PHYSIOLOGICAL AND GENETIC IMPLICATIONS TO CONSIDER IN TETRAPLOID BAHIAGRASS BREEDING

By

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To my wife, Lorena

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By

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Bahiagrass, *Paspalum notatum* Flüggé, is a warm-season, perennial grass extensively cultivated as forage and utility turf in southeastern USA. The tetraploid germplasm of this species constitutes an underexploited source of genetic variation. This variation has great potential for bahiagrass improvement because of the possibility of fixing superior hybrids by manipulating apomixis. Physiological and genetic variables that might have a major impact on the process of improving this species were studied. The objectives of this research were to evaluate the transmission of apomixis through generations, and estimate the genetic variability for growth habit, cool-season growth, and freeze resistance resulting from hybridization of sexual and apomictic F_1 clones. Additionally, estimates were made on the seasonal biomass yields and nitrogen and phosphorus accumulation and the genetic variability for the rate of root depth development, root mass and root length density among these novel F_1 hybrids. The relationship between root development and nitrogen uptake from deep soil layers, and the relationship between biomass yields and root characteristics were also determined.

Approximately 20% of the F_1 and F_2 were classified as apomictic. Eleven percent of the F_1 but only 3% of the F_2 were classified as highly apomictic. This variable expressivity might be

caused by the genetic background or epigenetic variation. The genetic variation observed for growth habit, cool-season growth, and freeze resistance remained relatively constant between the F_1 and F_2. Apomicitc F_1 hybrids accumulated more nitrogen and phosphorus in spring and produced higher cool-season and annual biomass yields compared to common bahiagrass cultivars. Little genetic variation was observed for nitrogen and phosphorus concentrations in foliage among these hybrids. Genetic variation for rate of root depth development was observed among hybrids. Higher rates of root depth development resulted in faster access and uptake of nitrogen, and higher root and shoot mass. Variation for root length density was found among apomictic hybrids during the second growing season. No relationship was found between root length density or root mass, and above-ground biomass production. The fertilization rate did not affect root mass or root length density. Root activity might play an important role in nutrient uptake and biomass production.

CHAPTER 1
INTRODUCTION

Bahiagrass, *Paspalum notatum* Flüggé, is a warm-season, perennial grass native to the Americas. It grows naturally in a vast region that extends from central Argentina to northern Mexico (Blount and Acuña, 2009). This species is especially abundant in native grasslands of southern Brazil, Uruguay, and northeastern Argentina. Bahiagrass has become widely distributed in almost all tropical and subtropical regions of the world, particularly in the western hemisphere. Bahiagrass germplasm was introduced into southeastern USA multiple times during the 20th century. Well adapted germplasm and locally released cultivars are now extensively cultivated as forage and utility turf in Florida and the southern Coastal Plain region of the USA.

Genetics and Breeding

As most *Paspalum* species, bahiagrass has a base chromosome number of x = 10 (Gates et al., 2004). Several ploidy levels are known occurring in this species, ranging from diploid (2n=2x=20) to pentaploid (2n=5x=50) types. Pozzobon and Valls (1997) examined 118 bahiagrass accessions collected in Brazil, of these, 108 were tetraploids and the other 10 were diploids. Dahmer et al. (2008) examined another 65 accessions collected in southern Brazil, of which 64 were tetraploid and 1 was diploid. In both studies the authors concluded that the rare diploid cytotypes escaped from cultivated Pensacola bahiagrass. Indigenous diploid accessions have only been collected in northeastern Argentina (Burton 1967; Daurelio et al., 2004). Thus, the tetraploid germplasm occupies most of the area where the species is distributed, while the diploid germplasm is restricted to a relatively small region of northeastern Argentina. Triploids and pentaploids have been occasionally collected (Gould, 1966; Quarin et al., 1989; Thischler and Burson 1995). Chromosome pairing behavior, and tetrasomic inheritance for most analyzed

loci indicated that tetraploid races originated by autoploidy (Forbes and Burton, 1961; Stein et al., 2004).

The genetic diversity of bahiagrass has been evaluated using simple sequence repeats markers (Cidade et al., 2008). After evaluating genetic polymorphisms among 91 accessions from Brazil, Argentina and Uruguay, the authors concluded that wide variability is present in this species. The genetic variability found in tetraploid populations growing in the vicinity of diploid populations was higher than that observed within isolated populations (Daurelio et al., 2004). These results indicate that the center of genetic diversity of this species is located in northeastern Argentina.

Ploidy levels in this species are linked to contrasting reproductive characteristics. Diploid cytotypes reproduce sexually and set low amounts of seed when self-pollinated due to self-incompatibility (Burton, 1955; Acuña et al., 2007). Tetraploid ecotypes reproduce asexually by apomixis and set the same amount of seed when self- or cross-pollinated (Burton, 1948; Acuña et al., 2007). Apomixis in bahiagrass includes the formation of unreduced embryo sacs from nucellar cells (apospory), the pathenogenetic development of embryos, and the development of the endosperm following fertilization of the polar nuclei (pseudogamy). These differences on mode of reproduction indicate that different breeding approaches are needed for diploid and tetraploid races.

The genetic control of apomixis in bahiagrass is still not well understood. Two out of three autotetraploids obtained by chromosome doubling of sexual diploids were classified as facultative apomictic (Quarin et al., 2001) indicating that the genetic determinants for apospory were present at the diploid level, or that novel variation resulting from chromosome doubling was responsible for the expression of the trait at the tetraploid level. Segregation ratios resulting

from crossing sexual induced and apomictic tetraploid clones always showed an excess of sexual progeny (Martínez et al., 2001; Acuña et al., 2009). Since self-pollination of sexual induced tetraploids, previously classified as sexual, only produced sexual individuals, it is believed that apospory is inherited as a single dominant Mendelian factor with distorted segregation. This factor is located in a large genomic region characterized by suppression of recombination and preferential chromosome pairing (Martínez et al., 2003; Stein et al., 2004; Stein et al., 2007). Results from a recent comprehensive transcriptome survey of genes differentially expressed in inflorescences of aposporous and sexual tetraploid genotypes, indicated that apomixis in *Paspalum* involves the altered expression of a signal transduction cascade that seems to be triggered by the silencing of a large genomic region, including the apospory locus (Laspida et al., 2008). It was also shown in this study that several genes, which are involved in aposporous development, are ploidy-regulated.

Although a remarkable amount of information about apomixis in *Paspalum* has been generated in the last decade, little is known about how this knowledge applies to breeding tetraploid bahiagrass. Theoretically, new apomictic genotypes could be readily produced through hybridization of sexual induced and apomictic tetraploids (Hanna and Bashaw, 1987). Continued breeding should be possible by repeated crossing of superior sexual plants with superior apomictic male pollinators. However, it is unknown if the segregation ratios observed for apomixis in the progeny of sexual induced and apomictic clones will remain constant across successive cycles of hybridization. This is especially unpredictable in a species showing distorted segregation and variable expressivity. Only 11% of a progeny resulting from crossing sexual induced and apomictic tetraploid clones was classified as highly apomictic (Acuña et al.,

2009). If this percentage is reduced through successive cycles of hybridization, this breeding approach would become impractical.

Sexual × apomictic crosses usually release a large amount of genetic variation because of the heterozygosity of the apomictic parents (Hanna and Bashaw, 1987). This phenomenon was observed when several induced sexual and apomictic bahiagrass clones were crossed (Acuña et al., 2009). However, it is questionable if this genetic variation is released from the apomictic parent or mainly results from crossing two isolated races (diploid and tetraploid). Variability for traits of agronomic importance, such as cool-season growth and freeze resistance are thought to be quantitatively inherited. The genetic variability for these traits is expected to decline through successive cycles of hybridization as other traits with quantitative inheritance (Poehlman and Sleper, 1995).

Ecological Determinants of Growth

Bahiagrass is a C_4 species adapted to tropical and subtropical regions. When grown in subtropical regions, it shows a marked seasonality of growth in response to photoperiod and temperature (Sinclair et al., 2001; Gates et al., 1999). In Florida, most of bahiagrass production occurs between April and October (Gates et al., 2004). Five months with no forage available to cover animal needs represents the most serious limitation for cattle production systems in southeastern USA. It is also a serious limitation when bahiagrass is cultivated as utility turf since its quality stays low with no growth. Plants with an extended growing season would be able to produce more biomass during the cool-season and probably during each year. An extended growing season would allow plants to capture solar radiation and utilize nutrients for a longer period each year resulting in higher annual biomass production.

Genetic variability for cool-season growth has been observed in the diploid bahiagrass germplasm (Gates et al., 2001). Several cycles of recurrent restricted phenotypic selection

(Burton, 1974) for higher forage yields has resulted in open-pollinated populations with greater cool-season growth. A large variation for cool-season growth and freeze resistance was observed among progeny generated by crossing sexual and apomictic tetraploid clones (Acuña et al., 2009). A relatively high proportion of this variation was attributed to genetic variability based on heritability estimates. However, variation for cool-season growth and freeze resistance was only observed among progeny cultivated as individual plants. An extended growing season could compromise the long-term survival of this crop in a real production system. Research is needed to determine if this new tetraploid germplasm can produce more biomass during the cool-season and annually when grown in swards.

Although rainfall is relatively high in the region where bahiagrass is cultivated, water is considered one of the main factors limiting its growth (Gates et al., 2004). Frequent droughts, high vapor pressure gradients between leaves and the environment during the growing season, and low water holding capacity of light textured soils result in water limited bahiagrass growth in most situations.

Availability of essential minerals in the soil also limits bahiagrass production. This crop is mainly cultivated in soils with inherent low fertility and low cation and anion exchange capacities. Therefore, fertilizer application is essential for crop production in most situations. The main essential nutrient limiting bahiagrass production is nitrogen (Gates et al., 2004). Bahiagrass production dramatically increased as annual rates of nitrogen fertilization increased (Beaty et al., 1960; Blue, 1973). A single annual application of fertilizer is a common and recommended practice for bahiagrass pastures (Gates et al., 2004). The high precipitation of the region and the low cation and anion exchange capacities of the soils could result in large amounts of fertilizer being lost in percolated water. A 7-year experiment with Pensacola

bahiagrass showed that the above-ground recovery of applied nitrogen increased from 30% during the first year to 70% during the 7th year. Part of the non-recovered nitrogen was used to build the rhizome-root system, but a considerable amount was lost, especially during the establishment years. Therefore, genotypes that can rapidly access stored soil water and nutrients from fertilization would have an advantage that could result in higher biomass yields, while better protecting the environment. Rapid establishment and utilization of available resources is especially important when bahiagrass is used in rotation with other crops that leave high amounts of residual fertilizer in the soil.

Genotypes with early vigor and good seedling establishment tend to enhance transpiration at the expense of direct soil evaporation (Ludlow and Muchow, 1990). Another characteristic that might be especially important for a quick establishment is the recovery of stored water due to rapid root penetration. Since nutrients readily migrate to deep soil layers in light textured soils, superior vigor and rapid root penetration would be also important for the recovery of nutrients left in the soil from previous crops or from early fertilizer applications. Thorup-Kristensen (2001) reported variation among plant families for rate of root penetration including crucifer and grass crops. The crucifer N-catch crops were faster in developing deep rooting and depleting nitrogen from the subsoil than grass crops. Only small differences for rates of root penetration were found within botanical groups. In contrast, variability for rate of root penetration was observed among perennial grass species (Burton et al., 1954). Higher rates of root penetration were observed for Coastal bermudagrass (*Cynodon dactylon* Pers.) than for other subtropical grasses like bahiagrass, Pangola (*Digitaria eriantha* Steud.), Dallis (*Paspalum dilatatum* Poir.), and carpetgrass (*Axonopus fissifolius* Raddi). Intraspecific variability for maximum root depth was also observed in annual crops such as rice (Shen et al., 2001), sunflower and soybean

(Dardanelli et al., 1997), and also for perennial crops such as Zoysiagrass (Marcum et al., 1995). It is unknown if there is genetic variability for rate of root penetration within the bahiagrass germplasm. However, Burton (1943) reported that two apomictic bahiagrass ecotypes differed for first year's root production. These results indicate that genetic variability for root characteristics may exist within the species.

Root mass and root length at different soil depths are other plant characteristics that might be related with bahiagrass biomass production. Selection for greater deep-root mass to shoot mass ratio in tall fescue (Bonos et al., 2004) resulted in genotypes with higher drought tolerance (Karcher et al., 2008). While root depth might be considered an important characteristic to capture nutrients that move readily with water flow (such as nitrogen), variation in root length density in the top soil layers is expected to be more important for the uptake of nutrients that have low mobility in the soil, such as phosphorus. It is crucial that the phosphorus absorbing surfaces in the soil are extensive and prolific to make contact with the available phosphorus (Sinclair and Valdez, 2002).

Ludlow and Muchow (1990) have questioned the relation between root depth or length, and biomass production under water stress situations because of the cost of root growth and maintenance represent clear diversions of assimilates, which might be used for shoot growth, and thus may decrease yield potential. The fact that root length densities can vary from 0.3 to 6 cm cm^{-3} among temperate cereals and legumes with no effect on soil water extraction suggest that root length densities may be in excess of requirement in some crops. The study of root mass and length at different soil depths, and their relationship with bahiagrass biomass production may add light to this issue. It might also indicate the potential use of these root traits for breeding bahiagrass.

Objectives

- Determine the stability of the inheritance and expression of apomixis across cycles of hybridization between sexual and apomictic tetraploid clones (Chapter 2).
- Quantify the genetic variability for growth habit, cool-season growth, and freeze resistance generated by a second cycle of hybridization between sexual and apomitic tetraploid clones (Chapter 2).
- Determine seasonal biomass production, nitrogen and phosphorus concentrations and contents of novel apomictic hybrids grown in swards (Chapter 3).
- Evaluate the genetic variation among novel apomictic hybrids for rate of root depth development (Chapter 4).
- Determine the relationship between rate of root depth development and nitrogen uptake (Chapter 5).
- Evaluate the genetic variability for root mass and root length density at different soil depths among novel apomictic hybrids (Chapter 6).
- Establish the relationship between root mass and length, and biomass production (Chapter 6).
- Determine the effect of nitrogen fertilization on root mass and length at different soil depths (Chapter 6).

CHAPTER 2
GENETIC VARIABILITY RESULTING FROM A SECOND CYCLE OF HYBRIDIZATION AMONG BAHIAGRASS TETRAPLOID CLONES

Introduction

Approximately 125 grass species form their seeds by an asexual process called apomixis (Bashaw and Hanna, 1990). This characteristic offers a unique opportunity for developing and using superior genotypes. Seed of any superior obligate apomict could be increased through open-pollination for an unlimited number of generations without loss of vigor (heterozigosity) or change in genotype (Hanna and Bashaw, 1987).

Apomixis is the predominant mode of reproduction among the polyploid germplasm of *Paspalum* (Quarin, 1992). Bahiagrass, *Paspalum notatum* Flüggé, has become one of the most economically important species of this genus mainly because of its use as forage and utility turf (Blount and Acuña, 2009). This species includes polyploids that reproduce by apomixis and diploids that reproduce sexually (Burton, 1948; Burton, 1955). Apomixis in bahiagrass includes the formation of unreduced embryo sacs from nucellar cells (apospory), the pathenogenetic development of embryos, and the development of the endosperm following fertilization of the polar nuclei (pseudogamy). Apospory in this species is inherited as a single dominant Mendelian factor with distorted segregation (Martínez et al., 2001). This factor is located in a genomic region characterized by suppression of recombination and preferential chromosome pairing (Martínez et al., 2003; Stein et al., 2004; Stein et al., 2007).

In nature, the tetraploid (2n=4x=40) cytotypes of bahiagrass are predominately facultative or obligate apomictic, and able to produce reduced pollen (n=2x=20) (Gates et al., 2004). Sexual tetraploids have been generated by treating both diploid (2n=2x=20) seed and tissue cultured calluses with chromosome duplication treatments (Burton and Forbes, 1960; Quarin et al., 2001; Quesenberry and Smith, 2003). Crosses between sexual induced tetraploid clones used as female

parents and apomictic tetraploid clones used as pollen donors resulted in the release of a large genetic variability for traits of agronomic importance among the F_1 hybrids (Acuña et al., 2009). When the progeny were classified by mode of reproduction, 80% of the hybrids were sexual, 11% highly apomictic, and 9% facultative apomictic. Continued improvement should theoretically be possible by repeated crossing of superior sexual plants with superior apomictic male pollinators. If the apospory locus has a pleitropic lethal effect with incomplete penetrance as stated by Martínez et al. (2001), the proportion between sexual and apomictic progeny could vary among hybridization cycles. The genetic variability for the selected agronomic traits with quantitative inheritance is expected to be reduced by each cycle of hybridization and selection.

The objectives of this research were to create a bahiagrass segregating F_2 population by crossing F_1 sexual hybrids and unrelated apomictic F_1 or natural hybrids, determine the segregation for mode of reproduction, and estimate the resulting genetic variation for growth habit, cool-season regrowth, production of inflorescences and freeze resistance.

Materials and Methods

Plant Material and Crosses

Crosses were made between sexual and apomictic tetraploid clones during the summer 2006 to generate a segregating bahiagrass population. Twelve sexual tetraploid clones were used as female parents (Table 2-1). These 12 clones were hybrids generated at the University of Florida by crossing induced tetraploids (derived from seeds of the diploid cultivar Tifton 9) and the apomictic tetraploid clones, Argentine and Tifton 7 (Acuña et al., 2009). The induced tetraploids were selected for this study because they were identified as sexual and cross-pollinated, based on two years of observations, and were selected out of several hundred clones based on growth habit, cool-season regrowth and freeze resistance. Seven highly apomictic clones were used as pollen donors (Table 2-1). Five of them were also hybrids generated by

crossing induced sexual and apomictic tetraploids, and later identified as highly apomictic (Acuña et al., 2009). They were also selected based on superior spreading, cool-season regrowth and freeze resistance. The other two were the cultivar Argentine, which is the best adapted tetraploid ecotype to southeastern USA, and the experimental hybrid Tifton 7.

Crosses were made by enclosing one inflorescence from the sexual female and one or two inflorescences from the apomictic male in a glassine bag prior to anthesis. Care was taken to select inflorescences at the same stage of maturity. All bags were shaken each day during anthesis. Five days after anthesis the inflorescences from the apomictic parent were removed from the bags, leaving only inflorescences from the sexual parent until seed maturity. At maturity, each head from the sexual parents was threshed separately, the number of florets was counted, empty florets were removed, and the number of florets containing caryopses was determined.

Seed were scarified using concentrated sulfuric acid for 10 min, and were sown in flats containing sterile germination medium in February 2007. Individual seedlings were later transplanted to seedling flats in a greenhouse. Plants were transplanted into a field located at the Agronomy Forage Research Unit near Hague, Florida on 11 May 2007. The soil classification at this location was a loamy, siliceous, subactive, thermic, Arenic Endoaquult. Parents were asexually propagated in the greenhouse and transplanted into the field with the progeny. While the apomictic parents were propagated by seeds, sexual parents were vegetatively propagated using short pieces of rhizomes containing the apical meristems. Progeny from each cross were planted in 55-plant rows where individual plants were spaced 1 m x 1 m. A row containing multiple replications of the two parents involved in the specific cross was planted next to their progeny. The field was fertilized with 290 kg ha^{-1} of 21-3.1-11.6 (N-P-K) in June 2007.

Progeny Evaluation

Mode of reproduction

Two hundred and eleven plants were selected from 2,700 total generated plants. Selection was based on good general vigor. This subsample was used to estimate the proportion of apomictic and sexual progeny. Although this selection based on vigor might introduce bias in the results because vigor could be linked to sexuality or apomixis, it was used to reduce the probability of including progeny resulting from accidental self pollination among the evaluated plants.

Inflorescences from this group were fixed at anthesis (when the embryo sacs are usually fully developed) in FAA (18 Ethanol 70 %: 1 Formaldehyde 37 %: 1 glacial acetic acid). Pistils were dissected out of the florets and cleared using the method of Young et al. (1979). Ovules were observed using a differential interference contrast microscope.

A minimum of 20 ovules were observed from at least two different inflorescences. Plants bearing ovules with single embryo sacs containing the egg apparatus, the bi-nucleated central cell, and a mass of antipodals at the chalazal end were classified as sexual. In contrast, plants bearing ovules with multiple or single embryo sacs with the egg apparatus, the central cell, no antipodals, and variable size and position, were classified as apomictic. Plants producing ovules with either sexual or apomictic embryo sacs were classified as facultative.

Field observations

Plant diameter was estimated using the average between the longest and the shortest diameter of a given plant on 2 October 2007, and 26 September 2008. Plant height was measured from the base of the plant to the top of the canopy on 21 September 2007 and 12 September 2008. The number of inflorescences per plant was counted on 22 September 2007.

On 23 September 2007 all plants were defoliated to approximately 6-cm above the soil level and regrowth was visually estimated on 28 October 2007, and 6 May 2008 using a 1 to 5 scale, where 1 = plants showing the lowest amount of herbage and 5 = plants showing the highest amount of herbage. Plants were defoliated on 19 September 2008 and regrowth was estimated on 7 November. Also, freeze resistance was visually estimated on 21 November 2008 after one freeze event on 19 November, with temperature of -6 °C, using a 1 to 5 scale, where 1 = the least freeze resistant, and 5 = the most freeze resistant plant.

Statistical Analysis

Chi-square tests were used to compare the observed apomixis/sexuality segregation ratio with expected ratios and with previously reported ratios. Field observations were analyzed using PROC GLM of PC SAS (SAS Institute, 2004) as a completely randomized design. When significant differences among families were detected for one variable, the Waller-Duncan Test was used for mean separations. Unless otherwise stated in the text, all differences refer to significance at $P< 0.05$.

Broad sense heritability (H^2) estimates were calculated using the following formula:

$$H^2 = \frac{\sigma^2_p - \sqrt{(\sigma^2_{sp} * \sigma^2_{ap})}}{\sigma^2_p}$$

Where σ^2_p equals the phenotypic variance among the progeny, σ^2_{sp} equals the phenotypic variance among clonal replications of the sexual parent, and σ^2_{ap} equals the phenotypic variance among replications of the apomictic parent. σ^2_p includes the additive, dominance, and epistatic genetic variance, variation due to interactions between genotypes and environment, and variation due to environmental effects. The environmental variation is estimated based on the variation among clonally propagated parents. Variances and means were obtained using PROC

UNIVARIATE of SAS. The variance used to calculate H^2 for fall regrowth was calculated across years, while the variance for plant diameter and height is the average variance between years.

Results

Hybridization Efficiency

In 2006, 2,700 progeny were generated using 12 combinations of selected sexual and apomictic bahiagrass clones. Parental clones were selected based on their mode of reproduction (highly sexual or highly apomictic) and superior agronomic characteristics including cool-season regrowth, spreading and leaf tissue freeze resistance (Acuña et al., 2009). Sexual clones were used as female parents and highly apomictic clones as pollen donors. The average seed set was 30% varying from 13 to 51% (Table 2-1). Great variation was observed among crosses for germination. The average germination was 82% varying from 0 to 99%. With the exception of seeds from FL-33 × FL-53 which did not germinate, germination can be considered high varying from 59 to 99%. The average reproductive efficiency resulting from these crosses was 25% varying from 9 to 46% indicating that the outcome from this type of hybridization is highly dependant on the genotypes selected as parents.

Segregation for Mode of Reproduction

Two hundred and eleven progeny were selected between 2007 and 2008 based on their superior vigor to study the segregation for mode of reproduction. The use of this approach was expected to reduce the probability of including progeny resulting from self-pollination of sexual parents in the study. Although different numbers of progeny were selected from each family, all families were represented in this group (Table 2-2). One hundred and seventy three plants were classified as sexual because only single reduced embryo sacs were observed in their ovules. Seven were classified as highly apomictic because aposporous embryo sacs were observed in no

less than 65% of their ovules. The remaining 31 plants were classified as facultative apomictic because aposporous embryo sacs were observed in no more than 30% of their ovules.

Segregation for Growth Habit, and Production of Inflorescences

Plant diameter and plant height were measured in 2007 and 2008 to characterize the growth habit of the progeny. Significant differences were observed among families for plant diameter in both years. A range of family means from 28 to 42 cm in diameter was observed in 2007 and from 35 to 60 cm in 2008 (Table 2-3). The highest variability resulted from cross 5 in 2007 and cross 6 in 2008 (Table 2-3). The average broad sense heritability for plant diameter was 0.8 varying from 0.47 to 0.97 (Table 2-4). Significant variation was also found among families for plant height in 2007 and 2008. Family means for height varied from 32 to 47 cm in 2007 and from 40 to 63 cm in 2008 (Table 2-3). The greatest amount of variability was observed for cross 5 in 2007 and for cross 3 in 2008 (Table 2-3). The average broad sense heritability for plant height was 0.79 varying among families from 0.61 to 0.95.

Since seed production is considered one important agronomic characteristic of bahiagrass, the total number of inflorescences per plant was counted at the end of the 2007 growing season. The mean number of inflorescences produced varied from 13 to 39 among families (Table 2-5). The highest variability was obtained from cross 9. Broad sense heritability varied from 0.19 to 0.82 among families, while the average was 0.48 (Table 2-4).

Segregation for Cool-season Regrowth and Freeze Resistance

The 12 families included in this studied were determined to be significantly different in terms of plant regrowth during fall 2007, spring 2007 and fall 2008. The largest variability for spring regrowth was contained in family 4, while the lowest was present in family 11 (Table 2-5). Broad sense heritability estimates varied greatly among families having an average of 0.64 (Table 2-4). Progeny within family 1, 3 and 4 exhibited the greatest amount of variability for fall

regrowth considering 2007 and 2008 data (Table 2-5). The average broad sense heritability for fall regrowth was 0.63 varying from 0.47 to 0.86 (Table 2-4). Significant differences were also observed among these 12 families for freeze resistance in 2008. The greatest amount of variability for this characteristic resulted from cross 11, while the lowest variation resulted from cross 12 (Table 2-5). The average broad sense heritability was 0.77, varying from 0.25 to 1.0 (Table 2-4).

