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SELECTION FOR REDUCED SEED DORMANCY
IN SEVEN NATIVE GRASS SPECIES

By

Kyle Bradley Holmberg

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Masters of Science
in Agriculture
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

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Kyle Bradley Holmberg

2007

SELECTION FOR REDUCED SEED DORMANCY IN SEVEN NATIVE GRASS SPECIES

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Lowland switchgrass (*Panicum virgatum*), big bluestem (*Andropogon gerardii*), indiangrass (*Sorghastrum nutans*), upland switchgrass (*Panicum virgatum*), little bluestem (*Schizachyrium scoparium*), beaked panicum (*Panicum capillare*), and purpletop (*Tridens flavus*) all show strong signs of seed dormancy which contributes to extremely poor field establishment. The objective of this work was to reduce seed dormancy by selecting individuals that exhibited reduced pre-stratification dormancy in laboratory tests. The classical breeding method of phenotypic recurrent selection was used to enhance germination. Of the three tall-stature species, lowland switchgrass made the greatest improvement in pre-stratification germination, followed by indiangrass and big bluestem. The four short stature species have shown various results after one cycle of selection at Starkville. A field emergence trial was also conducted to evaluate three cycles of breeding seed with five commercially available cultivars in which Cycle 3 seed produced more plants per hectare than any of the other cultivars or germplasm.

Key words: beaked panicum, big bluestem, biofuels, bluestem, indiangrass, little bluestem, lowland switchgrass, native grasses, purpletop, restoration, upland switchgrass.

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CHAPTER I

INTRODUCTION

Switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), indiangrass (Sorghastrum nutans [L.] Nash), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), beaked panicum (*Panicum capillare* L.), and purpletop (*Tridens flavus* [L.] Hitchc.) are warm-season grasses native to the prairie regions of North America. Within this group of grasses lies two categories. The first three listed are tall-stature plants including: lowland switchgrass, big bluestem, and indiangrass. The latter four listed are short-stature grasses including: upland switchgrass, little bluestem, peaked panicum, and purpletop. These C_4 plants begin their annual growth cycle in late spring and are generally most productive during the mid-to-late summer months. These native warm-season grasses are indigenous to the entire central region of the United States, but now are found growing naturally only in isolated pockets (Miller and Dickerson, 1999). Almost all important native prairie grasses are cross pollinated by wind (Hanson and Carnahan, 1956) and are generally self incompatible (Talbert, 1983; Taliaferro and Hopkins, 1996; Norman et al., 1997; Martínez-Reyna and Vogel, 2002). With cross pollinated progeny, a substantial degree of genetic variance exists in both native and cultivated populations. This genetic variance endows populations with sufficient diversity to have individuals that grow in a variety of locations within the United States and North America. Thus, unlike a monoculture of wheat (*Triticum aestivum* L.) or hybrid corn (*Zea mays* L.), a monoculture of any one of these grass species is still a heterogeneous population.

Tall-stature native grasses have the potential to reach 2m in height. The most extensive current use of tall stature native warm-season grasses is in prairie restoration projects where species are being re-introduced to enhance conservation. Although hundreds of grass species were native to the North American continent, only a few have the potential to become important forage, biomass, or turf crops (Vogel and Moore, 1993). The bulk of these species cannot survive the winters of the north-central and northern latitudes of the United States. Consequently, the few species that are able to survive (big bluestem, indiangrass, and lowland switchgrass) have, and are being, increasingly utilized in cultivated systems in these regions. These three grass species provide excellent pasture and forage during hot summer months when cool-season grasses are relatively unproductive and have poor forage quality (Vogel and Moore, 1993).

Another major area of interest is using native grasses as biomass fuel crops. Due to their tall heights and extensive biomass production, they have great potential to be used as biofuels. The use of native grasses for biofuels favors the southern United States because lowland switchgrass yields substantially greater than upland switchgrass and is easier to establish than big bluestem and indiangrass. The process of converting native grasses to biofuels would include: harvesting, drying, and then; either direct combustion in co-generation, or pyrolyzing the plant material in order to generate carbon dioxide, carbon monoxide and hydrogen (syn-gen) which is converted via anaerobic fermentation to ethanol.

Short-stature native grasses are widely used as habitat for ground-nesting birds in association with prairie restoration projects. The tall-stature grasses are not as acceptable as habitat for ground-nesting birds because the base of the plant produces a larger clump and denser canopy growth. These characteristics make it difficult for ground-nesting birds to flush from the habitat in the event of danger. The short-stature grasses produce less biomass needed for the industrial and energy sectors. However, they provide enough growth for wildlife habitat of birds while at the same time allowing for easier escape from grasses that only grow to a height of less than one meter.

In a series of studies conducted at the Jamie L. Whitten Plant Materials Center near Coffeeville MS, in conjunction with this research, selection for reduced seed dormancy in upland switchgrass was shown to be a viable method for cultivar development (USDA, 2005). Upon review of this literature, four other short stature species (upland switchgrass, beaked panicum, purpletop, and little bluestem) were added to the Starkville program for selection of reduced seed dormancy.

The main problem associated with native grasses is their inability to quickly establish stands capable of producing enough material to accommodate the needs of industrial consumers. Warm-season grasses are traditionally slow to establish, making the seedlings poor competitors with weeds (USDA, 1996; Robocker et al., 1953). The seed of these species is also hard to plant and clean due to the presence of hairs and awns. These hairs often make individual seed stick together causing bridging in the planter box, preventing seed from falling into the drop during planting. In addition, these species, like many native species, are known to have extensive seed dormancy, further decreasing establishment. All these obstacles pose a problem in making native warm-season grasses a viable crop. In an effort to make these grasses more appealing for use in both forage systems and biomass energy fuel prior research has selected individuals with shortened germination percentages (Jones, 2004). This project is a continuation of that study which was initiated and conducted between 2002 and 2004 and adds the four short stature grasses. The main objectives of this project remain the same and include: 1) to use classical breeding (phenotypic recurrent selection) to reduce seed dormancy in seven native grass species; 2) to compare the germination of existing populations of these species; and 3) to compare field emergence of the elite population to the original populations and existing commercial cultivars.

CHAPTER II

LITERATURE REVIEW

Overview of Seed Dormancy

Seed dormancy is a critical inhibitor of establishment and development among the native grass species. Seed dormancy is present in all native grass species and is considered a selective advantage under differing environmental conditions. The selective advantage of seed dormancy insures that some seed will remain viable but not germinate under short term environmentally favorable conditions (i.e. a warm period during winter months), and certain seed can revert to dormancy if unsuitable conditions are encountered. Seed imposed dormancy was defined by Chancellor (1984) when he stated that seed which are alive, but do not germinate are considered to be in a dormant state. This seed dormancy can be the result of actual seed characteristics or environmental factors where the seed is located. Chancellor gives a broad, yet simple, explanation for evaluating seed dormancy within this definition. In an effort to understand dormancy both as a product of the seed itself and a product of the environment Villiers (1972) gave more specific definitions and classified dormancy into two categories: seed dormancy and secondary dormancy. Villiers (1972) defined seed dormancy as a state of "abnormality" in which the seed may carry one or more mechanisms causing it not to germinate, which may be due to its chemical composition. This definition would indicate that dormancy is a result of its chemical makeup or organ structure. Thus, implying that the seed itself imposes dormancy by way of its organization. However, Villiers primary definition does not provide for a lack of germination due to the effects of unfavorable environmental conditions, which is called quiescence. Unfavorable environmental conditions play a major role in the germination capability of the seed. Conditions of adequate moisture, appropriate temperature, appropriate oxygen levels, and light, are all conditions which are required for small-seeded species to germinate. Upon exploring these factors, Villiers gives a definition explaining dormancy when imposed by environmental effects. He defines an environmentally imposed dormancy as secondary dormancy, which states that seed become dormant by imbibition under conditions unfavorable to germination, so that seed are not able to germinate under conditions which would be favorable of the species until a releaser stimulus is applied on the seed.

Dormancy could also be attributed to mechanisms within the seed in which requirements of these mechanisms are not satisfied. Crocker (1916) compiled a list of dormancy mechanisms that are separated into seven categories which are still used today. These categories cover a broad range of dormancy types and give investigators a starting point as they begin to determine what is causing dormancy in specific species. The mechanisms discussed by Crocker (1916) include:

1. Rudimentary embryos which must mature before germination can begin;
2. Complete imbibition of water absorption;
3. Mechanism resistance to the expansion of the embryo and seed contents by enclosing structures;
4. Encasing structures interfering with oxygen absorption by the embryo and

perhaps carbon dioxide elimination from it;

5. A state of dormancy in the embryo itself or some organ of it, in consequence of which it is unable to grow when naked and supplied with all ordinary germinative conditions;

6. Combination of two or more of these;

7. Assumption of secondary dormancy.

There are many potential causes of seed dormancy in these grass species. This research does not focus on physiological mechanisms, just selection of these individuals.

Characteristics of Species

The basic genetic makeup of lowland (LLSG) and upland switchgrass (ULSG) are the same. Switchgrass has an "x" chromosome of nine (x=9) with at least six ploidy levels (Nielsen, 1944). Most LLSG and ULSG cultivars are either tetraploids (2n=4x=36) or octaploids (2n=8x=72; Hopkins et al., 1996; Lu et al., 1998). The LLSG types are most often classified as tetraploids (2n=4x=36; Porter, 1966). Lowland switchgrass types are generally taller, ranging between 0.6 and 3 m tall, more coarse, and have a bunch-type growth due to the fact that they are naturally found within the flood plains. The ULSG types tend to be octaploids (2n=8x=72). They are typically shorter, ranging in height from 0.9-1.5 m tall, have fine stems, pubescent leaves, and are found in upland regions which tend to encounter droughts in the summer. Research conducted by Taliaferro and Hopkins (1996) shows a genetic barrier exists between the ploidy levels and there is a strong self incompatibility between these biotypes. When 6,242 crosses of octaploid and tetraploid plants were made, only 4 seeds were produced (0.06%). When

1,915 crosses were made using related plants with the same ploidy levels, the plants produced 82 seeds (4.28%). These data show that plants with varying ploidy levels are capable of crossing, but only in extremely rare instances. These biotypes should probably be considered separate species based on their inability to produce fertile offspring. A study by Talbert et al. (1983) gives additional strong evidence of the self incompatibility systems present in switchgrass. In the study, seed were obtained from 33 parents and allowed to open-pollinate or forced to self-pollinate (bagged). The results showed an average of over 99% greater seed yield in the open-pollinated when compared to the self-pollinated. Self-incompatibility systems in switchgrass have also been documented in work by Martínez-Reyna and Vogel (2002).

Big bluestem (BBS) has a base chromosome number of 10 (Gould, 1968). Two cytotypes have been reported by Norrmann et al. (1997): hexaploid ($2n=6x=60$) and enneaploid ($2n=9x=90$). Riley and Vogel (1982) tested two popular cultivars (Pawnee and Kaw) and determined them to be hexaploids. This species varies in height between 0.4 and 1.7 m and prefers well drained soils. Research conducted by Norrmann et al. (1997) showed that controlled self-pollination of BBS yielded less than 5% seed set in hexaploids, as compared to a greater than 50% in out crossings of different hexaploids. This research suggests that BBS depends on out crossing for pollination. This also suggests limited seed production capacity in that only 50-60% of flowers produce seed.

Recent research by Tiffany et al. (2006) was conducted to examine the benefits of using BBS as a biofuel. In the study, Conservation Reserve Program (CRP) areas of the northern Great Plains containing stands of BBS and LLSG were analyzed for yield and cost returns from biofuel production. It was observed that LLSG was faster to establish in the first year, but BBS began to become the dominant species in year two and subsequent years. It was also observed that BBS would be less expensive to process than LLSG and produce more bio-oil from pyrolysis. This higher bio-oil production is the result of lower amounts of resin found in processed BBS. The returns on profit from BBS ($17.58 per U.S. ton) also exceed LLSG ($9.50 per U.S. ton). This study would indicate the biofuel initiative should not focus solely on LLSG.

