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Selection and Breeding to Improve Commercial Germplasm and Increase Germination Percentage of Eastern Gamagrass [Tripsacum Dactyloides (L.) L.]

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Selection and breeding to improve commercial germplasm and increase germination percentage of eastern gamagrass [*Tripsacum dactyloides* (L.) L.]

By

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Perennial warm-season grasses constitute the backbone of many forage production systems, whether for grazing or harvested feed. North American native plants, specifically grasses, forbs and legumes offer unique ecosystem benefits along with forage quality and digestibility that are unmatched by introduced species. The disparity in breeding and research focused on improvement of introduced species as opposed to native genera has led to inflated use of introduced species as forage types in lieu of native options, due to their unimproved nature. Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is proven to be a widely adapted, highly productive forage species in the southeast, Great Plains and northeast United States. A major limitation to more widespread use of eastern gamagrass is high seed dormancy, which leads to increased seed cost. Here, research used recurrent phenotypic selection breeding methods to reduce seed dormancy, with the ultimate goal of developing a population of individuals that produce non-dormant eastern gamagrass seed.

DEDICATION

This is dedicated to my Grandmother, Vera. She wouldn't have been very interested in anything on these pages, but she would have listened intently.

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CHAPTER I

INTRODUCTION

The term native warm-season grass (NWSG) refers to a group of grasses – and occasionally forbs – that are historically native to the Great Plains states and most of North America, from California to the Southeast (Hamrick et al., 2007). This group is comprised of annual and perennial plants that grow during the late spring, through the warm months of the year, initiate jointing in the late summer, and typically go dormant through most of the fall and winter (Harper et al., 2004). Many of the native grasslands that existed in North America centuries ago were eventually developed as cultivated cropland or pasture for livestock, while native plants were replaced with exotic grasses in favor of their higher forage quality, ease of establishment and ability to withstand heavy grazing (Hamrick et al., 2007).

Despite lower nutritional value and slower rate of regrowth when compared to exotic species, native warm-season grasses have seen a resurgence in popularity over the last 25 years for use as forage and hay crops, wildlife habitat and in land reclamation sites. Another and perhaps larger interest in NWSG is in the field of renewable energy, in the form of cellulosic biofuel production and co-firing. While switchgrass [*Panicum virgatum* L. (Nash)] has become by far the most popular NWSG for consideration in the renewable energy forum, improved cultivars of eastern gamagrass [*Tripsacum dactyloides* (L.) L.], little bluestem [*Schizachyrium scoparium* (Michx.) (Nash)], big

bluestem [*Andropogon gerardii* (Vitman)] and indiangrass [*Sorghastrum nutans* (L.)(Nash)] have shown promise as feedstocks for renewable energy production as well as productive forage crops and high quality wildlife habitat. It is widely reported however, that further study is needed to optimize the commercial cellulosic fermentation process in order for NWSG to become economically viable sources of fuel for cellulosic ethanol production (Vogel and Masters, 1998; McLaughlin et al., 1999; Bouton, 2002; Anderson et al., 2008; Lemus and Parrish, 2009).

While NWSG are believed to have been growing on the central and southeastern plains of the United States since the Holocene era (between 11,500 and 2,000 years BP) (Casler, 2012), modern production of many species has been slow to evolve due to inherent dormancy characteristics that hinder germination and establishment. Modern research has shown that several species require long periods of cold or cold and moist stratification to break primary dormancy of seeds before they are planted. Chemical and mechanical scarification methods are also used to improve germination of seeds that exhibit physical or mechanical dormancy. Chemical treatments have included: strong acids and bases, reactive oxygen species (ROS), hot water, smoke, etc. (Geng and Barnett, 1969; Emal and Conrad, 1972; Fulbright, 1988; Jensen and Boe, 1991; Shen et al., 2001; Sarath et al., 2006; Sarath et al., 2007; Sarath and Mitchell, 2008; Huarte and Garcia, 2009).

While several studies have been published citing increases in germination and establishment of a variety of native warm-season grasses by utilizing chemical, mechanical or other laboratory treatment methods, gains in germination percentage when studying eastern gamagrass have been wholly insignificant and varied (Finneseth, 2010).

The need for improved breeding methods with increased selection pressure for seed quality and increased germination percentage is evident in many native warm-season crops, especially in the case of eastern gamagrass. This goal can be accomplished through establishment and continuous development of breeding populations.

Eastern gamagrass is a perennial bunch-type grass, with a habitat ranging geographically from the northern United States to as far south as Mexico (believed to be the center of origin), Central and South America (Aberle et al., 2003) and the Caribbean Islands (Springer et al., 2001). Gamagrass is a very productive, high quality and highly palatable forage, (Springer et al., 2001) that thrives in conditions where annual precipitation exceeds 89 cm (Dewald et al., 2006). Eastern gamagrass is commonly found in the diploid (2n=36) and tetraploid (2n=72) forms. Diploid cytotypes are the only sexual and reliably cross-pollinated types of eastern gamagrass, while there are many native and commercial cytotypes [triploid (2n=54), tetraploid (2n=72), pentaploid (2n=90) and hexaploid (2n=108)], that are predominantly apomictic (Farquharson, 1955; Burson et al., 1990; LeBlanc et al., 1995b). Gamagrass is monoclinous monoecious, with male and female flowers separated on the same raceme. The males compose the distal ¾ of the raceme, while the proximal ¼ of the raceme is made up of female flowers. Hybrids have been developed recently by utilizing a rare, genetically recessive gynomonoecious mutant, which produces female flowers on the proximal ¼ of the raceme and perfect flowers on the distal ¾ (Burson et al., 1990; Sherman et al., 1991; Dewald and Kindiger, 1994; Dewald and Kindiger, 1996; Kindiger and Dewald, 1997b).

The potential exists for utilizing corn [*Zea mays* (L.) subsp. *mays*] genetics to alter seed production morphology and improve germination and establishment (overcome

physical dormancy characteristics) in eastern gamagrass. Corn (the only domesticated taxon in the *Zea* genus) is a prolific seed producer and often exhibits no seed dormancy. Hybridization of corn and diploid eastern gamagrass, followed by backcrossing to diploid eastern gamagrass and subsequent selection for precocious germination (germination without need for stratification), increased seed production and forage quality could drastically increase the utility of eastern gamagrass as a forage crop. Corn and eastern gamagrass are both relatives of the ancestral *Zea* species 'teosinte'. Previous research indicates a strong likelihood of success in attempting to hybridize corn and eastern gamagrass (Kindiger and Beckett, 1990; Leblanc et al., 2009).

CHAPTER II

OBJECTIVES

Manipulation of inherent reproductive versatility in eastern gamagrass is vital to progressive domestication of the species. To achieve this goal, the proposed research will have the following main objectives: 1) build a breeding population of diverse eastern gamagrass individuals collected from across the southeast; 2) evaluate collection for forage quality characteristics and confirm ploidy level of desirable individuals using flow cytometry; 3) screen diploid commercial and wild-type eastern gamagrass germplasm for presence of seed that germinate without prior scarification or cold, moist stratification; 4) use classical breeding (phenotypic recurrent selection) to reduce seed dormancy in eastern gamagrass; 5) investigate timing of imbibition and effects of drying on seed germination using multiple cultivars.

CHAPTER III

LITERATURE REVIEW

Current Perspectives and Uses for North American Native Warm-Season Grass Species

Native warm-season grasses have received much attention over the last 25 years for their ability to persist under hot, dry conditions and thrive in marginal soils (Smith et al., 2009). With increased research in genetics and breeding, many species have shown promise for use as forage and hay crops, wildlife habitat and in land reclamation sites. Another (and perhaps larger) interest in NWSG has come from the bio-fuels/cellulosic ethanol market. Research shows that switchgrass [*Panicum virgatum* (L.) Nash.] and the warm-season Asian grass giant miscanthus [*Miscanthus* x *giganteus* (Keng)] have promise as fuel stock for cellulosic ethanol production (Lemus and Parrish, 2009). While these grasses have performed well in research trials, further improvement is needed to perfect the process of cellulosic ethanol fermentation before the industry can become economically sustainable. Further study is also needed in the area of germination and establishment of North American NWSG, as production of many species has been slow to evolve due to difficulties with embryo dormancy and the necessity for pre-planting manipulation of the seed coat, awns or glumes (Springer, 2005; Klein et al., 2007).

All NWSG do not exhibit the same types or levels of dormancy, nor do they maintain their dormancies with the same severity. Traditionally, after-ripening (dry storage of freshly harvested, mature seeds at room temperature for a period of several

months) is used to break primary dormancy and to promote germination in many grass species (Bewley, 1997; Leubner-Metzger, 2003; Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006). In laboratory tests, after-ripened seed display increased vigor and germination percentage when compared to freshly harvested seed, as well as an ability to germinate under a wider range of temperatures (Leubner-Metzger, 2005). This period of after-ripening is also reported to affect levels of and sensitivity to endogenous abscisic acid (ABA), increase gibberellic acid (GA) sensitivity and increase sensitivity and response to light treatment (Leubner-Metzger, 2002).

Eastern Gamagrass

History and Distribution

Eastern gamagrass (also called Fakahatchee grass and calamusgrass) is a warm-season, perennial, C4, bunch-type grass, native only to the Western Hemisphere (Polk and Adcock, 1964; Kindiger and Dewald, 1997b). The habitat for eastern gamagrass ranges geographically from the northern United States (generally no further north than the 40th latitude) to as far south as Mexico, Central and South America (Aberle et al., 2003) and the Caribbean Islands (Dewald, 2001; Springer et al., 2001). The *Tripsacum* genus is comprised of 15 species in two sections (Kindiger and Dewald, 1997b). A relative of corn [*Zea mays* (L.)] (tribe Andropogoneae, subtribe Tripsacinae, family Poaceae), gamagrass is a very productive, high quality and highly palatable forage, (Springer et al., 2001) that thrives in conditions where annual precipitation exceeds 89 cm (Dewald et al., 1996; Ball et al., 2002; USDA, 2007b). The exact path that led from ancestral *Tripsacum* genetics to modern maize is still unknown and highly debated, but

agreement can be reached on the impact of the role that human selection must have played (Dewald and Sims, 1990).

Many of the current commercial releases of conservation and forage-type eastern gamagrass are derived from wild populations in the Midwest (Oklahoma, Kansas, Arkansas, Missouri, and Texas). Sales of these cultivars are recommended for most of the southern Great Plains and southeastern states, including: Alabama, Arkansas, Florida, Georgia, Iowa, Illinois, Indiana, Kansas, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Nebraska, New Mexico, Ohio, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and West Virginia; as well as Maryland, New York, Pennsylvania and Washington (USDA, 2007b).

Uses

While eastern gamagrass is used in conservation plantings as well as in ornamental settings, it is most popularly utilized as a forage crop; suitable for green chop, hay, and silage or intensive pasture grazing. When compared to other NWSG, gamagrass often begins spring growth earlier in the year and recovers more rapidly (regrowth rates of up to 5 cm day^{-1}) following harvesting or defoliation (USDA, 2007b; Goldman and Springer, 2011). Gamagrass offers benefits for wildlife habitat and ornamental purposes, but is not recommended for use in mixed-species wildlife habitat plantings (Surrency et al., 1999), thus, its use as a livestock forage alone make it vital to the southern United States (Springer et al., 2001).

Forage, Silage and Hay

The successful introduction and use of most native grasses in livestock or pasture based production systems requires flexibility of use. The ability to graze, mow, cut and bale, or chop and store for later use is vital to the production of high-quality forage. Gamagrass is highly productive when intensively managed for pasture, hay and silage. A high leaf-to-stem ratio and highly digestible reproductive tillers make gamagrass evenly digestible throughout most of the growing season (USDA, 2007b). It is common for crude protein (CP) percentages of above-ground vegetation to exceed 12% up to the early reproductive stage of growth. With adequate nitrogen fertilizer, eastern gamagrass cut for hay at regular intervals maintains high quality across the growing season and CP levels can exceed 20%. In Mississippi, Missouri, New York and Oklahoma, percent CP for gamagrass hay commonly ranges from 7.0 – 14.4, 10.6 – 14.4, 10.0 – 16.4 and 10.3 – 13.7, respectively (Brejda et al., 1996; Douglas et al., 2002; Gillen et al., 2006; USDA, 2007b).

The high palatability of gamagrass often leads to overgrazing and stand loss in poor management (continuous grazing) scenarios. This preferential grazing by livestock is what many believe led to the initial decline of natural stands of eastern gamagrass (Rechenthin, 1951). Recommendations for grazing/harvesting suggest a minimum stubble height of 15-20 cm and rest periods of 30-45 days in order to maintain stand health and produce adequate regrowth (Edwards et al., 1999; USDA, 2007b; Goldman and Springer, 2011). Multiple harvests per season often produce higher seasonal yields and increased forage quality values than single harvest systems. In the years 2000 – 2002, a study at Coffeeville, MS reported mean yields of 14.8, 14.7 and 12.2 Mg ha^{-1},

respectively, of 'Highlander' eastern gamagrass (an apomictic tetraploid cultivar collected in northern Montgomery County, TN and developed in the Coffeeville region) when managed in a two-cut system (Table 3.1). These yields were significantly higher than their single-cut counterparts, which produced mean seasonal yields of 8.7, 12.8 and 7.8 Mg ha^{-1}, respectively (Grabowski et al., 2004).

Table 3.1 Comparison of harvest frequency effect on annual yield of eastern gamagrass in Coffeeville, Mississippi.

	Year			
	2000	2001	2002	Mean
	----------Dry matter forage (Mg ha^{-1})----------			
One-cut	8.7[†b]	12.8	7.8[b]	9.7
Two-cut	14.8[a]	14.7	12.2[a]	13.9

Harvests were conducted on the following dates: One-cut – 2000 (Sep. 14) 2001 (Aug. 8) 2002(Aug. 6); Two-cut – 2000 (Jun. 13, Sep. 14); 2001 (Jun. 18, Sep. 12); 2002 (Jun. 19, Sep. 9).
[†] Values with same letter within each year are not significantly different at $\alpha = 0.05$. (Adapted from Grabowski et al., 2004).

In a three-year study across Texas and the southeast, yields of Highlander and 'Jackson' (an apomictic tetraploid variety selected from wild-types in Jackson County, TX, for forage mass, persistence, seed production and vigor) eastern gamagrass were reported as high as 19.0 Mg ha^{-1} (Table 3.2), while in a separate study in three locations in Mississippi, maximum dry matter yields of Highlander, Jackson and 'Pete' (a sexual diploid developed in Manhattan, KS) were 21.5, 18.1 and 26.6 Mg ha^{-1}, respectively (Grabowski et al., 2003) (Table 3.3).

Table 3.2 Comparison of eastern gamagrass cultivars Highlander and Jackson for average annual forage yield across three years, at six locations.

	Location (State symbol)*					
Cultivar	AR	FL	GA	MS	East TX	West TX
	----------Dry matter forage (Mg ha^{-1})----------					
Highlander	14.3[†]a	7.6a	19.0a	18.1	12.7a	11.2
Jackson	7.84b	3.1a	19.0a	12.3[‡]	14.5a	[§]

[*] Booneville, AR; Brooksville, FL; Americus, GA; Coffeeville, MS; Nacogdoches, TX (east); Knox City, TX (west).
[†] Cultivar comparisons for average annual yield within a location followed by the same letter did not differ at $\alpha < 0.05$.
[‡] Plants of the cultivar Jackson were not harvested during the 3rd year of the study due to disease.
[§]Indicates that plants winterkilled during the first winter.
(Adapted from: Grabowski et al., 2003).

Table 3.3 Comparison of eastern gamagrass cultivars Highlander Jackson and Pete for mean annual forage yield at three locations in Mississippi.

	Location		
Cultivar	Coffeeville	Prairie	Raymond
	----------Dry matter forage (Mg ha^{-1})----------		
Highlander	13.8[†]	15.2ab	21.5
Jackson[‡]	12.3	17.4a	18.1
Pete	10.9	12.0b	26.6

[†] Cultivar comparisons for average annual yield within a location followed by the same letter did not differ at $P < 0.05$.
[‡] None of the plants of cultivar Jackson survived at Coffeeville after 2001. These plants showed signs of damage from Pythium ssp. and Rhizoctonia ssp. damage.
(Adapted from: Grabowski et al., 2003).

Reports of increased average daily gains for steers grazing eastern gamagrass in comparison to other popular forages (flaccidgrass [*Pennisetum flaccidum*], bermudagrass [*Cynodon dactylon*], tall fescue [*Schedonorus phoenix* (Scop.) Holub]) have been reported across the southeastern United States (Burns et al., 1992; Aiken, 1997; Edwards et al., 1999; Pingel, 1999; Burns and Fisher, 2000; USDA, 2007a).

It has been reported that gamagrass produces a suitable crop for silage production, and normally ensiles well, with pH ranges from 4.0 – 4.6. However, when compared to the same forage stored as hay, gamagrass chopped and preserved as silage has led to varying results in overall forage quality, dry matter digestibility and daily dry matter intake in beef steers and dairy cows (Eun et al., 2003; Burns and Fisher, 2006). When compared to corn silage, the lack of large amounts of grain in a gamagrass silage crop leads to lower levels of total digestible energy and higher values for acid detergent fiber (ADF) and neutral detergent fiber (NDF), forage quality parameters popularly used as indicators of digestibility and intake, respectively.

Ornamental and Wildlife

Native ornamental plantings (ornamental landscaping where native plants are intentionally included in abundance) have been shown to increase species richness, abundance and breeding pairs of avian and lepidopteran species over more conventional non-native mixtures (Burghardt et al., 2008). While many eastern gamagrass cultivars that have been developed and released for forage and conservation use also compliment wildlife habitats, ornamental cultivars such as the Florida ecotypes St. Lucie and Martin have been selected for aesthetic characteristics such as color, morphology, drought tolerance and strong perennial nature (Maura and Pfaff, 2006).

Because of its aggressive growth habit and tendency to form large bunches, eastern gamagrass is not generally recommended for mixed-species plantings for wildlife habitat (Surrency et al., 1999). Seed and vegetation of eastern gamagrass provide food and shelter for wildlife, as well as nesting habitat for upland wildlife such as bobwhite

quail [*Colinus virginianus*] and pheasant [*Phasianus colchicus*] (Krizek and Ritchie, 1999; USDA, 2007b; Zoller, 2011).

Nutrient Management

Native grasses have been utilized in field edges and areas of steep topographic decline to increase water infiltration and reduce fertilizer, sediment and chemical runoff. These sections of vegetation (often monoculture) are commonly referred to as vegetative filter strips (VFS) (Krutz et al., 2005). Eastern gamagrass proves useful in VFS thanks mostly to its stable root system and elevated crown (USDA, 2007b). In northern Mississippi, eastern gamagrass and switchgrass were evaluated for their ability to slow moving water and filter out sediment in series of one-, two-, three- and four-row VFS. The study found that switchgrass was more effective at removing sediment than eastern gamagrass. It was also reported that three- and four-row series of VFS were no more effective at removing sediment than a two-row series for both species (Becker, 2001). Foy (1997) found that eastern gamagrass was capable of surviving in acidic (aluminum toxic) Tatum-type subsoil, as well as in aluminum-rich nutrient solutions that prove lethal to most crop species. Gamagrass maintains a relatively high N/P uptake ratio, reported by McLaughlin et al. (2004) to be as high as 9.3 over a 3-year study, higher than 'Common' and 'Coastal' bermudagrass, johnsongrass [*Sorghum halepense* (Pers.)], 'Lometa' indiangrass and 'Alamo' switchgrass.

Biomass

Biofuels, bioenergy crops, biomass crops, and fuel crops are all terms used to designate crops with potential to produce energy in the form of transportation fuel or

electricity (Finneseth, 2010). Ethanol (CH_3CH_2OH; syn. EtOH) specifically, is easily produced from grains and roots high in starch, sugar and other fermentable carbohydrates. Popular ethanol crops to date include: grains (barley [*Hordeum vulgare* (L.)], wheat [*Triticum aestivum* (L.)], maize, rice [*Oryza sativa* (L.)], and sweet sorghum [*Sorghum vulgare* (L.)]), tubers (potato [*Solanum tuberosum* (L.)], sweetpotato [*Ipomea batatas* (L.) Lam.], cassava [*Manihot esculenta* (Crantz)], Jerusalem artichoke [*Helianthus tuberosus* (L.)], and sugar beet [*Beta vulgaris* (L.)]), sugarcane [*Saccharum officinarum* (L.)], fruits, and molasses. The process of converting starches to sugars via simple fermentation and distilling alcohol from the resultant product is centuries old, thus fuels derived from these sources are commonly referred to as 'first-generation' (Lemus and Parrish, 2009).

Native and non-native warm-season perennial grasses have also been popularized for this use via alternative forms of fermentation using specific enzymes, heat, catalysts or acids to derive simple sugars from plant cell constituents (Finneseth, 2010). Switchgrass, indiangrass, big bluestem, eastern gamagrass, kenaf [*Hibiscus cannabinus* (L.)], cotton [*Gossypium hirsutum* (L.)], giant miscanthus, and silphium [*Silphium asteriscus* (L.)] are all well suited to this production scheme.

The process of cellulosic fermentation is energy intensive and the net energetic and economic feasibilities of production have been highly debated, for review see Demirbas (2007). Long-term fossil fuel resources are becoming scarcer, while market supply and prices for fossil fuels are in continual flux. As such, the system of renewable energy production is being pushed to evolve at a greater rate. Economic feasibility of cellulosic ethanol production relies greatly on level of crop production, cost of crop

inputs (specifically nitrogen fertilizer and seed), efficiency of conversion/processing and costs associated with harvest and transport of cellulosic feedstock. When expressed in energy-profit ratios (amount of energy contained in processed fuel/amount of energy required to produce, transport, process and convert crops into fuel), Nelson et al. (1994) reported that ratios for polyculture big bluestem/indiangrass and monoculture eastern gamagrass ranged from 2.57 – 3.23 and 2.31 – 3.21, respectively, depending on crop yield, nitrogen fertilizer rate and frequency of application.

Biomass production of native warm-season perennial grasses is influenced by environment (precipitation, soil characteristics and pathogen abundance), cultivar and seed quality; increased biomass production is key to increasing production of fuel on a per unit area basis (Piper, 1998; Tober et al., 2008). This benefits crops that are capable of producing large amounts of aboveground biomass with minimal inputs and management. Tilman et al. (2006) reported that, when compared to monoculture crops (including corn) low-input polyculture crops of up to 16 native species produced significantly more energy (68.1 GJ ha^{-1} $year^{-1}$) and increased CO_2 sequestration.

Eastern gamagrass has little value in applications where plant material is used for direct combustion in small-scale boilers. Under optimum rainfall and fertilization, eastern gamagrass typically contains 2.24% nitrogen (N), 0.27% phosphorus (P) and 2.06% potassium (K) on a dry weight basis (Springer et al., 2003). High levels of potassium in standing vegetation cause slagging (the formation of molten or partially fused deposits on furnace walls or convection surfaces exposed to radiant heat). Overwintering standing vegetation in situ and harvesting in the spring allows natural leaching of potassium and other minerals from plant material, decreasing ash content as

well. Switchgrass, which remains erect during winter months, fits this purpose well, whereas eastern gamagrass which does not remain erect for the duration of most winter months and undergoes serious decay in the process, is less suitable (Staver, 2002).

Phytoremediation

As is common in reparation and decontamination of areas introduced to biological, metallic, chemical, or, most common in waterways, petroleum-based contaminants, bioremediation, or the use of biological processes to speed up the dissipation or catabolism of contaminants is relied on heavily. Phytoremediation (syn. phytoextraction, rhizofiltration) or, the use of higher-order plants – including forbs, grasses and woody species – to contain, neutralize, or increase the rate of natural degradation of contaminants in an ecosystem, is also a very popular topic in forage and grassland research (Hinchman et al., 1996; Euliss et al., 2007; Ryszka and Turnau, 2007; Nedunuri et al., 2009).

The effectiveness and feasibility of certain plants for phytoremediation of various toxic or non-toxic contaminants has been studied at length with species such as: switchgrass, canola [*Brassica napus* L.], indiangrass, broomssedge [*Andropogon virginicus* L.], arrowhead [*Sagittaria* sp.], gamagrass, willow [*Salix* sp.], Chinese cabbage [*Brassica rapa* L.], poplar [*Populus* sp.], kenaf, eastern redbud [*Cercis Canadensis* L.], Italian ryegrass [*Lolium perenne* L. subsp. m*ultiflorum* (Lam.) Husnot], orchardgrass [*Dactylis glomerata* L.] and tall fescue. Many of these species are utilized specifically to target areas where heavy metal contamination is an issue and the plant's natural affinity for metal hyperaccumulation is desirable (Hetrick et al., 1994; Trafas, 1996; Schat and Verkleij, 1998; Euliss et al., 2007).

In greenhouse studies, eastern gamagrass has been shown to decrease total petroleum hydrocarbons (approximately 70% reduction after one year) and residual pyrene concentrations in highly contaminated samples of lake sediment, although field trials did not result in the same findings, likely due to continuous contamination from a nearby waste-water source (Euliss et al., 2007).

Morphology

Mean height commonly ranges from 1.2 to 2.4 m, with flat leaves displaying a prominent midrib and rough margins, often 0.3 to 0.7 m in length and 1 to 3 cm in width (USDA, 2007b). Reproductive tillers typically exhibit a thick, flat base with a purplish color (Lemke et al., 2003). Mature crowns can grow to >1.5 m in diameter and, after 3-4 years begin to show signs of decline in the middle of the crown known as center dieback (Dewald and Louthan, 1979; Wright et al., 1983, Springer et al., 2003).

Young plants exhibit thick, flat stem bases that develop into a fan-shaped conformation, usually within 60-90 days after emergence. Similar to timothy [*Phleum pratense* (L.)] and blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], during expansion, eastern gamagrass develops stem base or crown structures known as proaxes. Proaxes are thick, knotty constructions comprised of basal phytomers with very short or non-elongating internodes that develop true leaves. A basal phytomer consists of a leaf, internode, axillary bud and a node (Dewald and Louthan, 1979).

Eastern gamagrass roots contain aerenchyma tissue (air spaces) that allow for gas exchange between the root and the shoot, even in anaerobic (flooded) conditions. Studies have shown that aerenchyma formation in eastern gamagrass roots is not a facultative response to oxygen (O_2) depravation, but rather an exploitable phenotypic characteristic

(Ray et al., 1999; Jatimliansky et al., 2004). Roots of long-lived stands of eastern gamagrass have shown excellent claypan penetrating ability, and have been reported to colonize with arbuscular mycorrhiza (AM) at depths > 120 cm in a variety of soil types (Clark et al., 1998). Plant root association with AM is believed to increase ability of plants to withstand water stress and increase nutrient uptake, specifically phosphorus (Bentivenga and Hetrick, 1992; Marschner and Dell, 1994).

Studies in cytological and morphological variability in wild-type eastern gamagrass have continually shown that populations of the species display wide phenotypic variation and that individual populations possess unique morphological characteristics, regardless of cytological attributes (Newell and de Wet, 1974). Dunfield (1988) reported that among 83 accessions (37 diploid, 46 tetraploid) collected from 30 northwest Texas counties, distinctions between diploid and tetraploid individuals could be made from visual estimation of leaf color, leaf width, date of spring green-up and date of flowering. Leaf width varied from 0.6-1.6 cm in diploid individuals and from 1.7-2.8 cm in tetraploid populations. Diploid individuals also consistently exhibited rolled leaves when moisture stressed. Upon visual evaluation of populations using a Munsell Color Chart for plant tissue (Munsell Color, X-Rite Inc., Grand Rapids, MI), diploid individuals appeared to have a darker green "bluish" leaf color (hue = 7.5 GY, value range 4 – 5, chroma = 4) while tetraploid individuals consistently exhibited a "green" leaf color (hue = 5 GY, value range 4 – 5, chroma range 4 – 6). Field transplanted diploid individuals generally began spring growth earlier and initiated flowering earlier than tetraploid individuals. The author of this document found these morphological characterizations to be inconsistent in wild-type and commercial germplasm collections.

Flower Structure

Similar to corn, gamagrass is monoecious; however, the two differ in that gamagrass is monoclinous, with male and female flowers found on the same raceme. The males compose the distal ¾ of the raceme, while the female flowers make up the proximal ¼. Eastern gamagrass is most often found in the diploid (2n=36) and tetraploid (2n=72) forms (Farquharson, 1955). Diploid populations reproduce sexually via cross-pollination, while polyploid individuals are predominantly, if not entirely apomictic (Burson et al., 1989; Burson et al., 1990; Leblanc et al., 1995a). Hybrids have been developed recently by utilizing a rare, genetically recessive gynomonoecious mutant (Dewald and Kindiger, 1994). The gynomonoecious form exhibits a spike with female flowers on the proximal ¼ of the raceme and perfect flowers on the distal ¾ (Burson et al., 1990; Orr et al., 2001).

Reproduction, Seed Head Formation and Seed Production

Gamagrass reproduces sexually as well as vegetatively from a thick, scaly rhizome-like construction known as a proaxis, which, along with the tiller base functions to store reserve carbohydrates (Jackson and Dewald, 1994). In the southern and central United States, flowering is normally initiated within a 20-day period in May. Flowering begins with the emergence of a single terminal inflorescence, followed 10-14 days later by a number (usually between one and four) of lateral inflorescences (Jackson and Dewald, 1994; USDA, 2007a; USDA, 2007b). This temporal division of seed maturity makes harvesting of quality seed very difficult to manage.

In northeast Texas, diploid populations have been found to begin spring growth and initiate flowering earlier than tetraploid populations (Dunfield, 1986). Eastern

gamagrass produces seed that is defined as a cupulate fruitcase (Galinat, 1956; Galinat and Craighead, 1964; Anderson, 1985). This structure (Figures 3.1 and 3.2) is an embryo protectively enclosed between the lemma, palea, hardened outer glume and hardened rachis internode of the spikelet (Hitchcock and Chase, 1950; Rogis et al., 2004a). The hardened cupule is also evident in other species of *Tripsacum* as well as teosinte (Dewald and Sims, 1990).

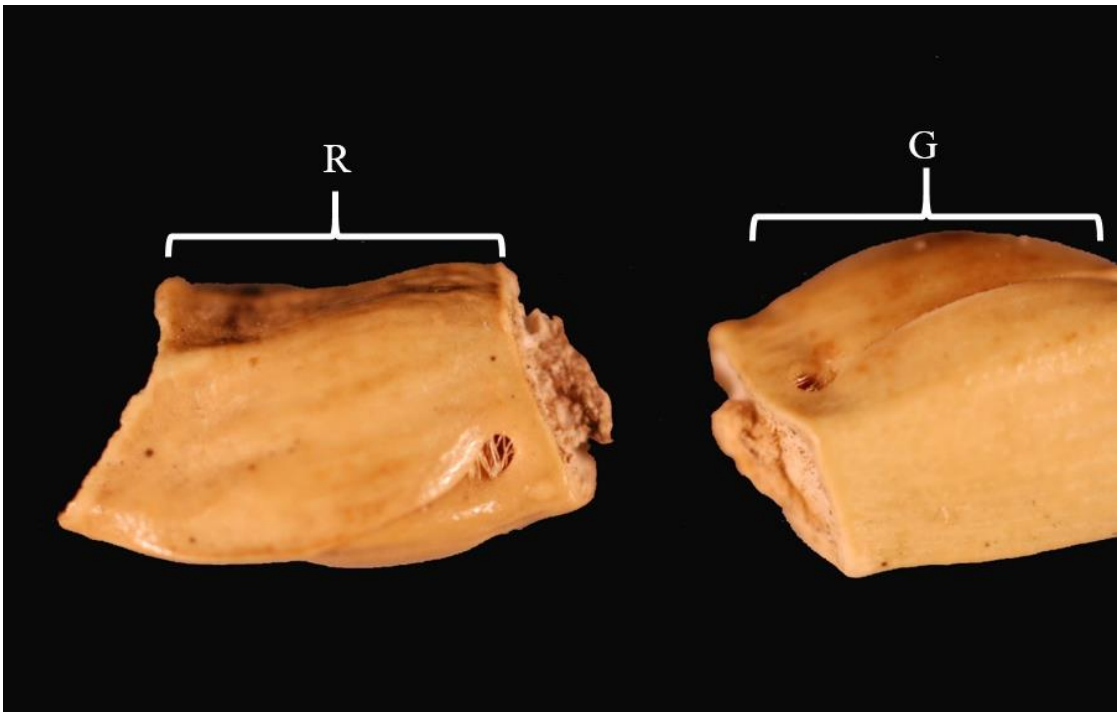

Figure 3.1 Pistillate eastern gamagrass [*Tripsacum dactyloides* (L.) L.] seed units. Labels: R, rachis; G, glume; (Courtesy of Dr. Alan Taylor: Cornell University; Geneva, NY)

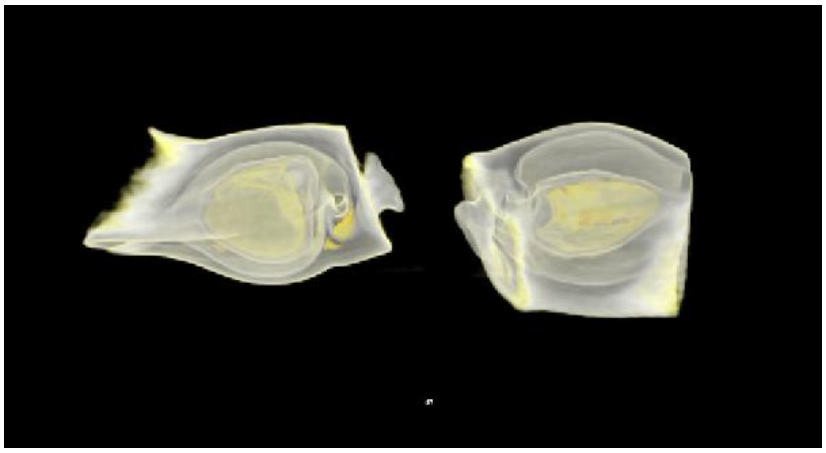

Figure 3.2 Pistillate eastern gamagrass [*Tripsacum dactyloides* (L.) L.] seed units scanned with x-ray Computed Tomography (CT) scanner (GE™ eXplore CT 120™ pre-clinical x-ray CT scanner: General Electric Company; Fiarfield, CT)

(Courtesy of Dr. Alan Taylor: Cornell University; Geneva, NY)

Seed Quality Issues

Along with the risk of overgrazing, other major factors affecting the popularity and widespread use of eastern gamagrass include its establishment difficulty and low seed production (Lemke et al., 2003). Several techniques for improved production of eastern gamagrass have been reported (Anderson, 1985; Kindiger, 1994; Mueller et al., 2000; Aberle et al., 2003), however, few address the issue of improving seed production. Eastern gamagrass matures slowly, often yielding seed only after the second or third season of production. Reproductive growth is apparently indeterminate, with floral initiation occurring over a long period (Jackson and Dewald, 1994; Lemke et al., 2003). Reproductive tillers typically initiate a single terminal inflorescence early in the spring season. This primary seed head is followed shortly (10 – 14 days) by secondary lateral inflorescences that originate from the axillary nodes (Lemke et al., 2003). Primary inflorescences typically reach heights of 1 – 3 m, while secondary inflorescences exhibit

a more prostrate confirmation and extend into the surrounding understory. This temporal delay in floral initiation is genetically controlled; however, environment and fertility can also affect characteristics of seed fill, seed maturation and dehiscence (shattering), making response phenotype characteristics unique per individual. Application of nitrogen fertilizer, late season defoliation (clipping) and early spring removal of plant debris (burning) have been reported to increase tiller number, inflorescence density, and seed production in eastern gamagrass and other native warm-season grasses (Vogel and Bjugstad, 1968; Hulbert, 1988; Hill and Loch, 1993; Masters et al., 1993; Lemke et al., 2003).

Uneven maturation makes timing of seed harvest difficult in eastern gamagrass. Cupules are often empty or only partially filled, making seed quality difficult to determine, leading to poor germination percentage in field and laboratory experiments (Ahring and Frank, 1968; Hauser, 1982, Douglas et al., 1997; Gibson et al., 2005). In studies to determine and improve seedlot quality a strong relationship has been reported linking seed unit weight with germination and vigor based on radicle and coleoptile length (Jackson et al., 1992; Springer et al., 2001). Ahring and Frank (1968) used air and flotation separation to remove empty cupules from seedlots, while Klein et al. (2007) sorted cupules by color and weight to improve germination percentage. Lemke et al., (2003) suggest an optimum harvest period of approximately two weeks after terminal spikelets begin to shatter. The authors noted a 5 – 15 % increase in immature seed harvested when harvesting one week after terminal spikelet shatter; there was also a 13 – 20 % decrease in overall seed yield noted when harvesting three weeks after the onset of spikelet shattering.