Discussion

The opportunity of fixing superior seed-propagated genotypes and the information generated in the last decade concerning the genetic control of apomixis have encouraged new attempts for the genetic improvement of apomictic bahiagrass as forage and turf. This research shows that it is feasible to generate a segregating population by crossing selected F_1 sexual and apomictic bahiagrass clones. In fact, F_2 hybrids can be created more efficiently than F_1 hybrids mainly because of the differences in fertility and vigor of the involved female parents. While F_1 sexual hybrids are superior genotypes selected from an original segregating population and have normal fertility, induced sexual tetraploid clones usually have reduced fertility and low vigor.

The ratio between sexual and apomictic progeny was of 4.6:1, which was different from the 1:1 segregation ratio [$\chi^2 = 86.4$, P(1 df) < 0.001] expected for a monogenic tetrasomic inheritance with apospory as a dominant trait. However, the observed segregation pattern was not different from the 4.3:1 ratio [$\chi^2 = 0.5$, P(1 df) = 0.48] observed for the F_1 population. These results would indicate that although there is a strong distortion with an excess of sexual progeny, the segregation patterns for apomixis remain constant through hybridization cycles. However, the proportion of highly apomictic progeny decreased from 11% in the F_1 to only 3% in the F_2, and the proportion of facultative apomictic increased from 9% in the F_1 to 15% in the F_2. These

findings would indicate that the probability of finding highly apomictic progeny decreases through hybridization cycles. This variation of gene expressivity between generations also suggests that epigenetic inheritance might be involved in the expression of aposporous apomixis. Moreover, Laspina et al. (2008) concluded that the expression of apospory in *Paspalum* might be triggered by the silencing of a large genomic region including the apospory locus. Environmental differences can also be responsible for the observed variable expressivity. Although Burton (1982) showed that the environment has little or no effect on the expression of apomixis in bahiagrass, seasonal variation of apospory was reported for *Paspalum cromiorrhyzon* Trin. (Quarin, 1985).

The most successful forages of southeastern USA have a prostrate growth habit that allows them to maintain their growing points without being defoliated by grazing animals. Marked variability for spreading and plant height was observed in the generated F_2 progeny (Figure 2-1 and Table 2-6). A considerably high proportion of this variability for growth habit was determined, using broad-sense heritability estimates, to be the result of genetic variation among the progeny (Table 2-6). These results indicate that selection can be efficiently used to develop clones with desirable growth habit. Previously reported broad sense heritability estimates for the F_1 progeny (Acuña et al., 2009) were similar to the average estimates now reported for the F_2 (Table 2-6). This is another indication that large genetic variation for growth habit was present in the F_2.

One of the advantages of bahiagrass as a forage and utility turf relies on seed propagation. Production of inflorescences is one trait that can be recorded early in the evaluation of a large segregating population as an indirect estimation of seed production. The variation observed for production of inflorescences in the F_2 was lower than that reported for the F_1 (Table 2-6). In

addition, a considerable portion of the variation observed in the F_2 was attributed to environmental differences. The decline in variance and heritability estimates indicates that one cycle of selection significantly reduced the genetic variability for this trait.

Warm-season grasses have a delimited growing season in subtropical areas mainly because of photoperiod responses (Sinclair et al., 2001). This physiological response, that seems to be a mechanism of freeze damage avoidance, reduces forage production during spring and fall. Efforts are being made to reduce this photoperiod sensitivity and increase freeze resistance by genetic manipulation of bahiagrass at the diploid and tetraploid level (Blount and Acuña, 2009). A large variation was observed in the F_2 for cool-season regrowth and freeze resistance (Table 2-6). A large part of this variation was attributed to genetic variation based on the obtained heritability estimates. A minimal reduction of genetic variance and heritability estimates for spring and fall regrowth was observed when comparing estimates for the F_1 and F_2 (Table 2-6). This small change reflects the effect of one cycle of phenotypic selection on the genetic variability for cool-season regrowth. In contrast, the genetic variance for freeze resistance was larger for the F_2 compared with that for the F_1. These results might relate to the fact that the F_2 was exposed to lower temperatures before the data was collected. While the F_1 was exposed to a minimal temperature of -2 °C, the F_2 was exposed to -6 °C before freeze resistance was estimated. Heritability estimates obtained in the F_1 and F_2 for freeze resistance did not change significantly.

In conclusion, hybridization between sexual and apomictic bahiagrass clones can be used efficiently to generate a large segregating population. Minimal reduction of the genetic variability for traits of agronomic interest can be expected after one cycle of selection. The low number of highly apomictic genotypes that can be found in each generation is a major limitation

of this breeding approach. Variation of apomixis expressivity can also be expected among generations.

Table 2-1. Seed set, germination and reproductive efficiency resulting from crosses between sexual and apomictic tetraploids clones of bahiagrass.

Cross			Seed Set†	Germination	Reproductive Efficiency‡
Sexual Female	Apomictic Male	Number	----------%----------		
FL-83	FL-13	1	32	98	32
FL-137	FL-13	2	51	89	46
FL-99	FL-3B	3	35	99	35
FL-14	FL-93	4	44	80	35
FL-16	FL-93	5	26	70	18
FL-41	FL-25	6	23	83	19
FL-3C	Argentine	7	39	97	38
FL-7	Tifton 7	8	24	92	22
FL-62	Tifton 7	9	26	72	19
FL-47	Tifton 7	10	23	84	20
FL-16	Argentine	11	13	66	9
FL-20	Argentine	12	20	59	12
	Average		30	82	25

† Seed set: percentage of obtained seed from the total number of pollinated florets.
‡ Reproductive efficiency: percentage of obtained plants from the total number of pollinated florets.

Table 2-2. Bahiagrass tetraploid progeny classification for method of reproduction based on embryo sacs observations.

Cross	Analyzed Progeny	Apomictic	Facultative	Sexual
1	22	0	3	19
2	33	0	4	29
3	22	0	3	19
4	43	2	12	29
5	23	3	3	17
6	12	1	0	11
7	4	0	2	2
8	19	1	1	17
9	16	0	0	16
10	3	0	0	3
11	2	0	0	2
12	12	0	3	9
Total	211	7	31	173

Table 2-3. Plant diameter and plant height for progeny of 12 combinations of sexual and apomictic clones.

Cross	Plant Diameter				Plant Height			
	2007		2008		2007		2008	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	cm							
1	33	8.9	50	13.9	40	8.6	51	8.6
2	42	11.0	59	22.6	34	7.7	48	8.9
3	35	8.0	47	11.2	43	7.9	53	31.6
4	34	8.4	50	12.4	47	8.0	63	9.0
5	40	14.4	60	15.7	40	9.4	52	21.2
6	40	7.7	60	39.2	40	8.2	48	11.1
7	28	8.3	35	15.6	40	7.9	51	15.4
8	40	8.7	59	28.4	36	7.7	48	10.7
9	37	9.4	49	14.6	36	8.4	47	10.2
10	37	10.0	54	20.3	32	7.6	42	11.8
11	38	7.8	59	33.0	36	7.5	40	11.5
12	37	6.4	59	10.6	43	8.1	57	8.8
MSD[†]	2.0		4.2		1.7		3.0	

[†] Minimum significant difference, Waller-Duncan means separation procedure.

Table 2-4. Broad-sense heritability estimates for several characteristics measured for 12 combinations of sexual and apomictic bahiagrass tetraploid clones.

Cross	Number of Inflorescences	Height	Diameter	Spring Regrowth	Fall Regrowth	Freeze Resistance
1	0.40	0.78	0.82	0.46	0.49	0.66
2	0.66	0.61	0.85	0.71	0.49	0.68
3	0.22	0.95	0.59	0.86	0.78	0.52
4	0.65	0.90	0.74	0.63	0.66	0.85
5	0.82	0.93	0.86	0.41	0.65	1.00
6	0.40	0.78	0.97	0.72	0.47	0.68
7	0.50	0.90	0.80	0.75	0.86	0.74
8	0.19	0.71	0.93	1.00	0.79	1.00
9	0.27	0.65	0.80	0.63	0.56	0.25
10	0.36	0.80	0.83	0.56	0.52	1.00
11	0.55	0.84	0.94	0.31	0.49	1.00
12	0.68	0.70	0.47	0.65	0.77	0.81
Mean	0.48	0.79	0.80	0.64	0.63	0.77

Table 2-5. Number of inflorescences, spring and fall regrowth, and freeze resistance of progeny resulting from 12 combinations of sexual and apomictic bahiagrass tetraploid clones.

Cross	Number of Inflorescences		Spring Regrowth		Fall Regrowth		Freeze Resistance	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	13	12.4	2.0	0.9	2.6	1.0	1.8	0.8
2	21	15.4	2.8	1.0	2.9	0.9	2.0	0.8
3	26	15.7	2.7	1.1	3.1	1.0	2.0	0.8
4	28	17.3	3.5	1.2	3.3	1.0	2.6	1.0
5	31	17.2	2.2	0.9	2.9	0.9	1.8	0.7
6	22	14.6	2.3	1.0	2.8	0.8	1.7	0.7
7	11	11.6	2.0	0.9	2.6	0.9	2.0	1.0
8	16	18.1	2.7	0.9	3.0	0.7	2.0	0.8
9	18	18.5	2.3	0.9	2.8	0.8	1.7	0.6
10	25	17.7	2.0	0.9	2.2	0.8	1.7	0.6
11	39	14.0	1.7	0.6	2.1	0.6	1.6	0.5
12	36	13.3	3.4	0.9	3.0	0.7	2.8	1.1
MSD[†]	3.2		0.2		0.1		0.2	

[†] Minimum significant difference, Waller-Duncan means separation procedure.

Table 2-6. Genetic variance and broad sense heritability (H^2) estimates for several agronomic characteristics for a F_1 population generated by crossing induced sexual and apomictic clones (Acuña et al., 2009) and a F_2 population generated by crossing sexual and apomictic F_1 clones.

Trait	F_1		F_2	
	Variance	H^2	Variance	H^2
Spreading	102.9	0.7	86.8	0.8
Height	55.5	0.8	68.2	0.8
Inflorescences	495.8	0.9	147.3	0.5
Spring Regrowth	0.88	0.8	0.73	0.6
Fall Regrowth	0.82	0.8	0.61	0.6
Freeze Resistance	0.25	0.7	0.58	0.8

Figure 2-1. Growth habit variation among bahiagrass hybrids generated by crossing F_1 sexual and apomictic clones.

CHAPTER 3
SEASONAL GROWTH, NITROGEN AND PHOSPHORUS UPTAKE OF NOVEL APOMICTIC BAHIAGRASS HYBRIDS

Introduction

Bahiagrass is widely adapted to sub-tropical and tropical regions around the world and is considered an important forage and utility turf for southeastern USA (Blount and Acuña, 2009). As forage, it is used as a long-term perennial pasture or in short-term rotations with row crops, such as cotton and peanut. As turf, it is sowed along road-ways, athletic fields and other recreational areas. Bahiagrass has potential as a bioenergy crop because of its long-term persistence, and, as other C_4 species, high water and nitrogen (N) use efficiency. It is also used as ground cover in different parts of the world to prevent soil erosion and ground water pollution resulting from nutrient runoff and leaching. This is especially important in areas with well drained, sandy soils (Brady and Weil, 2002).

Bahiagrass growth is restricted to a relatively short period in subtropical regions. In southeastern USA, most of its above-ground biomass is produced between June and August. This growth pattern is mainly the result of its sensitivity to photoperiod and low temperatures (Sinclair et al., 2001; Gates et al., 1999). Extending the growth period can increase its use as forage or turf. Genetic variability for extending the bahiagrass growth period has been observed in diploid and tetraploid bahiagrass germplasm (Blount and Acuña, 2009; Acuña et al., 2009). The possibility of fixing bahiagrass genotypes with an extended growing season by using apomixis makes the tetraploid germplasm a good candidate for genetic improvement of this crop.

Several apomictic hybrids exhibiting an extended growing season as individual plants were identified among a segregating population generated by crossing induced sexual and apomictic tetraploid clones (Acuña et al., 2009). However, it was unknown if these individual plant hybrids were able to exhibit the same response when grown in swards. It was also unknown how

selecting for an extended growing season would relate to root and rhizome mass. These organs are essential for the long-term survival and production of this crop. Hypothetically, if these hybrids produce more biomass in the cool-season, they will likely remove more total nutrients during this period. An extended growing season may also result in greater annual biomass and nutrient uptake. The objective of this study was to determine the seasonal biomass yield, N and P accumulation of novel apomictic bahiagrass hybrids grown in swards.

Materials and Methods

Plant Material

Thirteen tetraploid apomictic bahiagrass clones were selected for this study, based on expected variability in seasonal forage production. Two of these clones, Argentine and common, are natural ecotypes introduced from South America. Argentine is a productive tetraploid clone commonly grown in Florida. Both, Argentine and common were selected for this study because of their relatively short growing season. The other eleven clones were novel apomictic hybrids generated by crossing sexual and apomictic tetraploid clones. One of the better known clones, Tifton 7, was developed by Dr. Glenn Burton (USDA-ARS, Tifton, Georgia), and selected based on superior forage yields when grown in field plot trials. Three others, C-49, C-65 and C-92, were generated by Camilo Quarin (Corrientes, Argentina), and selected based on superior individual plant forage yields. The other seven clones (FL-3, FL-3B, FL-13, FL-14, FL-21, FL-93 and FL-122) were developed at the University of Florida, and selected, based on superior vigor, cool-season regrowth, and freeze resistance of spaced plants. Since these 13 clones are classified as highly apomictic, variation among replications was primarily restricted to environmental variation.

Experimental Design and Plot Management

Gainesville

Seeds were scarified (10 min) using concentrated sulfuric acid and rinsed with tap water. The scarified seed were sown in March 2006, in a greenhouse, using plastic trays containing a sterile germination mix. After two weeks, seedlings were transplanted to seedling flats containing multiple cells. Seedlings were transplanted into a field located at Gainesville (29°48'12" N, 82°24'47" W), Florida, on 15 May, 2006. The soil classification at this location was a loamy, siliceous, subactive, thermic, Arenic Endoaquult. Forty seedlings from individual clones were planted (40 cm x 40 cm) into 2-m x 3-m plots. Five blocks of 12 pure-stand plots were planted without alleys in a randomized complete block design. The ecotype common was not planted at this location. Plots were irrigated with approximately 20 mm of water after transplanting. Weeds were manually removed during the establishment year. In July 2006, plots were fertilized with 500 kg ha^{-1} of 16-4-8 (N-P_2O_5-K_2O). On 31 October, 2006, plots were harvested using a sickle bar mower leaving a 5-cm stubble height. A 2.35 m x 0.7 m strip was cut in the middle of each plot, the forage collected, weighed, and a subsample (approximately 700 g) immediately taken after harvest. The subsample was weighed and the material dried at 60 °C for 48 h, prior to reweighing for dry mass. Plots were harvested again on 4 May, 2007, and every four weeks during the rest of the 2007 growing season (Table A-1). With the exception of the last harvest of each year, plots were fertilized with 286 kg ha^{-1} of 21-7-14 (N-P_2O_5-K_2O) following each cutting. In 2008, plots were harvested for the first time on 13 May and every four weeks during the growing season (Table A-2). Plots were fertilized with 375 kg ha^{-1} of 16-4-8 (N-P_2O_5-K_2O) following each cutting.

Soil cores (4.7-cm diameter and 15-cm depth) were collected in spring, summer, and fall 2007, and spring and fall 2008. Samples were collected from the center of an original (or mother) plant located near the border of each plot. Soil samples were placed on a 2-mm mesh screen and washed with a gentle stream of tap water to recover rhizomes and roots. Samples where dried at 60°C for 48 h and dry mass recorded.

Dry forage and rhizome-root samples from each harvest were ground using a Willey mill, and passed through a 1-mm screen. Nitrogen and P concentrations were determined at the University of Florida Forage Evaluation Support Laboratory (Gainesville, FL). For N and P analyses, samples were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). Briefly, dry sample (0.25 g) was combined with 6 ml of H_2SO_4, 2 ml H_2O_2 and catalyst (1.5 g of 9:1 K_2SO_4:$CuSO_4$) and digestion conducted for at least 4 h at 375°C. Nitrogen and P in the digestate were determined by semiautomated colorimetry (Hambleton, 1977).

Live Oak and Quincy

Seeds from the 13 apomictic clones were germinated in March 2007, in a greenhouse located at Gainesville, FL. After two weeks, seedlings were transplanted to seedling flats containing multiple cells. Seedlings were transplanted into a field located at the North Florida Research and Education Center, Live Oak (32°18'03" N, 82°53'53" W), FL, on 9 May. The soil at is classified as a thermic, coated Typic Quartzipsamment. Four blocks (rows) were planted, containing 13 (1.2 m x 1.2 m) plots separated by a 1 m alley, as a randomized complete block design. Each plot contained 36 seedlings of each clone (20 cm x 20 cm), and clones were randomized within each block. Plots were irrigated with approximately 20 mm of water after transplanting, and weeds removed manually. Plots were fertilized with 530 kg ha^{-1} of 34-0-0 (N-

P_2O_5-K_2O) and 100 kg ha^{-1} of 0-0-60 (N-P_2O_5-K_2O) during 2007. A 70-cm wide strip was cut across each plot on 15 May, 2008, using a sickle bar mower, leaving a 5-cm stubble height. Plots were harvested every 4 weeks (Table A-3), and fertilized with 376 kg ha^{-1} of 16-4-8 (N-P_2O_5-K_2O) following each cutting, for the remainder of the growing season. Forage samples were dried at 60°C for 48 h, and dry mass recorded.

Seedlings from 13 apomictic clones germinated in March 2007 at Gainesville were also planted at the North Florida Research and Education Center, Quincy (30°32'59" N, 84°36'02" W), Florida, on 10 May, 2007. The soil in this location is classified as fine-loamy, kaolinitic, thermic, Typic Kandiudult. Forty seedlings were transplanted (40 cm x 40 cm) into each plot (2 m x 3.2 m). Five replication of each pure-stand plot were planted in a randomized complete block design. Plots were irrigated with 25 mm of water after transplanting. Weeds were controlled manually and mowed during 2007. On 2008, plots were harvested on 13 May for the first time, and every four weeks (Table A-4). On 22 May, plots were fertilized with 171 kg ha^{-1} of 35-0-0 (N-P_2O_5-K_2O) and 28 kg ha^{-1} of 0-0-60 (N-P_2O_5-K_2O). On 13 June, plots were fertilized with 20 kg P ha^{-1}, 67 kg N ha^{-1}, and 42 kg K ha^{-1}. In 11 July and after each subsequent harvest, plots were fertilized with 376 kg of 16-4-8 (N-P_2O_5-K_2O). A 2.35 m x 0.7-m strip was cut in the middle of each plot, the forage weighed, and a subsample was taken. The subsample fresh weight was obtained immediately after harvest. Subsamples were dried at 60 °C for 48 h, and dry mass recorded.

Statistical Analyses

Gainesville data for biomass, N concentration, P concentration, N content, and P content were analyzed as repeated measures using Proc Mixed procedure (SAS version 9.2, SAS Institute, Cary, NC). Clones and harvest dates were considered fixed, while replicates were

considered random. Biomass, N and P accumulated in spring, summer, and fall were also analyzed as repeated measures.

Biomass data collected every four weeks at Gainesville, Live Oak, and Quincy in 2008 were also analyzed as repeated measures using Proc Mixed of PC SAS. Locations, clones and harvest dates were considered fixed, while replicates were considered random. When significant treatment effects ($P = 0.05$) were found, the minimum significant difference (MSD) among means was calculated using the Waller-Duncan test.

Results

Seasonal Biomass Yields

Gainesville

Biomass yields among 12 bahiagrass tetraploid clones varied significantly among harvests in 2007 and 2008 (Figures 3-1 and 3-2). The highest biomass yields were observed in June 2007 and July 2008 (Tables A-1 and A-2). A significant interaction between harvests and clones resulted from differences on seasonal biomass production among clones, as illustrated with 4 of the clones (Figure 3-1). The largest genotypic differences were observed in the spring of both years (Table 3-1 and Figure 3-1). With few exceptions, most hybrids produced more biomass than Argentine during the spring of both years (Table 3-1). For example, clone FL-13 yielded 4.3 times more biomass than Argentine in spring 2007 and 1.9 times more in spring 2008. In contrast, Argentine was among the most productive clones during the summer of both years. Eight hybrids produced as much as Argentine in summer 2007, while only 3 hybrids were able to yield as much as Argentine in summer 2008. Although there were significant differences among clones, the fall biomass yield was significantly lower than that observed for spring and summer. Tifton 7 had among the greatest fall yields across years. There were significant differences among tetraploid clones for annual biomass accumulation in 2007 and 2008 (Table 3-1). While

Argentine accumulated less annual biomass than several clones in 2007, no clones produced more than Argentine in 2008.

Multi-location biomass production

A significant location effect and a significant interaction between location and clones were observed when the 2008 biomass data collected at Gainesville, Live Oak and Quincy were compared. There was also a significant harvest effect and an interaction between harvests and clones. Clones were significantly different for biomass production at each harvest for each of the three locations (Tables A-3 and A-4).

Spring biomass varied greatly among clones at Live Oak and Quincy (Tables 3-2 and 3-3, and Figures 3-3 and 3-4). FL-13 and FL-93 were among the highest spring yielding clones, while common and Argentine were among the lowest yielding clones at the two locations. However, some clones performed differently between locations. For example, C-49 was ranked low at Quincy and high at Live Oak. Regardless, most novel hybrids outperformed Argentine for spring biomass (Tables 3-2 and 3-3; Figures 3-3 and 3-4).

Significant differences were also observed among clones for summer biomass production at Live Oak and Quincy (Tables 3-2 and 3-3). However, no hybrid produced more biomass than Argentine. Common, FL-21 and FL-13 produced less biomass than Argentine at Live Oak, while only common produced less than Argentine at Quincy during the summer. Genotypic performance for fall biomass yield was different between Live Oak and Quincy. While seven hybrids produced more fall biomass than Argentine at Live Oak, no hybrid outperformed Argentine at Quincy.

Total annual biomass during 2008 differed among clones. While four hybrids accumulated more biomass than Argentine at Live Oak, five hybrids outperformed Argentine at Quincy

(Tables 3-2 and 3-3). FL-93, C-92 and C-49 produced more annual biomass than Argentine during 2008 at both locations.

Rhizome+Root Mass and Nutrient Concentration and Accumulation

Significant differences among clones for rhizome+root dry mass were observed in spring, 2007 (Figure 3-5 and Table A-5). Clone FL-13 had significantly greater mass than the C-65, C-49 and Argentine. No genotypic effect was detected for summer or fall 2007, nor spring or fall 2008. Rhizome+root mass decreased continuously from spring 2007 to fall 2008 (Table 3-4).

Only small genotypic differences in N concentration were observed in summer 2007 and fall 2008 (Table 3-5). No genotypic effect was observed for N concentration in spring 2007, spring 2008, or fall 2007. The lowest N values were observed in spring of both growing seasons (Table 3-4). Small differences were also observed for P concentrations in spring and summer 2007, and fall 2008 with the greatest P concentrations observed in fall 2007 and 2008 (Table 3-6).

Genotypic differences for rhizome+root N accumulation were observed in spring 2007 (Figure 3-6). These differences were mainly due to differences in dry mass (Figure 3-5). Nitrogen rhizome+root N accumulation increased during the 2007 growing season. However, N accumulation did not change between spring and fall 2008 (Table 3-4). Differences among genotypes were observed for P accumulation only in spring 2007 (Figure 3-7). While P accumulation increased during the 2007 growing season, it decreased during 2008 (Table 3-4).

Forage Nutrient Composition and Accumulation

Nitrogen concentration remained relatively constant during spring and summer of the 2007 and 2008 growing seasons (Figure 3-8 and 3-9). With the exception of the forage harvested at the end of October 2007, N concentration was higher during fall than spring and summer of both years. Minimal genotypic differences for N concentration were observed at the first spring

harvest of 2007 and 2008 and a few additional harvests during the 2-year study period (Tables A-6 and A-7). There was not a consistent pattern of N concentration differences among clones.

Phosphorus concentrations increased during spring and summer of 2007 and then decreased during the fall (Figure 3-10). A marked increase on P concentration was observed in spring 2008 (Figure 3-11). It continued increasing during summer and fall 2008 but at a lower rate. Significant differences were found among clones for P concentration at each harvest in 2007 (Table A-8). Differences were also observed at the summer and fall harvests of 2008 (Table A-9). Argentine consistently had the greatest forage P concentrations throughout all harvests (Figures 3-10 and 3-11).

A similar seasonal pattern was observed for N and P uptake in each year. In 2007, N and P uptake were low at the beginning of the spring (Figures 3-12 and 3-14). They increased during spring reaching a maximum at the end of this season, decreased and stayed low during the summer, increased at the end of the summer reaching another maximum, and decreased during fall reaching a minimum at the end of the season. In 2008, N and P uptake were also low at the beginning of spring (Figures 3-13 and 3-15). They increased continuously until reaching a maximum at the end of the summer. N and P uptake decreased during fall reaching a minimum at the end of the season.

Genotypic differences in N accumulation were observed for most harvests (Tables A-10 and A-11). The main differences were observed in spring of both years (Table 3-7). FL-3, FL-13 and FL-93 accumulated significantly more N than Argentine in spring 2007 and 2008. Clones also accumulated different amounts of N in summer 2007 and 2008. Any of the novel hybrids was able to accumulate more N than Argentine in summer. Differences for fall N accumulation were only observed in 2008. Tifton 7 was the only clone able to accumulate more N than

Argentine in fall 2008. There were no genotypic differences for annual N accumulation in 2007 or 2008. Genotypic differences for P uptake were also observed for most harvests (Tables A-12 and A-13). Several hybrids accumulated more P than Argentine in spring 2007 and 2008 (Table 3-8). In contrast, no other clone accumulated more P than Argentine in summer 2007 or 2008. No differences were detected among clones for P accumulation in fall 2007. However, Tifton 7 accumulated more P than most clones in fall 2008. There were differences among clones for annual P accumulation in 2007 and 2008. However, any of the novel hybrids was able to accumulate more P than Argentine in 2007 or 2008.

