ANNUAL AND PERENNIAL PEANUTS MIXED WITH ‘PENSACOLA’ BAHIA GRASS
(*Paspalum notatum* Flügge) OR ‘TIFTON 85’ BERMUDA GRASS (*Cynodon*
*Spp.*) IN NORTH FLORIDA

By

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To my mother, my sister, and my grandfather
ACKNOWLEDGMENTS

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<td>Acid detergent fiber</td>
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<td>BG</td>
<td>Bahiagrass</td>
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<td>BNF</td>
<td>Biological N\textsubscript{2}-fixation</td>
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<td>CEC</td>
<td>Cation exchange capacity</td>
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<td>CP</td>
<td>Crude protein</td>
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<td>DM</td>
<td>Dry matter</td>
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<td>EPA</td>
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<td>HA</td>
<td>Herbage accumulation</td>
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<td>IVOMD</td>
<td>In vitro organic matter digestibility</td>
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<td>NDF</td>
<td>Neutral detergent fiber</td>
</tr>
<tr>
<td>Ndfa</td>
<td>Nitrogen derived from the atmosphere</td>
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<tr>
<td>NS</td>
<td>Not significant</td>
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<tr>
<td>OM</td>
<td>Organic matter</td>
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<tr>
<td>PSV</td>
<td>Peanut stunt virus</td>
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<td>RP</td>
<td>Rhizoma peanut</td>
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<td>SOM</td>
<td>Soil organic matter</td>
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Nitrogen fertilization is among the largest expenses in Florida's beef cattle operations. Instances of grassland degradation have been observed due to producers opting out of applying N fertilizers. Promoting grass-legume mixed stands can reduce the demand for fertilizer use, while increasing productivity of a system. In this thesis, we evaluated one annual peanut species (*Arachis hypogaea* L. cv. TUFRunner '727') and two perennial peanut species: *A. pintoi* Krap. & W.C. Greg cv. ‘Amarillo’ and rhizoma peanut - RP (*A. glabrata* Benth cv. ‘Florigraze’ and germplasm Ecoturf). Peanut entries were planted into ‘Pensacola’ bahiagrass (*Paspalum notatum* Flügge) or ‘Tifton 85’ bermudagrass (*Cyndodon* spp.) in two separate studies and evaluated during 2014, 2015, and 2016. Responses measured in both experiments included herbage accumulation, botanical composition, N concentration, and biological N₂-fixation (BNF). Belowground root-rhizome responses were measured in the bahiagrass study only. In the bahiagrass study, TUFRunner 727 showed reseeding ability, illustrating its viability as a bi-annual legume. In the long-term, RP entries performed better than the others. Ecoturf herbage accumulation was greater and it appeared to be best suited for planting...
in bahiagrass pastures. For Tifton 85, rhizoma peanut cultivars, especially Ecoturf, performed better than the other entries, through greater herbage accumulation, participation in botanical composition, and BNF. Amarillo was largely underestimated due to its prostrate growth habit, and TUFRunner 727 had minimal evidence of reseeding. Future studies should focus on management practices to incorporate rhizoma peanut into pastures in North Florida and examine the long-term effects and benefits of these mixtures.
In 2016, Florida ranked among the top twelve states in beef cattle inventory with 915,000 cows, and ranked 19th in dairy cattle with 125,000 cows (USDA NASS, 2016). As a cow-calf production state, the vast majority of farms rely on grazing as a means of feeding animals. Bahiagrass (*Paspalum notatum* Flügge), having over one million hectares planted, is the most common perennial forage species in Florida (Newman et al., 2011). It was introduced by the Bureau of Plant Industry in 1913 from sub-tropical South America, where it grows abundantly in Southern Brazil, Argentina, and Eastern Bolivia (Newman et al., 2011).

Bahiagrass is a rhizomatous, perennial, warm-season grass that has adapted to a wide range of soil types (Houck, 2009; Newman et al., 2011), and has proven to thrive in the sandy soils of Florida. Bahiagrass is a versatile species and its uses can range from home and garden turf, land conservation, to a forage species for grazing and hay production. As a forage species, bahiagrass has thrived in Florida and it has been proven to be reliable and persistent when it comes to different management practices and climatic growing conditions (Vendramini et al., 2013). Bahiagrass is capable of tolerating heavy, continuous stocking, which is a management technique that often results in stand loss with other forage species (Sollenberger et al., 1988). This is attributed to its adaption to soils with low fertility, relatively low subtropical temperatures, and also because the plant is able to store its carbohydrate resources in belowground structures, called rhizomes (Newman et al., 2011), rather than aboveground organ structures. Bahiagrass can be vegetatively propagated; however, the plant is an
abundant seed producer and has a two-branched raceme inflorescence (Houck, 2009; Newman et al., 2011). In general, bahiagrass can have a canopy height between 30 and 40 cm tall with an upright or prostrate habit and the leaves are typically between 20 and 50 cm in length and stems can range between 20 and 76 cm (Houck, 2009).

‘Pensacola’ is the most predominant bahiagrass cultivar in Florida. Sollenberger et al. (1988) reported average daily gain (ADG) by yearling steers, grazing Pensacola bahiagrass in a 2-yr study, of 0.39 and 0.36 kg for each year respectively. Crude protein (CP) and in vitro organic matter digestibility (IVOMD) will typically vary from 99 to 140 g kg\(^{-1}\) and 459 to 505 g kg\(^{-1}\), respectively (Stewart et al., 2007). Nitrogen fertilizer and grazing management are two main factors that directly influence CP and IVOMD in bahiagrass (Stewart et al., 2007).

Bahiagrass forage production has greater during periods of combined high temperatures, high moisture, and long days (Newman et al., 2011). In Florida specifically, about 80 to 90% of bahiagrass herbage accumulation (HA) is produced between the months of April and September (Mislevy and Everett, 1981). Depending on the nutrient input levels and irrigation schemes, annual production has typically shown to range from 3.3 to 11.2 Mg ha\(^{-1}\) (Newman et al., 2011). In a low input system in which 40 kg N ha\(^{-1}\) yr\(^{-1}\) was applied, it was reported that 3.42 Mg DM ha\(^{-1}\) yr\(^{-1}\) were accumulated across a four-year grazing study (Stewart et al., 2007). When N was applied at 50 kg N ha\(^{-1}\), Mislevy et al. (2005) reported annual herbage accumulation of 10.3 Mg ha\(^{-1}\) across 3 yr of monthly harvests.