Decreased establishment rates that are often associated with eastern gamagrass can be linked to many factors, both internal (seed) and external (environment), however, the issue of seed dormancy is by far the most confounding. Light, temperature and moisture are the three most prominent protagonists, both individually and in concert, in the imposition of seed dormancy. This imposed dormancy leads to less than 10% of eastern gamagrass seed regularly exhibiting precocious germination (Ahring and Frank, 1968; Tian et al., 2002).

Mechanical and Physical Dormancy

Normally, eastern gamagrass seed are shed from the parent plant with native dormancy characteristics (physical, physiological, chemical) already established. This normal state is considered primary dormancy. Seed may also be shed from the parent plant and thereafter exposed to conditions not favorable for germination, such as drying or temperature extremes, these stimuli can lead to a decrease in sensitivity of receptors in the tissues of the seed which ultimately result in an imposed, or secondary dormancy.

Inconsistent stand establishment continues to limit the use of eastern gamagrass. Research has shown that difficulty in the establishment of gamagrass is likely due to several seed dormancy mechanisms. The most studied is the physical restriction of the seed coat (Anderson, 1985; Kindiger, 1994; Springer et al., 2001; Tian et al., 2002; Tian et al., 2003). The eastern gamagrass seed unit is a caryopsis enclosed in a hard fruit case known as a cupule (Galinat, 1956). Depending upon seedlot quality, the cupule of the gamagrass seed unit can make up approximately 70% of total seed weight, increasing as seed quality decreases (Dewald et al., 1996; Springer et al., 2001; Finneseth, 2010). The hard cupule surrounding the caryopsis often inhibits the seed's ability to imbibe water,

leading to poor germination percentage. The physical restriction of the seed coat also limits the ability of the elongating radicle and coleoptile to emerge from the cupule.

Physiological Dormancy

Physiological dormancy refers to endogenous chemical characteristics of seed that retard or prevent embryo elongation and emergence. The primary endogenous hormone acting in this role is abscisic acid (ABA). Gibberellins (GA) increase cell division and elongation by acting synergistically with auxins. Gibberellins have been shown to substitute for the requirement of seeds for red light, cold temperatures, and long photoperiods. The main role of gibberellin during and post- germination is to aid in the metabolism of stored carbohydrates into sugar. This is seen mainly in cereals, where the aleurone layer is principle for detection of embryonic GA and α-amylase secretion in response. Many seed germination studies that include experimental application of exogenous gibberellic acid (GA_3) do so to test for the presence or severity of physiological dormancy factors (Anderson, 1985; Tian et al., 2003; Rogis et al., 2004a).

Seed Germination and Dormancy Breaking Techniques

Seed quality is the most limiting factor in the popularity of eastern gamagrass as a crop species. Poor seed quality has been cited in numerous studies as a cause for poor germination results in laboratory settings. Poor seed quality coupled with seed dormancy factors that are commonly associated with eastern gamagrass marginalize its potential for widespread application. While there are many published stratification procedures for eastern gamagrass, to date there are no published purity or general germination standards from the Association of Official Seed Analysts or the International Seed Testing

Association (AOSA, 2009; ISTA, 2009). Finneseth and Geneve (2008) recommend increasing working weights for all purity and germination analyses conducted with eastern gamagrass, because of the relatively large-seeded nature of the crop (7,029-18,357 seed kg^{-1}). Also recommended were stratification standards (14-56 days at 5° or 10°C), daylength (short-day), and temperature (15/25, 15/35 or 20/30°C) for germination regimes. Many temperature combinations have been reported in the literature; however, alternating 20/30°C has been reported to produce superior germination results effectively and repeatedly (Springer et al., 2001; Tian et al., 2003; Rogis et al., 2004a; Rogis et al., 2004b; Finneseth, 2010).

Endogenous chemicals and structural barriers that promote dormancy in seed are environmentally weathered by factors such as fire, soil microbes, moisture and cold after the seed is shed from the mother plant (Sarath et al., 2006). In laboratory research with many native species, cold moist stratification, scarification (chemical and mechanical) and ectomycorrhizal fungi have been used to reduce or break these dormancies, resulting in accelerated early germination and improved overall germination percentage (Anderson, 1985; Grabowski and Douglas, 2000a, 2000b; Rogis et al., 2004a; Sarath and Mitchell, 2008; Ghimire et al., 2009).

Cold stratification, the process of storing seed at 2-5° C for 6-10 weeks, has been shown to break primary dormancy in gamagrass seed; however, this process requires large amounts of time and energy, and rarely yields a germination percentage over 60% (Ahring and Frank, 1968; Klein et al., 2007). Studies involving the use of gibberellins (Rogis et al., 2004a, 2004b) and hot water (Grabowski and Douglas, 1999) have been unsuccessful, while more recent studies have found that the process of soaking gamagrass

seed in a 15% solution of hydrogen peroxide (H_2O_2) for 18 hours can substitute for six weeks of stratification and yield higher germination percentage (Klein et al., 2007).

Stratification (Moist Chilling)

Stratification or moist chilling is the most commonly used process to reduce dormancy mechanisms in eastern gamagrass and other North American native grasses (Geng and Barnett, 1969; Emal and Conrad, 1972; Beckman et al., 1993; Shen et al., 2001; Adkins et al., 2002). This process (lengthy periods of cold, moist conditions that weaken the cupule and may leach some endogenous dormancy-inducing chemicals from the seed) artificially substitutes for the natural process of over-wintering seed on or close to the soil surface (Springer et al., 2001). Generally, stratification is performed at or around 4°C for 30 – 60 days.

In studying the impact of duration of stratification (0-10 weeks) on overall germination percentage of two eastern gamagrass seedlots, Ahring and Frank (1968) reported that cold moist stratification (5-10°C) for six weeks yielded maximum germination percentage. This treatment significantly improved germination of one eastern gamagrass seedlot from 14.6% to 58%, while germination percentage of the second seedlot improved from 28% to 66%. Increases in germination of eastern gamagrass subjected to at least four weeks of cold (4°C) moist stratification have been reported elsewhere (Anderson, 1985; Klein et al., 2007; Finneseth, 2010) with germination reaching a maximum after four weeks of stratification and plateauing thereafter (Rogis et al., 2004a). Rogis et al. (2004a) reported that moistened seed showed significantly lower dormancy than dry seeds after being subjected to the same temperature regime.

Seed Priming

Hydropriming involves hydrating seed in water and managing the moisture of the seedlot at a sub-optimum level that nears or even initiates germination. This is conducted during the pre-plant stage. In many species, seed are held at constant moisture for days or weeks before being dried down to near original moisture levels. This process is relied upon to initiate more even, rapid germination and establishment, resulting in decreased competition from non-desirable crop species or scavengers. Priming has been reported to increase germination of native warm-season grasses, specifically big bluestem and switchgrass (Beckman et al., 1993).

Anaerobic conditions in submersed seed vary widely between species and cultivar, but often regulate the duration of priming treatment before drying down is initiated. Primed seed may be stored for a short period before planting, but generally seed will not tolerate storage durations of more than a few weeks. Not all species respond to priming similarly, as some (including cereals) are highly tolerant of repeated cycles of wet and dry. For this reason, priming protocols should be designed specifically for the crop species and cultivar (Finneseth, 2010). Rogis et al., (2004b) reported that controlled hydration conditioning of Pete eastern gamagrass seed for one week using deionized water and GA_3 in solid carriers (Agro-Lig, MicroCel-E and Vermiculite #5) did not increase 28-day germination beyond that of seed that had been stratified at 4°C for six weeks.

Chemical Scarification

Chemical scarification involves soaking seed (generally for short periods of time) in strong acids or bases to weather the cupule and perhaps break down some endogenous

chemical factors that maintain dormancy. Chemicals such as potassium nitrate (KNO_3), sulfuric acid (H_2SO_4) sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) have been reported to increase germination in a number of native warm-season grasses (Emal and Conrad, 1972; Haynes et al., 1997; Sarath et al., 2007). Acid scarification must be managed delicately, as concentration and treatment duration can vary widely between species and cultivar (Finneseth, 2010). Ahring and Frank (1968) observed a decrease in fungal proliferation during germination, but no increase in germination when soaking eastern gamagrass in NaOCl at several concentrations for varying duration.

Mechanical Scarification

Mechanized equipment including: scarifiers, dehullers, tumblers, hammer mills, brushers and rubber abraders have been used successfully to separate caryopses of many grass, forb and legume species from seed coats and other appendages. This often leads to higher germination rates of crop species that regularly exhibit mechanical dormancy. The most popular form of mechanical scarification for eastern gamagrass involves removing the caryopsis from the cupulate fruit case and/or scarifying or piercing the pericarp over the embryo before germination (Simpson, 1990; Tian et al., 2002). This process has increased germination rates significantly in laboratory studies of many warm season grasses (Coukos, 1944; Sautter, 1962; Ahring and Todd, 1977; Anderson, 1985; Simpson, 1990; Tian et al., 2002), but is impractical for situations targeting the production of eastern gamagrass.

Cupule Removal

Cupule removal via forced mechanical separation has been reported to improve germination of several species that exhibit dormancy at seed maturity, including eastern gamagrass (Anderson, 1985; Simpson, 1990). However, this is by far the most time and labor intensive process currently used to increase germination and establishment (Anderson, 1985; Tian et al., 2003). No equipment has been designed for large-scale removal of eastern gamagrass caryopses from the cupulate fruit case (hulling). This process is also insensitive to embryo damage. Springer et al., (2001) reported that over 6N of force were required to open the cupulate fruit case of raw (untreated) Pete and 'Iuka' eastern gamagrass seed. The amount of force required for this process decreased to ≈4N as seed were exposed to moist chilling (0 to 5°C) for up to 8 weeks.

Hydrogen Peroxide and Other Reactive Oxygen Species

Hydrogen peroxide (H_2O_2) is a naturally occurring endogenous phytochemical that, along with nitric oxide (NO), is also a large reserve of endogenous reactive oxygen species (ROS) (Sarath et al., 2007). Studies have shown exogenous application of ROS to illicit various responses from both mature and juvenile plant tissues (Neill et al., 2002). The effects of ROS on dormancy and seed germination have also garnered much interest (Gidrol et al., 1994; Ogawa and Iwabuchi 2001; Zhang et al., 2003; Gechev and Hille 2005; Sarath et al., 2007). Also, the role that ROS may play in activating or altering different signaling pathways in plants has been extensively studied in species like maize [Zea mays (L.)], arabidopsis [*Arabidopsis thaliana* (L.) Heynh.], wheat [*Triticum aestivum* (L.)], and fava bean [*Vicia faba* (L.)] (Prasad et al., 1994; Zhang et al., 2001; Neill et al., 2002; Li et al., 2010).

Recent literature indicates that the use of hydrogen peroxide as a treatment for after-ripened seed can enhance germination and establishment percentages in North American native warm season grasses, while eliminating the need for time and input intensive stratification and scarification (Keeley and Fotheringham, 1998; Sarath et al., 2007). In the case of eastern gamagrass, Klein et al. (2007) concluded that while cold stratification has been shown to alleviate dormancy by leading to the degradation of endogenous ABA and the promotion of GA, it does little to break down the hard coat that surrounds the germinating seed. In this study, stratified cupules had much lower germination percentages than those treated with 15% H_2O_2, however radicle lengths were much greater in stratified cupules; leading to the hypothesis that H_2O_2 may have little, if any effect on levels of ABA and GA.

Indiangrass and switchgrass seed, as reported by Sarath et al. (2007), were significantly responsive to treatment with H_2O_2. Peroxide was shown to reverse the inhibitory effects of diphenyleneiodonium (DPI) and ABA on germination and coleoptile elongation of switchgrass seed. At 25°C, imbibition of both switchgrass and indiangrass seed in 20 mM H_2O_2 caused significant increase in germination when compared to seed imbibed in water alone. This research concluded that exogenous peroxide could increase germination of NWSG through many avenues; the most intriguing being enhanced tissue oxygenation, interaction of endogenous and exogenous ROS in gibberellin/abscisic acid signaling cascades, and the oxidative degradation of germination inhibitors.

In germination of eastern gamagrass, H_2O_2 is believed to primarily affect the structural integrity of the seed cupule, softening the complex fused lemma-palea and glume-rachis internode structures enough to allow imbibition and emergence (Tian et al.,

2002; Klein et al., 2007). Beyond physical alterations to the seed unit, H2O2 may also increase germination rates by disrupting ABA production at the genetic level, as demonstrated in other crops such as switchgrass, arabidopsis and barley (Wang et al., 1998; Meinhard et al., 2002; Sarath et al., 2007).

Exogenous Hormones

Exogenous application of plant hormones – specifically gibberellic acid (GA), but also cytokinins (CK), ethylene (E) and brassinosteriods (BS) – in concert or individually can mitigate dormancy in some plant seeds. Not all species or cultivars within species respond to exogenous hormonal application similarly. Hormones must be imbibed into the tissues of the seed in order to be recognized by and act on the seed's natural biochemical processes.

Gibberellin can diminish the degree of primary or secondary dormancy in grasses, and has been used in several studies with eastern gamagrass and other native warm-season species (Anderson, 1985; Grabowski and Douglas, 2000a, 2000b; Tian et al., 2003; Rogis et al., 2004a, 2004b; Huarte et al., 2007; Huarte and Garcia, 2009), however, in the case of eastern gamagrass, with mostly unsuccessful results (Springer et al., 2001).

Huarte et al., (2007) reported a significant increase in germination of eastern gamagrass caryopses (cupule removed) from 37% to 71% following pericarp scarification when imbibed on water. The same study reported no significant change in germination (79%) when scarified caryopses were imbibed in solutions containing GA_3.

Tian et al., (2003) reported that three concentrations of GA_3 (0.001, 0.005 and 0.01 M) in citrate phosphate buffered solutions (pH 3.5) were equally effective at increasing germination of eastern gamagrass caryopses when soaked for 24-hours prior to

beginning of germination test. Among six commercial seedlots (three Pete, three Iuka) increases in germination due to treatment ranged from 25% up to 47% compared to controls, where all treatments elicited a significant response. The most effective (numerically) treatment from this study (buffered 0.001 M GA_3) was used along with distilled H_2O and 0.001 M GA_3 solution (un-buffered) to test efficacy on germination of whole (intact cupule) gamagrass seed when exposed to treatment for 24 or 48 hours. There were no significant increases in germination due to treatment for either time regime. This study supports the importance of the role of the cupule in dormancy of eastern gamagrass.

Huarte and Garcia (2009) reported that when testing effects of incubation of intact (non-scarified) eastern gamagrass caryopses in solutions of: H_2O_2 (0.8 M), GA_3 (2.88 mM), fluridone (an inhibitor of ABA biosynthesis) (30µM) and fluridone (30µM) + GA_3 (1.44 mM) on germination rate and percentage, that significant increases in germination were observed for all treatments when compared to intact caryopses treated with water alone. Two of these treatments (GA_3 and fluridone + GA_3) produced 100% germination in 20.5 and 11.5 days, respectively. Also in this study, it was shown that scarified caryopses incubated in water generated 100% germination in 19.9 days. This provides support for leaching and antagonism of endogenous abscisic acid (ABA) and ABA biosynthesis as a primary form of dormancy breakage and germination hastening.

Use of Eastern Gamagrass [*Tripsacum dactyloides* (L.) L.] in Maize [*Zea mays* (L.) subspecies *mays*] Improvement

Since its discovery, eastern gamagrass has most widely been used as a forage crop for all classes of livestock, specifically cattle. Early improvement programs (ca. 1830 –

1914) focused on selection breeding for seed quality, time to and uniformity of maturity and apparent forage quality (Magoffin, 1831; Killebrew, 1878; Dewald, 2001). Later (ca. 1914 – 1970), focus turned to utilizing the *Tripsacum* genome – which has been described as having no economic importance – for the improvement of maize, a crop with major global economic importance (Collins and Kempton, 1916).

Eastern gamagrass and corn have been referred to in the literature as distant relatives (Dewald and Kindiger, 1994; Kindiger and Dewald, 1996, 1997b), wild relatives (Duvick et al., 2003, 2006) and sister genera (Chia et al., 2012). Modern Mexican farmers refer to native eastern gamagrass as "madre del maíz" or, "mother of maize" (Denise Costich, personal communication). The history to date of *Zea* x *Tripsacum* crosses has primarily focused on the creation of *Tripsacum* introgressed maize lines (transferring genetics from gamagrass to maize). Gamagrass genetics for resistance to diseases including: anthracnose (*Colletotrichum graminicola*), fusarium (*Fusarium graminearum*), Stewart's bacterial blight (*Erwinia stewartii*), southern corn leaf blight (*Bipolaris maydis* [anamorph] = *Helminthosporium maydis*), Southern corn rust (*Puccinia polysora*) and tolerance to acidic soil have been targeted extensively and, in some cases recovered, in maize improvement research (Collins, 1930; Weatherwax, 1930; Longley, 1941; Simone and Hooker, 1976; Berquist, 1977; de Wet, 1979).

The first reported successful hybridization of maize and *Tripsacum* occurred in 1913 when Collins and Kempton (1916) utilized *T. dactyloides* and *Euchlaena mexicana* [Syn. *Zea mays* (L.) subspecies *mexicana* (Schrad.) H.H. Iltis] in a greenhouse experiment. This original cross produced four seed on the mother (*Tripsacum*) plant, of which one germinated and reached maturity.

Later studies by Mangelsdorf and Reeves (Mangelsdorf and Reeves, 1930, 1931; Reeves and Mangelsdorf, 1931, 1935; Mangelsdorf, 1934, 1935) utilized multiple successful crossing schemes between teosinte, maize and *Tripsacum*. Successive backcrosses to maize often eliminated any cytotypic traces of *Tripsacum* genetics. Soon thereafter, Mangelsdorf and Reeves proposed a theory of shared ancestry between the three relatives, where a hybridization event between early domesticated maize and wild *Tripsacum* generated wild maize (teosinte) (Mangelsdorf and Reeves, 1939). A hybrid origin for teosinte was generally rejected by contemporaries at the time (Galinat, 1970; de Wet et al, 1971; Newell and de Wet, 1973; de Wet and Harlan, 1974) and more recent literature still strives to define the role of teosinte as a direct progenitor of modern maize (Harlan, 1992; Orr et al., 2001, 2002).

Russian scientists at the Institute of Cytology and Genetics at Novosibirsk in Siberia, Russia continued this process in the 1960's, using dated technology to identify the *Tripsacum* chromosome carrying apomixis (asexual reproduction without fertilization) genes. Their first hybrid with apomictic characteristics possessed 20 corn (race unknown, but likely *Z. mays*) and 36 *Tripsacum* chromosomes. Successive backcrosses to corn lines yielded individuals that possessed 38 chromosomes (20 corn, 18 *Tripsacum*) and 39 chromosomes (30 corn, 9 *Tripsacum*). All retained the apomixis genes. The ability to reduce the chromosomal contribution of *Tripsacum* to 9 chromosomes and still retain apomictic characteristics was viewed as a huge achievement. Researchers at the University of Illinois carried this work forward, and in 1977 released a report stating that through seven successive backcrosses of aneuploid maize (20 *Z. mays* chromosomes, 2 *T. dactyloides*) to 'UI 1974' (an inbred maize line)

many abnormalities were observed. These included: endosperm alteration, fascinated and branched ears, tassel seed, tassel tipped ears and male spikelets between rows of kernels (Stalker et al., 1977).

In 2003, Pollak, Duvick and White at Iowa State University utilized *Tripsacum* introgressed corn cultivars bred to traditional Corn Belt inbred lines to produce 14 corn lines with higher percentages of oleic acid, a "heart-friendlier" monounsaturated fatty acid (Duvick et al., 2003; Pollak and Scott, 2005).

Dr. B. K. Kindiger, a cytogeneticist with USDA ARS in Woodward, Oklahoma and retired ARS cytogeneticist J. B. Beckett have successfully crossed cultivars of popcorn with eastern gamagrass that produce ears with up to 50 "plump" kernels (Kindiger and Beckett, 1990, 1991). Dr. C. A. Blakely, a USDA ARS molecular genetics researcher at the University of Missouri – Columbia (also a member of the Woodward, OK research team) has utilized RFLP and PCR technology to identify homeologous genes in gamagrass and corn and has found similarities that provide the opportunity to utilize the traits of each. It appears that although the chromosome number of the parental species differ, the two share a large number of DNA polymorphisms, and the total length of the 10 chromosomes of *Z. diploperennis* is virtually equal to that of the 18 gamagrass chromosomes (Becker, 1998).

Dr. M. Eubanks at Duke University has successfully produced viable progeny from crosses of *Tripsacum* x *Z. diploperennis* that were cross-fertile with maize (var. Tripsacorn, var. Sun Star) (Eubanks, 1997, 1998, 2001, 2002, 2003, 2006; Prischmann et al., 2009). Tripsacorn has a tetraploid *T. dactyloides* as the seed parent (Source: Indiana University, Bloomington, IN; originally collected from Santa Claus, Spencer County, IN)

and *Z. diploperennis* as the pollen parent (Source: Upper las Joyas, Sierra de Manantlan, Jalisco, Mexico; Iltis, Nee and Guzman Acc. # 1250; 1979); whereas Sun Star has *Z. diploperennis* as the seed parent (Source: Jalisco, Mexico; R. Guzman M. Acc. #777) and a diploid *T. dactyloides* as the pollen parent (Source: Manhatttan, KS; K. Anderson) (Prischmann et al., 2009). Most of these efforts were in order to express corn rootworm resistance in maize or to develop suitable refuge partners for transgenic maize (Eubanks, 2002, 2006; Prischmann et al., 2009). Ten experimental Sun Dance Genetics maize lines (SDG6, SDG7, SDG9, SDG10, SDG11, SDG12, SDG15, SDG17, SDG19 and SDG20) have been crossed with introgressed Tripsacorn and/or Sun Star genetics. SDG6, 9, 15 and 19 have *T. dactyloides* cytoplasmic genes, whereas SDG7, 10, 11, 12 and 17 have *Z. diploperennis* cytoplasmic genes. Three SDG20 individuals have been evaluated, with two possessing *T. dactyloides* cytoplasmic genes and the third with *Z. diploperennis* cytoplasm. The experimental lines listed ranged from 72% maize 28% exotic, to 97% maize 3% exotic (Prischmann et al., 2009).

Selection and Breeding for Improvement of Eastern Gamagrass [*Tripsacum dactyloides* (L.) L.] at the Diploid, Triploid and Tetraploid Levels

Current Breeding Issues

Studies of variability in agronomic and morphological traits of wild-type eastern gamagrass populations in the Midwest and neighboring states have found that diploid and tetraploid populations exist in widespread sympatric distributions, while triploid, pentaploid and hexaploid wild-types are rare (Newell and de Wet, 1974; Wright et al., 1983). Manipulation of inherent reproductive versatility in eastern gamagrass is vital to progressive domestication of the species. Fertile triploid individuals have been used in

cross pollination with diploids and apomictic polyploids to exchange valuable genetic information and enrich the *Tripsacum* genetic base in breeding programs (Dewald, 2001).

Seed generated from tetraploid and other polyploid individuals have been reported to be entirely, predominantly, or only partially apomictic, but it is generally believed that facultative apomictic reproduction is the rule in polyploid forms (Burson et al., 1990; Sherman et al., 1991; Dewald and Kindiger, 1994; Leblanc et al., 1995a; Bantin et al., 2001). While this limits the utility of tetraploid individuals in sexual recombination breeding, apomixis is highly desirable in commercial germplasm development and release, as it ensures true breeding lines.

Apomixis in Eastern Gamagrass

Apomixis is a genetically controlled form of asexual reproduction that is common in many genera of angiosperms (Voigt et al., 1989; Grimanelli et al., 2003), especially Poaceae (Brown and Emery, 1958). Apomixis is marked by the formation of an unreduced embryo without the sexual union of a sperm and egg cell (Naumova, 1992). This process yields progeny that are genetic clones of the maternal parent plant.

Normal embryonic formation during seed development involves the meiotic reduction of the megaspore mother cell into a tetrad of reduced (haploid) megaspores. In most angiosperms, three of these megaspores degenerate, and a single haploid megaspore enlarges and divides mitotically to produce two polar nuclei (a single egg cell and a central cell), two synergids and three (or more) antipodals. Plants that reproduce via apomixis forego the meiotic reduction of the megaspore mother cell as well as fertilization of the egg cell by the male sperm cell.

Diplospory

There are several avenues by which apomixis functions, but the major classifications of these mechanisms are apospory, diplospory, and adventitious embryony (Burson et al., 1989). The form of gametic apomixis that has been observed most commonly in eastern gamagrass is diplospory, or generative apospory, whereby the megagametophyte arises from a cell of the archesporium (pre-megaspore cell). While pollen is not essential to embryogenesis in diplosporous plants, eastern gamagrass is pseudogamous, meaning that fertilization of the polar nuclei must still occur in order for the seed to produce endosperm (Grimanelli et al., 2003). Polyploid gamagrass plants tend to display facultative apomictic characteristics, as opposed to diploids, which are where sexual reproduction is exclusively observed in the species (Kindiger and Vierling, 1994, Kindiger and Dewald, 1997a, 1997b). Contradictory reports on apomixis in polyploid (especially tetraploid) gamagrass plants are common; as some state that tetraploids are obligatory apomicts, and are classified as entirely such; but research shows that some sexual recombination still occurs in all polyploid eastern gamagrass (Burson et al., 1989, 1990; Kindiger and Dewald, 1997a).

Selection and Breeding

From the late 1960's into the 1970's there was renewed interest in eastern gamagrass as a forage crop in the central and southeastern United States. Issues with poor stand establishment due to fluctuating seed quality and severe dormancy still hindered the crop's wide acceptance. Cold, moist stratification was effectively used at the time to alleviate dormancy mechanisms, yet establishment of *Tripsacum* remained inconsistent (Ahring and Frank, 1968; Anderson, 1985).

Along with research in proper grazing management of eastern gamagrass, in 1981, the discovery of a gynomonoecious sex form (GSF) mutant, *T. dactyloides* (L.) forma *prolificum*, Dayton et Dewald (Dewald and Dayton, 1985a) provided researchers with a genetic breakthrough for increasing seed production and potential for enriching breeding programs (Dewald, 2001). The gynomonoecious mutant (GSF-I) was discovered in a germplasm collection at the NRCS Plant Materials Center near Manhattan, KS. The plant's origin was traced to a collection from Ottawa County, KS, where an additional similar GSF mutant (GSF-II) was discovered in a wild population (Dewald and Dayton, 1985b). These mutants displayed highly feminized inflorescences rather than the classical monoecious form. The increased ratio of female to male flowers set a potential for increasing seed production 20 to 25-fold (Dewald et al., 1987; Salon and Dewald, 2000; Dewald, 2001). The GSF trait was associated only with diploid populations and was later found to be controlled by a recessive gene at a single locus.

Early research utilizing the GSF mutants revealed a lack of sufficient pollen production for use in breeding lines and seed production (Salon and Dewald, 2000). Later studies focused on transferring GSF traits from the diploid level to polyploid by crossing GSF mutants with apomictic tetraploid or hexaploid pollen parents and inter-mating progeny. Breeding programs attempting these crosses produced fertile triploids, improved gynomonoecious diploids and fertile, gynomonoecious triploids (Dewald and Dayton, 1985b, Dewald and Kindiger, 1994; Dewald and Kindiger, 1996).

The majority of improvement breeding in eastern gamagrass has taken place since the 1980's. Perhaps the most prolific has been research conducted by Mr. Chester "Chet" L. Dewald and fellow researchers at the USDA-ARS Southern Plains Range Research

Station (SPRRS) at Woodward, OK (Dewald and Dayton, 1985a, 1985b; Dewald et al., 1987; Voigt et al., 1989; Dewald and Sims, 1990; Kindiger and Beckett, 1990; Dewald and Kindiger, 1994, 1996; Kindiger and Dewald, 1997a, 1997b; Dewald, 2001; Gillen et al., 2006; Springer et al., 2006; Goldman and Springer, 2011) and their collaborations with government and university researchers at neighboring institutions including: Cornell University (Jackson et al., 1992), USDA-ARS, Temple, TX (Burson et al., 1989, 1990; Sherman et al., 1991) and the USDA-NRCS Big Flats Plant Materials Center in Corning, NY (Salon and Dewald, 2000).

Ploidy Level of Commercial Cultivars

Gamagrass is commonly found in the diploid (2n=36), triploid (2n=54) and tetraploid (2n=72) forms. Somatic chromosome numbers of 45, 90 (pentaploid) and 108 (hexaploid) have also been reported (Farquharson, 1955).

Diploid Commercial Cultivars

Pete

One of the principle commercial gamagrass releases, the variety Pete (PI-421612; formerly designated PMK-24) is a composite of 70 accessions collected from Oklahoma and Kansas in 1958. Pete was developed by the USDA-NRCS Plant Materials Center at Manhattan, KS as a cultivar suited best for conservation and forage production. Three generations of open pollination bulking were used to advance the release, which was presumably selected for uniform maturation qualities during the bulking process. Pete was originally released in 1974, and formally released to certified growers in 1989 (USDA, 2007b; Goldman and Springer, 2011).

Iuka IV

Developed by the USDA-ARS Southern Plains Range Research Station in Woodward, Oklahoma, the gamagrass variety Iuka IV was produced from 21 individuals selected based on forage value from over 500 accessions. The geographic area of selection ranged from Oklahoma, Texas, Kansas, Arkansas and included one individual from the variety Pete (USDA, 2007b). These selections were transplanted in 1979, along with Oklahoma individuals in a 0.4 ha block near Iuka, KS for seed increase via open pollination. Increases were made for four generations before release, which was made through the Grass Variety Review Board in 1995 (Dewald, 2001).

St. Lucie

St. Lucie eastern gamagrass (USDA NRCS accession number: 9059278) is an ornamental type of gamagrass that was found and collected vegetatively in St. Lucie County, Florida in 1990. From 114 accessions in a test at the USDA-NRCS Plant Materials Center in Brooksville, FL, St. Lucie was selected based on its desirable growth habit, blue color and colorfastness following light frost. St. Lucie is an open pollinated diploid (2n=36), and thus, must be vegetatively propagated in order to maintain its color characteristics.

Martin

The eastern gamagrass variety Martin (USDA NRCS accession number: 9056069) was developed at the USDA-NRCS Plant Materials Center in Brooksville, Florida. Germplasm for Martin was from a vegetative collection in 1989 from Martin County, Florida. Similar to St. Lucie, Martin was also selected for based on growth

habit, color and color retention following light frost. Popular uses for Martin include ornamental landscape or vegetative buffer strips.

Triploid Commercial Cultivars

Verl

Verl eastern gamagrass (PI-543890; FT-II) is a fertile triploid (2n = 3x = 54), released from the USDA-ARS in cooperation with the USDA-NRCS and the Oklahoma Agricultural Experiment Station in Woodward, Oklahoma (Springer et al., 2006). Verl was produced by a forced cross of a diploid gynomonoecious sex form individual (GSF-1: PI-483447) with a monoecious tetraploid (WW-1724). Verl was selected from over 230 F1 progeny from the diploid x tetraploid cross. It was released by the Oklahoma Agricultural Experiment Station and the USDA-ARS in February, 2005. It is recommended for use as a pasture and hay crop.

Tetraploid Commercial Cultivars

Nacogdoches

The eastern gamagrass variety Nacogdoches (PI-595898) was developed by the East Texas Plant Materials Center in Nacogdoches, Texas. Nacogdoches has potential for use as conservation buffer as well as forage uses including pasture, hay, green chop and silage. The variety is suited to many soil types, however, deep, sandy soils are not recommended. Nacogdoches is best adapted to areas that receive >63 cm of rainfall throughout USDA hardiness zones 8 and 9 (excluding Florida) (USDA, 2007b).

Highlander

The eastern gamagrass variety Highlander (PI-634941) was released in 2003 by the USDA NRCS PMC in Coffeeville, Mississippi, with cooperation from the Mississippi Agricultural and Forestry Experiment Station (MAFES) Mississippi State, Mississippi, and the Jimmy Carter PMC, Americus, Georgia. Highlander was originally collected along Woodland Road on the Fort Campbell Army Base, Montgomery County, Tennessee in 1990 (USDA, 2007b). Highlander progeny were selected for vigor of germination and growth, yield, resistance to disease and forage-related morphology characteristics, including number of female flowers per inflorescence (Randy Seymour, Joel Douglas, personal communication). Highly recommended as a forage crop, primarily for hay or grazing, Highlander shows promise as a silage and bio-energy crop, and is also often utilized for many types of conservation plantings. Highlander is best suited to well drained, fertile soils, but will tolerate poorly drained soils and has good flood and drought tolerance.

Bumpers

The eastern gamagrass variety Bumpers (USDA NRCS Accession Number: 9058495) was developed by the USDA NRCS Plant Materials Center in Booneville, Arkansas. The original collection of seed for Bumpers was taken from a native stand near a roadside in Yell County, AR. Bumpers was selected from a collection of 250 accessions from Oklahoma and Arkansas in 1989 based on forage production, resistance to disease, seed quality and vigor of germination and establishment.

Medina

Eastern gamagrass variety Medina (PI-595897) was developed at the USDA-NRCS Plant Materials Center in Nacogdoches, TX. Medina was produced from seed collected in Medina County, TX in bottom land along the Hondo Creek. Medina was among three collections selected from a test in Nacogdoches, TX which included 86 accessions being evaluated for forage mass, seed production, stand persistence and vigor of germination and establishment.

San Marcos

The eastern gamagrass variety San Marcos (PI-434493) was collected from a native tetraploid stand located near San Marcos, in Hays County, Texas. Selections were made based on seed production and forage mass and were developed for release at the USDA-NRCS James E. "Bud" Smith Plant Materials Center in Knox City, TX.

Jackson

Eastern gamagrass variety Jackson (PI-595896), developed by USDA-NRCS Plant Materials Center, Nacogdoches, TX, was collected in 1986 from a landrace in Jackson County, TX. Jackson is an apomictic tetraploid variety that was multiplied and increased for seed production via vegetative propagation. In a test of 93 eastern gamagrass selections from approximately 60 counties in Texas, Jackson was one of three selections chosen for performance based on forage mass, persistence, seed production and vigor of germination and establishment.

Critique of Efficacy of Flow Cytometry in Predicting Ploidy Level in Grasses

Introduction to Flow Cytometry

Flow cytometry (FCM) is a prevalent technique gaining popularity for its use in measuring nuclear DNA in a wide range of materials, both plant and animal. Though more popular in the biomedical research industry, many plant scientists, plant breeders and agronomists are now using FCM as a means to estimate genome size, identify and confirm polyploidy in research populations. Flow cytometry has been used to confirm ploidy level in induced tetraploids of eastern gamagrass (Salon and Earle, 1998), and was also used by Bantin et al. (2001) to refine previous estimates of genome size (C-value) in diploid and polyploid eastern gamagrass accessions: (2C) 7.37pg, (4C) 14.74pg, and (6C) 22.39pg, respectively.

The basis of FCM involves DNA fluorochromes such as DAPI (4',6-diamidino-2-phenylindole), Hoechst dyes, mithramycin, and propidium iodide (PI, $C_{27}H_{34}I_2N_4$), which bind to specific base pairs (A-T specific, in the case of DAPI) or to DNA base pairs with little or no sequence preference (in the case of PI). These fluorescent dyes are excited when exposed to energy from ultra-violet (UV) lamps or ion lasers, rendering measurable reflectance in an electronic detection apparatus. To determine ploidy level or estimate C-value, liquid suspensions of intact nuclei are stained and analyzed along with either internal or external standards of known ploidy and genome size (see examples in Doležel et al., 2007).

Popular Techniques

Widely regarded as the primer for any FCM study, Doležel et al., (2007) gives basic guidelines of many intact cell nuclei suspension preparation protocols, which build

on the solid foundation previously laid by D. Galbraith (Galbraith et al., 1983). Variations on preparation protocols are numerous, but are generally straight-forward, with careful attention paid to sample quality and size, as well as preparation and maintenance of standard samples used for confirmation of nuclear DNA contents. Popular techniques for use in grasses involve excising small (usually 1 cm^2) tissue samples from the actively photosynthetic area of a leaf, placing the sample in a dish and chopping to a very fine consistency with a razor blade in a hypotonic buffer, thus releasing cellular contents into the solution. Very fine mesh (≈50μm) filters are used to strain particles from the homogenate, which is collected and stained using a DNA fluorochrome. The flow instrument injects the stained sample in a stream of fluid moving past a detector, which records the fluorescence of nuclei as they move past. In this manner, the relative DNA content of thousands of cells can be rapidly observed and averaged for a single sample. The protocol used in this study was adapted from that of Sysmex (Appendix A).