Native stands and commercial cultivars of indiangrass (IG) (Oto, Holt, Nebraska 54, Osage) were examined by Church (1929), Bragg (1964), and Riley and Vogel (1982), their research shows that all were tetraploid (2n=4x=40). However, diploid (2n=2x=20) members of the species have been reported in South America by Saura (1948). Indiangrass inflorescence's can reach heights up to 1.8 m and the species is best suited for light sandy soils which tend to be drought prone. Newell (1936) examined seed production affects on plants in which inflorescences were selfed (bagged together) and compared them to plants which open-pollinated. His results concluded that there is no significant difference in seed producing capability between the self-pollinated and open pollinated plants, suggesting that self-fertility alleles may be present in IG.

Archer and Bunch (1953), and Gould (1956) describe little bluestem (LBS) as a highly out-crossing, perennial bunch grass that is a tetraploid (2n=4x=32) with a base chromosome number of eight. These plants range in height from 0.5 to 1.5 m. Douglas et al. (USDA, 2005) have reported some progress in selecting seed that have early germination. The process includes establishing a mother plant nursery and selecting early germinating seedlings to advance cycles.

Beaked panicum (BP) (also known as beaked panicgrass) is classified as a diploid (2n=2x=18) and tetraploid (2n=4x=36). Its height ranges between 0.3 and 1.3 m. The ploidy levels give explanation for the slow progress of the species. Relying on out-crossing to produce offspring the out-cross of the diploid and tetraploid would generate a sterile triploid. This would re-inforce the isolation of the two cytotypes, causing them to diverge into separate species. Grabowski et al. (USDA, 2005) studied the effects of seed dormancy in BP by means of selection. Seed was collected in 2004 from 29 seed lots of plants established in 2002 and 9 seed lots established in 2003 after a process of selecting reduced dormancy plants. The germination percentages were 2.6% for the 2002 seed lot and 1% for the 2003 seed lot. These seed were then stratified and germinated, showing germination percentages of 12% in 2002 and 36% in 2003, respectively. The stratified seed showed increased germination due to the release of dormancy by the cold treatment. They concluded that the progress of BP would be much slower than that of the other short-stature species due to no progress being made with unstratified seed lots.

Purpletop (PT) is a native tufted grass that has a height range between 1.2 and 1.5 meters when flowering. To date, the base chromosome number cannot be determined. Snider (USDA, 1996) has conducted research on PT for establishment techniques. While conducting this research several environmental and cultural factors were observed to affect establishment of a stand, such as: season, seeding rate, and mulching. He concluded these factors had little or no influence on establishment of the species; suggesting instead that slow establishment of the species was not an effect of seeding times or techniques, but rather a result of seed imposed dormancy.

Genetic Variation

Genetic variation within native plant species can influence their long-term chances of survival and growth. Sometimes specialized ecotypes within a species develop to specific local environmental conditions. An ecotype is a certain isolated population of plants within a species that, due to different genetics, has a different physical form, flowering time, or hardiness that is adapted to certain environmental conditions. Genetic variation is known to exist within germplasm and populations on three different levels. It can exist as ecotypic variation when germplasm is collected from specific regions, as population variation among accessions of a specific ecotype, and variation among populations when all plants are collected from a specific site (Eberhart and Newell, 1959).

Currently there is considerable discussion among conservation groups concerning the genetic value of ecotypes and whether or not planting cultivars/populations generated in other geographic locations represents “genetic pollution”. Conventional wisdom would suggest that this would be true. As mentioned previously, material from northern latitudes would be mal-adapted for southern plantings (Eberhart and Newell, 1959; Moser and Vogel, 1995; Vogel, 2000). Such plantings would result in a substantial percentage of seed produced by the native population yielding offspring less adapted to that specific location. Molecular analysis (Gustafson et al., 2001; Gustafson et al., 2004; Gustafson et al., 2005) indicates that introducing material from other locations can change allelic frequencies of the offspring of native ecotypes making the offspring less fit than their parents (outbreeding depression). This work on Illinois prairie sites indicates that this contamination can be especially persistent, lasting 12-20 years, or longer.

Calser et al. (2006) assessing molecular markers among northern switchgrass (ULSG) populations has found that while specific populations do differ in their allelic frequencies leading to identifiable ecotypes, all alleles are conserved among all populations tested (to date). Casler's data suggests that any population introduced to any non-native area has the genetic ability to adapt over time (generations) and match the allelic frequency of the native ecotype. While introduction of a new population may initially contaminate the total gene pool causing outbreeding depression of the original population's initial offspring; the same process is true of the native ecotype. Pollen from the ecotype is also contaminating the introduced population, enhancing the fitness of the offspring and increasing the frequency of adaptive alleles. Over time, only the offspring that are most adapted to that location will persist to replace parental genotypes. Parental genotypes that are mal-adapted will be the first to be replaced by offspring that are adapted. Gustafson (2005) even suggests this conversion may be accelerated by light disking or harrowing after burning, to create voids in the field in which seed of better adapted offspring could establish. The result is a virtual restoration, but more importantly an expansion of the original ecotype.

Native warm-season grasses are photoperiod sensitive because of genetic control in the plant. (Vogel, 2000). According to Moser and Vogel (1995), photoperiod requirements and fall senescence differ depending on the origin of latitude. This is the primary ecotype-adaptation determining factor by maximizing vegetative growth within the allowable season. When populations with southern origins are moved north and exposed to longer photoperiods for a given date (due to latitude) they will stay vegetative longer and produce more forage due to longer days late in the season, (Cornelius and Johnston, 1941; McMillian, 1959, 1965; Newell, 1968) but are often damaged by cold temperatures. Just the opposite is true when northern types are moved south and subjected to earlier shorter day lengths, inducing an earlier flowering date and a cessation of growth even though temperatures are still suitable for growth.

Benedict (1941) determined that switchgrass has a short day requirement to induce flowering. Flowering in all warm-season native grass species is induced by increased duration of darkness (skotoperiod). Photoperiod is also indicative of winter survival. Plants that are moved to far north develop longer vegetatively in the fall and will not survive winters (Vogel, 2000). This is most commonly seen in LLSG, as there is currently only one cultivar adapted to the humid Southeast (Alamo). As other cultivars of LLSG, that are adapted to the North and northcentral areas, are brought further south, the desirable traits which make them productive, diminish when they are grown in the southern regions. Moser and Vogel (1995) determined that precipitation and humidity are also factors in disease and drought pressure in grasses. They determined that cultivars native to the arid central states are more susceptible to foliar disease when grown in the more humid eastern states, conversely, plants with eastern origin are not as well adapted to drought stress when grown in the western states.

The conclusions from these studies are summarized in the following sentences.

Substantial genetic variation exists both within and between populations for agronomically important traits, including traits that affect seed yield, forage yield, and forage quality. Heritability values for the important traits range from 20 to 40% and breeding should make it possible to improve these grasses. Also, the correlations among desirable traits are usually positive, but are not large when negative, indicating that it should be possible to improve several traits without adversely affecting others (Vogel, 2000).

Evaluation of Seed Germination

The Association of Official Seed Analysts (AOSA, 1993) has outlined procedures for testing the germination of seeds. The procedure recommendations for native grasses include moistening the seed for 2 weeks at 5°C prior to testing germination percentages as a stratification treatment in an effort to reduce seed dormancy. This moist chilling of the seed serves as a simulation of conditions native grasses encounter prior to spring germination. After this stratification, it is recommended that the seed be placed in petri dishes on top of sand and in an alternating temperature of 20°C/30°C for IG and BBS, and 15°C/30°C for switchgrass. To compensate for light requirement of the seed, the test should be conducted with a cool white fluorescence light at an illuminance of 75 -125 ft-c (750-1250 lux) for a period of at least 8 hours when the temperature range is 30°C. In an effort to provide moisture for the seed AOSA guidelines advise that the sand should be dampened with a 0.2% solution of KNO_3.

Extensive research has been conducted to test the effects of chemical and environmental conditions and how they relate to germination percentages of native grasses. Work directly related to the seed in the form of seed treatments, storage techniques, and temperature conditions has been shown to improve germination percentages in many native grasses. The following paragraphs summarize and highlight this research conducted with pre-chilled, scarified, and chemically treated seed.

Hsu et al. (1985) conducted research with un-chilled seed of several native grass species under different germination temperatures in an effort to identify an ideal germination temperature. The study consisted of BBS (a native population, Rountree, and Caucasian), IG (IG-2C-F and Osage), and ULSG (Blackwell and Cave-in-Rock) under the temperatures of 15°C, 20°C, 25°C, and 30°C. These data suggest that ideal germination temperatures not only varied between species, between cultivars, but also varied within the species. The following includes the: species, cultivar, and temperature at which the highest percentage germination was observed in BBS; native population 15°C, Rountree 15°C, Caucasian 30°C. IG: IG-2C-F 12°C, Osage 15°C, LLSG: Blackwell 15°C, Cave-in-Rock 25°C. This research suggests that ideal temperature for germination is most likely a result of environmental conditions paralleling where each cultivar (within the species) is most suitably adapted.

Stratification of seed has also proven to be an effective treatment in reducing seed dormancy. Shen et al. (2001) studied the effects of dormancy reversion as influenced by duration of stratification. The experiment consisted of stratifying seed lots of Cave-in-Rock ULSG for varying time periods of 14, 28, 42, and 56 days at 5°C. They also studied the effects of seed that were completely dried post stratification to determine if detrimental effects occurred. Potentially, the seed could become dormant after the drying process and cause lower germination percentages. The results indicate that stratification for 14 days increased germination from 5% (original germination pre-stratification) to 81%. When the same seed were dried, the germination percentages fell to 45%. To study the effects on the seed after stratification and drying, the authors restratified the seed lot for an additional 14 days. The germination percentages were restored to 83% after restratification concluding that seed viability had not been decreased from the drying procedures and that the seed reverted to a dormant state upon drying. The results also showed that dormancy reversion was eliminated with longer stratification periods. The 28 day stratification period germinated at 81% with 76% germination after drying. Ninety-one percent of the seed germinated after 42 days and 93% after 56 days. This research showed that stratification can be a useful procedure to get high germination percentages after only 14 days.

Chemical treatments were seen as a faster alternative to stratification that could potentially ameliorate seed dormancy. Tischler et al. (1994) explored this hypothesis and performed an experiment using a seed treatment of sulphuric acid and chloroethanol on Alamo LLSG. The purpose of the study was to compare chemical treatment against the already accepted procedure of stratification. The test consisted of 4 levels of chemical treatment - concentrated (16.8 *M*) sulphuric acid at 5, 10, 15, and 20 minutes. The results concluded that sulphuric acid had a significant effect on germination. It was found that a 10 minute acid treatment (92% germination) was comparable to the four week (94% germination) and six week stratification (89% germination), and superior to the two week stratification (82% germination). It was also the best treatment when compared between the four time intervals: 0 min. (51%), 5 min. (88%), 10 min. (92%), 15 min. (85%), and 20 min. (89%).

A summary of the literature suggests that physical methods to alleviate seed dormancy either are not permanent or involve the use of caustic chemicals. Domestication of these native grasses is an important first step in expanding their use in agricultural or peri-agriculture systems. The current methods of commercial seed harvest (early September harvest) for these species preclude natural stratification as a method of seed dormancy amelioration. Stratification, to be effective, must be conducted while seed is wet (embryo is active). Drying the seed to plant substantially reverses this treatment (Shen et al., 2001). Scarification with acid has also been show to be successful (Tischler et al., 1994), but neutralization of the acidic seed is necessary, adding substantial cost to already expensive seed. Breeding to reduce seed dormancy would seem to be the best option to aid in germination and establishment of these species, and a vital first step in domestication and expanded use.