‘Tifton 85’ Bermudagrass in Florida

Hay production is a significant enterprise in Florida’s agricultural commodity sector with a value of $130 million in sales per year and having over 117,000 ha in
production (USDA NASS, 2016). Feeding hay is an attractive option during the cool season because forage growth is limited during late fall, winter, and early spring throughout Florida. A challenge to hay conservation is high humidity and frequent rainfall that occur during the summer months and that hinders the hay drying process (Chambliss et al., 2006). Bermudagrass \( \text{Cynodon dactylon} \) (L.) Pers.] is the most commonly planted species in Florida hay production systems. In north Florida, specifically, r improved bermudagrass hybrids are among the most popular grasses given they are highly productive and tend to dry faster than other similar grasses (Chambliss et al., 2006).

Bermudagrass is an introduced plant species from South Africa. ‘Tifton 85’ bermudagrass was released in 1992 by the USDA-ARS in cooperation with the University of Georgia (Burton et al., 1993). It is a hybrid of ‘Tifton 68’ bermudagrass and PI 290884 and produces greater herbage accumulation, greater digestibility, and overall greater nutritive value compared to other bermudagrass entries (Agyin-Birikoran et al., 2012; Burton et al., 1993). Several studies evaluating Tifton 85 in Florida have shown that fertilized swards can have crude protein (CP) concentrations ranging from 160 to 180 g kg\(^{-1}\) (Michelangeli et al., 2010; Agyin-Birikoran et al., 2012). Agyin-Birikoran et al. (2012) reported, in a two-year study, that unfertilized Tifton 85 had CP concentrations of 109 and 104 g kg\(^{-1}\), over two consecutive yr, highlighting the importance of fertilization in Tifton 85 swards since fertilized swards were greater in CP. In the same study, Agyin-Birikoran et al. (2012) also concluded there was no significant differences in CP or IVDOM in plots treated with 50, 70, or 90 kg N ha\(^{-1}\). These authors also found that
plots with 90 kg N ha\(^{-1}\) were reported to have groundwater NO\(_3\)-N levels greater than allowed by the Environmental Protection Agency (EPA) regulations.

One of the main attributes of Tifton 85 is its high productivity when compared to other bermudagrass entries (Hill et al., 1993; Mandebvu et al., 1999; Michelangeli et al., 2010). Hill et al. (1993) compared annual DM yield among four bermudagrass entries (Tifton 85, Tifton 68, Tifton 44, and Coastal) and concluded that Tifton 85 produced significantly greater DM (18.6 t ha\(^{-1}\)) than the others, including 26% more than Coastal (Hill et al., 1993). More specific to hay production systems, Michelangeli et al. (2010) described a study in North Central Florida, where the relationship of stubble height (7.6 and 10 cm) and harvest interval (21, 24, 27, and 35 d) were investigated. They concluded that greatest herbage accumulation was obtained at lower stubble heights and when harvests occurred at 35 d. However, harvesting at such short stubble height increased weed encroachment and therefore, was not recommended. Nevertheless, data showed that harvesting at a 10-cm stubble heights with 24 to 27 d harvest intervals increased CP and IVDOM (Michelangeli et al., 2010).

Importance of Grass-Legume Mixed Stands

Legumes are significant contributors to the overall sustainability of pasture ecosystems through the addition of protein in animal diets, as well as through biological nitrogen fixation (BNF) (Russelle, 2008). Although dinitrogen (N\(_2\)) is the most abundant atmospheric gas, it is largely unavailable for plant use since the N\(_2\) molecule is very stable. The triple bond holding the two N atoms together requires a high amount of energy to break apart. Legumes have a symbiotic association with rhizobia (the six recognized genera: \textit{Allorhizobium}, \textit{Azorhizobium}, \textit{Bradyrhizobium}, \textit{Mesorhizobium}, \textit{Sinorhizobium}, and \textit{Rhizobium}) that involves the infection of the host plant through
various entry points in root tissue (Sprent, 1992; Russelle, 2008). Most commonly, legumes used in agriculture are infected through root hairs, however peanut (*Arachis* spp.) and *Stylosanthes* are exceptions in which they are infected at the sites of lateral root emergence (Russelle, 2008). Once infected, rhizobia form nodules, which are specific tissue structures where BNF occurs through various biochemical reactions (Russelle, 2008). Nitrogenase is the enzyme responsible for the conversion of N\(_2\) into NH\(_3\), which yields two NH\(_3\) molecules and one H\(_2\), and requiring 16 ATP for every N\(_2\) molecule (Russelle, 2008). In the absence of legumes, N\(_2\) can also be fixed via free living rhizosphere diazotrophs, facultative endophytic diazotrophs, or obligate endophytic diazotrophs, which are found within the rhizosphere (Baldani et al., 1997). It is estimated that legumes, which are grown on about 250 million ha worldwide, are responsible for fixing an estimated 40 Tg of N yr\(^{-1}\) with an average of 160 kg N ha\(^{-1}\) yr\(^{-1}\) (Kinzing and Socolow, 1994). It is also important to note that BNF is directly influenced by factors such as soil nutrition, the strain of *Rhizobium*, plant species and cultivar, season, environment, and climate (Russelle, 2008; Rouquette and Smith, 2010). In all, BNF serves to reduce the overall nitrogen inputs into a production system through reducing demand for synthetic fertilizers, which also translates into reduced economic costs for producers in purchasing these synthetic fertilizers.

An additional benefit of BNF for grass-legume mixed stands is the N transfer from the legume to the grass. Dinitrogen fixed by legumes in binary stands can be transferred to the grass components through active N transfer (the release of N compounds from the legume into the soil), which in turn has a direct impact on growth and production of the grass component of the specific stand (Rouquette and Smith,
It is important to note that the amount of N transferred in this manner is significantly less than the amount of N\textsubscript{2} fixed from the atmosphere (Dubach and Russelle, 1994). The dominant pathway for the release of fixed N from living legume plants is through the decomposition of dead roots and nodules (Dubach and Russelle, 1994). It is difficult to quantify the exact amount of N transfer from legume to grasses grown in association due to large background fluxes affecting the net effect measurements and also the fact that excluding one species from a system fundamentally alters a system (Høgh-Jensen, 2006).