Summary and Discussion

Difficulties in germination and establishment of NWSG are many and varied. More importantly, while the control pathways of these dormancies (plant hormones imposing dormancy and/or promoting germination) are known, overcoming, or trying to modify their control has been slow. Abating seed dormancy factors with the use of chemical, mechanical or hormonal treatments, regardless of efficacy, is generally relegated to laboratory practice only. The need for simplicity in on-site establishment of perennial crop species is substantial enough that only the most efficient and sustainable practices should be entertained and developed further.

Crop improvement is necessary to increase the utility, acceptance and production of native warm-season grasses, especially eastern gamagrass. The highly variable phenotypic characteristics (observed even in improved cultivars) that hinder the potential of *Tripsacum* include: uneven maturation, low seed production (seed per raceme), seed shattering, poor seedlot quality, and highly dormant seed. In order to improve the utility of the species as a forage, conservation, or biomass crop, vast improvements must first be achieved. Genetic diversity of the *Tripsacum* genus is very high across the United States and further south, closer to the center of origin (Li et al., 1999, 2000). With the proper exploitation of that diversity, these non-desirable characteristics can be managed through effective selection breeding.

CHAPTER IV

RESEARCH EXPERIMENT 1: REGIONAL GERMPLASM COLLECTION AND EVALUATION OF GENOMES OF EASTERN GAMAGRASS ACCESSIONS VIA FLOW CYTOMETRY

Introduction

Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is widely distributed throughout the central and eastern United States, ranging predominantly from 40°N latitude to as far south the US-Mexico border. Eastern gamagrass shares ancestry with corn [*Zea mays* (L.)] and naturally occurs in several chromosomal factors. Diploid (2n = 36) and tetraploid (2n = 72) are the predominant ploidy levels commonly found in nature, although triploid (2n = 54), pentaploid (2n = 90) and hexaploid (2n = 108) cytotypes also exist. It is widely believed that polyploidy confers apomictic traits to eastern gamagrass, but it is still unknown whether apomixis in eastern gamagrass is obligate or facultative (Burson et al., 1990; Jackson and Dewald, 1994; Dewald and Kindiger, 1994; Kindiger and Dewald, 1996, 1997a, 1997b; Grimanelli et al., 1998, 2003; Blakey et al., 2001, 2007; Leblanc et al., 2009; Finneseth, 2010). As apomictic germplasm affords little utility in breeding programs focused on genetic improvement of the species, it is important to understand the spatial distribution and richness of variant cytotypes across the area of interest. Research suggests that ploidy level in eastern gamagrass is dependent on geographic range, with most of the diploid cytotypes inhabiting the central

United States, while the far eastern and southern United States are predominantly tetraploid (Anderson, 1944, Newell and de Wet, 1974; de Wet et al., 1982).

Objectives

This experiment involved the collection of germplasm from several sub-regions of the southeastern and Atlantic United States. These individuals were evaluated for ploidy level using flow cytometry, a standard laboratory procedure for estimating nuclear DNA content. Primary objectives of this study were to chronicle occurrence and cytotypic variation among eastern gamagrass ecotypes, and to build on previous research to better understand the effects of geographic region, terrain, or soil type on cytotype distribution and richness. Also, elite individuals from these populations will be identified – regardless of cytotype – to build a breeding nursery population with increased forage quality characteristics.

Materials and Methods

Germplasm Collection

Germplasm was collected during scouting endeavors throughout the southeast, mid-Atlantic, and Atlantic coast regions from April, 2012 to August, 2012. Stands of eastern gamagrass believed to be native – based on location, proximity to other stands, and relative abundance (minimum of 30 individuals per population) – were assessed *in situ* for stand health before subsequent collection. Samples were harvested as vegetative stem bases (proaxes) and transported to Mississippi State University (Starkville, Mississippi). Vegetative samples were transplanted into a nursery block in a 1m x 1m grid arrangement at Mississippi State University Henry H. Leveck Animal Research

(South) Farm [(33.423756, -88.791594), soil type: Savannah fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Fragiudults)]. A total collection of 171 individuals was compiled (Appendix B).

Screening and Evaluation of Individuals for Forage Use Characteristics

Throughout 2013 and 2014, all individuals in nursery were screened for desirable forage use characteristics, including disease resistance, cold hardiness and onset of reproduction. These data were taken through visual ratings, and compiled to identify individuals for future use in forage variety development systems. All ratings were made in triplicate, on a 1-5 scale, and are presented in Appendix C. Forage quality measurement – reported here as in vitro true dry matter degradability (IVTDMD) – were also taken to further assess individual merit for forage-use.

From 28 October to 1 November, 2014, individuals in nursery block were visually evaluated for foliar disease, specifically southern corn rust (*Puccinia polysora*). Individual plants were ranked on a scale of 1 to 5 with: 1) No disease present 2) Very few, faint lesions totaling less than 25% coverage of plant 3) Moderate rust infestation, total coverage not exceeding 50% of plant material 4) Abundant rust and fungal lesions, covering more than 50% of leaf tissue 5) Complete infestation, entire plant displaying rust spores or fungal lesions. This was an initial screening to determine elite germplasm for continued breeding fitness evaluation.

Individuals were assessed for cold hardiness on two separate dates, 20- November and 5- December, 2013. Each daytime evaluation took place five days after extended overnight periods of -5°C ambient air temperatures. Individual plants were ranked on a scale of 1 to 5 with: 1) Severe leaf tissue damage, complete loss of tissue structure 2)

Widespread leaf tissue damage and loss of color to greater than 50% of total leaf area 3) Moderate tissue damage and loss of color on up to 50% of total leaf area 4) Minimal leaf tip damage and some loss of color to less than 25% of total leaf area 5) No presence of damage to leaves. Onset of reproduction was defined as the first visible reproductive tiller with an emerged inflorescence. This criteria was divided into and reported by week during the months of May and June (Week 1= 15-May – 22-May, Week 2 = 22-May – 29-May, Week 3 = 29-May – 5-June, Week 4 = 5-June – 12-June, Week 5= 12-June – 19-June, Week 6 = 19-June – 26-June).

Forage quality was assessed in separate harvesting events during 2014 and 2015. Entire plants were harvested at a height of 30 cm using a hand-held electric sickle-bar type harvester. Whole plant samples were homogenized and divided into subsamples for analysis. Subsamples were dried in a forced air oven at 60°C for 96 hours, then ground to pass a 2 mm mesh in a Thomas® model 4 Wiley® mill (Thomas Scientific, Swedesboro, NJ). Samples were processed for IVTDMD according to ANKOM Technology Dietary Fiber Analysis Method 3 using the DAISY^{II} Incubator (ANKOM Technology, Macedon, NY) (Methods and protocol: Appendix D). All samples were analyzed in duplicate at the Mississippi State University H.W. Essig Nutrition Lab in the James W. Scales Building (Mississippi State, MS).

In vitro true dry matter degradability was calculated as:

$$\frac{100 - (W_2 - (W_1 \times C_1))}{(W_2 \times DM)} \times 100 \qquad \text{Eq. (4.1)}$$

Where W_1 = Bag tare weight

W_2 = Sample weight

W_3 = Final bag weight after in vitro and sequential neutral detergent treatment

C_1 = Blank bag correction

Cytological Analysis

Using standard protocols refined by Dr. Timothy Rinehart (Research Plant Molecular Geneticist, USDA ARS Thad Cochran Southern Horticultural Research Laboratory, Poplarville, MS), individuals were screened for ploidy using a BD Accuri C6 cytometer (BD Biosciences: Becton, Dickinson and Company, Franklin Lakes, NJ). Two buffers were used during plant material preparation, both supplied by Sysmex (Sysmex Partec, Görlitz, Germany). Live plant material was used for each analysis. Cytometer was programmed to run without limits based on length of sample collection (time) or overall amount of sample collected (volume). Fluidics parameters were set to run on the slowest flow rate setting of 14 µL per minute, with a 10 µm core size.

Approximately 0.5 cm^2 of turgid, clean, healthy, leaf tissue was excised from donor plants and placed in a 90 mm round polystyrene petri dish along with 250 µL of nuclei extraction buffer (actual buffer is proprietary, but many use a hypotonic, neutral-pH $MgSO_4$ solution containing a small-molecule redox reagent such as dithiothreitol (DTT), and a non-ionic surfactant (similar to Triton X-100)). A double-sided razor blade was used to firmly chop leaf material into a fine, even consistency. Chopping events lasted no longer than 60 seconds. Razors were replaced after four samples (two samples per side) or sooner if necessary.

After chopping, dish contents were washed together using 1 ml of staining solution, which was made in bulk daily (each milliliter contains 6 µl propidium iodide (PI) stock solution (fluorescent stain) and 3 µl RNase stock solution (to avoid RNA staining), diluted to 1ml with staining buffer (proprietary, Sysmex)). Nuclei suspension

was filtered through a clean 50 µm mesh filter cap into a 3.5 ml (12 x 75 mm) test tube. Plates and filter caps were discarded after each use. Filtered samples were labeled and placed in a test tube rack in dark refrigerated storage for 30 minutes before being analyzed.

Individuals were analyzed for ploidy by comparing to external eastern gamagrass standards (Figure 4.1) which were previously karyotyped by root tip chromosome counts. These standards (diploid and tetraploid) were reanalyzed regularly during screening of unknown individuals (every 20-30 samples) to insure flow cytometer readings did not fluctuate (drift) in ploidy estimation. Garden pea (*Pisum sativum* L.) was also used as an external standard in flow evaluations. Every screening was allowed to progress until at least 1,000 events had been recorded within a predetermined gate-width. Numerical data, statistical analysis and histograms (similar to Figure 4.1) for each individual screened are located in Appendix E.

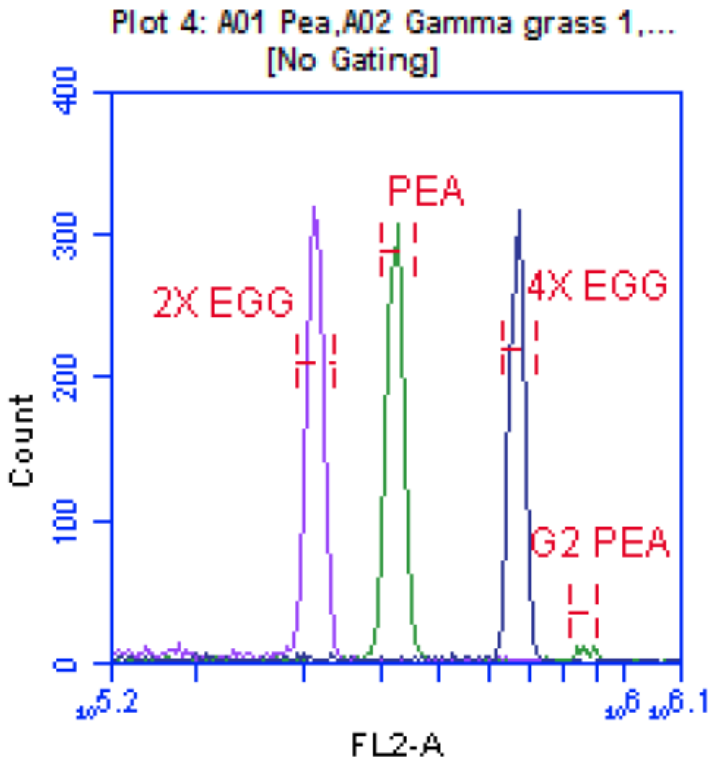

Figure 4.1 Two-parameter frequency histogram as generated by BD Accuri C6 flow cytometry analysis software.

2X EGG – Diploid eastern gamagrass [*Tripsacum dactyloides* (L.) L.] standard in G_1interphase
Mean FL2-A value = 311,611 (CV = 2.89)
PEA – Garden pea (*Pisum sativum* L.) standard in G_1 interphase (used as reference)
Mean FL2-A value =423,389 (CV = 2.80)
4X EGG – Tetraploid eastern gamagrass standard in G_1 interphase
Mean FL2-A value =672,850 (CV = 2.50)
G2 PEA – Garden pea standard in G_2/metaphase
Mean FL2-A value =842,324 (CV = 2.44)
X-axis is logarithmic scale area of the FL2 (propidium iodide fluorescence) channel
Y-axis is linear count of events as determined by acceptable thresholds

Results and Discussion

Germplasm Collection

A total of 171 individual plants were collected from across the Southeast and Atlantic coast regions of the United States during the time period mentioned (Appendix B). Of these individuals, seven failed to survive through the 2014 growing season and subsequent winter in Starkville. Surviving individuals were evaluated for several forage quality characteristics including rust resistance, onset of flowering, and in vitro true dry matter degradability (IVTDMD).

During germplasm collection, it was observed that populations of eastern gamagrass became increasingly scarce in southern Alabama and throughout the southern Atlantic coastal plain and the gulf coastal plain of Georgia. There appeared to be a defined limit to eastern gamagrass populations in the coastal plain regions of Alabama, Georgia and South Carolina, specifically at the junction of the piedmont and coastal plain regions, along the fall line, when soil texture became predominantly shallow, infertile sand, a result of the sedimentary parent material. This line follows the remnant Black Belt and Jackson Prairie regions of Mississippi in a crescent shape throughout southern Alabama, northeast through the city of Montgomery and extends east-northeast to Columbus, GA. Continuing through Macon, Milledgeville and Augusta, GA before continuing into Columbia, South Carolina, this line created a boundary for native eastern gamagrass populations.

Populations were heavy along the northern piedmont of Alabama and Georgia, but began to decline going north and east, as the piedmont region gave way to the ridge

and valley terrain of the Blue Ridge region. Populations were increasingly rare at higher altitudes, with none found over 610 meters above sea level.

Screening and Evaluation of Individuals for Forage Use Characteristics

Visual ratings for rust resistance were significantly affected ($P< 0.0001$) by individuals in the population (Appendix C). Mean raw data values ranged from 5.0 – 1.0 (LSD = 0.9278), with an overall raw mean of 2.9 and a median of 3. Southern corn rust is regarded as the most likely bacterial and fungal disease that could potentially affect the forage production of eastern gamagrass. Very little is known about presence of or breadth of genetic resistance in eastern gamagrass to these pathogens. With increased corn production, plant diseases common to corn are likely to have substantial effects on eastern gamagrass production. Visual confirmation of incidence, virulence and persistence of these pathogens is vital to isolation, identification, and further study of these diseases.

Mississippi accessions were among the most severely infested individuals in the entire collection. Of the lowest ranked (highest rust infestation) mean separation, Mississippi accessions comprise 71% (10 of 14) of that group, while the highest ranked (lowest rust infestation) mean separation group is 35% (7 of 20) Mississippi accessions. The absence of fungal lesions on accessions from other sub-regions of the Southeast and Atlantic United States argue for the possibility of resistance present in the collected population.

Evaluation of cold tolerance ratings showed that individual plant had no significant effect ($P=0.7264$) on cold tolerance rating (LSD=2.168). Mean cold tolerance ratings ranged from 1.0 to 4.0, with a mean reading of 2.5 and 1.3 in November and

December, respectively. Median rating for November was a 3, while December was a 1. The lack of main effects in the model removes the ability to discuss cold tolerance of individual plants, however, these data suggest that eastern gamagrass may keep its structural canopy form and leaf integrity well into the winter months at this latitude. This can lend well to forage based stockpiling scenarios where plant material is allowed to remain in situ, for use at a later date. It should also be noted that these temperatures were abnormally low for the site location. National Weather Service records (since 1948) have the record low for the month at -9°C.

Onset of reproduction began in May and continued through June. By the 5th week of June, all entries had initiated reproduction. Overall mean date of initiation of reproduction was week 3.07. Results were divided by state of origin and week of maturity initiation. A total of 21 entries were recovered from the state of Alabama. Of those individuals, mean flowering date occurred later (0.93 weeks) than the overall population mean date of initiation. Accessions from Arkansas (5) had a mean date of initiation of reproduction of 1.8 weeks, 1.27 weeks earlier than the overall population mean. Georgia accessions (16) had a mean date of initiation of reproduction of 3.125 weeks, nearly identical with the overall population mean. The single Kentucky individual began reproduction in week two (22-29 May). Individuals collected from Mississippi (96) had a mean initiation of reproduction of 2.85 weeks, with over 50% of those individuals becoming reproductive within the first two weeks of observation. North Carolina (5) and South Carolina (10) accessions were later maturing than most other collections, averaging 3.8 and 4.1 weeks to maturity, respectively. Lastly,

Tennessee accessions (9) had a mean onset of maturation date of 2.1 weeks, much earlier than the overall population mean.

Individual nursery plants showed significant differences ($P < 0.0001$) in forage digestibility, represented as IVTDMD (LSD = 2.67). Forage IVTDMD values ranged from 50.2%. – 76.3% with an overall mean of 64.5 and a median of 65.2. In vitro true dry matter degradability is calculated by measuring the undigested, fibrous portion of a feed source left over following a laboratory simulation of rumen degradation over a 48 hour period. The value reported is the portion of the sample that is degraded away during the simulation. Values of 70% or greater are considered very high quality (similar to alfalfa and other legumes).

Cytological Analysis

All surviving 164 individuals were screened for ploidy level in February, 2016. Histograms for each individual are presented in Appendix E. All screened individuals were classified as either diploid (0), or tetraploid (164). All cytometric screenings recorded over 1,000 events per peak and coefficients of variation were maintained below 5.0%, with an overall mean CV of 2.95%.

Because all of the collected accessions were determined to be greater than diploid, all flow cytometry screenings were reevaluated for fit and value. Of the total population, 19 individuals (11.5% of population) were evaluated for aneuploidy, by the strictest definitions of aneuploidy, there was not enough variation in DNA index (10% deviation is sufficient) to justify further analysis of individuals for ploidy level.

These findings lend substance to previous speculation that the southeastern and mid-Atlantic sub-regions of the United States contain a large proportion of tetraploid

germplasm. The reason for this unbalanced relationship, whether it was altered in an historic geological event, or is the result of more recent natural or human-mediated occurrences is unknown and remains vexing. The difficulty remains, that in these regions of polyploid dominated populations, there is, in turn, great lack of potential for genetic flow (gene migration) due to apomictic seed production in polyploid eastern gamagrass.

In published reports (Dunfield, 1986) of similar germplasm collections, diploid populations tended to be located in open fields without grazing pressure, as opposed to tetraploid populations, which were generally found to populate protected waterways and other areas where encroaching water may leave a debris layer upon its retreat. There were several meadow areas sampled for the germplasm collection described above, however, as intensified agriculture has altered the landscape of a large portion of the southeast, a lack of beneficial habitat (i.e. open fields without grazing pressure) may have contributed to the absence of diploid individuals in the collection. Literature sources also cite leaf size, seed size (rachis segment), leaf width and color as identifiable characteristics separating diploid and polyploid eastern gamagrass individuals (Farquharson, 1955; Dunfield, 1986, 1991). These criteria were considered during germplasm collection, and prove to not hold true of southeastern ecotypes of eastern gamagrass.

CHAPTER V

RESEARCH EXPERIMENT II: SELECTION BREEDING FOR RAPID, EARLY GERMINATION WITHOUT STRATIFICATION

Introduction

Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] shows great promise as a forage crop; however, its widespread use has been negated due to intrinsic anti-germination and establishment characteristics. These seed dormancies are difficult and expensive to bypass or remove, either mechanically or by way of exogenous chemical and hormonal treatment. Genetic improvement of eastern gamagrass germplasm is necessary to commercially promote the species as a viable forage, biomass and conservation crop. Germplasm collection, screening and recurrent phenotypic selection are proven methods of increasing the utility of crop species without sacrificing genetic heritage.

Objectives

The objective of this multi-year experiment was to develop a diploid germplasm line of eastern gamagrass with significantly increased mean percentage germination of non-stratified, untreated raw seed. This would be in comparison to currently available commercial diploid cultivars. Also, while increasing mean percent germination of untreated seed, the implied goal would be that onset of germination of this developed line would begin significantly fewer days after imbibition (DAI) as compared to that of other

commercially available cultivars when similarly treated. The limit for selection criteria was set at seven days or less.

Materials and Methods

Seedlot Evaluation

Seedlots of two commercial diploid cultivars, Pete and Iuka were obtained from Johnston Seed Company (Johnston Enterprises, Enid, OK) in July, 2012. Seed were purchased as untreated, unprimed, raw seed. Entire seedlots were cleaned using an impeller-type fractionating aspirator (Bulldog Brand, Carter Day International; Minneapolis, MN) to remove small debris, chaff and empty seedcases. Initial germination screenings were performed to assess overall germination percentage of each seedlot. Standard clear Lucite 'crisper box' style containers (17.7Lx12.7Wx3.1H cm) lined with Steel Blue™ germination blotter paper (Anchor Paper Company; St. Paul, MN) were used in all standard germination studies described herein.

Six replications of 100 seed from each cultivar were placed in crisper boxes in between two layers of blotter paper, wetted with 50ml of distilled H_2O and placed in a cold chamber at 4°C for 48 hours. Boxes were removed, any excess water was allowed to drain and boxes were placed back in refrigeration for 8 weeks. After 8 weeks, boxes were removed from refrigeration, seed were rinsed and placed in clean crisper boxes on one layer of blotter. Simultaneously, six replications of 100 untreated seed from both Pete and Iuka seedlots were placed in individual crisper boxes on one layer of blotter. All boxes were wetted with 25ml of a solution containing 4g Captan™ ((3aR,7aS)-2-[(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione) microfine wettable powder fungicide per liter distilled H_2O. Germination boxes were then placed in

plant growth chambers set at 30°/20°C and exposed to 8/16hr light/dark in accordance with standards published by Finneseth and Geneve (2012). Germination counts were made daily for 7 days, and every two days thereafter until 28 days. Extremely low percentage germination (< 1.0%) in untreated boxes after 28 days required mass screening (procedure below) of untreated germplasm for initial selection process.

Because germination percentages were so low at the end of the 28-day germination period, all remaining ungerminated seed from untreated controls and stratified treatment were removed, rinsed with distilled H_2O and returned to a clean crisper box, on a single layer of blotter. Blotter was moistened with 10ml of Captan™ fungicide solution and crisper boxes were placed in cold chamber at 4°C to stratify for 28 days. Following stratification, crisper boxes were returned to the germination chamber for a second 28-day germination. This process was repeated until no remaining seed germinated, establishing a base germination velocity when seed are stratified.

Mass Screening and Germination Testing

Mass screening was carried out in the same manner as previously described, however, larger covered trays were used in place of crisper boxes in order to accommodate the large number of seed needed for selection. One screening of cold, moist stratified germplasm yielded enough seedlings to proceed to the steps outlined ahead. Several screenings of untreated seed were necessary to obtain 100 desired seedlings.

Subsamples (200g, ≈3,500 seed) of both commercial seedlots were placed on 0.6 x 0.6m stainless steel trays lined with two layers of regular, 17.2kg creped seed germination paper (Anchor Paper Company; St. Paul, MN), and initially wetted with a

solution containing 4g Captan™ microfine wettable powder fungicide per liter distilled H_2O. Seed trays were covered with a 3mm clear Lucite plate, placed in plant growth chambers set at 30°/20°C and exposed to 8/16hr light/dark. Seed that germinated within the 7-day selection criteria period were removed from trays and placed in seed germination boxes lined with Steel Blue™ germination blotter paper, wetted with distilled H_2O and returned to the growth chamber. At 7 days after germination, or when appropriate, seedlings were transferred from crisper boxes to small pots (4.1 x 4.4 x 7cm) filled with 1:1:2 ratio (v/v) of coarse pine bark, potting soil (Sunshine #1) and fine play sand. Pots were then placed in a greenhouse under long-day conditions to promote vegetative growth. Following selection, all un-germinated seed from screening trays were counted to allow for heritability calculations.

Realized heritability was calculated as:

$$H_R = \text{gain/reach} \qquad \text{Eq. (5.1)}$$

Where: gain = $\bar{x}$ selected individuals - $\bar{x}$ starting population

And: reach = $\bar{x}$ new population - $\bar{x}$ starting population

All statistical analyses were carried out using SAS analytics software (SAS Institute, Cary, NC). All tests were run at $\alpha = 0.05$ level of significance unless otherwise noted. Means separations were conducted using the MEANS statement in the General Linear Model (GLM). All germination testing was evaluated using a chi squared test and, where appropriate, Fisher's Exact Test.

Short-cycle Vernalization

In December, 2012, all screened germplasm was moved to a greenhouse and exposed to a short season regime. The process was as follows: Long-day conditions at

25°C through January, 2013 (30 days), then ambient daylength, maintaining 25°C (30 days), then combined ambient daylength and temperature (6-7°C) to simulate vernalization and promote the onset of synchronous flowering in early spring 2013.

All selections (vernalized and non-vernalized) were moved from greenhouse and transplanted to establish initial nurseries (referred to as 'crossing blocks' or 'cycles') stratified germplasm (90 individuals) was used to establish Cycle0 and untreated germplasm selections (90 individuals) were used to establish Cycle1.

Crossing Block Establishment

Cycles 0 and 1 were established in separate areas of Mississippi State University R.R. Foil Plant Science Research Center (33.471283, -88.783133). The dominant soil types at Cycle0 and Cycle1 were Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquepts) and Marietta fine sandy loam (Fine-loamy, siliceous, active, thermic Typic Endoaquults) respectively. Because eastern gamagrass is a cross pollinated species, and pollen is readily transported by wind, subsequent crossing blocks were established at ≥ 500m distances, to avoid undesired cross-pollination. Prior to establishment of crossing blocks, each location was scouted for wild gamagrass plants, which were subsequently removed or destroyed.

All cycles were laid out on a 1 x 1m grid, with a single individual planted at each intersection. Alleys between plants were maintained in a weed-free fashion with applications of glyphosate herbicide or hand weeding, when necessary. All cycles were fertilized and amended according to soil test recommendations prior to planting, and 56kg ha^{-1} nitrogen fertilizer was applied each spring following green-up. Blocks were burned in February each year to remove dead leaf material and pre-emergent herbicide

[Prowl H_2O ACS; (pendimethalin: N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) BASF Corporation; Research Triangle Park, NC] was applied at 4.64 L ha^{-1} (0.86kg ai) following burning to reduce early season annual weed infestation. After anthesis and pistil senescence, entire spikes were covered with cone-shaped or rectangular tulle fabric bags (Paper Mart; Orange, CA) to avoid shattered seed loss. Cone-shaped bags were 20.3cm long and 10.8cm wide at the opening, while rectangular bags measured 22.8L x 16.2W cm, both bag styles were hemmed with a double drawstring closure. Bags were placed on one or several spikes together, and were secured to a segment of steel reinforcing bar which was driven into the ground at each plant.

Seed Harvest

Ripe seed were harvested from crossing blocks on a weekly basis. Whole seed heads were harvested, as opposed to stripping easily dehiscent seed one at a time. All seed heads were covered with ivory-white tulle fabric bags, which allowed most, if not all seed to be harvested. Harvested mature seed from each cycle was air-dried on a laboratory bench indoors for 14 days. Seed were placed in a standard freezer (-21°C) for 24 hours to eliminate eggs and larvae of the *Lepidoptera* species *Ostrinia nubilalis* (European corn borer) that were identified and observed feeding on seed.

All seed were cleaned using a forced air fractionating aspirator set at the highest possible air speed and smallest allowable column volume (see Figure 5.1, C). Fractions were weighed and counted to assess seedlot quality. All fractions were bagged and stored at room temperature until November, when germination screening would begin again.

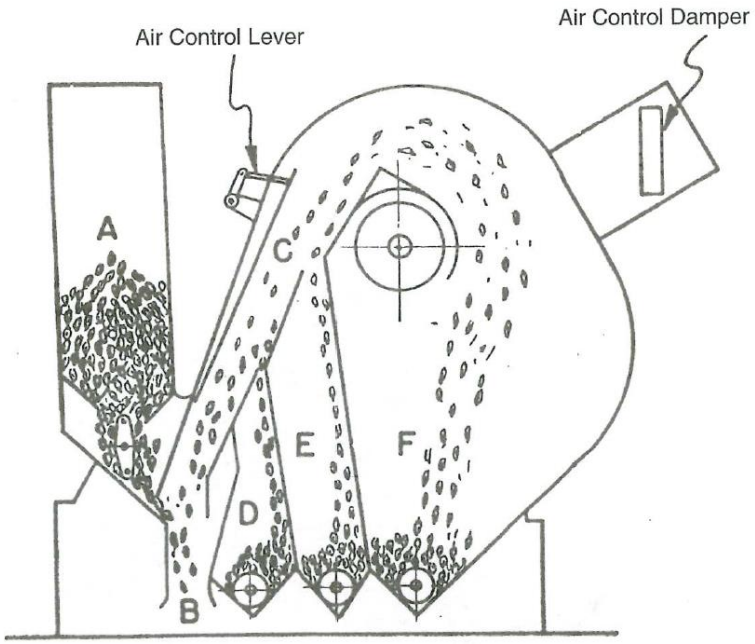

Figure 5.1 Fractionating aspirator, cross-section view.

(A) Seed hopper; (B) air column through which heavy seed fall against air flow; (C) column into which lighter seeds and chaff are lifted; (D) section that receives heaviest liftings; (E) section that receives second heaviest fraction; (F) section into which extremely light waste materials are delivered
(From Vaughn, C. 1968 p. 180)

Cycle2 Establishment

Seed from Cycle1 was screened beginning in December, 2013 (in similar fashion as mentioned before). Individual seedlings were selected based on velocity of germination, and advanced. Cycle0 and each successional generation crossing block (Cycle1, Cycle2 etc.) were maintained to measure and define the progress of selection. Each successive cycle of selection was evaluated for selection pressure. Selection pressure (i) is an implied measurement of the proportion of the population actually selected for advancement, and is calculated as:

$$i = (\Sigma_s / \Sigma_p) * 100 \qquad \text{Eq. (5.2)}$$

Where: s = selected germplasm, And: p = population germplasm

Germination was evaluated by several calculations, with care taken to measure the dispersion of germination over time (relating to establishment), without confusing the effects described by Scott et al. (1984).

Mean daily germination was calculated as

$$(\Sigma N_j / (V*S)) * 100 \qquad \text{Eq. (5.3)}$$

Where N = the number of seed germinated on the jth day

V = the number of replications used in the study

S = the number of seed in each replication at the beginning of the study

Mean Cumulative Germination was calculated as:

$$D_i [(\Sigma N_i / (V*S)) * 100] + D_{i+1}[(\Sigma N_{i+1} / (V*S)) * 100] + \ldots \qquad \text{Eq. (5.4)}$$

Where the mean daily germination is compiled for each day in the study to develop an accumulating germination percentage that accounts for temporal fluctuations in germination percentage.

Mean total germination was calculated as

$$(\Sigma N_i..N_f / (V*S)) * 100 \qquad \text{Eq. (5.5)}$$

Where N_i = the number of seed germinated on the initial day of the study

N_f = the number of seed germinated on the final day of the study

These equations are critical to the calculation of realized heritability, a measurement of the extent to which phenotype is affected by genes inherited from parents, also called 'narrow sense' heritability.

Results and Discussion

Seedlot Evaluation

Initial screening of untreated Pete and Iuka seedlots yielded very low percentage germination for both cultivars (Figure 5.2). In a standard (6 replications of 100 seed) germination test, both cultivars had overall 28-day percentage germination values of 0.67%. This was followed with a 28-day stratification, to assess potential germination of the seedlots. Following the first stratification, untreated seedlots were again germinated for a period of 28 days. Pete and Iuka had a mean post-stratification percentage germination of 12% and 9.67%, respectively. There were no significant differences observed between cultivars for either of these germination experiments ($\alpha = 0.05$). However, during the second germination period (Post-Strat1), percentage germination increased significantly for both cultivars compared to the first germination period (Pre-Strat) ($p < 0.0001$). Further stratification and germination procedures on this treatment group yielded no additional germination (Post-Strat2, Post-Strat3).

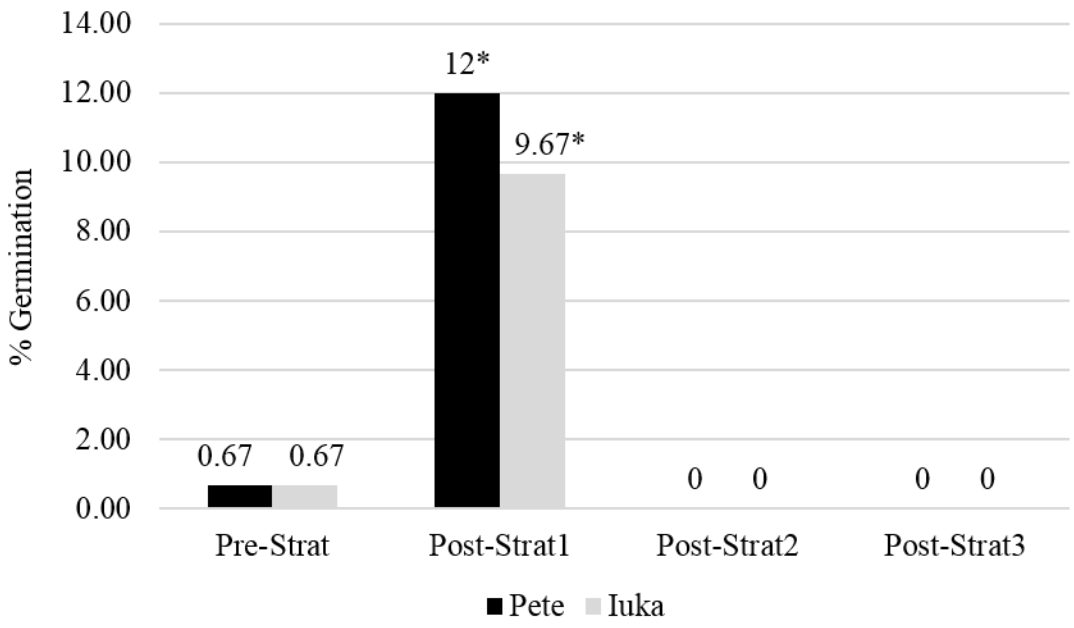

Figure 5.2 Comparison of untreated control groups of eastern gamagrass cultivars Pete and Iuka mean 28-day germination at initial screening.

Six replications of 100 seed were used for each cultivar
Pre-Strat: 1st germination period: 28-Sept. 2012 – 26-Oct. 2012
1st Stratification period: 26-Oct. – 23-Nov.
Post-Strat1: 2nd germination period: 23-Nov. – 21-Dec.
2nd Stratification period: 21-Dec. – 18-Jan.
Post-Strat2: 3rd germination period: 18-Jan. – 15-Feb.
* Denotes significant difference between bars of the same color
LSD for Pete = 4.18; Iuka = 3.13

To determine if seed viability was an issue, identical replications of Pete and Iuka were stratified for 8 weeks, then germinated in a standard (6 replications of 100 seed each) 28-day germination test (Post Strat1, Figure 5.3). Percentage germination during this period increased for both cultivars, which was expected. This stratified treatment group had mean 28-day percentage germination of 4.0% (Pete) and 10.33% (Iuka). Mean germination of Iuka was significantly greater than germination of Pete (P = 0.0009). This treatment group was also re-evaluated with repeated 28-day stratification/germination

cycles (Post Strat2, Post Strat3, etc.). No further germination was observed from the pre-stratified treatment group following a second stratification.