Inbreeding Constraints

When using a breeding system that involves repeated selection of cross pollinated species resulting in a limited population, the issue of inbreeding depression arises. Inbreeding depression is defined as a reduction of fitness and vigor in progeny derived from inbreeding, relative to those derived from freely pollinated individuals/populations (Husband and Schemske, 1996). Furthermore, the potential for decreased vigor and infertility arises with inbreeding depression and can cause inbred lines to become less fit and can ultimately cause complete loss of the population due to sterility issues unrelated to self incompatibility.

All of the species in this study have the potential to succumb to inbreeding depression as a result of strong selection pressure with the exception of IG. It has been shown in work by Husband and Schemske (1997) that polyploids can buffer the effects of inbreeding better than diploid species because of their increased innate heterozygosity. Grasses are genetically different from other angiosperms because of a two-loci system of incompatibility common to the family *Poaceae* (Poehlman and Sleper, 1995). Therefore, in a diploid if any of the four alleles in the pollen are the same as those in the ovule, fertilization will be precluded. When working with higher ploidy species (4x and 8x), the number of incompatibility alleles affecting self fertility are increased (4 and 8 fold, respectively) (Briggs and Knowles, 1977). Meaning that the potential for inbreeding depression resulting from crosses of related individuals would be greatly reduced (because the fertilization of the ovule would be nearly impossible).

Diploids more often show the effects of inbreeding depression and precautionary measures must be taken when working with the species classified in that category and the species with unknown ploidy levels (IG, BP, and PT). Additional precautions, such as maintaining high population numbers, must be taken for IG because of its ability to self pollinate (Newell, 1936).

Breeding Systems

Though there has been limited work to improve native grass populations, specific breeding systems are used to enhance some agronomically and horticulturally important traits. These breeding systems can be used to manipulate medium to high heritability traits such as: biomass production (Vogel and Pedersen, 1993), forage quality (Vogel and Pedersen, 1993), height (Burton, 1974), and in this case, reduced seed dormancy (Jones, 2004). However, improvements that can be made to these traits are dependent upon genetic variability within the species, a characteristics ability to be inherited, the breeder's ability to recognize genetically superior genotypes, and the breeding program's effectiveness (Vogel, 2000; Tischler and Young, 1987; Schaaf and Rogler, 1960).

Several breeding methods were examined by Vogel and Pedersen (1993) for cross-pollinated grasses and evaluated for yield increase. These breeding techniques included: recurrent restricted phenotypic selection (RRPS) and a modified form of between and within half-sib family selection (B&WFS). The objective in using any recurrent selection breeding system was to change the frequency of desirable alleles in a population and thus changing the population mean for the characteristic. In both breeding systems (RRPS and B&WFS), seedlings of the appropriate species were produced in the greenhouse and transplanted into field evaluation nurseries to represent a mother plant nursery. Limited data were taken during the establishment year. Data collected in growing seasons following the establishment year were used to determine individuals with superior biomass yields for forage. These individuals were then moved to a separate isolated polycross nursery. Seed collected from the polycross nursery was then used to initiate the next cycle of selection. In this case, each breeding cycle required 4-5 years. Their data showed a 16% improvement in biomass yield from base population through cycle 3.

Other breeding systems for plants are available, although their affects have not been studied in native warm-season grasses. Phenotypic recurrent selection (PRS) is a system designed to improve a plants quantitative characteristics based on visual observation or physical measurement of the character (Poehlman and Sleper, 1995). This method worked well for native grasses requiring no tests of combining ability, and each cycle may be as short as one year. Phenotypic recurrent selection is an extension of mass selection with two refinements. The refinements include screening and isolating the selected plants and crossing those plants before the next round of selection (Briggs and Knowles, 1977). Phenotypic recurrent selection is also seen as a selection method with exceptional outcomes, which was designed to increase the ratio of favorable alleles while not compromising genetic variability and therefore minimizing inbreeding depression within populations (Casler, 1999). Improved disease resistance, forage nutritive quality, and forage yield have been observed in warm-season grasses using PRS (Burton, 1974; Casler and Pedersen, 1996; Casler and Vogel, 1999). Genetic advances demonstrated by these authors, in other grass species would seem to indicate that PRS was the most suitable breeding system for reducing seed dormancy in native warm-season grasses. The breeding methods mentioned were performed on native grasses that are consistent with the present work in this research: LLSG, BBS, and IG.

Genotypic recurrent selection (GRS), also known as half sib progeny test (HSPT), is a selection system used to improve quantitative character, but parental selection is based on progeny performance as measured by the results of test crosses, or other means (Poehlman and Sleper, 1995). GRS is typically used to improve more complex polygenic traits with lower heritability such as, combining ability in corn inbred lines or forage yields in alfalfa. Jones (2004) determined GRS to be more effective, but logistically impractical for reducing seed dormancy in IG and BBS due to limited success during the first cycle of selection. Though significant advancement was observed in the first cycle of GRS selection, progression to the second cycle of GRS would: 1. substantially reduce genetic diversity of the population and, 2. require establishment of another isolated 300-500 individual mother plant nursery.

Improved Establishment

Appropriate selection of species and cultivar for a region, along with seed bed preparation, planting depth, and weed control are all necessary in order to successfully establish native warm-season grasses. Seedling establishment occurs when a plant reaches a stage of development in which it can continue to maintain plant growth (Wolf et al., 1996). Getting plants to this stage requires that establishment techniques be suitable for the species in use.

Rapid establishment is essential for the implementation of native grasses to become widely used. This is a major concern due to weed competition (especially warm-season annual grasses) and environmental factors that affect juvenile seedlings during the first year of growth. Establishment of native grasses can be improved through breeding techniques and the alteration of the environment through cultural practices (USDA, 1996). An increased competitive edge over annual grasses is the desired outcome of our breeding program for reduced seed dormancy in that, seedlings that have faster germination rates will establish more rapidly and be able to compete for essential nutrients.

Researchers (Trupp and Carlson, 1971; Wright 1977; Clements and Latter, 1973), have determined that breeding for larger seed size can result in populations with improved seedling growth and establishment. Selection on native warm-season grasses for seedling vigor and emergence has focused on high seed weight. Sebolai and Vogel (1989) selected individual seedlings of IG, Switchgrass, and BBS based on seed weight. Seedlings were grown in micro-pots for 6 weeks and the heaviest seedlings of each species were allowed to continue growth in field polycross nurseries, collecting the seed from the elite plants, and testing the resulting seedlings vigor. Three cycles of selection have been completed in the three species and the data suggests that heavier seedling weights directly correspond with improved seedling vigor, but not enhanced germination.

Boe and Johnson (1987) conducted similar research using mass selection of switchgrass seed dependent on seed weight. They based their study on the principle that heavier seed would confer greater seedling vigor. The authors separated seed into heavy and medium weight categories using a seed blower and found that selection for heavier seedling weight and size directly correlates with other improved traits such as forage quality. These studies showed a direct correlation of higher seed weights and better establishment. However, this breeding program, for reduced seed dormancy, is expected to generate rapid germination and establishment affecting seed weight.

CHAPTER III

MATERIALS AND METHODS

Germplasm Assembly

This breeding project, for reduced seed dormancy, originated in 2001 with Jones (2004) conducting the first three years research on three species of tall-stature native grasses (LLSG, BBS, and IG). The procedures of this study are consistent with the research performed during 2001-2004 (Jones, 2004) and was a continuation of that work. Four short-stature native grass species (ULSG, LBS, PT, and BP) were introduced to the project in 2005, the first year for evaluation for reduced seed dormancy of these species at Starkville, MS.

Germplasm of all seven species originated from the Jamie L. Whitten Plant Materials Center (PMC), near Coffeeville, MS, either as individual clones from the "native prairie", or from accessions or composites of accessions in their collections. Crown divisions of IG and BBS were selected from the PMC native prairie in 2001 and established on the R.R. Foil Plant Research Center (North Farm) in Starkville, MS (Figure 1). LLSG was obtained from open pollinated polycross seed derived form 100 accessions planted at the PMC. The short-stature species, which were tested on site at the PMC for two years, and clones of existing crossing blocks were introduced to the North Farm during the summer of 2006. Suspected problems with

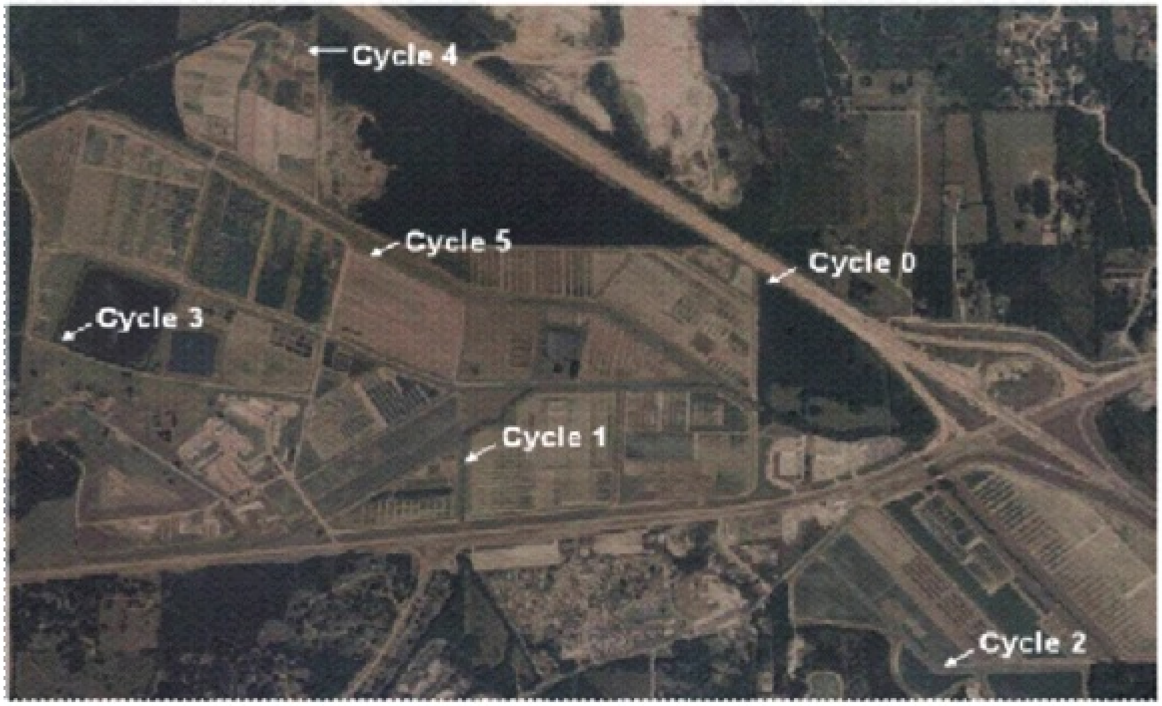

Figure 1. Aerial photograph of R.R. Foil Plant Research Center (North Farm and Ramsey Bottom) near Starkville, MS. and placement of original cycle (Cycle 0) and advanced cycles of selection with reduced seed dormancy (Cycles 1-5) for all species. All blocks are separated from each other by no less than 400 meters. (Scale: 1cm=160m)

pollen contamination of the blocks at the PMC (Figure 2), due to a stalling in progress, and differences in environments between Coffeeville and Starkville made it necessary to assemble all species at the same location.

Prior to 2005, three cycles of selection for LLSG, and two cycles of selection for IG and BBS had been completed at Starkville (Jones, 2004). Selection for improved pre-stratification germination (PSG) was accomplished by collecting seed from established species blocks (LLSG-C3, IG-C2, and BBS-C2). Seed were collected in equal quantities from each mother plant in each block in October / November of the appropriate year and stored under low humidity (60 ± 4% RH) at room temperature (22 ± 2°C) until evaluation in January. All seed of the short-stature species was collected from the appropriate crossing blocks and mother plant nurseries at the PMC at Coffeeville and sent to Starkville for evaluation. All species were advanced using PRS.