Discussion

Genetic variability for seasonality of above ground biomass production is present in the tetraploid bahiagrass germplasm. The main differences can be expected to occur in spring and fall when bahiagrass is grown in subtropical zones. Hybridization of sexual and tetraploid bahiagrass clones selected for superior cool-season re-growth and freeze resistance (Acuña et al., 2009) resulted in hybrids with an extended growing season. The heterosis observed for these characteristics of hybrids grown in a space-plant nursery have been fixed by their high degree of inherent apomixis. The growing season extension is especially important when bahiagrass is used as forage. Cultivars with an extended growing season can provide forage for grazing livestock longer during the year. Increasing the growing season of bahiagrass lines can also be exploited for turf. Turf cultivars with an extended growing season will maintain good sod quality longer during each season. Further research is needed to determine if these bahiagrass hybrids exhibiting a longer growing season are less photoperiod sensitive, as reported by Sinclair et al. (2001). Anatomical or physiological characteristic behind their higher freeze resistance should also be further investigated.

The observed decline in rhizome-root mass throughout the experiment could be the result of frequent defoliation, or the transition between individual plants in a plot to a well developed sward. Since samples were always collected from the center of the original plants, this decline in mass might result from spreading and independence of new rhizomes. The lack of genotypic differences for rhizome+root mass would support this hypothesis since the cultivar Argentine is well know for its robust rhizome-root system and tolerance to intense defoliation. The decline in N and P concentrations from fall to spring seems to be the result of utilization of stored nutrients for spring regrowth. Blue (1973) reported that the bahiagrass rhizome-root system can be a source of nutrients for new growth. Although remobilization of N and P stored in these organs would have little relevance for forage yields in intensive production systems, it seems to be an important survival mechanism.

Hybrids that produced more biomass in spring also removed more N and P. For example, FL-93 produced consistently more spring biomass than Argentine across years and locations, and it also removed more N and P in spring 2007 and 2008. This indicates that hybrids with an extended growing season would reduce the amount of nutrient losses in runoff water in years with high precipitation in the spring. Hybrids showing this characteristic will be more appropriate for crop rotation with row crops, since remnant fertilizer would be extracted and converted into forage sooner during the crop cycle.

Seasonal biomass yield differences affected total biomass in 2007 at Gainesville. For example, C-92 produced more biomass in spring and fall than Argentine, resulting in higher annual biomass accumulation. FL-13 producing more spring biomass than Argentine resulted in a higher annual biomass yield. Some of these novel hybrids with an extended growing season were also able to accumulate more biomass than Argentine in Live Oak and Quincy in 2008. In

contrast, superior spring or fall biomass yields did not result in higher annual biomass accumulation at Gainesville in 2008. These results indicate that in most years and locations across north Florida hybrids showing superior spring and fall growth will accumulate annually higher biomass. However, long term studies will be needed to determine the consistency of annual forage accumulation of these novel apomictic hybrids across multiple years. Based on our data, hybrids with an extended growing season would be also more appropriate for hay or biomass production systems with one or two harvests per year.

Greater spring and fall biomass yield did not result in higher annual N and P accumulation. This is because clones with a shorter growing season tended to have greater N and P tissue concentrations which offset the lower biomass yields. This was particularly true with Argentine, which had higher P concentrations than other clones for most of 2007 and 2008. If annual nutrient removal is the considered objective for planting bahiagrass on nutrient impacted land, available cultivars should be considered appropriate. The possibility of transferring genes for superior P and N accumulation present in available cultivars should be further evaluated.

In this study we reported seasonal biomass production, N and P concentrations and accumulation of several apomictic bahiagrass clones. Genetic variability for seasonality of biomass yields was reported. Higher cool-season biomass production and N and P removal was observed for a few novel hybrids. Greater cool-season biomass resulted in higher annual biomass accumulation of newly developed clones.

Table 3-1. Seasonal and annual biomass accumulation of 12 apomictic bahiagrass clones grown at Gainesville, FL.

	2007				2008			
Clone	Spring	Summer	Fall	Total	Spring	Summer	Fall	Total
	---------- g m^{-2} ----------							
FL-13[†]	483	657	94	1233	353	720	257	1330
C-92	455	835	71	1360	319	953	248	1520
C-65	428	835	85	1347	356	926	292	1574
FL-14	398	952	30	1380	337	887	319	1543
Tifton 7	379	893	79	1350	240	981	401	1622
FL-3	354	887	63	1303	353	886	287	1526
FL-93	353	879	44	1276	366	873	278	1517
FL-3B	331	788	42	1162	298	908	253	1460
FL-122	278	805	50	1133	217	842	231	1291
FL-21	261	775	42	1078	334	728	214	1276
C-49	217	808	44	1069	247	841	289	1377
Argentine	112	940	37	1089	188	1022	253	1464
MSD[‡]	107	138	29	183	89	100	75	207

[†]Clones were ordered based on spring 2007 biomass.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-2. Seasonal biomass accumulation of 13 apomictic bahiagrass clones grown at Live Oak, FL.

Clone	Spring	Summer	Fall	Total
	---------- g m^{-2} ----------			
FL-93[†]	260	916	90	1265
C-92	229	984	115	1328
FL-13	226	750	97	1073
C-65	210	835	127	1172
FL-3	195	867	97	1159
FL-14	195	888	144	1226
FL-3B	188	880	136	1204
FL-21	157	763	74	994
Tifton 7	149	870	109	1127
FL-122	137	894	114	1145
C-49	135	930	167	1231
Argentine	121	895	82	1099
Common	0	563	55	618
MSD[‡]	38	106	23	123

[†]Clones were ordered based on spring biomass.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-3. Seasonal biomass accumulation of 13 apomictic bahiagrass clones grown at Quincy, FL.

Clone	Spring	Summer	Fall	Total
	----------g m^{-2}----------			
FL-13[†]	215	525	129	776
C-49	212	651	185	972
C-92	206	631	136	882
FL-14	193	577	155	841
FL-93	176	642	144	869
FL-3B	161	638	155	880
FL-3	153	636	148	875
C-65	137	635	153	868
Tifton 7	135	542	169	790
FL-21	129	542	146	747
FL-122	110	642	156	853
Argentine	86	570	160	769
Common	1	460	123	583
MSD[‡]	44	80	43	99

[†]Clones were ordered based on spring biomass.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-4. Rhizome+root dry mass, N and P concentrations and accumulation (Gainesville).

Season	Rhizome+root mass	Nitrogen	Phosphorus	Nitrogen	Phosphorus
	mg cm^{-3}	g kg^{-1}	g kg^{-1}	mg cm^{-3}	mg cm^{-3}
Spring 2007	41	10.2	0.68	0.42	0.028
Summer 2007	36	11.0	0.85	0.38	0.030
Fall 2007	32	15.7	1.20	0.50	0.040
Spring 2008	18	9.3	0.88	0.17	0.020
Fall 2008	14	12.6	0.97	0.18	0.010
MSD[†]	4	1.0	0.09	0.06	0.005

[†] Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-5. Nitrogen concentration in rhizome+roots of 12 bahiagrass clones (Gainesville).

	2007			2008	
Clone	Spring	Summer	Fall	Spring	Fall
	---------- g kg^{-1} ----------				
FL-21[†]	9.4	14.0	15.1	9.5	16.8
FL-13	11.6	12.7	13.7	12.2	15.2
C-65	11.8	11.4	15.5	9.5	12.6
FL-122	8.3	7.4	12.6	7.4	12.6
FL-3B	11.0	14.0	17.9	9.6	12.3
FL-3	10.0	12.2	14.7	10.7	12.1
C-49	10.0	11.4	14.6	8.8	12.1
FL-93	9.5	9.7	16.2	10.1	12.1
Argentine	10.7	11.3	15.9	9.3	11.7
FL-14	9.5	7.2	15.0	6.6	11.6
C-92	10.9	9.9	18.2	8.4	11.2
Tifton 7	9.1	11.3	18.8	9.6	10.4
MSD[‡]	ns	4.1	ns	ns	4.4

[†]Clones were ordered based on N concentration for fall 2008.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-6. Phosphorus concentration in rhizome+roots of 12 bahiagrass clones (Gainesville).

	2007			2008	
Clone	Spring	Summer	Fall	Spring	Fall
	---------- g kg^{-1} ----------				
Argentine[†]	0.91	0.97	1.19	1.02	1.33
FL-122	0.83	1.02	1.12	1.20	1.22
FL-21	0.69	0.83	1.05	0.87	1.04
C-65	0.81	1.02	1.45	0.69	0.97
FL-93	0.57	0.68	1.27	0.79	0.94
FL-3	0.65	0.83	1.34	0.88	0.93
C-49	0.63	0.87	1.10	0.67	0.92
FL-13	0.69	0.75	1.56	0.68	0.91
FL-14	0.47	0.60	1.13	1.20	0.90
Tifton 7	0.66	0.97	1.16	1.06	0.87
C-92	0.70	0.97	1.31	0.82	0.84
FL-3B	0.53	0.68	1.32	0.69	0.73
MSD[‡]	0.26	0.33	ns	ns	0.26

[†]Clones were ordered based on P concentration for fall 2008.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-7. Seasonal and annual forage N uptake of 12 apomictic bahiagrass clones (Gainesville).

	2007				2008			
Clone	Spring	Summer	Fall	Total	Spring	Summer	Fall	Total
	----------	----------	----------	g m^{-2}	----------	----------	----------	----------
C-92[†]	7.9	11.8	6.1	25.8	4.6	14.6	5.6	24.9
FL-13	7.6	9.1	6.2	22.9	5.4	11.4	5.8	22.7
C-65	7.6	11.3	6.3	25.2	5.2	14.1	6.2	25.5
FL-3	6.4	11.3	7.1	24.7	5.4	13.4	6.1	25.0
Tifton 7	6.4	11.6	6.9	24.9	3.9	14.3	8.4	26.4
FL-93	5.8	11.5	7.2	24.5	5.4	13.8	6.0	24.7
FL-14	5.6	12.0	7.3	25.0	4.6	14.1	6.5	25.3
FL-3B	5.5	10.2	6.0	21.7	4.3	14.5	5.4	24.2
FL-122	5	11.1	6.3	22.4	3.5	13.4	5.2	22.1
FL-21	4.3	9.7	6.6	20.6	5.3	12.5	4.9	22.7
C-49	4.2	11.7	5.6	21.5	3.5	12.6	6.2	22.2
Argentine	2.0	11.0	7.8	20.7	2.9	16.2	5.6	24.7
MSD[‡]	3.0	2.4	ns	ns	2.3	2.6	1.8	ns

[†]Clones were ordered based on spring 2007 N accumulation.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table 3-8. Seasonal and annual forage P uptake of 12 apomictic bahiagrass clones grown at Gainesville, FL.

	2007				2008			
Clone	Spring	Summer	Fall	Total	Spring	Summer	Fall	Total
	----------	----------	----------	g m^{-2}	----------	----------	----------	----------
C-92[†]	0.7	1.4	0.5	2.6	0.4	2.0	0.6	2.9
FL-13	0.7	1.1	0.6	2.3	0.4	1.5	0.6	2.5
Tifton 7	0.6	1.6	0.7	2.9	0.3	2.2	1.0	3.5
C-65	0.6	1.3	0.6	2.5	0.4	1.9	0.6	2.9
FL-93	0.6	1.4	0.6	2.5	0.4	1.8	0.6	2.8
FL-14	0.5	1.4	0.7	2.7	0.4	1.8	0.8	3.0
FL-3	0.5	1.3	0.7	2.5	0.4	1.8	0.7	2.9
FL-3B	0.5	1.2	0.6	2.3	0.3	1.7	0.5	2.6
FL-122	0.4	1.4	0.6	2.4	0.3	1.9	0.5	2.8
C-49	0.4	1.2	0.5	2.1	0.3	1.7	0.6	2.6
FL-21	0.4	1.2	0.6	2.2	0.4	1.5	0.5	2.4
Argentine	0.2	1.7	0.8	2.7	0.2	2.6	0.7	3.6
MSD[‡]	0.2	0.3	ns	0.6	0.1	0.3	0.2	0.4

[†]Clones were ordered based on spring 2007 P accumulation.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

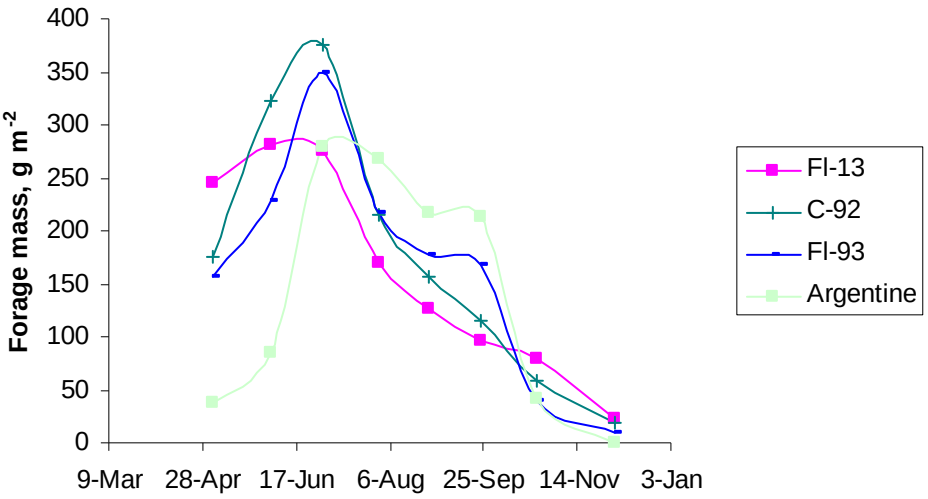

Figure 3-1. Biomass of 4 bahiagrass clones grown at Gainesville in 2007.

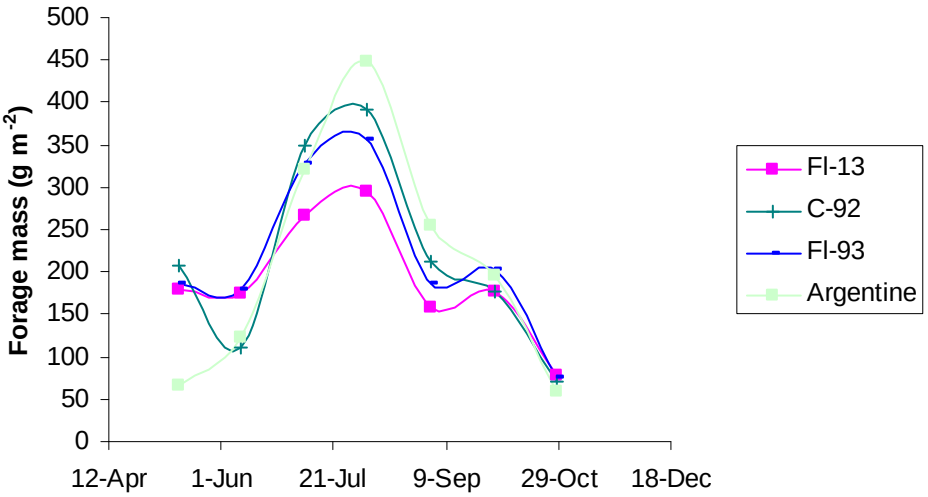

Figure 3-2. Biomass of 4 bahiagrass clones grown at Gainesville in 2008.

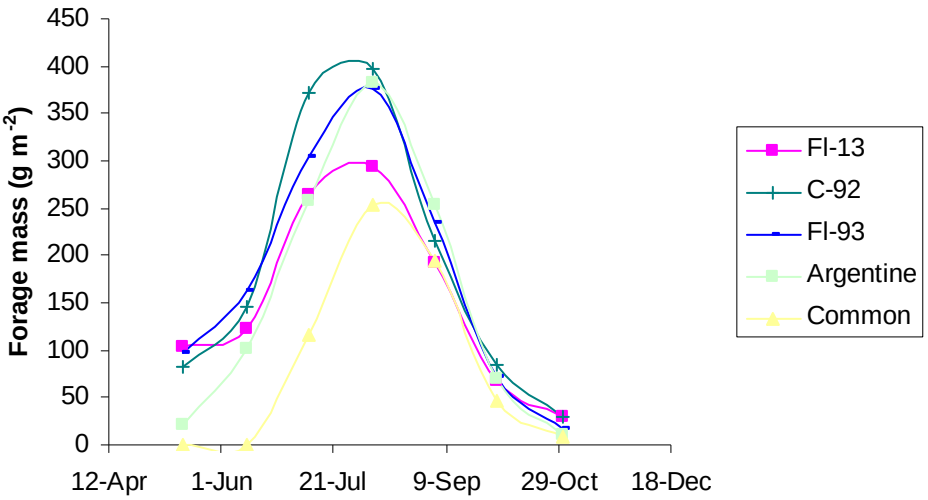

Figure 3-3. Biomass of 5 bahiagrass clones grown at Live Oak in 2008.

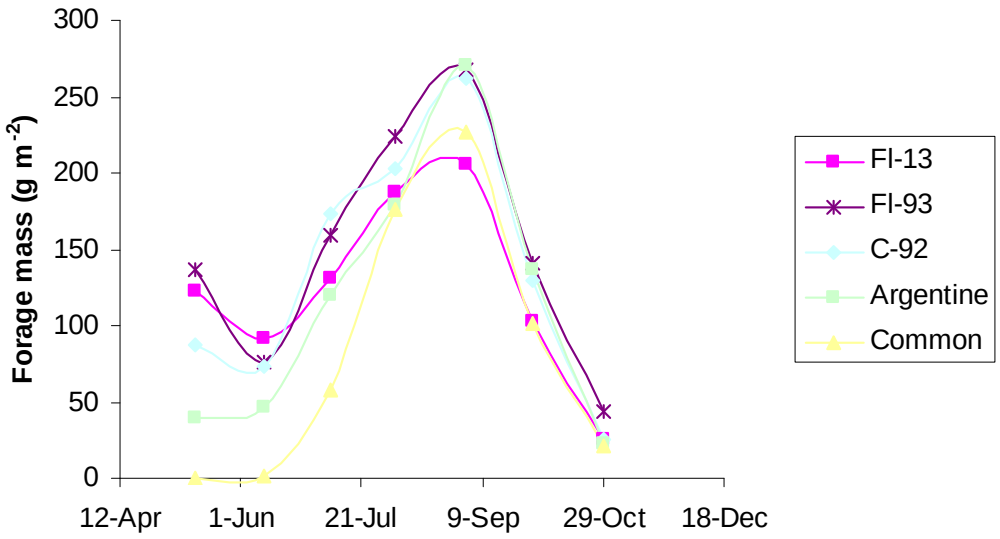

Figure 3-4. Biomass of 5 bahiagrass clones grown at Quincy in 2008.

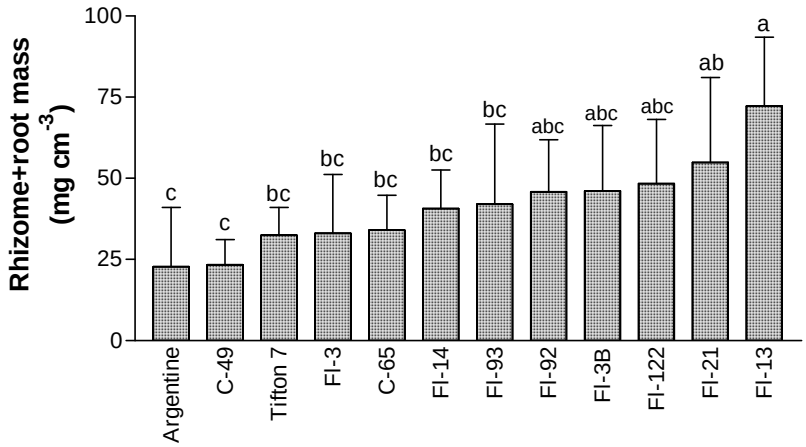

Figure 3-5. Spring rhizome-root mass of 12 bahiagrass clones grown at Gainesville, FL. Error bars represent the standard deviations. Clone means followed by the same letter are not different.

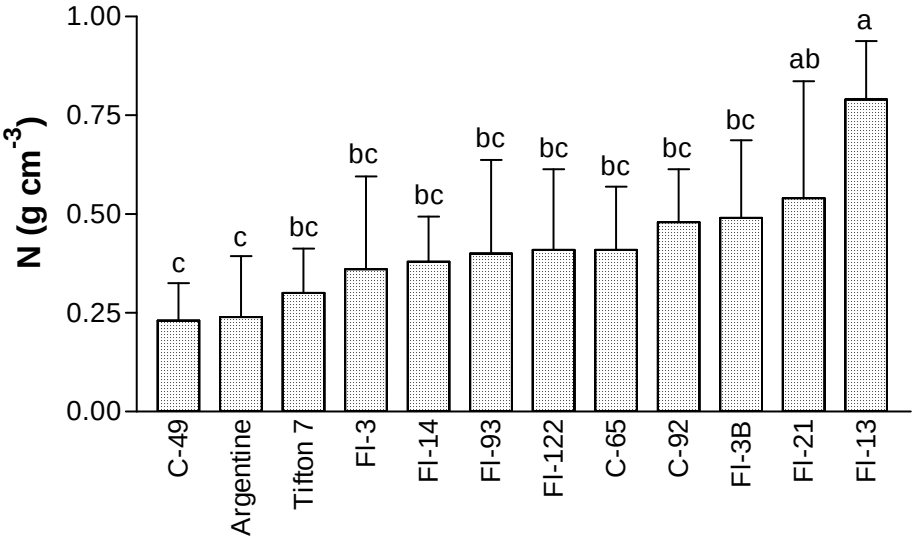

Figure 3-6. Spring 2007 rhizome+root nitrogen accumulation of 12 bahiagrass clones grown at Gainesville, FL. Error bars represent the standard deviations. Clone means followed by the same letter are not different.

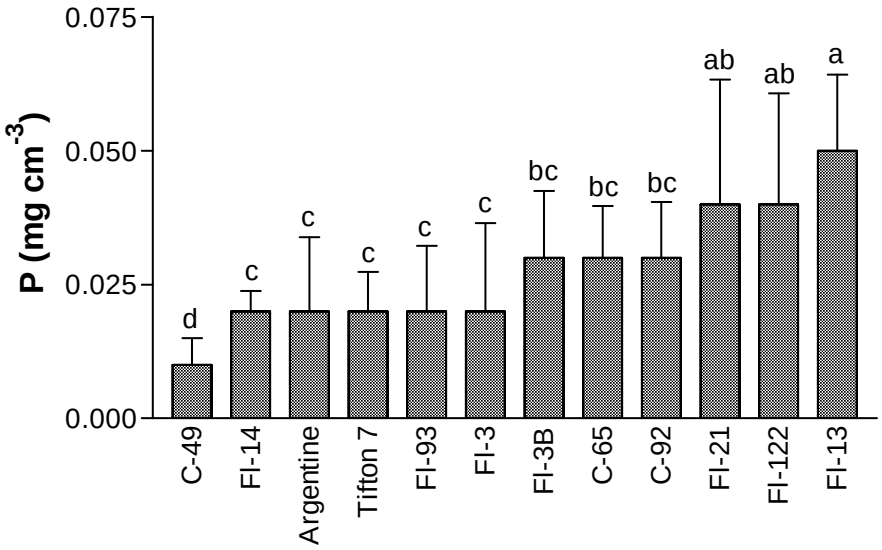

Figure 3-7. Spring 2007 rhizome+root phosphorus accumulation of 4 representative bahiagrass clones grown at Gainesville, FL. Error bars represent the standard deviations. Clone means followed by the same letter are not different.

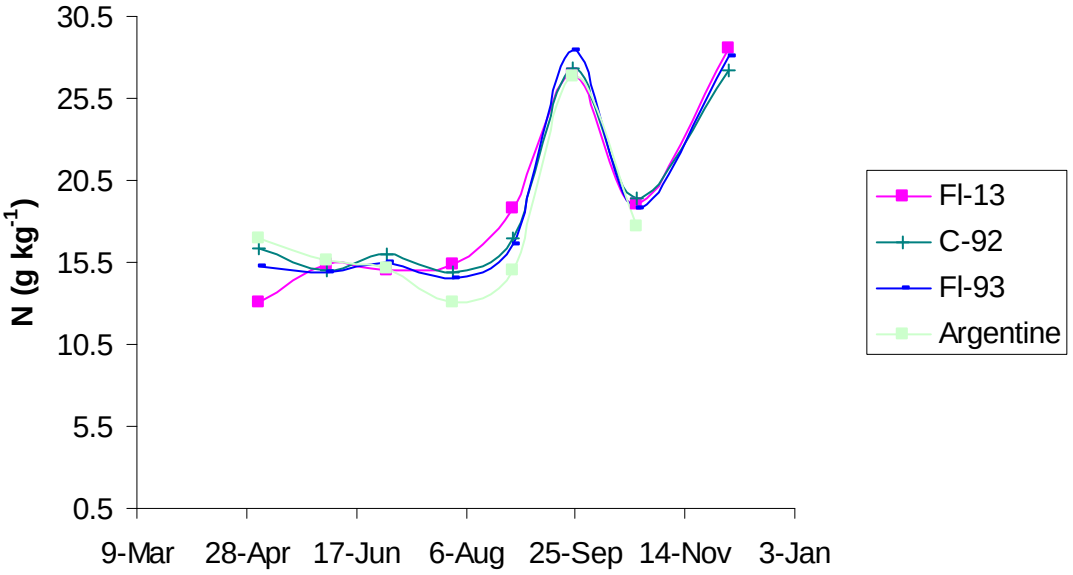

Figure 3-8. Nitrogen concentration of 4 representative bahiagrass clones during the 2007 growing season.

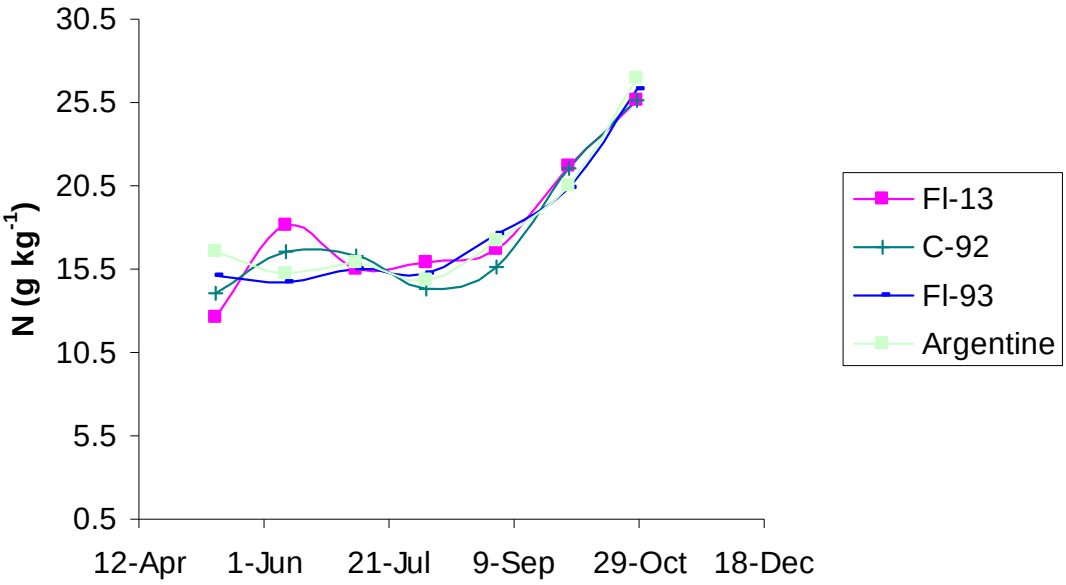

Figure 3-9. Nitrogen concentration of 4 bahiagrass clones during the 2008 growing season.