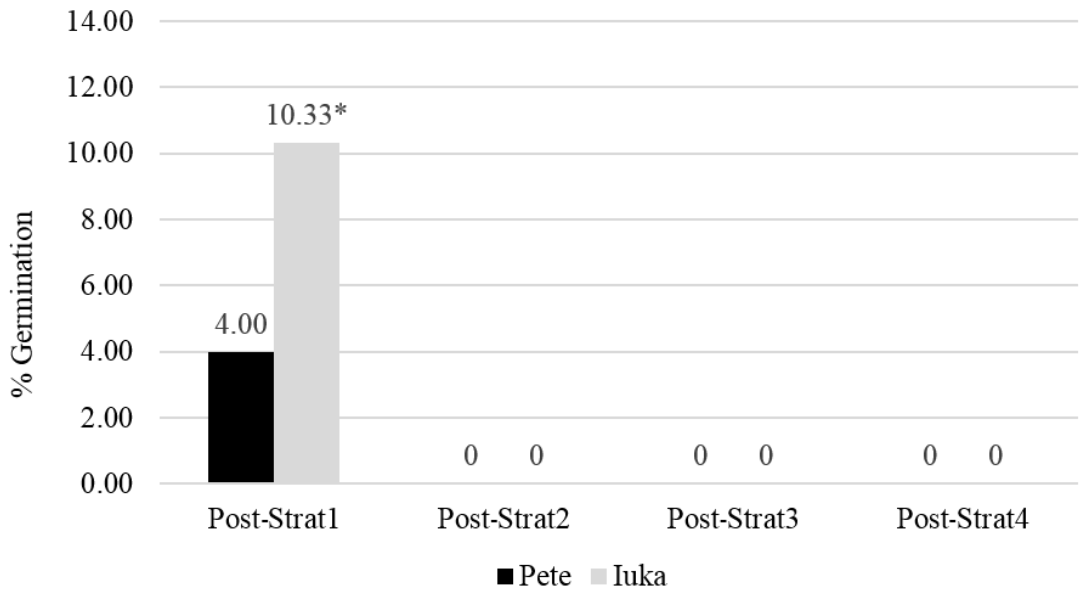

Figure 5.3 Comparison of stratified eastern gamagrass cultivars Pete and Iuka mean 28-day germination at initial screening.

Six replications of 600 seed were used for each cultivar.
1st Stratification period: 31-Aug, 2012
Post Strat1: 1st germination period: 28-Sept. 2012 – 26-Oct. 2012
2nd Stratification period: 26-Oct. – 23-Nov.
Post Strat2: 2nd germination period: 23-Nov. – 21- Dec.
* Denotes significant difference between bars in same cluster (LSD = 1.75)

Due to seemingly abnormally low overall germination percentages from both treatment groups, and in order to develop criteria for phenotypic recurrent selection (PRS), initial assessment data from pre-stratified treatment group were divided by week (Figure 5.4). There were no significant differences observed between weeks for germination percentage nor cumulative germination. Because of the relatively high germination percentage that occurred during the first week of testing (no significant

differences), it was determined that selection criteria for the PRS segment of this experiment would be germination in 7 days or less. It is also due in part to this uneven temporal distribution of germination that most germination tests throughout this experiment focus on the first 14 days of germination, as opposed to the traditional 28.

Mass Screening

Mass screening of all available diploid germplasm began in October, 2012 and continued until February, 2013. During that time, seedlots of Pete, Iuka and 74 diploid individuals from the USDA collection at Cornell University [Ithaca, NY; Courtesy of Denise E. Costich, Head, Maize Germplasm Collection, International Maize and Wheat Improvement Center (CIMMYT) El Batán, Texcoco, Mexico.], were evaluated for rapid germination without prior treatment or stratification. Selection pressure for each cycle of selection was well below 1.0% (Table. 5.2, Table 5.3). All seed that germinated in less than 7 days were not represented in the subsequent established cycle, as some seedlings developed abnormally, and were deemed unfit for use.

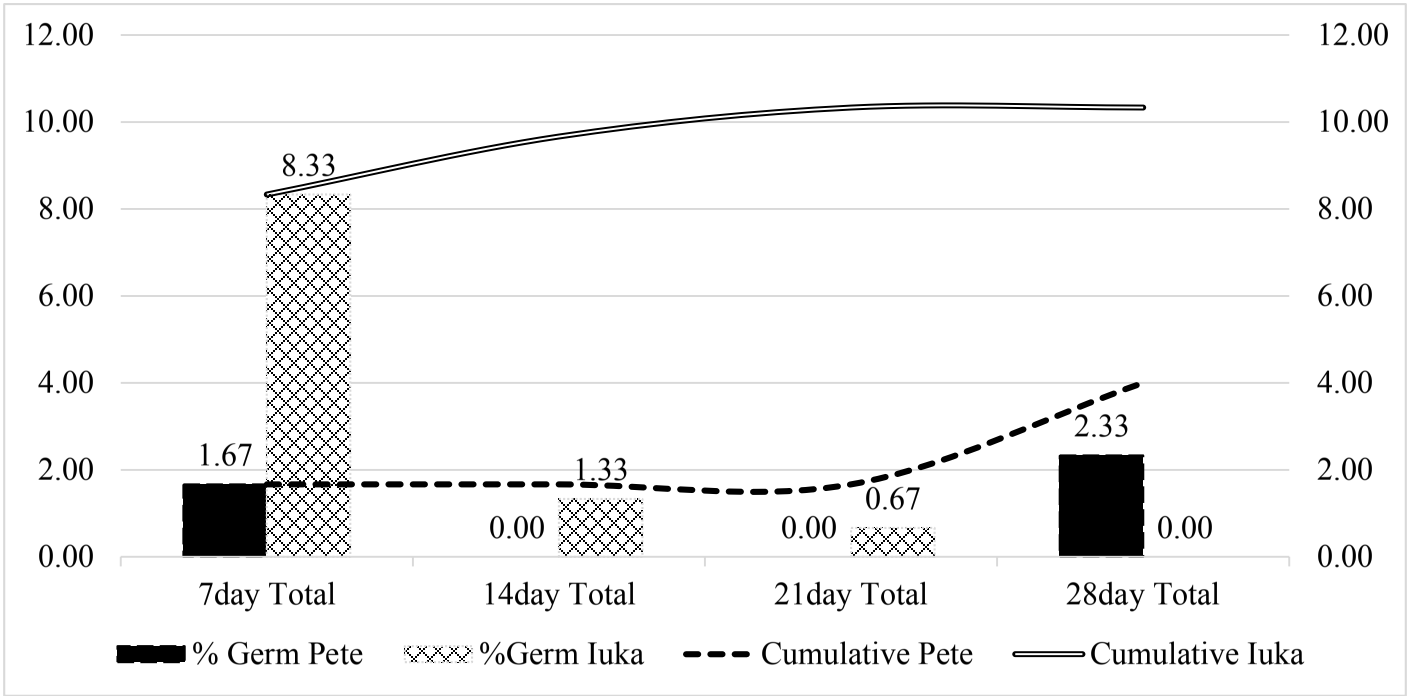

Figure 5.4 Comparison of stratified eastern gamagrass cultivars Pete and Iuka mean 28 day percent germination and cumulative percent germination by week

Six replications of 100 seed were used for each cultivar

Table 5.2 Seedlot size and intensity of selection of eastern gamagrass cultivar Pete seedlot during 2012 mass screening for establishment of phenotypic recurrent selection breeding Cycle1

		Days									
Event	Category	1	2	3	4	5	6	Row Total	Lot Size	Percentage Germination	Selection Pressure
-------	----------	---------Number of seed---------								----%----	-----%-----
1	Germinated					3	5	8	3086	0.26	0.19
	Selected					3	3	6			
2	Germinated					1	9	10	3459	0.29	0.12
	Selected					1	3	4			
3	Germinated					2	6	8	2891	0.28	0.17
	Selected					2	3	5			
4	Germinated				2	3	2	7	3055	0.23	0.16
	Selected				1	2	2	5			
5	Germinated				1	3	13	17	6079†	0.28	0.16
	Selected				0	2	8	10			
6	Germinated					2		2	2971	0.07	0.03
	Selected					1		1			
7	Germinated				2	4	4	10	3057	0.33	0.29
	Selected				1	4	4	9			
8	Germinated				4	5	3	12	3959	0.30	0.30
	Selected				4	5	3	12			
									Σ	$\bar{x}$	$\bar{x}$
Totals	Germinated	0	0	0	9	23	42	74	28557	0.25	0.18
	Selected	0	0	0	6	20	26	52			

† Denotes screening event with two replicates of seedlot

Table 5.3 Seedlot size and intensity of selection of eastern gamagrass cultivar Iuka seedlot during 2012 mass screening for establishment of phenotypic recurrent selection breeding Cycle1

		Days									
Event	Category	1	2	3	4	5	6	Row Total	Lot Size	Percentage Germination	Selection Pressure
-------	----------	----------Number of seed----------								----%----	-----%-----
1	Germinated					3	2	5	3453	0.14	0.09
	Selected					3	0	3			
2	Germinated					2	7	9	3550	0.25	0.14
	Selected					2	3	5			
3	Germinated					2	4	6	3420	0.18	0.12
	Selected					2	2	4			
4	Germinated				1	1	5	7	3376	0.21	0.12
	Selected				1	1	2	4			
5	Germinated					2	3	5	3311	0.15	0.09
	Selected					2	1	3			
6	Germinated					8	0	8	6795†	0.12	0.06
	Selected					4	0	4			
7	Germinated				1	4	3	8	6725	0.12	0.09
	Selected				1	3	2	6			
8	Germinated				2	5	4	11	3624	0.30	0.30
	Selected				2	5	4	11			
									Σ	$\bar{x}$	$\bar{x}$
Totals	Germinated	0	0	0	4	27	28	59	34254	0.18	0.13
	Selected	0	0	0	4	22	14	40			

† Denotes screening event with two replicates of seedlot

Mass screening for establishment of PRS Cycle1 resulted in a total of 62,811 seed from commercial cultivars (Pete = 28,557; Iuka = 34,254) and 5,275 from USDA collections being screened. Data from USDA seedlots is not presented here as there was no germination which met the 7-day criteria established during screening.

Cycle1 Seed Harvest and Selection for PRS Cycle2

Ninety individual eastern gamagrass plants were transplanted in May, 2013 to establish PRS Cycle1. Of those plants, 10 failed to flower in 2013. Establishment year seed harvest yielded 9,410 heavy seed (Table 5.4), of which half (by weight) was allotted for mass screening and selection for the establishment of PRS Cycle2. All seed collected from Cycle1 was bulked, without maintaining parental identity. In total, 5,275 heavy seed from Cycle1 were screened in 2013, resulting in 24 individual seedlings that germinated in less than 7 days. Selection pressure for establishment of Cycle2 was therefore 0.41%.

These 24 individuals were transplanted in the spring of 2014 to establish PRS Cycle2. All but two of the Cycle2 plants flowered during the 2014 season. Seed yields for 2014 showed numerical increases for Cycle0 and Cycle1 (Table 5.4), and a total of 1,766 heavy seed produced by Cycle2.

Cycle2 Seed Harvest and Selection for PRS Cycle3

As with previous studies, half (by weight) of 2014 Cycle2 seed was set aside for PRS selection screening, however, six replications of 100 seed are required in order to conduct proper tests for germination, leaving only a few hundred seed for selection screening. It was decided by the author that use of the entire 2014 Cycle2 seedlot was

not advantageous, so selection screening took place concurrently with germination testing. Any seed from germination test that met 7-day or less selection criteria was bulked with any individuals selected from mass screening and was considered acceptable for PRS breeding.

This act yielded only three germinated individuals from germination testing, and it was decided that the medium fraction of seed from Cycle2 would not be utilized for further PRS screening. These seedlings (Cycle3) were maintained as previously described, but were all lost to damping off during the greenhouse vernalization period. The causal organism was not identified. Because of this, there was no establishment of a Cycle3 crossing block in 2015. Screening for Cycle3 is currently under way for establishment in 2016.

Table 5.4 Seed harvest weight (total and individual fractions) from PRS Cycles during 2013-2015

		2013			2014			2015		
Cycle	Fraction†	Number	Weight‡	Hundred seed weight§	Number	Weight	Hundred seed weight	Number	Weight	Hundred seed weight
Cycle0	Heavy	8131	877	9.71§	9779	1136	8.75	3392	319	9.49
	Medium	1629	57		2744	83		1504	106	
	Light	991	19		2010	54		1695	37	
	Total	10751	953		14533	1273		6591	461	
Cycle1	Heavy	9410	1008	7.89	14875	1408	9.56	1060	107	9.22
	Medium	442	22		3816	146		751	52	
	Light	619	6		1280	28		1100	28	
	Total	10471	1036		19971	1582		2911	186	
Cycle2	Heavy				1766	160	6.76	5139	594	11.51
	Medium				1226	46		2191	164	
	Light				710	20		2472	63	
	Total				3702	226		9802	820	

† Fractions as determined by forced air fractionating aspirator.
‡ All weights measured in grams
§ Mean of ten replications of one hundred seed

Germination Screening

2014

Germination screening for 2014 seedlots from Cycles 0, 1 and 2 began in November, 2014. This germination screening represented the second year of seed production for Cycles 0 and 1, and the first year of production (establishment year) for Cycle2. In this screening (Figure 5.5, Table 5.5), the statistical model (General Linear Model) showed no significant effect due to cycle of selection on any of the germination parameters at 7 DAI. There were also no significant effects on mean daily germination percentage ($P = 0.091$) after 14 days, however, cycle of selection did significantly affect cumulative germination ($P = 0.026$) and mean total germination percentage ($P < 0.0001$) after 14 days. At 28 DAI, mean cumulative germination and mean total germination each showed significant effects due to cycle of selection ($P < 0.0001$, $P = 0.0141$, respectively) though mean daily germination was not affected ($P = 0.3328$, Table 5.5).

Using Fisher's LSD, a significant increase in mean daily germination percentage and cumulative germination between Cycle0 and Cycle2 was observed at 14 days, but neither Cycle0 nor Cycle2 were statistically different from Cycle1 in either measurement criteria after 14 days of germination. After 28 days, there were no differences in mean daily germination, however, Cycle1 and Cycle2 had significantly greater mean cumulative germination than Cycle0 and Cycle2 had a significantly greater mean total germination than either Cycle0 or Cycle1.

Table 5.5 Germination testing results for seedlots harvested from phenotypic recurrent selection breeding Cycles0, 1 and 2 during the 2014 seed production season.

	7 DAI			14 DAI			28 DAI		
	Germination Percentage								
Seedlot	Mean Daily	Mean Cumulative	Mean Total	Mean Daily	Mean Cumulative	Mean Total	Mean Daily	Mean Cumulative	Mean Total
	----%---	-----%-----	---%---	----%----	-----%-----	----%----	----%----	-----%-----	----%----
Cycle0	0[† NS]	0[NS]	0.17[NS]	0.05[b]	0.26[b]	0.33[b]	0.09[NS]	1.08[b]	3.17[b]
Cycle1	0.11	0.13	0.17	0.21[ab]	1.19[ab]	0.17[b]	0.19	2.91[a]	4.33[b]
Cycle2	0.03	0.06	0.50	0.33[a]	1.66[a]	4.83[a]	0.19	3.41[a]	6.83[a]
LSD (0.05)	0.17	0.20	0.72	0.25	1.02	0.71	0.16	0.96	2.35

Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) based on Fisher's LSD using SAS PROC GLM.

[NS] Means within a column were not significantly different ($P \leq 0.05$) based on Fisher's LSD using SAS PROC GLM.

When analyzing germination data at 28 DAI, the trend continued that cycle of selection had no significant effect on mean daily germination percentage (P=0.332), but had a significant effect on cumulative germination (P<0.0001), with Cycle1 and Cycle2 showing significantly greater cumulative germination percentage than Cycle0. Based on the response to selection, the realized heritability (h^2) of mean percent germination for Cycle2 was calculated as 0.05 in 2014 seed production year. Realized heritability for Cycle1 and Cycle0 were 0.01 and 0.02, respectively. Generally, h^2 values < 0.01 are considered low, while $h^2 > 0.6$ are considered high. The calculated heritability values for mean percent germination in Cycles 0, 1 and 2 show that the offspring of the selected parents vary little from the original population. It can be argued that the value of this measurement is not significant at such an early stand age. Published realized heritability for selection of morphological characteristics in other North American Native species have ranged from 0.20 (switchgrass seed yield), 0.23 (switchgrass seedling tiller number), 0.26 (big bluestem seedling tiller number), 0.55 (switchgrass high in vitro dry matter degradability), 0.56 (switchgrass forage index), 0.58 (switchgrass seed weight, cv. 'Summer'), 0.59 (switchgrass low in vitro dry matter degradability), 0.62 (switchgrass rust index), 0.88 (switchgrass seed weight, cv. 'Sunburst') 0.92 (switchgrass late panicle emergence), 1.00 (switchgrass early panicle emergence) (Eberhart and Newell, 1959; Newell and Eberhart, 1961; Vogel et al., 1981; Van Esbroeck et al., 1998; Boe, 2003; Smart et al., 2003).

The data sets used in these analyses contain several cells with values less than 5.0, which can make categorical relationship (chi-squared) analysis troublesome. For that reason, these data were also analyzed using Fisher's Exact Test, which uses no

assumption of categorical data value. These results suggest that there was a statistically significant relationship between cycle of selection and germination percentage at 14 days ($P = 0.0015$), however, there is no difference at 28 days ($P = 0.2993$). Note that the Fisher's exact test does not have a "test statistic", but computes the P-value directly.

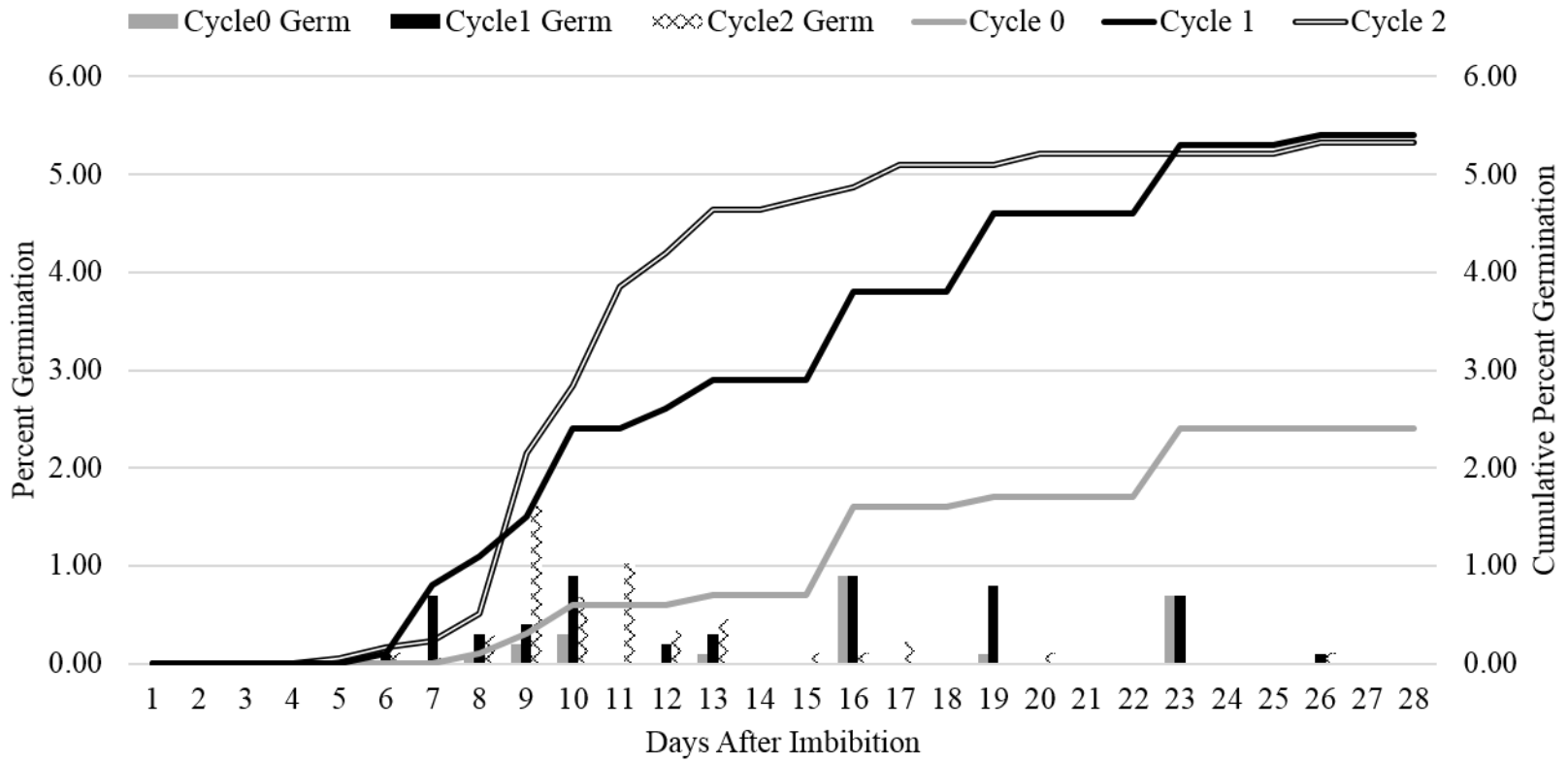

Figure 5.5 Daily germination percentage and cumulative germination percentage for standard 28-day germination test of 2014 seedlots harvested from phenotypic recurrent selection breeding cycles 0, 1 and 2.

Columns represent percentage germination (left axis) by day after imbibition (DAI). Lines represent cumulative percentage germination (right axis).

2015

Germination screening for 2015 seedlots from Cycles 0, 1 and 2 began in November, 2015. This germination screening represented the third year of seed production for Cycles 0 and 1, and the second year of production for Cycle2. There was a statistically significant ($P< 0.0001$) difference in percent daily germination, cumulative germination and mean total germination from 2014 seed production year to 2015 production year. As such, data were analyzed separately.

In this screening (Figure 5.6, Table 5.6), the statistical model (General Linear Model) showed no significant effect due to cycle of selection on mean daily germination percentage ($p = 0.0829$) after 14 days, however, cycle of selection did significantly affect ($P < 0.0001$) cumulative germination after 14 days. Cycle of selection also had a significant effect on mean total germination at 7 ($P = 0.147$), 14 ($P < 0.0001$) and 28 ($P < 0.0001$) DAI. This difference in effect is explained by the fluctuations in daily germination percentages, specifically days that record zero germination.

Means separation using Fisher's LSD showed no significant differences in mean daily germination at 7 DAI, however, Cycle2 mean daily germination was significantly greater than Cycle0 at 14 and 28 DAI (Table 5.6). Cycle2 also showed significant increases in cumulative germination percentage at 7, 14 and 28 DAI, as compared to Cycles 0 and 1. This trend was reflected in mean total germination also, where Cycle2 had significantly greater mean total germination at 7, 14 and 28 DAI in contrast to Cycle0 and Cycle1, which were not significantly different from one another at either time period.

Table 5.6 Germination testing results for seedlots harvested from phenotypic recurrent selection breeding Cycles0, 1 and 2 during the 2015 seed production season.

	7 DAI			14 DAI			28 DAI		
	Germination Percentage								
Seedlot	Mean Daily	Mean Cumulative	Mean Total	Mean Daily	Mean Cumulative	Mean Total	Mean Daily	Mean Cumulative	Mean Total
	---%---	-----%-----	---%---	----%----	-----%-----	---%---	---%---	---%---	---%---
Cycle0	0.0$^{\dagger NS}$	0.0^{b}	0.0^{b}	0.14^{b}	0.50^{b}	2.0^{b}	0.21^{b}	2.18^{b}	6.00^{b}
Cycle1	0.07	0.40^{b}	0.5^{b}	0.26^{ab}	1.41^{b}	3.67^{b}	0.27^{ab}	3.69^{b}	7.67^{b}
Cycle2	0.52	1.78^{a}	3.67^{a}	0.81^{a}	4.85^{a}	11.33^{a}	0.60^{a}	9.41^{a}	16.83^{a}
LSD (0.05)	0.67	1.07	2.51	0.62	1.89	2.07	0.36	2.14	3.42

†Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) based on Fisher's LSD using SAS PROC GLM.

^{NS} Means within a column were not significantly different ($P \leq 0.05$) based on Fisher's LSD using SAS PROC GLM.

Analysis of full 28-day germination testing indicated that cycle of selection had no significant effect on the statistical model for mean daily percent germination ($P = 0.0775$), but did significantly affect cumulative germination ($P < 0.0001$) over three cycles of selection. Mean daily percent germination for Cycle2 seedlot was significantly greater than Cycle0, but neither Cycle was significantly different from Cycle1. Cycle2 had a significantly greater mean cumulative germination over the 28-day period than either Cycle 0 or 1. Realized heritability was calculated for Cycle0, 1 and 2 for the 2015 seed production year using Equation 5.1. Realized heritability for Cycle2 was calculated as 0.12, which is more than a two-fold increase from 2014 to 2015. Realized heritability for Cycle1 and 0 were 0.04 and 0.03, respectively.

Analysis of these data sets using Fisher's Exact Test, suggest that there was a statistically significant relationship between cycle of selection and germination percentage at 14 DAI ($P = 0.0034$), as well as at 28 DAI ($P = 0.0370$). Note that the Fisher's Exact Test does not have a "test statistic", but computes the P-value directly.

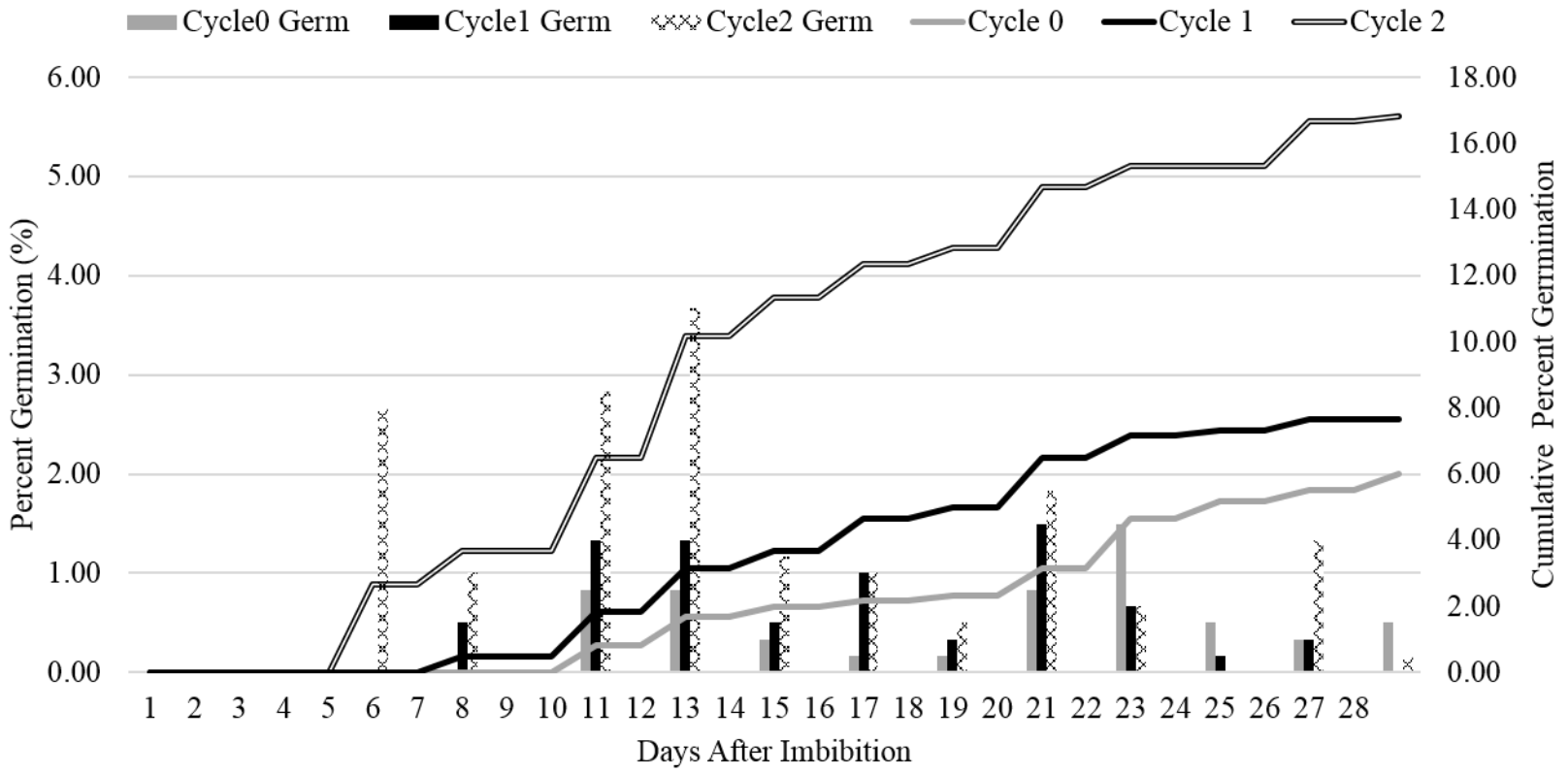

Figure 5.6 Daily germination percentage and cumulative germination percentage for standard 28-day germination test of 2015 seedlots harvested from phenotypic recurrent selection breeding cycles 0, 1 and 2.

Columns represent percentage germination (left axis) by day after imbibition (DAI). Lines represent cumulative percentage germination (right axis).

CHAPTER VI

RESEARCH EXPERIMENT 3: HYDRATION AND DESICCATION EFFECTS ON GERMINATION OF EASTERN GAMAGRASS SEED

Introduction

Diploid eastern gamagrass [*Tripsacum dactyloides* (L.) L.] represents a reliable candidate for selection breeding via sexual recombination. Abundant research has been done to elucidate specific characteristics of physical dormancy associated with eastern gamagrass seed units. Those studies have proven that large amounts of force are required to open the cupule, and that cold, moist stratification works to weaken the seed case, thus improving chances for seedling germination and establishment. The practice of cold, moist stratification may be the industry standard for seed production, but may also be detrimental to the future of the species in a genetic sense.

As generation upon generation of eastern gamagrass seed is produced and subjected to cold, moist stratification prior to germination, common sense dictates that only those seed capable of withstanding such treatment will survive to planting, and potentially produce the next generation seed crop. This is less a hazard in a perennial crop, but a hazard all the same if withstanding 6-8 weeks of cold, moist stratification (and responding to such stimuli) is hereditary. This raises the question if there are other methods by which the eastern gamagrass cupule can be manipulated in order to

successfully increase germination and establishment, without causing major disruption to the genetic pool.

Objectives

During previous germination experiments, the author observed that after a period of 7 days in a controlled environment chamber, mass screening trays, if allowed to dry slowly, at room temperature (20°C), would show flushes of germinating seed, inconsistently elevated from the germination rates exhibited by analogous trays kept in moistened conditions.

It is hypothesized that structural destabilization of the glume-rachis interface during prolonged drying periods may allow for easier or more rapid radicle or coleoptile emergence. If this is true, it may be possible to remove one of the standard treatments (i.e. cold temperatures, or prolonged hydration) as an inadvertent selection factor and eliminate its impact on the future of eastern gamagrass genetic variation.

The primary objective of this study was to investigate the effects of hydration and subsequent drying on the eastern gamagrass cupule and to define what associations those effects may have with percent germination of a particular seed lot.

Materials and Methods

Seed Imbibition

To test these hypotheses, a preliminary study was designed to establish the time to complete imbibition of eastern gamagrass seed. Previous research suggests that seed with permeable to semi-permeable membranes are fully hydrated within 24 hours (in high water potential environments) (Bewley and Black, 1994). This, of course fluctuates

depending on many factors, mainly species, water source and seed contact with hydrating medium. In a laboratory, analogous 0.6 x 0.6 m stainless steel trays lined with two thicknesses of regular, 17.2 kg creped seed germination paper (Anchor Paper Company; St. Paul, MN), with a single 288-cell seedling plug liner on top of the paper toweling were used to evaluate eastern gamagrass seed imbibition while ensuring the ability to maintain seedlot identity.

Individual seed from four varieties, two tetraploids (Meadowcrest, San Marcos) and two diploids (Iuka, Pete) were weighed and placed in individual cells of each 288-cell plug liner. Trays contained one liner each, liners were 12 cells wide by 24 cells long. Each individual cell measured 1.9L x 1.9W x 2.8H cm and liners were perforated on the bottom to allow water to infiltrate each cell evenly. Each 24-cell row was considered a replication. Trays were initially wetted with enough distilled water to cover the seed in the bottom of the liner cells by half. Seed trays were covered with a 3mm clear Lucite plate, placed on a bench top in the laboratory and allowed to imbibe for 96 hours.

Seed were weighed every 24 hours to quantify overall water content and percent weight gain. Seed were removed from cells, weighed, and returned to cells in a one-at-a-time fashion to reduce any potential for water loss due to evaporation. After removal, seeds were lightly wiped with a Kimwipe™ (Kimtech Science, Kimberly-Clark Professional, GA) to remove surface moisture, placed in a weigh boat inside an enclosed analytical balance, weighed and quickly returned to tray.

Ten seed of each variety were placed in each replicated row. Study was allowed to continue until complete hydration (< 3% weight gain per day) level was reached. This

study was repeated three times over the period of 60 days, each replication using the same seedlots.

Hydration and Drying Effects on Germination

Following results of seed imbibition study, it was evident that cultivar had a significant impact on total percentage seed weight gain during imbibition, and that all cultivars began a significant decline in rate of imbibition (as expressed in percentage of original seed weight gained per 24 hours) from 24 hours until the period between 48 and 72 hours after imbibition began. Using this information, another study was designed to test the hypothesis that a short period of hydration (7 days) followed by drying would initiate germination response in eastern gamagrass seed without prior cold, moist stratification.

This experiment utilized three cultivars of eastern gamagrass, two diploids (Pete, Iuka) and one tetraploid (Nacogdoches). This experiment was designed in similar fashion to the imbibition study, using analogous 0.6 x 0.6 m stainless steel trays lined with two thicknesses of regular, 17.2 kg creped seed germination paper, with a single-288-cell seedling plug liner on top of the paper toweling. In total, 6 trays were used in this study for each treatment combination of cultivar and level of hydration. In wet treatment (T_W), trays were maintained in a hydrated state during 7-day incubation period, and, when removed from germination chamber, remained hydrated throughout the duration of this study, similar to conditions during a normal germination screening. For dry treatment (T_D), trays were maintained in a hydrated state for 7 days and were then removed from germination chamber and allowed to dry down naturally at room

temperature (20°C) with no additional hydration following removal from germination chamber.

Individual seed were weighed and placed in individual cells of each 288-cell plug liner. Trays contained one liner each, liners were 12 cells wide by 24 cells long. Each individual cell measured 1.9L x 1.9W x 2.8H cm and liners were perforated on the bottom to allow water to infiltrate each cell evenly. Each 24-cell row was considered a replication. Twenty four seed of each variety were placed in each replicated row, one seed per cell. Trays were initially wetted with enough distilled water to cover the seed in the bottom of the liner cells by half. Seed trays were covered with a 3mm clear Lucite plate, placed in an artificial condition chamber set to 25°C/20 °C (day/night) with 8hr light for 7 days. This study was replicated twice in the period of 6 months due to very limited seed availability, and different seedlots were used for the two replications.

Seed were monitored for germination daily for a standard period of 28 days from beginning of imbibition (7 days while in artificial growth conditions, 21 days after removal from chamber). Seed were weighed every 24 hours for a period of 72 hours to quantify overall water content and percentage weight gain. Seed were removed from cells, weighed, and returned to cells in a one-at-a-time fashion to reduce any potential for water loss due to evaporation. After removal, seeds were lightly wiped with a Kimwipe™ to remove surface moisture, placed in a weigh boat inside an enclosed analytical balance, weighed and quickly returned to tray.

During 28-day monitoring period, any seed found to be germinated (root radicle or coleoptile emerged from cupule) was removed from cell, wiped dry and weighed to determine total seedling water content.

Unless otherwise stated, all statistical analyses were conducted using Proc GLM and Proc CORR in SAS statistical analysis software at the $\alpha = 0.05$ level of significance.

Results and Discussion

Seed Imbibition

Starting seed weights were significantly affected by cultivar ($P < 0.0001$) and date of study ($P = 0.0029$), however, there was no significant cultivar*date interaction ($P = 0.067$). The cultivar Meadowcrest had significantly heavier dry seed weights to begin the study, and, as such, continued to have consistently heavier ($P < 0.0001$) seed than the other cultivars at 24, 48 and 72 hours after imbibition (Table 6.1).

Table 6.1 Mean eastern gamagrass seed weight and percentage weight gain at 0, 24, 48 and 72 hours after imbibition.