Population Evaluation

Populations were evaluated by screening seed from the appropriate crossing block. Six replications of 100 seed of each species and each generation (cycle of selection) were placed into petri dishes (100 x 20 mm). A solution of fungicide-wetted (9.67 g of 50% benomyl per 3.78 l of water) germination paper was placed in the dishes for moisture requirements. The petri dishes containing the seed were placed into the germination chamber (GR-371. Percival Scientific Inc., Boone, Iowa) under a 30°C light (16 hr) / 20°C dark (8 hr) temperature and light regime (Shaidaee et al., 1969). Minimum light levels in the growth chambers were 55 micro-Einsteins as measured by LI-188B

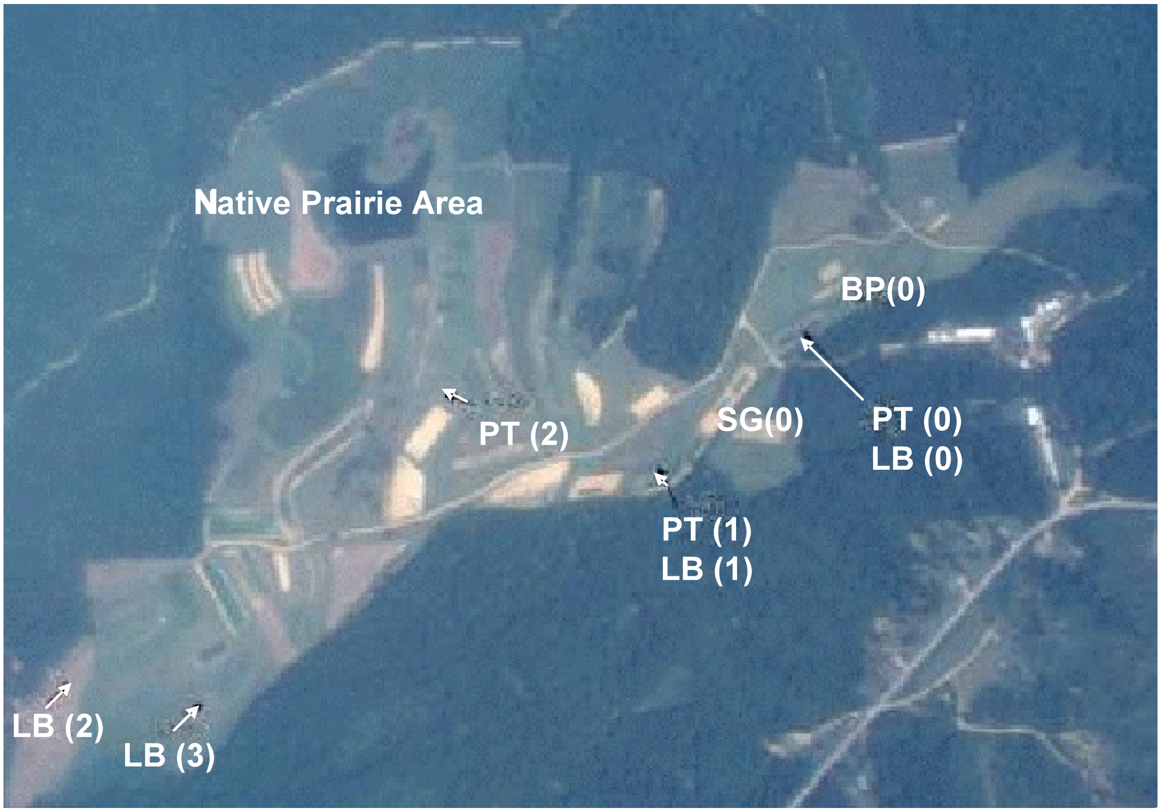

Figure 2. Aerial photograph of Jamie L. Whitten Plant Materials Center near Coffeeville, MS. and placement of original cycle (cycle 0) and advanced cycles of selection with reduced seed dormancy (cycles 1-3) during the years 2002-2005 for individual species of SG-lowland switchgrass, PT-purpletop, LBS-little bluestem, and BP-beaked panicum. (Scale: 1cm=92m)

Integrated Quantum Radiometer/Photometer (LICor, Lincoln, NE). Seed were checked for radical emergence (germination) every second day for a total of 14 days. Seed was considered germinated when radical emergence was ≥2 mm and coleoptile/1st leaf was ≥3mm. All seedlings that germinated during this period were counted and recorded by day. To determine progress of selection, germination percentages were compared. Population means were evaluated by PROC GLM (SAS, 2005) because population numbers were unbalanced.

Mass Screening of Populations

To obtain the next generation, mass screening of seed of individual populations originating from the most advanced cycles of selection (crossing blocks) were placed on top of double thickness germination paper and placed in stainless steel trays (56 x 64 cm). The germination paper was wetted with 9.67 g of 50% benomyl solution per 3.78 liters of water. Approximately 30,000 seed were then spread onto the paper ensuring the seed became wet. Clear lucite covers were used to allow light to the seed, keep humidity high in the tray, and keep the germination paper and seed from drying out. This method adapted itself well to species that had extremely low germination percentages in its ability to screen large numbers of seed at once. The first 100 seedlings of each species to germinate (either from the population evaluation test or mass seed screening) were removed and placed together into species-labeled clear plastic crisper boxes containing fungicide (9.67 g of 50% benomyl per 3.78 l of water) wetted germination paper and fertilizer (2g 30-15-15 Osmocote®). The first 100 seedlings were collected to insure 64 plants for the crossing blocks of the next cycle. The labeled crisper boxes were maintained under the same conditions as the petri dishes and served as an “incubator” for growth before plants were transferred to potting medium. After one week, the seedlings were placed into plastic six-packs (3.8 x 3.8 cm) containing potting medium (Baccto® potting soil) and transferred to a second growth chamber at a constant 22°C and 16 hour light to act as an intermediate between the conditions of the growth chamber and greenhouse. Seedlings surviving two weeks in potting medium were moved to a growth chamber intermediate to the germination chamber and the greenhouse to acclimatize them. Once the plants established (as indicated by new growth) in the second growth chamber (~2 weeks) they were transferred to the greenhouse (27°C / 16 hr supplemental light) until repotting was necessary.

During the first week in May, the plants were taken from the greenhouse and planted in the field. Placement of individual blocks were selected so that different cycles/generations were far enough (no less than 400 m) to prevent cross pollination, but close enough so all blocks endured the same basic environmental conditions (Figure 1). For most wind-pollinated crops 200 meters separation is recommended (Ireland et al., 2006). Elite populations consisted of a minimum eight by eight block on 1 m centers, for a total of 64 elite mother plants per species. All species of a given generation (cycle) were planted as blocks in close proximity to one another. Upland switchgrass and LLSG polycross blocks were also adjacent, as research by Martínez-Reyna and Vogel (2002) indicated that crossing between these biotypes (really different species) was less than 0.05%. All blocks were established on the edges of row crop fields as to be easily accessible for maintenance and isolated from any other native grass stands. Once plants were transplanted into the field, efforts were made to ensure maximum growth during the first year. A regiment of irrigation twice a week (approximately 2 inches simulated rainfall per application), with one application of fertilizer (Peters® 20-20-20 at 200 ppm N) was applied to maximize the transplanted seedlings growth. Once established, crossing blocks were manually weeded three times annually and treated with a granular form of trifluralin (Preen at 0.9 kg per 64 m^2 = 4.5 kg a.i. per hectare) twice annually to suppress weeds and volunteer grass seedlings. Blocks were checked weekly for emerged weeds and volunteer seedlings and hoed as needed. All plants were allowed to grow under ambient conditions and produce seed. Because of its excessive height, LLSG was mowed to 20 cm each July. Regrowth produced panicles at 1.5 m. Seed was harvested as it ripened, usually October-November.

Inbreeding Depression

While inbreeding was not expected in these populations, we did conduct simple testing for inbreeding depression. LLSG seedlots of all cycles of selection were aspirated (Bulldog VDMC aspirator, Carter Day International Inc.) at the same air velocity into three weight categories: heavy (positively viable seed), medium (empty sheaths or compromised embryos resulting from inbreeding depression), and light (blank florets, putatively resulting from self incompatibility) to determine the percentage of heavy viable seed compared to blanks. Each seed lot was then weighed by category to obtain percentages of heavy seed and empty sheaths. The seed lots were then analyzed by generation using Chi Square analysis to determine similarities (or dissimilarities) between generations as it relates to symptoms of inbreeding depression.

Realized Heritability

Calculations were performed to evaluate the realized heritability between each cycle of selection to provide an estimate of narrow sense heritability and therefore measure phenotypic change between cycles of selection. Research by Vogel (2000) indicated that switchgrass heritability characteristics of important traits are between 20 and 40% (0.2-0.4). Lowland switchgrass (C1-4), Indiangrass (C2-3), Upland switchgrass (C1-2) were the only generations in which data could be analyzed because all other species did not produce enough seedlings prior to stratification in the petri dishes (within the first 14 days) to allow a calculation of heritability for the cycles available. Realized heritability (H_R) was calculated as:

H_R = gain/reach,

where:

gain = $\bar{x}$ new population - $\bar{x}$ original population and

reach = $\bar{x}$ selected population - $\bar{x}$ original population

Once calculated, the realized heritability between cycles of selection is an indication of transmission of alleles associated with PSG as it relates to phenotypic change of the species. After analysis of this phenotypic change, the values of H_R can be compared between cycles of selection to show the actual heritability of traits being selected for (non-dormancy).

Field Emergence Trial

A single field emergence trial was initiated on June 12, 2006 on the Leveck Animal Science Research Center (South Farm) near Starkville on a catalpa silty clay loam, to test LLSG seed from this breeding program (C2, C3, C4) with commercially available cultivars (Alamo, Cave-in-Rock, Kanlow, Shawnee, and Trailblazer) obtained from the Noble Foundation in Ardmore, OK. Seed from other species of this project were not advanced enough to begin emergence trials. Testing of the Breeder's Seed under field conditions is necessary to evaluate actual progress as it relates to commercially grown cultivars and the trial served in this capacity to evaluate the progress. Single rows 6.1 m in length of each cultivar and germplasm line, for a total of eight, were planted 50.8 cm apart. The test was arranged as a completely randomized block containing 4 replications. A preemergence, s-triazine herbicide (Simazine 1.47% a.i. at 89 ml per 600 m^2 = 2.24 kg per hectare) was applied at the labeled rate over-the-top. The test was irrigated twice (on July 12 and 14) to incorporate the herbicide and induce seed germination. Numerical ratings (1-5, with 1 indicating no seedlings, 3 indicating intermediate growth, and 5 indicating exceptional growth determined by density and thickness of seedlings in the row as it would relate to a full row of seedlings) on visual seedling emergence began after two weeks and continued bi-weekly for a total of five ratings to test emergence of each cultivar with first emergence being seen at 6 days. On October 4, 2006, sub-samples of plants in a random 0.3m section of each 6.1m row (0.6m row spacing) were hand counted and multiplied up to reflect an estimated number plants per hectare. The purpose of the emergence trial was to monitor seed from the breeding program with seed that are considered the standard for switchgrass production.

CHAPTER IV

RESULTS AND DISCUSSION

Tall Stature Species

For all tall stature species, data collected were analyzed both across cycles of selection and within cycles across years. The purpose of analyzing across different cycles of selection was to determine progress of the species using PRS. To determine environmental impacts on seed dormancy, analysis within each cycle across years was assessed.

Comparison of mean germination percentages in selected populations of lowland switchgrass

Effect of Selection:

The PSG were analyzed across cycles of selection to examine improvements in PSG using PRS. Data collected by Jones (2004) in the years 2002-2003 showed improvements in mean germination between C1 and C2 of LLSG. She observed that mean germination with only one cycle of selection improved from 4.2% for C1 to 25.5% in C2, a significant increase (Table 1). An attempt was made by Jones to compare C1 with C0 seed. However, C0 plants were at Coffeeville PMC and grew under favorable

Table 1. Effect of selection on lowland switchgrass (*Panicum virgatum*) prestratified seed germination (PSG) percentages by year and cycle of selection. Seed were bulked from each plant and a representative sub-sample was taken to conduct the germination test. There were six replications of 100 seed tested for each population. Percentages represent 14-day totals.

