $92^{\circ}21'21.25''\text{W}$ ), and site #4 ( $30^{\circ}0'25.50''\text{N}$ ;  $92^{\circ}27'43.38''\text{W}$ ).

## Germination

Each treatment consisted of 4 replicate clear plastic dishes 8.9 x 8.9 cm (3.5 in x 3.5 in) with lids containing 100 randomly assigned seeds each (Figure 4). All dishes were lined with two layers of 7.6 cm x 7.6 cm (3 in x 3 in) indented germination pads purchased from Seedburo Equipment Company in Chicago, Illinois and moistened as needed with distilled water throughout the germination period. Quantification of germination rates consisted of 28 days with seed counts performed every 7 days as outlined by Association of Official Seed Analysts seed testing manual (AOSA 2007). Seeds were considered germinated if the radicle was visible and all germinated seeds were discarded after being counted to reduce crowding in the dishes. Germination tests were conducted in 0.3048 m<sup>3</sup> (1 cubic ft) incubators at mean temperatures of 19.8 ± 2.3°C, 24.5 ± 1.8°C, and 28.3 ± 3.6°C for each seed lot. The target temperatures were 20, 25, and 30°C respectively, and these temperatures will be used to refer to the germination temperatures throughout the thesis (Figure 4). Seeds were exposed to 12 hours light/ 12 hours dark photoperiod for all treatments with light provided by 4 – 122 cm (4 ft) long fluorescent bulbs at 5000 Kelvin, the color temperature of natural daylight. The commercially obtained cultivars served as the control groups; therefore, germination rates of the local ecotypes were compared to the germination rates of the commercial cultivars.

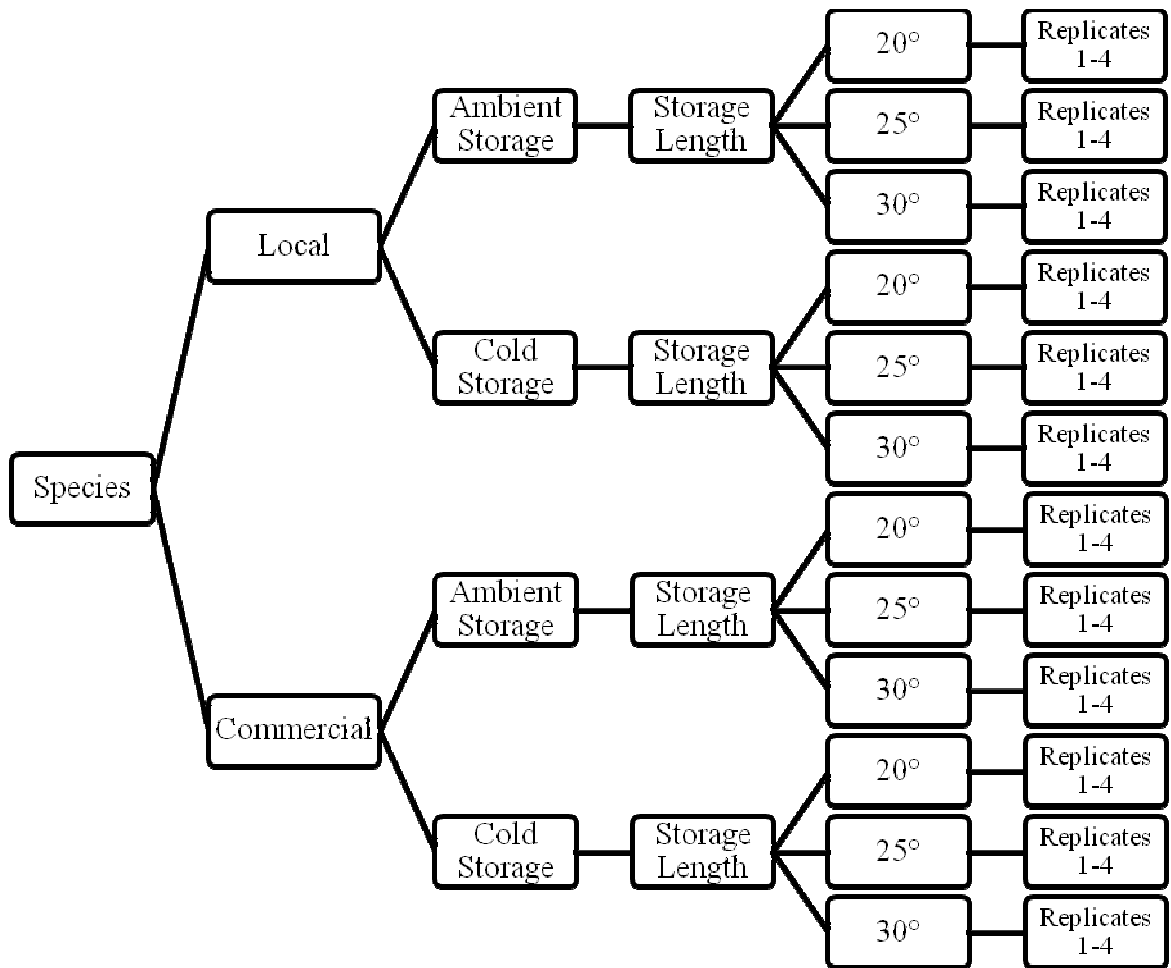


Figure 4. Experimental design outlining how treatments were analyzed and compared. Local and commercial represent the origin of the seed lots for each species. Three (3) germination temperatures were used for all seed lots. There are seven (7) storage lengths (0, 60, 120, 180, 240, 300, and 360 days) at which a germination test was conducted. Each replicate (1-4) contained 100 seeds of the specified seed lot.

## **Tetrazolium Testing**

Tetrazolium testing was performed on each seed lot as outlined by the Tetrazolium Testing Committee of the Association of Official Seed Analysts (AOSA 2000) to provide an estimate of seed viability for each seed lot. For each seed lot, three (3) replicate dishes with 100 seeds in each dish were tested. The tetrazolium derivative used was 2, 3, 5-triphenyl tetrazolium chloride (TTC) purchased from Seedburo Equipment Company in Chicago, Illinois, at a concentration of 0.25%. Seeds were placed in saturated paper towels overnight at room temperature to be preconditioned until fully imbibed with water and soft enough to allow for a clean slice through the embryo. Once conditioned, the seeds were bisected longitudinally with a razor blade near the center of the caryopsis above the embryo (Figure 5). The embryo end of the seeds were placed in petri dishes and soaked in TTC solution for 12 hours at approximately 22° C (room temperature) in the absence of light. The seeds were soaked in the dark to prevent nonembryonic material or dead embryos from absorbing the dye and creating false positives (AOSA 2000).

Once soaking was complete, excess tetrazolium was removed with a medicine dropper leaving just enough liquid to prevent the seeds from drying out. Seed viability was evaluated immediately after soaking was complete. Seeds were separated into germinable and non-germinable groups. Seeds were considered germinable when (AOSA 2000):

1. The embryo structures are well developed, non-fractured and of a “normal” cherry red color.
2. The embryo contains no more than the maximum listed for one or more of the following types of deterioration:

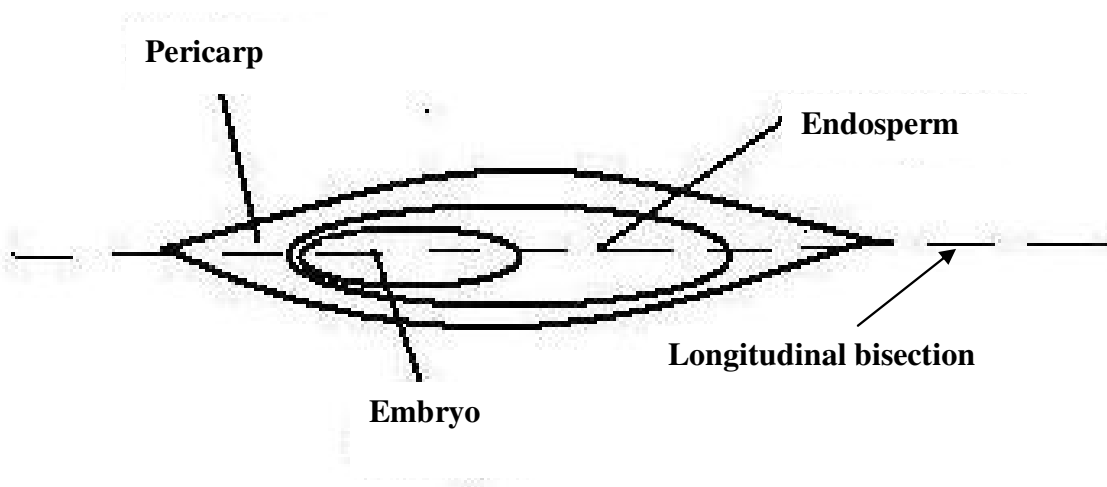


Figure 5. Seeds were bisected longitudinally to expose embryos to 2, 3, 5-triphenyl tetrazolium chloride solution when soaked in Petri dishes.

- a. Necroses within the upper or lower ends of the scutellum. No more than 1/3 of the scutellum should be unstained at either end.
- b. Radicle is unstained.
- c. Shallow layer of white, blurred tissue over the cut surfaces of the embryo structures.
- d. Insect, rodent, mechanical, or other injuries that do not involve essential structures to a critical extent.

### **Statistical Analyses**

Mean ( $\pm$  standard deviation) was calculated for tetrazolium test results for each treatment.

Means for tetrazolium tests were subjected to analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis to compare commercial viability to local viability (SAS 2003).

Mean percent germinated was calculated for all seed treatments for each 60 day interval.

Means for each 60 day interval were subjected to ANOVA followed by Tukey's *post hoc* analysis (SAS 2003). All tests were based on  $\alpha = 0.05$ .

## RESULTS

### Big Bluestem

Tetrazolium tests conducted in April 2008 (two months after start of germination trials), resulted in a mean of 52% ( $\pm 7.5$ ) viable seeds for commercial big bluestem and 18% ( $\pm 3.2$ ) viable seeds for the local ecotype indicating a higher percent of viable seeds in the commercial source ( $P = 0.0022$ ; Figure 6).

Average ambient storage temperature at the Nicholls State University farm was 21.3°C ( $\pm 6.3$ ) ranging from a high of near 30°C in the summer months to a low of about 7°C in the winter months (Figure 7). Mean humidity for ambient storage was 73.7% ( $\pm 8.3$ ) ranging from a high near 90% to a low near 30% (Figure 8). All commercial and local seed lots for all species were stored in the same ambient storage and exposed to the same ambient conditions.

Percent germination was higher in commercial seed than in local seed ( $P < 0.0001$ ). Based on ANOVA and Tukey's *post hoc* analyses, percent germination was similar in local and commercial seed up to 120 days of storage for both ambient and cold stored seeds (Figure 8). Percent germination of ambient stored seeds decreased after 120 days of storage for both local and commercial seeds (Figure 9; Tables 1B and 2B). No commercial or local big bluestem seeds stored at ambient temperature germinated after 180 days of storage (Figure 9; Tables 1 and 2).