	Time (hours) after imbibition						
	0	24		48		72	
	Mean seed weight†	Mean seed weight	Percent weight gain	Mean seed weight	Percent weight gain	Mean seed weight	Percent weight gain
Meadowc.	0.0903‡[a]	0.1402[a]	57.5[NS]	0.1446[a]	3.5[a]	0.1463[a]	1.2[b]
San Marc.	0.0832[b]	0.1289[b]	56.5	0.1330[b]	3.6[a]	0.1348[b]	1.5[b]
Pete	0.0836[b]	0.1307[b]	58.1	0.1332[b]	2.1[b]	0.1352[b]	1.6[b]
Iuka	0.0774[c]	0.1199[c]	56.5	0.1220[c]	1.9[b]	0.1250[c]	2.6[a]
LSD ($\alpha = 0.05$)	0.0045	0.0067	4.77	0.0066	0.93	0.0066	0.71

† All weights in grams
‡ Values within column with same letter are not significantly different
[NS] Values in column are not significantly different

Statistically, percent weight gain over time did not follow the same trend as mean seed weight, as there was no significant effect on the model due to cultivar at 24 hours after imbibition ($P = 0.8959$) or in total percentage weight gain ($P = 0.9509$)(data not

shown). There was no effect due to replication (row) in mean seed weight at any time period, however, there was a significant effect due to replication (row) on percent weight gain at 48 hours (0.0305) and for total percent weight gain (P = 0.0202). Effect of replication (date) was significant for every time point and seed weight/ percent gain measurement except for percentage weight gain at 72 hours (P = 0.5210).

When divided by replication (date), there was a significant effect due to cultivar on seed weights at all time points in date 1 a single time point in date 2, and no effect of cultivar on seed weights in date 3 (Table 6.2). There was a significant effect of cultivar on percentage weight gain at one time point in date 1 (48 hours), two time points in date 2 (48 and 72 hours) and no effect on percentage weight gain in date 3.

Table 6.2 Mean eastern gamagrass seed weight and percentage weight gain at 0, 24, 48 and 72 hours after imbibition.

	Time (hours) after imbibition						
	0	24		48		72	
	Mean seed weight[†]	Mean seed weight	Percent weight gain	Mean seed weight	Percent weight gain	Mean seed weight	Percent weight gain
Date 1							
Meadowc.	0.0954[‡a]	0.1408[a]	51.9[NS]	0.146[a]	4.0[a]	0.1480[a]	1.3[b]
San Marc.	0.0787[b]	0.1188[b]	52.1	0.1232[b]	4.9[a]	0.1248[b]	1.3[b]
Pete	0.0769[b]	0.1178[b]	57.8	0.1206[b]	1.8[b]	0.123[b]	1.9[ab]
Iuka	0.072[b]	0.1071[c]	50.8	0.1105[c]	3.2[b]	0.1135[c]	2.7[a]
LSD ($\alpha = 0.05$)	0.0078	0.0096	9.45	0.0096	1.96	0.0098	0.71
Date 2							
Meadowc.	0.0915[a]	0.1459[a]	60.3[NS]	0.1497[a]	2.8[a]	0.1513[a]	1.2[b]
San Marc.	0.0871[ab]	0.136[ab]	57.4	0.1394[a]	2.8[a]	0.1409[a]	1.3[b]
Pete	0.0898[a]	0.1434[a]	59.3	0.1442[a]	0.6[b]	0.1461[a]	1.5[b]
Iuka	0.0810[b]	0.1259[ab]	56.7	0.1268[b]	0.7[b]	0.1306[b]	3.2[a]
LSD ($\alpha = 0.05$)	0.0074	0.0127	7.79	.0125	1.21	0.0122	1.18
Date 3							
Meadowc.	0.084[NS]	0.133[NS]	61.9[NS]	0.138[NS]	3.6[a]	0.139[NS]	3.61[a]
San Marc.	0.0837	0.1328	60.5	0.1364	2.9[ab]	0.1387	2.8[ab]
Pete	0.0848	0.1298	60.1	0.1346	3.7[a]	0.1360	3.7[a]
Iuka	0.0790	0.1265	57.0	0.1287	1.8[b]	0.1310	1.8[b]
LSD ($\alpha = 0.05$)	0.0078	0.0115	7.57	0.0115	1.46	0.0116	1.46

[†] All weights in grams

[‡] Values within column with same letter are not significantly different

Increased seed weight is often positively correlated with germination and establishment, and seed or caryopsis weight is commonly used as a measure of seed quality. Seed weight is affected by cultivar, year and location of production, and variation in seedlot can be substantial (Boe, 2003, Finneseth, 2010). It is generally found that seed count per unit weight and ploidy level share an inverse relationship, (as ploidy level increases, number of seed kg^{-1} decreases). This rule was evident with the cultivar

Meadowcrest, which significantly outweighed other cultivars on numerous occasions before and during the imbibition study. However, this rule did not hold true throughout imbibition studies, as evidenced by tetraploid cultivar San Marcos and diploid cultivar Pete, which were consistently chained together statistically in weight categories (Table 6.2).

The standard commercial practice of cold, moist stratification of eastern gamagrass seed is still the most reliable way to counter the effects of physical and physiological dormancy on germination. When planted dry, the hard, woody cupule of eastern gamagrass represents a potential barrier to seed water uptake and coleoptile and/or radicle emergence that the majority of other forage grass species do not encounter. These studies have shown that appreciable imbibition for eastern gamagrass is reached between 48 and 72 hours after imbibition begins. In the future, with a more stable supply of reliably germinating seedlots, producers can potentially bypass negative field conditions, hydrate seedlots for 48-72 hours, then plant at their leisure. This allows potential eastern gamagrass producers to secure a dry seedlot (without paying for shipping wet seed) and hydrate it prior to planting, without a 6-8 week waiting period.

Hydration and Drying Effects on Germination

Similar to initial results in imbibition study, there was a significant effect ($P < 0.0001$) due to variety on beginning seed weight in this experiment, with Nacogdoches, a tetraploid cultivar, significantly outweighing Iuka and Pete (diploid cultivars). There was no effect due to replication (row) ($P = 0.0916$) on seed weight at the onset of the experiment. After 72 hours, Nacogdoches seedlot had a significantly greater ($P < 0.0001$)

percentage seed weight gain (63.31%) than either diploid cultivar (36.16% Iuka, 40.14 Pete) (LSD = 3.559).

In total, 21 seed germinated from the six experimental trays during the study. There was a significant effect (P = 0.0159) due to replication (date) on beginning seed weight, as well as overall percentage germination. Nineteen seed germinated during the first experiment (14 from dry treatment (T_D) and five from wet treatment (T_W)). During the second experiment, only two seed from T_W germinated during the 28-day period. None of these results were significantly different at $\alpha = 0.05$ level of significance.

As has been documented in multiple research reviews, using multiple seedlots in germination-based studies can be problematic, especially where native species are concerned (Blake, 1935; Cole and Johnston, 2006; Finneseth, 2010). Seedlot variation can be extreme in some cases. New seedlots of each cultivar were used for the imbibition and drying studies, solely due to extremely limited seed availability. To illustrate seedlot variability, the diploid cultivar Pete often had mean seed weights that were not significantly different than the tetraploid cultivar San Marcos (Table 6.1). This is unusual, as tetraploid varieties are well documented for producing larger seed (as evidenced by lesser seed per kg values). This brings into question the purity and ploidy level of each seedlot utilized in these studies. Seedlots were procured from the Gamagrass Seed Company (Iuka), Johnston Enterprises (Pete) and East Texas Plant Materials Center (Nacogdoches, San Marcos). Considerable care was taken to avoid statistical pitfalls associated with variation in seedlot quality, however, because of gross disparity among cultivars, sources and subsamples from individual seedlots, evaluated criteria results are statistically confounding.

CHAPTER VII

SUMMARY

Observational notes on geographic distribution of ploidy in eastern gamagrass have been published from Anderson (1944) to Newell and de Wet (1974) and Dunfield (1986) among others. Most hypothesize that the extent of diploid gamagrass populations in the United States is limited to the Great Plains region. They generally agree that the Atlantic seaboard is dominated by tetraploid genotypes. There had been no real botanical collections including the southeast region until this study. Obviously, germplasm collections would have been redirected had it been evident during the course of collection that ploidy variation among collected genotypes was so low. There are confirmed diploid populations in Florida and Texas (Newell and de Wet, 1974; de Wet et al., 1982), and published discernable morphologic characteristics of diploid and tetraploid populations (Dunfield, 1986, Kindiger et al, 1996a, 1996b) which were used during collection. Additionally, given the extremely diverse phenotypic characteristics of many populations encountered during the germplasm collection, it was generally accepted that some portion of the collection would, in fact, be diploid. It was discovered that published morphological characteristics that can be used to differentiate diploid from polyploid individuals, such as leaf width, leaf color and earliness of spring growth did not hold true for eastern gamagrass individuals found in the southeastern and Atlantic states.

In evaluating germplasm collection for ploidy level using flow cytometry, there were few findings involving confirmation of tetraploid level in wild-type gamagrass individuals that were unusual. The tetraploid eastern gamagrass standard used for comparison during cytological analysis was karyotyped by an independent company (Agrigensol, Córdoba, Spain) and confirmed tetraploid. When compared using flow cytometry to an eastern gamagrass diploid standard (which was confirmed diploid by the same company and USDA ARS, Cornell, NY), the two individuals align as expected, with the tetraploid sample mean FL2 reflectance value at approximately double that of the diploid standard. Of the collection, there are twenty individuals that will be further evaluated for ploidy level. These individuals fall outside of the acceptable window for ploidy confirmation calculated as [$\bar{x}$ tetraploid control FL2-A reflectance value) ± 10%]. Four of these individuals were analyzed as greater than tetraploid, while 16 were analyzed as lesser than. Multiple replications of each individual must be analyzed before any determination can be made confirming aneuploidy.

Two generations of phenotypic selection breeding was successful in generating eastern gamagrass seedlots that germinate more reliably and vigorously without stratification. Significant increases in mean daily germination percentage as well as overall cumulative germination percentage were made after only two cycles of selection. Even though, germination percentages are still too low to be considered satisfactory for use as a modern commercial gamagrass seed source. The realized heritability calculations prove that while little of the variance in the starting population was attributable to additive genetic variation, there is real gain yet to be made in response to selection pressure for more rapid germination without prior stratification.

As evidenced by the results of this selection breeding experiment, the gains made in two cycles of selection breeding are strong enough to necessitate the continuation of this research. The utility of eastern gamagrass in forage use scenarios cannot be argued against, especially given the current trends of climatic extremes becoming more commonplace. Eastern gamagrass is proven to be drought tolerant and high yielding in a variety of production scenarios across the southeast including high temperatures and low rainfall. With increased germination rates (whether measured as velocity of germination or total germination), the majority of which are occurring at ≤ 14 days (Figure 5.6), climatic impacts on germination and establishment can be minimized. This would make eastern gamagrass arguably the most versatile North American native grass species offered today.

The question remains whether the same gains in improvement breeding are possible when dealing with a polyploid gamagrass population. Selection for increased velocity of germination and increased germination percentage have been successful with other polyploid species, specifically polyploid native grasses (Jones, 2004, Burson et al., 2009). However, selection for increased velocity of germination or precocious germination in polyploid eastern gamagrass has not yet been reported. It is assumed that apomictic traits in polyploid populations will negate any attempts at making appreciable gains due to sexual recombination and additive trait selection.

The major drawback to eastern gamagrass is a combination of poor seed quality and germination percentage, combined with a marked lack of convenience afforded to the potential seed buyer. Cold, moist stratified seed, which is the market standard today, must be shipped or otherwise transported from the seed producer to the buyer and either

maintained cold and wet, or planted immediately to avoid damaging emerging root radicles and coleoptiles as temperatures rise and seed begin to germinate in the transportation vessel. In most situations, this distinct hindrance outweighs the potential benefits provided by eastern gamagrass to the end user. Cost is another major drawback to adopting eastern gamagrass into a production scenario. Currently, internet-based seed prices range from $14.80 – $18 per pound (February, 2016).

The forage industry and producers would benefit from further breeding programs focused on improving diploid populations of eastern gamagrass, specifically those that target increasing retention of seed, increasing number of seed per plant, and identifying any benefits achievable by deliberately introducing pollinator species in seed production fields. When compared to other grasses, the staggeringly low amounts of seed produced per eastern gamagrass plant make retaining harvestable seed of optimum importance. There has been no published report concerning the importance of seed size in eastern gamagrass as it relates to germination or establishment. Compound with that the alarmingly low germination percentages in these studies, regardless of seed size, and it appears to be a trait of little importance currently.

As Cycle0 and Cycle1 became more established stands, seed quality and germination increased. This is not uncommon, and can be expected from Cycle2 and future seed production blocks as they establish further. There has not yet been a noticeable physical change in the cupulate fruit case of the eastern gamagrass seed harvested from PRS breeding blocks that would explain the significant increase in germination from Cycle0 to Cycle1 and Cycle2. The most encumbering facet of the cupule is the rachis-glume interface, which, at its apex, must be manipulated to create

enough volume to allow the expanding embryo and coleoptile to emerge in order to complete germination. Without altering this specific region of the seed unit's physical characteristics, the only other feasible option is to alter the glume as a whole unit. The hypothesized potential drawbacks from this deliberate alteration of the physical form of the cupule are decreased seed viability and storage time and, as noted by Dewald and Kindiger (2000) predation.

The possible inadvertent phenotypic effects of selection for rapid, early germination may include the lessening of concentrations of endogenous anti-germination chemicals and phytohormones like abscisic acid (ABA) and ethylene, cytokinins, or brassinosteriods in the seed. For each standard (6 replication of 100 seed) germination test performed in the evaluation of these seed production cycles (Table 5.5, 5.6), a companion group of 600 seed have been cold, moist stratified for 8 weeks, and then germinated in the same manner as the untreated seedlots. Germination percentages of Cycle2 were numerically higher than Cycle0 and Cycle1 in 2014 and 2015 seed production year (data not presented). Further work will be required in this study to attempt to prove a phenotypic shift toward physical or physiological dormancy reduction through selection.

Significant phenotypic variation is prevalent in eastern gamagrass populations, regardless of geographic region or chromosome number. Variation is expressed in a variety of ways and is quantifiable, but this variation is no better evidenced than in the case of quantity and quality of seed produced, which can be judged by the quantity and quality of commercial seed available for purchase. Commercial seed sources for eastern gamagrass are few, and seedlot quality is not of paramount importance. To exacerbate

the problem, many current sources for seed do not provide information on Pure Live Seed (PLS) or Pure Actual Germination (PAG) for marketed seedlots.

In a search of the literature cited in this document, when referring to characteristics of eastern gamagrass seed, the word "inconsistent", or one of its variants, appears over 400 times, and nearly 70 times in one single document related to eastern gamagrass seed quality and germination. Inconsistencies associate with eastern gamagrass laboratory germination protocol, seed treatments (chemicals, hormones), seedlot quality descriptions (PLS PAG), pollen shed, pistil receptivity, seed ripening and dehiscence, emergence and establishment, laboratory germination results, seedlot quality, response in laboratory testing, response to seed stratification and other treatments have been well documented in the literature. This research is no different, and because of this, the need for a stable, dependable, desirable seed source of eastern gamagrass is more important now than ever before.

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APPENDIX A

PROTOCOL FOR PREPARING PLANT SAMPLES FOR EVALUATION OF NUCLEAR DNA USING FLOW CYTOMETRY

Polyploidy assessment in plants using Flow Cytometry

From: 05-5022 CyStain® PI Absolute P
05-5022_Product Data Sheet_Rev 005_2011-08-11
Partec GmbH, D-48161 Münster, Germany

Principle

CyStain PI absolute P is a reagent kit for nuclei extraction and DNA staining of nuclear DNA from plant tissues in order to determine absolute or relative genome size and ploidy level. CyStain PI absolute P is well suited for the preparation of nuclear DNA staining of plenty of different plant species and a variety of different tissues. Samples can be analyses with standard flow cytometers with 488, 514 or 532 nm laser excitation or with mercury arc lamp flow cytometers using green excitation.

Equipment and Reagents

Machine/Product	**Reference (Company, Type, …)**
Crushed Ice	
CyStain® PI Absolute P Kit *This kit contains:* • *Propidium Iodide (PI) stock solution* • *RNase stock* • *Staining Buffer solution* • *Extraction Buffer solution*	Partec 05-5022 CyStain® PI Absolute P
Razorblades	
55 mm plastic Petri dishes	
CellTrics 50µm filters	Partec 04-0042-2317 50 µm CellTrics Filters
3.5 ml test tubes (12x75 mm)	
Pipettes	
Flow Cytometer	Accuri C-6

Preparing RNase stock solution:

- **RNase stock solution:** Add 1.5 ml H2O to 5 mg RNase (Eppendorf tube) and mix well.
- **Staining solution:** Mix 2.0 ml Staining Buffer per sample with 12 µl of PI stock solution and 6 µl of RNase stock solution. E.g. to prepare 10, mix 20 ml of staining Buffer with 120 µl of PI stock solution and 60 µl of RNase Stock solution. The staining solution with PI is stable for one day.
- Storage of the reagents:

Extraction Buffer	4°C
Staining Buffer	4°C
PI Stock Solution	4°C (protected from light)
RNase stock solution	-20°C (allow to warm up to RT before use.)

GENERAL NOTE: If using samples with little plant material (like Lemna, or just one Arabidopsis leaf), you can use 250 µl Extraction buffer and stain with 1.0 ml staining buffer to obtain the same results.

Nuclei isolation and staining procedure:

- Due to the big variations, make sure to have at least 6 replicates for each sample.
- Put approximately 0.5 cm² (or less) of leaf tissue or other plant material in a 55 mM plastic Petri dish
- Add 250 µl Extraction buffer
- Chop the material using a sharp razor blade for 30 to 60 seconds. Do not over-chop! 2 samples can be done with each side of a razor blade.
- After chopping, incline the Petri dish and collect all plant material and solution to one side using a pipette ***NOTE***: Chopping yields better results when using a "*used / scratched"* Petri dish: a dish where the cutting surface has been scratched and indented with a razor blade repeatedly.
- Leave the sample for 30 - 90 seconds of incubation in the extraction buffer (During this waiting time, the next sample can already be chopped)
- Rinse the plate again with the extraction buffer using a pipette.
- Transfer the sample to a Partec 50 µm CellTrics disposable filter with a pipette.
- Add 1.0 ml Staining solution (with PI and RNAse) to the test tube.
- Incubate on ice and in the dark for at least 30 to 60 minutes.
- Samples are stable for at least 12 hours at 4°C. Store stained samples at 4°C protected from light. Staining for several hours may improve the result. If samples oxidize add PVP (1%) or mercaptoethanol.

Polyploidy assessment by flow cytometry

- Analyze in your flow cytometer in the red channel.
- Nuclei size is compared to PI intensity.
- Settings strongly depend on the used flow cytometer. Following procedure is specifically for the Accuri C-6 flow cytometer:

Before running samples:
1) Place an Empty 12x75mm tube on the SIP.
2) Click BACKFLUSH.
3) Place a fresh tube with 2 ml of filtered, DI H2O on the SIP.
4) Set Time Limit for 2 minutes and Fluidics Speed to FAST.
5) Click RUN.
6) Once the time limit is reached, Click DELETE SAMPLE DATA.
7) Remove the tube and run samples.

Measuring a batch of samples:
1) Click File - open file or template.
2) Select "Lemna template" or "Arabidopsis template."
3) Set Time Limit for 4 minutes, click RUN.
4) When the sum of events in the regions of interest (P1, P2, P3, P4,...) reach 100, the measurement can be stopped.

After Running Samples:
1) Place a tube with 2 ml of diluted cleaning solution (#KR-225) on the SIP.

2) Select an ampty data well.
3) Set Time Limit for 2 minutes and Fluidics Speed to FAST.
4) Click RUN.
5) Once the time limit is reached, remove the tube with cleaning solution.
6) Place a tube with 2 ml of filtered, DI H2O on the SIP.
7) Set Time Limit for 2 minutes .
8) Click RUN. The C-6 will stop automatically when the time limit is reached.
9) Leave the tube on the SIP until the C-6 is used again.
10) Push the ON/OFF button for no more than 1 second to shut it down.

Analysis of the results:
After exporting the results to Excel, the relative percentages of the counts targeted zones (P1, P2, P3,...) containing the different polyploidy levels (2n, 4n, 8n, ...) are compared with each other.

APPENDIX B

EASTERN GAMAGRASS COLLECTION ORIGINS

Table B.1 Identification tag number, state, county, GPS coordinates, physical description, city of origin and determined ploidy level for all entries in eastern gamagrass collection

Plant	State	County	Latitude	Longitude	Location Description, City	Ploidy Level
101	MS	Oktibbeha	33.447400	-88.772969	Blackjack Road @ Campus View Drive, Starkville	4x
102	MS	Oktibbeha	33.444763	-88.844370	Pollard Road @ Airport Road and RR Tracks, Starkville	4x
103	MS	Oktibbeha	33.476961	-88.744060	Hwy. 182 E @ Tabernacle Church Drive, Starkville	4x
105	MS	Newton	32.329687	-89.046049	Hwy. 80 @ Wilbur Road, Hickory	4x
106	MS	Oktibbeha	33.332997	-89.037742	Louisville Road @ Morgantown Bottom, Sturgis	4x
107	MS	Jones	31.507162	-89.278309	Moselle Road @ Moselle Oak Grove Road, Moselle	4x
109	MS	Jones	31.433958	-89.283171	1396 Leeville Road @ Eastabuchie Road, Petal	4x
110	MS	Jones	31.465883	-89.283794	2687 Hwy. 11, Moselle	4x
111	MS	Oktibbeha	33.448156	-88.698320	Hickory Grove Road @ Curtis Chapel Road, Starkville	4x
112	MS	Hinds	32.228432	-90.516143	Oakley Road @ Fourteenmile Creek Bridge SE Corner, Raymond	4x
113	MS	Oktibbeha	33.477096	-88.707370	Hwy. 182 East @ Hickory Grove Road, Starkville	4x
114	MS	Winston	33.260912	-89.047443	265 Sturgis Road @ Haimes Road, Sturgis	4x
115	MS	Oktibbeha	33.371409	-88.991184	Hwy. 12 @ Smith Road, Sturgis	4x
201	MS	Oktibbeha	33.371369	-88.991246	Hwy. 12 @ Smith Road, Sturgis	4x
202	MS	Oktibbeha	33.419323	-88.790078	South Farm Road, Mississippi State	4x
203	MS	Hinds	32.228689	-90.516544	Oakley Road @ Fourteenmile Creek Bridge NW Corner, Raymond	4x
204	AL	Perry	32.619203	-87.515838	5620 Hwy. 48, Newbern	4x
205	MS	Oktibbeha	33.412507	-88.949135	2286 Dido Road, Starkville	4x
206	MS	Oktibbeha	33.419259	-88.790085	South Farm Road, Mississippi State	4x
208	MS	Oktibbeha	33.478508	-88.749793	1340 Cannon Lane @ Pond, Starkville	4x
210	MS	Oktibbeha	33.341725	-88.757876	2700 Oktoc Road @ Mactoc Dairy, Starkville	4x

Table B.1 (Continued)

211	MS	Lowndes	33.425764	-88.658175	1988 Sessums Road @ Catalpa Creek Bridge, Starkville	4x
212	MS	Oktibbeha	33.486274	-88.741512	1450 16 Section Road, Starkville	4x
213	MS	Leake	32.717739	-89.620707	Hwy. 25 @ Wiggins Loop Road, Carthage	4x
214	MS	Oktibbeha	33.381952	-88.697417	2027 Crawford Road, Starkville	4x
215	MS	Oktibbeha	33.485281	-88.777093	948 Pat Station Road, Starkville	4x
301	MS	Winston	33.255274	-89.051480	747 Sturgis Road, Sturgis	4x
303	MS	Winston	33.256073	-89.050985	747 Sturgis Road, Sturgis	4x
305	MS	Oktibbeha	33.486196	-88.741520	1466 16 Section Road, Starkville	4x
306	MS	Jones	31.472834	-89.203283	3089 Augusta Road, Ellisville	4x
307	MS	Oktibbeha	33.460156	-88.773577	1201 Bardwell Road, Starkville	4x
308	MS	Hinds	32.228258	-90.516349	Oakley Road @ Fourteenmile Creek Bridge SW Corner, Raymond	4x
309	MS	Jones	31.433949	-89.323805	1347 Monroe Road, Hattiesburg	4x
310	MS	Yalobusha	33.983102	-89.804955	20563 Hwy. 330 Jamie L. Witten Plant Materials Center, Tillatoba	4x
311	AL	Perry	32.619114	-87.511101	5591 Hwy. 48, Newbern	4x
312	MS	Oktibbeha	33.434588	-88.885859	1476 Old Hwy. 12, Starkville	4x
313	MS	Oktibbeha	33.427095	-88.911849	1400 New Hope Church Road, Starkville	4x
314	MS	Hinds	32.228763	-90.516326	Oakley Road @ Fourteenmile Creek Bridge NE Corner, Raymond	4x
315	MS	Oktibbeha	33.334521	-89.038524	Louisville Road @ Morgantown Bottom, Sturgis	4x
401	MS	Oktibbeha	33.474143	-88.750440	Hwy 182 @ Endotha Road Pond, Starkville	4x
403	MS	Oktibbeha	33.369611	-88.994110	3166 Hwy. 12 @ Cypress Creek, Sturgis	4x
404	MS	Neshoba	32.765104	-89.140114	Hwy. 21 @ Funny Yockana Creek Canal, Philadelphia	4x
405	MS	Winston	32.957653	-89.085922	12618 Hwy. 15, Noxapater	4x
406	MS	Neshoba	32.861657	-89.272757	Hooper Mill Creek Road @ Lukfapa Creek Bridge, Carthage	4x

Table B.1 (Continued)

407	MS	Choctaw	33.295976	-89.173431	Hwy 15 @ McMinn Road, Ackerman	4x
408	MS	Oktibbeha	33.484168	-88.779139	Pat Station Road @ Hwy. 82 Overpass, Starkville	4x
409	MS	Lowndes	33.480568	-88.635634	3329 S. Frontage Road, Mayhew	4x
410	MS	Yazoo	32.758303	-90.090958	252 Moore Road, Vaughan	4x
411	MS	Grenada	33.798594	-89.839330	I-55 North @ Yalobusha River Bridge, Grenada	4x
412	MS	Leake	32.797247	-89.335819	2566 River Road @ Pearl River, Carthage	4x
413	MS	Neshoba	32.789133	-89.303504	1059 Hwy 16 @ Bihhi Ayashi Bottom, Philadelphia	4x
415	MS	Winston	33.131173	-89.086269	Hwy 15 @ Miller Avenue, Louisville	4x
501	MS	Winston	33.100213	-89.071482	Hwy. 15 @ Smyth Lake Road, Louisville	4x
502	MS	Choctaw	33.338877	-89.123220	Airy Rd @ Hwy 12, Ackerman	4x
503	MS	Clay	33.636610	-88.594852	Barton Ferry Road, West Point	4x
504	GA	Laurens	32.560977	-83.157513	4332 Wade Street, US 80, Montrose	4x
505	GA	Columbia	33.495460	-82.306580	I-20 @ Old Appling Harlem Hwy., Harlem	4x
506	GA	Columbia	33.481730	-82.315540	1313 Appling Harlem Road, Harlem	4x
507	GA	Jefferson	32.992170	-82.429860	1948 Hwy. 24, Louisville	4x
508	GA	Twiggs	32.690800	-83.358210	105 Watson Dairy Road, Jeffersonville	4x
509	GA	Bibb	32.834170	-83.540750	4267 Jeffersonville Road @ Donnan Road and Sawyer Lake, Macon	4x
510	GA	Crawford	32.786910	-83.913350	Hwy. 22 Between Girl Scout Road and Causey Road, Lizella	4x
511	GA	Crawford	32.759950	-84.114530	Hwy. 22 @ Mt. Carmell Road, Roberta	4x
512	GA	Taylor	32.715780	-84.273460	Hwy. 22 @ Riley Road, Butler	4x
513	GA	Talbot	32.688890	-84.499880	Hwy. 22 @ Beckman Street, Talbotton	4x
514	GA	Talbot	32.552290	-84.671500	Hwy. 22 @ Baker Creek Bridge, Box Springs	4x
515	GA	Muscogee	32.538770	-84.839260	Macon Road @ Technology Pkwy. and Railroad Tracks, Midland	4x
603	AL	Lee	32.642890	-85.344870	1301 Columbus Pkwy., Opelika	4x

Table B.1 (Continued)

604	AL	Autauga	32.517320	-86.599000	2150 Hwy. 6 @ Blueberry Hill Road, Prattville	4x
605	AL	Montgomery	32.378610	-86.017910	I-85 @ Line Creek, Shorter	4x
606	AL	Fayette	33.661588	-87.925236	6533 Hwy 96, Fayette	4x
607	AL	Tuscaloosa	33.045160	-87.400660	13605 Hwy. 82 E @ Bear Creek, Duncanville	4x
608	AL	Pickens	33.401470	-88.075510	Hwy. 82 @ Sentell Road and Coal Fire Circle, Reform	4x
609	AL	Pickens	33.318840	-87.907650	Hwy. 82 @ 1st Ave. W and 2nd St. SW, Gordo	4x
610	AL	Pickens	33.336730	-87.932850	Hwy. 82 @ Hargrove Church Road, Gordo	4x
611	AL	Lamar	33.581510	-88.028590	Clearcut @ Weyerhaeuser and Luxapalila Creek, Hwy. 17, Millport	4x
612	AL	Lamar	33.581530	-87.992820	17171 Hwy. 96, Kennedy	4x
613	AL	Bibb	33.014970	-87.248940	Hwy. 82 E @ Mooney Creek and Horse Branch, Centreville	4x
614	AL	Tuscaloosa	33.246100	-87.674390	McFarland Blvd. @ Falls Cutoff Road, Coker	4x
615	AL	Tuscaloosa	33.068950	-87.449270	12101 Hwy. 82 E, Duncanville	4x
701	AL	Lamar	33.559620	-88.107927	10280 Hwy. 96, Mile Marker 10, Millport	4x
702	AL	Walker	33.966990	-87.606230	58073 Hwy. 13 @ Fern Springs Rd, Nauvoo	4x
703	AL	Winston	34.139560	-87.510020	Hwy. 74 E @ W.K. Wilson Lake, Haleyville	4x
704	AL	Morgan	34.500380	-86.898270	5133 Marsha Ave., Decatur	4x
705	AL	Jackson	34.623770	-86.305040	Hwy. 72 W @ Paint Rock River Bridge, Woodville	4x
706	AL	Madison	34.743650	-86.446090	3844 Hwy. 72 E @ Flint River Bridge, Brownsboro	4x
707	AL	Jackson	34.882660	-85.811980	Hwy 72 @ Will Chitty Ave., Stevenson	4x
708	SC	Edgefield	33.684990	-81.870870	54 Edgefield Road, Trenton	4x
709	SC	Edgefield	33.738430	-81.860330	Augusta Road @ Oscar Drive, Trenton	4x
710	SC	Edgefield	33.810130	-81.941860	1083 Bauskett Street @ Bacskel St., Edgefield	4x
711	SC	Edgefield	33.810130	-81.941860	1081 Bauskett Street, Edgefield	4x
712	SC	Greenwood	34.023000	-82.040400	8103 U.S. Hwy. 25 @ Horsepen Creek, Ninety Six	4x
713	SC	Greenwood	34.186800	-82.134640	U.S. Hwy. 178 @ Marshall Road, Greenwood	4x

Table B.1 (Continued)

714	SC	Abbeville	34.415260	-82.380470	1432 U.S. Hwy. 178 @ Olin Kay Road, Honea Path	4x
715	SC	Anderson	34.671010	-82.687620	6601 Liberty Hwy. @ Six and Twenty Road, Pendleton	4x
801	SC	Oconee	34.913840	-82.919490	Cherokee Falls Scenic Hwy. @ Blue Water Trail, Salem	4x
802	SC	Pickens	34.946300	-82.729470	3387 Moorefield Memorial Hwy., Pickens	4x
803	NC	Macon	35.170820	-83.364120	1637 U.S. 441 Bypass @ Cullasaja River Bridge, Franklin	4x
804	NC	Macon	35.157660	-83.313500	4595 Highlands Road @ Cullasaja River, Franklin	4x
805	AR	Union	33.215596	-92.922154	10000 Magnolia Hwy. @ Mt. Sinai Road, El Dorado	4x
806	NC	Cherokee	35.024244	-84.222921	U.S. Hwy. 64 @ Holiness Church Road, Murphy	4x
807	NC	Clay	35.026187	-83.707146	6612 U.S. Hwy. 64 @ Ash Loop Road, Hayesville	4x
808	NC	Clay	35.047795	-83.882243	4214 U.S. Hwy. 64 @ Dyer Cove Road, Hayesville	4x
809	TN	Polk	35.022207	-84.317831	5849 Hwy. 40 @ NC/TN Line, Copperhill	4x
810	TN	Bradley	35.133472	-84.746797	6501 Waterlevel Hwy. @ Old Parksville Road, Cleveland	4x
811	TN	Bradley	35.131446	-84.872614	1055 King Street SE @ U.S. Hwy 64 Bypass, Cleveland	4x
812	TN	Bradley	35.149250	-84.957190	I-75 S @ Mile Marker 19.8, Cleveland	4x
813	MS	Pearl River	30.812120	-89.611512	1799 Hwy. 26 @ Hartfield Rd, Poplarville	4x
814	MS	Pearl River	30.816512	-89.599446	1648 Hwy. 26, Poplarville	4x
815	MS	Clay	33.665740	-88.579190	1966 Old Vinton Road @ Town Creek Bridge, West Point	4x
901	AR	Union	33.246314	-92.982693	7714 U.S. Hwy. 82 @ Columbia Road 36, Magnolia	4x
902	AR	Columbia	33.247806	-93.011813	6901 U.S. Hwy. 82 @ Columbia Road 437, Magnolia	4x
903	AR	Union	33.226338	-92.954104	10984 Magnolia Hwy. @ Braswell Corner Road, Magnolia	4x
904	AR	Union	33.219975	-92.868483	8198 Magnolia Hwy. @ Lisbon Road, El Dorado	4x
905	KY	Hart	37.299009	-85.911241	127 Raider Hollow Road @ Railroad Tracks, Munfordville	4x
906	MS	Madison	32.548110	-90.192240	Livingston Drive @ Persimmon Creek, Madison	4x
907	MS	Madison	32.632010	-89.928520	2670 Hwy. 16, Canton	4x
908	MS	Madison	32.611400	-89.993480	1785 E Peace Street, Canton	4x
909	MS	Madison	32.647700	-89.876020	3191 Hwy. 16, Canton	4x

Table B.1 (Continued)

910	GA	Coweta	33.330570	-84.703460	70 J.Y. Carmichael Road @ Hwy. 16, Sharpsburg	4x
911	GA	Spalding	33.247880	-84.454850	60 Clayton Road @ Newnan Road, Brooks	4x
912	GA	Coweta	33.410840	-84.842150	Carlton Hwy/Hwy 16 W, Newnan	4x
913	GA	Coweta	33.329420	-84.644580	5869 Hwy. 54 @ Hwy. 16, Sharpsburg	4x
914	MS	Clay	33.630590	-88.596740	47 Barton Ferry Road, West Point	4x
915	MS	Clay	33.515350	-88.640020	Lummus Road @ Old Mayhew, West Point	4x
1001	MS	Oktibbeha	33.539566	-88.742193	2125 16th Section Road, Starkville	4x
1002	MS	Montgomery	33.438540	-89.520510	2524 Hwy 82, Kilmichael	4x
1003	MS	Carroll	33.488420	-89.816180	25786 Hwy 82, Winona	4x
1004	MS	Grenada	33.722280	-89.514920	3198 Pleasant Grove Road @ Sand Hill Road, Gore Springs	4x
1005	MS	Grenada	33.764600	-89.960320	10158 Hwy 8, Holcomb	4x
1006	MS	Grenada	33.778450	-90.055520	4742 Hwy 8, Holcomb	4x
1007	MS	Leflore	33.510400	-90.151440	2795 US 82, Greenwood	4x
1008	MS	Leflore	33.514680	-90.332800	19266 US 82, Itta Bena	4x
1009	MS	Sunflower	33.738070	-90.507200	2094 Hwy 8, Ruleville	4x
1010	MS	Oktibbeha	33.496350	-89.027680	1600 J.Y. Turner Road @ Lick Creek Bridge, Maben	4x
1011	MS	Leflore	33.746117	-90.308544	Hwy. 8 @ U.S. Hwy 49E, Minter City	4x
1012	MS	Webster	33.482490	-89.365330	2692 Hwy. 182, Eupora	4x
1013	MS	Oktibbeha	33.364221	-88.969549	2237 Sliver Ridge Road, Starkville	4x
1014	MS	Oktibbeha	33.386598	-88.852685	315 South Gate Road, Starkville	4x
1015	TN	Weakley	36.279810	-88.838940	7391 U.S. Hwy. 45E @ Rowlett Road, Martin	4x
1101	TN	Weakley	36.304680	-88.759070	10570 Hwy. 22 @ Latta Road, Dresden	4x
1102	MS	Calhoun	33.775860	-89.364110	1230 Hwy. 9 South, Calhoun City	4x
1103	MS	Calhoun	34.082200	-89.344730	1134 Hwy. 9 North Bruce, MS	4x
1104	MS	Lee	34.258562	-88.652596	1977 E. Main Street, Tupelo	4x
1105	MS	Itawamba	34.285860	-88.461400	2345 Hwy. 363, Mantachie	4x

Table B.1 (Continued)

1106	MS	Lee	34.090930	-88.720570	197 County Road 130, Shannon	4x
1107	MS	Tishomingo	34.717120	-88.224080	1848 Hwy. 25, Iuka	4x
1108	MS	Lee	34.120413	-88.710336	6391 Noah Curtis Road, Shannon	4x
1109	MS	Lee	34.267030	-88.563590	3698 Hwy. 178, Mooreville	4x
1110	TN	Hardeman	35.003450	-88.893960	834 Hwy. 125S, Middleton	4x
1111	TN	McNairy	35.077950	-88.558640	706 Hwy. 57, Eastview	4x
1112	TN	Hardeman	35.047990	-88.807560	22366 Hwy. 57, Pocahontas	4x
1113	MS	Tippah	34.871168	-88.917593	22240 Hwy. 15, Tiplersville	4x
1114	MS	Calhoun	33.811990	-89.347550	363 Hwy. 8N, Calhoun City	4x
1115	MS	Union	34.541690	-88.990290	1059 Hwy. 15 N, New Albany	4x
1201	MS	Tippah	34.689090	-89.003770	4780 Hwy. 15 N, Blue Mountain	4x
1202	MS	Calhoun	33.775860	-89.364400	312 Hwy. 9 S, Calhoun City	4x
1203	MS	Pontotoc	34.235430	-89.091570	4494 Hwy. 9 S, Pontotoc	4x
1204	MS	Calhoun	33.673480	-89.333940	220 Hwy. 9 S, Calhoun City	4x
1205	MS	Hinds	32.511060	-90.135230	507 Bozeman Road, Madison	4x
1206	MS	Hinds	32.549400	-90.147250	158 Caroline Pointe Blvd. @ Lake Caroline, Madison	4x

Place all detailed caption, notes, reference, legend information, etc here\

APPENDIX C

EASTERN GAMAGRASS REGIONAL GERMPLASM COLLECTION FORAGE USE

FITNESS EVALUATION DATA

Table C.1 Visual ratings of rust and/or fungal pathogen infestation in eastern gamagrass accessions collected from southeastern and Atlantic United States.