Nutrient cycling, specifically N cycling, differs in grazing and hay systems. In grazing systems there are more opportunities for nutrient re-cycling through litter deposition and animal excretion (Dubeux et al., 2007). Plant litter deposition is a significant N pool because it is the direct link between N in metabolically active plants and N available for plant uptake, while also affecting the net balance between immobilization and mineralization of soil nutrients (Thomas and Asakawa, 1993; Myers et al., 1994; Dubeux et al., 2007). Plant litter decomposition occurs in a continuous manner under grazing, whereas in hay systems litter decomposition is minimal since there is inherently less material available for decomposition (Eilitta et al., 2003; Dubeux et al., 2007). Plant litter quality is also an important factor to consider in nutrient cycling (Dubeux et al., 2007). It is especially important in Florida’s pastures since most of the litter pool consists of poor-quality plant material, thus acting primarily as a sink of soil available N (Thomas and Asakawa, 1993; Myers et al., 1994; Dubeux et al., 2007). One way of increasing plant litter quality is through establishing legume-grass mixtures, as
these can potentially improve the nutrient turnover and overall nutrient bioavailability during litter mineralization (Dubeux et al., 2007).

Animal excretion is an important contributor to nutrient cycling and redistribution within grazing systems (Dubeux et al., 2007). In hay systems, nutrient redistribution is minimal since fertilization events tend to distribute nutrients in a more homogenous manner, whereas in grazing systems nutrients in urine and feces tend to be deposited and recycled in distinct areas within a pasture (Dubeux et al., 2007). Both feces and urine are important in nutrient recycling since retention of ingested nutrients is low within adult animal body tissue; as a result, mineral nutrients are excreted in feces and urine (Rotz et al., 2005; Dubeux et al., 2007). Feces tend to be rich in P, Ca, Mg, Fe, Cu, Mn, and Zn, while urine tends to be rich in K and Na (Dubeux et al., 2007). Nitrogen and S are excreted in both, urine and feces, although N proportion tends to increase in urine as dietary N and S concentrations increase (Matthews et al., 1994; Dubeux et al., 2007). In all, nutrient content within fecal and urinary excretions offer the most significant pathway for nutrient transfer and nutrient re-cycling.

When grown in mixtures with C₄ grasses, N transfer from the legume to grass component helps to increase the overall nutritive value of the grass (Sleugh et al., 2000; Muir et al., 2011). With only a few exceptions, CP concentrations of legumes rarely fall below 70 g kg⁻¹ and digestibility tends to range between 600 and 800 g kg⁻¹ (Muir et al., 2011). In low-input systems, mixed stands serve to contribute to overall animal performance through increased nutritive value and increased DM intake (Stobbs, 1975). Foster et al. (2009) quantified this process, showing mixed stand systems having increases in DM and N intake, as well as improved microbial N-synthesis and
digestibility in lambs fed bahiagrass hay and supplemented with annual peanut (*Arachis hypogaea* L.) and rhizoma peanut (RP; *Arachis glabrata* Benth) hays when compared with lambs consuming only bahiagrass hay. Furthermore, growing legume-grass mixed stands has also been shown to increase the overall productivity of a system through increases in DM yields, forage quality, and improvements in seasonal forage distribution (Baylor, 1974; Sleugh et al., 2000; Muir et al., 2011). Sleugh et al. (2000) evaluated the effects of alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and kura clover (*Trifolium ambiguum* Bieb.) grown in binary mixtures with orchardgrass (*Dactylis glomerata* L.), smooth bromegrass (*Bromus inermis* L.), and intermediate wheatgrass (*Thinopyrum intermedium* Barkworth & D.R. Dewey) on forage yield and nutritive value characteristics. Results showed lower CP, herbage accumulation, and IVOMD for grasses grown in monoculture and greater values resulted for grass-legume mixtures (Sleugh et al., 2000). Additionally, it was also reported that mixtures containing legumes increased the season distribution of forage yield (Sleugh et al., 2000), which further points out the benefits from inclusion of mixed-stands.

Though legumes have made large contributions in temperate-climate regions, their potential in tropical regions has yet to be realized (Shelton et al., 2005). In northeastern US and Canada, pastures have been historically developed through incorporating temperate grasses in mixtures with temperate legumes, most commonly using alfalfa, white clover (*Trifolium repens* L.), or red clover (*T. pretense* L.) (Ahlgren, 1949; Muir et al., 2011). In the U.S. Southern Coastal Plain, however, warm-season legume implementation has been rather limited. Trannin et al. (2000) suggested legume
persistence in mixed tropical-grass legume pastures has been poor due to strong competitiveness of the grass associated with its extensive root system, high N and P utilization efficiency, and its grazing tolerance. In Florida, aeschynomene (Aeschynomene americana L.) and carpon desmodium (Desmodium heterocarpon (L.) DC.) are the most commonly used tropical legumes planted on bahiagrass pastures and are both found on approximately 80,000 ha in the state, though this quantity is largely decreasing (Sollenberger and Kalmbacher, 2005). Reduced rates of legume establishment have led to increased fertilizer use since fertilizing can often improve stand establishment, while also producing greater herbage accumulation (Sollenberger and Kalmbacher, 2005). Butler et al. (2012) also express that certain legumes can be viable replacements for commercial fertilizers (within specific systems studied) but adoption of legumes among producers is largely limited due by the ease of N fertilization practices and weed control.

Peanut as a Forage in Florida

Rhizoma Peanut

Rhizoma peanut (RP; Arachis glabrata Benth) is a warm-season perennial legume introduced to Florida from Mato Grosso, Brazil in 1936 (French et al., 1993; Prine et al., 2010). In 1949 this early plant introduction, originally cataloged as accession PI 118457, was planted and assessed at the Brooksville and Arcadia Plant Materials Centers and was eventually named ‘Arb’ (French et al., 1993). Research efforts continued through the 1960s and 1980s which focused on developing RP for forage and ornamental use (French et al., 1993; Prine et al., 2010; Quesenberry et al., 2010). In present day, there are several cultivars and germplasms commercially available for both forage and ornamental use.
A. glabrata is unlike the commercial edible peanut (A. hypogaea L.) in that it is a perennial that produces very few viable seeds and is propagated vegetatively through belowground rhizomes (Quesenberry et al., 2010). The common name rhizoma peanut was suggested with aims of differentiating between other perennial, but stoloniferous Arachis spp. such as A. pintoi Krapov & W.C. Greg (Quesenberry et al., 2010). Rhizoma peanut varieties have adapted well to the well-drained soils and warmer climates of the US Southern Coastal Plain (French et al., 1993). More recent research efforts in RP have been focused on improvement of establishment and grazing tolerance (Quesenberry et al., 2010).