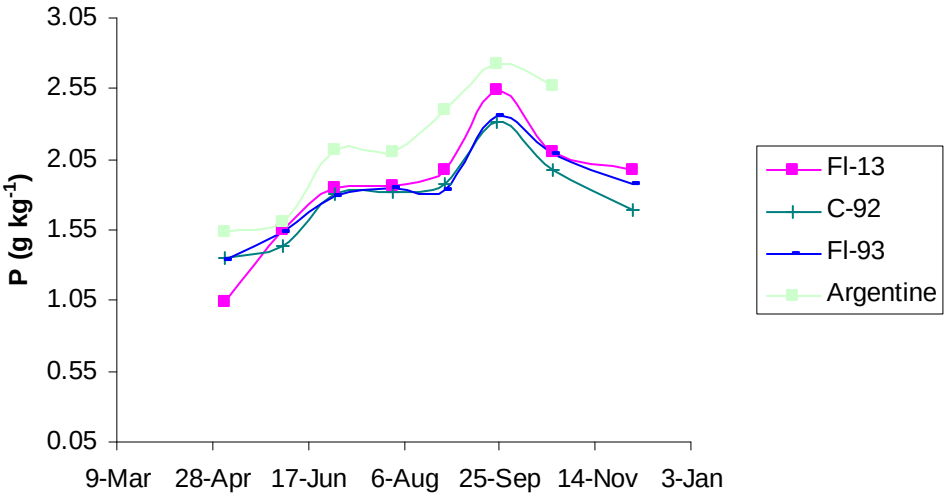

Figure 3-10. Phosphorus concentration of 4 bahiagrass clones during the 2007 growing season.

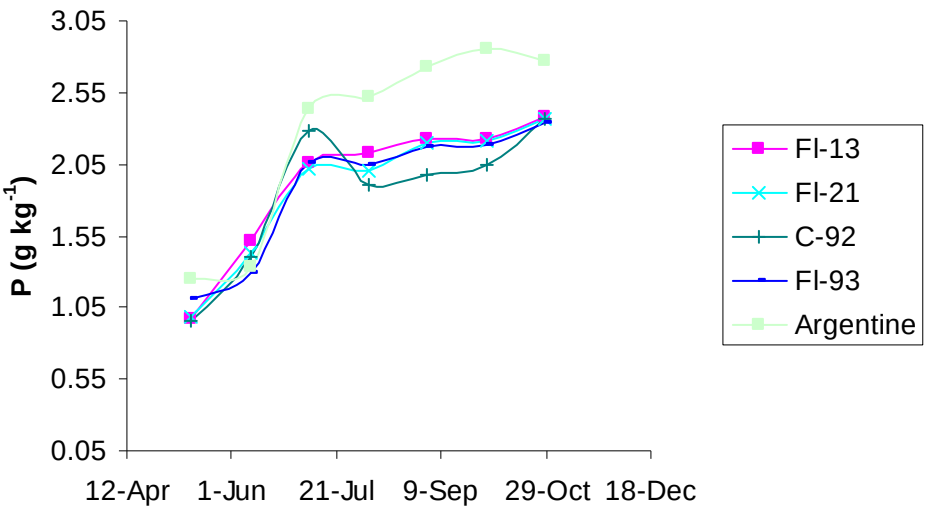

Figure 3-11. Phosphorus concentration of 4 bahiagrass clones during the 2008 growing season.

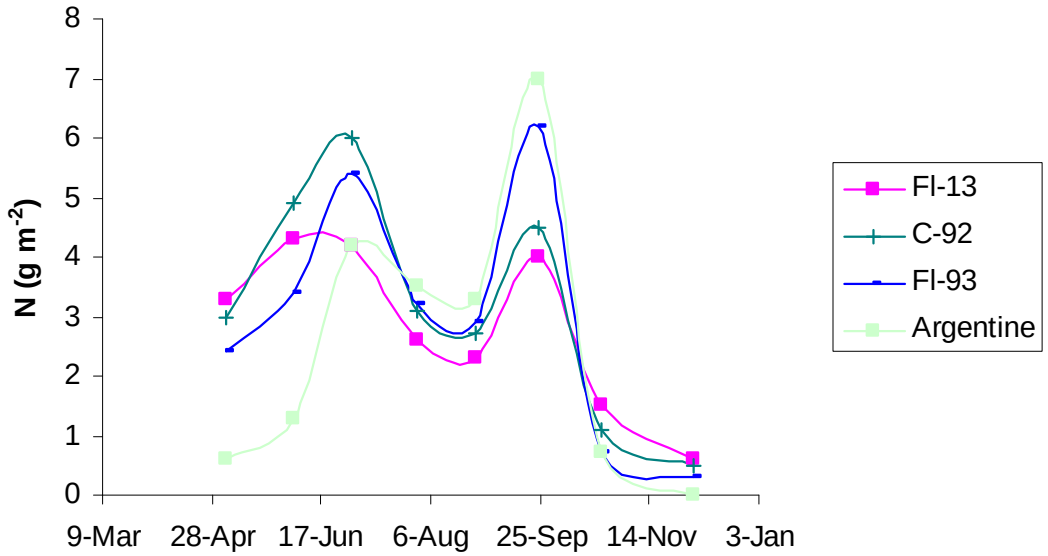

Figure 3-12. Nitrogen accumulation of four bahiagrass clones during the 2007 growing season.

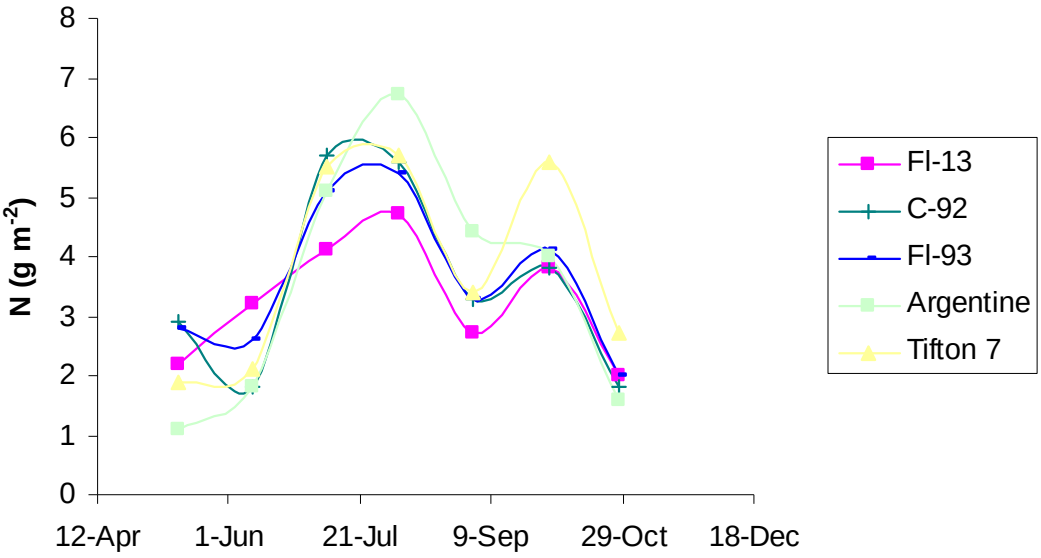

Figure 3-13. Nitrogen accumulation of four bahiagrass clones during the 2008 growing season.

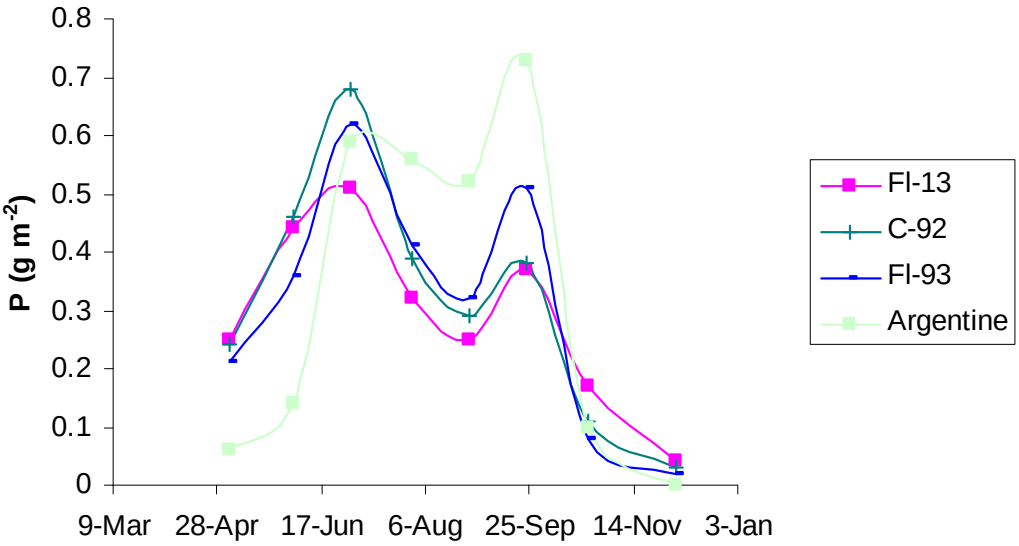

Figure 3-14. Phosphorus accumulation of four bahiagrass clones during the 2007 growing season.

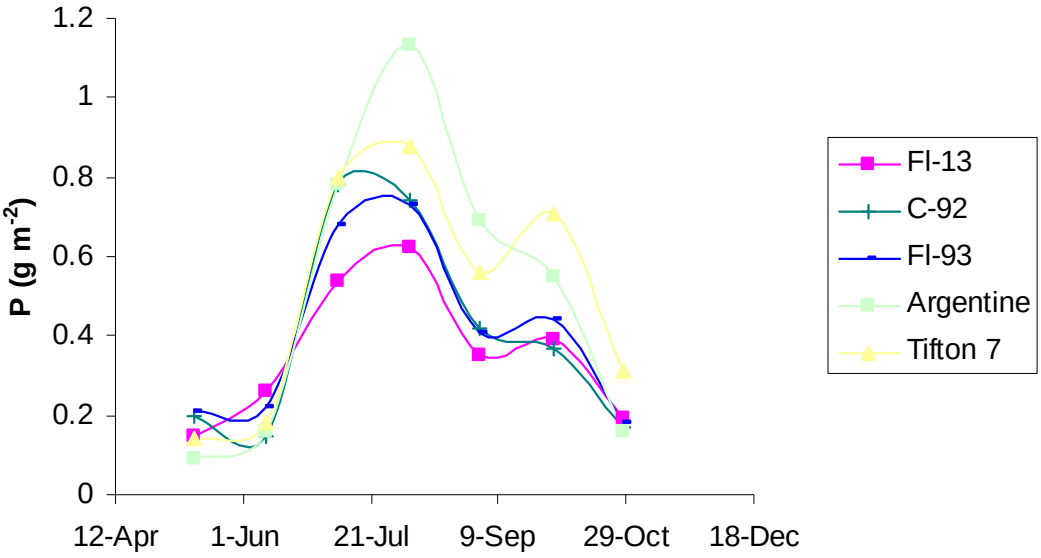

Figure 3-15. Phosphorus accumulation of four bahiagrass clones during the 2008 growing season.

CHAPTER 4
ROOT DEPTH DEVELOPMENT IN APOMICTIC BAHIAGRASS

Introduction

Drought is an occasional hazard in all types of dry-land agriculture. In wet regions, crops are especially vulnerable to sudden dry spells resulting in more severe damage than crops grown in dry regions. Success with dry-land farming depends upon appropriate crop and cultivar selections (Loomis and Connor, 1992). Higher yielding genotypes could be bred more efficiently if attributes that confer yield under water-limited conditions are identified and used as selection criteria. The general principle is that crops yield best when their developmental cycle avoids or tolerates periods of water shortage and they make the best use of the available water supply (Ludlow and Muchow, 1990).

One such plant characteristic that may optimize productivity under water-limited conditions is rapid vertical root development for recovering soil water (Ludlow and Muchow, 1990; Sinclair, 1999). Rapid vertical root development is expected to result in deeper rooting. Studies with subtropical grasses have indicated benefits of deep rooting. Burton et al. (1954) observed marked differences for early root penetration among subtropical forages. Forages with deeper roots were classified as more drought tolerant. In contrast, total root dry mass was not correlated with drought tolerance. In another experiment, Marcum et al. (1995) compared maximum root depth of 25 Zoysiagrass (*Zoysia sp.*) cultivars and found that cultivar rooting depth correlated with the drought tolerance of the cultivars. Genotypic variation for rooting depth was also found within grain crops, such as rice (Shen et al., 2001), sunflower and soybean (Dardanelli et al., 1997). Deep rooting was also considered important for deep nitrogen uptake of vegetable crops, especially in areas where nitrate can be leached out of the soil profile, potentially contaminating ground water (Kristensen and Thorup-Kristensen, 2004).

Bahiagrass, *Paspalum notatum* Flüggé, is a warm-season grass cultivated in southeastern USA as forage and utility turf. It is considered a drought tolerant species because of its deep and dense root system (Burton, 1943). The bahiagrass tetraploid germplasm reproduces asexually by apomixis, which allows the perpetuation of superior hybrids. The genetic variability of a variety of agronomically important traits has been reported for this species (Gates et al., 2004; Acuña et al., 2007; Acuña et al., 2009). However, it is unknown if there is genetic variability for root architecture and performance. The evaluation of the bahiagrass germplasm for rate of root development could indicate genotypes that can reach stored water in deep soil layers more rapidly. A screening technique would be advantageous to evaluate a large number of genotypes in less space and more timely. Since bahiagrass is frequently defoliated mechanically or by livestock the effect of defoliation on the rate of root depth development also needs to be considered.

The aim of this research was to generate a screening technique that can be readily used to detect genotypic variation for rate of root depth development (RRDD) among tetraploid cultivars or breeding lines of bahiagrass, analyze the variability for RRDD among novel tetraploid hybrids, and determine the effect of stress (i.e., defoliation) on RRDD.

Materials and Methods

Plant Material

Thirteen bahiagrass tetraploid clones were used for this research. The cultivar Argentine, an ecotype known as Common and the unreleased clone Tifton 7 (obtained from Dr. G. Burton, USDA-ARS, Tifton, GA) were selected for this study because they were considered well adapted clones in the southeastern USA (Burton, 1992), and because they were classified as highly apomictic, based on embryo sac observations and field progeny tests (Acuña et al., 2007). The other ten clones were novel hybrids generated by crossing sexual induced autotetraploid clones

as female parents and apomictic clones as pollen donors. Seven of these hybrids (FL-3, FL-3B, FL-13, FL-14, FL-21, FL-93 and FL-122) were generated at the University of Florida, and selected for this study because of their extended growing season, cold tolerance, and high apomictic expression (Acuña et al., 2009). The other 3 hybrids (C-49, C-65, and C-92) were generated at Universidad Nacional del Nordeste, Argentina, and selected for their high herbage accumulation (Quarin, personal communication).

Development of a Screening Technique

The rate of root depth development (RRDD), which is the slope of the linear function between time and depth of the deepest root, was monitored by growing Argentine and Tifton 7 plants in clear acrylic tubes (Figure 4-1). Tubes 100-cm length and either 3.5-cm or 10-cm diameter were tested. These two tube sizes were used to evaluate the effect of soil volume and plant competition on RRDD. All tubes were filled with a sandy soil (Thermic, coated Typic Quartzipsamment) collected at Live Oak, Florida, or with commercial potting mix (Jungle Growth, Professional Grower Mix, Piedmont Pacific Inc., Statham, GA). These two soils were used to test the potential effect of organic and inorganic soils on RRDD. The field soil was collected from three successive 30.5-cm thick layers, the bulk density of each layer was determined, and columns were filled with material from these three layers maintaining thickness and density. Seeds from the two genotypes were scarified using concentrated sulfuric acid for 10 min, washed, and sown on the substrate surface of each column. A single plant was grown in 3.5-cm diameter tubes, while 5 plants were grown in the 10-cm diameter tubes. The growing medium was maintained at field capacity by watering every other day. The depth of the deepest visible root was recorded three times per week. Above- and below-ground plant dry mass were determined at the end of each trial. Ten tubes were used for each treatment and these were

considered replicates. Two trials in 2006 (from 16 May to 17 July, and from 7 August to 7 October) were located outdoors in Gainesville, FL.

Effect of Defoliation on the Rate of Root Depth Development

Using the same procedure described above, Argentine and Tifton 7 plants were grown in clear acrylic columns from 7 August to 7 October, 2006. Plants were grown in 3.5-cm or 10-cm diameter tubes filled with inorganic or organic soil. Plants were manually defoliated with scissors at 3 cm from the soil surface weekly or biweekly starting on 8 September. Ten replications were used for each treatment.

Genotypic Variation for Rate of Root Depth Development

Ten novel apomictic hybrids, Argentine, Tifton 7 and Common were grown as described above in 3.5-cm diameter columns filled with sandy soil from 21 May to 5 July 2007. Eight replications of each clone were used for this trial. The depth of the deepest visible root was recorded three times per week. Above- and below-ground plant dry mass were determined at the end of the experiment.

Statistical Analyses

Regression analysis was used to estimate the slope (RRDD) of the linear function between time and depth of the deepest visible root. The RRDD, above and below ground mass data were analyzed using PROC GLM (PC SAS version 9.2, SAS Institute, Cary, NC) as a randomized complete block design. When significant differences were detected for one variable, the Fisher's least significant difference (LSD) was used for comparing two means and the Duncan's Multiple Range Test was used for mean separations when comparing more than two means.

Results

Development of a Screening Technique

A linear relationship between root depth and time was observed when Argentine and Tifton 7 were grown in clear acrylic tubes (Figures 4-2 and 4-3). The increase in root depth proved to be highly linear under all circumstances over the entire observation period. The RRDD values, (i.e., slope) were similar between genotypes (Figures 4-4 and 4-5). There were no interactions between genotypes and the growing medium or tube size. There were no RRDD differences when the experiment was carried out in spring or summer. The RRDD of plants grown in potting mix or soil were similar, indicating that bulk density and other soil attributes (within the range tested) had no measurable effect on RRDD. Therefore, either medium can be used to screen a larger number of bahiagrass genotypes. RRDD were similar when plants were grown in small or large tube diameters indicating that small tubes can be used efficiently to test a large number of genotypes.

Tifton 7 produced more above ground mass (AGM) than Argentine (Figures 4-4 and 4-5), and field results supported these findings (Chapter 3). Tifton 7 also produced more below ground mass (BGM) than Argentine during the spring, but they were not significantly different during the summer. In general, plants produced more biomass when grown in potting mix, which might be the result of its higher nitrogen content, compared with the sandy soil used in this experiment.

Effect of Defoliation on the Rate of Root Depth Development

Weekly defoliation significantly reduced the RRDD of plants grown in small tubes containing soil (Figure 4-6). Argentine's RRDD was equally reduced by both defoliation treatments (weekly and biweekly). However, defoliation did not have an effect on RRDD of plants growing in small tubes with potting mix or big tubes with soil. The lack of response for plants grown in the potting mix might again result from readily available nutrients to the roots.

Since the bigger tubes contained more soil, they too offered a greater amount of nutrients than when plants were grown in the small tubes.

Genotypic Variation for Rate of Root Depth Development

Since there were no appreciable differences among growing medium, tube size or date during the growing season, the novel apomictic hybrids were grown in small tubes filled with soil and were grown at the end of the spring 2008. Hybrid FL-122 had a greater RRDD than the other 12 apomictic hybrids in this study (Figure 4-7) including its male parent, Argentine, or other hybrids sharing this same male parent. Unfortunately, there were not any other hybrids sharing the same female parent (Q-4188). The superiority of FL-122 for RRDD might be related to genes present in Q-4188 or it could also be related to heterosis resulting from this combination of parents. No differences were observed among the other 12 hybrids for RRDD. The low variation among the eight replications included in this experiment indicates the consistency of these results. Hybrid FL-122 also produced more above and below ground dry mass compared with the other hybrids (Figure 4-7).

Discussion

These experiments showed that clear acrylic tubes can be effectively used to screen bahiagrass germplasm for RRDD. Of particular interest was the lack of variation resulting from the use of two contrasting growing media. This indicates that mineral or organic growing media can be used indifferently to screen bahiagrass genotypes for RRDD. The results also indicated that small tubes (3.5-cm diameter) can be used as efficiently as big tubes (10-cm diameter) to screen genotypes for RRDD differences during early growth. These results are important because small tubes are lighter, use less growing medium, and require less space, allowing for the screening of a larger number of genotypes. The results also indicate that the screening for RRDD can be carried out at the beginning or middle of the bahiagrass growing season without a

significant effect on the results. Kramer and Boyer (1995) stated that a RRDD of 10 to 12 mm per day was considered typical among grasses. In our experiment, the average RRDD was 18 mm per day. However, it is important to recognize that these values are expected to be affected by environmental variables, such as temperature, soil available water, and nutrient concentration in the growing medium.

Although bahiagrass is able to tolerate severe and frequent grazing or mowing (Gates et al., 2004), our results indicated that defoliation decreases the RRDD. It seems that defoliation limits the plant's ability to mobilize carbohydrates for root depth development. These results indicate that frequent defoliation would compromise bahiagrass ability for water and nutrient uptake, and probably establishment.

Genotypic variability for RRDD was found among a group of novel apomictic bahiagrass hybrids with different genetic backgrounds. Although most root studies have been based on measurements of root mass and maximum root depth instead of root depth development, some indirect comparisons can be made with studies involving maximum root depth. Genotypic variation for maximum root depth was reported for other grasses, such as zoysiagrass (Marcum et al., 1995), fescue and perennial ryegrass (Bonos et al., 2004). Subsequent studies have shown that selection for maximum rooting depth can result in drought tolerant plants, and that selection based on root characteristics can be more effective than selection based on field screening (Karcher et al., 2008). In our experiment, hybrid FL-122 not only showed greater RRDD but also greater above and below ground mass. This might be the result of its ability for exploring deeper soil layers by extending its roots faster. However, further experiments are needed to examine this hypothesis. These characteristics observed with line FL-122 may result in faster establishment,

which is highly desirable in perennial grasses. The genetic variability for RRDD observed by this experiment indicates the potential use of this trait for bahiagrass genetic improvement.

Figure 4-1. Bahiagrass plants growing in clear acrylic tubes. A) 3.5-cm diameter tubes filled with potting mix. B) 10-cm diameter tubes filled with sandy soil.

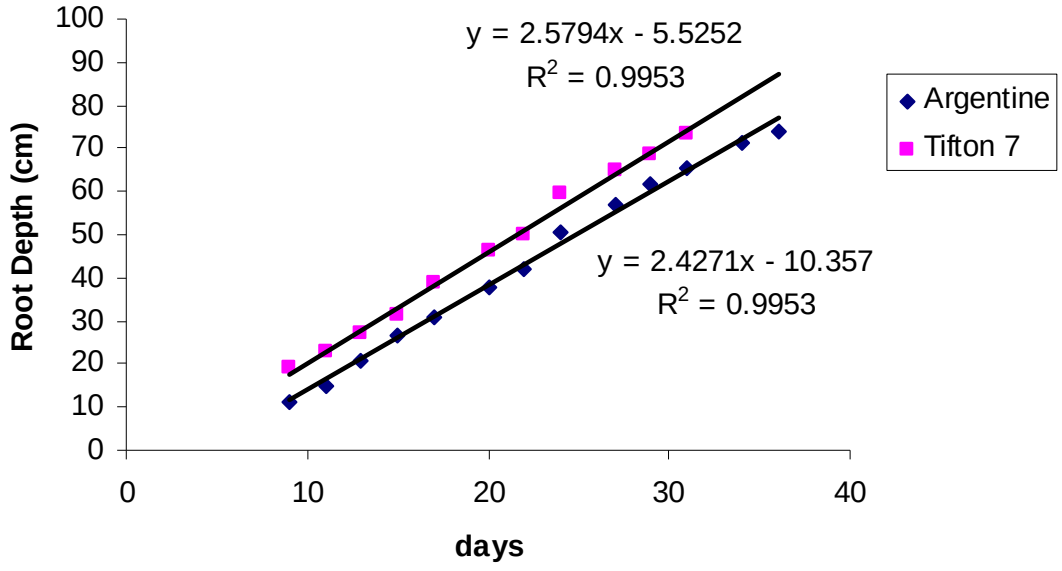

Figure 4-2. Rate of root depth development for Argentine and Tifton 7 growing in clear acrylic tubes (10-cm diameter) filled with potting mix.