In commercial big bluestem seeds stored at ambient temperature for 120 days, mean percent germination was lowest 6.0% ( $\pm 1.83$ ) at 30°C (Figure 9A; Table 1B). At 180 days, commercial seeds from cold storage had a higher germination percentage than seeds from ambient storage with the exception of those germinated in the 30°C incubator

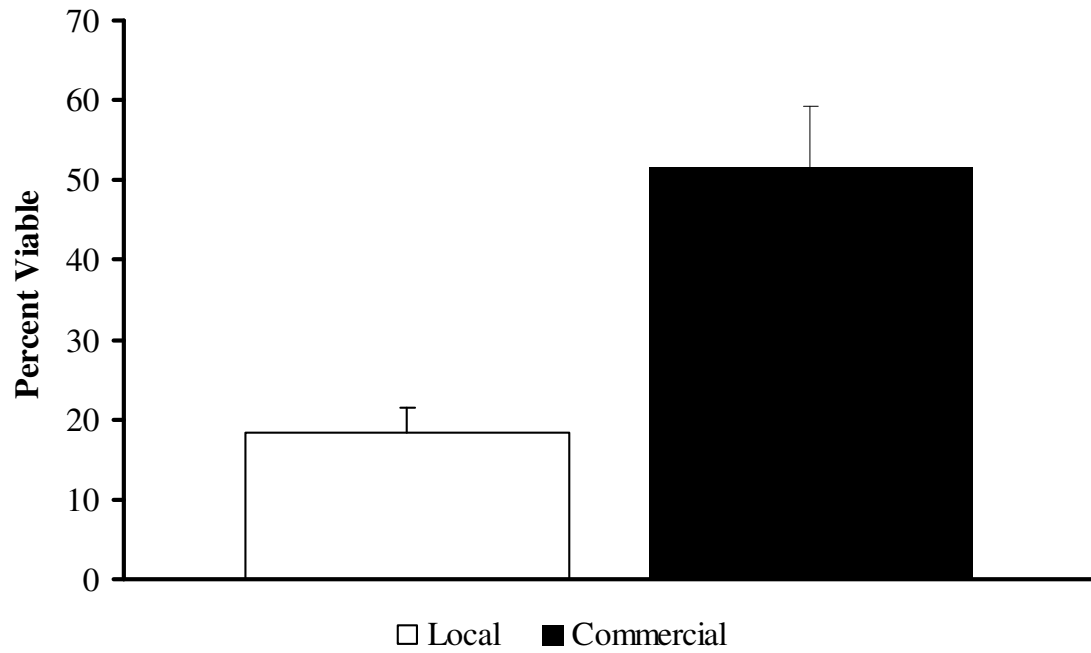


Figure 6. Mean ( $\pm$  standard deviation) percent viable seeds based on tetrazolium tests for commercial and local sources of big bluestem. Percent of viable seeds was higher for the commercial source ( $\alpha = 0.05$ ). Mean percent viable for local seed was  $18.3 \pm 3.22\%$  and for commercial seed was  $51.7 \pm 7.57\%$ .

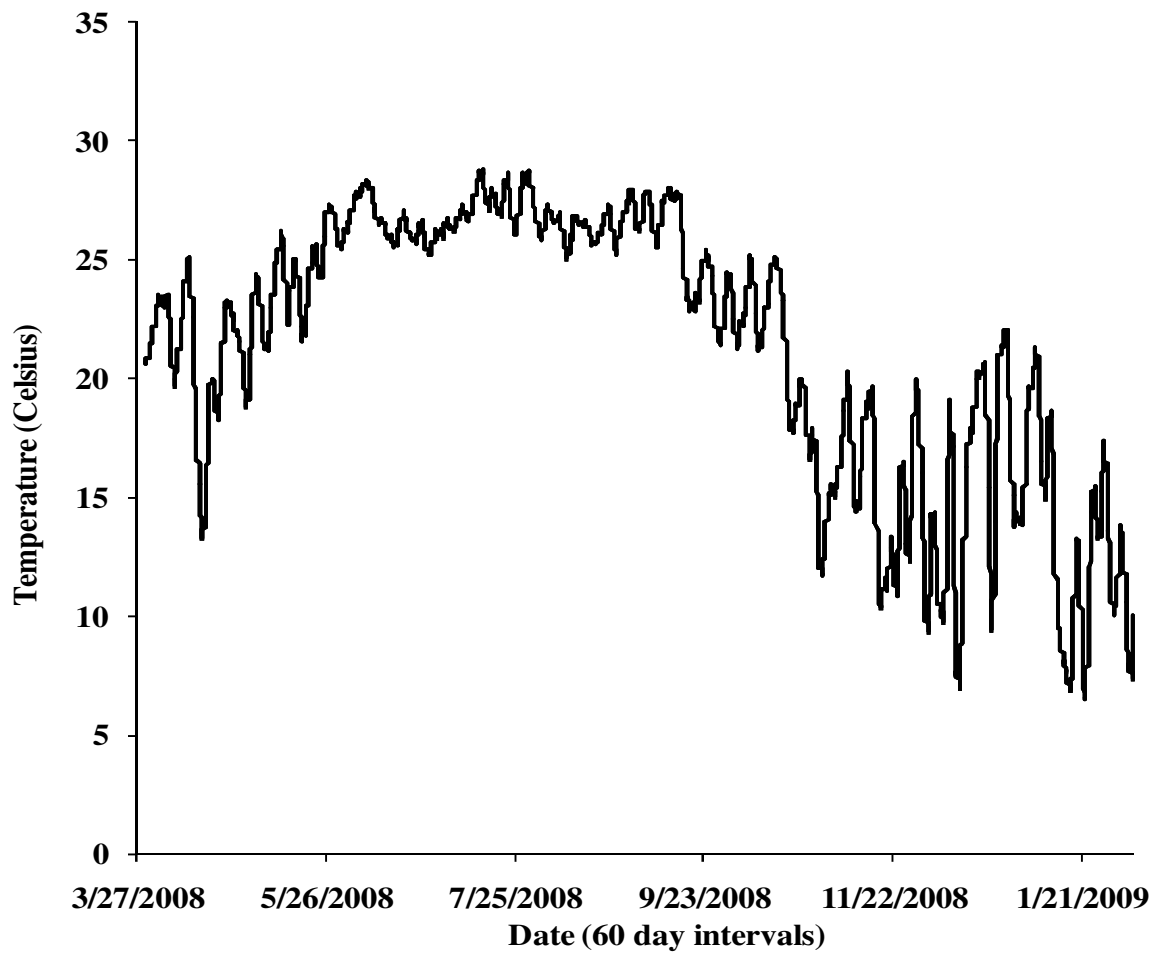


Figure 7. Average daily temperature in ambient storage location ( $21.3^{\circ}\text{C} \pm 6.3$ ) at the Nicholls State University farm in Thibodaux, Louisiana. Data were collected hourly.

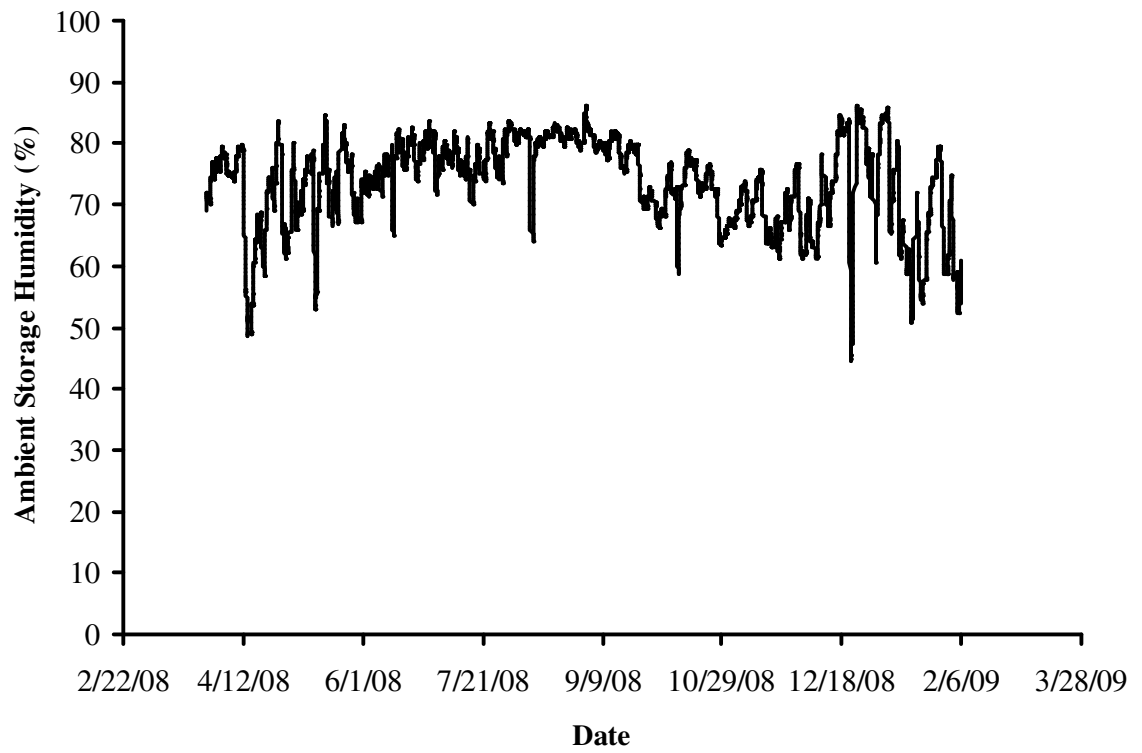


Figure 8. Average daily humidity in ambient storage location ( $73.7\% \pm 8.3$ ) at the Nicholls State University farm in Thibodaux, Louisiana. Data were collected hourly.

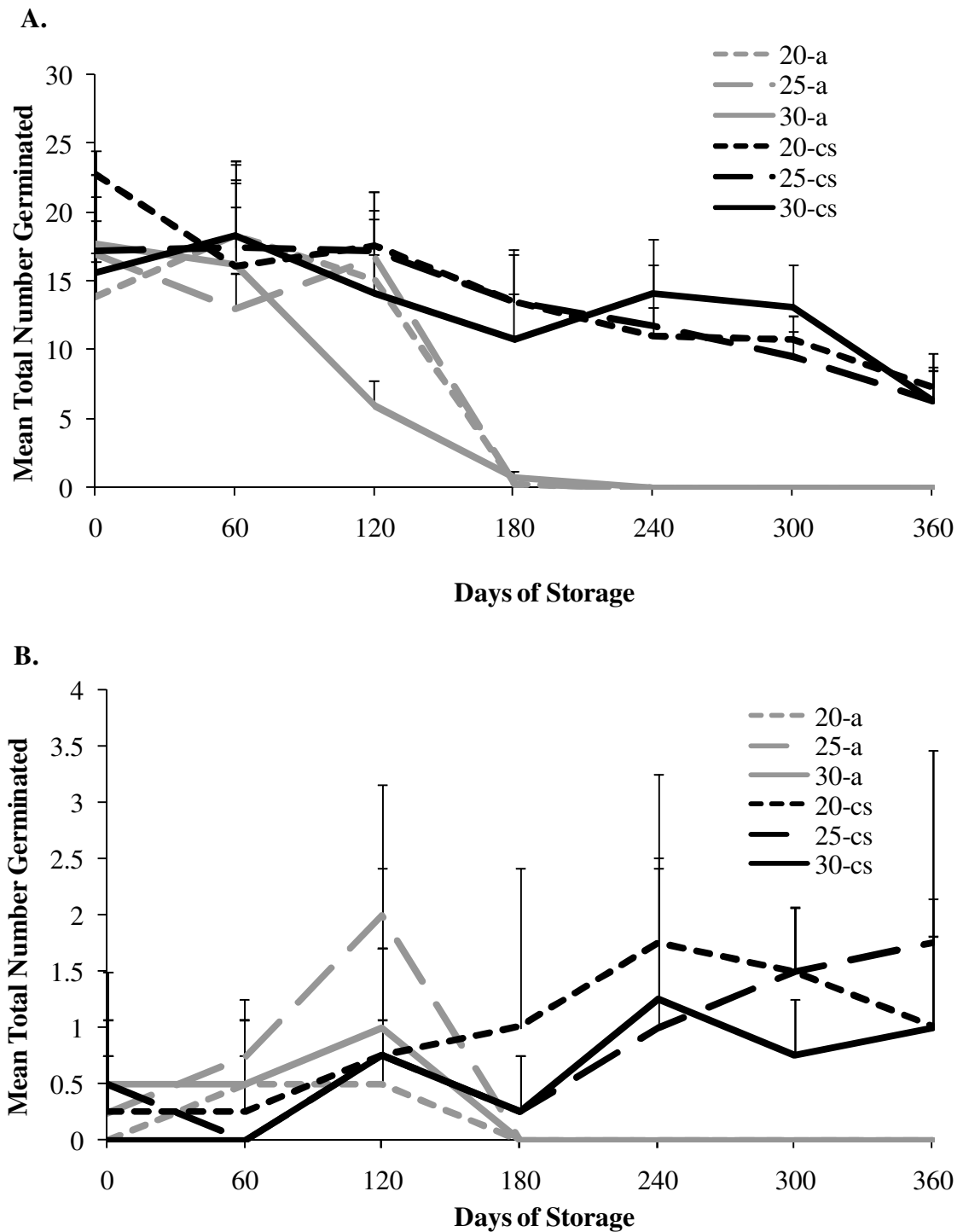


Figure 9. (A.) Mean ( $\pm$  SD) total number of germinated seeds of commercial big bluestem at 60 day intervals of storage for 1 year. (B.) Mean ( $\pm$  SD) total number of germinated seeds of local big bluestem at 60 day intervals of storage for 1 year. Grey lines are ambient storage seed treatments and black lines are cold storage treatments. In the legend, 20, 25, and 30 represent the germination temperatures and cs = cold storage and a = ambient storage.