Adoption of RP has been limited among pasture producers in Florida mainly due to high costs related to establishment (approximately $1000 ha⁻¹), weed management, and having to take land out of production for multiple growing seasons (Williams et al., 1997; Prine et al., 2010; Quesenberry et al., 2010; Castillo et al., 2013b). The recommended planting time for RP throughout Florida is February and March, as it is during this time that aboveground biomass and new sprout growth is restricted by freezing temperatures, which minimize losses during mechanical digging (Williams et al., 1997). Williams et al. (1997) examined the interactions of cultivar, planting date, and location within FL, on the establishment of RP. In this study, Williams et al. (1997) expressed that establishment of RP is more variable than once believed and noted that having ample soil moisture content for 60 to 90 d post planting should be the first criterion for deciding when to plant. Ample soil moisture during this period is critical for preventing sprout desiccation and death (Castillo et al., 2013b). Since there can be multiple periods in a year that meet these criteria, the planting date should be chosen
with the longest frost-free period after planting (Williams et al., 1997). Late spring rainfall is typically abundant in North Florida thus April planting proved to be advantageous when compared with summer plantings (Williams et al., 1997).

Strip planting of RP has been shown to be an effective, relatively low-cost, and long-term solution to the problems related with RP adoption among producers (Cook et al., 1993; Whitbread et al., 2009; Castillo et al., 2013a). This technique involves planting vegetative material in strips, typically 4-m wide (although this can vary from different tractor implements), leaving certain portions of warm-season grass sod unplanted (Castillo et al., 2013a). By implementing this strategy, the producer will spend less money since less area is planted, and there is the added benefit of RP spreading into the surrounding grass areas with proper management, to become a binary mixture (Castillo et al., 2013b). Castillo et al. (2014) examined the effects of four seedbed preparation techniques in strip-planted RP: 1) glyphosate+tillage, 2) tillage only, 3) glyphosate + no-till, and 4) sod removal; and four post-emergence weed control strategies: 1) control (no herbicides, no mowing), 2) mowing (every 28 d to 10-cm stubble height), 3) imazapic (0.29 and 0.58 L ha⁻¹), and 4) imazapic + 2,4-D amine (0.29 and 0.58 L ha⁻¹). Results from this study indicate that the most cost effective manner of seedbed preparation for RP strip planting is through an application of glyphosate + no-till followed by an application of imazapic with or without 2, 4-D amine (Castillo et al., 2014).

Since it is recommended for planted areas to be taken out of production for multiple growing seasons, Castillo et al. (2013a) determined the best defoliation management strategy during the first year of planting RP strips onto warm-season grass
sod is through hay production. Although it is best to avoid defoliation in the first year of establishment, it is advised that the pasture not be grazed but cut for hay (Castillo et al., 2013a). Hay harvests within the period of establishment can potentially increase the commodity value of hay, which helps with the overall economics of the system.

Rhizoma peanut cultivars: *A. glabrata* cv. ‘Florigraze’, registered in 1978, is a joint release by the University of Florida and USDA Soil Conservation Service (Prine et al., 1986). Dr. G.M. Prine observed the rapid spread of RP plants between the germplasm Arb and PI151982 RP plots in 1962, and later released Florigraze (Prine et al., 1986). In its morphology, Florigraze has a finer stem and narrower leaflets when compared to Arb and Arblick (Prine et al., 1986). Florigraze grows well in moderately to well-drained soils, but it is not adapted to “flatwoods” soils having poor drainage (Prine et al., 1986). Mislevy et al. (2007) reported a 5-yr average (from 2002 through 2006 growing seasons) of 10,590 kg ha⁻¹ yr⁻¹ herbage yield for Florigraze. Crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) were reported as 180, 340, 450, and 550 g kg⁻¹, respectively (Quesenberry et al., 2010). Florigraze is the most commonly planted RP species in Florida (Quesenberry et al., 2010). Peanut stunt virus (PSV) has been identified in commercial Florigraze fields, generating concerns on the genetic vulnerability of this cultivar (Quesenberry et al., 2010).

Ecoturf was released as a dual purpose RP germplasm by Dr. G.M. Prine of the University of Florida; it can be used as an ornamental, or ground-cover species since it has a more prostrate growth habit compared with Florigraze, or it can be used as a forage (Prine et al., 2010). Ecoturf has been valued as an ornamental species given it
produces a daily abundance of bright, yellow flowers (Prine et al., 2010). Leaflets tend to be longer but not as wide as Arblick leaflets (Prine et al., 2010). Dry matter yield, on a 5-yr average (from 2002 through 2006 growing seasons) has been reported as 8310 kg ha\(^{-1}\) yr\(^{-1}\) (Mislevy et al., 2007). Crude protein, ADF, NDF, and TDN are reported as 200, 340, 430, and 570 g kg\(^{-1}\) grown in North Florida (Mislevy et al., 2007). Unlike Florigraze, there were no PSV incidences reported, indicating a probable genetic resistance to PSV (Quesenberry et al., 2010). Although herbage accumulation is not quite as high as with other RP entries under clipping management, Ecoturf was shown to be an excellent ground cover species and, with proper management, can reach acceptable forage yields and nutritive values (Prine et al., 2010; Quesenberry et al., 2010). Mullenix et al. (2016) found no difference in herbage accumulation among Florigraze, Ecoturf, 'UF-Tito', and ‘UF-Peace’ when grazed and intensity of grazing was expressed as a percentage of pre-grazing sward height removed during the grazing period.

**Other Peanut Species**

*Arachis pintoi* is another multi-use legume in that it can be used as forage, ground cover, erosion control, and ornamental purposes (Carvalho and Quesenberry, 2009). It is a stoloniferous, low-growing species with axonomorphous roots, and cylindrical, angular, hollow stems (Carvalho and Quesenberry, 2009). *A. pintoi* produces small fruit in an underground articulated legume form which contains a single seed (Cook et al., 1990). *A. pintoi* has been an important legume in the tropics. Fisher and Cruz (1993) noted that *A. pintoi* is close to being an ideal pasture legume ideotype given it has procumbent stolons, which allow for its grazing tolerance and resistance to animal treading. Mixed stand production with bahiagrass in Australia has been reported by Bowman and Wilson (1996), though most *A. pintoi* production remains in South
America. In Florida, *A. pintoi* adoption remains low due to high costs of seed ($100 kg\(^{-1}\)) and uncertainty about cold tolerance. Carvalho and Quesenberry (2012) assessed several *A. pintoi* entries in Florida and concluded an average HA of 4.35 Mg ha\(^{-1}\), for 2003, with CP and IVOMD of 180 and 670 g kg\(^{-1}\), respectively (Carvalho and Quesenberry, 2012).