	Evaluation / Screening Year				
Cycle of Selection	2002[‡]	2003[‡]	2004	2005	% Mean Germination
	Mean Germination Percentage by Year				
0	#	#	1.0 a [†]	0.5 a	0.8 a
1	1.2	4.2 a	2.83 a	27.8 b	9.0 a
2		25.5 b	7.0 b	48.0 c	26.8 a
3			22.2 c	45.8 c	34.0 a
4				34.5 b	34.5 a

Seed not produced at Starkville

[‡] Data collected by Jones (2004)

[†] Comparisons made between cycles (within column). Values followed by the same letter are not significantly different at the 0.05 level of probability.

environmental conditions while less favorable conditions existed during the growing season at Starkville. PSG of C0 seed grown at Coffeeville PMC greatly exceeded those of C1 from Starkville. This was the first indication that environment plays a significant role in PSG and clones of C0 plants would have to be moved to the Starkville test site for realistic comparisons. As with Jones' research, the overall trend of this research was an increase in PSG as cycles of selection progressed. Seed collected from LLSG C2 in 2003 was screened and those seed showing reduced dormancy were used as elite parents for C3. Cycle 3 seed were then collected from field plants in 2004 along with previous generations and screened to monitor improvements across the cycles. Data from 2004 indicated the mean PSG of each cycle of LLSG continued to improve with continued selection (Table 1). In 2004, the first year of this research, C2 showed mean PSG of 7.0%, while C3 had a 22.2% mean PSG. This improvement represents a 3-fold increase that is substantially and significantly better than the previous cycle.

Screening for all cycles/generations in 2005 showed unexpected results. Germination percentages of LLSG that year decreased numerically in C3 and C4. While C2 had a mean PSG of 48.0%, the more advanced C3 and C4 showed a PSG of 45.8% and 34.5%, respectively. These changes in values were slight, but not a significant decline in PSG for C3 as compared to C2, but a significant decline for C4 compared to C2 and C3. There could be a number of reasons for this variable progression. We suspect the selection criterion. In previous years, selection for the next generation (first 100 individuals to germinate) was exemplified by a slow progression in germination and limited number of responding seedlings. In C2, screening took 5 days to obtain 100 seedlings as elite parents for the next generation (Table 2; Figure 3). In the earlier cycles of selection, no problems were incurred in the determination and selection of the first 100 seedlings because numbers were low and germination was slow. As the generations progressed, there were hundreds of germinated seedlings present at the four day observation (when none were observed at two days), making it virtually impossible to identify the first 100 seedlings that germinated. Repeating the screening and making observations at day 3 was no better. No germinated seed were observed at day 3, but hundreds were present the morning of the fourth day. Every effort was made to select the largest leafed seedlings, on the assumption they were the first to germinate, but that may not have been successful.

A second possibility for the decline in PSG in the advanced cycles might be due to inbreeding depression. Inbreeding depression is always a concern with cross pollinated crops and limited population numbers. In these species it would be expected to manifest as blank seed/sheaths due to fatal genetic load and self incompatibility. To determine if inbreeding depression was occurring, seedlots of all LLSG cycles were fractionally aspirated (Bulldog VDMC aspirator, Carter Day International Inc.) into three weight categories: heavy (viable seed), medium (empty sheaths or compromised embryos putatively resulting from inbreeding depression), and light (blank florets resulting from self incompatibility). Each seedlot was then weighed by category to obtain percentage of heavy seed, medium and empty sheaths (Table 3). Because of the extremely small percentages of the light category (<5% = default α), medium and light aspirated lots were combined for analysis. If inbreeding depression were occurring, we would have expected a substantial decrease in the percentage of heavy seed and increases in the medium and

Table 2. Days to acquire 100 seedlings measured by days as a secondary measure of progress.

	Species						
Cycle of Selection	LLSG	IG	BBS	ULSG	LBS	PT	BP
	Days to acquire 100 seedlings						
0	16 (3x)[†]	25 (2x)	28 (3x)	17 (2x)	20	14	21[‡] (5x)
1	6	16	20	9	7	11	
2	5	9	9		7	9	
3	5	5	12				
4	5						

LLSG - lowland switchgrass, IG - indiangrass, BBS - big bluestem, ULSG - upland switchgrass, LBS - little bluestem, PT - purpletop, BP - beaked panicum

[†] Number of times mass screening was perfomed to acquire 100 seedlings. Mass screenings were for 14 days of germination under 30 / 20°C , 16 / 18 light / dark. Seed not germinated after 14 days was discarded and the screening was repeated (number in parentheses)

[‡] BP-21 days only yielded 19 seedlings. This process was repeated 4 times for a total of 41 seedlings

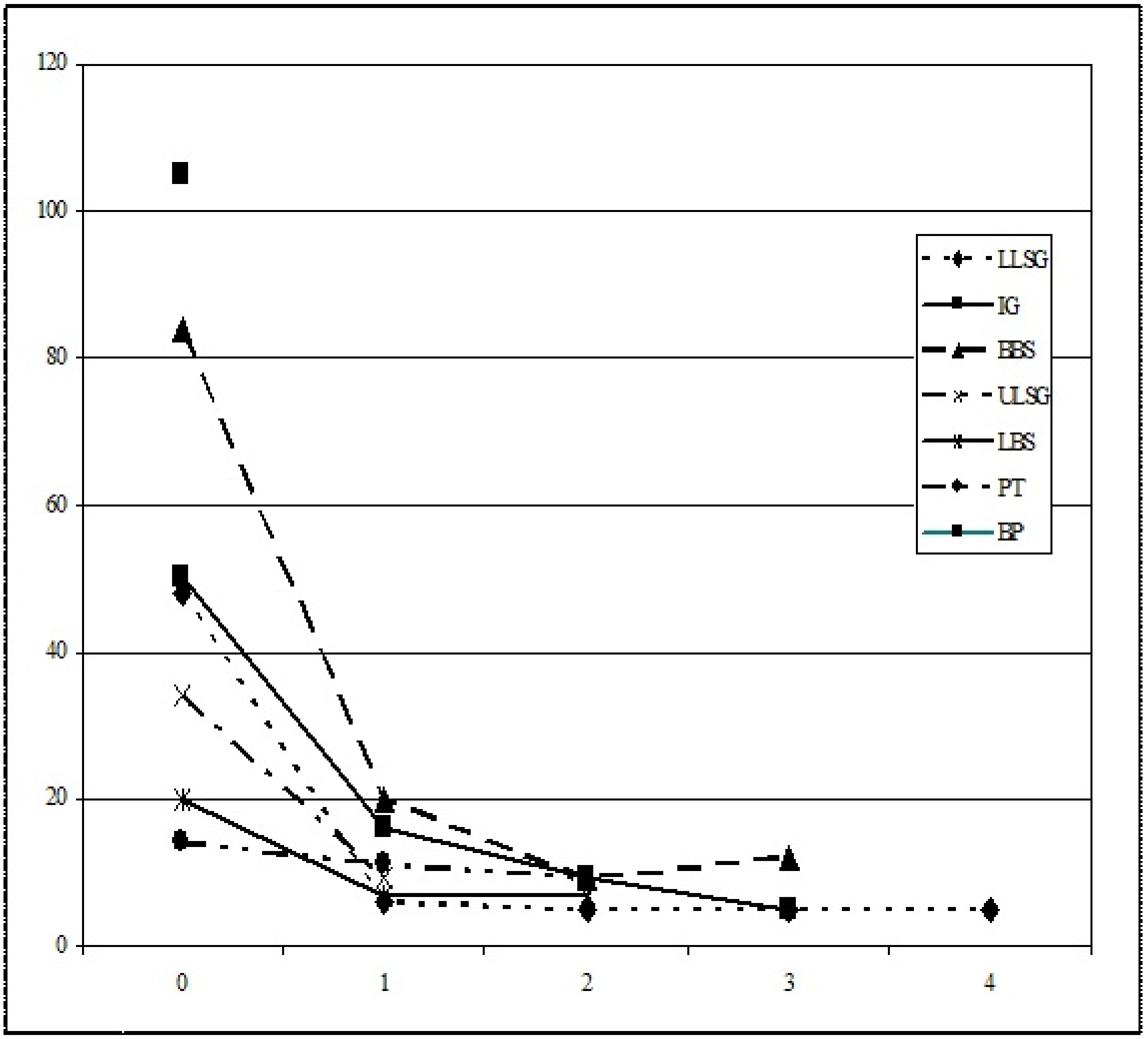

Figure 3. Total days to 100 seedlings for seven species by cycle of selection. LLSG - lowland switchgrass, IG - indiangrass, BBS - big bluestem, ULSG - upland switchgrass, LBS - little bluestem, PT - purpletop, BP - beaked panicum

Table 3. Percentage of viable seed compared to empty sheaths in seed lots of all cycles of lowland switchgrass based on three weight categories, to determine the effects of inbreeding depression across cycles of selection. Chi Square analysis indicated no difference in percentage heavy : light seed across generations.

	Weight Category		
Cycle of Selection	Heavy (viable seed)	Medium (empty sheaths)	Light (empty sheaths)
	Percentage for each weight category		
0	79	19	2
1	75	20	5
2	84	14	1
3	76	23	1
4	87	12	2

Chi Square = 7.67, $P \geq 0.17$, at $\alpha = 0.05$. Not significant.

light categories. The C0 seed lot contained 79% heavy and 21% medium and light fractions, compared to 75% heavy and 25% medium and light fractions in C1. After one cycle of selection a small reduction in the heavy fraction is observed (from 79% to 75%). Following this small reduction in percentage heavy fraction after one cycle of selection, the heavy fraction percentage increases to 84% in C2. Another reduction in heavy fraction is seen between C2 and C3, as heavy seed in C3 accounted for 76% of the seedlot and light seed/empty sheaths accounted for 24%. Ultimately, the highest percentage of viable seed was observed in C4 as 84% of the seed lot was heavy seed. If inbreeding depression were the actual cause for a decrease in PSG, the percentage of heavy seed as compared to the whole seed lot would decrease and light seed/empty sheaths would increase in percentage with advancing cycles of selection. Ultimately, the Chi Square Analysis indicated that there were no significant differences between populations (X^2=7.67 $P \geq 0.17$).

A further refutation of the inbreeding hypothesis resulting in variable germination would be supported by the fact that grasses possess a two-loci gametophytic system of multiple alleles for self-incompatibility (Poehlman and Sleper, 1995: see also Review of Literature). This system offers insight on why inbreeding depression would not be expected as an issue in LLSG. In the gametophytic self incompatibility system (believed to be genetically conserved in the family *Poacea*), if any of the self incompatibility alleles in the pollen or ovules were shared, fertilization and seed production would be precluded, causing empty sheaths. In a polyploid species, self incompatibility systems are enhanced by the multiple of ploidy, precluding multiple numbers of potentially common alleles and therefore more pollen from any genetic relation. Ultimately, any ovule which failed to be fertilized (signs of incompatibility and inbreeding) would result in aborted seed or blank florets. We would not see inbreeding depression as weakened progeny, but as a reduction of seed portion, which again is not the case.

The only other possible explanation for such variable PSG would be differences due to environment. While clones of all species are planted at Starkville, there may be subtle differences in soil, soil moisture retention, and moisture availability at the specific location of each crossing block. The effect of environment is evident in the year by year comparison of the exact same clones of each crossing block discussed in the next section. A stepwise regression comparison comparing rainfall or temperature around the time of anthesis with percentage germination may offer some insight, but is beyond the scope of this research.