Table 1. Mean percent germinated, standard deviations, and results of one-way ANOVA comparisons for each 60 day interval of commercial big bluestem cold stored seeds and ambient stored seeds. Means with the same letter indicate no difference (Tukey's multiple range test). Dashed lines indicate zero germinated seeds.

Days of Storage	Cold Storage			Ambient Storage		
	20°C	25°C	30°C	20°C	25°C	30°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>0</b>	22.8 ± 1.71 <sup>A</sup>	17.3 ± 3.86 <sup>AB</sup>	15.5 ± 1.00 <sup>AB</sup>	13.8 ± 3.30 <sup>B</sup>	17.0 ± 5.77 <sup>AB</sup>	17.8 ± 1.71 <sup>AB</sup>
<b>60</b>	16.0 ± 6.16 <sup>A</sup>	17.5 ± 6.24 <sup>A</sup>	18.3 ± 4.19 <sup>A</sup>	18.3 ± 5.31 <sup>A</sup>	13.0 ± 2.58 <sup>A</sup>	16.3 ± 4.19 <sup>A</sup>
<b>120</b>	17.5 ± 4.04 <sup>A</sup>	17.3 ± 2.87 <sup>A</sup>	14.0 ± 1.94 <sup>AB</sup>	15.0 ± 6.48 <sup>A</sup>	16.8 ± 2.75 <sup>A</sup>	6.0 ± 1.83 <sup>B</sup>
<b>180</b>	13.5 ± 3.41 <sup>A</sup>	13.5 ± 3.79 <sup>A</sup>	10.8 ± 3.10 <sup>AB</sup>	1.0 ± 0.50 <sup>B</sup>	1.0 ± 0.50 <sup>B</sup>	1.0 ± 0.50 <sup>B</sup>
<b>240</b>	11.0 ± 2.16 <sup>A</sup>	11.8 ± 4.50 <sup>A</sup>	14.0 ± 4.08 <sup>A</sup>	-	-	-
<b>300</b>	10.8 ± 1.71 <sup>A</sup>	9.5 ± 1.91 <sup>A</sup>	13.0 ± 3.16 <sup>A</sup>	-	-	-
<b>360</b>	7.3 ± 1.50 <sup>A</sup>	6.3 ± 3.50 <sup>A</sup>	6.3 ± 2.22 <sup>A</sup>	-	-	-

Table 2. Mean percent germinated, standard deviations, and results of one-way ANOVA comparisons for each 60 day interval of local big bluestem cold stored seeds and ambient stored seeds. Means with the same letter indicate no difference (Tukey's multiple range test). Dashed lines indicate zero germinated seeds.

Days of Storage	Cold Storage			Ambient Storage		
	20°C	25°C	30°C	20°C	25°C	30°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>0</b>	1.0 ± 0.50 <sup>B</sup>	1.0 ± 0.58 <sup>B</sup>	-	-	1.0 ± 0.50 <sup>B</sup>	2.0 ± 1.00 <sup>A</sup>
<b>60</b>	1.0 ± 0.50 <sup>A</sup>	-	-	1.0 ± 0.58 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	1.0 ± 0.58 <sup>A</sup>
<b>120</b>	1.5 ± 0.96 <sup>A</sup>	1.5 ± 0.96 <sup>A</sup>	1.5 ± 0.96 <sup>A</sup>	1.0 ± 0.58 <sup>A</sup>	2.0 ± 1.15 <sup>A</sup>	2.0 ± 1.41 <sup>A</sup>
<b>180</b>	2.0 ± 1.41 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	-	-	-
<b>240</b>	2.3 ± 1.50 <sup>A</sup>	2.0 ± 1.41 <sup>A</sup>	1.7 ± 1.26 <sup>A</sup>	-	-	-
<b>300</b>	1.5 ± 0.58 <sup>A</sup>	1.5 ± 0.58 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	-	-	-
<b>360</b>	2.0 ± 1.15 <sup>A</sup>	2.3 ± 1.71 <sup>A</sup>	1.3 ± 0.82 <sup>A</sup>	-	-	-

( $P = 0.01$ ; Figure 9A; Table 1). After 240 days of storage, however, cold storage seeds germinated at 30°C also had a higher percent germination than ambient treatments (Figure 9A).

### **Indiangrass**

Tetrazolium tests conducted in April 2008 (two months after start of germination trials), resulted in a mean of 70.0% ( $\pm 10.5$ ) viable seeds for commercial indiagrass and 38.0% ( $\pm 2.1$ ) viable seeds for the local ecotype indicating a higher percent of viable seeds in the commercial source ( $P = 0.0062$ ; Figure 10).

Percent germination was higher in commercial seed than in local seed ( $P < 0.0001$ ). Based on ANOVA and Tukey's *post hoc* analyses, percent germination was similar in commercial seed up to 120 days of storage for both ambient and cold stored seeds (Figure 11A). After 120 days of storage, commercial seed from ambient storage had a lower mean germination percent than seed from cold storage excluding cold stored seed germinated at 30°C ( $P < 0.05$ ; Figure 11A; Table 3). After 240 days of storage, no commercial seed from ambient storage germinated (Figure 11A; Table 3). At 180 days of storage, commercial seed from cold storage had higher percent germination at 20° and 25°C ( $P = 0.0004$ ; Figure 11A; Table 3A). At 240 days of storage, commercial seed from cold storage had higher percent germination at 30°C and was similar to the other cold storage germination temperatures ( $P < 0.0001$ ; Figure 11A).

Percent germination was similar in all local seed for all storage days except for 300 days (Figure 11B; Table 4). At 300 days of storage, local seed from cold storage had higher percent germination than seed from ambient storage ( $P < 0.0002$ ; Figure 11B; Table 4). At 240 days of storage, no local seeds from ambient storage germinated at 20°

or 30°C (Figure 11B; Table 4B). After 300 days of storage, no local seeds from ambient storage germinated at 25°C (Figure 11B; Table 4B).

### **Switchgrass**

Tetrazolium tests conducted in April 2008 (two months after the start of germination trials), resulted in a mean of 86.0% ( $\pm 2.0$ ) viable seeds for commercial switchgrass and 6.3% ( $\pm 4.0$ ) viable seeds for the local ecotype indicating a higher percent of viable seeds in the commercial source ( $P < 0.0001$ ; Figure 12).

Percent germination was higher in commercial seed than in local seed ( $P < 0.0001$ ; Tables 5 and 6). Based on ANOVA and Tukey's *post hoc* analyses, percent germination for commercial seeds was dependent on germination temperature and varied at different lengths of storage. At 60 days of storage the higher germination temperature (30°C) resulted in higher percent germination regardless of storage type ( $P = 0.0078$ ; Figure 13A). At 120 days of storage, seeds from cold storage had a higher percent germination at 30°C than all seeds germinated at 20°C ( $P = 0.0078$ ; Figure 13A; Tables 5 and 6). At 180 days of storage, seeds from ambient storage had a higher percent germination at 25°C than all seeds germinated at 20°C ( $P = 0.0078$ ; Figure 13A; Tables 5 and 6). At 240 days of storage, percent germination for commercial seeds from cold storage germinated at 30°C was higher than percent germination for all ambient stored seeds ( $P = 0.0078$ ; Figure 13A). Also at 240 days of storage, percent germination at 25° and 30°C was higher than percent germination at 20°C with the exception of ambient seed germinated at 30°C ( $P = 0.0078$ ; Figure 13A). At 300 days of storage, percent germination of commercial seed from cold storage germinated at 30°C was higher than ambient stored seed germinated at temperatures below 30°C ( $P = 0.0078$ ; Figure 13A).

Also at 300 days of storage, percent germination of commercial cold stored seeds germinated at 25°C was higher than percent germination of ambient stored seeds germinated at 20°C ( $P = 0.0078$ ; Figure 13A). For commercial seeds stored for 360 days, percent germination of cold stored seeds germinated at 25°C was significantly higher than the percent germination of ambient stored seeds germinated at 20°C ( $P = 0.0078$ ; Figure 13A; Table 5).

Local switchgrass exhibited low germination percentages with the highest mean percentage 4.0% ( $\pm 2.4$ ) occurring in cold stored seeds germinated at 30°C at 300 days of storage (Figure 13B; Table 6). The highest percent germination 3.8% ( $\pm 6.8$ ) for ambient stored seeds occurred at 25°C at 120 days of storage (Figure 13B; Table 6).

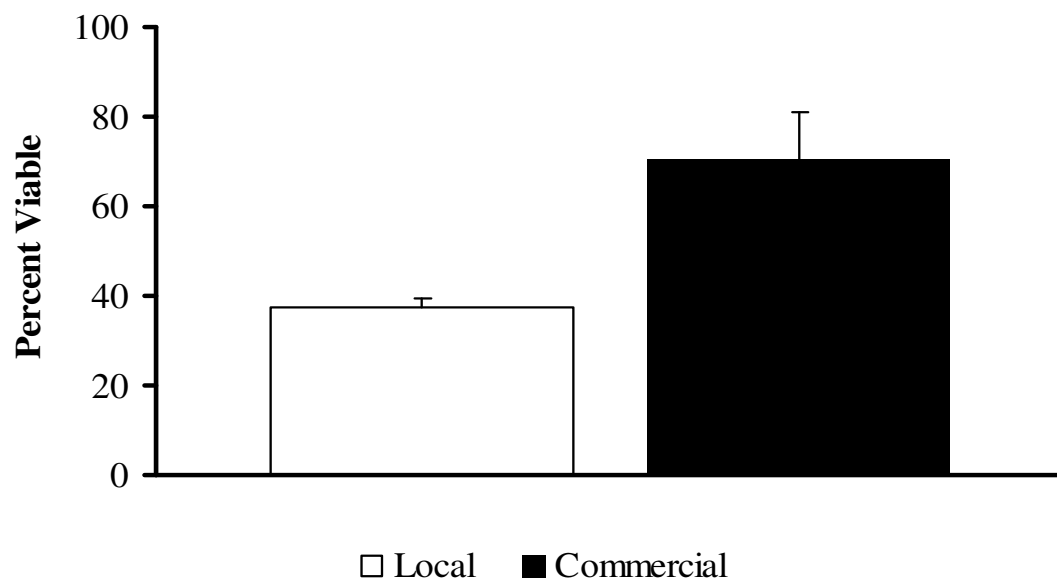


Figure 10. Mean ( $\pm$  SD) percent viable seeds based on tetrazolium test for commercial and local sources of indiagrass. Percent of viable seeds was higher for the commercial source ( $\alpha = 0.05$ ). Mean percent viable for local seeds was  $37.7 \pm 2.1\%$  and for commercial seeds was  $70.3 \pm 10.5\%$ .