*A. hypogaea*, or common, annual peanut, is an important food crop throughout the world. The forage use of this species has been limited due to two main reasons: 1) it is planted primarily for its seed, for human consumption and 2) the fungicides applied during growth make its forage use illegal according to the fungicide label (Myer et al., 2010b). Fungicides are commonly used in annual peanut production to prevent late leaf spot (Myer et al., 2010b). Gorbet et al. (1994) evaluated the potential hay forage use of annual peanut with the assumption that without harvesting the seed, the plant would be able to self-seed and re-emerge over successive growing season. Two cuttings were obtained each year for three growing seasons, in which 4,000 kg DM ha\(^{-1}\) was recorded for the first year, a 27% decrease was noted for the second year, and a further 6% decrease for the third year (Myer et al., 2010b). From various *A. hypogaea* accessions grown in North Florida, CP and IVOMD averaged at 15.7 and 646 g kg\(^{-1}\), respectively (Gorbet et al., 1994). Myer et al. (2010) conducted a grazing study in which an annual peanut pasture was rotationally stocked for two subsequent growing seasons. For the first year, the annual peanut provided sufficient forage yield (6,078 kg ha\(^{-1}\)) early in the grazing season; however, forage quality began to decline later in the grazing season (Myer et al., 2010b). There was a lack of re-growth for the second year and the grazing season was cut short due to a lack of forage (Myer et al., 2010b).
CHAPTER 2
ANNUAL AND PERENNIAL PEANUT MIXED WITH ‘PENSACOLA’ BAHIAGRASS IN NORTH FLORIDA

Introduction

Nitrogen fertilization is among the largest expenses in forage based cow-calf operations in Florida. Carbon emissions from production, transportation, storage, and distribution of N fertilizers range from 3.3-6.6 kg CO₂ equivalent per kg of N produced (Lal, 2004). In addition, over-fertilization of agricultural land has led to groundwater pollution through nitrate leaching (cite – you can make a good case for this particularly for FL); therefore, decreasing overall N fertilizer inputs in pastures can bring environmental benefits while simultaneously increasing economic gains for producers. Because of high fertilizer costs, producers are opting out of fertilization. A lack of maintenance fertilization has led to pasture and grassland decline (Boddey et al., 2004). A possible mode of mitigation for excess N fertilizer use leading to leaching is through promoting grass-legume mixed stands since they have the potential to improve overall land productivity through increases in herbage yield and forage quality, while also making improvements in seasonal forage distribution (Baylor, 1974; Sleugh et al., 2000; Muir et al., 2011).

Even with these benefits in mind, legume adoption among producers is minimal. Butler et al. (2012) mentioned that although annual legumes are viable replacements for commercial N fertilizer in cool-season rye (Secale cereale L.)- annual ryegrass (Lolium multiflorum Lam.) mixed pastures, the adoption rates of legumes has been limited due to the ease of N fertilizer application. Similarly, Sollenberger and Kalmbacher (2005) state that in Florida specifically, low legume adoption rates have been a direct result of producer’s past experiences with poor establishment and stand failure of the mixed
species systems, and producers will thus opt for fertilizing as it can be a more dependable mode of producing increased forage mass.

There are a wide selection of cool-season legumes available in the market; however, when it comes to perennial warm-season legumes, the options are limited. Aeschynomene (an annual species; *Aeschynomene americana* L.), and carpon desmodium [a perennial species; *Desmodium heterocarpon* (L.) D.C.] are viable species most commonly planted in central and south Florida pastures, though adoption rates among producers have steadily decreased through the years mostly due to issues with establishment (Sollenberger and Kalmbacher, 2005). Rhizoma peanut (RP; *Arachis glabrata* Benth), a vegetatively propagated perennial legume, is another viable option. Rhizoma peanut was introduced to Florida from Brazil in 1939 (French et al., 1993; Prine et al., 2010). Adoption rates of RP in grazing systems have been minimal, mostly due to high costs of vegetative establishment, and the long establishment period which often requires land to be taken out of production for up to three growing seasons (Williams et al., 1997; Prine et al., 2010; Quesenberry et al., 2010; Castillo et al., 2013a). Rhizoma peanut is used extensively as a high value hay crop in Florida.

Seeded peanut may provide another option for incorporating legumes to grass stands. Two potential species are *Arachis hypogaea* L. and *Arachis pintoi* Krap. & W.C. Greg. *A. hypogaea*, otherwise known as the commercial edible peanut, is an annual species commonly planted commercially in the southeastern U.S for its seed production. Several cultivars of *A. hypogaea* planted in Florida were developed and selected for high content of oleic acid, which is beneficial not only in terms of human nutrition, but also of extended shelf life (Tillman, 2013). Of these cultivars, the
‘TUFRunner’ line has been among the highest performers in terms of disease resistance and having high yield potential (Tillman, 2013). One specific cultivar, TUFRunner ‘727’ is rated among the best in terms of leaf spot, *Tomato spotted wilt virus* resistance, and white mold disease resistance when compared with similar cultivars commonly planted in the southeast (Tillman, 2013). However, from the forage perspective, Myer et al. (2010) reported declining herbage accumulation (HA) for TUFRunner 727. Accessions of other *A. hypogaea* grown in North Florida are reported to have crude protein (CP) and *in vitro* organic matter digestibility (IVOMD) of 157 and 646 g kg\(^{-1}\), respectively (Gorbet et al., 1994).