With the decline in germination in 2004 (Table 1) across all cycles and a decline in germination among advanced cycles in 2005, significant improvements were not observed among mean PSG between any of the cycles (C0-C4) across all 4 years. Though percent improvement over C0 LLSG is greater than any other species, the statistical analysis was unable to detect significant differences between the means pooled over years due to extreme variation between years. However, LLSG has been improved using PRS and is easily observed as mean PSG increase from 0.8% in the original population (C0) to 34.5% in C4.

Effect of Year:

It is important to remember that these are perennial grasses and exactly the same genotypes contribute to the offspring each year. Since there is no change in the genetics of the parents within a cycle, changes in the PSG of the progeny of any given cycle must be due to the environment of the production year. Variability in germination as it relates to environment was seen in previous research by Schaaf and Rogler (1960) when they discovered that dormancy was greatly influenced by interactions between the genotypes with environmental conditions. Examination of seed germination data of LLSG C1, 2, and 3 for three years revealed strong environmental (year) effects on pre-stratification germination percentages (PSG). These environmental impacts can be observed as PSG in C1 moves from 4.2% in 2003, to a much lower 2.83% in 2004, only to increase to 27.8% in 2005 (Table 4). This trend is varied within a cycle, but was however observed in C2. Cycle 2 had a PSG of 25.5% in 2003, which decreased to 7.0% in 2004, followed by an increase to 48.0% in 2005 (same trend in C1).

It was not the intent to model environment and seed germination. However the strong decrease in PSG of the LLSG cycles in 2004 would seem to implicate environment as having a strong effect on PSG. Other researchers (Panciera et al., 1987; Fenner, 1991; Sanderson and Wolf, 1995) have also remarked on the effect of seed production environment on subsequent germination. Panciera et al. (1987) was among the first to note that seed of a single switchgrass cultivar grown at different locations had a wide range of germination (from 16 to 78%) and suggested this was due to climatic conditions during seed development. Fenner (1991) suggested higher than average temperatures during seed development were positively correlated with germination rates and dormancy reduction. Sanderson and Wolfe (1995) suggest a narrower assessment of seed dormancy in switchgrass, attributing differences in germination specifically to cumulative degree days and discounted precipitation. Review of temperature and precipitation during the

Table 4. Effect of year on lowland switchgrass (*Panicum virgatum*) prestratified seed germination (PSG) percentages by year and cycle of selection. Seed were bulked from each plant and a representative sub-sample was taken to conduct the germination test. There were six replications of 100 seed tested for each population. Percentages represent 14-day totals.

	Evaluation / Screening Year				
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	Germination Percentage				
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3			22.2 a	45.8 b	34
4				34.5	34.5

Seed not produced at Starkville

[‡] Data collected by Jones (2004)

[†] Comparisons made between production years (within rows). Values followed by the same letter are not significantly different at the 0.05 level of probability.

months of August through October (Table 5) (the months most influential on seed development) indicate substantial differences in temperature across the three months when observed over several years. It would seem that rainfall would be the factor affecting PSG. When looking at precipitation amounts for three years; in 2003, rainfall totaled 17.25 cm for August. which was crucial for seed production at the onset of anthesis. In a crop that produces seed sequentially, heavy rain late in the season may have the potential to dislodge early maturing seed. The later months showed a lower rainfall total which we expect was not sufficient to dislodge viable non-dormant seed from the LLSG plant. The year 2004 brought much less rainfall in August (9.01 cm) with higher totals in September (12.60 cm) and October (11.40 cm). These totals show that rainfall was lower during seed set (August) in 2004 and may have caused stress on the mother plants when producing seed. Fenner (1991) suggests that stress is induced on plants with changes in abscisic acid (ABA) known for its ability to enhance seed dormancy. While species with mechanical dormancy were more difficult to germinate following drought seasons due to thicker seed coats, drought usually reduced biochemical seed dormancy by limiting the production of germination inhibitors. The higher rainfall totals in September and October of 2004 could have dislodged viable seed from the LLSG plant, causing a much narrower seedlot to be harvested and screened. These explanations for varying PSG are also supported by climatological data in 2005, where PSG for C1 and 2 were the greatest of the three years examined. The year 2005 showed adequate rainfall during August (14.27 cm) with lower totals in September (9.55 cm) and October (0.28 cm). It superficially appears that "adequate" (higher than mean) rainfall in August is crucial to produce viable seed and rainfall in October is less desirable because ripe LLSG seed

Table 5. Climatological data for Missisippi State University (August-October / 2002-2005) used to determine the effects of weather patterns on growth rates and reduced seed dormancy of native grasses

	Year					
Month	2003	2004	2005	2003	2004	2005
	Mean Temperature (°C)			Mean Precipitation (cm)		
August	27.27	24.92	27.55	17.25	9.01	14.27
September	22.85	23.07	24.64	6.35	12.6	9.55
October	17.47	20.38	16.97	8.33	11.4	0.28
Total	67.59	68.37	69.16	31.93	33.01	24.1

would be more susceptible to dislodging or fungal infestation as reported by Jones (2004) during this month.

The data analyzed for LLSG over the two years of this study are consistent with observations by Jones (2004). Numerical values for PSG of LLSG continue to improve between cycles although no significance is seen for switchgrass. Environmental effects are substantial causing differences in prestratification germination to be varied sufficiently so that actual progress can be seen but not detected statistically when examined as a result of mean germination. However, statistical progress is made between cycles within the same year.

Comparison of mean germination percentages in selected populations of indiangrass

Effect of Selection:

When looking at the PSG data from IG the first thing that is most obvious is the confounding effect of year (Table 6). Unlike LLSG, the PSG of IG was higher in 2004 than in 2003 and 2005. This is not seen as uncommon when attempting to discern progress due to selection. Each species tested is adapted to specific niches. Jones (2004), in 2002, observed an increase in PSG of IG between C0 and C1 as germination in this year increased from 5.2% in C0 to 10.5% in C1. In 2003, differences were masked by environment, as there was no indication of improvement, IG C0 PSG=0.2% and C1 PSG=0.3% (Table 6). This lack of improvement may be attributed to a limited seed lot and difficulties in establishment of seedlings after selection.

Table 6. Effect of selection on indiangrass (*Sorghastrum nutans*) prestratified seed germination (PSG) percentages by year and cycle of selection. Seed were bulked from each plant and a representative sub-sample was taken to conduct t the germination test. There were six replications of 100 seed tested for each population. Percentages represent 14-day totals.

	Evaluation / Screening Year				
Cycle of Selection	2002[‡]	2003[‡]	2004	2005	%Mean Germination
	Germination Percentage				
0	5.2	0.2	1.7 a[†]	0.3 a	1.9 a
1	10.5	0.3	2.7 a	2.2 a	3.9 a
2			1.0 a	7.5 b	4.3 a
3				17 c	17.0 b

[‡] Data collected by Jones (2004)

[†] Comparisons made between cycles. Values followed by the same letter are not significantly different at the 0.05 level of probability

When compared in 2004, there was a slight numerical (but not statistical) increase in germination between C0 (1.7%) and C1 (2.7%). However, the most advanced IG cycle of 2004 (C2) actually shows a numerical (though not statistical) decline in PSG. After two years research and one advanced cycle of IG (C1) when comparing mean PSG across cycles, Jones (2004) failed to show significant progress towards reduced seed dormancy using PRS. Also, when mean PSG for IG C2 was observed, significant progress was still unseen with an increase from only 3.9% (C1) to 4.3% (C2). However, with the advancement to C3 in this study, significant progress was shown as mean PSG increased to 17%. This significant increase in mean PSG was thought to be the result of the advanced cycle in conjunction with a year favorable for seed production. The data from LLSG and some cycles of IG would lead one to believe that there are overriding differences (high variability) between cycles on the North Farm, however the 2005 results will dispute this because variability between IG cycles were low enough to detect statistical differences.

Assessment of the means over years indicate the original population (C0) had a mean PSG of 1.9%. Selection was able to advance mean PSG to 3.9% in C1, though this was not a significant improvement. Progress made in this study advanced the next generation (C2) to 4.3% mean PSG (also not significant). In 2005, C3 seed was screened and clear progress was made as a significant increase to 17% mean PSG for IG C3 was observed. Again, the extreme variation in PSG of field produced seed increases the variance around each mean interfering with detection of differences.

Effect of Year:

When environmental conditions on IG were observed, actual progress was made from C0 to C1 selection, but undetectable (Table 7; 2002) until germination percentages were compressed in 2003. These differences disappeared in 2004 and 2005 screenings. When advanced cycles of selection for IG (C1 and C2) were compared for reduced seed dormancy, environmental conditions affecting germination were still evident. In 2004, C1 (2.7%) had a greater PSG than C0 (1.7%), however C2 (1.0%) was lower than both of the previous cycles. This decline in PSG for C2 is the result of environmental conditions. This result is confirmed in 2005 when IG C2 (7.5%) had a greater PSG than the C0 (0.3%) and C1 (2.2%). When IG C3 was observed, it was determined that significant progress in reducing seed dormancy had been accomplished. Cycle 3 in 2005 had a PSG of 17.0%, which was significantly better than all others cycles (C0=0.3%, C1=2.2%, and C2=7.5%) in that year.

One trait that separates IG from LLSG is the ease upon which seed is dislodged from the plant (personal observation). Indiangrass produces seed that is not easily lost under adverse environmental conditions, such as raining and moderate winds. While heavy rains in October 2004 may have caused seed loss in LLSG, the structure of the IG seed head (seed held tightly in the glume) may have prevented this, and therefore not caused a detrimental effect on PSG. This explains the variance between PSG's of different species.

Ultimately, 2002 was an excellent year for IG as it relates to PSG, however during the two years of this study (2004-2005) neither year is clearly better than the other as was seen with LLSG. The reason for this is that different cycles occupy different niches in the environment. For example, 2004 was the establishment year for C2 and in 2005 these plants

Table 7. Effect of year on indiangrass (*Sorghastrum nutans*) prestratified seed germination (PSG) percentages by year and cycle of selection. Seed were bulked from each plant and a representative sub-sample wastaken to conduct the germination test. There were six replications of 100 seed tested for each population. Percentages represent 14-day totals.

	Evaluation / Screening Year				
Cycle of Selection	2002[‡]	2003 [‡]	2004	2005	%Mean Germination
	Germination Percentage				
0	5.2 a	0.2 c	1.7 b	0.3 bc	1.9
1	10.5 a	0.3 b	2.7 b	2.2 b	3.9
2			1.0 b	7.5 a	4.3
3				17	17

[‡] Data collected by Jones (2004)

[†] Comparisons made between cycles. Values followed by the same letter are not significantly different at the 0.05 level of probability

were bigger with stronger root systems. This increased growth can be attributed to the species itself which has been known to be drought resistant (Newell, 1936). While C3 would be in the establishment year in 2005 and be less developed than previous cycles when compared in the same year.

Indiangrass has been slow to respond to PRS (as compared to LLSG), due in part to slow establishment of the species and alternating environmental conditions during the steps of development which compromise its growth. Unextended photoperiod caused senescence of seedlings of earlier cycles of IG delaying progress. While 2005 was clearly an excellent year for LLSG seed production from a PSG standpoint, it is unclear if the same can be said about IG in 2005. It was better for C2, but the same for C0 and poorer for C1. It should be noted, IG is the only species in this study suspected to posses self-fertility alleles (Newell, 1936), which may be altering the expected outcome of selection. These self fertile species are generally known to inhabit drier regions where potential pollen sources may be limited, and flowering earlier to compensate for a shorter growing season offers a selective advantage (Solbrig and Rollins, 1977). However, self fertility in this species must offset the negative aspects of increased homozygosity and inbreeding depression. Manifestation of selfing from self-fertility alleles would be expected in distinctly smaller populations (Nasrallah et al., 2004), such as those in this study. Without the assumption of random mating, the expected results cannot be predicted.

This lack of clarity is due to the limited numbers of generations being evaluated, and perhaps will become clearer in subsequent years as replicates of the same generations can be evaluated.