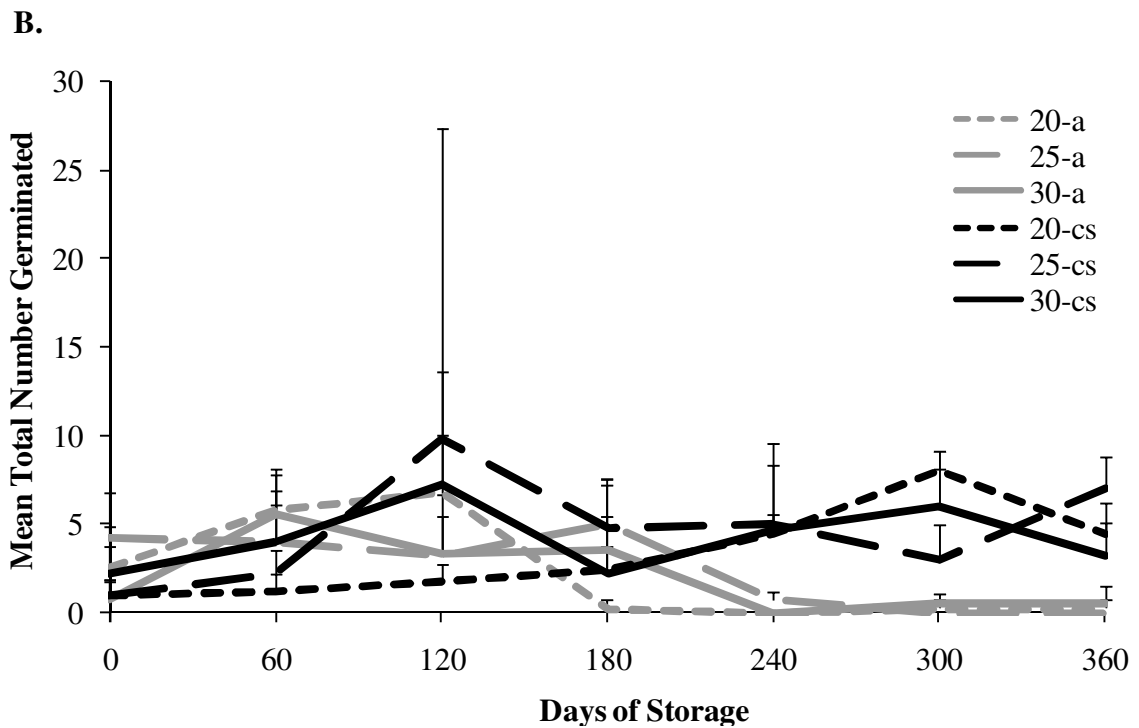
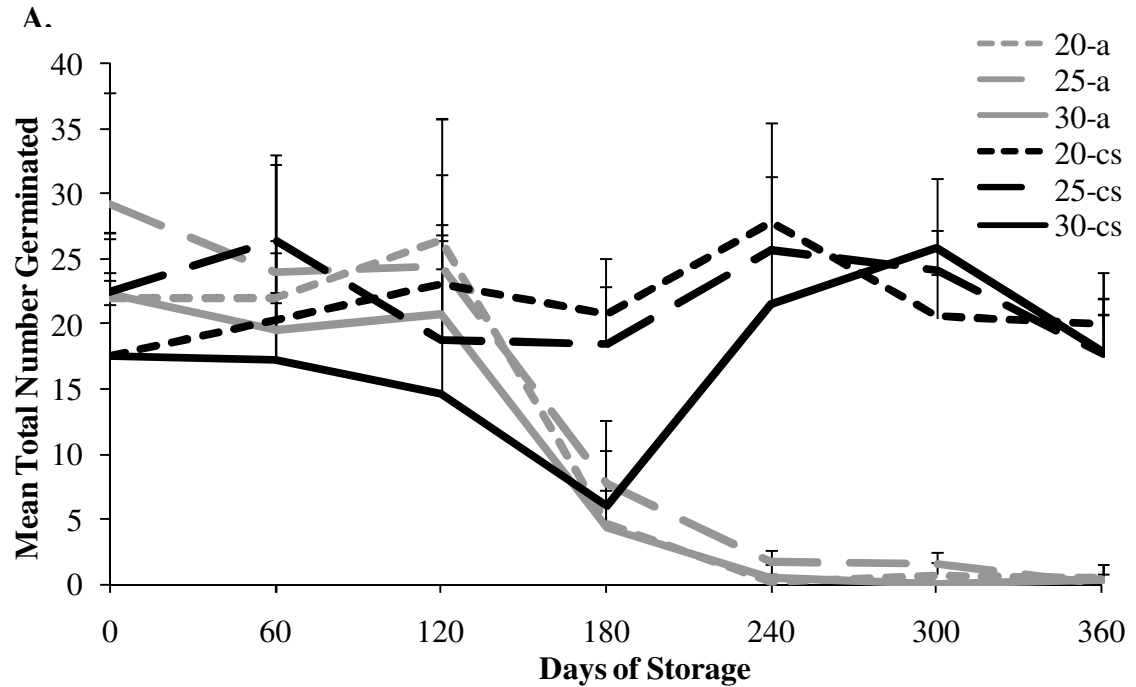


Figure 11. (A.) Mean ( $\pm$  SD) total number of germinated seeds of commercial indiagrass at 60 day intervals of storage for 1 year. (B.) Mean ( $\pm$  SD) total number of germinated seeds of local indiagrass at 60 day intervals of storage for 1 year. Grey lines are ambient storage seed treatments and black lines are cold storage treatments. In the legend, 20, 25, and 30 represent the germination temperatures and cs = cold storage and a = ambient storage. After 240 days of storage there was a difference in the total number of germinated seeds between ambient (a) stored seeds and seeds placed in cold storage (cs) for commercial seeds.

Table 3. Mean percent germinated, standard deviations, and results of one-way ANOVA comparisons for each 60 day interval of commercial indiangrass cold stored seeds and ambient stored seeds. Means with the same letter indicate no difference (Tukey's multiple range test). Dashed lines indicate zero germinated seeds.

Days of Storage	Cold Storage			Ambient Storage		
	20°C	25°C	30°C	20°C	25°C	30°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>0</b>	17.5 ± 4.04 <sup>A</sup>	22.5 ± 4.51 <sup>A</sup>	17.5 ± 6.56 <sup>A</sup>	22.0 ± 1.41 <sup>A</sup>	29.3 ± 8.54 <sup>A</sup>	22.3 ± 4.27 <sup>A</sup>
<b>60</b>	20.3 ± 1.50 <sup>A</sup>	26.5 ± 6.61 <sup>A</sup>	17.3 ± 5.19 <sup>A</sup>	22.0 ± 3.46 <sup>A</sup>	24.0 ± 8.29 <sup>A</sup>	19.5 ± 6.95 <sup>A</sup>
<b>120</b>	23.0 ± 3.83 <sup>A</sup>	25.0 ± 12.82 <sup>A</sup>	14.5 ± 9.75 <sup>A</sup>	26.5 ± 9.33 <sup>A</sup>	24.5 ± 1.91 <sup>A</sup>	20.8 ± 6.99 <sup>A</sup>
<b>180</b>	20.8 ± 4.27 <sup>A</sup>	18.5 ± 4.36 <sup>A</sup>	8.0 ± 6.68 <sup>B</sup>	4.8 ± 2.50 <sup>B</sup>	7.8 ± 2.50 <sup>B</sup>	4.3 ± 2.22 <sup>B</sup>
<b>240</b>	27.8 ± 3.59 <sup>A</sup>	25.8 ± 9.71 <sup>A</sup>	21.5 ± 4.04 <sup>A</sup>	1.0 ± 0.50 <sup>B</sup>	1.8 ± 0.96 <sup>B</sup>	2.0 ± 1.00 <sup>B</sup>
<b>300</b>	20.5 ± 3.32 <sup>A</sup>	24.3 ± 6.95 <sup>A</sup>	25.8 ± 1.50 <sup>A</sup>	1.5 ± 0.96 <sup>B</sup>	1.5 ± 1.00 <sup>B</sup>	-
<b>360</b>	20.0 ± 0.82 <sup>A</sup>	17.8 ± 6.18 <sup>A</sup>	17.8 ± 4.27 <sup>A</sup>	2.0 ± 1.00 <sup>B</sup>	-	1.0 ± 0.50 <sup>B</sup>

Table 4. Mean percent germinated, standard deviations, and results of one-way ANOVA comparisons for each 60 day interval of local indiangrass cold stored seeds and ambient stored seeds. Means with the same letter indicate no difference (Tukey's multiple range test). Dashed lines indicate zero germinated seeds.

Days of Storage	Cold Storage			Ambient Storage		
	20°C	25°C	30°C	20°C	25°C	30°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>0</b>	1.3 ± 0.82 <sup>A</sup>	1.3 ± 0.82 <sup>A</sup>	3.0 ± 2.63 <sup>A</sup>	2.5 ± 1.29 <sup>A</sup>	4.3 ± 2.50 <sup>A</sup>	1.5 ± 0.96 <sup>A</sup>
<b>60</b>	1.7 ± 0.96 <sup>A</sup>	2.3 ± 1.26 <sup>A</sup>	4.0 ± 2.16 <sup>A</sup>	5.8 ± 2.06 <sup>A</sup>	4.0 ± 2.94 <sup>A</sup>	5.5 ± 2.65 <sup>A</sup>
<b>120</b>	1.8 ± 0.96 <sup>A</sup>	19.5 ± 17.56 <sup>A</sup>	7.3 ± 6.39 <sup>A</sup>	6.8 ± 3.30 <sup>A</sup>	4.3 ± 2.22 <sup>A</sup>	4.3 ± 3.40 <sup>A</sup>
<b>180</b>	2.5 ± 1.29 <sup>A</sup>	4.8 ± 2.50 <sup>A</sup>	3.0 ± 2.63 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	5.0 ± 2.58 <sup>A</sup>	3.5 ± 1.91 <sup>A</sup>
<b>240</b>	4.5 ± 1.00 <sup>A</sup>	5.0 ± 3.37 <sup>A</sup>	4.8 ± 4.79 <sup>A</sup>	-	1.0 ± 0.50 <sup>A</sup>	-
<b>300</b>	8.0 ± 1.15 <sup>A</sup>	3.0 ± 2.00 <sup>AB</sup>	6.0 ± 2.16 <sup>AB</sup>	1.0 ± 0.50 <sup>B</sup>	-	1.0 ± 0.58 <sup>B</sup>
<b>360</b>	4.5 ± 1.73 <sup>A</sup>	7.0 ± 1.83 <sup>A</sup>	3.3 ± 1.89 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	-	2.0 ± 1.00 <sup>A</sup>