*A. pintoi* on the other hand, is not commonly found in Florida. *A. pintoi* is a perennial, stoloniferous, prostrate growing species that is especially important legume in tropical regions due to its grazing tolerance (Cook et al., 1990; Fisher and Cruz, 1993). In South America, special attention has been given to *A. pintoi*, a multi-purpose legume, that can be used for forage, ground cover, and as an ornamental (Valentim et al., 2001). Throughout the world, several *A. pintoi* cultivars have been released for forage, including ‘Amarillo’ in Australia, ‘Mani Forrajero’ in Colombia and Bolivia, ‘Mani Mejorador’ in Costa Rica, ‘Pico Bonito’ in Mexico and Honduras, and ‘Golden Glory’ in USA (Valentim et al., 2001). Carvalho and Quesenberry (2012) evaluated *A. pintoi* accessions introduced to Florida and reported an average herbage yield of 4.35 Mg ha\(^{-1}\) and CP and IVOMD of 180 and 670 g kg\(^{-1}\), respectively. Cultivars of these peanut species, *A. hypogaea* and *A. pintoi*, have been minimally studied as part of mixed stands with warm-season grasses in North Florida.
One common warm-season grass utilized in systems within this region is Bahiagrass. It is the most commonly planted perennial forage species in Florida, occurring on over one million hectares (Newman et al., 2011). Bahiagrass is persistent and reliable as a forage species in that it is capable of tolerating intensive, continuous stocking (Sollenberger et al., 1988). Depending on nutrient management, typical CP concentrations can range from 99 to 140 g kg\(^{-1}\), and IVOMD can range from 459 to 505 g kg\(^{-1}\) (Stewart et al., 2007). Herbage accumulation can range from 3.3 to 11.2 Mg DM ha\(^{-1}\), depending on nutrient management practices (Newman et al., 2011). Stewart et al. (2007) reported herbage accumulation of 3.42 Mg DM ha\(^{-1}\) when 40 kg N ha\(^{-1}\) yr\(^{-1}\) was applied under continuous stocking. Several studies have examined the integration of RP into bahiagrass swards. Williams et al. (1991) reported average daily gains (ADG) of 0.79 kg on steers grazing RP-bahiagrass pastures and 0.51 kg when grazing only bahiagrass. The authors also reported no apparent decline in the mixed swards in the subsequent years (Williams et al., 1991). Based on these results, there is potential for developing other mixed forage systems utilizing bahiagrass in combination with other *Arachis* species that would decrease the need for N fertilizer inputs.

To assess other mixed systems, we evaluated the potential use of *A. hypogaea* L. and its re-seeding potential in subsequent growing seasons. We also evaluated the performance of *A. pintoi* and two *A. glabrata* entries in mixtures with bahiagrass. The overall objectives were to: i) determine viability of *A. hypogaea* for forage use in mixed swards; ii) evaluate the performance of *A. pintoi* and *A. glabrata* in North Florida during the establishment period when mixed with bahiagrass; and iii) to make recommendations about which peanut species is best suited for incorporation into
bahiagrass pastures in low-input systems in order to decrease the overall need for N fertilization in North Florida.

**Materials and Methods**

**Experimental Site**

The study was conducted during the 2014, 2015, and 2016 growing seasons at the University of Florida - North Florida Research and Education Center (NFREC), in Marianna, Florida (30°52’ N, 85°11’ W, 35 m altitude). The soil at the experimental site is an Orangeburg loamy sand (fine-loamy- kaolinitic, thermic Typic Kandiudults). Initial composite soil samples (0-15 cm) were collected in May 2014 and results indicated a soil pH of 6.4 and Mehlich-1 extractable P, K, Mg, and Ca concentrations of 22, 67, 80, and 370 mg kg⁻¹, respectively. Soil organic matter (SOM) was 8.6 g kg⁻¹, and CEC was 3.5 meq 100g⁻¹. Total rainfall for 2014, 2015, and 2016 was 1,573, 1,403, and 1,378 mm, respectively (Figure 2-1).

**Treatments, Plot Establishment, and Design**

Plots were allocated on a well-established (10+ yr) ‘Pensacola’ bahiagrass (Paspalum notatum Flügge) pasture. Treatments consisted of unfertilized Pensacola bahiagrass monoculture, one RP (*Arachis glabrata* Benth) cultivar (‘Florigraze’) and one germplasm (‘Ecoturf’), *Arachis hypogaea* cv. TUFRunner‘727’ and *Arachis pintoi* cv. ‘Amarillo’. Rhizoma peanut entries were planted on 17 Apr. 2014, using 1,300 kg rhizomes ha⁻¹, and a Holden Sodmaster No-till Bermudagrass Sprigger (Holden-Sodmaker, Southwest City, MO) with four rows spaced 71 cm apart. The area was cultivpapped using a Brillion cultipacker (Landoll Corp.; Marysville, KS) after planting. Plots with *A. hypogaea* and *A. pintoi* were planted 5 May 2014. The plots were strip-tilled before planting with rows 71 cm apart and eight rows per plot before planting.
Seeds were planted using a twin-row planter (Burch Farm Implements Inc.; Evansville, IN) with rows 71 cm apart and eight rows per plot, placing 10 seeds per linear meter, resulting in seeding rates for *Arachis pintoi* and *A. hypogaea* of 12 and 110 kg ha\(^{-1}\), respectively. Seeds were treated with a fungicide, Dynasty PD (Syngenta Crop Production) (a.i. Azoxystrobin (C\(_{22}\)H\(_{17}\)N\(_3\)O\(_5\)), Fludioxonil (C\(_{12}\)H\(_6\)F\(_2\)N\(_2\)O\(_2\)), Mefenoxam (C\(_{15}\)H\(_{21}\)NO\(_4\)), at 2.6 mL kg\(^{-1}\). *Arachis hypogaea* seeds were inoculated with Graph-EX SA for peanuts [ABM: Advanced Biological Marketing (5 x 10\(^9\) Rhizobia spp. + 1 x 108 cfu gm\(^{-1}\) Trichoderma spp.)] at 15 mL per 11 kg seed (1.36 mL kg\(^{-1}\)), and *A. pintoi* seeds were inoculated with CIAT 3103 *Arachis pintoi* inoculant [Centro de Investigacion de Agricultura Tropical (1 x 10\(^9\) Bradyrhizobium spp.) at 25 g of inoculant per 25 kg seed]. For weed control, Prowl H\(_2\)O [BASF Corporation (pendimethalin; C\(_{13}\)H\(_{19}\)N\(_3\)O\(_4\))] was applied on 24 Mar. 2015 at 2.33 L ha\(^{-1}\). Each plot was fertilized with 7 kg P ha\(^{-1}\) and 56 kg K ha\(^{-1}\), following each harvest (336 kg ha\(^{-1}\) of the commercial formula 0-5-20), except for 24 Sept. 2015, 20 May 2015, and 6 May 2016, where 336 kg ha\(^{-1}\) of Kmag (183 g K kg\(^{-1}\), 108 g Mg kg\(^{-1}\), 220 g S kg\(^{-1}\)) was applied, which is equivalent to 61 kg K ha\(^{-1}\), 36 kg Mg ha\(^{-1}\), 74 kg S ha\(^{-1}\). Irrigation (29 mm) was applied 13 May 2014 using a lateral irrigation system. Each plot measured 6 x 6 m, with no alleys between adjacent plots. Experimental design was a randomized complete block design with four replications per treatment.