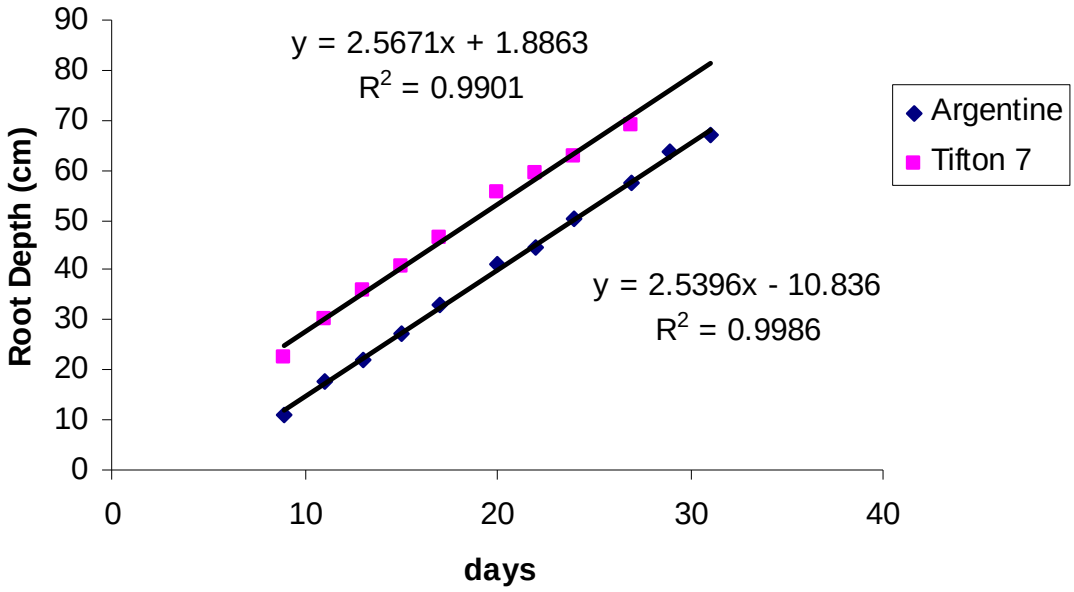

Figure 4-3. Rate of root depth development for Argentine and Tifton 7 growing in clear acrylic tubes (3.5-cm diameter) filled with soil.

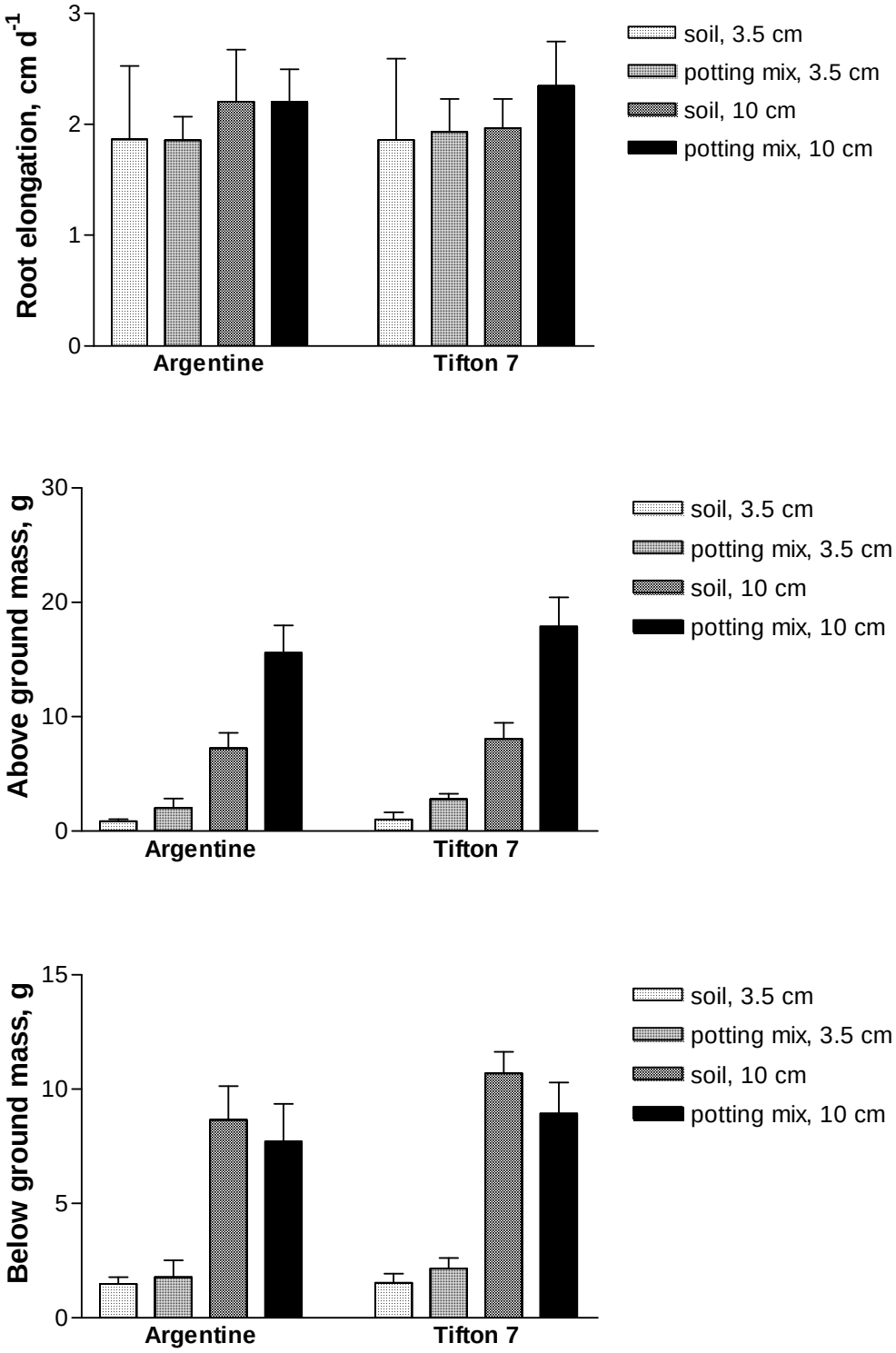

Figure 4-4. Rate of root depth development, and above and below ground mass for Argentine and Tifton 7 grown in clear acrylic tubes during spring 2006.

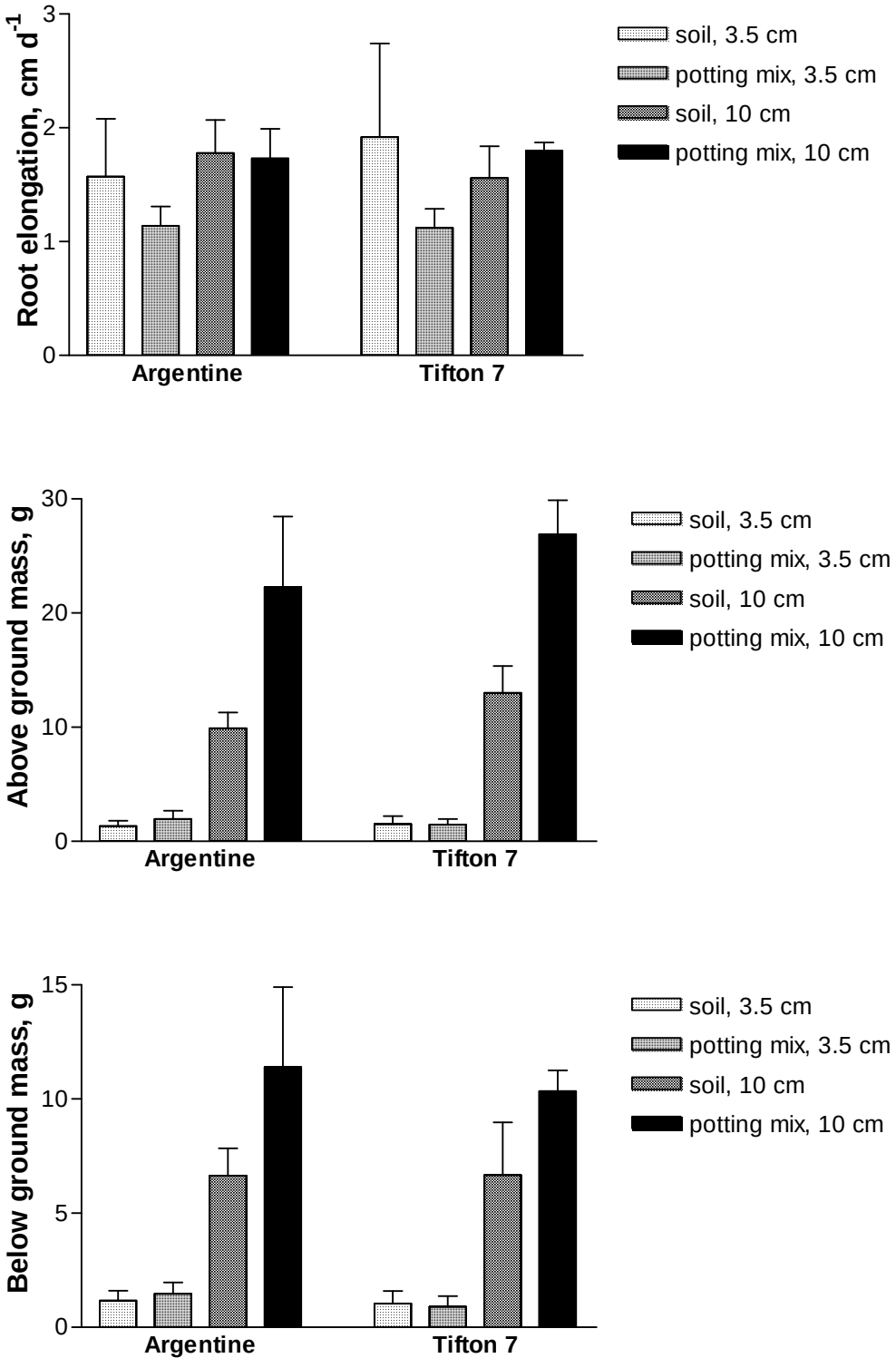

Figure 4-5. Rate of root depth development , and above and below ground mass for Argentine and Tifton 7 grown in clear acrylic tubes during summer 2006.

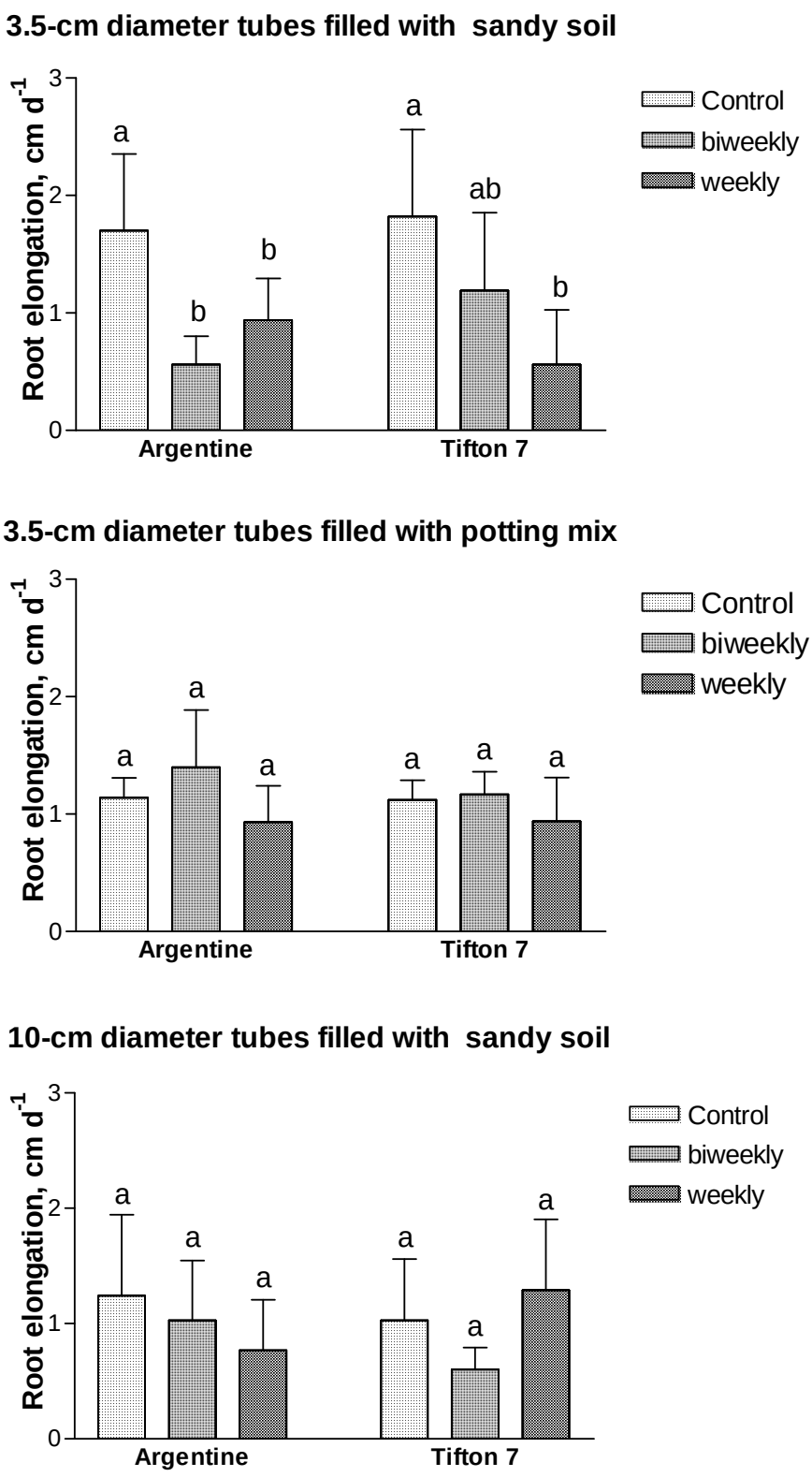

Figure 4-6. Effect of defoliation on root depth development for Argentine and Tifton 7.

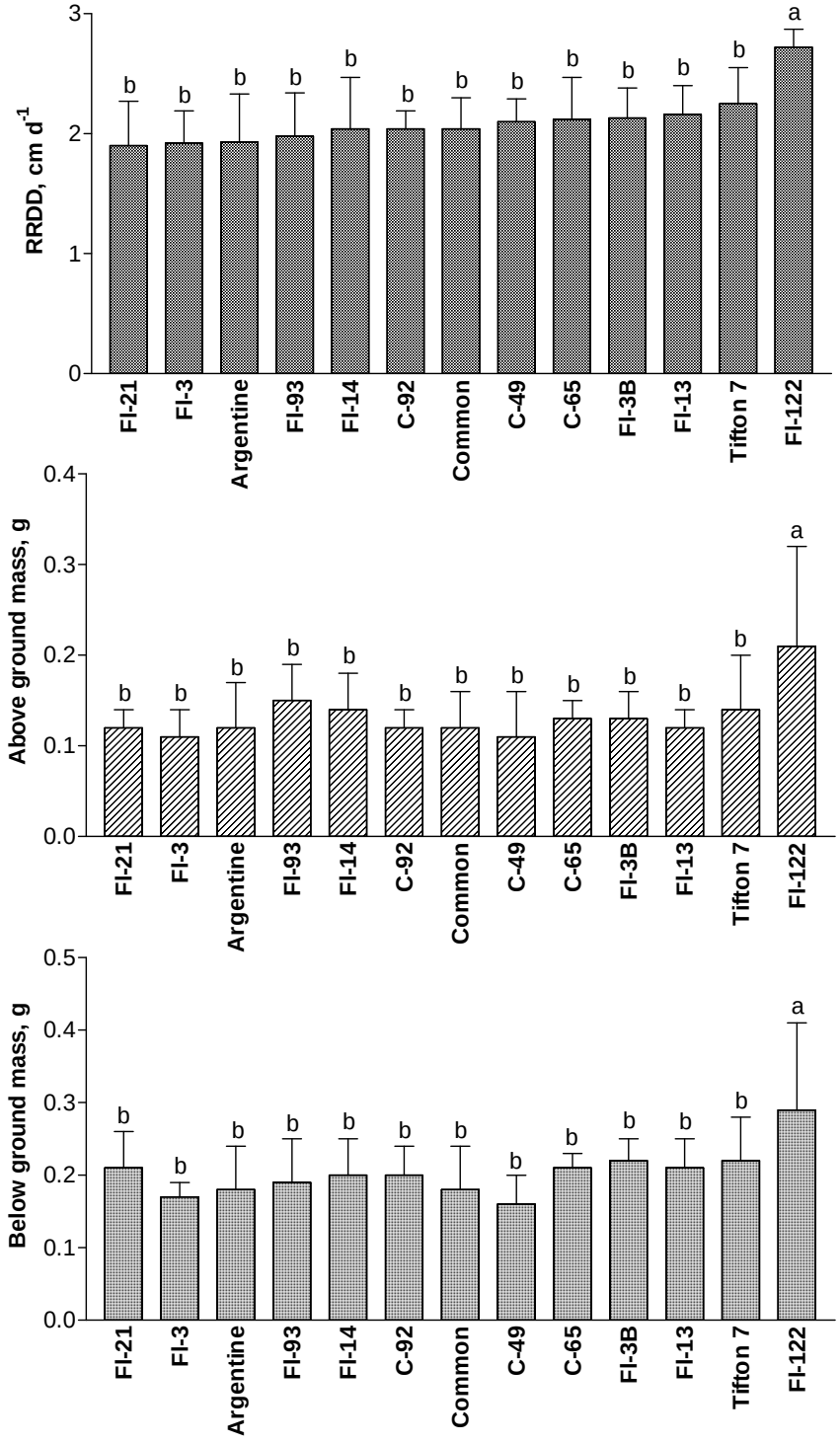

Figure 4-7. Rate of root depth development (RRDD), and above and below ground mass for 13 novel apomictic bahiagrass hybrids growing in clear plastic tubes during spring 2007.

CHAPTER 5
IMPORTANCE OF RAPID VERTICAL ROOT DEVELOPMENT FOR RECOVERING OF SOIL NITROGEN IN TETRAPLOID BAHIAGRASS

Introduction

Most of the physiological processes contributing to crop yield are quantitatively dependent on plant nitrogen (N) availability (Sinclair and Valdez, 2002). Therefore, the addition of organic or inorganic sources of N is essential for crop production in most environments. The highly weathered soils of the U.S. Southern Coastal Plain require relatively high N inputs for adequate crop production. Therefore, concerns exist that the region's subtropical environment (warm temperatures and heavy rains) may contribute to significant N contamination of surface and groundwaters (Hubbard et al., 2004). Several studies have shown that deep-rooted crops can reduce the potential for N leaching losses by capturing N from deep soil or subsoil horizons (Peterson et al., 1979; Huang et al., 1996; Thorup-Kristensen, 2001; Kristensen and Thorup-Kristensen, 2004). Rapid vertical root growth was theoretically proposed as an important trait for developing deep root systems and for recovering stored water (Ludlow and Muchow, 1990; Sinclair, 1999). Moreover, N catch crops showing more rapid rates of vertical root development were able to deplete more N from deep soil layers than those showing slower rates (Thorup-Kristensen, 2001). The crucifer catch crops were faster in developing deep rooting and depleting nitrogen from the subsoil than grass crops. Only small differences for rates of root penetration were found within botanical groups.

Bahiagrass, *Paspalum notatum* Flüggé, is a warm season perennial grass used as a forage and turf in the southeastern United States. It is also utilized in crop rotations, particularly with row crops and various vegetable crops (Blount and Acuña, 2009). Higher yields of row crops, great reductions in insect, nematode, and disease problems associated with peanut, cotton and soybean have been linked to short-term crop rotation with bahiagrass. In addition, bahiagrass is a

good candidate for mitigating excess soil N since it has a large, deep fibrous root system (Burton, 1943). Different rates of root depth development have been reported for novel apomictic bahiagrass hybrids (Chapter 4). The objective of this research was to determine if velocity differences in vertical root development are important for the uptake of N present in deep soil layers.

Materials and Methods

Plant Material

Four bahiagrass clones were used for this experiment: Argentine (PI 148996), FL-3, FL-122 and C-65. Clones FL-3 and FL-122 were obtained by hybridizing sexual and apomictic tetraploid clones as part of a breeding program conducted at the University of Florida, and selected based on superior cool-season regrowth, and freeze tolerance when grown as individual plants (Acuña et al., 2009). Clone C-65 was also generated by crossing sexual and apomictic clones, but it was selected based on superior biomass yields when grown as individual plant (Quarin, personal communication). These four clones were selected for this experiment because variation for rate of root depth development was previously observed among them (Chapter 4). The four clones were classified as highly apomictic (Acuña et al., 2007; Acuña et al., 2009), therefore, plant variation among replications was expected to be restricted to the environment.

Root Development and Harvest Measurements

The rate of root depth development (RRDD) was monitored by growing the bahiagrass clones in clear acrylic tubes. Tubes (3.5-cm diameter and 100-cm length) were filled with a sandy soil (Thermic, coated Typic Quartzipsamments) collected at Live Oak, Florida. The field soil was collected from three consecutive 30.5-cm thick layers, the bulk density of each layer was determined, and columns were filled with material from these three layers maintaining thickness and density. Seeds were scarified using concentrated sulfuric acid for 10 min, rinsed

with tap water, and sown at the top of each column. The growing medium was maintained at field capacity by watering every other day. No fertilizer was added to the growing media. Clear acrylic tubes where kept in vertical position inside a PVC pipe. The tops of the acrylic tubes were painted white to exclude light. The depth of the deepest observable root was recorded two times per week. At the end of each trial, plants and growing media were placed on a 2-mm mesh and plants were washed with a stream of water. Roots and shoots (rhizomes+leaves) were separated by cutting the roots at the base of the rhizomes, dried at 60°C for 48 h, and dry weights were determined. Eight tubes (replications) were used for each clone and the study was conducted twice in 2008 (from 2 May to 7 June, and from 31 July to 16 September), outdoors in Gainesville, Florida.

^{15}N Uptake and Partitioning between Roots and Shoots

To determine if the four bahiagrass clones differ in their ability to uptake N with soil depth, ^{15}N label was applied to four replicates. To demonstrate, when at least one clone within each replicate was observed having roots deeper than 60 cm, a solution containing ^{15}N was prepared by dissolving 1444 mg of 10% enriched ^{15}N as KNO_3 in 200 ml of distilled water. A 2-mm hole was drilled through the tube wall where the deepest root of each plant was observed, and 5 ml of labeled solution was injected into the soil.

The label was also imposed to the remaining four replicates by injecting these tubes at the depth where the deepest root of FL-122 (clone having the fastest vertical root development) was observed for that replicate. These data helped to determine if differences in RRDD resulted in differences in N uptake. Three days after the injection, plants were washed and roots and shoots were separated. Samples were dried at 60 °C for 48 h and ground with a cyclone mill to 1-mm particle size. Determinations of ^{15}N atom abundance were conducted at the Soil and Water

Science Department Stable Isotope Mass Spectrometry Laboratory at the University of Florida. The atom % 15N/14N ratios were determined using a Costech model 4010 elemental analyzer (Costech Analytical Industries, Valencia, Calif.) coupled to a Finnigan MAT DeltaPlusXL mass spectrometer (continuous flow isotope ratio mass spectrometry; Thermo Finnigan, San Jose, Calif.) via a Finnigan Conflo III interface. Results were standardized using Ammonium Sulfate at 0.5, 1.0, 1.5, 2.0 and 5.0 atom percent 15N.

Statistical Analyses

Regression analysis was used to estimate the slope (RRDD) of the linear function between time and depth of the deepest visible root. Data were analyzed using PROC GLM (SAS version 9.2, SAS Institute, Cary NC) as a randomized complete block design. When significant differences were detected, the Duncan's Multiple Range Test was used for mean separations.

Results

Root Depth Development

As reported for other bahiagrass clones (Chapter 4), root depth increased linearly with time (Figure 5-1). The rates of root depth development (RRDD), which is the slope of the root depth development plots, were significantly different among clones. Clone FL-122 had higher RRDD than the other 3 apomictic clones (Figure 5-2). Although there was a significant season (spring vs. summer) effect for RRDD, the apomictic clones were ranked in a similar order in both seasons (Figure 5-2). Clone C-65 showed greater RRDD than FL-3 and Argentine during spring. This superiority of FL-122 for early RRDD confirmed the results reported previously (Chapter 4).

Plant Mass and Tiller Number

There were no significant differences among clones for the number of tillers produced by the end of the experiment in either trial. Significant differences were observed among the 4

bahiagrass clones for above-ground (AGM) and below-ground (BGM) mass during spring 2008 and only for BGM during summer 2008 (Figure 5-3). While FL-65 produced more AGM than FL-3 and Argentine during the spring, it did not differ from FL-122. C-65 and FL-122 produced more BGM during the spring than FL-3, but they did not produce significantly more than Argentine. During the summer, FL-122 produced significantly more BGM than the other 3 clones.

^{15}N Uptake and Partitioning between Roots and Shoots

Atom % ^{15}N abundance in roots and shoots was not significantly different among clones when ^{15}N-enriched KNO_3 was applied where the deepest visible root of each plant was observed (Figure 5-4). Results were similar in spring and summer (Figure 5-4). These results indicate that roots from these four clones have the same ability for N uptake once they reach a particular soil depth. The atom % ^{15}N abundance was significantly greater in shoots than in roots indicating that the N absorbed after the injection of ^{15}N-enriched KNO_3 was preferentially mobilized to shoots.

Atom % ^{15}N abundance in roots and shoots was significantly different among clones when ^{15}N-enriched KNO_3 was applied to all clones at the point of the single deepest visible root of FL-122 (Figure 5-5). Roots and shoots from clone FL-122 had higher atom % ^{15}N abundance three days after the injection than the other three test clones. Clone C-65 had higher ^{15}N abundance than FL-3 and Argentine, but only for the spring trial. Shoots of FL-122 had higher ^{15}N abundance than roots of the same clone. However, there were no differences for ^{15}N abundance between roots and shoots of C-65, FL-3 or Argentine. Correlations between ^{15}N abundance in roots and RRDD were high for the spring ($r = 0.91$) and summer ($r = 0.80$). The correlations between ^{15}N abundance in shoots and RRDD were also high for spring ($r = 0.90$) and summer ($r = 0.80$).

Discussion

Genetic variability for RRDD exists within the bahiagrass tetraploid germplasm. Our results confirm the findings of our previous experiments (chapter 4) showing that clone FL-122 had greater RRDD as compared to other apomictic bahiagrass clones. It indicates that genetic improvements for rapid root penetration by tetraploid bahiagrass are possible. Lehman and Englke (1991) reported relatively high narrow-sense heritability for root extension of creeping bentagrass (*Agrostis palustris* Huds.). However, further studies are needed to evaluate the possibility of using this characteristic in a bahiagrass breeding program. The presence of apomixis in tetraploid bahiagrass cytotypes (Gates et al., 2004) would make feasible the perpetuation of new hybrids showing this characteristic.

No genotypic differences were observed for ^{15}N abundance in shoots or roots when ^{15}N was injected at rooting depth (Figure 5-4). These results indicate that the rate of nitrogen uptake of actively growing roots was not different among genotypes. These results also indicate that once N is taken up by bahiagrass plants, it is mobilized to shoots at a constant rate. Since the N uptake capacity of roots is constant, the amount of N that a bahiagrass clone can accumulate at establishment mainly depends on soil exploration by actively growing roots.