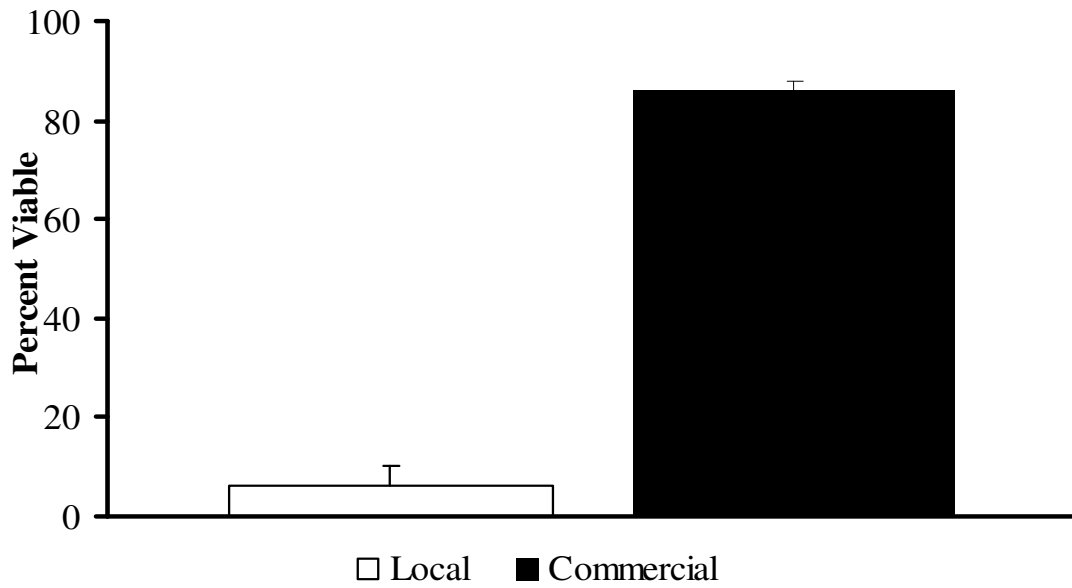


Figure 12. Mean ( $\pm$  standard deviation) percent viable seeds based on tetrazolium test for commercial and local sources of switchgrass. Percent of viable seeds is greater for the commercial source ( $\alpha = 0.05$ ). Mean percent viable for local seeds was  $6.3 \pm 4.0\%$  and for commercial seeds was  $86.0 \pm 2.0\%$

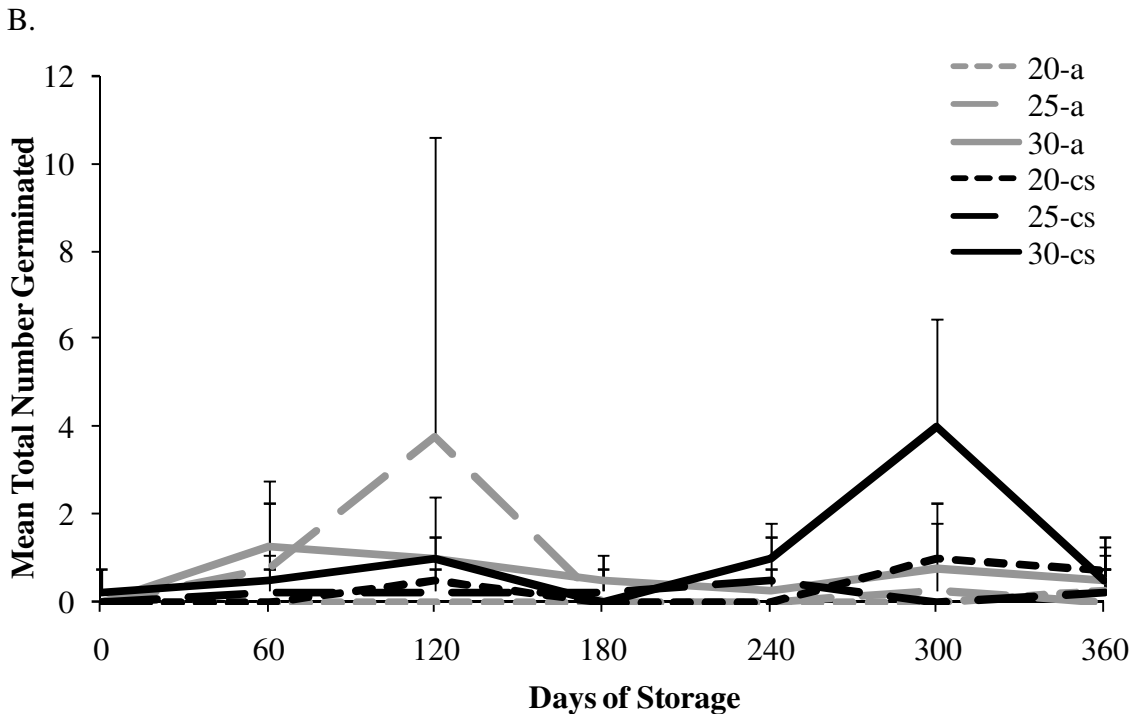
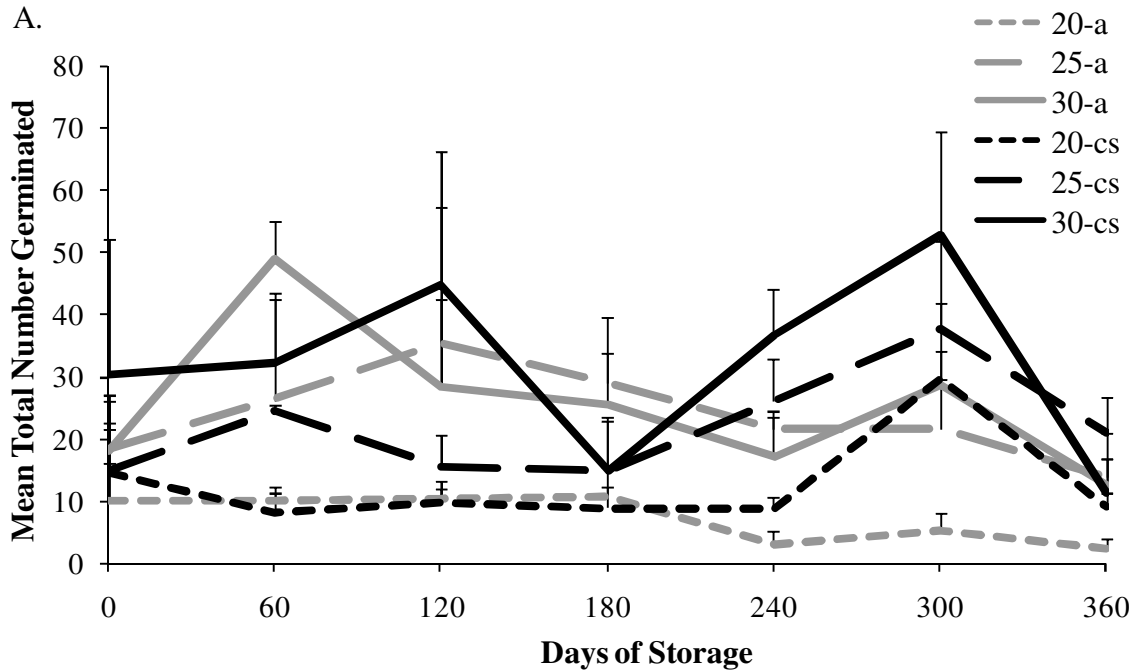


Figure 13. (A.) Mean ( $\pm$  standard deviation) total number of germinated seeds of commercial switchgrass at 60 day intervals of storage for 1 year. (B.) Mean ( $\pm$  SD) total number of germinated seeds of local switchgrass at 60 day intervals of storage for 1 year. Grey lines are ambient storage seed treatments and black lines are cold storage treatments. In the legend, 20, 25, and 30 represent the germination temperatures and cs = cold storage and a = ambient storage. Percent germination for commercial seeds was dependant on germination temperature and varied at different storage lengths.

Table 5. Mean percent germinated, standard deviations, and results of one-way ANOVA comparisons for each 60 day interval of commercial switchgrass cold stored seeds and ambient stored seeds. Means with the same letter indicate no difference (Tukey's multiple range test). Dashed lines indicate zero germinated seeds.

Days of Storage	Cold Storage			Ambient Storage		
	20°C	25°C	30°C	20°C	25°C	30°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>0</b>	14.8 ± 6.85 <sup>A</sup>	15.0 ± 7.62 <sup>A</sup>	30.3 ± 21.79 <sup>A</sup>	10.3 ± 5.96 <sup>A</sup>	18.5 ± 8.50 <sup>A</sup>	18.0 ± 8.04 <sup>A</sup>
<b>60</b>	8.3 ± 3.95 <sup>C</sup>	24.5 ± 1.00 <sup>BC</sup>	32.3 ± 11.12 <sup>A</sup>	10.3 ± 0.96 <sup>C</sup>	26.3 ± 16.34 <sup>BC</sup>	48.8 ± 6.29 <sup>AB</sup>
<b>120</b>	9.8 ± 2.22 <sup>B</sup>	15.5 ± 5.20 <sup>AB</sup>	44.8 ± 21.52 <sup>A</sup>	10.5 ± 2.65 <sup>B</sup>	35.3 ± 22.04 <sup>AB</sup>	28.3 ± 14.17 <sup>AB</sup>
<b>180</b>	9.0 ± 3.16 <sup>B</sup>	15.0 ± 8.37 <sup>AB</sup>	14.8 ± 7.98 <sup>AB</sup>	10.8 ± 4.79 <sup>B</sup>	29.0 ± 4.69 <sup>A</sup>	28.3 ± 14.27 <sup>AB</sup>
<b>240</b>	9.0 ± 1.83 <sup>CD</sup>	26.3 ± 6.60 <sup>AB</sup>	36.8 ± 7.23 <sup>A</sup>	2.8 ± 2.36 <sup>D</sup>	21.5 ± 3.11 <sup>B</sup>	17.0 ± 6.63 <sup>BC</sup>
<b>300</b>	29.5 ± 12.48 <sup>ABC</sup>	37.5 ± 14.34 <sup>AB</sup>	52.8 ± 16.74 <sup>A</sup>	5.3 ± 2.75 <sup>C</sup>	21.5 ± 8.19 <sup>BC</sup>	28.5 ± 5.74 <sup>ABC</sup>
<b>360</b>	9.3 ± 2.06 <sup>AB</sup>	21.0 ± 5.89 <sup>A</sup>	11.3 ± 5.56 <sup>B</sup>	3.0 ± 1.70 <sup>B</sup>	14.0 ± 2.83 <sup>AB</sup>	12.5 ± 8.35 <sup>AB</sup>

Table 6. Mean percent germinated, standard deviations, and results of one-way ANOVA comparisons for each 60 day interval of local switchgrass cold stored seeds and ambient stored seeds. Means with the same letter indicate no difference (Tukey's multiple range test). Dashed lines indicate zero germinated seeds.