**Sampling Techniques**

Plots were harvested every 5 wk (Table 2.1) at 10-cm stubble height. Herbage accumulation was determined by cutting a 3 x 1 m area using a flail style mower (Carter Mfg. Co. Brookston, IN). Peanut height was measured by averaging 10 random measurements of peanut plants within each plot. Peanut stand per linear meter was
measured by placing a 1-m ruler parallel to the planted peanut rows and counting the number of plants within that length. An average of three measurements was obtained for each plot during each sampling period.

Botanical composition was determined by harvesting all herbage above a 10-cm stubble within one 0.25-m² quadrat. After removal of samples for herbage accumulation and botanical composition, the entire plot was staged to the same stubble height using a rotary chopper (John Deere 972; Deere Company, Moline, IL). Hand-harvested samples were hand-separated for determination of botanical composition on a dry matter basis. All samples were dried at 55°C to constant weight, then ground to pass through a 1-mm stainless steel screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill; Thomas Scientific, Swedesboro, NJ). Samples were ball milled using a Mixer Mill MM400 (Retsch, Newton, PA, USA) at 25 Hz for 9 min. Samples were then analyzed for total N using a CHNS analyzer through the Dumas dry combustion method (Vario Micro Cube; Elementar, Hanau, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK). In-vitro organic matter digestibility (IVOMD) was determined using the two-stage technique described by Moore and Mott (1974).

Roots and rhizomes were collected using a 20-cm deep x 10.8-cm diameter golf hole cutter (Standard Gold Company, Cedar Falls, IA, USA). Two cores per sampling were obtained from each plot on November 2015 and November 2016. Samples were dried at 55°C to a constant weight and final biomass was determined. Root and rhizome samples were washed using an 850-µm sieve and dried once again to a constant weight, and final biomass determined. Dried root and rhizome samples were ground to
pass through a 2-mm stainless steel screen using a Willey Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ) then ball milled using a Mixer Mill MM400 (Retsch, Newton, PA, USA) at 25 Hz for 9 min. Samples were analyzed for total N using the same protocols for shoot samples. Soil bulk density was determined using the undisturbed core method described by Grossman and Reinsch (2002), in order to estimate the root and rhizome mass per hectare from 0- to 20-cm soil depth. The results were expressed on an ash-free basis (i.e., OM) to avoid contamination with soil particles.

Biological N\textsubscript{2}-fixation (BNF) was determined using the natural abundance technique (Freitas et al., 2010). Reference plant samples were obtained from adjacent, unfertilized, 'Tifton 85' bermudagrass (Cynodon spp.) plots that were harvested on the same sampling dates. Proportion of plant N derived from atmosphere (\%Nd\textsubscript{fa}) was estimated using the equation described by Shearer and Kohl (1986):

\[
\text{%Nd\textsubscript{fa}} = \frac{(\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{N\textsubscript{2}-fixing legume}})}{\delta^{15}\text{N}_{\text{reference}} - B} \times 100
\]

In which \(\delta^{15}\text{N}_{\text{reference}}\) is the \(\delta^{15}\text{N}\) value for the non N\textsubscript{2}-fixing reference plant, \(\delta^{15}\text{N}_{\text{N\textsubscript{2}-fixing legume}}\) is the \(\delta^{15}\text{N}\) value for the N\textsubscript{2}-fixing rhizoma or seeded peanut cultivar in this study, and \(B\) is the \(\delta^{15}\text{N}\) value for the N\textsubscript{2}-fixing plant grown in the absence of inorganic N. The \(B\) value used in this study was reported by Okiko et al. (2004) for A. hypogaea L. The \(\delta^{15}\text{N}\) from reference plants ranged from -0.15 to 1.44‰ with confidence interval (\(P < 0.05\)) of 0.82 ± 0.37‰, with values changing with sampling date. Shoot N yield was estimated by multiplying aboveground biomass by total N concentration. Shoot BNF was estimated by multiplying shoot N yield by \%Nd\textsubscript{fa}. 
The proportion of legume in the root and rhizomes was estimated by using $\delta^{15}$N two pool mixing model explained by Fry (2008):

$$f_{\text{total}} = \frac{(\delta_{\text{sample}} - \delta_{\text{source 2}})}{(\delta_{\text{source 1}} - \delta_{\text{source 2}})}$$

Where $f_{\text{total}}$ is the contribution (percentage) of the source (in this case, the peanut percentage in the root and rhizomes), $\delta_{\text{sample}}$ is the $\delta^{15}$N of the sample, and $\delta_{\text{source 2}}$ is the $\delta^{15}$N of peanut roots in pure stand. Pure peanut root samples were obtained from dried root material as to obtain a standard value across all harvests. The $\delta_{\text{source 1}}$ is the $\delta^{15}$N from unfertilized bahiagrass.

**Statistical Analyses**

Data were analyzed using the PROC MIXED procedure in SAS (SAS for Windows V 9.4, SAS Institute, 2009, Cary, NC, USA). Random effects includes blocks and block x treatment. Fixed effects included peanut cultivar and species, harvest date, and year for root and rhizome data. Repeated measures procedure was used for herbage responses and harvest date was the repeated measure. The LSMEANS were compared with the PDIFF procedure adjusted for Tukey’s test. Differences were declared significant at $P \leq 0.05$.

**Results and Discussion**

**Herbage Responses**

There was a treatment by harvest date interaction for herbage accumulation (HA; $P < 0.0001$). The yearly total herbage accumulation is reported in Table 2-2. Herbage accumulation did not differ across treatments for 2014 and 2015 growing seasons (Figure 2-2). However, in 2016 rhizoma peanut-bahiagrass (BG) mixtures produced greater herbage accumulation than unfertilized BG ($P = 0.01$), producing an average of 693 and 541 kg DM ha$^{-1}$ harvest$^{-1}$, respectively. Both rhizoma peanut treatments
(Florigraze and Ecoturf) showed greater herbage accumulation in the 2016 growing season than the other treatments. These results are in accordance with previous literature stating that it can take up to three growing seasons for rhizoma peanut to establish before becoming productive (Adjei and Prine, 1976; Prine et al., 1986). When establishing peanut-grass mixtures, it is important to harvest at appropriate periods in order to prevent grasses from shading out the peanut since grasses often grow to a greater height than peanut species. Castillo et al. (2013b) studied defoliation effects during the year if planting rhizoma peanut in strips in bahiagrass swards. They concluded that hay production is the best option for defoliation management during that year. Grazing was not a useful option because cattle heavily grazed the rhizoma peanut strips before starting to graze bahiagrass. The authors concluded that if swards are to be grazed, successful establishment would depend upon removing cattle before they overgrazed the RP (Castillo et al., 2013b).