When ^{15}N was injected at the rooting depth, higher ^{15}N abundance was observed in shoots of bahiagrass plants compared to roots. This result indicates that nitrogen is preferentially mobilized to shoots after it is taken up by bahiagrass plants. Shoots are the predominant site of nitrate reduction in grasses (Scheurwater et al., 2002). The observed rapid accumulation of ^{15}N in bahiagrass shoots could be the result of absorbed nitrate being preferentially translocated to shoots for reduction and incorporation in organic forms. This new hypothesis needs to be tested by analyzing xylem sap after nitrate absorption, or by measuring the activity of nitrogen reductase in roots and shoots.

Our results showed that rapid root penetration was determinant for rapid access and uptake of N present in deep soil layers (Figure 5-5). Bahiagrass is typically characterized as having slow establishment due, in part, to weak seedlings that may be outcompeted by weeds (Gates et al, 2004). Early rapid root penetration would result in a faster establishment due to rapid access to soil N and probably soil water. Utilization of bahiagrass cultivars with rapid root penetration in crop rotations would lead to better soil N mitigation by reducing the chance for N leaching. Further research is needed to determine the interaction of this trait with soil water and nutrient stresses.

The ^{15}N injection technique used for this experiment can be utilized with other species to discriminate between N uptake capacity of roots and consequences of deeper rooting. In this experiment, the technique was successfully used to determine that the N uptake capacity of several bahiagrass clones did not differ, and that higher RRDD was critical for rapid access to N in deep soil layers.

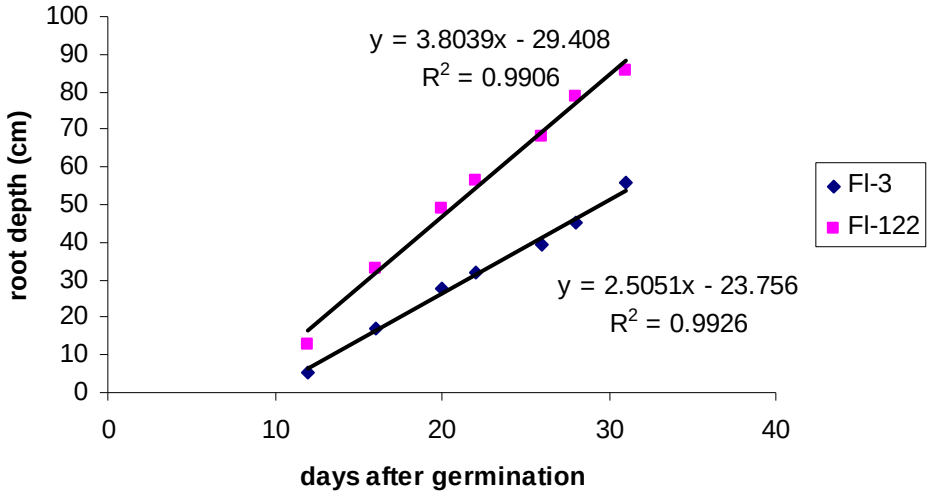

Figure 5-1. Root depth development for FL-3 and FL-122 bahiagrass growing in clear acrylic tubes filled with sandy soil.

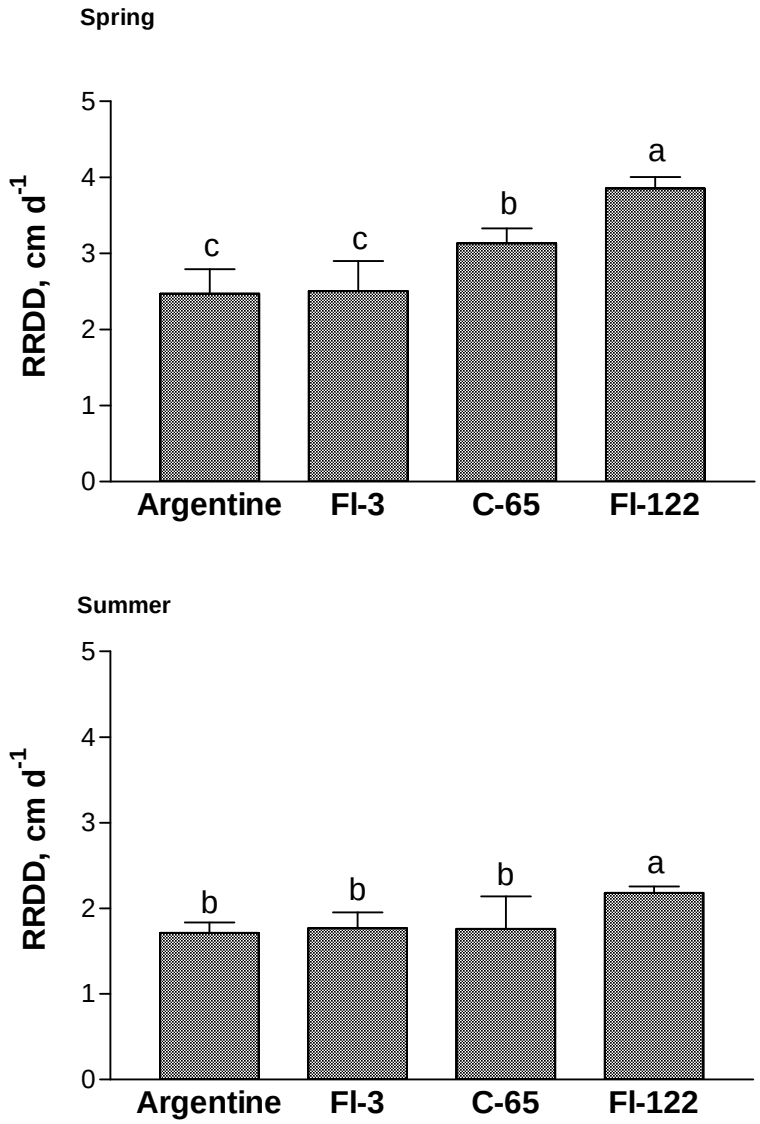

Figure 5-2. Rate of root depth development for Argentine, FL-3, C-65 and FL-122 bahiagrass grown in clear acrylic tubes filled with soil during the spring and summer 2008. Different letters indicate significant differences between means. Error bars represent standard deviations.

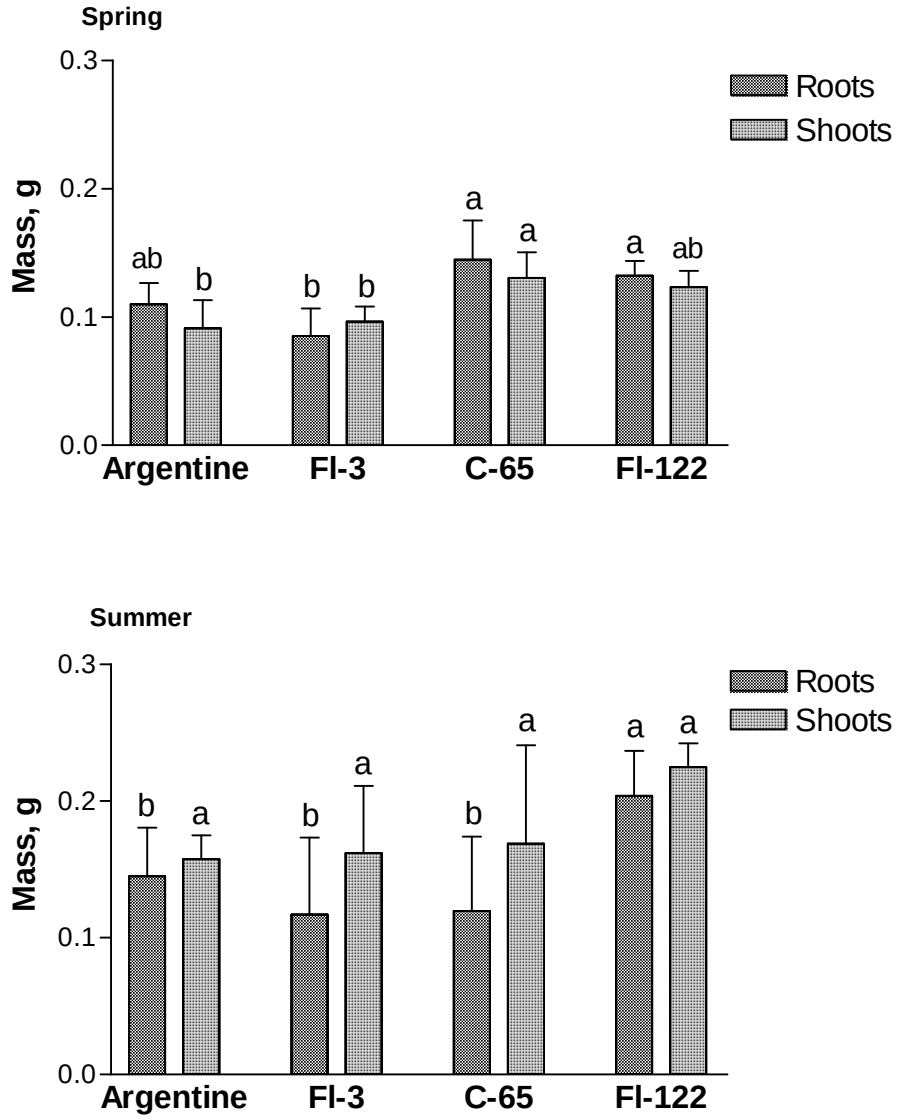

Figure 5-3. Root and shoot mass for Argentine, FL-3, C-65 and FL-122 bahiagrass grown in clear acrylic tubes filled with soil during the spring and summer 2008. Different letters represent significant differences among clones. The error bars represent standard deviation.

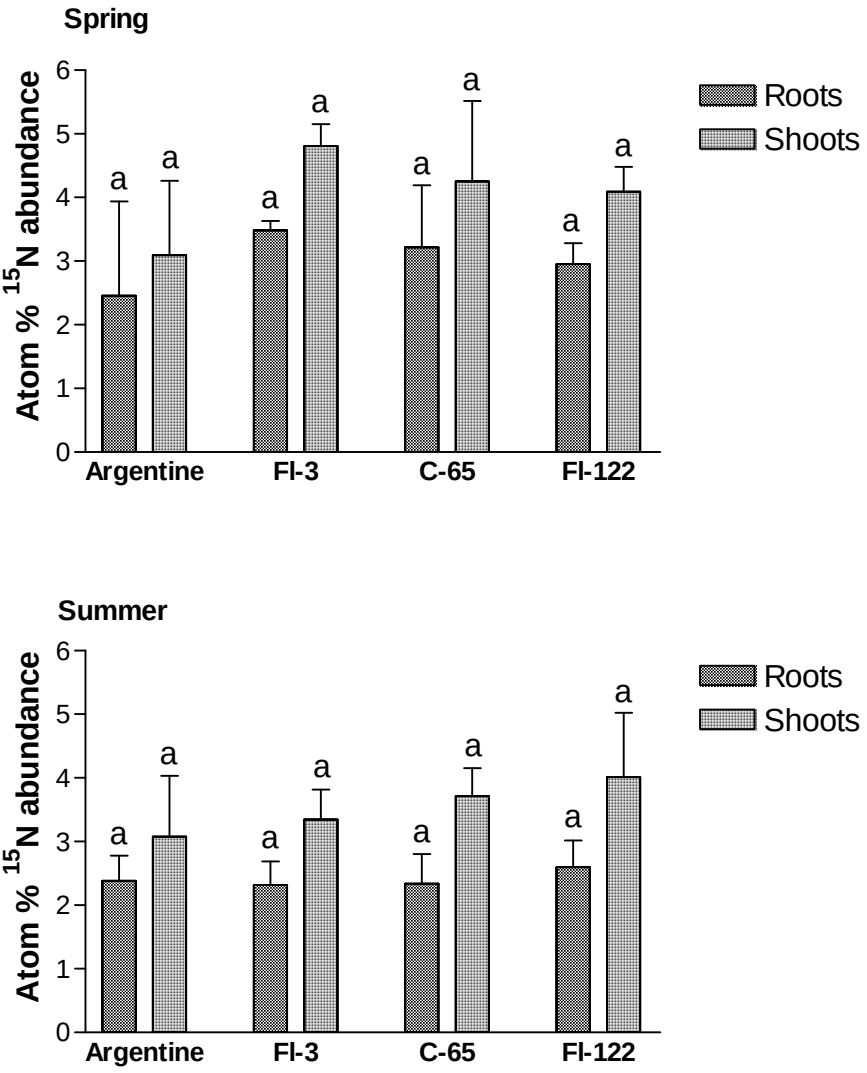

Figure 5-4. Atom % ^{15}N abundance in roots and shoots of Argentine, FL-3, C-65 and FL-122 bahiagrass grown in acrylic tubes during the spring and summer 2008. ^{15}N-enriched KNO_3 was injected where the deepest root of each clone was observed. Different letters represent significant differences among clones. The error bars represent standard deviations.

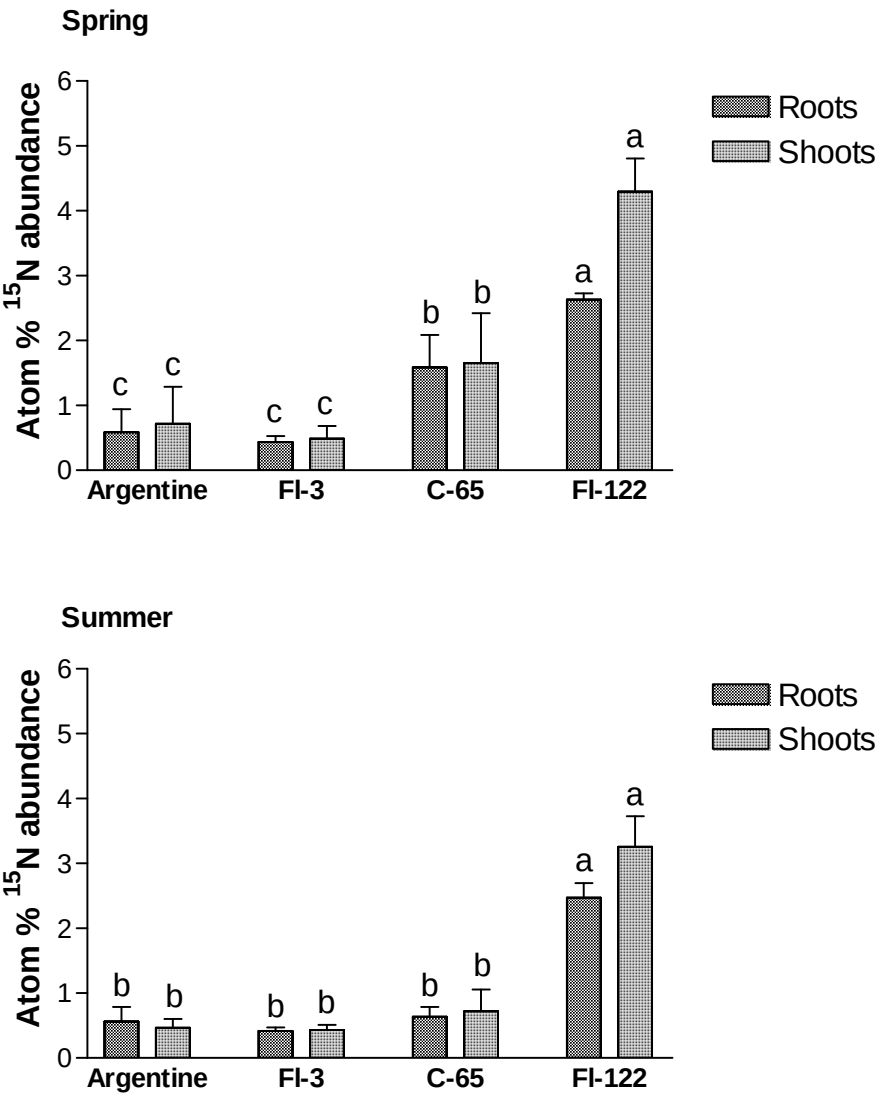

Figure 5-5. Atom % ^{15}N abundance in roots and shoots of Argentine, FL-3, C-65 and FL-122 bahiagrass grown in acrylic tubes during the spring and summer 2008. ^{15}N-enriched KNO_3 was injected where the deepest root of FL-122 was observed. Different letters represent significant differences among clones. The error bars represent standard deviations.

CHAPTER 6
IMPORTANCE OF ROOT MASS AND ROOT LENGTH DENSITY ON FORAGE PRODUCTION OF NOVEL APOMICTIC BAHIAGRASS HYBRIDS

Introduction

Bahiagrass, *Paspalum notatum* Flüggé, is a warm-season, perennial grass extensively cultivated as forage in Florida and the southern Coastal plain region of USA. Recent breeding efforts have resulted in high yielding experimental lines and cultivars of this species (Blount and Acuña, 2009). The identification of traits responsible for the observed differences in forage yields could be utilized to enhance the genetic improvement of bahiagrass and other subtropical grasses.

Although rainfall is relatively high in the region where bahiagrass is cultivated, water is considered one of the main factors limiting its growth (Gates et al., 2004). Regular water shortages, high vapor pressure gradients during the growing season, and low water holding capacity of light textured soils often result in growth being limited by water availability. Availability of essential minerals in the soil also limits bahiagrass forage production. This crop is mainly cultivated in soils with inherent low fertility and low cation and anion exchange capacity.

The study of root mass and root length density (RLD) in superficial soil layers might indicate the capacity of a genotype for uptake of nutrients with low mobility in the soil, such as phosphorus. Sinclair and Valdez (2002) indicated that an extensive root system is especially advantageous for plant production in phosphorus-limited environments. The study of root mass and RLD in deep soil layers is expected to indicate the ability of a genotype for water and nitrogen uptake under limited conditions. Plant species with deep roots are expected to yield more in water-limited environments due to their capacity to recover stored soil water (Ludlow and Muchow, 1990). Selection for higher deep-root to shoot mass ratio in tall fescue (Bonos et al., 2004) resulted in genotypes with higher drought tolerance (Karcher et al., 2008). Deep

rooting has been also been associated with the capacity of certain crops to deplete nitrogen (N) from N-enriched subsoils (Thorup-Kristensen, 2001). In this study, crucifer crops removed more nitrogen from the subsoil than grass crops because they developed a deeper root system.

There are a limited number of reports about root characteristics of forage grasses that can be grown in subtropical regions. Burton (1943) reported marked differences among warm-season grass species during the first year of root production. Variation among warm-season grasses was also reported for root penetration, root yields, and root activity (Burton et al., 1954). Intraspecific variability for root characteristics was reported for Zoysiagrass (Marcum et al., 1995). Genetic variability for maximum root depth, root mass and root number at different soil depths was reported for this species.

Bahiagrass is a diverse species that contains races with different ploidy levels and linked reproductive characteristics (Acuña et al., 2007). Asexual reproduction through seeds (apomixis) is characteristic of the tetraploid germplasm, and it can be manipulated to fix genotypes with superior agronomic characteristics. Several hundred bahiagrass hybrids were recently generated by crossing induced sexual and apomictic tetraploid clones (Acuña et al, 2009). Several of these progeny were selected, based on their high apomixis expression, improved vigor, and freeze resistance when grown as individual plants. Since they were classified as highly apomictic, it should be possible to establish pure-stand plots of each hybrid by seed propagation. However, it is unknown if selection based on phenotypic characteristics of individual plants will result in higher forage production when these novel hybrids are grown in swards. If genetic variability exists for forage production among novel tetraploid hybrids and tetraploid ecotypes, variability for root mass and RLD might also be present among them. Moreover, variation in root mass and RLD among tetraploid clones might explain variation in forage production.

Our objectives were to determine the genetic variability for forage and root production among apomictic bahiagrass clones grown under different fertilization rates, and to evaluate the relationship between root mass and RLD, and forage production.

Materials and Methods

Plant Material

Thirteen tetraploid apomictic bahiagrass clones were selected for this study, based on expected variability in forage production. Two of these clones, Argentine (PI 148996) and Common, are natural ecotypes introduced from South America. Argentine is a productive tetraploid clone commonly grown in Florida. The other eleven clones were novel apomictic hybrids generated by crossing sexual and apomictic tetraploid clones. One of the better known clones, Tifton 7, was generated by Dr. Glenn Burton (Tifton, Georgia). Selection was based on superior forage yields, based on field plot trials. Three others, C-49, C-65 and C-92, were generated by Camilo Quarin (Corrientes, Argentina), and selected, based on superior individual plant forage yields. The other seven clones (FL-3, FL-3B, FL-13, FL-14, FL-21, FL-93 and FL-122) were developed by University of Florida forage breeders, and selected, based on superior vigor, cool-season regrowth, and cold tolerance of spaced plants. Since these 13 clones are classified as highly apomictic, variation among replications was primarily restricted to environmental variation.

Experimental Design and Plot Management

Seeds were scarified using concentrated sulfuric acid for ten minutes, and sown on 3 March, 2007, in a greenhouse, using plastic flats containing a sterile germination mix. After two weeks, seedlings were transplanted to seedling flats containing multiple cells. Seedlings were transplanted into a field located at the North Florida Research and Education Center, Live Oak, FL, on 9 May. Eight rows were planted, containing 13 (1.2 m x 1.2 m) pure-stand plots separated

by a 1 m alley. The clones were randomized within each row. Pure-stand plots were established by planting 36 plants (20-cm apart) of each clone in each plot. Plots were irrigated with approximately 25 mm of water immediately after planting. Weeds were removed manually on 15 June and plots were fertilized with 60 or 20 kg N ha^{-1} (34-0-0) and 20 kg P ha^{-1} (0-0-60). The fertilizer treatment was randomly assigned to each of four blocks containing 2 rows. The experiment was a 2 (fertilizer rates) by 13 (clones) factorial in a completely randomized split-plot design, where fertilizer rate was the whole plot and clones the subplot. A 70 cm wide strip was cut across each plot on 30 July using a sickle bar mower, leaving a 5 cm stubble height. The harvested material was dried at 60 °C for 48 h and weighed. After harvest, plots were fertilized with 120 or 40 kg N ha^{-1} and 40 kg P ha^{-1}. Plots were harvested again on 13 November 2007. Plots were harvested on 15 May for the first time in 2008, and every four weeks. Plots were harvested seven times during 2008. At the first and fourth harvest date for 2008, all plots were fertilized with 60 kg N ha^{-1} (16-4-8). At the second, third, fifth and sixth harvest dates only plots originally assigned for the high fertilization treatment were fertilized with 60 kg N ha^{-1}. The resulting rates of fertilization were 180 or 60 kg N ha^{-1} $year^{-1}$ in 2007, and 360 or 120 kg N ha^{-1} $year^{-1}$ in 2008.

Collection and Analyses of Root Samples

Between 19 July and 21 July 2007, single soil cores were taken from each of the 104 plots using a trailer-mounted, hydraulic soil coring machine (Giddings, model HDGSRPST) (Figure 6-1). Soil cores were obtained by inserting a 5.1-cm-diam steel tube with plastic liner to a depth of 120 cm. On 12 and 13 August 2008, two additional soil cores were taken from plots containing clones FL-3, FL-122, C-65, and Argentine. After sample collection, the internal plastic liners were removed from the steel tubes, capped and the samples transported to Gainesville, FL, and

kept in a cold room overnight. Plastic liners were then marked and the sample divided in 3 sections: 0-40 cm, 40-80 cm, and 80-120 cm. Each section was placed on a 1.5-mm mesh screen and washed with a gentle stream of water to recover roots. Samples were placed in plastic bags and kept refrigerated (4 °C). Roots were spread on clear plastic trays and images generated and analyzed using WinRHYZO (Regent Instruments Inc., Quebec, Canada) scanner and software (WinRHIZO Pro v2002c). Root length density was calculated by dividing sample root length (cm) by sample soil volume (cm^{-3}). After the images were generated, roots were dried at 60 °C for 48 h, and weighed.

Statistical Analysis

Data was analyzed separately by year because plants were establishing in year one and fertilization practice differed between years. Root data and annual forage accumulation were analyzed as a split-plot design where fertilizer rate was considered the whole plot and clones were subplots. Seasonal biomass data was analyzed as a split-plot design with repeated measures. Fertilizer rate, clone, and harvest dates were considered fixed, while replicates where considered random. The data was analyzed using Proc mixed of SAS (SAS version 9.2, SAS Institute, Cary NC). When significant differences were detected ($P = 0.05$), the least significant difference (LSD) was computed. Correlations between root variables and forage production were calculated using the Pearson product-moment correlation coefficient (r).

Results

Forage Production

Significant differences were observed among hybrids for forage annual accumulation (FAA) in 2007 (Table 6-1). As expected, there was a significant N effect on forage production. No significant interaction between hybrids and N occurred in 2007, so hybrids were ranked in similar order for FAA (Table 6-1). With the exception of FL-21, artificially generated hybrids

produced more forage than the natural ecotypes, Argentine and Common, in 2007 and the trend continued in 2008 (Table 6-2). There was a significant interaction between hybrids and N in 2008. Some hybrids, such as FL-14 and FL-13, were ranked higher under the low fertilization treatment (Table 6-3), while others, such as C-92, were ranked higher under the high fertilization treatment (Table 6-2).

When the data were analyzed to consider forage production from each harvest date, fertilizer rate had a significant effect on forage production in both years. Genotypic differences were observed for forage production in both years. There was a significant interaction between fertilizer rate and hybrid only in 2008. In addition, the effect of harvest date on forage yield indicated the marked seasonal grow habit of bahiagrass. Since there was a significant interaction between harvest date and cultivar in 2007 and 2008, the data were further analyzed at each harvest. Although significant differences were detected among hybrids for forage mass at each harvest (Tables 6-1, 6-2 and 6-3), the chief differences were observed at the beginning and at the end of the growing season (Tables 6-2 and 6-3; Figures 6-2 and 6-3). Most of the artificially generated hybrids included in this study had a more extended growing season than the natural ecotypes (Figures 6-2 and 6-3). At the peak of the growing season hybrids did not out-produce Argentine, however most of them produced more forage than Argentine in May and October (Tables 6-2 and 6-3).