Days of Storage	Cold Storage			Ambient Storage		
	20°C	25°C	30°C	20°C	25°C	30°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>0</b>	-	-	1.0 ± 0.50 <sup>A</sup>	-	-	-
<b>60</b>	-	1.0 ± 0.50 <sup>A</sup>	1.0 ± 0.58 <sup>A</sup>	-	3.0 ± 1.50 <sup>A</sup>	2.5 ± 1.50 <sup>A</sup>
<b>120</b>	2.0 ± 1.00 <sup>A</sup>	1.0 ± 0.50 <sup>A</sup>	1.0 ± 0.00 <sup>A</sup>	-	3.8 ± 6.85 <sup>A</sup>	2.0 ± 1.41 <sup>A</sup>
<b>180</b>	-	1.0 ± 05.0 <sup>A</sup>	-	-	-	1.0 ± 0.58 <sup>A</sup>
<b>240</b>	-	2.0 ± 1.00 <sup>A</sup>	1.3 ± 0.82 <sup>A</sup>	-	-	1.0 ± 0.50 <sup>A</sup>
<b>300</b>	1.3 ± 0.82 <sup>A</sup>	-	4.0 ± 2.45 <sup>A</sup>	-	1.0 ± 0.50 <sup>A</sup>	3.0 ± 1.50 <sup>A</sup>
<b>360</b>	1.0 ± 0.50 <sup>B</sup>	1.0 ± 0.50 <sup>B</sup>	1.0 ± 0.58 <sup>B</sup>	1.0 ± 0.50 <sup>B</sup>	-	2.0 ± 1.00 <sup>A</sup>

## DISCUSSION

Seed dormancy often reduces initial germination rates in big bluestem, indiagrass, switchgrass and other warm-season grasses. Several treatments break seed dormancy in big bluestem, thus increasing germination rates when compared to untreated seeds (Beckman et al. 1993; Madakadze et al. 2003). Treatments have been shown to break seed dormancy in switchgrass thus increasing germination rates when compared to untreated seeds (Haynes et al. 1997; Sarath et al. 2006; Zarnstoff et al. 1994). Treatments may break seed dormancy in indiagrass thus increasing germination rates when compared to untreated seeds (Sarath et al. 2006; Watkinson 1998). The purpose of this study was to determine what effects germination temperature, storage temperature, and storage length have on commercially and locally obtained seeds for each of these species. Prior to completing the germination tests, tetrazolium (TZ) testing was conducted on both the local and commercial sources to estimate the percentage of viable seed in both commercial and local seed lots.

Based on the TZ test, there was a higher percentage of viable seeds from the commercial seed lots compared to the local seed lots for all three species. A higher percentage of viable seed was expected from the commercial source because of management practices associated with commercial production. Weed management via herbicides, fertilizer application, and seedbed preparation are some of the common practices employed in the commercial production of warm-season grasses (Harper et al. 2007). These grow out practices reduce competition with other species and provide plants with optimal nutrients and moisture which natural conditions may not always provide. Also, selection practices associated with commercial production select for

increased seed production and higher germination rates. Wild stands often produce less viable seed because these common practices are not applied. Another possible reason for a lower percentage of viable seed from the local source was a poor crop year. Viable seed production of many local warm-season grasses throughout the Kaplan area was low in 2007 (Scott Edwards, NRCS, personal communication).

### **Big Bluestem**

Storage temperature significantly impacted the total number of germinated seeds over time (Figure 9). For both local and commercial seed, no or fewer seeds germinated after 120 days of ambient storage (Figure 9). Cold storage for more than 120 days may extend the life of viable seeds that perish after 120 days in ambient storage. Therefore, cold storage may not increase germination rates but may extend the life of the seed lot. A similar study supports this hypothesis. Kentucky bluegrass *Poa pratensis*, red fescue *Festuca rubra*, orchardgrass *Dactylis glomerata*, and chewings fescue *Festuca rubra* var. *commutata* stored at sub-freezing temperatures for up to 8 years maintained their original germination rates (Rincker 1986). These same species, however, experienced declines in germination rate when stored in an unheated warehouse where temperature fluctuated with weather conditions ranging from -20° to 40°C (Rincker 1986).

Germination temperature also influenced percent germination in commercial seed after 120 days of storage. Commercial big bluestem seed stored at ambient temperature for 120 days may have a lower germination percent at temperatures > 25°C (Figure 9A). Also, the ambient stored seed averaged 20-30°C for most of the first 180 days of storage (Figure 7). This suggests that the lack of cold stratification may require a lower germination temperature to break dormancy and/or promote germination.

## **Indiangrass**

Storage temperature significantly impacted total number of germinated seeds over time ( $P < 0.0001$ ; Figure 11). Emal and Conard (1973) found that dry indiagrass seeds stored at room temperature (8-28°C) remained dormant for 7 months before dormancy began to decline. Indiangrass seed dormancy has also been reduced 65% and 47% in dry seeds stored at 5°C for 5 or 11 months, respectively, when combined with a two week prechill (Watkinson and Pill 1998).

In local seeds, the only difference occurred at 300 days of storage ( $P = 0.0002$ ; Figure 11B). Local cold stored seed germinated at 20°C had a higher percent germination at 300 days of storage than all ambient stored seed germinated at 300 days of storage ( $P = 0.0002$ ; Figure 11B). There appeared to be a difference in mean percent germination of local cold stored seed germinated at 25°C at day 120 when compared to other treatments at 120 days of storage; however, there was no difference because of a high standard deviation for 25°C ( $9.8\% \pm 17.6$ ; Figure 11B). This indicates that cold storage for 120 days may extend the life of viable seeds that perish after 120 days in ambient storage. Therefore, cold storage may not increase germination rates but may extend the life of the seed lot.

## **Switchgrass**

Percent germination was dependant on germination temperature for commercial switchgrass seed. For most storage lengths, germination percentages were higher for commercial seed germinated at 30°C than for commercial seed germinated at 20°C, regardless of storage temperature (Figure 13A). Hanson and Johnson (2005) also found

that switchgrass germination was highest at temperatures between 25-35°C. This indicates that switchgrass may germinate best in soils  $\geq 25^{\circ}\text{C}$ .

When commercial switchgrass was compared at each 60 day interval of storage results varied. At 60 days of storage, percent germination was higher at 30°C regardless of storage temperature (Figure 13A). At 120 days of storage, only commercial seed from cold storage germinated at 30°C had a significantly higher percent germination than seeds germinated at 20°C (Figure 13A). At 180 days of storage, only commercial seed from ambient storage germinated at 25°C had a significantly higher percent germination than seeds germinated at 20°C (Figure 13A). These results indicate that cold storage may not increase germination rate in switchgrass. Zarnstoff et al. (1994) also found storage temperature to not affect germination rate in certain cultivars of switchgrass.

For commercial seeds at 240 days of storage, all seeds germinated at 25 and 30°C with the exception of (30-a) had a higher percent germination than seeds germinated at 20°C (Figure 13A). Commercial seed from cold storage germinated at 30°C had the highest percent germination at 240 and 300 days of storage (Figure 13A). This indicates that cold storage may break dormancy in some switchgrass seed when stored for 240-300 days. Percent germination for this same treatment, however, decreased by over 50% at 360 days of storage. This suggests that cold storage may only break dormancy or sustain viable switchgrass seed for about 1 year. Overall, cold storage did not appear to improve percent germination in commercial switchgrass. Zarnstoff et al. (1994) found that storage at 23°C resulted in higher germination rates than seed stored at 7°C. These results, as well as the results of this study, suggest that equal or better field germination may be obtained by storing seed at ambient temperature in Louisiana.

Local switchgrass seed germinated at 30°C had a similar trend at 300 days of storage as the same treatment for commercial seeds (Figure 13). This indicates that possibly the same effects cold storage and 30°C germination temperature have on commercial seeds may occur in local seeds, however, germination percentages were too low for this spike to be significant. Local switchgrass seed germinated at 25°C had a similar trend at 120 days of storage as the same treatment for commercial seeds (Figure 13). This indicates that possibly the same effects cold storage and 25°C germination temperature have on commercial seeds may exist in local seeds, however, germination percentages were too low and standard deviation was too high for this spike to be significant.

## RECOMMENDATIONS

Despite having lower viability, I recommend the use of local seed in restoration projects as this will better ensure the genetic diversity and ecological integrity of future populations. Even though establishment is typically slower, local seed will perform better in regards to long-term above ground growth and competitive ability (Gustafson et al. 2004).

The results of this thesis may be skewed because of a poor crop of local seeds that were collected in 2007. If locally adapted seeds could have been collected from another year, the percent germination for commercial and local seeds may have been more comparable. A multi-year study that would assess consecutive year classes of seeds would better determine the actual germination rates for each respective seed type.

I recommend storing big bluestem and indiangrass seed at cold temperatures (~4°C) if seeds will not be used for an extended period (> 180 days). Based on this study, cold storage would increase the length of time these seeds will remain viable. More research is needed to determine which storage treatment is best for switchgrass.

This study could be improved by using germinators instead of incubators, which would provide more consistent temperatures. More replications would also provide more accurate germination percentages. Studying the effects of these treatments when used in combination with other dormancy reducing treatments would provide a better understanding of what treatments yield the highest percent germination; however, the use of other treatments may not be feasible on a commercial scale. The commercial seed had less seed coat structures present than the local seed which was cleaned at the Golden Meadow Plant Material Center. The equipment used at the Golden Meadow PMC was

limited in its ability to remove seed coat structures and could not remove them as efficiently as methods used by the commercial producers. The seed coat structures in warm-season grasses act as mechanical barriers delaying germination (Fulbright and Flenniken 1988). Removal of seed coat structures such as the lemma and palea in local seed may have increased percent germination as well.

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**Appendix I.** Mean number ( $\pm$ SD) of germinated seeds for all seed treatments.

<b>Species</b>	<b>Origin</b>	<b>Storage Type</b>	<b>Days of Storage</b>	<b>Germination Temperature</b>	<b>Mean Number Germinated <math>\pm</math> SD</b>
Big Bluestem	Commercial	Ambient	0	20	13.7 $\pm$ 3.3
Big Bluestem	Commercial	Ambient	60	20	18.2 $\pm$ 5.3
Big Bluestem	Commercial	Ambient	120	20	15.0 $\pm$ 6.5
Big Bluestem	Commercial	Ambient	180	20	0.2 $\pm$ 0.5
Big Bluestem	Commercial	Ambient	240	20	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Ambient	300	20	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Ambient	360	20	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Cold Storage	0	20	22.7 $\pm$ 1.7
Big Bluestem	Commercial	Cold Storage	60	20	16.0 $\pm$ 6.2
Big Bluestem	Commercial	Cold Storage	120	20	17.5 $\pm$ 4.0
Big Bluestem	Commercial	Cold Storage	180	20	13.5 $\pm$ 3.4
Big Bluestem	Commercial	Cold Storage	240	20	11.0 $\pm$ 2.2
Big Bluestem	Commercial	Cold Storage	300	20	10.7 $\pm$ 1.8
Big Bluestem	Commercial	Cold Storage	360	20	7.3 $\pm$ 1.5
Big Bluestem	Commercial	Ambient	0	25	17.0 $\pm$ 5.8
Big Bluestem	Commercial	Ambient	60	25	13.0 $\pm$ 2.6
Big Bluestem	Commercial	Ambient	120	25	16.8 $\pm$ 2.8
Big Bluestem	Commercial	Ambient	180	25	0.3 $\pm$ 0.5
Big Bluestem	Commercial	Ambient	240	25	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Ambient	300	25	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Ambient	360	25	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Cold Storage	0	25	17.3 $\pm$ 3.9
Big Bluestem	Commercial	Cold Storage	60	25	17.5 $\pm$ 6.2
Big Bluestem	Commercial	Cold Storage	120	25	17.3 $\pm$ 2.9
Big Bluestem	Commercial	Cold Storage	180	25	13.5 $\pm$ 3.8
Big Bluestem	Commercial	Cold Storage	240	25	11.8 $\pm$ 4.5
Big Bluestem	Commercial	Cold Storage	300	25	9.5 $\pm$ 1.9
Big Bluestem	Commercial	Cold Storage	360	25	6.3 $\pm$ 3.5
Big Bluestem	Commercial	Ambient	0	30	17.8 $\pm$ 1.7
Big Bluestem	Commercial	Ambient	60	30	16.3 $\pm$ 4.2
Big Bluestem	Commercial	Ambient	120	30	6.0 $\pm$ 1.8
Big Bluestem	Commercial	Ambient	180	30	0.8 $\pm$ 0.5
Big Bluestem	Commercial	Ambient	240	30	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Ambient	300	30	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Ambient	360	30	0.0 $\pm$ 0.0
Big Bluestem	Commercial	Cold Storage	0	30	15.5 $\pm$ 1.0
Big Bluestem	Commercial	Cold Storage	60	30	18.3 $\pm$ 4.2
Big Bluestem	Commercial	Cold Storage	120	30	14.0 $\pm$ 2.9
Big Bluestem	Commercial	Cold Storage	180	30	10.8 $\pm$ 3.4
Big Bluestem	Commercial	Cold Storage	240	30	14.0 $\pm$ 4.1
Big Bluestem	Commercial	Cold Storage	300	30	13.0 $\pm$ 3.2
Big Bluestem	Commercial	Cold Storage	360	30	6.3 $\pm$ 2.2
Big Bluestem	Local	Ambient	0	20	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	60	20	0.5 $\pm$ 0.6
Big Bluestem	Local	Ambient	120	20	0.5 $\pm$ 0.6
Big Bluestem	Local	Ambient	180	20	0.0 $\pm$ 0.0