Plant ID†	Rating 1	Rating 2	Rating 3	Rating 4	Mean ($\bar{x}$)	Standard Deviation (σ)
101	4	3	4	3	3.5	0.58
102	5	4	4	4	4.3	0.50
103	2	1	2	2	1.8	0.50
104	3	2	3	3	2.8	0.50
105	3	3	2	3	2.8	0.50
106	4	3	2	3	3.0	0.82
107	2	1	1	1	1.3	0.50
108	3	2	3	2	2.5	0.58
109	3	2	4	4	3.3	0.96
110	3	2	3	3	2.8	0.50
111	4	3	2	2	2.8	0.96
112	3	2	1	2	2.0	0.82
113	5	4	4	4	4.3	0.50
114	3	2	3	3	2.8	0.50
115	2	2	3	2	2.3	0.50
201	3	2	2	2	2.3	0.50
202	3	2	2	2	2.3	0.50
203	2	1	1	1	1.3	0.50
204	4	3	3	2	3.0	0.82
205	4	3	1	1	2.3	1.50
206	3	2	2	2	2.3	0.50
207	5	3	4	4	4.0	0.82
208	4	2	3	3	3.0	0.82
209	4	3	4	3	3.5	0.58
210	4	3	2	2	2.8	0.96
211	3	3	2	3	2.8	0.50
212	4	3	3	3	3.3	0.50
213	5	3	5	5	4.5	1.00
214	4	3	4	4	3.8	0.50
215	3	2	3	3	2.8	0.50
301	3	2	3	2	2.5	0.58
302	4	3	4	4	3.8	0.50

Table C.1 (Continued)

303	3	2	3	2	2.5	0.58
304	.	.	.	.	.	.
305	4	2	2	2	2.5	1.00
306	2	1	1	1	1.3	0.50
307	5	5	4	5	4.8	0.50
308	1	1	1	1	1.0	0.00
309	3	2	2	2	2.3	0.50
310	3	2	3	3	2.8	0.50
311	3	1	2	2	2.0	0.82
312	4	3	3	3	3.3	0.50
313	5	4	5	4	4.5	0.58
314	3	1	1	1	1.5	1.00
315	4	3	3	3	3.3	0.50
401	3	2	3	3	2.8	0.50
402	3	3	4	3	3.3	0.50
403	2	1	3	3	2.3	0.96
404	4	3	1	3	2.8	1.26
405	4	2	2	2	2.5	1.00
406	3	3	4	3	3.3	0.50
407	2	2	2	2	2.0	0.00
408	3	2	3	3	2.8	0.50
409	4	2	2	2	2.5	1.00
410	4	2	3	3	3.0	0.82
411	4	3	4	4	3.8	0.50
412	3	2	2	2	2.3	0.50
413	4	3	3	3	3.3	0.50
414	.	.	.	.	.	.
415	3	2	4	4	3.3	0.96
501	4	3	4	3	3.5	0.58
502	2	1	2	2	1.8	0.50
503	3	2	3	2	2.5	0.58
504	2	2	2	2	2.0	0.00
505	1	1	1	1	1.0	0.00
506	2	2	2	2	2.0	0.00
507	3	2	2	2	2.3	0.50
508	3	2	3	2	2.5	0.58

Table C.1 (Continued)

509	3	2	4	4	3.3	0.96
510	3	2	3	3	2.8	0.50
511	3	2	3	2	2.5	0.58
512	4	2	3	3	3.0	0.82
513	3	2	2	2	2.3	0.50
514	4	2	2	3	2.8	0.96
515	3	2	3	3	2.8	0.50
601	.	.	.	.	.	.
602	.	.	.	.	.	.
603	4	2	4	4	3.5	1.00
604	3	2	2	2	2.3	0.50
605	3	3	4	4	3.5	0.58
606	2	2	3	3	2.5	0.58
607	1	2	1	1	1.3	0.50
608	3	3	3	3	3.0	0.00
609	1	1	2	1	1.3	0.50
610	3	2	5	4	3.5	1.29
611	4	3	5	5	4.3	0.96
612	3	3	5	4	3.8	0.96
613	3	3	3	3	3.0	0.00
614	3	4	4	4	3.8	0.50
615	2	2	1	2	1.8	0.50
701	5	3	5	5	4.5	1.00
702	4	3	4	4	3.8	0.50
703	4	3	3	4	3.5	0.58
704	3	3	3	3	3.0	0.00
705	2	1	2	2	1.8	0.50
706	4	3	3	3	3.3	0.50
707	2	2	2	2	2.0	0.00
708	2	1	2	2	1.8	0.50
709	2	1	1	1	1.3	0.50
710	2	2	3	3	2.5	0.58
711	3	2	4	2	2.8	0.96
712	2	2	2	2	2.0	0.00
713	2	2	3	3	2.5	0.58
714	3	2	2	2	2.3	0.50

Table C.1 (Continued)

715	3	3	5	4	3.8	0.96
801	4	2	4	2	3.0	1.15
802	2	2	2	2	2.0	0.00
803	3	2	2	2	2.3	0.50
804	3	1	3	3	2.5	1.00
805	2	1	2	2	1.8	0.50
806	3	3	1	3	2.5	1.00
807	3	4	3	3	3.3	0.50
808	2	1	2	2	1.8	0.50
809	2	2	1	2	1.8	0.50
810	4	4	3	4	3.8	0.50
811	4	3	4	4	3.8	0.50
812	3	3	3	3	3.0	0.00
813	2	3	2	3	2.5	0.58
814	2	1	1	1	1.3	0.50
815	3	4	4	4	3.8	0.50
901	5	2	4	4	3.8	1.26
902	2	2	3	2	2.3	0.50
903	3	1	3	2	2.3	0.96
904	3	2	2	2	2.3	0.50
905	3	3	3	3	3.0	0.00
906	3	2	3	2	2.5	0.58
907	4	3	5	4	4.0	0.82
908	4	3	5	5	4.3	0.96
909	3	3	4	4	3.5	0.58
910	3	2	2	2	2.3	0.50
911	5	5	5	5	5.0	0.00
912	3	3	3	3	3.0	0.00
913	3	3	4	4	3.5	0.58
914	4	4	4	5	4.3	0.50
915	4	3	3	3	3.3	0.50
1001	5	3	4	3	3.8	0.96
1002	3	3	4	4	3.5	0.58
1003	3	2	4	3	3.0	0.82
1004	3	2	3	3	2.8	0.50
1005	2	2	1	2	1.8	0.50

Table C.1 (Continued)

1006	3	2	3	3	2.8	0.50
1007	5	4	4	4	4.3	0.50
1008	4	2	4	3	3.3	0.96
1009	4	3	3	3	3.3	0.50
1010	3	3	3	3	3.0	0.00
1011	2	2	1	2	1.8	0.50
1012	4	3	3	3	3.3	0.50
1013	4	3	3	3	3.3	0.50
1014	5	4	3	4	4.0	0.82
1015	4	3	5	4	4.0	0.82
1101	5	4	5	5	4.8	0.50
1102	2	3	3	3	2.8	0.50
1103	3	2	3	3	2.8	0.50
1104	5	3	3	3	3.5	1.00
1105	5	3	2	3	3.3	1.26
1106	5	3	4	3	3.8	0.96
1107	4	3	3	3	3.3	0.50
1108	5	3	4	3	3.8	0.96
1109	3	2	3	3	2.8	0.50
1110	3	3	3	3	3.0	0.00
1111	3	4	4	4	3.8	0.50
1112	3	2	3	3	2.8	0.50
1113	4	2	3	3	3.0	0.82
1114	.	.	.	.	.	.
1115	3	2	5	5	3.8	1.50
1201	4	3	4	4	3.8	0.50
1202	.	.	.	.	.	.
1203	3	3	3	3	3.0	0.00
1204	3	3	3	3	3.0	0.00
1205	5	3	5	4	4.3	0.96
1206	5	4	4	4	4.3	0.50

Ratings were taken in fall, 2014.

Key: 1 = No disease, 2 = Very few, faint lesions, 3 = Some lesions, but covering less than 50% of leaf tissue, 4 = Abundant rust or fungal lesions, covering more than 50% of the tissue, 5 = Entirely covered with rust and/or fungal lesions

† For Plant ID, reference table B.1

Table C.2 Analysis of variance and means separations of rust and/or fungal pathogen infestation of eastern gamagrass accessions collected from southeastern and Atlantic United States.

t Grouping				Mean	N	Plant ID
		A		5	4	911
		A				
B		A		4.75	4	1101
B		A				
B		A		4.75	4	307
B		A				
B		A	C	4.5	4	701
B		A	C			
B		A	C	4.5	4	313
B		A	C			
B		A	C	4.5	4	213
B		A	C			
B	D	A	C	4.25	4	1007
B	D	A	C			
B	D	A	C	4.25	4	102
B	D	A	C			
B	D	A	C	4.25	4	1206
B	D	A	C			
B	D	A	C	4.25	4	1205
B	D	A	C			
B	D	A	C	4.25	4	914
B	D	A	C			
B	D	A	C	4.25	4	113
B	D	A	C			
B	D	A	C	4.25	4	611
B	D	A	C			
B	D	A	C	4.25	4	908
B	D		C			
B	D	E	C	4	4	1014
B	D	E	C			
B	D	E	C	4	4	907
B	D	E	C			
B	D	E	C	4	4	207
B	D	E	C			
B	D	E	C	4	4	1015
	D	E	C			

Table C.2 (Continued)

F	D	E	C	3.75	4	901
F	D	E	C			
F	D	E	C	3.75	4	1106
F	D	E	C			
F	D	E	C	3.75	4	715
F	D	E	C			
F	D	E	C	3.75	4	1111
F	D	E	C			
F	D	E	C	3.75	4	214
F	D	E	C			
F	D	E	C	3.75	4	302
F	D	E	C			
F	D	E	C	3.75	4	612
F	D	E	C			
F	D	E	C	3.75	4	811
F	D	E	C			
F	D	E	C	3.75	4	614
F	D	E	C			
F	D	E	C	3.75	4	1115
F	D	E	C			
F	D	E	C	3.75	4	1201
F	D	E	C			
F	D	E	C	3.75	4	815
F	D	E	C			
F	D	E	C	3.75	4	411
F	D	E	C			
F	D	E	C	3.75	4	702
F	D	E	C			
F	D	E	C	3.75	4	1001
F	D	E	C			
F	D	E	C	3.75	4	1108
F	D	E	C			
F	D	E	C	3.75	4	810
F	D	E				
F	D	E	G	3.5	4	603
F	D	E	G			
F	D	E	G	3.5	4	605
F	D	E	G			
F	D	E	G	3.5	4	1002

Table C.2 (Continued)

F	D	E	G			
F	D	E	G	3.5	4	913
F	D	E	G			
F	D	E	G	3.5	4	101
F	D	E	G			
F	D	E	G	3.5	4	703
F	D	E	G			
F	D	E	G	3.5	4	209
F	D	E	G			
F	D	E	G	3.5	4	610
F	D	E	G			
F	D	E	G	3.5	4	909
F	D	E	G			
F	D	E	G	3.5	4	501
F	D	E	G			
F	D	E	G	3.5	4	1104
F		E	G			
F	H	E	G	3.25	4	406
F	H	E	G			
F	H	E	G	3.25	4	1008
F	H	E	G			
F	H	E	G	3.25	4	415
F	H	E	G			
F	H	E	G	3.25	4	1107
F	H	E	G			
F	H	E	G	3.25	4	312
F	H	E	G			
F	H	E	G	3.25	4	109
F	H	E	G			
F	H	E	G	3.25	4	413
F	H	E	G			
F	H	E	G	3.25	4	1013
F	H	E	G			
F	H	E	G	3.25	4	509
F	H	E	G			
F	H	E	G	3.25	4	402
F	H	E	G			
F	H	E	G	3.25	4	915
F	H	E	G			

Table C.2 (Continued)

F	H	E	G	3.25	4	315
F	H	E	G			
F	H	E	G	3.25	4	1009
F	H	E	G			
F	H	E	G	3.25	4	1105
F	H	E	G			
F	H	E	G	3.25	4	1012
F	H	E	G			
F	H	E	G	3.25	4	807
F	H	E	G			
F	H	E	G	3.25	4	212
F	H	E	G			
F	H	E	G	3.25	4	706
F	H		G			
F	H	I	G	3	4	1003
F	H	I	G			
F	H	I	G	3	4	1110
F	H	I	G			
F	H	I	G	3	4	812
F	H	I	G			
F	H	I	G	3	4	704
F	H	I	G			
F	H	I	G	3	4	1010
F	H	I	G			
F	H	I	G	3	4	801
F	H	I	G			
F	H	I	G	3	4	905
F	H	I	G			
F	H	I	G	3	4	410
F	H	I	G			
F	H	I	G	3	4	1113
F	H	I	G			
F	H	I	G	3	4	912
F	H	I	G			
F	H	I	G	3	4	1204
F	H	I	G			
F	H	I	G	3	4	512
F	H	I	G			
F	H	I	G	3	4	208

Table C.2 (Continued)

F	H	I	G			
F	H	I	G	3	4	613
F	H	I	G			
F	H	I	G	3	4	608
F	H	I	G			
F	H	I	G	3	4	1203
F	H	I	G			
F	H	I	G	3	4	106
F	H	I	G			
F	H	I	G	3	4	204
	H	I	G			
J	H	I	G	2.75	4	1004
J	H	I	G			
J	H	I	G	2.75	4	1103
J	H	I	G			
J	H	I	G	2.75	4	711
J	H	I	G			
J	H	I	G	2.75	4	404
J	H	I	G			
J	H	I	G	2.75	4	104
J	H	I	G			
J	H	I	G	2.75	4	105
J	H	I	G			
J	H	I	G	2.75	4	401
J	H	I	G			
J	H	I	G	2.75	4	1109
J	H	I	G			
J	H	I	G	2.75	4	114
J	H	I	G			
J	H	I	G	2.75	4	211
J	H	I	G			
J	H	I	G	2.75	4	310
J	H	I	G			
J	H	I	G	2.75	4	514
J	H	I	G			
J	H	I	G	2.75	4	111
J	H	I	G			
J	H	I	G	2.75	4	215
J	H	I	G			

Table C.2 (Continued)

J	H	I	G	2.75	4	110
J	H	I	G			
J	H	I	G	2.75	4	1112
J	H	I	G			
J	H	I	G	2.75	4	515
J	H	I	G			
J	H	I	G	2.75	4	1006
J	H	I	G			
J	H	I	G	2.75	4	210
J	H	I	G			
J	H	I	G	2.75	4	408
J	H	I	G			
J	H	I	G	2.75	4	1102
J	H	I	G			
J	H	I	G	2.75	4	510
J	H	I				
J	H	I	K	2.5	4	713
J	H	I	K			
J	H	I	K	2.5	4	305
J	H	I	K			
J	H	I	K	2.5	4	301
J	H	I	K			
J	H	I	K	2.5	4	906
J	H	I	K			
J	H	I	K	2.5	4	806
J	H	I	K			
J	H	I	K	2.5	4	813
J	H	I	K			
J	H	I	K	2.5	4	409
J	H	I	K			
J	H	I	K	2.5	4	508
J	H	I	K			
J	H	I	K	2.5	4	511
J	H	I	K			
J	H	I	K	2.5	4	710
J	H	I	K			
J	H	I	K	2.5	4	606
J	H	I	K			
J	H	I	K	2.5	4	108

Table C.2 (Continued)

J	H	I	K			
J	H	I	K	2.5	4	503
J	H	I	K			
J	H	I	K	2.5	4	405
J	H	I	K			
J	H	I	K	2.5	4	303
J	H	I	K			
J	H	I	K	2.5	4	804
J		I	K			
J	L	I	K	2.25	4	910
J	L	I	K			
J	L	I	K	2.25	4	803
J	L	I	K			
J	L	I	K	2.25	4	902
J	L	I	K			
J	L	I	K	2.25	4	201
J	L	I	K			
J	L	I	K	2.25	4	309
J	L	I	K			
J	L	I	K	2.25	4	412
J	L	I	K			
J	L	I	K	2.25	4	513
J	L	I	K			
J	L	I	K	2.25	4	115
J	L	I	K			
J	L	I	K	2.25	4	604
J	L	I	K			
J	L	I	K	2.25	4	904
J	L	I	K			
J	L	I	K	2.25	4	903
J	L	I	K			
J	L	I	K	2.25	4	205
J	L	I	K			
J	L	I	K	2.25	4	403
J	L	I	K			
J	L	I	K	2.25	4	714
J	L	I	K			
J	L	I	K	2.25	4	206
J	L	I	K			

Table C.2 (Continued)

J	L	I	K	2.25	4	202
J	L	I	K			
J	L	I	K	2.25	4	507
J	L		K			
J	L	M	K	2	4	504
J	L	M	K			
J	L	M	K	2	4	712
J	L	M	K			
J	L	M	K	2	4	506
J	L	M	K			
J	L	M	K	2	4	407
J	L	M	K			
J	L	M	K	2	4	311
J	L	M	K			
J	L	M	K	2	4	112
J	L	M	K			
J	L	M	K	2	4	802
J	L	M	K			
J	L	M	K	2	4	707
	L	M	K			
N	L	M	K	1.75	4	809
N	L	M	K			
N	L	M	K	1.75	4	708
N	L	M	K			
N	L	M	K	1.75	4	1011
N	L	M	K			
N	L	M	K	1.75	4	1005
N	L	M	K			
N	L	M	K	1.75	4	502
N	L	M	K			
N	L	M	K	1.75	4	705
N	L	M	K			
N	L	M	K	1.75	4	615
N	L	M	K			
N	L	M	K	1.75	4	808
N	L	M	K			
N	L	M	K	1.75	4	103
N	L	M	K			
N	L	M	K	1.75	4	805

Table C.2 (Continued)

N	L	M				
N	L	M		1.5	4	314
N		M				
N		M		1.25	4	709
N		M				
N		M		1.25	4	107
N		M				
N		M		1.25	4	814
N		M				
N		M		1.25	4	607
N		M				
N		M		1.25	4	306
N		M				
N		M		1.25	4	609
N		M				
N		M		1.25	4	203
N						
N				1	4	308
N						
N				1	4	505

Means with the same letter are not significantly different
LSD = 0.927

Table C.3 Visual ratings for cold tolerance in eastern gamagrass accessions collected from across the southeastern and Atlantic United States.

Plant ID	Origin (State)	Rating 1	Rating 2
101	MS	3	2
102	MS	2	2
103	MS	3	1
104	MS	2	2
105	MS	4	1
106	MS	3	2
107	MS	1	2
108	MS	4	1
109	MS	2	2
110	MS	3	1
111	MS	3	1
112	MS	4	1
113	MS	4	1
114	MS	3	2
115	MS	4	1
201	MS	4	2
202	MS	4	2
203	MS	5	3
204	AL	3	1
205	MS	3	1
206	MS	2	1
207	MS	1	1
208	MS	1	1
209	MS	2	1
210	MS	1	1
211	MS	3	1
212	MS	1	1
213	MS	3	1
214	MS	2	1
215	MS	1	1
301	MS	3	2
302	MS	2	1
303	MS	2	1
305	MS	1	1
306	MS	4	3

Table C.3 (Continued)

307	MS	1	1
308	MS	4	3
309	MS	3	2
310	MS	3	1
311	AL	4	2
312	MS	2	1
313	MS	1	1
314	MS	3	2
315	MS	1	1
401	MS	3	1
402	MS	2	1
403	MS	1	1
404	MS	2	1
405	MS	2	1
406	MS	2	1
407	MS	4	3
408	MS	1	1
409	MS	2	1
410	MS	2	2
411	MS	3	1
412	MS	1	1
413	MS	1	1
415	MS	3	2
501	MS	4	2
502	MS	5	2
503	MS	2	1
504	GA	3	1
505	GA	4	2
506	GA	4	1
507	GA	3	2
508	GA	2	2
509	GA	2	1
510	GA	4	2
511	GA	3	1
512	GA	4	1
513	GA	4	1
514	GA	3	1
515	GA	3	2
603	AL	3	1

Table C.3 (Continued)

604	AL	4	2
605	AL	2	1
606	AL	1	1
607	AL	3	1
608	AL	2	1
609	AL	4	3
610	AL	3	1
611	AL	1	1
612	AL	3	1
613	AL	4	1
614	AL	4	1
615	AL	3	2
701	AL	4	2
702	AL	3	2
703	AL	4	1
704	AL	3	1
705	AL	2	1
706	AL	2	1
707	AL	3	1
708	SC	3	2
709	SC	3	1
710	SC	4	3
711	SC	3	1
712	SC	4	2
713	SC	4	2
714	SC	4	3
715	SC	4	3
801	SC	4	2
802	SC	4	2
803	NC	3	1
804	NC	3	2
805	AR	3	2
806	NC	2	1
807	NC	3	1
808	NC	2	1
809	TN	2	1
810	TN	1	1
811	TN	2	1
812	TN	1	1

Table C.3 (Continued)

813	MS	5	3
814	MS	5	3
815	MS	3	1
901	AR	3	1
902	AR	3	1
903	AR	3	1
904	AR	3	1
905	KY	3	1
906	MS	1	1
907	MS	2	1
908	MS	3	1
909	MS	3	1
910	GA	2	1
911	GA	3	1
912	GA	3	1
913	GA	4	1
914	MS	1	1
915	MS	3	1
1001	MS	1	1
1002	MS	2	1
1003	MS	1	1
1004	MS	1	1
1005	MS	1	1
1006	MS	3	1
1007	MS	2	1
1008	MS	2	1
1009	MS	1	1
1010	MS	2	1
1011	MS	3	1
1012	MS	3	1
1013	MS	3	1
1014	MS	1	1
1015	TN	2	1
1101	TN	2	1
1102	MS	1	1
1103	MS	3	1
1104	MS	2	1
1105	MS	3	1
1106	MS	2	1

Table C.3 (Continued)

1107	MS	1	1
1108	MS	1	1
1109	MS	1	1
1110	TN	2	1
1111	TN	1	1
1112	TN	2	1
1113	MS	3	1
1114	MS	1	1
1115	MS	2	1
1201	MS	2	1
1202	MS	2	1
1203	MS	3	1
1204	MS	1	1
1205	MS	1	1
1206	MS	1	1

Rankings were taken five days following overnight low temperatures of -5°C.
Key: 1 = Severely damaged, 100% leaf damage, from leaf tips to crown, 2 = Over 75% of entire plant is heavily damaged from leaf tips to crown, 3 = Considerable central leaf damage, at least 50% of entire plant, 4 = Some central leaf damage, but less than 50% of entire plant, 5 = Minimal damage to leaves, completely relegated to leaf tips.
Rating 1: 20-November
Rating 2: 5-December

Table C.4 Analysis of variance and means separations for visual ratings of cold tolerance in eastern gamagrass accessions collected from across the southeastern and Atlantic United States.

T Grouping			Mean	N	Plant ID
	A		4	2	814
	A				
	A		4	2	813
	A				
	A		4	2	203
	A				
B	A		3.5	2	715
B	A				
B	A		3.5	2	308
B	A				
B	A		3.5	2	710
B	A				
B	A		3.5	2	306
B	A				
B	A		3.5	2	714
B	A				
B	A		3.5	2	407
B	A				
B	A		3.5	2	609
B	A				
B	A		3.5	2	502
B	A				
B	A	C	3	2	202
B	A	C			
B	A	C	3	2	505
B	A	C			
B	A	C	3	2	801
B	A	C			
B	A	C	3	2	802
B	A	C			
B	A	C	3	2	712
B	A	C			
B	A	C	3	2	701
B	A	C			
B	A	C	3	2	311
B	A	C			
B	A	C	3	2	604

Table C.4 (Continued)

B	A	C			
B	A	C	3	2	510
B	A	C			
B	A	C	3	2	501
B	A	C			
B	A	C	3	2	201
B	A	C			
B	A	C	3	2	713
B	A	C			
B	A	C	2.5	2	708
B	A	C			
B	A	C	2.5	2	804
B	A	C			
B	A	C	2.5	2	615
B	A	C			
B	A	C	2.5	2	614
B	A	C			
B	A	C	2.5	2	112
B	A	C			
B	A	C	2.5	2	913
B	A	C			
B	A	C	2.5	2	805
B	A	C			
B	A	C	2.5	2	703
B	A	C			
B	A	C	2.5	2	613
B	A	C			
B	A	C	2.5	2	507
B	A	C			
B	A	C	2.5	2	309
B	A	C			
B	A	C	2.5	2	115
B	A	C			
B	A	C	2.5	2	702
B	A	C			
B	A	C	2.5	2	106
B	A	C			
B	A	C	2.5	2	415
B	A	C			
B	A	C	2.5	2	301

Table C.4 (Continued)

B	A	C			
B	A	C	2.5	2	114
B	A	C			
B	A	C	2.5	2	515
B	A	C			
B	A	C	2.5	2	512
B	A	C			
B	A	C	2.5	2	113
B	A	C			
B	A	C	2.5	2	506
B	A	C			
B	A	C	2.5	2	513
B	A	C			
B	A	C	2.5	2	101
B	A	C			
B	A	C	2.5	2	314
B	A	C			
B	A	C	2.5	2	105
B	A	C			
B	A	C	2.5	2	108
B	A	C			
B	A	C	2	2	603
B	A	C			
B	A	C	2	2	707
B	A	C			
B	A	C	2	2	807
B	A	C			
B	A	C	2	2	102
B	A	C			
B	A	C	2	2	508
B	A	C			
B	A	C	2	2	909
B	A	C			
B	A	C	2	2	1203
B	A	C			
B	A	C	2	2	401
B	A	C			
B	A	C	2	2	103
B	A	C			
B	A	C	2	2	104

Table C.4 (Continued)

B	A	C			
B	A	C	2	2	109
B	A	C			
B	A	C	2	2	110
B	A	C			
B	A	C	2	2	211
B	A	C			
B	A	C	2	2	709
B	A	C			
B	A	C	2	2	213
B	A	C			
B	A	C	2	2	511
B	A	C			
B	A	C	2	2	504
B	A	C			
B	A	C	2	2	111
B	A	C			
B	A	C	2	2	1103
B	A	C			
B	A	C	2	2	711
B	A	C			
B	A	C	2	2	1006
B	A	C			
B	A	C	2	2	1113
B	A	C			
B	A	C	2	2	815
B	A	C			
B	A	C	2	2	901
B	A	C			
B	A	C	2	2	607
B	A	C			
B	A	C	2	2	903
B	A	C			
B	A	C	2	2	1011
B	A	C			
B	A	C	2	2	905
B	A	C			
B	A	C	2	2	1013
B	A	C			
B	A	C	2	2	915

Table C.4 (Continued)

B	A	C			
B	A	C	2	2	908
B	A	C			
B	A	C	2	2	610
B	A	C			
B	A	C	2	2	902
B	A	C			
B	A	C	2	2	911
B	A	C			
B	A	C	2	2	912
B	A	C			
B	A	C	2	2	1012
B	A	C			
B	A	C	2	2	1105
B	A	C			
B	A	C	2	2	204
B	A	C			
B	A	C	2	2	205
B	A	C			
B	A	C	2	2	411
B	A	C			
B	A	C	2	2	410
B	A	C			
B	A	C	2	2	612
B	A	C			
B	A	C	2	2	904
B	A	C			
B	A	C	2	2	310
B	A	C			
B	A	C	2	2	704
B	A	C			
B	A	C	2	2	514
B	A	C			
B	A	C	2	2	803
B		C			
B		C	1.5	2	214
B		C			
B		C	1.5	2	808
B		C			
B		C	1.5	2	503

Table C.4 (Continued)

B		C			
B		C	1.5	2	1002
B		C			
B		C	1.5	2	303
B		C			
B		C	1.5	2	406
B		C			
B		C	1.5	2	705
B		C			
B		C	1.5	2	1015
B		C			
B		C	1.5	2	1115
B		C			
B		C	1.5	2	402
B		C			
B		C	1.5	2	608
B		C			
B		C	1.5	2	1008
B		C			
B		C	1.5	2	1104
B		C			
B		C	1.5	2	1112
B		C			
B		C	1.5	2	806
B		C			
B		C	1.5	2	605
B		C			
B		C	1.5	2	206
B		C			
B		C	1.5	2	910
B		C			
B		C	1.5	2	1010
B		C			
B		C	1.5	2	302
B		C			
B		C	1.5	2	405
B		C			
B		C	1.5	2	107
B		C			
B		C	1.5	2	1106

Table C.4 (Continued)

B		C			
B		C	1.5	2	706
B		C			
B		C	1.5	2	409
B		C			
B		C	1.5	2	809
B		C			
B		C	1.5	2	1202
B		C			
B		C	1.5	2	404
B		C			
B		C	1.5	2	312
B		C			
B		C	1.5	2	1201
B		C			
B		C	1.5	2	907
B		C			
B		C	1.5	2	1110
B		C			
B		C	1.5	2	1007
B		C			
B		C	1.5	2	1101
B		C			
B		C	1.5	2	509
B		C			
B		C	1.5	2	209
B		C			
B		C	1.5	2	811
		C			
		C	1	2	1204
		C			
		C	1	2	1005
		C			
		C	1	2	305
		C			
		C	1	2	1001
		C			
		C	1	2	215
		C			
		C	1	2	403

Table C.4 (Continued)

		C			
		C	1	2	1114
		C			
		C	1	2	210
		C			
		C	1	2	313
		C			
		C	1	2	1206
		C			
		C	1	2	307
		C			
		C	1	2	606
		C			
		C	1	2	1205
		C			
		C	1	2	208
		C			
		C	1	2	315
		C			
		C	1	2	812
		C			
		C	1	2	611
		C			
		C	1	2	810
		C			
		C	1	2	408
		C			
		C	1	2	1009
		C			
		C	1	2	207
		C			
		C	1	2	1102
		C			
		C	1	2	412
		C			
		C	1	2	413
		C			
		C	1	2	906
		C			
		C	1	2	1014

Table C.4 (Continued)

		C			
		C	1	2	1107
		C			
		C	1	2	1108
		C			
		C	1	2	1109
		C			
		C	1	2	1003
		C			
		C	1	2	1004
		C			
		C	1	2	1111
		C			
		C	1	2	914
		C			
		C	1	2	212

Means with the same letter are not significantly different
LSD = 2.17
Rankings were taken five days following overnight low temperatures of -5°C.

Table C.5 Visual ratings for onset of maturity in eastern gamagrass accessions collected from across the southeastern and Atlantic United States.

Plant ID	Origin (State)	Date of Maturity (week)	Subsample mean ($\bar{x}$)
204	**AL**	5	
603	**AL**	5	
604	**AL**	5	
605	**AL**	5	
606	**AL**	5	
607	**AL**	2	
608	**AL**	5	
609	**AL**	5	
610	**AL**	4	
611	**AL**	4	
612	**AL**	4	
613	**AL**	4	
614	**AL**	4	
615	**AL**	1	
701	**AL**	4	
702	**AL**	4	
703	**AL**	1	
704	**AL**	5	
705	**AL**	2	
706	**AL**	5	
707	**AL**	5	
311	**AL**	6	
805	**AR**	2	4.0
901	**AR**	1	
902	**AR**	3	
903	**AR**	2	
904	**AR**	1	
504	**GA**	2	1.8
505	**GA**	4	
506	**GA**	5	
507	**GA**	4	
508	**GA**	5	

Table C.5 (Continued)

509	**GA**	1	
510	**GA**	5	
511	**GA**	5	
512	**GA**	2	
513	**GA**	4	
514	**GA**	2	
515	**GA**	2	
910	**GA**	2	
911	**GA**	6	
912	**GA**	2	
913	**GA**	2	
905	**KY**	2	3.13
101	**MS**	1	2.0
102	**MS**	4	
103	**MS**	1	
104	**MS**	3	
105	**MS**	5	
106	**MS**	1	
107	**MS**	2	
108	**MS**	3	
109	**MS**	1	
110	**MS**	3	
111	**MS**	3	
112	**MS**	2	
113	**MS**	2	
114	**MS**	4	
115	**MS**	4	
201	**MS**	4	
202	**MS**	2	
203	**MS**	4	
205	**MS**	4	
206	**MS**	1	
207	**MS**	5	
208	**MS**	1	
209	**MS**	5	

Table C.5 (Continued)

210	**MS**	4	
211	**MS**	2	
212	**MS**	1	
213	**MS**	2	
214	**MS**	5	
215	**MS**	4	
301	**MS**	1	
302	**MS**	5	
303	**MS**	2	
305	**MS**	5	
307	**MS**	3	
308	**MS**	2	
309	**MS**	2	
310	**MS**	1	
312	**MS**	6	
313	**MS**	5	
314	**MS**	1	
315	**MS**	1	
401	**MS**	4	
402	**MS**	6	
403	**MS**	6	
404	**MS**	4	
405	**MS**	4	
406	**MS**	3	
407	**MS**	2	
408	**MS**	3	
409	**MS**	1	
410	**MS**	2	
411	**MS**	6	
412	**MS**	6	
413	**MS**	4	
415	**MS**	4	
501	**MS**	2	
502	**MS**	2	
503	**MS**	4	
813	**MS**	3	
814	**MS**	4	

Table C.5 (Continued)

815	**MS**	3	
906	**MS**	2	
907	**MS**	5	
908	**MS**	5	
909	**MS**	2	
914	**MS**	5	
915	**MS**	5	
1001	**MS**	1	
1002	**MS**	1	
1003	**MS**	1	
1004	**MS**	2	
1005	**MS**	2	
1006	**MS**	2	
1007	**MS**	3	
1008	**MS**	2	
1009	**MS**	2	
1010	**MS**	2	
1011	**MS**	4	
1012	**MS**	2	
1013	**MS**	1	
1014	**MS**	5	
1102	**MS**	3	
1103	**MS**	1	
1104	**MS**	2	
1105	**MS**	2	
1106	**MS**	3	
1107	**MS**	1	
1108	**MS**	2	
1109	**MS**	1	
1113	**MS**	2	
1115	**MS**	2	
1201	**MS**	2	
1203	**MS**	1	
1204	**MS**	1	
1205	**MS**	5	
1206	**MS**	2	
			2.85

Table C.5 (Continued)

803	**NC**	1	
804	**NC**	5	
806	**NC**	5	
807	**NC**	4	
808	**NC**	4	
			3.8
708	**SC**	5	
709	**SC**	6	
710	**SC**	4	
711	**SC**	5	
712	**SC**	5	
713	**SC**	4	
714	**SC**	4	
715	**SC**	4	
801	**SC**	2	
802	**SC**	2	
			4.1
809	**TN**	2	
810	**TN**	5	
811	**TN**	4	
812	**TN**	2	
1015	**TN**	1	
1101	**TN**	4	
1110	**TN**	1	
1111	**TN**	2	
1112	**TN**	1	
			2.1

Rankings were taken weekly following first observation of reproductive meristem with emergent inflorescence (15-May, 2015).