Root Mass and Root Length Density

No significant differences were observed among hybrids for root mass or RLD from any of the three analyzed soil layers in 2007. Nitrogen fertilization did not have an effect on root mass or RLD from any of the analyzed soil layers in 2007. The average root mass (across hybrids and fertilization rates) was 0.50 mg cm^{-3} at the 0- to 40-cm depth, 0.13 mg cm^{-3} at the 40- to 80-cm depth, and 0.08 mg cm^{-3} at the 80- to 120- cm depth. The average root length density was 1.1 cm

cm^{-3} at the 0- and 40-cm depth, 0.15 cm cm^{-3} at the 40- and 80-cm depth, and 0.08 cm cm^{-3} at the 80- to 120-cm depth.

There were no significant differences between hybrids for root mass from any of the analyzed soil layers in 2008. Additionally, the fertilizer rate did not have a significant effect on root mass from any soil layer. The average root mass (across hybrids and fertilizer rates) was 0.87 mg cm^{-3} at the 0- to 40-cm depth, 0.24 mg cm^{-3} at the 40- to 80-cm depth, and 0.18 mg cm^{-3} at the 80- to 120-cm depth. Significant differences for RLD were observed among hybrids in 2008 (Figure 6-4). However, genotypic differences for RLD at the 0- to 40-cm and 40- to 80-cm depths were only observed for the high fertilizer treatment. In contrast, genotypic differences for RLD at the 80- to 120-cm depth were only observed for the low fertilizer treatment (Figure 6-4).

A significant positive correlation was found between RLD and root mass at the 0- to 40-cm depth in 2007 (Figure 6-5) and 2008. Positive correlations were also found between RLD and root mass at the 40- to 80-cm depth ($r^2 = 0.7$), and for the 80- to 120-cm depth ($r^2 = 0.6$) in 2007. Results were similar for the 2008 growing season. Positive correlations were found between RLD and root mass at the 0- to 40-cm depth ($r^2 = 0.4$), 40- to 80-cm depth ($r^2 = 0.7$), and 80- to 120-cm depth ($r^2 = 0.7$).

There were no significant correlations between root mass at the 0- to 40–cm depth and forage annual accumulation (Figure 6-6) or for the forage harvested at the time of root collection in 2007 or 2008. Although significant, the correlation between root mass at the 40- to 80-cm and 80- to 120-cm depths and forage annual accumulation were low (Figures 6-7 and 6-8). No correlations were found between root mass at these two depths and forage annual accumulation nor forage harvested at the time of root collection in 2008.

No significant correlations were found between RLD at the 0- to 40-cm depth and forage annual accumulation (Figure 6-9) nor forage harvested at the time of root collection for either year. Significant but low correlations were found between RLD at the 40- to 80-cm and 80- to 120-cm depths and forage annual accumulation (Figures 6-10 and 6-11) only in 2007. There were not significant correlations between RLD at the 40- to 80-cm and 80- to 120-cm depths and forage harvested at the time of root collection for either year.

Discussion

Most of the hybrids included in this study produced more forage than the cultivar Argentine, which is the most commonly used bahiagrass tetraploid cultivar in the southeastern USA. Differences in forage production were mainly observed during spring and fall. These findings indicate that phenotypic selection of individual progeny for cool-season regrowth and freeze resistance (Acuña et al., 2009) can successfully result in hybrids with extended growing season when grown in swards. Although the physiological reasons for these differences were not part of this research, previous reports indicate that the two major factors influencing the extension of the bahiagrass growing season were photoperiod (Sinclair et al., 2001) and temperature (Gates et al., 1999). The results also indicate that the genetic variability for photoperiod sensitivity and freeze resistance can be successfully fixed through apomixis. However, it is important to mention that apomictic bahiagrass plants are occasionally able to sexually reproduce resulting in a low degree of segregation for these characteristics.

Once established, the apomictic hybrids included in this study responded differently to the application of different amounts of fertilizer. This response indicates that some lines, such as FL-13, may be more appropriate for systems with minimal fertilizer inputs, while others, such as C-92, may be more appropriate for intensive production systems receiving high fertilizer inputs.

Further research is needed to investigate the tolerance of these hybrids to grazing stress, and associated nutritive value.

The lack of genetic variability for root mass in 2007 and 2008, in addition to the lack of significant (or low) correlations between root mass and forage mass would indicate that root mass can not explain the genotypic differences observed for forage production. These results indicate that root activity of perennial grasses might play an important role for plant production.

Genotypic differences for forage production may be more greatly affected by differences in above-ground growth habits (i.e., rhizome production, prostate vs. upright). These attributes were not evaluated in this study. In addition, further research is needed to analyze the seasonal (year-long) root production, since the major differences for forage production were observed in spring and fall, while, root sampling (mid July) best represented the mid-season production.

Although genotypic variation for RLD was observed in 2008, this variation was not significantly correlated with the genotypic variation observed for forage production. This would indicate that RLD was not limiting those hybrids producing less forage, even under lower soil fertility. Passioura (1983) indicated that crop RLD appears much larger than what is required to extract water at reasonable rates, and that selecting for less massive root systems (particularly in the topsoil) may be beneficial in water-limited environments. Since water can be expected to be the most limiting factor to many production systems in southeastern USA, selection of a smaller root system in the topsoil should be considered for bahiagrass breeding especially for intensive production systems. Increasing the fertilizer rate resulted in greater forage production but it did not result in greater root mass or root length density. This lack of root response to the 2 fertilizer rates is further evidence for the presence of an excessively big root system in bahiagrass. However, comparisons with lower fertility soils need to be evaluated. Additionally, a more

massive root system might be an advantage for bahiagrass long-term survival under very low input systems, such as rangelands in Florida, southern Brazil, Uruguay, northeastern Argentina and other parts of the world.

Table 6-1. Average biomass produced by of 13 apomictic bahiagrass clones in 2007.

Clone	Biomass Yield					
	Low Fertilization			High Fertilization		
	30 July	13 November	Annual	30 July	13 November	Annual
	----------g m^{-2}----------					
C-92[†]	245	325	570	345	348	693
C-49	225	360	585	319	372	692
C-65	182	273	456	351	336	688
FL-13	262	283	545	338	343	681
FL-93	241	249	491	327	331	658
FL-3B	257	320	576	328	310	638
Tifton 7	202	344	546	261	363	624
FL-122	198	285	482	310	306	616
FL-3	241	290	531	315	299	615
FL-14	236	342	578	287	310	597
FL-21	202	237	439	247	230	477
Argentine	141	190	331	237	207	443
Common	154	128	283	201	113	314
LSD[‡]	70	62	113	75	87	119

[†]Clones were ranked based on annual biomass accumulation with high fertilization.
[‡]Least significant difference.

Table 6-2. Average biomass produced by 13 bahiagrass clones and a high fertilizer rate in 2008.

Hybrid	Biomass Yield							
	15 May	12 June	10 July	7 August	4 September	2 October	31 October	Annual
	----------g m^{-2}----------							
C-92[†]	82	147	373	396	216	85	30	1328
FL-93	97	162	304	377	235	72	18	1266
C-49	41	94	298	376	255	115	52	1231
FL-14	88	107	305	346	237	106	38	1226
FL-3B	66	121	323	338	219	98	38	1204
C-65	85	125	274	317	244	97	30	1172
FL-3	80	115	276	377	213	69	28	1159
FL-122	37	100	258	395	241	80	34	1145
Tifton 7	52	96	273	369	229	76	33	1127
Argentine	21	101	258	383	254	71	12	1099
FL-13	103	124	264	294	191	67	30	1073
FL-21	70	87	276	279	209	58	16	994
Common	0	0	116	253	194	46	8	618
LSD[‡]	28	21	46	80	43	19	10	133

[†]Clones were ranked based on annual biomass accumulation.
[‡]Least significant difference.

Table 6-3. Average biomass produced by 13 bahiagrass clones and low fertilizer rate in 2008.

Hybrid	Biomass Yield							
	15 May	12 June	10 July	7 August	4 September	2 October	31 October	Annual
	----------	----------	----------	----------	g m^{-2} ----------	----------	----------	----------
FL-14[†]	73	117	252	227	229	82	14	993
FL-13	96	138	235	216	217	49	9	960
FL-93	68	126	249	204	225	45	3	919
C-92	54	124	263	196	213	58	5	913
C-65	67	118	223	192	205	75	14	893
FL-3	64	112	207	212	197	63	9	863
FL-122	44	108	204	190	219	80	14	859
FL-3B	46	111	218	202	206	65	7	855
C-49	34	94	215	183	221	81	16	843
FL-21	54	108	214	193	183	55	9	816
Tifton 7	69	100	160	209	207	60	6	810
Argentine	16	80	178	201	201	50	6	732
Common	0	0	82	152	157	40	2	432
LSD[‡]	31	21	47	28	41	17	6	117

[†]Clones were ranked based on annual biomass accumulation.
[‡]Least significant difference.

Figure 6-1. Root sample collection on bahiagrass plots. A hydraulic soil sampling equipment can be seen at front. A person removing an internal plastic liner can be seen in the back.

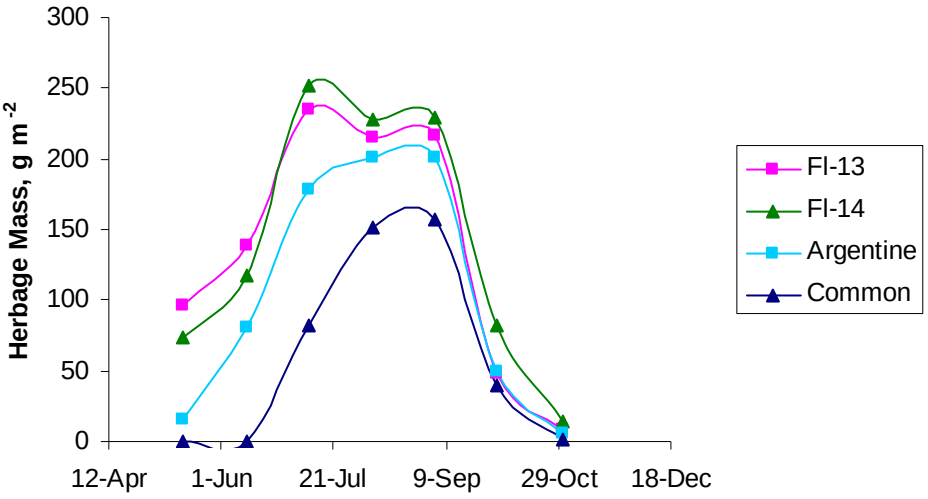

Figure 6-2. Seasonal biomass yields of the cultivar Argentine, the ecotype Common and hybrids FL-13 and FL-14 during 2008. Plots were fertilized with 120 kg N ha^{-1} $year^{-1}$.

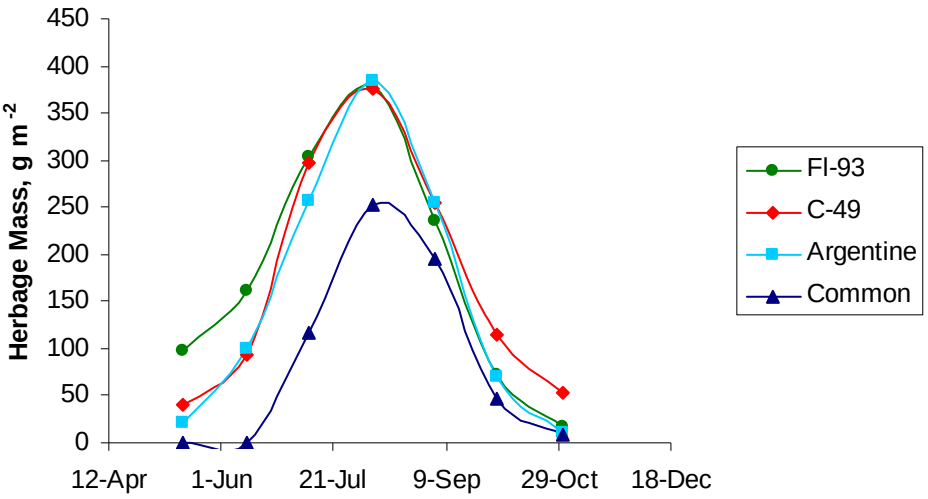

Figure 6-3. Seasonal biomass yields of the cultivar Argentine, the ecotype Common and hybrids C-49 and C-93 during 2008. Plots were fertilized with 360 kg N ha^{-1} $year^{-1}$.

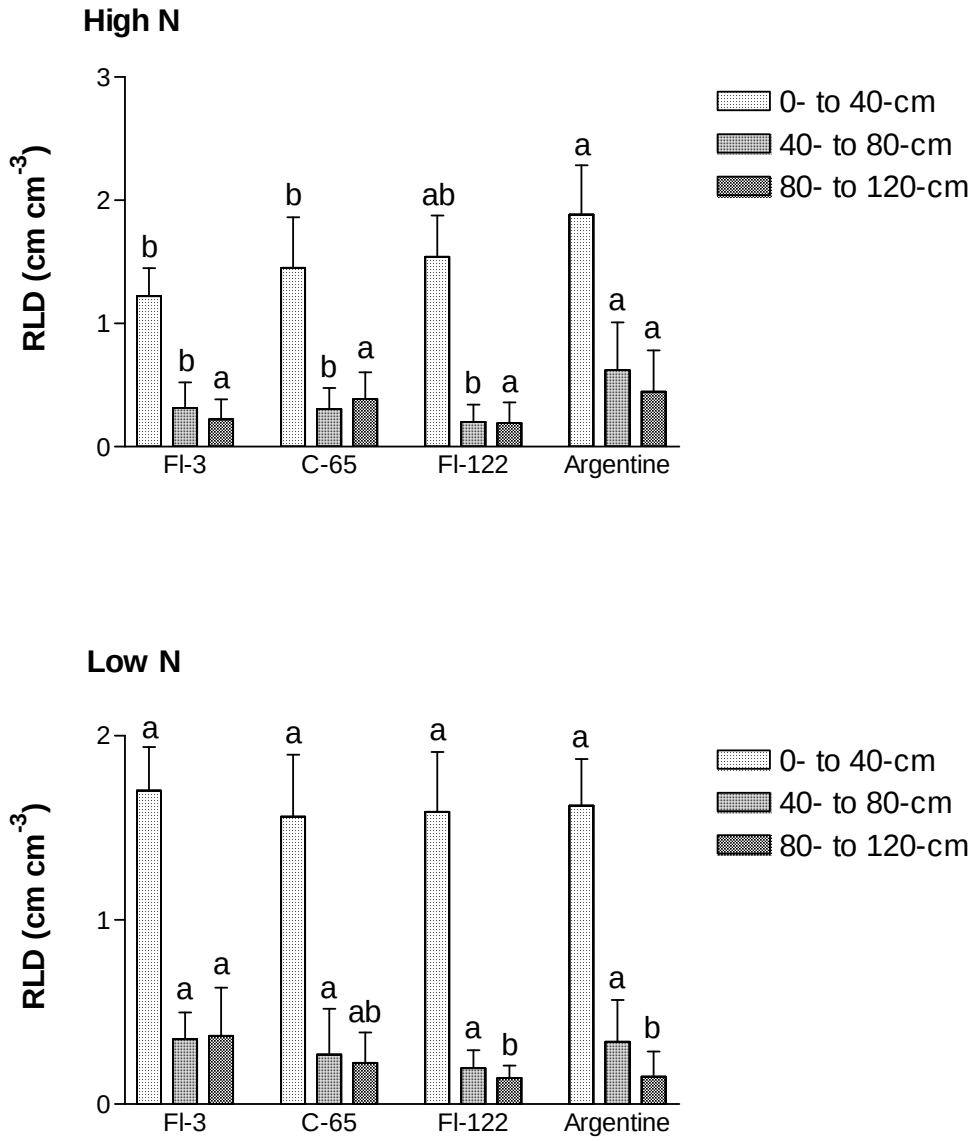

Figure 6-4. Root length density of 4 bahiagrass hybrids fertilized with 360 kg N ha^{-1} yr^{-1} (high N) or 360 kg N ha^{-1} $year^{-1}$(low N). Bars at specific depths having different letters have significance at alpha = 0.05.

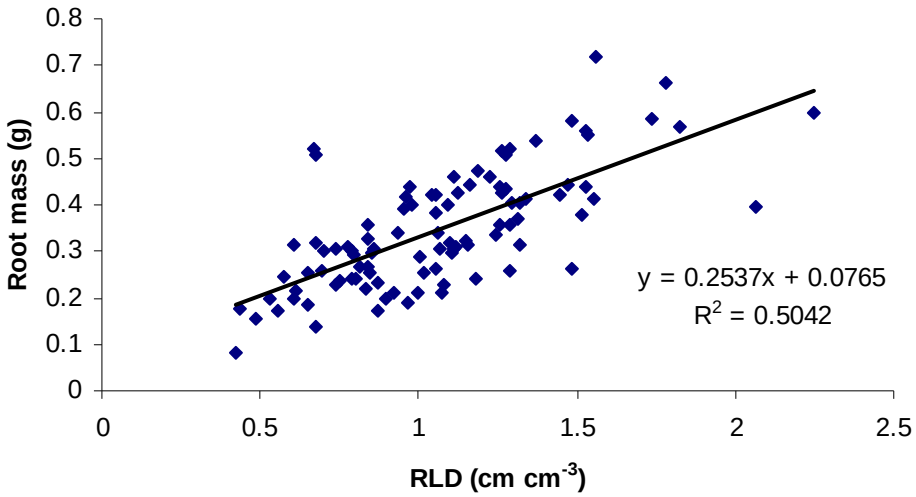

Figure 6-5. Relationship between root mass and RLD at the 0- to 40-cm soil layer of bahiagrass hybrids in 2007.

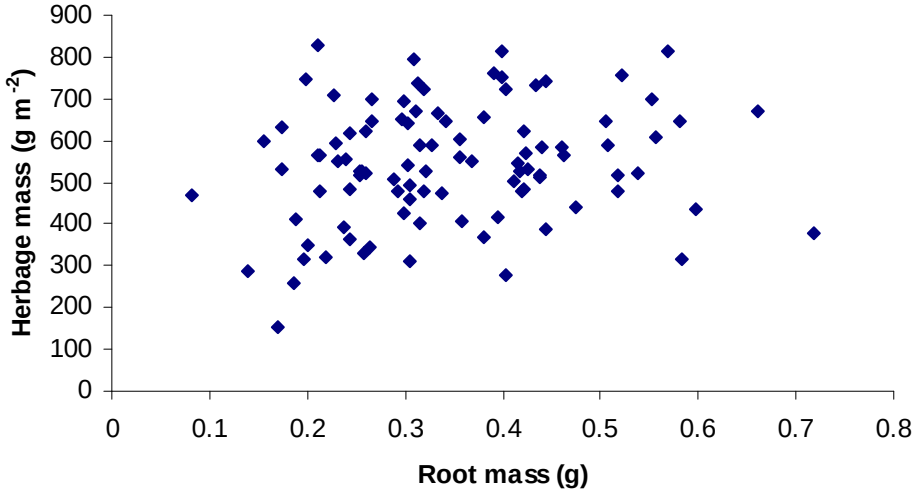

Figure 6-6. Relationship between root mass at the 0- to 40-cm soil layer and forage annual accumulation of bahiagrass hybrids in 2007.

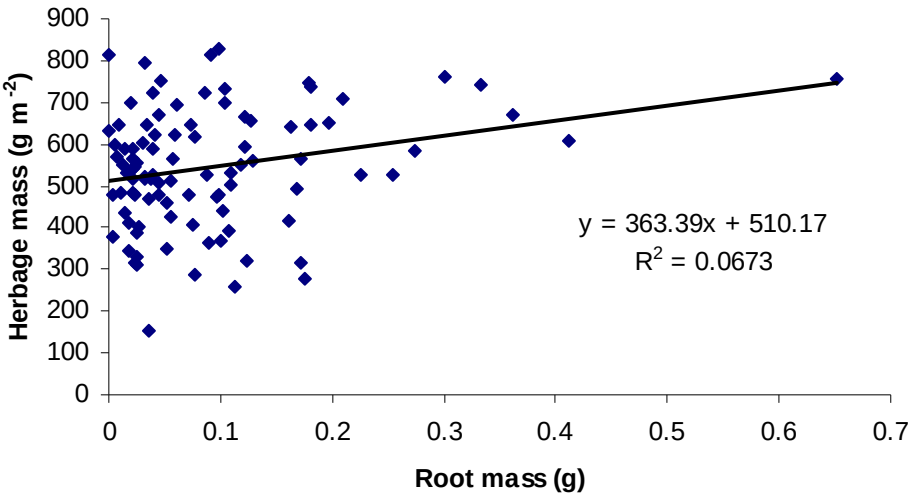

Figure 6-7. Relationship between root mass at the 40- to 80-cm soil layer and forage annual accumulation of bahiagrass hybrids in 2007.

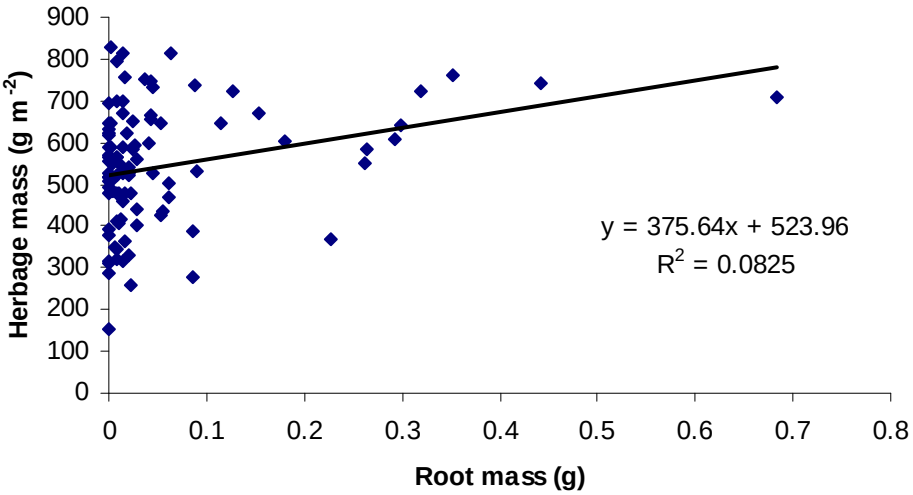

Figure 6-8. Relationship between root mass the 80- to 120-cm soil layer and forage annual accumulation of bahiagrass hybrids in 2007.

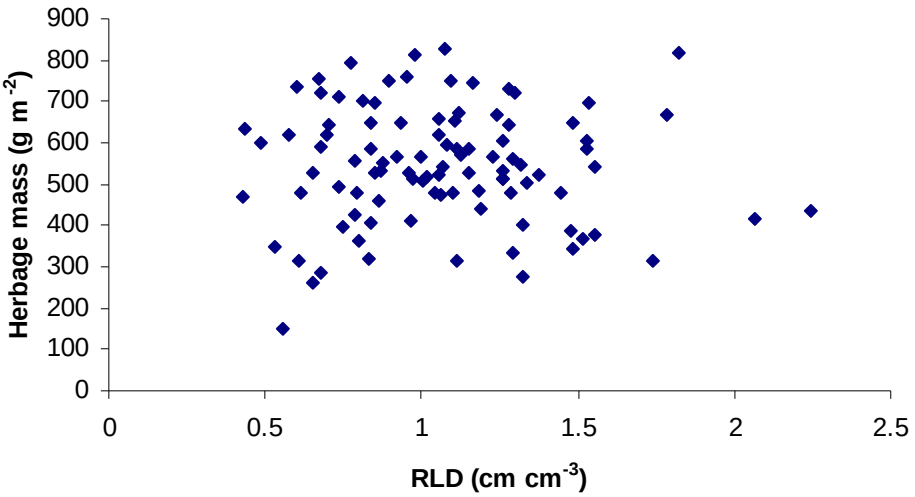

Figure 6-9. Relationship between RLD at the 0- to 40-cm soil layer and forage annual accumulation of bahiagrass hybrids in 2007.

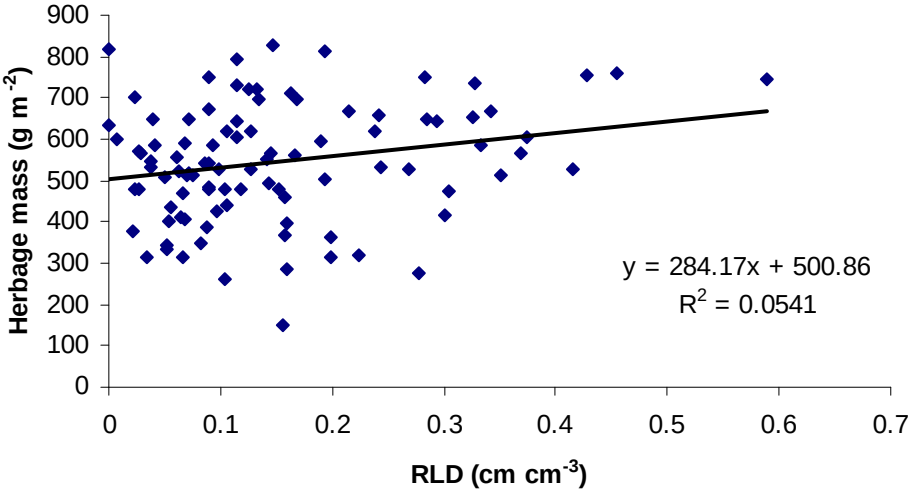

Figure 6-10. Relationship between RLD at the 40- to 80-cm soil layer and forage annual accumulation of bahiagrass hybrids in 2007.

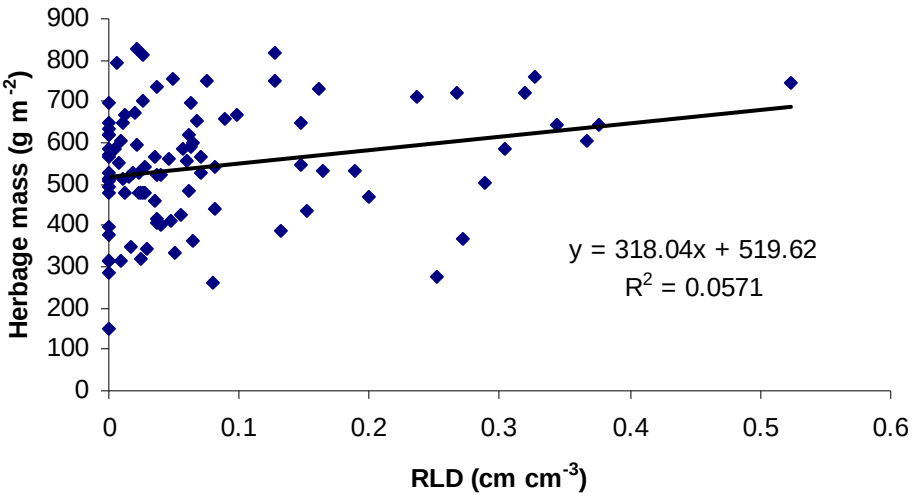

Figure 6-11. Relationship between RLD at the 80- to 120-cm soil layer and forage annual accumulation of bahiagrass hybrids in 2007.