<b>Species</b>	<b>Origin</b>	<b>Storage Type</b>	<b>Days of Storage</b>	<b>Germination Temperature</b>	<b>Mean Number Germinated <math>\pm</math> SD</b>
Big Bluestem	Local	Ambient	240	20	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	300	20	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	360	20	0.0 $\pm$ 0.0
Big Bluestem	Local	Cold Storage	0	20	0.3 $\pm$ 0.5
Big Bluestem	Local	Cold Storage	60	20	0.3 $\pm$ 0.5
Big Bluestem	Local	Cold Storage	120	20	0.8 $\pm$ 1.0
Big Bluestem	Local	Cold Storage	180	20	1.0 $\pm$ 1.4
Big Bluestem	Local	Cold Storage	240	20	1.8 $\pm$ 1.5
Big Bluestem	Local	Cold Storage	300	20	1.5 $\pm$ 0.6
Big Bluestem	Local	Cold Storage	360	20	1.0 $\pm$ 1.2
Big Bluestem	Local	Ambient	0	25	0.3 $\pm$ 0.5
Big Bluestem	Local	Ambient	60	25	0.8 $\pm$ 0.5
Big Bluestem	Local	Ambient	120	25	2.0 $\pm$ 1.2
Big Bluestem	Local	Ambient	180	25	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	240	25	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	300	25	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	360	25	0.0 $\pm$ 0.0
Big Bluestem	Local	Cold Storage	0	25	0.5 $\pm$ 0.6
Big Bluestem	Local	Cold Storage	60	25	0.0 $\pm$ 0.0
Big Bluestem	Local	Cold Storage	120	25	0.8 $\pm$ 1.0
Big Bluestem	Local	Cold Storage	180	25	0.3 $\pm$ 0.5
Big Bluestem	Local	Cold Storage	240	25	1.0 $\pm$ 1.4
Big Bluestem	Local	Cold Storage	300	25	1.5 $\pm$ 0.6
Big Bluestem	Local	Cold Storage	360	25	1.8 $\pm$ 1.7
Big Bluestem	Local	Ambient	0	30	0.5 $\pm$ 1.0
Big Bluestem	Local	Ambient	60	30	0.5 $\pm$ 0.6
Big Bluestem	Local	Ambient	120	30	1.0 $\pm$ 1.4
Big Bluestem	Local	Ambient	180	30	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	240	30	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	300	30	0.0 $\pm$ 0.0
Big Bluestem	Local	Ambient	360	30	0.0 $\pm$ 0.0
Big Bluestem	Local	Cold Storage	0	30	0.0 $\pm$ 0.0
Big Bluestem	Local	Cold Storage	60	30	0.0 $\pm$ 0.0
Big Bluestem	Local	Cold Storage	120	30	0.8 $\pm$ 1.0
Big Bluestem	Local	Cold Storage	180	30	0.3 $\pm$ 0.5
Big Bluestem	Local	Cold Storage	240	30	1.3 $\pm$ 1.3
Big Bluestem	Local	Cold Storage	300	30	0.8 $\pm$ 0.5
Big Bluestem	Local	Cold Storage	360	30	1.0 $\pm$ 0.8
Indiangrass	Commercial	Ambient	0	20	22.0 $\pm$ 1.4
Indiangrass	Commercial	Ambient	60	20	22.0 $\pm$ 3.5
Indiangrass	Commercial	Ambient	120	20	26.5 $\pm$ 9.3
Indiangrass	Commercial	Ambient	180	20	4.8 $\pm$ 2.5
Indiangrass	Commercial	Ambient	240	20	0.3 $\pm$ 0.5
Indiangrass	Commercial	Ambient	300	20	0.8 $\pm$ 1.0
Indiangrass	Commercial	Ambient	360	20	0.5 $\pm$ 1.0
Indiangrass	Commercial	Cold Storage	0	20	17.5 $\pm$ 4.0
Indiangrass	Commercial	Cold Storage	60	20	20.8 $\pm$ 1.5

<b>Species</b>	<b>Origin</b>	<b>Storage Type</b>	<b>Days of Storage</b>	<b>Germination Temperature</b>	<b>Mean Number Germinated <math>\pm</math> SD</b>
Indiangrass	Commercial	Cold Storage	120	20	23.0 $\pm$ 3.8
Indiangrass	Commercial	Cold Storage	180	20	20.8 $\pm$ 4.3
Indiangrass	Commercial	Cold Storage	240	20	27.8 $\pm$ 3.6
Indiangrass	Commercial	Cold Storage	300	20	20.5 $\pm$ 3.3
Indiangrass	Commercial	Cold Storage	360	20	20.0 $\pm$ 0.8
Indiangrass	Commercial	Ambient	0	25	29.3 $\pm$ 8.5
Indiangrass	Commercial	Ambient	60	25	24.0 $\pm$ 8.3
Indiangrass	Commercial	Ambient	120	25	24.5 $\pm$ 1.9
Indiangrass	Commercial	Ambient	180	25	7.8 $\pm$ 2.5
Indiangrass	Commercial	Ambient	240	25	1.8 $\pm$ 1.0
Indiangrass	Commercial	Ambient	300	25	1.5 $\pm$ 1.0
Indiangrass	Commercial	Ambient	360	25	0.0 $\pm$ 0.0
Indiangrass	Commercial	Cold Storage	0	25	22.5 $\pm$ 4.5
Indiangrass	Commercial	Cold Storage	60	25	26.5 $\pm$ 6.6
Indiangrass	Commercial	Cold Storage	120	25	18.8 $\pm$ 12.8
Indiangrass	Commercial	Cold Storage	180	25	18.5 $\pm$ 4.4
Indiangrass	Commercial	Cold Storage	240	25	25.8 $\pm$ 9.7
Indiangrass	Commercial	Cold Storage	300	25	24.3 $\pm$ 6.9
Indiangrass	Commercial	Cold Storage	360	25	17.8 $\pm$ 6.2
Indiangrass	Commercial	Ambient	0	30	22.3 $\pm$ 4.3
Indiangrass	Commercial	Ambient	60	30	19.5 $\pm$ 7.0
Indiangrass	Commercial	Ambient	120	30	20.8 $\pm$ 7.0
Indiangrass	Commercial	Ambient	180	30	4.3 $\pm$ 2.2
Indiangrass	Commercial	Ambient	240	30	0.5 $\pm$ 1.0
Indiangrass	Commercial	Ambient	300	30	0.0 $\pm$ 0.0
Indiangrass	Commercial	Ambient	360	30	0.3 $\pm$ 0.5
Indiangrass	Commercial	Cold Storage	0	30	17.5 $\pm$ 6.6
Indiangrass	Commercial	Cold Storage	60	30	17.3 $\pm$ 5.2
Indiangrass	Commercial	Cold Storage	120	30	14.5 $\pm$ 9.7
Indiangrass	Commercial	Cold Storage	180	30	6.0 $\pm$ 6.7
Indiangrass	Commercial	Cold Storage	240	30	21.5 $\pm$ 4.0
Indiangrass	Commercial	Cold Storage	300	30	25.8 $\pm$ 1.5
Indiangrass	Commercial	Cold Storage	360	30	17.8 $\pm$ 4.3
Indiangrass	Local	Ambient	0	20	2.5 $\pm$ 1.3
Indiangrass	Local	Ambient	60	20	5.8 $\pm$ 2.1
Indiangrass	Local	Ambient	120	20	6.8 $\pm$ 3.3
Indiangrass	Local	Ambient	180	20	0.3 $\pm$ 0.5
Indiangrass	Local	Ambient	240	20	0.0 $\pm$ 0.0
Indiangrass	Local	Ambient	300	20	0.3 $\pm$ 0.5
Indiangrass	Local	Ambient	360	20	0.3 $\pm$ 0.5
Indiangrass	Local	Cold Storage	0	20	1.0 $\pm$ 0.8
Indiangrass	Local	Cold Storage	60	20	1.3 $\pm$ 1.0
Indiangrass	Local	Cold Storage	120	20	1.8 $\pm$ 1.0
Indiangrass	Local	Cold Storage	180	20	2.5 $\pm$ 1.3
Indiangrass	Local	Cold Storage	240	20	4.5 $\pm$ 1.0
Indiangrass	Local	Cold Storage	300	20	8.0 $\pm$ 1.2
Indiangrass	Local	Cold Storage	360	20	4.5 $\pm$ 1.7

<b>Species</b>	<b>Origin</b>	<b>Storage Type</b>	<b>Days of Storage</b>	<b>Germination Temperature</b>	<b>Mean Number Germinated <math>\pm</math> SD</b>
Indiangrass	Local	Ambient	0	25	4.3 $\pm$ 2.5
Indiangrass	Local	Ambient	60	25	4.0 $\pm$ 2.9
Indiangrass	Local	Ambient	120	25	3.3 $\pm$ 2.2
Indiangrass	Local	Ambient	180	25	5.0 $\pm$ 2.6
Indiangrass	Local	Ambient	240	25	0.8 $\pm$ 0.5
Indiangrass	Local	Ambient	300	25	0.0 $\pm$ 0.0
Indiangrass	Local	Ambient	360	25	0.0 $\pm$ 0.0
Indiangrass	Local	Cold Storage	0	25	1.0 $\pm$ 0.8
Indiangrass	Local	Cold Storage	60	25	2.3 $\pm$ 1.3
Indiangrass	Local	Cold Storage	120	25	9.8 $\pm$ 17.6
Indiangrass	Local	Cold Storage	180	25	4.8 $\pm$ 2.5
Indiangrass	Local	Cold Storage	240	25	5.0 $\pm$ 3.4
Indiangrass	Local	Cold Storage	300	25	3.0 $\pm$ 2.0
Indiangrass	Local	Cold Storage	360	25	7.0 $\pm$ 1.8
Indiangrass	Local	Ambient	0	30	0.8 $\pm$ 1.0
Indiangrass	Local	Ambient	60	30	5.5 $\pm$ 2.6
Indiangrass	Local	Ambient	120	30	3.3 $\pm$ 3.4
Indiangrass	Local	Ambient	180	30	3.5 $\pm$ 1.9
Indiangrass	Local	Ambient	240	30	0.0 $\pm$ 0.0
Indiangrass	Local	Ambient	300	30	0.5 $\pm$ 0.6
Indiangrass	Local	Ambient	360	30	0.5 $\pm$ 1.0
Indiangrass	Local	Cold Storage	0	30	2.3 $\pm$ 2.6
Indiangrass	Local	Cold Storage	60	30	4.0 $\pm$ 2.2
Indiangrass	Local	Cold Storage	120	30	7.3 $\pm$ 6.4
Indiangrass	Local	Cold Storage	180	30	2.3 $\pm$ 2.6
Indiangrass	Local	Cold Storage	240	30	4.8 $\pm$ 4.8
Indiangrass	Local	Cold Storage	300	30	6.0 $\pm$ 2.2
Indiangrass	Local	Cold Storage	360	30	3.3 $\pm$ 1.9
Switchgrass	Commercial	Ambient	0	20	10.3 $\pm$ 6.0
Switchgrass	Commercial	Ambient	60	20	10.3 $\pm$ 1.0
Switchgrass	Commercial	Ambient	120	20	10.5 $\pm$ 2.6
Switchgrass	Commercial	Ambient	180	20	10.8 $\pm$ 4.8
Switchgrass	Commercial	Ambient	240	20	2.8 $\pm$ 2.4
Switchgrass	Commercial	Ambient	300	20	5.3 $\pm$ 2.8
Switchgrass	Commercial	Ambient	360	20	2.3 $\pm$ 1.7
Switchgrass	Commercial	Cold Storage	0	20	14.8 $\pm$ 6.8
Switchgrass	Commercial	Cold Storage	60	20	8.3 $\pm$ 3.9
Switchgrass	Commercial	Cold Storage	120	20	9.8 $\pm$ 2.2
Switchgrass	Commercial	Cold Storage	180	20	9.0 $\pm$ 3.2
Switchgrass	Commercial	Cold Storage	240	20	9.0 $\pm$ 1.8
Switchgrass	Commercial	Cold Storage	300	20	29.5 $\pm$ 12.5
Switchgrass	Commercial	Cold Storage	360	20	9.3 $\pm$ 2.1
Switchgrass	Commercial	Ambient	0	25	18.5 $\pm$ 8.5
Switchgrass	Commercial	Ambient	60	25	26.3 $\pm$ 16.3
Switchgrass	Commercial	Ambient	120	25	35.3 $\pm$ 22.0
Switchgrass	Commercial	Ambient	180	25	29.0 $\pm$ 4.7
Switchgrass	Commercial	Ambient	240	25	21.5 $\pm$ 3.1