Comparison of mean germination percentages in selected populations of big bluestem

Big bluestem was analyzed for advancement toward PSG and effect of the environment, in the same manner as the other tall stature plants. Advancement toward seed non-dormancy using PRS in BBS progressed little, but significant differences were observed in 2005 (Table 8).

Effect of selection:

During the first two years of this work, Jones (2004) was not able to determine improvements from the original population (C0). After one cycle of selection in 2002, PSG actually numerically decreased, from 2.3% (C0) to 1.5% (C1) PSG, though this was not significant. The same trend was observed the next growing season as C1 decreased to 0.2% from the original population which had a PSG of 0.3%. The decline in PSG was due in part to a limited seed lot and establishment problems of seedlings after selection (consistent with IG). It appears that both IG and BBS are extremely sensitive to photoperiod as seedlings, a characteristic not seen in LLSG. During the first years, seed and seedlings were screened under long-day conditions. However, after transplanting to soil they were moved to a greenhouse (March-April). Ambient day length at that time of year is shorter than the screening conditions. Since these are perennial grasses, the seedlings prepared for the onset of winter, but with no crown developed, the leaves senesced and the seedlings died. In 2004, we were able to advance to the second cycle of selection. Improvement in PSG was observed for C1 (1.5%) over C0 (0.0), which had not been evident the year before, however, the newest generation (C2) demonstrated a PSG of 0.5%, not different from the base

Table 8. Effect of selection on big bluestem (*Andropogon gerardii*) prestratified seed germination (PSG) percentages by year and cycle of selection. Seed were bulked from each plant and a representative sub-sample was taken to conduct the germination test. T here were six replications of 100 seed tested for each population. Percentages represent 14-day totals

	Evaluation / Screening Year				
Cycle of Selection	2002[‡]	2003[‡]	2004	2005	% Mean Germination
	Germination Percentage				
0	2.3 a[†]	0.3 a	0.0 a	0.3 a	0.7 a
1	1.5 a	0.2 a	1.5 b	1.0 a	1.5 a
2			0.5 a	3.3 b	1.9 a
3				3.2 b	3.2 b

[‡] Data collected by Jones (2004)

[†] Comparisons made between cycles with a single year (within a column). Values followed by the same letter are not significantly different at the 0.05 level of probability.

population, but a significant decrease when compared to its parental population (C1). In 2005, C3 showed no progress in advancement in PSG over its parental population (C2), 3.2% vs 3.3% PSG, respectively. The two most advanced cycles showed significantly higher PSG than the two earliest cycles of selection (C0=0.3%, C1=1.0% PSG).

Mean PSG for all years shows numerical improvement. Over all cycles of selection there is an incremental increase in PSG, but significant advancement occurred only in the most advanced generation. After three cycles of selection (mean PSG of C3=3.2%), significant progress was finally observed. The stalling of progress in several of the generations may be the result of slow seedling establishment. In 2004, only four plants of BBS were selected for C3 and transplanted into the field causing a reduced PSG resulting in failed seed set due to isolation with limited members with which to cross. However, by the 2005 seed harvest all blocks had been filled out to their appropriate minimum populations. The factor with the largest effect on confounding differentiation of generations is environment, as well as the limited genetics (for 1 year) of the cycles being assessed. Ideally, we would narrow variability by replicating the test, in this case over time/years. However, soil types, plant locations, rainfall, and temperature vary from crossing block to crossing block and year to year, and seem to be making discrete differentiation difficult.

Effect of Year:

When evaluated within a cycle across years, no trends in PSG as affected by environment (year) are evident (Table 9). Cycle 0, with the greatest number of evaluation years shows that 2002 was the best year with regard to PSG and has not performed as well since. The base population was moved from Coffeeville in 2001 and 2002 as crown

Table 9. Effect of year on big bluestem (*Andropogon gerardii*) prestratified seed germination (PSG) percentages by year and cycle of selection. Seed were bulked from each plant and a representative sub-sample was taken to conduct the germination test. There were six replications of 100 seed tested for each population. Percentages represent 14-day totals.

	Evaluation / Screening Year				
Cycle of Selection	2002[‡]	2003[‡]	2004	2005	% Mean Germination
	Germination Percentage				
0	2.3 a[†]	0.3 b	0.0 b	0.3 b	0.7
1	1.5 a	0.2 b	1.5 a	1.0 ab	1.5
2			0.5 b	3.3 a	1.9
3				3.2	3.2

[‡] Data collected by Jones (2004)

[†] Comparisons made between years within a single cycle of selection (within a row). Values followed by the same letter are not significantly different at the 0.05 level of probability.

divisions. It appears to be a characteristic for BBS, as extremely low PSG has occurred across all four years research (2.3% in 2002, 0.3% in 2003, 0.0% in 2004, and 0.3% in 2005).

Cycle 1, also with four years evaluation seems even more variable. Like the C0, it had a good PSG in 2002 (even though PSG was numerically less than the base population), but also showed good PSG in 2004 (1.5%), when C2 demonstrated poorer PSG (0.5%). One might suspect the establishment year of each new cycle to be a causal factor for lower PSG. Cycle 1 crossing block was completed in 2004 and C2 in 2005, both these cycles in their respective year of completion (when they would have the greatest number of new seedlings) produced higher PSG.

Big bluestem also closely relates to IG as far as seed dislodging is concerned. BBS seed is held tightly in the glume, attached to the spike. It has the ability to withstand rain and moderate winds without losing seed. However, with regard to years 2002 and 2004, PSG differs between IG and BBS. This may be the result of adaption and maturity of established plants during times which they encounter specific environmental conditions.

Short Stature Species

The short stature grass species were only analyzed for improvements in PSG due to cycles of selection. An analysis of improvement due to selection within a species was not possible due to environmental effects, because all preliminary data on these grasses was conducted at the Jamie L. Whitten PMC, Coffeeville, MS (Figure 2). During the first two years of selection, minimal progress was observed because native stands of these species were located near the crossing blocks causing pollen contamination of the elite parents and the production of wild-type offspring. To better determine improvements due to selection, clones (as crown divisions) of all C0 and C1 plants of ULSG, LBS, PT, and BP were brought to Starkville, MS in 2006.

Comparison of germination percentages after one cycle of selection in upland switchgrass

Upland switchgrass showed significant improvement over one cycle of selection at Coffeeville, MS. The original population (C0) exhibited a PSG of 0.7% (Table 10). This PSG advancement is parallel to the LLSG biotype, and improvements in advanced cycles were expected to be comparable to those of LLSG; with one cycle of selection there was improvement to 4.0%. As with LLSG, seedlings responded well to handling and greenhouse conditions. Upland switchgrass was advanced to C2 at Coffeeville PMC, but clones of C2 were pooled together with C1 when they were moved to Starkville because of lack of advancement.

Comparison of germination percentages between two cycles of selection in little bluestem

Little bluestem was advanced to C2 at Coffeeville, MS, although limited progress in PSG was observed. The original population (C0) had a germination percentage of 0.8% (Table 10). After one cycle of selection, a significant improvement to 2.7% was made for C1. Cycle 2 showed a PSG of 1.7% which was numerically (not statistically) lower than C1 (2.7%). After observing a loss of progress in this species, seed from C1 and C2 were pooled

Table 10. Pre-stratification germination (PSG) percentages of short stature grasses 2005 counts. All seed produced at Jamie L. Whitten Plant Materials Center (Coffeeville, MS). (upland switchgrass, little bluestem, purpletop, and beaked panicum) prior to stratification. Seed were bulked from each plant and a representative sub-sample was taken to conduct the germination test. There were six replications of 100 seed tested for each population. Percentages represent 14-day totals.

	Cycle of Selection		
Species	Cycle 0	Cycle 1	Cycle 2
	Germination Percentage		
Upland Switchgrass	0.7 a [†]	4.0 b	(4.0 b) *
Little Bluestem	1.3 a	1.7 ab	2.7 b
Purpletop	1.2 a	(1.2 a)**	15.2 b
Beaked Panicum	0		

[†] Comparisons made between years within a single cycle of selection (within a row). Values followed by the same letter are not significantly different at the 0.05 level of probability

* Advanced generation of Cycle 2 was pooled with Cycle 1 when clones were moved to Starkville

** Advanced generation of Cycle 1 was pooled with Cycle 0 when clones were moved to Starkville together and seedling selections were made for a new C2 at Starkville. This failure to progress is believed to be the result of contaminating pollen from native stands of LBS on the prairie region (plants were derived form this region to obtain C0) at the PMC at Coffeeville, MS (Figure 2).

The staff at Coffeeville made no attempt to protect their crossing blocks from pollen contamination in the first years of selection. During the second year they surrounded crossing blocks with sorghum/sudangrass to reduce pollen contamination and PSG progress was observed. Clones of all LBS cycles have been moved to Starkville, MS. It is interesting to note that the C0 population (300) had 22 individuals which were prostrate when mature (a subspecies of LBS). These phenotypes were moved with other clones to Starkville in 2005. In the 2 cycles of selection for PSG that have occurred at Starkville the prostrate phenotype is increasing in the percentage of the total block. This phenotype is undesirable for conservation purposes and re-selection will have to take place to remove it.

Comparison of germination percentages between two cycles of selection in purpletop

After two cycles of selection, PT has shown the greatest improvement among the short stature grasses. The PSG in the original population (1.2%) has been improved to 15.2% in C2 (Table 10). This improvement comes after C1 failed to advance and showed a germination percentage of 1.2% (same as C0). In an effort to make improvements with fewer cycles of selection, plants from C0 and C1 at Coffeeville, MS were pooled together and make a new C0 in Starkville, MS. The rapid jump in PT advancement is reminiscent of IG in this study, and would seem to suggest the presence of self fertility alleles in the PT population. Like IG, PT is a species adapted to droughty conditions; which would limit time available for flowering as well as alternative pollen sources and therefore create a positive selection environment for self fertility alleles.

Evaluation of original population of beaked panicum

Evaluation of the original population (C0) of BP shows that many obstacles are to be overcome if progress is to be made with this species. Beaked panicum was the most recalcitrant species we have worked with to date. The characteristics of this species were consistent with information reported by Grabowski et al. (USDA, 2005) when they suggested slow advances would be expected in this species. These characteristics were limited seed production and slower growth. The original population of BP showed a mean PSG of 0.0% after 2 weeks (mean of 6 reps of 100 seed). This in itself is a sign that selection for advanced cycles of BP would be difficult. Mass screening of all the seed harvested from the C0 block (307g of seed) produced only 30 individuals.

Secondary Measure of Progress

As a secondary measure of progress, assessed on the mass screening of seedlings, an evaluation on the number of days it required to acquire one hundred seedlings which were to be used in the next cycle was conducted (Table 2). This measurement assessed by days, could also be used as a gross indicator of progress for reducing seed dormancy, and help determine how the population was changing with selection toward PSG. This comparison also allows a visual comparison between species and shows the recalcitrant nature of some species. Since 2005 was the first year of observation for this characteristic, there are no statistics, replication will have to be established through years.

The mass screening procedure provided visual confirmation that demonstrated substantial improvements in germination from C0 to C1 in six of the seven species (no comparable C1 for BP). This is negatively related to PSG for all species that showed progress i.e., if PSG advanced throughout cycles of selection, days to obtain 100 seedlings from the mass screening decreased with advancing cycles.

Lowland switchgrass:

The original population (C0) of LLSG required 3 screenings of 16 days (each of approximately 30,000 seed) for a total of 48 screening days to produce 100 seedlings (Table 2; Figure 3). Evaluation of C1 showed that only 6 days were needed to obtain seedlings for C2. This shows substantial progress in screening time. However, with significant progress in germination percent between C1 and C2, progress with days to obtain 100 seedlings was minimal (improving only 1 day). Similar results were seen for C3 and C4 of LLSG in that, there is advancement in cycles, but no progress towards reduced seed dormancy or rapid germination as progress to obtain 100 seedlings. Stalling of progress in the later cycles was not seen as uncommon. The same results were seen when screening LLSG for PSG when it appeared that LLSG required 4 days to germinate. When screening for PSG, no seedlings were seen at days 3 and 4. However, when seed were examined on day 5, hundreds had germinated in the later cycles, causing an effect that could be seen as stalling.