CHAPTER 7
CONCLUSIONS

Genetics and Breeding

Naturally occurring tetraploid bahiagrass races reproduce asexually by aposporous apomixis. Crosses between sexual female parents and apomictic male pollinators allowed the generation of segregating populations for the genetic improvement of this species. Two populations (F_1 and F_2) were generated in the last 4 years and evaluated for mode of reproduction, growth habit, cool-season regrowth, freeze resistance, and production of inflorescences. The F_1 was generated by crossing sexual induced tetraploid clones and apomictic tetraploid ecotypes, while the F_2 was generated by crossing selected and sexual and apomictic F_1 progeny.

The proportion of progeny able to form aposporous embryo sacs remained constant between the F_1 and F_2 (Chapter 2). Approximately 20% of the progeny from a sexual x apomict cross can be expected to inherit the apospory locus. The proportion of highly apomictic progeny was reduced from 11% in the F_1 to only 3% in the F_2. This variation in expressivity suggests that epigenes are involved in the expression of aposporous apomixis in bahiagrass. Since the fixation of superior hybrids is the ultimate goal of manipulating apomixis for breeding purposes, this low proportion of obligate apomicts within the progeny seriously compromise the practicability of this breeding approach. More research is needed to investigate possible methods to increase the proportion of obligate apomicts in the progeny. The possibility of using sexual clones that show a minimum expression of apomixis as female parents should be evaluated as a potential breeding approach.

The genetic variability and heritability estimates for growth habit, cool-season regrowth, and freeze resistance remained relatively constant between the F_1 and F_2 indicating that

phenotypic mass selection could be efficiently used for continuous improvement of this species (Chapter 2). Seven apomictic F_1 hybrids were selected based on superior spreading, cool-season regrowth and freeze tolerance. These hybrids were cultivated in pure-stand plots in three locations across north Florida. Five hybrids produced higher cool-season biomass yields compared with commercial cultivars when grown in swards (Chapter 3). The main differences for cool-season biomass production were observed at the beginning of the growing season. Higher cool-season growth resulted in most cases in higher annual biomass accumulation. These F_1 hybrids were also able to remove more nitrogen and phosphorus from the soil during the spring than commercial tetraploid cultivars (Chapter 3). The genetic variability observed for nitrogen and phosphorus concentration in foliage of these novel hybrids was very low. Rhizomes and roots of these novel hybrids were not compromised by frequent defoliation during the three years of evaluation.

It is necessary to further investigate the physiological and anatomical characteristics that might be responsible for the extended growing season of these novel tetraploid hybrids. Best management practices need to be determined for the use of these hybrids for hay production or as a bioenergy crop. The potential use of these hybrids for grazing needs to be determined in larger trials where they will be evaluated for persistence under grazing by livestock. Performance of these novel lines will need further evaluation in regards to animal performance, palatability and preference.

Ecological Determinants of Growth

Clear acrylic tubes were used to screen several bahiagrass tetraploid clones for rate of root depth development during the first 6 to 8 weeks after germination (Chapter 4). This trait can be evaluated using different growing media and tube sizes with minimal variation in the results. The rate of root depth development was also shown to be independent of the growing season when

plants are frequently irrigated. The technique was determined to be adequate for the screening of a large number of plants in a reduced spaced (3.5-cm diameter tubes). The rate of root depth development in bahiagrass is not significantly affected by close and frequent defoliation. . Bahiagrass is typically known to withstand close defoliation, and the speed of root depth development and recovery following defoliation may be related to this plant's success when cultivated as grazed forage or used for hay production.

The bahiagrass germplasm contains genetic variability for rate of root depth development (Chapter 4). Faster root development was observed for clone FL-122. This clone is one of the novel F_1 apomictic hybrids generated by crossing induced sexual and apomictic tetraploid clones. Since the female parent of this clone was derived from an induced autotetraploid, the genes behind this superiority could be present in the diploid or tetraploid germplasm. This trait always resulted in higher above- and below-ground biomass when grown in tubes. We hypothesized that higher biomass results from rapid exploration of water and nutrients present in deep soil layers. Field observations confirmed the rates of root depth development observed for plants grown in acrylic tubes. Plants growing in the field were able to explore a depth of 100 cm after 60 days which reflects a rate of depth increase approximately the same as observed for plants grown in clear acrylic tubes (1.8 cm d^{-1}).

Higher rate of root depth development resulted in faster access and uptake of label nitrogen deposited in deep soil layers (Chapter 5). This observation indicated that high biomass yield observed for FL-122 was associated with faster soil depth exploration and water and nutrient uptake. Although FL-122 was not one of the highest yielding apomictic clones, it could be used in a breeding program as a source of genes for early vigor and quick establishment. Clone F-122 was generated by crossing Q-4188 as female parent and Argentine as male parent. Since

Argentine showed lower rates of root depth development than FL-122, the source of superior genes for this trait might also be present in Q-4188. Other apomictic male pollinators could be crossed with Q-4188 to test this hypothesis.

Genetic variability for root length density is present among the bahiagrass tetraploid germplasm (Chapter 6). This variability can be detected in the second growing season once a sward is established. Genotypic variation for root length density varies depending on the amount of fertilizer applied. Clones like Argentine are able to produce a prolific root system at the soil surface when high rates of fertilizer are applied. In contrast, other clones like FL-3 are able to produce a more extensive root system in the subsoil when low amounts of fertilizer are applied. The potential advantages of the observed genetic variability need to be further investigated. The prolific root system observed for Argentine is probably related with its superior ability for phosphorus uptake when high amounts of fertilizer were applied. The relationship between higher root length density in the subsoil under the low fertilization treatment of FL-3 should be investigated further for a potential superior drought tolerance and deep soil mineral recovery.

The observed genetic variability for root length density was not related to differences in above-ground biomass yields (Chapter 6). This result indicates that lower root length density observed for some clones did not limit their productivity. Although the extensive root systems of these clones should have contributed to the success of this species in native grasslands, it might be excessively high for a semi-intensive production system. It would be essential to complement these results by studying the seasonal variation for root length density since tetraploid clones have shown a marked variability for seasonality of above-ground biomass production. It would be particularly important to determine the root length density of these clones in spring and fall since the main differences for biomass production were detected in these two seasons.

Genetic variability for root mass was not observed among the bahiagrass tetraploid germplasm at any of the evaluated soil depths (Chapter 6). Variation in above ground biomass was not related with variation on below-ground biomass. Selection based on root mass should not be considered appropriate for bahiagrass if the breeding objective is to increase above-ground biomass production.

Root length density and root mass were not affected by the amount of fertilizer applied (Chapter 6). Although increasing the amount of fertilizer increased the amount of foliage that was harvested, it did not have a significant effect on the root system. These results are further evidence that the bahiagrass root system might be in excess of what is needed to uptake nutrients in a semi-intensive production system. Our results would also indicate that root activity instead of root architecture is probably involved in the uptake of nutrients and biomass yields. Further research will be needed to test how root activity is correlated to biomass production, and if this is a major genotypic trait responsible for differences found in biomass yields.

Perspective on Future Research

The genetic variability contained in the bahiagrass germplasm should be further investigated to enhance future breeding. Molecular markers can be used to identify relationships among tetraploid populations growing in the wild or contained in a germplasm bank. The identification of heterotic groups using molecular techniques can be tested by hybridization of individuals from related and unrelated populations. One of the most important aspects to improve upon is the proportion of highly apomictic progeny that can be generated by hybridization. Since the apomixis locus is inherited as a dominant Mendelian factor, identification of self-incompatible sexual mother plants will enhance future breeding.

The relationship between seasonal herbage production and root development and activity should also be further investigated. These root parameters can be estimated at beginning and end

of the growing season by quantifying the uptake of phosphorus isotopes (^{32}P) placed at different soil depths. Genotypes exhibiting contrasting growing patterns may be utilized for this purpose.

Apomictic hybrids exhibiting an extended growing season should be further evaluated for tolerance to defoliation by livestock. The utilization of mob grazing on small pure-stand plots can be considered for this purpose. Additionally, the potential of using these novel hybrids in crop rotation with row crops should be investigated. Field trials evaluating the seasonal growing patterns of novel hybrids with current cultivars should determine a best fit for crop rotation systems. From the efforts of this project we believe there exists great potential for the genetic improvement of bahiagrass through manipulation and exploitation of apomixis in this genus.

APPENDIX
SEASONAL FORAGE AND RHIZOME+ROOT MASS, AND NITROGEN AND PHOSPHORUS CONCENTRATIONS AND ACCUMULATIONS IN HERBAGE

Table A-1. Biomass of 12 bahiagrass clones grown at Gainesville in 2006 and 2007.

Hybrid	Biomass								
	10/31/06	05/04/07	06/04/07	07/01/07	07/31/07	08/27/07	09/24/07	10/23/07	12/04/07
	---g m^{-2}---								
FL-3	375	153	234	341	239	177	214	52	16
FL-13	383	246	282	275	170	126	147	80	22
FL-14	287	171	265	375	244	174	249	30	3
FL-21	173	129	157	312	182	152	202	39	7
C-49	512	66	172	296	229	174	184	42	6
C-65	499	187	281	384	209	157	163	72	20
C-92	370	175	323	376	215	157	166	59	19
FL-93	348	157	229	349	217	177	218	39	9
FL-122	339	114	191	318	232	137	194	48	7
FL-3B	298	127	236	283	214	173	192	36	11
Argentine	239	37	85	280	268	217	263	41	0
Tifton 7	359	214	201	342	248	185	202	71	16
MSD†	118	64	72	77	55	40	137	25	11

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-2. Biomass of 12 bahiagrass clones grown at Gainesville in 2008.

Hybrid	Biomass						
	13 May	10 June	8 July	5 August	2 September	1 October	28 October
	---g m^{-2}---						
FL-3	176	177	338	361	187	200	87
FL-13	179	174	267	295	159	178	79
FL-14	243	94	343	360	184	217	102
FL-21	143	191	270	310	148	151	63
C-49	171	76	313	350	179	197	92
C-65	214	142	381	343	202	204	88
C-92	207	112	349	392	213	177	71
FL-93	186	180	329	357	187	202	76
FL-122	77	140	328	351	163	161	71
FL-3B	158	140	372	362	174	156	98
Argentine	66	122	321	447	254	195	58
Tifton 7	124	116	362	398	221	288	113
MSD†	79	58	62	59	53	88	9

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-3. Biomass of 13 bahiagrass clones grown at Live Oak in 2008.

Hybrid	Biomass						
	15 May	12 June	10 July	7 August	4 September	2 October	31 October
	----------g m^{-2}----------						
FL-3	80	115	276	377	213	69	28
FL-13	103	124	264	294	191	67	30
FL-14	88	107	305	346	237	106	38
FL-21	70	87	276	279	209	58	16
FL-93	97	162	304	377	235	72	18
FL-122	37	100	258	395	241	80	34
FL-3B	66	121	323	338	219	98	38
C-49	41	94	298	376	255	115	52
C-65	85	125	274	317	244	97	30
C-92	82	147	373	396	216	85	30
Argentine	21	101	258	383	254	71	12
Common	0	0	116	253	194	46	8
Tifton 7	52	96	273	369	229	76	33
MSD†	28	21	46	80	43	19	10

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-4. Biomass of 13 bahiagrass clones grown at Quincy in 2008.

Hybrid	Biomass						
	13 May	10 June	8 July	5 August	2 September	1 October	28 October
	----------g m^{-2}----------						
FL-3	90	63	156	221	259	110	39
FL-13	122	92	131	188	206	103	26
FL-14	108	85	151	179	247	116	39
FL-21	59	70	114	174	255	119	27
C-49	136	76	159	224	268	141	44
C-65	80	56	145	209	280	120	33
C-92	114	92	178	189	264	114	22
FL-93	84	92	157	209	275	120	24
FL-122	55	55	137	204	301	123	32
FL-3B	87	73	173	203	262	129	26
Argentine	39	47	120	179	271	137	23
Common	0	1	58	176	227	101	21
Tifton 7	78	56	124	176	242	134	36
MSD†	29	31	28	57	46	42	11

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-5. Rhizome+root mass of 12 bahiagrass clones (Gainesville).

Clone	2007 Spring	2007 Summer	2007 Fall	2008 Spring	2008 Fall
	----------mg cm^{-3}----------				
FL-13[†]	72.2	49.0	25.7	23.9	16.4
FL-21	54.9	30.7	36.1	14.1	11.4
FL-122	48.4	40.3	31.4	13.2	10.8
FL-3B	46.1	33.1	25.0	16.6	15.6
C-92	45.8	44.2	32.8	18.7	13.7
FL-93	42.1	39.9	37.7	23.6	13.7
FL-14	40.7	39.4	28.5	18.7	13.6
C-65	34.0	27.9	32.4	17.8	16.2
FL-3	33.1	30.7	26.6	21.5	15.3
Tifton 7	32.5	43.2	41.3	14.8	15.5
C-49	23.4	21.6	30.3	13.9	11.3
Argentine	22.8	25.5	30.5	17.3	15.7
MSD[‡]	28.0	ns	ns	ns	ns

[†]Clones were ordered based on rhizome+root mass for Spring 2007.
[‡]Minimum significant difference, Waller-Duncan means separation procedure.

Table A-6. Nitrogen concentration in forage of 12 bahiagrass clones grown at Gainesville in 2006 and 2007.

Hybrid	Nitrogen 10/31/06	05/04/07	06/04/07	07/01/07	07/31/07	08/27/07	09/24/07	10/23/07	12/04/07
	----------g kg^{-1}----------								
FL-3	11.30	15.63	16.41	14.70	13.79	16.51	26.14	17.93	27.83
FL-13	12.24	13.06	15.36	15.04	15.39	18.74	27.01	19.07	28.52
FL-14	11.81	13.4	12.79	14.02	13.88	19.45	27.08	18.1	25.48
FL-21	12.04	13.6	16.35	14.19	15.15	16.64	28.34	17.81	28.41
C-49	9.50	16.76	18.92	16.54	15.49	18.97	25.05	19.48	27.08
C-65	10.26	15.62	16.08	14.70	13.96	17.18	25.23	19.25	31.58
C-92	10.79	16.4	15.01	16.01	14.93	16.94	27.28	19.46	27.21
FL-93	12.05	15.28	14.83	15.44	14.56	16.56	28.44	18.83	28.11
FL-122	11.56	17.18	16.1	16.90	14.30	17.32	26.71	18.35	27.57
FL-3B	10.03	13.79	15.91	14.47	15.09	17.15	26.05	19.32	27.83
Argentine	12.20	17.01	15.64	15.09	13.02	15.01	26.84	17.64	.
Tifton 7	11.16	14.49	15.88	15.04	13.51	16.54	25.71	18.39	25.74
MSD[†]	1.9	3.3	ns	ns	ns	3	ns	ns	ns

[†] Minimum significant difference, Waller-Duncan means separation procedure.

Table A-7. Nitrogen concentration in forage of 12 bahiagrass clones grown at Gainesville in 2008.

Hybrid	Nitrogen						
	13 May	10 June	8 July	5 August	2 September	1 October	28 October
	---------- g kg^{-1} ----------						
FL-3	14.59	15.99	14.27	15.22	16.49	20.02	24.26
FL-13	12.62	18.15	15.49	15.88	16.75	21.62	25.61
FL-14	12.99	16.23	17.67	14.68	15.11	18.48	24.48
FL-21	15.05	16.97	17.96	16.37	17.68	21.92	24.72
C-49	12.44	17.28	15.74	14.45	14.41	19.78	24.91
C-65	13.46	16.87	16.20	14.25	15.18	19.83	24.05
C-92	13.99	16.50	16.32	14.37	15.63	21.53	25.57
FL-93	15.15	14.77	15.55	15.25	17.58	20.37	26.29
FL-122	14.48	16.95	16.54	15.30	17.19	20.76	26.82
FL-3B	12.28	17.32	17.64	14.75	15.02	19.30	24.22
Argentine	16.53	15.29	15.93	14.90	17.22	20.47	27.00
Tifton 7	14.88	17.52	15.23	14.28	15.21	19.57	24.30
MSD†	1.6	ns	2.3	ns	0.9	1.7	2.1

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-8. Phosphorus concentration in forage of 12 bahiagrass clones grown at Gainesville in 2007

Hybrid	Phosphorus							
	05/04/07	06/04/07	07/01/07	07/31/07	08/27/07	09/24/07	10/23/07	12/04/07
	---------- g kg^{-1} ----------							
FL-3	1.30	1.38	1.72	1.66	1.94	2.55	2.14	2.15
FL-13	1.04	1.55	1.85	1.86	1.98	2.54	2.10	1.97
FL-14	1.25	1.21	1.60	2.01	2.01	2.49	2.05	2.36
FL-21	1.41	1.42	1.55	1.97	2.02	2.59	2.00	1.92
C-49	1.70	1.64	1.69	1.77	1.98	2.36	2.00	1.98
C-65	1.24	1.41	1.66	1.81	1.90	2.26	2.05	1.71
C-92	1.35	1.43	1.80	1.82	1.87	2.32	1.97	1.69
FL-93	1.34	1.54	1.79	1.85	1.83	2.36	2.09	1.88
FL-122	1.16	1.24	2.08	2.01	2.20	2.45	2.18	1.81
FL-3B	1.13	1.67	1.72	1.84	1.90	2.46	2.02	2.16
Argentine	1.54	1.61	2.11	2.10	2.40	2.73	2.57	.
Tifton 7	1.34	1.68	1.90	2.01	2.21	2.58	2.28	2.51
MSD†	0.20	0.20	0.30	ns	0.30	0.20	0.20	0.50

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-9. Phosphorus concentration in forage of 12 bahiagrass clones grown at Gainesville in 2008

Hybrid	Phosphorus						
	13 May	10 June	8 July	5 August	2 September	1 October	28 October
	---------- g kg^{-1} ----------						
FL-3	1.12	1.28	1.98	1.90	2.34	2.33	2.53
FL-13	0.97	1.51	2.06	2.13	2.23	2.22	2.38
FL-14	1.07	1.53	2.02	1.98	2.07	2.32	2.75
FL-21	0.98	1.43	2.02	2.00	2.20	2.21	2.37
C-49	1.06	1.66	2.21	1.80	2.01	2.03	2.26
C-65	1.03	1.48	2.16	1.95	2.15	2.02	2.05
C-92	0.96	1.41	2.28	1.90	1.98	2.05	2.36
FL-93	1.11	1.29	2.06	2.05	2.17	2.19	2.34
FL-122	1.05	1.48	2.33	2.23	2.36	2.26	2.34
FL-3B	0.97	1.36	1.98	1.81	1.95	1.94	2.39
Argentine	1.25	1.34	2.43	2.52	2.73	2.86	2.77
Tifton 7	1.14	1.58	2.21	2.22	2.47	2.46	2.77
MSD†	0.60	0.80	0.60	0.20	0.20	0.30	0.30

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-10. Nitrogen accumulation in forage of 12 bahiagrass clones grown in Gainesville in 2006 and 2007.

Hybrid	Nitrogen								
	10/31/06	05/04/07	06/04/07	07/01/07	07/31/07	08/27/07	09/24/07	10/23/07	12/04/07
	---------- g m^{-2} ----------								
FL-3	4.2	2.4	4.0	5.0	3.3	2.9	5.7	1.0	0.5
FL-13	4.6	3.3	4.3	4.2	2.6	2.3	4.0	1.5	0.6
FL-14	3.4	2.3	3.4	5.3	3.4	3.4	6.7	0.5	0.1
FL-21	2.0	1.8	2.6	4.4	2.8	2.5	5.7	0.7	0.2
C-49	4.9	1.1	3.2	4.8	3.5	3.3	4.7	0.8	0.2
C-65	5.1	3.0	4.6	5.6	3.0	2.7	4.2	1.4	0.6
C-92	3.9	3.0	4.9	6.0	3.1	2.7	4.5	1.1	0.5
FL-93	4.1	2.4	3.4	5.4	3.2	2.9	6.2	0.7	0.3
FL-122	3.9	2.0	3.1	5.4	3.3	2.4	5.2	0.9	0.2
FL-3B	3.0	1.8	3.7	4.1	3.2	2.9	5.0	0.7	0.3
Argentine	2.8	0.6	1.3	4.2	3.5	3.3	7.0	0.7	0.0
Tifton 7	4.0	3.2	3.3	5.2	3.4	3.0	5.2	1.3	0.4
MSD†	1.2	2.3	1.9	1.6	ns	0.8	ns	0.5	0.4

† Minimum significant difference, Waller-Duncan means separation procedure.

Table A-11. Nitrogen accumulation in forage of 12 bahiagrass clones grown in Gainesville in 2008.

Hybrid	Nitrogen						
	13 May	10 June	8 July	5 August	2 September	1 October	28 October
	----------g m^{-2}----------						
FL-3	2.6	2.8	4.9	5.5	3.1	4.0	2.1
FL-13	2.2	3.2	4.1	4.7	2.7	3.8	2.0
FL-14	3.2	1.5	6.1	5.3	2.8	4.0	2.5
FL-21	2.1	3.3	4.9	5.1	2.6	3.3	1.6
C-49	2.1	1.3	4.9	5.1	2.6	3.9	2.3
C-65	2.9	2.3	6.2	4.9	3.1	4.0	2.1
C-92	2.9	1.8	5.7	5.6	3.3	3.8	1.8
FL-93	2.8	2.6	5.1	5.4	3.3	4.1	2.0
FL-122	1.1	2.4	5.3	5.3	2.8	3.3	1.9
FL-3B	1.9	2.5	6.6	5.3	2.6	3.0	2.4
Argentine	1.1	1.8	5.1	6.7	4.4	4.0	1.6
Tifton 7	1.9	2.1	5.5	5.7	3.4	5.6	2.7
MSD[†]	1.2	1.4	1.3	ns	1.0	ns	0.3

[†] Minimum significant difference, Waller-Duncan means separation procedure.

Table A-12. Phosphorus accumulation in forage of 12 bahiagrass clones grown in Gainesville in 2007.

Hybrid	Phosphorus							
	4 May	4 June	1 July	31 July	27 August	24 September	23 October	4 December
	----------g m^{-2}----------							
FL-3	0.20	0.33	0.59	0.39	0.34	0.55	0.11	0.04
FL-13	0.25	0.44	0.51	0.32	0.25	0.37	0.17	0.04
FL-14	0.21	0.32	0.60	0.49	0.35	0.62	0.06	0.01
FL-21	0.18	0.22	0.48	0.36	0.31	0.52	0.08	0.01
C-49	0.11	0.27	0.48	0.40	0.34	0.44	0.08	0.01
C-65	0.23	0.40	0.64	0.37	0.30	0.37	0.15	0.03
C-92	0.24	0.46	0.68	0.39	0.29	0.38	0.11	0.03
FL-93	0.21	0.36	0.62	0.41	0.32	0.51	0.08	0.02
FL-122	0.13	0.23	0.66	0.47	0.30	0.48	0.10	0.01
FL-3B	0.14	0.39	0.49	0.39	0.33	0.47	0.07	0.02
Argentine	0.06	0.14	0.59	0.56	0.52	0.73	0.10	0.00
Tifton 7	0.29	0.33	0.66	0.50	0.41	0.52	0.16	0.04
MSD[†]	0.10	0.10	0.20	0.10	0.10	0.30	0.10	0.03

[†] Minimum significant difference, Waller-Duncan means separation procedure.

Table A-13. Phosphorus accumulation in forage of 12 bahiagrass clones grown in Gainesville in 2008.

Hybrid	Phosphorus						
	13 May	10 June	8 July	5 August	2 September	1 October	28 October
	---------- g m^{-2} ----------						
FL-3	0.19	0.22	0.67	0.69	0.44	0.47	0.22
FL-13	0.15	0.26	0.54	0.62	0.35	0.39	0.19
FL-14	0.27	0.14	0.69	0.71	0.38	0.50	0.28
FL-21	0.13	0.27	0.54	0.62	0.33	0.34	0.15
C-49	0.18	0.13	0.69	0.63	0.36	0.40	0.21
C-65	0.21	0.20	0.83	0.66	0.44	0.41	0.18
C-92	0.20	0.15	0.78	0.74	0.42	0.37	0.17
FL-93	0.21	0.22	0.68	0.73	0.41	0.44	0.18
FL-122	0.09	0.21	0.76	0.78	0.38	0.36	0.16
FL-3B	0.14	0.19	0.74	0.65	0.34	0.30	0.23
Argentine	0.09	0.16	0.78	1.13	0.69	0.55	0.16
Tifton 7	0.14	0.18	0.80	0.88	0.56	0.71	0.31
MSD[†]	0.09	0.15	0.21	0.12	0.10	0.18	0.04

[†] Minimum significant difference, Waller-Duncan means separation procedure.

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BIOGRAPHICAL SKETCH

Carlos Alberto Acuna was born in Corpus Christy, northeastern Argentina in 1977. In 1995, he moved to Corrientes, Argentina, and began his undergraduate studies at the University of the Northeast. In 2001, he received his Agronomy Engineer degree, after defending his thesis entitle: “The relevance of a triploid in the evolution of *Paspalum*”. He worked for 4 years, including the last 2 years of his undergraduate studies, with reproductive systems of warm-season grasses at Botany Institute of the Northeast (IBONE), Corrientes, Argentina. In May 2004 he began his graduate studies at the University of Florida under the direction of Dr. Ann Blount and Dr. Kenneth Quesenberry. In May 2006, he was awarded a Master of Science degree in agronomy with an emphasis in genetics and plant breeding. Immediately after graduation, he began his Ph.D. studies in agronomy working with genetics and plant breeding also under the direction of Dr. Ann Blount. He received his Ph.D. from the University of Florida in May 2009. He plans to return to Argentina, working in the area of plant breeding, and teaching at the Universidad Nacional del Nordeste.