<b>Species</b>	<b>Origin</b>	<b>Storage Type</b>	<b>Days of Storage</b>	<b>Germination Temperature</b>	<b>Mean Number Germinated <math>\pm</math> SD</b>
Switchgrass	Commercial	Ambient	300	25	21.5 $\pm$ 8.2
Switchgrass	Commercial	Ambient	360	25	14.0 $\pm$ 2.8
Switchgrass	Commercial	Cold Storage	0	25	15.0 $\pm$ 7.6
Switchgrass	Commercial	Cold Storage	60	25	24.5 $\pm$ 1.0
Switchgrass	Commercial	Cold Storage	120	25	15.5 $\pm$ 5.2
Switchgrass	Commercial	Cold Storage	180	25	15.0 $\pm$ 8.4
Switchgrass	Commercial	Cold Storage	240	25	26.3 $\pm$ 6.6
Switchgrass	Commercial	Cold Storage	300	25	37.5 $\pm$ 14.3
Switchgrass	Commercial	Cold Storage	360	25	21.0 $\pm$ 5.9
Switchgrass	Commercial	Ambient	0	30	18.0 $\pm$ 8.1
Switchgrass	Commercial	Ambient	60	30	48.8 $\pm$ 6.3
Switchgrass	Commercial	Ambient	120	30	28.3 $\pm$ 14.2
Switchgrass	Commercial	Ambient	180	30	25.3 $\pm$ 14.3
Switchgrass	Commercial	Ambient	240	30	17.0 $\pm$ 6.6
Switchgrass	Commercial	Ambient	300	30	28.5 $\pm$ 5.7
Switchgrass	Commercial	Ambient	360	30	12.5 $\pm$ 8.3
Switchgrass	Commercial	Cold Storage	0	30	30.3 $\pm$ 21.8
Switchgrass	Commercial	Cold Storage	60	30	32.3 $\pm$ 11.1
Switchgrass	Commercial	Cold Storage	120	30	44.8 $\pm$ 21.5
Switchgrass	Commercial	Cold Storage	180	30	14.8 $\pm$ 8.0
Switchgrass	Commercial	Cold Storage	240	30	36.8 $\pm$ 7.2
Switchgrass	Commercial	Cold Storage	300	30	52.8 $\pm$ 16.7
Switchgrass	Commercial	Cold Storage	360	30	11.3 $\pm$ 5.6
Switchgrass	Local	Ambient	0	20	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	60	20	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	120	20	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	180	20	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	240	20	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	300	20	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	360	20	0.3 $\pm$ 0.5
Switchgrass	Local	Cold Storage	0	20	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	60	20	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	120	20	0.5 $\pm$ 1.0
Switchgrass	Local	Cold Storage	180	20	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	240	20	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	300	20	1.0 $\pm$ 0.8
Switchgrass	Local	Cold Storage	360	20	0.8 $\pm$ 0.5
Switchgrass	Local	Ambient	0	25	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	60	25	0.8 $\pm$ 1.5
Switchgrass	Local	Ambient	120	25	3.8 $\pm$ 6.8
Switchgrass	Local	Ambient	180	25	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	240	25	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	300	25	0.3 $\pm$ 0.5
Switchgrass	Local	Ambient	360	25	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	0	25	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	60	25	0.3 $\pm$ 0.5
Switchgrass	Local	Cold Storage	120	25	0.3 $\pm$ 0.5

<b>Species</b>	<b>Origin</b>	<b>Storage Type</b>	<b>Days of Storage</b>	<b>Germination Temperature</b>	<b>Mean Number Germinated <math>\pm</math> SD</b>
Switchgrass	Local	Cold Storage	180	25	0.3 $\pm$ 0.5
Switchgrass	Local	Cold Storage	240	25	0.5 $\pm$ 1.0
Switchgrass	Local	Cold Storage	300	25	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	360	25	0.3 $\pm$ 0.5
Switchgrass	Local	Ambient	0	30	0.0 $\pm$ 0.0
Switchgrass	Local	Ambient	60	30	1.3 $\pm$ 1.5
Switchgrass	Local	Ambient	120	30	1.0 $\pm$ 1.4
Switchgrass	Local	Ambient	180	30	0.5 $\pm$ 0.6
Switchgrass	Local	Ambient	240	30	0.3 $\pm$ 0.5
Switchgrass	Local	Ambient	300	30	0.8 $\pm$ 1.5
Switchgrass	Local	Ambient	360	30	0.5 $\pm$ 1.0
Switchgrass	Local	Cold Storage	0	30	0.3 $\pm$ 0.5
Switchgrass	Local	Cold Storage	60	30	0.5 $\pm$ 0.6
Switchgrass	Local	Cold Storage	120	30	1.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	180	30	0.0 $\pm$ 0.0
Switchgrass	Local	Cold Storage	240	30	1.0 $\pm$ 0.8
Switchgrass	Local	Cold Storage	300	30	4.0 $\pm$ 2.4
Switchgrass	Local	Cold Storage	360	30	0.5 $\pm$ 0.6

**Appendix II.** Results of tetrazolium (TZ) tests for seed viability.

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<b>Species</b>	<b>Seed Origin</b>	<b>Replicate</b>	<b>Percent Viable</b>
big bluestem	local	1	22
big bluestem	local	2	17
big bluestem	local	3	16
big bluestem	commercial	1	43
big bluestem	commercial	2	55
big bluestem	commercial	3	57
indiangrass	local	1	40
indiangrass	local	2	37
indiangrass	local	3	36
indiangrass	commercial	1	70
indiangrass	commercial	2	81
indiangrass	commercial	3	60
switchgrass	local	1	7
switchgrass	local	2	10
switchgrass	local	3	2
switchgrass	commercial	1	86
switchgrass	commercial	2	84
switchgrass	commercial	3	88

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## **BIOGRAPHICAL SKETCH**

Trevis Olivier was born on 18 July 1984, in Houma, Louisiana. After graduating from H.L. Bourgeois High School in 2002, Trevis attended Nicholls State University. Trevis graduated from Nicholls State University in December of 2006 with a B.S. in Biology with a concentration in Marine Biology. Trevis then continued his education by enrolling in the graduate program in Marine and Environmental Biology at Nicholls State University in spring of 2007. Trevis conducted research on the effects of different temperature and storage regimes on the germination rates of three native warm-season grasses. Trevis is scheduled to complete his degree requirements in the fall of 2009. Trevis currently works as an Environmental Professional at a professional consulting firm in Houma, Louisiana.

## CURRICULUM VITAE

**Trevis Olivier**

Graduate Student  
Nicholls State University

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### EDUCATION

**M. S. Marine and Environmental Biology, December, 2009**, Nicholls State University, Thibodaux, Louisiana, 70310. Thesis title: Effects of temperature and storage regimes on the germination rates of three native warm-season grasses.

**B.S. in Biology with a concentration in Marine Biology, December 2006**, Nicholls State University, Thibodaux, Louisiana, 70310.

### WORK EXPERIENCE

2008 – Present T. Baker Smith, Inc., Houma, LA. Environmental Professional: conduct oyster assessments and wetland delineations for environmental permitting.

Jan 2007 – May 2008 Nicholls State University, Biology Department, Thibodaux, LA. Graduate Assistant: assists in various research and fieldwork tasks associated with the Louisiana Native Plant Initiative.

Jan 2008 - March 2008 U.S. Fish and Wildlife Service, Mandalay National Refuge, Houma, LA. Intern: assisted in various duties involved with maintaining the refuge.

Sept 2006 - Jan 2007 Nicholls State University, Biology Department, Thibodaux, LA. Research Assistant: assisted in collecting and identifying fish samples.

2005 - 2007 Dinger's Outdoors, Houma, LA. Sales and warehouse management: responsible for sales/service, delivery and installation, organizing inventory.

2003 - 2005 Weatherford Gemeco, Houma, LA. Batchplant Operator: duties included cementing, finish work, and crating/shipping of various oilfield products.

## **FIELD EXPERIENCE**

Boat and trailer operation (LDWF-Vessel Operator Certification), seine sampling, gillnet sampling, electrofishing, water quality monitoring (pH, dissolved oxygen, temperature, specific conductance, salinity, Secchi disk depth, ammonia, and nitrite), oyster sampling and measuring, wetland delineations, soil sampling, collecting and processing seed for planting, tractor and agriculture equipment operation, Microsoft Word, Excel, and Power Point as well as internet and HTML knowledge and some experience with ArcGIS, AutoCAD, and SAS.

## **LABORATORY EXPERIENCE**

Processing seed for planting, analyzing oyster samples, conducting germination experiments, and conducting seed viability tests

## **MEMBERSHIPS**

Nicholls State University Biology Society  
Natural Resources Conservation Service Earth Team Volunteer  
Louisiana Chapter of the American Fisheries Society

## **PRESENTATIONS**

Nicholls State University Research Week, 2008, Nicholls State University, "Germination rates of locally versus non-locally obtained ecotypes of big bluestem, indiangrass, and switchgrass under different stratification and storage regimes"

Calypseaux, 2007, LUMCON, Cocodrie, LA, "Germination rates of locally versus non-locally obtained ecotypes of big bluestem, indiangrass, and switchgrass under various stratification and storage regimes"

Nicholls State University Research Week, 2006, Nicholls State University, "The Allelopathic Effects of Red Maple, Box Elder, Eucalyptus, and Broccoli extract on the Germination of Lettuce and Radish Seeds"

## **AWARDS**

2007/08 Rockefeller State Wildlife Scholarship

2006 Second place poster, Undergraduate, Nicholls State University Research Week  
Poster Competition

2006 First place abstract, Undergraduate, Nicholls State University Research Week  
Abstract Competition