Indiangrass:

Progress was evident in all cycles of IG in that, days to acquire 100 seedlings decreased as cycles advanced (C0 - 2 screenings of 25 days for a total of 50 screening days, C1 - 16 days, C2 - 9 days, and C3 - 5 days). This progression shows that this species is, in fact, showing a positive response to PRS both as a measure of germination percent and days to germinate.

Big bluestem:

This method also allowed clarification of "stalled" progress. Progress was limited in later generations of LLSG. Germination percentages of these cycles varied, but the days to obtain the first 100 seedlings remained the same. However, selection for PSG in BBS was hindered because the overall germination rate (velocity of germination) declined. Substantial advancements were made between C0 (3 screenings of 28 days), C1 (20 days), and C2 (9 days), only to see C3 take 12 days.

Short stature species:

Due to limited cycles in the short stature species, this secondary measure may not be a good indicator of progress as actual late cycle PSG. Three of the four short stature grasses (ULSG, LBS, and PT) responded similarly. As cycles advanced within the species, days to acquire 100 seedlings decreased. While these species are very diverse taxonomically, all responded similarly to selection (except BP which has yet to undergo selection). Cycle 0 seed of all species reflected the taxonomic diversity in seed dormancy, but respond to selection by becoming more similar in C1 and tightly clustered in C2 with a range of five to nine days for all species. Only LBS, between C1 and C2, showed an increase in the number of days to obtain 100. After mass screening of seed of the original population of BP, only 19 seedlings emerged after 21 days from the first 30,000 seed that were screened. This process was repeated 4 times to obtain a total of 40 elite seedlings that were planted as C1 crossing block. Similar difficulties were observed during the early cycles of BBS, but not this severe. Carrying the test out for such a long time (5 x 21 days = 105 days) means screening continued until late May, substantially reducing selection pressure on the original population. This will affect the subsequent rate of success, but still enable selection.

Portrayal of this data in this manor should modulate the extreme variability seen with percentage germination because the seedlots used in the mass screening are, in some cases, greater than a 500 fold increase (30,000 vs 600) in the seedlot being tested. However, it does seem to have a four day floor, in that there is a minimum amount of time even the most non-dormant seed takes to germinate (Figure 3).

Evaluation of realized heritability (H_R) among tall stature grasses

Cycles of LLSG (C1-4), IG (C2-3), and ULSG (C1-2) were evaluated for heritability. For LLSG H_R between C1 and C2 was -0.22. A negative number was expected as efforts were focused on moving the population towards reduced seed dormancy. Comparisons of the other LLSG cycles showed lower values for H_R at -0.03 (C2 to C3) and -0.04 (C3 to C4). The lower values for later generations are explained in part by the observed PSG. With a substantial 18% change in PSG between C1 and C2 the H_R was -0.22. This large value is common especially in early cycles of selection as the population moves away from seed imposed dormancy. As the later cycles are examined, changes in germination percent are not as substantial between C2 and C3 where H_R was -0.03, and between C3 and C4 where H_R was -0.04. This is an observation of slower progress in moving the population further away from the original population, and thus, lower H_R values were expected.

When H_R in IG was calculated between C2 and C3, it was determined to be -0.01 and for ULSG a H_R of 0.0 was observed between C1 and C2. For ULSG a H_R of 0.0 is expected as there was no change in PSG between C2 and C3 (16.83% for both cycles).

In retrospect, we needed to record data from the seedlings of the mass screening. However, mass screening is only used for advancement of the most advanced cycle and these produce greater than 50 individuals (elite base population for the next cycle). Additional selection may result in a second substantial jump in H_R (and progress) if alleles were being accumulated on the various genomes. Once critical genome saturation is reached, continued crossing would assemble multiple alleles on the ploidy genomes together into the next generation. Because of strong self incompatibility (SI) systems (further strengthened by polyploidy) selection causes accumulation of desirable alleles to be limited, i.e. the individuals that have accumulated the non-dormancy alleles are limited in crossing during the next generation because of a degree of relationship. This can limit progress after the first generation (larger increase in C1, but limited in C2 and C3). The resulting effect saturates the various genomes with non-dormancy alleles sequentially (because SI alleles prevent the individuals with similar SI alleles from mating). After all the genomes have non-dormancy alleles built up and there is a thorough mix of SI alleles, there should be a large increase in germination and heritability.

Evaluation of Field Emergence Trial

Statistical analysis of the field emergence trial was calculated to compare establishment rates between LLSG seed in this breeding program to that of commercially available cultivars (Alamo, Cave-in-Rock, Kanlow, Shawnee, Trailblazer). Analyses were run on biweekly establishment ratings and a final number of plants per hectare at week 12.

Ratings over the 12 weeks of the test indicate that C3 is in the top statistical category for all 12 weeks evaluated (Table 11). Cultivars Cave-in-Rock and Trailblazer had similar final populations to C3 with 88,139 and 81,529 plants per hectare, respectively (Table 12) and had similar ratings throughout most of the test, except Cave-in-Rock at the two week rating. Cycles 2, 3, and 4 at the two week rating were also significantly better established than Cave-in-Rock, but only numerically better than Trailblazer. After comparison of the five commercially available cultivars with the three cycles of seed from this breeding program (C2, C3, C4), it was determined that C3 performed significantly better for number of plants established (Table 12), than the other two breeding lines and three of the commercial cultivars, with 116,785 plants per hectare. However, by week eight, both cultivars had become as well established as C3 and kept pace throughout the remainder of the12 week test period. Cycle 4, Shawnee, C2, and Alamo had significantly less plants per hectare (70,511; 67,206; 66,104; and 36,357, respectively) than C3. Alamo also had significantly less plants per hectare than Cave-in-Rock and Trailblazer. This was not seen as unexpected considering the results based on weekly ratings showed Alamo was significantly less than three of the other seven cultivars at week two and four. Kanlow, which performed poorest overall was significantly lower than six of the seven cultivars and cycles, with only 18,729 plants observed per hectare. Kanlow was also in the lowest statistical category for

Table 11. Field emergence trial - ratings by week (and t-grouping)

	Average Emergence Rating by Week*					
Cultivar	Week 2	Week 4	Week 6	Week 8	Week10	Week 12
Cycle 2	2.5 a [†]	2.5 abc	2.0 b	2.75 bc	2.75 b	2.5 bc
Cycle 3	2.0 ab	3.25 ab	2.75 ab	3.25 ab	3.25 ab	3.25 ab
Cycle 4	1.75 ab	1.75 c	2.5 bc	2.5 bc	2.75 b	3.0 abc
Shawnee	1.25 ab	3.75 a	4.0 a	4.25 a	3.75 ab	2.75 abc
Trailblazer	1.25 ab	2.25 bc	2.25 bc	3.0 abc	2.75 b	2.5 bc
Cave-in-Rock	1.0 b	3.25 ab	4.0 a	4.25 a	4.25 a	4.0 a
Alamo	1.0 b	1.5 c	1.25 c	1.0 d	1.25 c	2.0 bc
Kanlow	1.0 b	1.75 c	1.5 bc	1.75 cd	1.25 c	1.75 c

* Ratings displayed in this chart are an average rating of each cultivar or breeding line from the 4 reps in the test.

[†] Comparisons made between cycles. Values followed by the same letter are not significantly different at the 0.05 level of probability.

Table 12. Comparison of lowland switchgrass existing cultivars and breeding program seed for improved emergence.

Cultivar	Mean Number of Plants per Hectare
	12 Weeks
Cycle 2	163,342 bc [†]
Cycle 3	288,572 a
Cycle 4	174,232 bc
Shawnee	166,065 bc
Trailblazer	201,456 ab
Cave-in-Rock	217,790 ab
Alamo	89,837 cd
Kanlow	46,280 d

[†] Comparisons made between cycles and cultivars of LLSG. Values followed by the same letter are not significantly different at the 0.05 level of probability.

establishment ratings throughout the 12 week study.

Advanced cycles of LLSG from the breeding program outperformed or paralleled current readily available cultivars, which is considered a success, as the original population is wild material. However, C3 was significantly better than C4 for establishment as measured by plants per hectare. This may be attributed to the establishment date of the C3 crossing block as compared to the C4 crossing block. The maturity and development of C3 plants as compared to the more recently established C4 plants in this case seems to have an effect on seed and seedling establishment. If this proves true, C4 seed should perform better in the next field emergence trial. It should be noted that all breeding cycles were significantly better than the cultivar Kanlow, which is a recognized, and widely planted cultivar for the Southcentral United States. More importantly, C3 established better than Alamo, the premier cultivar for the South.

CHAPTER V

CONCLUSIONS

After all species were advanced by PRS for reduced seed dormancy, it was evident that LLSG made the most appreciable improvement. In working with these seven native species it is apparent why the biofuel/biomass initiatives focus on switchgrass, especially LLSG. Of all the species tested in this research, switchgrass lent itself to “domestication” from completely wild stocks most easily. The other tall grass species (BBS and IG), though no progress was made by Jones (2004) after one cycle of selection, showed significant improvement for reduced seed dormancy after three cycles of selection. With slow establishment and difficulties in handling of BBS and IG, results in the earlier cycles were minimal. However, as this researcher became more adapted to the conditions required by these species, progress towards improvement were more forth coming. Continued progress towards greater PSG is expected as all tall stature species undergo more cycles of selection.

The short stature species of this study were less responsive to PRS in part to constraints on the early cycles of selection. Limited germplasm, crossing block location, and environmental conditions seemed to work against our attempts at selection for reduced seed dormancy. Procedures to address these situations were taken with the expectations for a greater subsequent response to the breeding program. Upland switchgrass made the most improvement toward reduced seed dormancy after one cycle of selection. With an additional selection (C2), PT achieved a substantial advance in PSG. The other short stature species (LBS and BP) were less responsive to this breeding program due to the certain constraints.

It was also recognized that selection for quantitative traits such as reduced seed dormancy is a slow process, due not only to idiosyncracies of the particular species, but also the fact that there can only be one cycle of selection per year. The selection progress can be further hindered by several factors which may induce stalling of progress of the species, such as; loss of germplasm during the many procedures that are required before crossing blocks are established, or unfavorable or annually changing environmental conditions. These factors were evident in all species at different stages of the program.

When this study was compared with previous research, it was evident that some of the species in this breeding program outperformed those evaluated by other researchers from the standpoint of cycles of selection and days to achieve maximum germination. This is true when compared with blue panicgrass evaluated by Wright (1978), in which he observed that after six cycles of selection, five and a half days were required to generate a maximum germination rate of 30 percent. This study was able to achieve 34.5 mean PSG in LLSG after four cycles in 14 days. Schaaf and Rogler (1960), who studied *Stipa* (needlegrass) were able to achieve a high rate of germination in 18 days after seven cycles when they observed an increase from 42% (C0) to 82% (C7). However, needlegrass is a self pollinated species and producing a preponderance of low seed dormancy progeny would be expected to cause a rapid reduction in seed dormancy. Although Schaaf and Rogler were able to make dramatic increases in reducing seed dormancy, we believe that if the mode of reproduction of the species in this study were parallel with needlegrass (self pollinated) that advancement of our species would have been similar if not substantially greater. However, if that were the case the area of adaptation of these species would have greatly reduced. Working with these species is a tradeoff, in that the extensive cross pollinated nature of these species provides greater genetic variability which, in turn, provides for more extensive adaptability. However, this increased variability slows progress for selecting such traits as reduced seed dormancy.

With the knowledge obtained from this program, additional research in reducing seed dormancy remains important for development of commercial cultivars for all native grass species. Development of these cultivars would be applicable in many markets, including biofuels, restoration initiatives, habitat development, wildlife enhancement, and forage production.

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