Table C.6 In vitro true dry matter degradability (IVTDMD) of eastern gamagrass accessions collected from across the southeastern and Atlantic United States.

ID	Rep	Bag + Sample Weight	Dry Bag Weight	Net Sample Weight	Dry Bag + Sample Weight	Dry Sample Residue. Weight	IVTDMD	Mean IVTDMD	CV
101	1	0.8021	0.5108	0.2913	0.6299	0.1191	59.1143		
	2	0.7911	0.5197	0.2714	0.6296	0.1099	59.5063	59.3103	0.4673
102	1	0.7615	0.4966	0.2649	0.5993	0.1027	61.2307		
	2	0.788	0.5239	0.2641	0.6222	0.0983	62.7793	62.0050	1.7660
103	1	0.7992	0.5088	0.2904	0.6377	0.1289	55.6129		
	2	0.7843	0.5067	0.2776	0.6368	0.1301	53.1340	54.3735	3.2238
104	1	0.805	0.5351	0.2699	0.635	0.0999	62.9863		
	2	0.7564	0.4933	0.2631	0.5976	0.1043	60.3573	61.6718	3.0143
105	1	0.821	0.5366	0.2844	0.6475	0.1109	61.0056		
	2	0.7779	0.5051	0.2728	0.6072	0.1021	62.5733	61.7895	1.7940
106	1	0.7958	0.5145	0.2813	0.6349	0.1204	57.1987		
	2	0.8249	0.5182	0.3067	0.6398	0.1216	60.3521	58.7754	3.7938
107	1	0.8044	0.5207	0.2837	0.6431	0.1224	56.8558		
	2	0.7782	0.5105	0.2677	0.6231	0.1126	57.9380	57.3969	1.3332
108	1	0.7962	0.5101	0.2861	0.6195	0.1094	61.7616		
	2	0.8046	0.5120	0.2926	0.6253	0.1133	61.2782	61.5199	0.5556
109	1	0.8038	0.5318	0.272	0.6471	0.1153	57.6103		
	2	0.8248	0.5285	0.2963	0.6482	0.1197	59.6018	58.6060	2.4028
110	1	0.8111	0.5130	0.2981	0.6414	0.1284	56.9272		

Table C.6 (Continued)

	2	0.7872	0.5043	0.2829	0.6172	0.1129	60.0919	58.5096	3.8246
111	1	0.7839	0.4939	0.29	0.6303	0.1364	52.9655		
	2	0.8215	0.5293	0.2922	0.668	0.1387	52.5325	52.7490	0.5804
112	1	0.8117	0.5154	0.2963	0.6577	0.1423	51.9744		
	2	0.7975	0.5111	0.2864	0.6489	0.1378	51.8855	51.9299	0.1210
113	1	0.7757	0.5098	0.2659	0.6316	0.1218	54.1933		
	2	0.777	0.4972	0.2798	0.6263	0.1291	53.8599	54.0266	0.4364
114	1	0.7783	0.5044	0.2739	0.6197	0.1153	57.9043		
	2	0.7969	0.5174	0.2795	0.6376	0.1202	56.9946	57.4495	1.1197
115	1	0.7724	0.5151	0.2573	0.5965	0.0814	68.3638		
	2	0.756	0.4903	0.2657	0.5777	0.0874	67.1058	67.7348	1.3133
201	1	0.7677	0.5114	0.2563	0.5968	0.0854	66.6797		
	2	0.788	0.5278	0.2602	0.6197	0.0919	64.6810	65.6803	2.1517
202	1	0.7935	0.5117	0.2818	0.6227	0.111	60.6104		
	2	0.8082	0.5356	0.2726	0.6344	0.0988	63.7564	62.1834	3.5775
203	1	0.7655	0.5068	0.2587	0.6283	0.1215	53.0344		
	2	0.7831	0.5151	0.268	0.6352	0.1201	55.1866	54.1105	2.8124
204	1	0.7604	0.5018	0.2586	0.6115	0.1097	57.5793		
	2	0.7591	0.5039	0.2552	0.6071	0.1032	59.5611	58.5702	2.3927
205	1	0.7978	0.5252	0.2726	0.6401	0.1149	57.8503		
	2	0.7942	0.5221	0.2721	0.6461	0.124	54.4285	56.1394	4.3100
206	1	0.8152	0.5382	0.277	0.6414	0.1032	62.7437		
	2	0.7815	0.5149	0.2666	0.6114	0.0965	63.8035	63.2736	1.1843

Table C.6 (Continued)

207	1	0.8261	0.5399	0.2862	0.6658	0.1259	56.0098		
	2	0.8498	0.5529	0.2969	0.6843	0.1314	55.7427	55.8762	0.3380
208	1	0.7879	0.5240	0.2639	0.6236	0.0996	62.2584		
	2	0.7783	0.5255	0.2528	0.6125	0.087	65.5854	63.9219	3.6804
209	1	0.8112	0.5407	0.2705	0.6703	0.1296	52.0887		
	2	0.7875	0.5309	0.2566	0.6496	0.1187	53.7412	52.9150	2.2083
210	1	0.8215	0.5347	0.2868	0.6472	0.1125	60.7741		
	2	0.8234	0.5359	0.2875	0.6488	0.1129	60.7304	60.7522	0.0508
211	1	0.8091	0.5425	0.2666	0.6419	0.0994	62.7157		
	2	0.8326	0.5627	0.2699	0.6663	0.1036	61.6154	62.1655	1.2515
212	1	0.7779	0.5159	0.262	0.6033	0.0874	66.6412		
	2	0.7756	0.5132	0.2624	0.5924	0.0792	69.8171	68.2291	3.2914
213	1	0.8335	0.5366	0.2969	0.682	0.1454	51.0273		
	2	0.8188	0.5432	0.2756	0.6827	0.1395	49.3832	50.2052	2.3156
214	1	0.8216	0.5535	0.2681	0.654	0.1005	62.5140		
	2	0.8325	0.5477	0.2848	0.6555	0.1078	62.1489	62.3314	0.4142
215	1	0.7999	0.5420	0.2579	0.6437	0.1017	60.5661		
	2	0.8088	0.5396	0.2692	0.6411	0.1015	62.2957	61.4309	1.9909
301	1	0.7983	0.5200	0.2783	0.6283	0.1083	61.0852		
	2	0.8378	0.5379	0.2999	0.657	0.1191	60.2868	60.6860	0.9303
302	1	0.7831	0.5247	0.2584	0.6257	0.101	60.9133		
	2	0.8165	0.5432	0.2733	0.6472	0.104	61.9466	61.4299	1.1894
303	1	0.8235	0.5154	0.3081	0.6257	0.1103	64.1999		
	2	0.8338	0.5407	0.2931	0.6459	0.1052	64.1078	64.1539	0.1015

Table C.6 (Continued)

305	1	0.7907	0.5316	0.2591	0.6479	0.1163	55.1139		
	2	0.7868	0.5200	0.2668	0.6414	0.1214	54.4978	54.8058	0.7949
307	1	0.8181	0.5469	0.2712	0.6607	0.1138	58.0383		
	2	0.7946	0.5291	0.2655	0.6407	0.1116	57.9661	58.0022	0.0881
308	1	0.7868	0.5327	0.2541	0.6344	0.1017	59.9764		
	2	0.8054	0.5315	0.2739	0.6393	0.1078	60.6426	60.3095	0.7811
309	1	0.7984	0.5418	0.2566	0.6371	0.0953	62.8605		
	2	0.7953	0.5336	0.2617	0.6295	0.0959	63.3550	63.1077	0.5541
310	1	0.8022	0.5263	0.2759	0.6258	0.0995	63.9362		
	2	0.8133	0.5376	0.2757	0.6292	0.0916	66.7755	65.3558	3.0719
311	1	0.7897	0.5144	0.2753	0.6058	0.0914	66.7999		
	2	0.7968	0.5118	0.285	0.6191	0.1073	62.3509	64.5754	4.8717
312	1	0.809	0.5364	0.2726	0.6226	0.0862	68.3786		
	2	0.7936	0.5304	0.2632	0.6198	0.0894	66.0334	67.2060	2.4674
313	1	0.828	0.5299	0.2981	0.6491	0.1192	60.0134		
	2	0.8026	0.5287	0.2739	0.6423	0.1136	58.5250	59.2692	1.7757
314	1	0.8081	0.5532	0.2549	0.6609	0.1077	57.7481		
	2	0.8371	0.5363	0.3008	0.6736	0.1373	54.3551	56.0516	4.2805
315	1	0.7958	0.5324	0.2634	0.6379	0.1055	59.9468		
	2	0.818	0.5532	0.2648	0.6601	0.1069	59.6299	59.7884	0.3748
401	1	0.8237	0.5304	0.2933	0.6562	0.1258	57.1088		
	2	0.7927	0.5412	0.2515	0.6376	0.0964	61.6700	59.3894	5.4307
402	1	0.8223	0.5329	0.2894	0.6319	0.099	65.7913		
	2	0.7984	0.5371	0.2613	0.631	0.0939	64.0643	64.9278	1.8808

Table C.6 (Continued)

403	1	0.8264	0.5244	0.302	0.6695	0.1451	51.9536		
	2	0.8105	0.5358	0.2747	0.6642	0.1284	53.2581	52.6059	1.7534
404	1	0.8221	0.5523	0.2698	0.6866	0.1343	50.2224		
	2	0.8159	0.5319	0.284	0.6669	0.135	52.4648	51.3436	3.0882
405	1	0.8133	0.5599	0.2534	0.6723	0.1124	55.6433		
	2	0.8401	0.5430	0.2971	0.6729	0.1299	56.2773	55.9603	0.8012
406	1	0.8182	0.5601	0.2581	0.664	0.1039	59.7443		
	2	0.7782	0.5267	0.2515	0.6259	0.0992	60.5567	60.1505	0.9550
407	1	0.8097	0.5432	0.2665	0.637	0.0938	64.8030		
	2	0.7887	0.5224	0.2663	0.6259	0.1035	61.1341	62.9685	4.1200
408	1	0.8288	0.5284	0.3004	0.6524	0.124	58.7217		
	2	0.7888	0.5278	0.261	0.6374	0.1096	58.0077	58.3647	0.8651
409	1	0.8026	0.5323	0.2703	0.6227	0.0904	66.5557		
	2	0.7816	0.5148	0.2668	0.6037	0.0889	66.6792	66.6174	0.1311
410	1	0.8088	0.5397	0.2691	0.6334	0.0937	65.1802		
	2	0.8036	0.5378	0.2658	0.6358	0.098	63.1302	64.1552	2.2595
411	1	0.8111	0.5473	0.2638	0.6439	0.0966	63.3813		
	2	0.8	0.5413	0.2587	0.6338	0.0925	64.2443	63.8128	0.9562
412	1	0.7878	0.5098	0.278	0.6064	0.0966	65.2518		
	2	0.8429	0.5350	0.3079	0.6437	0.1087	64.6963	64.9741	0.6045
413	1	0.8124	0.5174	0.295	0.6183	0.1009	65.7966		
	2	0.8239	0.5406	0.2833	0.6398	0.0992	64.9841	65.3904	0.8786
415	1	0.7955	0.5284	0.2671	0.6054	0.077	71.1718		
	2	0.7976	0.5243	0.2733	0.6061	0.0818	70.0695	70.6207	1.1037

Table C.6 (Continued)

501	1	0.8142	0.5134	0.3008	0.6431	0.1297	56.8816		
	2	0.8287	0.5301	0.2986	0.6618	0.1317	55.8942	56.3879	1.2383
502	1	0.7597	0.4952	0.2645	0.6003	0.1051	60.2647		
	2	0.791	0.4940	0.297	0.6141	0.1201	59.5623	59.9135	0.8289
503	1	0.8104	0.5250	0.2854	0.6054	0.0804	71.8290		
	2	0.803	0.5372	0.2658	0.6109	0.0737	72.2724	72.0507	0.4351
504	1	0.8123	0.5474	0.2649	0.6393	0.0919	65.3077		
	2	0.8299	0.5330	0.2969	0.6293	0.0963	67.5648	66.4362	2.4024
505	1	0.7877	0.5137	0.274	0.635	0.1213	55.7299		
	2	0.8211	0.5336	0.2875	0.658	0.1244	56.7304	56.2302	1.2582
507	1	0.7714	0.5132	0.2582	0.5928	0.0796	69.1712		
	2	0.804	0.5459	0.2581	0.6273	0.0814	68.4618	68.8165	0.7289
508	1	0.8292	0.5515	0.2777	0.6644	0.1129	59.3446		
	2	0.7999	0.5300	0.2699	0.641	0.111	58.8737	59.1091	0.5634
509	1	0.7947	0.5286	0.2661	0.6099	0.0813	69.4476		
	2	0.8178	0.5322	0.2856	0.6319	0.0997	65.0910	67.2693	4.5794
510	1	0.818	0.5385	0.2795	0.631	0.0925	66.9052		
	2	0.8058	0.5149	0.2909	0.6113	0.0964	66.8615	66.8833	0.0462
511	1	0.7966	0.5339	0.2627	0.6106	0.0767	70.8032		
	2	0.8192	0.5524	0.2668	0.6331	0.0807	69.7526	70.2779	1.0570
512	1	0.8034	0.5321	0.2713	0.6177	0.0856	68.4482		
	2	0.7717	0.5033	0.2684	0.5799	0.0766	71.4605	69.9544	3.0449
513	1	0.807	0.5292	0.2778	0.6195	0.0903	67.4946		
	2	0.8322	0.5697	0.2625	0.6569	0.0872	66.7810	67.1378	0.7516

Table C.6 (Continued)

514	1	0.7885	0.5229	0.2656	0.6324	0.1095	58.7726		
	2	0.7843	0.5242	0.2601	0.6356	0.1114	57.1703	57.9715	1.9544
515	1	0.7769	0.5243	0.2526	0.6085	0.0842	66.6667		
	2	0.7846	0.5277	0.2569	0.6201	0.0924	64.0327	65.3497	2.8500
603	1	0.7876	0.5181	0.2695	0.6284	0.1103	59.0724		
	2	0.7633	0.5071	0.2562	0.613	0.1059	58.6651	58.8687	0.4892
604	1	0.7737	0.5204	0.2533	0.5969	0.0765	69.7987		
	2	0.8311	0.5456	0.2855	0.6329	0.0873	69.4221	69.6104	0.3825
605	1	0.7927	0.5334	0.2593	0.6178	0.0844	67.4508		
	2	0.7879	0.5226	0.2653	0.609	0.0864	67.4331	67.4420	0.0186
607	1	0.7734	0.4972	0.2762	0.5912	0.094	65.9667		
	2	0.8092	0.5230	0.2862	0.6254	0.1024	64.2208	65.0938	1.8965
608	1	0.7971	0.5335	0.2636	0.6026	0.0691	73.7860		
	2	0.7917	0.5214	0.2703	0.5934	0.072	73.3629	73.5745	0.4066
609	1	0.8004	0.5242	0.2762	0.5992	0.075	72.8458		
	2	0.8111	0.5230	0.2881	0.6062	0.0832	71.1211	71.9835	1.6941
610	1	0.776	0.5223	0.2537	0.6329	0.1106	56.4052		
	2	0.8307	0.5504	0.2803	0.6661	0.1157	58.7228	57.5640	2.8469
611	1	0.8438	0.5392	0.3046	0.6638	0.1246	59.0939		
	2	0.8	0.5424	0.2576	0.6518	0.1094	57.5311	58.3125	1.8951
612	1	0.8209	0.5210	0.2999	0.6319	0.1109	63.0210		
	2	0.8217	0.5431	0.2786	0.6496	0.1065	61.7732	62.3971	1.4141
613	1	0.7972	0.5285	0.2687	0.6186	0.0901	66.4682		
	2	0.8067	0.5159	0.2908	0.6186	0.1027	64.6836	65.5759	1.9243

Table C.6 (Continued)

614	1	0.8197	0.5182	0.3015	0.6117	0.0935	68.9884		
	2	0.786	0.5278	0.2582	0.6062	0.0784	69.6359	69.3122	0.6606
615	1	0.7888	0.5258	0.263	0.6005	0.0747	71.5970		
	2	0.7626	0.5073	0.2553	0.5856	0.0783	69.3302	70.4636	2.2747
701	1	0.7618	0.5099	0.2519	0.5894	0.0795	68.4399		
	2	0.7668	0.5144	0.2524	0.5987	0.0843	66.6006	67.5202	1.9261
702	1	0.7599	0.5040	0.2559	0.5644	0.0604	76.3970		
	2	0.84245	0.5724	0.27005	0.6461	0.0737	72.7088	74.5529	3.4982
703	1	0.7924	0.5266	0.2658	0.604	0.0774	70.8804		
	2	0.7977	0.5376	0.2601	0.6138	0.0762	70.7036	70.7920	0.1766
704	1	0.7907	0.5349	0.2558	0.6069	0.072	71.8530		
	2	0.7671	0.5106	0.2565	0.5811	0.0705	72.5146	72.1838	0.6481
705	1	0.7785	0.5251	0.2534	0.6026	0.0775	69.4159		
	2	0.7886	0.5232	0.2654	0.5996	0.0764	71.2133	70.3146	1.8074
706	1	0.7857	0.5233	0.2624	0.609	0.0857	67.3399		
	2	0.7718	0.5177	0.2541	0.5961	0.0784	69.1460	68.2430	1.8714
707	1	0.7697	0.5012	0.2685	0.585	0.0838	68.7896		
	2	0.7812	0.5229	0.2583	0.6058	0.0829	67.9055	68.3476	0.9146
708	1	0.7824	0.5289	0.2535	0.618	0.0891	64.8521		
	2	0.7883	0.5344	0.2539	0.6194	0.085	66.5223	65.6872	1.7979
709	1	0.7827	0.5318	0.2509	0.6145	0.0827	67.0387		
	2	0.7769	0.5100	0.2669	0.5941	0.0841	68.4901	67.7644	1.5145
710	1	0.7869	0.5212	0.2657	0.6204	0.0992	62.6647		
	2	0.783	0.5240	0.259	0.6225	0.0985	61.9691	62.3169	0.7892

Table C.6 (Continued)

711	1	0.8164	0.5541	0.2623	0.6432	0.0891	66.0313		
	2	0.8308	0.5622	0.2686	0.6551	0.0929	65.4133	65.7223	0.6649
712	1	0.7943	0.5395	0.2548	0.6243	0.0848	66.7190		
	2	0.8151	0.5445	0.2706	0.6293	0.0848	68.6622	67.6906	2.0299
713	1	0.7701	0.5106	0.2595	0.5886	0.078	69.9422		
	2	0.8474	0.5521	0.2953	0.6397	0.0876	70.3353	70.1387	0.3963
714	1	0.7982	0.5277	0.2705	0.6041	0.0764	71.7560		
	2	0.7881	0.5261	0.262	0.6041	0.078	70.2290	70.9925	1.5209
715	1	0.8048	0.5353	0.2695	0.6226	0.0873	67.6067		
	2	0.7804	0.5250	0.2554	0.6072	0.0822	67.8152	67.7109	0.2178
801	1	0.7877	0.5203	0.2674	0.6214	0.1011	62.1915		
	2	0.8238	0.5260	0.2978	0.6378	0.1118	62.4580	62.3247	0.3024
802	1	0.8067	0.5441	0.2626	0.6464	0.1023	61.0434		
	2	0.832	0.5571	0.2749	0.6583	0.1012	63.1866	62.1150	2.4398
803	1	0.7817	0.5213	0.2604	0.5981	0.0768	70.5069		
	2	0.7894	0.5260	0.2634	0.6004	0.0744	71.7540	71.1304	1.2397
804	1	0.7832	0.5265	0.2567	0.6096	0.0831	67.6276		
	2	0.7988	0.5183	0.2805	0.6016	0.0833	70.3030	68.9653	2.7432
805	1	0.7881	0.5202	0.2679	0.5999	0.0797	70.2501		
	2	0.7927	0.5309	0.2618	0.6043	0.0734	71.9633	71.1067	1.7037
806	1	0.7891	0.5248	0.2643	0.6139	0.0891	66.2883		
	2	0.7927	0.5118	0.2809	0.6053	0.0935	66.7141	66.5012	0.4528
807	1	0.8014	0.5405	0.2609	0.6219	0.0814	68.8003		
	2	0.7904	0.5264	0.264	0.6099	0.0835	68.3712	68.5858	0.4424

Table C.6 (Continued)

808	1	0.7898	0.5017	0.2881	0.6159	0.1142	60.3610		
	2	0.8281	0.5335	0.2946	0.6549	0.1214	58.7916	59.5763	1.8627
809	1	0.8007	0.5271	0.2736	0.6179	0.0908	66.8129		
	2	0.8124	0.5331	0.2793	0.6208	0.0877	68.6001	67.7065	1.8665
810	1	0.7557	0.4953	0.2604	0.568	0.0727	72.0814		
	2	0.7845	0.4907	0.2938	0.5811	0.0904	69.2308	70.6561	2.8528
811	1	0.8014	0.5446	0.2568	0.6326	0.088	65.7321		
	2	0.7679	0.5075	0.2604	0.596	0.0885	66.0138	65.8730	0.3024
812	1	0.7753	0.5147	0.2606	0.6011	0.0864	66.8457		
	2	0.7856	0.5232	0.2624	0.6121	0.0889	66.1204	66.4831	0.7714
813	1	0.7866	0.5231	0.2635	0.6264	0.1033	60.7970		
	2	0.7983	0.5411	0.2572	0.6412	0.1001	61.0809	60.9389	0.3294
814	1	0.7661	0.5106	0.2555	0.6028	0.0922	63.9139		
	2	0.7707	0.5023	0.2684	0.6043	0.102	61.9970	62.9555	2.1530
815	1	0.8248	0.5179	0.3069	0.6335	0.1156	62.3330		
	2	0.8002	0.5311	0.2691	0.6296	0.0985	63.3965	62.8648	1.1962
901	1	0.8008	0.5375	0.2633	0.6077	0.0702	73.3384		
	2	0.7859	0.5218	0.2641	0.5845	0.0627	76.2590	74.7987	2.7610
902	1	0.7836	0.5141	0.2695	0.6014	0.0873	67.6067		
	2	0.8225	0.5142	0.3083	0.6192	0.105	65.9423	66.7745	1.7625
903	1	0.7658	0.5131	0.2527	0.5877	0.0746	70.4788		
	2	0.8355	0.5301	0.3054	0.6252	0.0951	68.8605	69.6697	1.6425
904	1	0.7739	0.5183	0.2556	0.5797	0.0614	75.9781		
	2	0.7773	0.5137	0.2636	0.5749	0.0612	76.7830	76.3805	0.7452

Table C.6 (Continued)

905	1	0.7966	0.5006	0.296	0.5848	0.0842	71.5541		
	2	0.76	0.5050	0.255	0.5743	0.0693	72.8235	72.1888	1.2435
906	1	0.7595	0.4919	0.2676	0.5552	0.0633	76.3453		
	2	0.7472	0.4968	0.2504	0.5637	0.0669	73.2827	74.8140	2.8946
907	1	0.7792	0.4829	0.2963	0.5762	0.0933	68.5116		
	2	0.7695	0.4960	0.2735	0.5806	0.0846	69.0676	68.7896	0.5715
908	1	0.7567	0.4979	0.2588	0.5964	0.0985	61.9397		
	2	0.7849	0.5254	0.2595	0.6253	0.0999	61.5029	61.7213	0.5005
909	1	0.7614	0.4963	0.2651	0.5893	0.093	64.9189		
	2	0.7696	0.4980	0.2716	0.5811	0.0831	69.4035	67.1612	4.7216
911	1	0.76	0.5013	0.2587	0.5977	0.0964	62.7368		
	2	0.8101	0.5503	0.2598	0.6489	0.0986	62.0477	62.3922	0.7809
912	1	0.7681	0.5095	0.2586	0.5971	0.0876	66.1253		
	2	0.8166	0.5250	0.2916	0.6248	0.0998	65.7750	65.9502	0.3755
913	1	0.811	0.5281	0.2829	0.6351	0.107	62.1774		
	2	0.7847	0.5143	0.2704	0.6058	0.0915	66.1612	64.1693	4.3899
914	1	0.8284	0.5217	0.3067	0.6525	0.1308	57.3525		
	2	0.81	0.5333	0.2767	0.6466	0.1133	59.0531	58.2028	2.0661
915	1	0.7799	0.5229	0.257	0.6074	0.0845	67.1206		
	2	0.8227	0.5363	0.2864	0.6311	0.0948	66.8994	67.0100	0.2334
916	1	0.8268	0.5379	0.2889	0.6467	0.1088	62.3399		
	2	0.8033	0.5222	0.2811	0.6298	0.1076	61.7218	62.0309	0.7046
1001	1	0.8119	0.5555	0.2564	0.6467	0.0912	64.4306		
	2	0.8714	0.5716	0.2998	0.6716	0.1	66.6444	65.5375	2.3886

Table C.6 (Continued)

1002	1	0.8372	0.5374	0.2998	0.6331	0.0957	68.0787		
	2	0.8027	0.5292	0.2735	0.6112	0.082	70.0183	69.0485	1.9863
1003	1	0.8211	0.5122	0.3089	0.5926	0.0804	73.9722		
	2	0.786	0.5283	0.2577	0.603	0.0747	71.0128	72.4925	2.8866
1004	1	0.7858	0.5292	0.2566	0.6043	0.0751	70.7327		
	2	0.8075	0.5231	0.2844	0.6137	0.0906	68.1435	69.4381	2.6367
1005	1	0.8012	0.5387	0.2625	0.6268	0.0881	66.4381		
	2	0.8294	0.5652	0.2642	0.6602	0.095	64.0424	65.2402	2.5966
1006	1	0.7807	0.5253	0.2554	0.6082	0.0829	67.5411		
	2	0.8609	0.5523	0.3086	0.6531	0.1008	67.3364	67.4387	0.2147
1007	1	0.8192	0.5447	0.2745	0.6183	0.0736	73.1876		
	2	0.8555	0.5574	0.2981	0.6455	0.0881	70.4462	71.8169	2.6992
1008	1	0.7934	0.5364	0.257	0.6138	0.0774	69.8833		
	2	0.7774	0.5237	0.2537	0.6027	0.079	68.8609	69.3721	1.0421
1009	1	0.7907	0.5257	0.265	0.6139	0.0882	66.7170		
	2	0.848	0.5568	0.2912	0.6616	0.1048	64.0110	65.3640	2.9273
1010	1	0.8034	0.5437	0.2597	0.6206	0.0769	70.3889		
	2	0.7935	0.5337	0.2598	0.6028	0.0691	73.4026	71.8958	2.9640
1011	1	0.8255	0.5549	0.2706	0.6402	0.0853	68.4775		
	2	0.8194	0.5542	0.2652	0.6472	0.093	64.9321	66.7048	3.7582
1012	1	0.8314	0.5455	0.2859	0.6335	0.088	69.2200		
	2	0.7762	0.5195	0.2567	0.5981	0.0786	69.3806	69.3003	0.1639
1013	1	0.8202	0.5493	0.2709	0.645	0.0957	64.6733		
	2	0.8439	0.5455	0.2984	0.6547	0.1092	63.4048	64.0391	1.4006

Table C.6 (Continued)

1014	1	0.7921	0.5036	0.2885	0.6146	0.111	61.5251		
	2	0.7667	0.5084	0.2583	0.6151	0.1067	58.6914	60.1083	3.3335
1015	1	0.7843	0.5208	0.2635	0.612	0.0912	65.3890		
	2	0.7937	0.5160	0.2777	0.6165	0.1005	63.8099	64.5994	1.7285
1101	1	0.7961	0.5323	0.2638	0.6241	0.0918	65.2009		
	2	0.7944	0.5081	0.2863	0.6127	0.1046	63.4649	64.3329	1.9081
1102	1	0.8132	0.5391	0.2741	0.6247	0.0856	68.7705		
	2	0.7359	0.4849	0.251	0.5565	0.0716	71.4741	70.1223	2.7263
1103	1	0.8019	0.4944	0.3075	0.5847	0.0903	70.6341		
	2	0.8199	0.5291	0.2908	0.6236	0.0945	67.5034	69.0688	3.2051
1104	1	0.8022	0.5321	0.2701	0.6203	0.0882	67.3454		
	2	0.8163	0.5089	0.3074	0.6069	0.098	68.1197	67.7326	0.8083
1105	1	0.8203	0.5118	0.3085	0.646	0.1342	56.4992		
	2	0.7824	0.5140	0.2684	0.6254	0.1114	58.4948	57.4970	2.4542
1106	1	0.7791	0.5203	0.2588	0.5857	0.0654	74.7295		
	2	0.7886	0.5190	0.2696	0.5879	0.0689	74.4436	74.5866	0.2710
1107	1	0.768	0.5158	0.2522	0.5985	0.0827	67.2086		
	2	0.7659	0.4963	0.2696	0.5783	0.082	69.5846	68.3966	2.4564
1108	1	0.7934	0.5373	0.2561	0.6405	0.1032	59.7032		
	2	0.8111	0.5169	0.2942	0.6333	0.1164	60.4351	60.0692	0.8615
1109	1	0.7723	0.5084	0.2639	0.595	0.0866	67.1845		
	2	0.7823	0.5013	0.281	0.5893	0.088	68.6833	67.9339	1.5600
1110	1	0.7967	0.4931	0.3036	0.5925	0.0994	67.2596		
	2	0.7789	0.5103	0.2686	0.5995	0.0892	66.7908	67.0252	0.4946

Table C.6 (Continued)

1111	1	0.7991	0.5233	0.2758	0.5978	0.0745	72.9877		
	2	0.7704	0.5067	0.2637	0.586	0.0793	69.9279	71.4578	3.0277
1112	1	0.7757	0.5038	0.2719	0.5668	0.063	76.8297		
	2	0.801	0.4981	0.3029	0.5746	0.0765	74.7441	75.7869	1.9459
1113	1	0.7832	0.5181	0.2651	0.6037	0.0856	67.7103		
	2	0.831	0.5245	0.3065	0.6366	0.1121	63.4258	65.5680	4.6206
1115	1	0.7696	0.5088	0.2608	0.6021	0.0933	64.2255		
	2	0.806	0.5243	0.2817	0.6315	0.1072	61.9453	63.0854	2.5557
1201	1	0.7849	0.5183	0.2666	0.6271	0.1088	59.1898		
	2	0.8363	0.5335	0.3028	0.6481	0.1146	62.1532	60.6715	3.4538
1203	1	0.7954	0.5097	0.2857	0.583	0.0733	74.3437		
	2	0.8107	0.5074	0.3033	0.5888	0.0814	73.1619	73.7528	1.1331
1204	1	0.7424	0.4902	0.2522	0.558	0.0678	73.1166		
	2	0.7757	0.5192	0.2565	0.5912	0.072	71.9298	72.5232	1.1571
1205	1	0.8	0.5187	0.2813	0.6199	0.1012	64.0242		
	2	0.8319	0.5229	0.309	0.6294	0.1065	65.5340	64.7791	1.6481
1206	1	0.7771	0.5218	0.2553	0.5987	0.0769	69.8786		
	2	0.8141	0.5166	0.2975	0.6136	0.097	67.3950	68.6368	2.5587

Subsamples taken from 30-day regrowth, harvested at 30-cm stubble height during August, 2015.
All weights in grams

Table C.7 Analysis of variance and means separations for in vitro true dry matter degradability of eastern gamagrass accessions collected from across the southeastern and Atlantic United States.

T Grouping																Mean	N	Plant
								A								76.38	2	904
								A										
			B					A								75.785	2	1112
			B					A										
			B					A				C				74.815	2	906
			B					A				C						
			B					A				C				74.8	2	901
			B					A				C						
			B		D			A				C				74.585	2	1106
			B		D			A				C						
	E		B		D			A				C				74.555	2	702
	E		B		D			A				C						
	E		B		D			A				C		F		73.75	2	1203
	E		B		D							C		F				
	E		B		D			G				C		F		73.575	2	608
	E				D			G				C		F				
	E		H		D			G				C		F		72.525	2	1204
	E		H		D			G				C		F				
	E		H		D			G				C		F		72.49	2	1003
	E		H		D			G				C		F				
	E		H		D			G		I		C		F		72.185	2	905

Table C.7 (Continued)

	E		H		D		G		I		C		F						
	E		H		D		G		I		C		F		72.18	2	704		
	E		H		D		G		I				F						
	E		H		D		G		I		J		F		72.05	2	503		
	E		H		D		G		I		J		F						
	E		H		D	K	G		I		J		F		71.985	2	609		
	E		H			K	G		I		J		F						
	E		H		L	K	G		I		J		F		71.895	2	1010		
			H		L	K	G		I		J		F						
			H		L	K	G		I		J		F		71.82	2	1007		
			H		L	K	G		I		J		F						
	M		H		L	K	G		I		J		F		71.46	2	1111		
	M		H		L	K	G		I		J		F						
	M		H	N	L	K	G		I		J		F		71.13	2	803		
	M		H	N	L	K	G		I		J		F						
	M		H	N	L	K	G		I		J		F		71.105	2	805		
	M		H	N	L	K	G		I		J								
	M		H	N	L	K	G		I		J		O		70.995	2	714		
	M		H	N	L	K			I		J		O						
	M		H	N	L	K	P		I		J		O		70.79	2	703		
	M		H	N	L	K	P		I		J		O						
	M		H	N	L	K	P		I		J		O		70.655	2	810		
	M		H	N	L	K	P		I		J		O						
	M		H	N	L	K	P		I		J		O		70.62	2	415		

Table C.7 (Continued)

	M		H	N	L	K		P		I		J		O				
	M	Q	H	N	L	K		P		I		J		O		70.465	2	615
	M	Q	H	N	L	K		P		I		J		O				
R	M	Q	H	N	L	K		P		I		J		O		70.315	2	705
R	M	Q	H	N	L	K		P		I		J		O				
R	M	Q	H	N	L	K		P		I		J		O		70.275	2	511
R	M	Q	H	N	L	K		P		I		J		O				
R	M	Q	H	N	L	K		P		I		J		O	S	70.14	2	713
R	M	Q	H	N	L	K		P		I		J		O	S			
R	M	Q	H	N	L	K		P		I		J		O	S	70.12	2	1102
R	M	Q	H	N	L	K		P		I		J		O	S			
R	M	Q	H	N	L	K		P		I		J	T	O	S	69.955	2	512
R	M	Q		N	L	K		P		I		J	T	O	S			
R	M	Q	U	N	L	K		P		I		J	T	O	S	69.67	2	903
R	M	Q	U	N	L	K		P		I		J	T	O	S			
R	M	Q	U	N	L	K		P		I		J	T	O	S	69.61	2	604
R	M	Q	U	N	L	K		P				J	T	O	S			
R	M	Q	U	N	L	K		P		V		J	T	O	S	69.435	2	1004
R	M	Q	U	N	L	K		P		V			T	O	S			
R	M	Q	U	N	L	K		P		V		W	T	O	S	69.37	2	1008
R	M	Q	U	N	L	K		P		V		W	T	O	S			
R	M	Q	U	N	L	K		P		V		W	T	O	S	69.315	2	614
R	M	Q	U	N	L			P		V		W	T	O	S			
R	M	Q	U	N	L			P		V		W	T	O	S	69.3	2	1012

Table C.7 (Continued)

R	M	Q	U	N			P		V		W	T	O	S				
R	M	Q	U	N	X		P		V		W	T	O	S	69.065	2	1103	
R	M	Q	U	N	X		P		V		W	T	O	S				
R	M	Q	U	N	X		P		V		W	T	O	S	69.05	2	1002	
R	M	Q	U	N	X		P		V		W	T	O	S				
R	M	Q	U	N	X		P		V		W	T	O	S	68.965	2	804	
R	M	Q	U	N	X		P		V		W	T	O	S				
R	M	Q	U	N	X		P		V		W	T	O	S	68.815	2	507	
R	M	Q	U	N	X		P		V		W	T	O	S				
R	M	Q	U	N	X		P		V		W	T	O	S	68.79	2	907	
R		Q	U	N	X		P		V		W	T	O	S				
R		Q	U	N	X		P		V		W	T	O	S	68.635	2	1206	
R		Q	U	N	X		P		V		W	T	O	S				
R	Y	Q	U	N	X		P		V		W	T	O	S	68.585	2	807	
R	Y	Q	U		X		P		V		W	T	O	S				
R	Y	Q	U		X	Z	P		V		W	T	O	S	68.395	2	1107	
R	Y	Q	U		X	Z	P		V		W	T	O	S				
R	Y	Q	U	A	X	Z	P		V		W	T	O	S	68.35	2	707	
R	Y	Q	U	A	X	Z	P		V		W	T		S				
R	Y	Q	U	A	X	Z	P		V		W	T	B	S	68.245	2	706	
R	Y	Q	U	A	X	Z	P		V		W	T	B	S				
R	Y	Q	U	A	X	Z	P		V		W	T	B	S	68.23	2	212	
R	Y	Q	U	A	X	Z			V		W	T	B	S				
R	Y	Q	U	A	X	Z	C		V		W	T	B	S	67.93	2	1109	

Table C.7 (Continued)

R	Y		U	A	X	Z		C		V		W	T	B	S			
R	Y	D	U	A	X	Z		C		V		W	T	B	S	67.765	2	709
R	Y	D	U	A	X	Z		C		V		W	T	B	S			
R	Y	D	U	A	X	Z		C		V		W	T	B	S	67.735	2	115
R	Y	D	U	A	X	Z		C		V		W	T	B	S			
R	Y	D	U	A	X	Z		C		V		W	T	B	S	67.735	2	1104
R	Y	D	U	A	X	Z		C		V		W	T	B	S			
R	Y	D	U	A	X	Z		C		V		W	T	B	S	67.715	2	715
R	Y	D	U	A	X	Z		C		V		W	T	B	S			
R	Y	D	U	A	X	Z		C		V		W	T	B	S	67.705	2	809
R	Y	D	U	A	X	Z		C		V		W	T	B	S			
R	Y	D	U	A	X	Z		C		V		W	T	B	S	67.69	2	712
	Y	D	U	A	X	Z		C		V		W	T	B	S			
E	Y	D	U	A	X	Z		C		V		W	T	B	S	67.52	2	701
E	Y	D	U	A	X	Z		C		V		W	T	B				
E	Y	D	U	A	X	Z		C		V		W	T	B	F	67.44	2	605
E	Y	D	U	A	X	Z		C		V		W	T	B	F			
E	Y	D	U	A	X	Z		C		V		W	T	B	F	67.44	2	1006
E	Y	D	U	A	X	Z		C		V		W		B	F			
E	Y	D	U	A	X	Z		C		V		W	G	B	F	67.27	2	509
E	Y	D	U	A	X	Z		C		V		W	G	B	F			
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F	67.205	2	312
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F			
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F	67.16	2	909

Table C.7 (Continued)

E	Y	D	U	A	X	Z		C		V	H	W	G	B	F					
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F	67.135	2	513		
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F					
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F	67.025	2	1110		
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F					
E	Y	D	U	A	X	Z		C		V	H	W	G	B	F	67.01	2	914		
E	Y	D		A	X	Z		C		V	H	W	G	B	F					
E	Y	D	I	A	X	Z		C		V	H	W	G	B	F	66.885	2	510		
E	Y	D	I	A	X	Z		C		V	H	W	G	B	F					
E	Y	D	I	A	X	Z		C	J	V	H	W	G	B	F	66.775	2	902		
E	Y	D	I	A	X	Z		C	J		H	W	G	B	F					
E	Y	D	I	A	X	Z		C	J	K	H	W	G	B	F	66.705	2	1011		
E	Y	D	I	A	X	Z		C	J	K	H		G	B	F					
E	Y	D	I	A	X	Z		C	J	K	H		G	B	F	66.62	2	409		
E	Y	D	I	A	X	Z		C	J	K	H		G	B	F					
E	Y	D	I	A	X	Z		C	J	K	H	L	G	B	F	66.5	2	806		
E	Y	D	I	A	X	Z		C	J	K	H	L	G	B	F					
E	Y	D	I	A	X	Z	M	C	J	K	H	L	G	B	F	66.485	2	812		
E	Y	D	I	A	X	Z	M	C	J	K	H	L	G	B	F					
E	Y	D	I	A	X	Z	M	C	J	K	H	L	G	B	F	66.435	2	504		
E	Y	D	I	A		Z	M	C	J	K	H	L	G	B	F					
E	Y	D	I	A		Z	M	C	J	K	H	L	G	B	F	65.955	2	911		
E		D	I	A		Z	M	C	J	K	H	L	G	B	F					
E		D	I	A	N	Z	M	C	J	K	H	L	G	B	F	65.87	2	811		

Table C.7 (Continued)

E		D	I	A	N	Z	M	C	J	K	H	L	G	B	F			
E	O	D	I	A	N	Z	M	C	J	K	H	L	G	B	F	65.72	2	711
E	O	D	I	A	N		M	C	J	K	H	L	G	B	F			
E	O	D	I	A	N		M	C	J	K	H	L	G	B	F	65.685	2	708
E	O	D	I	A	N		M	C	J	K	H	L	G	B	F			
E	O	D	I	A	N		M	C	J	K	H	L	G	B	F	65.68	2	201
E	O	D	I		N		M	C	J	K	H	L	G	B	F			
E	O	D	I		N	P	M	C	J	K	H	L	G	B	F	65.575	2	613
E	O	D	I		N	P	M	C	J	K	H	L	G	B	F			
E	O	D	I		N	P	M	C	J	K	H	L	G	B	F	65.57	2	1113
E	O	D	I		N	P	M	C	J	K	H	L	G		F			
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F	65.535	2	1001
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F			
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F	65.39	2	413
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F			
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F	65.365	2	1009
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F			
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F	65.36	2	310
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F			
E	O	D	I		N	P	M	C	J	K	H	L	G	Q	F	65.35	2	515
E	O	D	I		N	P	M		J	K	H	L	G	Q	F			
E	O	D	I		N	P	M		J	K	H	L	G	Q	F	65.24	2	1005
E	O	D	I		N	P	M		J	K	H	L	G	Q	F			
E	O	D	I		N	P	M		J	K	H	L	G	Q	F	65.095	2	607

Table C.7 (Continued)

E	O		I		N	P	M		J	K	H	L	G	Q	F			
E	O		I		N	P	M	R	J	K	H	L	G	Q	F	64.975	2	412
E	O		I		N	P	M	R	J	K	H	L	G	Q	F			
E	O		I		N	P	M	R	J	K	H	L	G	Q	F	64.925	2	402
	O		I		N	P	M	R	J	K	H	L	G	Q	F			
	O		I	S	N	P	M	R	J	K	H	L	G	Q	F	64.775	2	1205
	O		I	S	N	P	M	R	J	K	H	L	G	Q				
	O	T	I	S	N	P	M	R	J	K	H	L	G	Q		64.6	2	1015
	O	T	I	S	N	P	M	R	J	K	H	L		Q				
	O	T	I	S	N	P	M	R	J	K	H	L		Q		64.575	2	311
	O	T	I	S	N	P	M	R	J	K		L		Q				
U	O	T	I	S	N	P	M	R	J	K		L		Q		64.33	2	1101
U	O	T		S	N	P	M	R	J	K		L		Q				
U	O	T	V	S	N	P	M	R	J	K		L		Q		64.17	2	912
U	O	T	V	S	N	P	M	R	J	K		L		Q				
U	O	T	V	S	N	P	M	R	J	K		L		Q		64.155	2	303
U	O	T	V	S	N	P	M	R	J	K		L		Q				
U	O	T	V	S	N	P	M	R	J	K		L		Q		64.155	2	410
U	O	T	V	S	N	P	M	R		K		L		Q				
U	O	T	V	S	N	P	M	R		K		L		Q	W	64.035	2	1013
U	O	T	V	S	N	P	M	R				L		Q	W			
U	O	T	V	S	N	P	M	R				L		Q	W	63.925	2	208
U	O	T	V	S	N	P	M	R						Q	W			
U	O	T	V	S	N	P	M	R						Q	W	63.81	2	411

Table C.7 (Continued)

U	O	T	V	S	N	P		R				X		Q	W					
U	O	T	V	S	N	P		R				X		Q	W	63.27	2	206		
U	O	T	V	S		P		R				X		Q	W					
U	O	T	V	S		P		R				X		Q	W	63.105	2	309		
U	O	T	V	S		P		R				X		Q	W					
U	O	T	V	S		P		R				X		Q	W	63.09	2	1115		
U		T	V	S		P		R				X		Q	W					
U		T	V	S	Y	P		R				X		Q	W	62.965	2	407		
U		T	V	S	Y	P		R				X		Q	W					
U		T	V	S	Y	P		R				X		Q	W	62.955	2	814		
U		T	V	S	Y			R				X		Q	W					
U		T	V	S	Y			R				X		Q	W	62.865	2	815		
U		T	V	S	Y			R				X			W					
U	Z	T	V	S	Y			R				X			W	62.395	2	612		
U	Z	T	V	S	Y			R				X			W					
U	Z	T	V	S	Y			R				X			W	62.395	2	910		
U	Z	T	V	S	Y			R				X			W					
U	Z	T	V	S	Y			R				X			W	62.33	2	214		
U	Z	T	V	S	Y			R				X			W					
U	Z	T	V	S	Y			R				X			W	62.325	2	801		
U	Z	T	V	S	Y			R				X			W					
U	Z	T	V	S	Y			R				X			W	62.315	2	710		
U	Z	T	V	S	Y							X			W					
U	Z	T	V	S	Y			A				X			W	62.185	2	202		

Table C.7 (Continued)

U	Z	T	V	S	Y		A				X			B	W				
U	Z	T	V	S	Y		A				X				W	62.17	2	211	
U	Z	T	V	S	Y		A				X				W				
U	Z	T	V	S	Y		A				X				W	62.115	2	802	
U	Z	T	V		Y		A				X				W				
U	Z	T	V		Y		A				X			B	W	62.03	2	915	
U	Z	T	V		Y		A				X			B	W				
U	Z	T	V		Y		A				X			B	W	62.005	2	102	
U	Z	T	V		Y		A				X			B	W				
U	Z		V		Y		A			C	X			B	W	61.79	2	105	
U	Z		V		Y		A			C	X			B	W				
U	Z		V		Y	D	A			C	X			B	W	61.72	2	908	
U	Z		V		Y	D	A			C	X			B	W				
U	Z		V		Y	D	A			C	X			B	W	61.675	2	104	
	Z		V		Y	D	A			C	X			B	W				
	Z		V	E	Y	D	A			C	X			B	W	61.52	2	108	
	Z			E	Y	D	A			C	X			B	W				
	Z		F	E	Y	D	A			C	X			B	W	61.435	2	215	
	Z		F	E	Y	D	A			C	X			B	W				
	Z		F	E	Y	D	A			C	X			B	W	61.43	2	302	
	Z		F	E	Y	D	A			C	X			B					
	Z	G	F	E	Y	D	A			C	X			B		60.94	2	813	
	Z	G	F	E	Y	D	A			C	X			B					
H	Z	G	F	E	Y	D	A			C	X			B		60.75	2	210	

Table C.7 (Continued)

H	Z	G	F	E	Y	D		A		C		X		B									
H	Z	G	F	E	Y	D		A		C		X		B		60.69	2	301					
H	Z	G	F	E	Y	D		A		C		X		B									
H	Z	G	F	E	Y	D		A		C		X		B	I	60.67	2	1201					
H	Z	G	F	E	Y	D		A		C				B	I								
H	Z	G	F	E	Y	D		A		C		J		B	I	60.31	2	308					
H	Z	G	F	E		D		A		C		J		B	I								
H	Z	G	F	E	K	D		A		C		J		B	I	60.15	2	406					
H	Z	G	F	E	K	D		A		C		J		B	I								
H	Z	G	F	E	K	D		A		C		J	L	B	I	60.11	2	1014					
H	Z	G	F	E	K	D		A		C		J	L	B	I								
H	Z	G	F	E	K	D		A		C	M	J	L	B	I	60.07	2	1108					
H	Z	G	F	E	K	D		A		C	M	J	L	B	I								
H	Z	G	F	E	K	D		A		C	M	J	L	B	I	59.91	2	502					
H	Z	G	F	E	K	D		A		C	M	J	L	B	I								
H	Z	G	F	E	K	D		A		C	M	J	L	B	I	59.79	2	315					
H		G	F	E	K	D		A		C	M	J	L	B	I								
H		G	F	E	K	D		A		C	M	J	L	B	I	59.575	2	808					
H		G	F	E	K	D				C	M	J	L	B	I								
H		G	F	E	K	D				C	M	J	L	B	I	59.39	2	401					
H		G	F	E	K	D				C	M	J	L		I								
H		G	F	E	K	D				C	M	J	L		I	59.31	2	101					
H		G	F	E	K	D				C	M	J	L		I								
H		G	F	E	K	D				C	M	J	L		I	59.27	2	313					

Table C.7 (Continued)

H		G	F	E	K	D				M	J	L		I					
H		G	F	E	K	D				M	J	L		I	59.105	2	508		
H		G	F	E	K					M	J	L		I					
H		G	F	E	K			N		M	J	L		I	58.87	2	603		
H		G	F		K			N		M	J	L		I					
H	O	G	F		K			N		M	J	L		I	58.775	2	106		
H	O	G			K			N		M	J	L		I					
H	O	G	P		K			N		M	J	L		I	58.605	2	109		
H	O	G	P		K			N		M	J	L		I					
H	O	G	P		K			N		M	J	L		I	58.57	2	204		
H	O	G	P		K			N		M	J	L		I					
H	O	G	P		K			N		M	J	L	Q	I	58.51	2	110		
H	O	G	P		K			N		M	J	L	Q	I					
H	O	G	P		K			N		M	J	L	Q	I	58.365	2	408		
H	O	G	P		K			N		M	J	L	Q	I					
H	O	G	P		K			N		M	J	L	Q	I	58.31	2	611		
H	O		P		K			N		M	J	L	Q	I					
H	O		P		K			N		M	J	L	Q	I	58.2	2	913		
	O		P		K			N		M	J	L	Q	I					
	O		P		K			N		M	J	L	Q	I	58.005	2	307		
	O		P		K			N		M	J	L	Q						
	O		P		K			N		M	J	L	Q		57.97	2	514		
	O		P		K			N		M		L	Q						
	O		P		K			N		M		L	Q		57.565	2	610		

Table C.7 (Continued)

	O		P		K			N			M		L	Q						
	O		P		K			N			M		L	Q		57.495	2	1105		
	O		P					N			M		L	Q						
	O		P					N			M	R	L	Q		57.445	2	114		
	O		P					N			M	R		Q						
	O		P					N			M	R		Q		57.4	2	107		
	O		P					N				R		Q						
	O		P		S			N				R		Q		56.385	2	501		
	O		P		S			N				R		Q						
	O		P		S			N				R		Q		56.23	2	505		
	O		P		S							R		Q						
	O		P		S							R		Q		56.14	2	205		
			P		S							R		Q						
			P		S							R		Q		56.055	2	314		
			P		S							R		Q						
			P		S							R		Q		55.96	2	405		
					S							R		Q						
					S							R		Q		55.875	2	207		
					S							R								
					S			T				R				54.805	2	305		
					S			T												
			U		S			T								54.37	2	103		
			U		S			T												
			U		S			T								54.11	2	203		

Table C.7 (Continued)

			U		S			T										
			U		S			T								54.025	2	113
			U					T										
			U					T				V				52.915	2	209
			U					T				V						
			U		W			T				V				52.75	2	111
			U		W			T				V						
			U		W			T				V				52.605	2	403
			U		W							V						
			U		W							V				51.93	2	112
					W							V						
					W							V				51.34	2	404
					W													
					W											50.205	2	213

Subsamples were collected from 30-day regrowth, harvested at 30-cm stubble height during August, 2015.
Means with the same letter are not significantly different
LSD = 2.68

APPENDIX D

ANKOM PROCEDURE FOR DETERMINATION OF IN VITRO TRUE DRY MATTER DEGRADABILITY

ANKOM Technology Method 3

In Vitro True Digestibility using the DAISY^{II} Incubator

ANKOM Technology **- 08/05**

A. Reagents

(a) Buffer Solution A:	g/liter
KH_2PO_4	10.0
$MgSO_4 \cdot 7H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2H_2O$	0.1
Urea (reagent grade)	0.5

(b) Buffer Solution B:	
Na_2CO_3	15.0
$Na_2S \cdot 9H_2O$	1.0

(c) Neutral Detergent Solution

B. Apparatus

(a) DAISY^{II} Incubator
(b) Filtration device - F57 Filter Bags
(c) Impulse bag sealer - 1915/1920 Heat Sealer
(d) Thermos
(e) ANKOM$^{200/220}$ Fiber Analyzer

C. Procedure

Preparation of Filter Bags and Sample:
Pre-rinse F57 filter bags in acetone for three to five minutes and completely air-dry. The acetone rinse removes a surfactant that inhibits microbial digestion. Weigh each F57 filter bag and record weight (W_1). Zero the balance and weigh 0.25g of sample (W_2) **directly** into filter bag. NOTE: For 48 hr studies a sample size of 0.5 g is acceptable. Heat seal bag closed and place in the **Daisy^{II} Incubator** digestion jar (up to 25 samples per jar). Samples should be evenly distributed on both sides of the digestion jar divider. Include at least one sealed blank bag for correction factor (C_1).

Preparation of (combined) Buffer Solution: *(For each digestion jar)*

a) Pre-warm at 39°C both buffer solutions (A & B). In separate container add ~266 ml of solution B to 1330 ml of solution A (1:5 ratio). The exact amount of A to B should be adjusted to obtain a final pH of 6.8 at 39°C. No further adjustment of pH is necessary. Add 1600 ml of combined A/B mixture to each digestion jar.
b) Place the digestion jars with samples and buffer solution into **Daisy^{II} Incubator** and turn on heat and agitation switches. Allow temperature of digestion jars to equilibrate for at least twenty to thirty minutes.

Preparation of Inoculum and Incubation:

Maintain all glassware at 39°C

a) Preheat two 2L thermos bottles by filling with 39° C water. Empty heated water just prior to collection of rumen inoculum. Using the appropriate collection procedure, remove at least 2000 ml of rumen inoculum and place in thermos. Include approximately two "fistfuls" of the fibrous mat from the rumen with your collection in one thermos.

b) Preheat a blender by filling with 39° C water. Empty the heated water just prior to pouring the rumen inoculum from the thermos into the blender. Purge the blender container with CO_2 gas and blend at a high speed for 30 seconds. The blending action serves to dislodge microbes that are attached to the mat and assure a representative microbial population for the *in vitro* fermentation. Filter the blended digesta through four layers of cheesecloth into a five-liter flask (pre-heated 39° C). Filter the remaining rumen fluid in the other thermos through four fresh layers of cheesecloth into the same five-liter flask. NOTE: Allow for extra cheesecloth around the edges to facilitate squeezing contents of filtered mat. The flask should be continually purged with CO_2 and continued during the transfer of the inoculum.

c) Remove one digestion jar from the **Daisy^{II} Incubator** and add the 400ml of inoculum to the buffer solution and samples. Purge the digestion jar with CO_2 gas for thirty seconds and secure lid.

d) Repeat process for all digestion jars to be used. NOTE: Do not allow CO_2 gas to bubble through the buffered inoculum, rather use the CO_2 to form a gaseous blanket over the contents of the jar.

e) Incubate for 48 hours. The **DAISY^{II} Incubator** will maintain a temperature of 39.5°C ± 0.5. If temperature of jars varies greater than one degree then move incubator to a warmer location or place blanket or similar insulator over incubator.

f) At completion of incubation, remove jars and drain fluid. Rinse bags thoroughly with cold tap water until water is clear. Use a minimum of mechanical agitation.

g) When determining True Digestibility it is necessary to remove microbial debris and any remaining soluble fractions using Neutral Detergent Solution. After rinsing the bags in water place them in the **ANKOM^{200} Fiber Analyzer** and follow the procedure for determining NDF. Record the post *in vitro* NDF weight as W_3. NOTE: Bags can be stored in the refrigerator or freezer until NDF determinations can be performed.

APPENDIX E

GENOME EVALUATION OF EASTERN GAMAGRASS REGIONAL GERMPLASM COLLECTION ACCESSIONS VIA FLOW CYTOMETRY (RESULTS)

Table E.1 Accession identification tag number, custom, tetraploid and diploid evaluation parameters for each individual.

	Custom			4x			2x		
ID	Count	Mean FL2-A	CV FL2-A	Count	Mean FL2-A	CV FL2-A	Count	Mean FL2-A	CV FL2-A
101	1,009	647,759.91	2.83%	321	623,047.65	2.37%	45	319,843	4.22%
102	1,006	544,558.68	3.65%	34	592,922.88	2.36%	28	337,400.43	5.18%
103	1,524	627,928.17	2.74%	1,629	628,257.42	3.17%	70	337,680.61	5.43%
104	1,030	661,932.22	2.67%	1,026	657,601.30	2.85%	21	338,632.71	5.18%
105	1,245	639,782.76	2.62%	1,499	633,766.71	3.23%	31	337,546.23	4.81%
106	1,127	621,420.54	2.75%	1,199	622,825.45	3.18%	37	339,573.68	5.82%
108	1,258	597,322.51	3.41%	1,233	596,393.20	3.27%	52	321,949	4.64%
111	1,299	592,908.82	3.03%	950	602,443.00	2.32%	38	339,417.47	5.29%
112	1,005	582,571.03	3.34%	985	583,321.79	3.15%	35	322,720	4.53%
113	1,133	598,157.85	2.97%	1,130	597,867.52	2.96%	49	320,125	4.07%
114	1,330	676,315.32	3.36%	89	609,047.06	4.44%	39	318,923	3.90%
115	1,042	649,823.60	2.96%	291	617,461.65	3.25%	39	321,292	4.43%
201	1,323	545,285.33	3.41%	724	560,161.06	2.30%	62	324,516	4.42%
202	1,411	599,433.36	2.64%	1,282	606,761.35	2.74%	65	337,905.38	5.27%
203	1,175	570,980.59	3.10%	344	593,516.02	1.53%	39	341,953.64	5.88%
204	1,715	569,852.46	2.89%	426	594,909.50	2.27%	57	342,103.46	5.20%
205	1,009	588,721.25	3.01%	1,007	588,813.91	2.99%	30	320,886	4.43%
206	1,332	671,280.86	2.83%	1,185	660,669.57	2.93%	68	342,841.16	5.44%
207	1,338	584,252.53	3.18%	703	601,100.44	2.57%	77	334,794.51	5.82%
208	1,326	597,563.26	4.21%	1,233	595,725.10	3.71%	43	321,840	4.35%

Table E.1 (Continued)

209	1,007	558,919.98	3.28%	823	564,647.08	2.69%	43	321,641	4.37%
210	1,007	629,799.95	2.69%	688	618,271.11	2.32%	20	323,673	4.46%
212	1,033	620,071.70	3.73%	789	610,618	2.99%	41	322,907	4.40%
213	1,012	558,090.83	3.11%	829	563,596.21	2.42%	21	316,900	4.96%
214	1,273	535,694.02	3.21%	504	557,359.99	2.30%	68	320,582	4.17%
215	1,004	581,008.47	3.78%	950	583,269.01	3.29%	50	318,589	4.58%
302	1,244	628,254.19	3.02%	1,253	628,159.38	3.07%	31	343,835.77	5.06%
303	1,028	541,004.75	3.31%	30	600,998.67	3.62%	23	334,179.43	6.13%
307	1,007	595,488.64	2.96%	1,003	595,408.63	2.92%	32	320,912	3.98%
309	1,035	661,129.40	3.40%	158	616,403.03	3.58%	42	320,351	3.77%
312	2,080	603,293.92	3.05%	2,030	602,105.99	2.91%	62	321,911	4.14%
313	1,655	648,495.77	2.72%	1,751	647,226.02	3.08%	70	339,661.51	5.71%
314	1,005	523,131.43	3.34%	16	617,937.62	4.90%	59	338,019.95	5.55%
401	1,164	632,264.48	2.99%	1,174	632,453.61	3.07%	59	340,867.97	5.53%
402	1,106	561,345.38	3.13%	940	566,397.54	2.52%	29	319,159	3.64%
403	1,500	594,334.58	2.65%	1,180	603,210.18	2.60%	80	338,825.61	6.12%
404	1,075	569,037.91	2.83%	233	594,481.81	2.23%	55	332,477.07	5.48%
405	1,117	667,273.70	2.85%	1,036	660,286.48	3.01%	75	339,091.72	5.52%
406	1,237	527,563.16	2.93%	55	609,919.07	4.36%	51	336,087.73	5.21%
407	1,391	588,581.55	2.54%	1,046	600,775.70	2.32%	65	343,991.38	4.77%
408	1,104	594,624.62	2.46%	918	601,889.63	2.19%	73	343,085.60	5.37%
409	1,045	599,831.56	2.77%	932	606,625.67	2.61%	59	337,199.88	5.25%
412	1,018	635,895.96	2.68%	1,047	635,679.35	2.91%	21	342,767.33	5.06%

Table E.1 (Continued)

413	1,175	577,361.21	3.11%	1,149	578,373.07	2.95%	33	318,331	3.97%
415	1,500	572,598.47	3.33%	1,418	575,352.04	2.96%	49	322,066	4.00%
501	1,279	596,558.54	2.55%	1,293	596,047.92	2.67%	48	320,365	4.06%
502	1,127	566,866.88	2.87%	219	592,298.91	2.12%	36	342,799.61	5.03%
504	1,022	556,727.69	2.56%	78	594,030.17	3.13%	34	338,862.85	5.07%
508	1,000	567,270.94	3.30%	917	570,498.64	2.76%	27	320,431	4.52%
509	1,220	600,813.54	2.64%	1,081	605,381.89	2.44%	40	335,118.68	4.81%
510	1,460	548,083.34	2.76%	41	602,435.37	4.07%	67	337,848.06	5.70%
511	1,000	608,098.25	2.75%	971	605,496.46	2.71%	22	319,801	3.77%
513	1,170	574,833.62	2.73%	401	593,464.19	1.86%	46	343,278.43	5.88%
514	1,592	561,177.13	2.61%	204	593,489.60	2.89%	57	337,868.47	5.20%
515	1,181	611,764.90	3.32%	1,069	607,921.11	2.96%	33	319,830	4.60%
603	1,134	601,936.31	3.31%	1,093	600,478.12	3.08%	31	322,540	4.17%
604	1,168	656,754.45	2.97%	1,156	653,692.60	3.07%	63	345,525.41	5.62%
605	1,336	614,285.38	3.24%	1,165	607,822.79	2.79%	39	316,768	4.05%
605	1,006	624,942.84	2.72%	773	617,133.14	2.17%	29	317,675	3.98%
607	1,003	562,191.68	3.31%	867	567,018.11	2.57%	32	315,672	4.03%
608	1,106	592,533.88	2.99%	1,119	592,242.55	3.09%	25	319,885	3.80%
609	1,634	616,706.71	3.24%	1,572	620,030.81	3.09%	103	338,808.42	5.61%
610	1,490	628,528.82	2.65%	1,496	628,601.41	2.69%	68	341,240.00	5.78%
611	1,182	692,404.78	2.55%	640	671,406.11	2.60%	49	340,754.98	6.04%
612	1,311	562,363.56	2.91%	305	598,918.23	3.08%	50	337,264.04	5.39%
614	1,003	495,849.75	5.12%	53	558,580.89	3.19%	22	316,871	3.96%

Table E.1 (Continued)

615	1,113	610,004.32	3.02%	1,059	612,791.63	2.90%	54	339,582.57	5.42%
701	1,007	634,312.12	3.55%	565	618,361.50	2.71%	41	318,668	4.48%
703	1,308	576,565.42	3.83%	1,215	580,097.53	3.37%	30	320,288	4.73%
704	1,003	614,396.24	3.19%	892	609,899.93	2.76%	39	322,046	4.29%
705	1,014	609,538.25	3.12%	944	605,758.32	2.92%	51	320,298	4.01%
706	1,000	582,109.49	3.50%	973	583,253.21	3.17%	31	323,431	4.21%
707	1,004	620,599.38	3.12%	811	612,937.13	2.75%	20	317,048	4.54%
708	2,942	621,160.72	2.55%	3,017	621,054.08	2.73%	99	336,716.80	5.10%
709	1,634	623,795.81	2.58%	1,705	624,209.35	2.88%	62	336,513.42	4.63%
710	1,078	626,490.87	3.42%	744	614,867.93	2.59%	30	320,711	3.88%
711	1,411	626,881.04	2.78%	1,025	617,757.84	2.35%	30	321,293	4.15%
713	1,004	666,632.74	3.57%	114	617446.35	3.55%	23	323,926	4.71%
714	1,407	629,195.10	2.72%	1,415	629,222.18	2.78%	38	340,496.79	5.13%
715	1,000	653,413.52	2.77%	215	621,056.91	3.00%	22	325,340	4.87%
801	1,147	643,700.24	2.78%	1,243	642,531.53	3.31%	67	336,395.66	5.55%
802	1,008	572,122.56	3.28%	947	574,468.49	2.92%	30	315,801	3.92%
803	1,006	542,464.12	3.81%	490	560,269.27	2.22%	32	320,531	4.32%
805	1,004	640,875.18	3.35%	464	619,545.55	2.70%	46	322,778	4.11%
806	1,125	694,327.77	2.70%	48	592,507	4.59%	34	318,969	4.45%
809	1,400	589,103.10	2.89%	931	600,098.28	2.38%	68	335,627.69	5.50%
811	1,059	564,370.34	3.14%	946	568,283.89	2.68%	27	316,265	3.90%
812	1,302	575,448.65	3.74%	1,224	578,301.93	3.40%	50	320,469	3.86%
813	1,225	477,590.90	4.01%	9	582,193.78	5.00%	29	321,251	4.03%

Table E.1 (Continued)

814	1,223	577,235.79	3.49%	1,180	579,063	3.29%	50	321,045	3.90%
815	1,724	706,504.35	2.49%	417	675,398.65	2.87%	60	339,304.90	5.70%
901	1,076	606,111.19	3.67%	987	602,557.01	3.20%	44	322,373	4.39%
902	1,008	641,591.23	3.61%	452	618,233.40	2.77%	33	324,493	4.17%
903	1,037	486,997.49	4.81%	24	573,782.25	6.14%	29	320,418	3.58%
906	1,189	628,534.50	2.63%	1,262	628,106.85	2.97%	32	340,893.97	6.23%
907	1,154	560,713.15	2.65%	141	592,793.30	2.63%	28	336,725.18	4.94%
908	1,032	569,596.92	3.58%	937	573,170.69	3.09%	29	321,259	4.67%
909	1,124	660,295.89	2.58%	1,129	656,852.53	2.79%	38	335,028.47	4.77%
912	1,001	622,459.10	3.35%	790	614,065.94	2.73%	61	320,186	4.35%
913	1,376	652,466.07	2.79%	329	621,731.56	2.96%	64	321,789	4.43%
914	1,501	650,515.60	2.71%	1,619	647,560.44	3.26%	76	340,640.61	5.25%
915	1,195	562,975.05	3.10%	194	591,419.16	1.56%	58	339,107.47	5.40%
1002	1,635	618,035.68	3.37%	1,353	609,623.68	2.91%	71	322,125	4.31%
1003	1,264	532,889.49	4.00%	421	556,825	2.21%	36	321,349	4.00%
1004	1,250	600,044.71	3.00%	1,219	599,011.78	2.80%	26	318,514	4.55%
1005	2,361	648,142.79	2.76%	2,550	645,652.91	3.27%	96	341,264.95	5.10%
1007	1,367	557,002.58	2.71%	123	596,144.41	3.33%	79	335,561.43	5.15%
1008	1,461	569,994.31	3.34%	1,341	573,046.02	2.96%	39	319,347	4.79%
1009	1,019	523,986.78	3.16%	145	553,359.34	2.28%	29	319,295	4.19%
1010	1,446	614,970.79	2.98%	1,306	610,397.74	2.75%	27	320,725	3.98%
1011	1,113	600,073.97	2.97%	939	606,163.31	2.63%	65	340,787.98	6.22%
1012	1,005	663,697.54	2.96%	111	614,661.89	4.01%	45	318,834	3.95%

Table E.1 (Continued)

1013	1,003	597,454.60	3.47%	982	596,437.86	3.31%	33	316,304	4.28%
1014	1,005	532,755.80	4.05%	360	555,421.46	2.01%	41	321,288	4.00%
1015	1,241	624,507.37	3.80%	875	610,480.99	3.07%	33	318,200	4.28%
1101	1,195	574,848.03	2.68%	471	595,328.79	1.88%	62	337,504.44	5.67%
1102	1,223	656,559.72	3.77%	1,265	643,625.35	4.08%	86	341,398.97	5.45%
1103	1,029	558,566.05	3.54%	169	594,892.46	2.69%	60	343,933.85	5.00%
1104	1,006	578,096.49	3.05%	984	578,636.30	2.81%	56	322,484	4.08%
1105	1,083	546,692.03	2.56%	18	604,683.83	4.33%	44	339,292.25	5.32%
1107	1,394	596,958.59	3.39%	1,110	610,089.79	3.24%	73	338,796.84	5.52%
1108	1,212	643,319.45	2.73%	1,274	645,020.30	2.96%	42	336,358.83	5.36%
1110	1,005	583,483.01	3.12%	551	599,312.41	2.23%	31	339,782.52	5.43%
1111	1,003	615,985.27	3.57%	834	609,682.89	2.92%	31	321,520	5.25%
1112	1,018	588,828.31	2.88%	1,014	588,720.32	2.83%	37	322,444	4.63%
1113	2,068	712,224.40	2.69%	456	667,606.29	3.95%	130	336,741.46	5.32%
1115	1,095	618,541.35	3.08%	1,081	620,461.70	3.06%	35	345,415.26	5.47%
1201	1,162	595,541.08	2.87%	921	602,791.51	2.24%	46	343,952.07	5.58%
1203	1,373	572,731.17	3.19%	458	596,264.47	2.21%	70	335,059.13	4.95%
1204	1,070	634,278.30	3.37%	662	614,759.05	2.74%	47	316,876	3.96%
1205	1,035	537,015.36	3.25%	401	556,354.70	2.29%	30	324,951	4.25%
1206	1,008	710,612.59	3.16%	44	584,090	5.08%	45	325,409	4.04%
2x Control (1)	1,024	322,748.84	3.23%	3	568,904.67	2.43%	1004	322,532	3.02%
2x Control (2)	2,100	344,798.40	2.97%	4	672,363.00	1.66%	2184	343,465.60	3.22%
2x Control (3)	1,079	331,512.61	2.69%	9	655,116.67	4.54%	1165	332,059.18	3.28%

Table E.1 (Continued)

2x Control (4)	1,120	330,611.91	2.92%	2	654,671.40	2.11%	1641	332,059.18	3.15%
4x Control (1)	1,050	588,047.65	4.00%	1,011	588,822.12	3.73%	45	320,301	3.86%
4x Control (2)	1,008	586,832.33	3.22%	989	587,374.53	3.00%	32	322,683	4.21%
4x Control (3)	1,228	633,229.25	3.31%	1,248	633,739.45	3.44%	109	337,864.84	5.38%
4x Control (4)	1,113	635,597.50	3.34%	1,185	634,696.36	3.73%	90	337,147.97	5.61%
Super Gold PC	678	243,618.76	4.28%	17	564,086.29	3.83%	52	321,204.54	4.37%
White Pearl PC	1,097	246,403.47	4.72%	62	559,174.44	3.73%	45	319,771.82	3.76%

All fitted gates were made using BD Accuri C6 software

† All diploid estimations are based on mean of diploid controls

‡ All tetraploid estimations are based on mean of tetraploid controls