Botanical composition had a treatment by harvest date interaction ($P < 0.0001$; Figure 2-3). Presence of rhizoma peanut cultivars (Florigraze and Ecoturf) was minimal for the first two growing seasons (2014 and 2015), but they showed increased participation in botanical composition during the 2016 growing season. Ecoturf had a greater participation in each evaluation, with an average increase from 6 to 24%, from 2014 to 2016. Although not examined in this present study, Florigraze has been reported to be affected by the peanut stunt virus. whereas Ecoturf exhibits greater tolerance; which might partly explain why Florigraze did not perform as well as Ecoturf (Quesenberry et al., 2010). In addition, Ecoturf has a more prostrate growth habit and spreads aggressively (Mullenix et al., 2015, 2016) which may make it more competitive
with bahiagrass than Florigraze. TUFRunner 727 had an 11% average participation during 2014, 8% average participation in 2015, and 5% participation in 2016. TUFRunner 727 showed a 43% decrease on average, from 2014 to 2016. TUFRunner 727 did re-seed naturally, however, the re-seeding was minimal and the stand was not able to reach the same population size from the previous year. Further research should be conducted to determine the best harvesting frequency or best stubble height for the use of annual peanut in forage systems. Since there was evidence of re-seeding by this cultivar, it can potentially be planted on a bi-annual basis with the idea that a seed bank will be created, and therefore the costs of planting can be spread out over multiple growing seasons. The other benefit of using this species in the North Florida area is that planting equipment is widely available among local producers, since peanuts are commonly planted within the region. Amarillo showed a positive increase in participation in botanical composition. Its presence showed an increase, especially during the last four evaluations in 2016, having an average presence of 17% in the last harvest of 2016. Even though Amarillo and TUFRunner 727 mixtures had minimal legume participation in the botanical composition, the overall herbage yield was still influenced by the small amount of plant material present within the plots, as mixtures outperformed bahiagrass monocultures during the 2016 growing season.

Peanut shoots per linear meter exhibited a treatment by harvest date interaction ($P < 0.001$). There was a significant difference between rhizoma peanut and seeded peanut types ($P = 0.002$, SE = 1.97). Rhizoma peanut cultivars averaged 12 shoots m$^{-1}$, and seeded peanut averaged 8 shoots m$^{-1}$ across the three growing seasons. Height of peanut plants also showed a treatment by harvest date interaction ($P < 0.001$, SE=...
Average height for each peanut cultivar across all growing seasons was 15, 16, 11, and 15 cm (SE = 1.3), for Ecoturf, Florigraze, Amarillo, and TUFRunner 727, respectively. At the 10-cm stubble height used in this experiment, Amarillo was underrepresented since the average shows the plants to be only 1-cm above the harvesting stubble height. Amarillo is a prostrate growing species (Cook et al., 1990). Visually, there was *A. pintoi* plant biomass present within the plots, however, the plants grew close to the soil surface and most biomass was not harvested at the given stubble height.

Mislevy et al. (2007) examined the effects of stubble heights (2.5 and 10 cm) on herbage accumulation of rhizoma peanut entries grown on flatwood soils in south Florida. Across four growing seasons and harvesting every 8 wk, average herbage accumulation was greater at 2.5 (11.8 Mg DM ha\(^{-1}\) year\(^{-1}\)) than 10 cm (9.0 Mg DM ha\(^{-1}\) year\(^{-1}\)) (Mislevy et al., 2007) and became equal for both stubble heights during the fourth growing season, which totaled 5.8 Mg DM ha\(^{-1}\) year\(^{-1}\) (Mislevy et al., 2007). Likewise, Interrante et al. (2009a) studied stubble height effects on bahiagrass herbage accumulation and concluded there was minimal effect when bahiagrass was harvested at 4 or 8 cm stubble heights. Since evidence exists that both RP and BG are tolerant of low stubble heights, further research should be focused on finding the optimal harvest frequency and intensity in order to increase the overall productivity of RP-BG mixtures.

In vitro organic matter digestibility (IVOMD) was analyzed for each component of the mixtures (rhizoma or seeded peanut and bahiagrass). Grass components showed a treatment by harvest date interaction (Figure 2-4; \(P = 0.0003\)). On average, unfertilized BG had IVOMD concentrations of 495 g kg\(^{-1}\) which is similar to what is reported in the
literature (Stewart et al., 2007). Overall, bahiagrass in grown in association with either annual or perennial peanut (507 g kg$^{-1}$) had greater digestibility than bahiagrass grown in monoculture (493 g kg$^{-1}$) ($P = 0.026$); furthermore TUFRunner 727 appeared to have the greatest effect in increasing the digestibility of the grass component (524 vs. 494 g kg$^{-1}$) when compared with the unfertilized monoculture ($P = 0.003$). Since TUFRunner 727 is an annual species, root and nodule turnover occurs to a greater extent, therefore more nutrients, especially N, is transferred to grasses. Although the effects on digestibility of N fertilization are not consistent in all species, forage digestibility might be positively impacted by N fertilization through increases in production of new tissue (Coleman et al., 2004).

The rhizoma or seeded peanut components of the mixtures also had a treatment by harvest date interaction (Figure 2-4; $P = 0.0006$). Amarillo was the least digestible peanut cultivar (668 g kg$^{-1}$). Carvalho and Quesenberry (2012) reported similar average digestibility values (670 g kg$^{-1}$) for various Amarillo accessions grown in Florida; and these values are also in accordance with Fernandes et al. (2014). It is important to note that in both cases, the entries were grown in monoculture, and not in mixtures with grasses, as was the case in the current experiment. Digestibility of TUFRunner 727 was within the previously reported ranges by Gorbet et al. (1994) of 596 to 720 g kg$^{-1}$; on average, this treatment had IVOMD of 686 g kg$^{-1}$. Digestibility of Ecoturf and Florigraze was 700 g kg$^{-1}$, for both, which is in accordance with previous literature (Prine et al., 1986, 2010). The overall average digestibility for all rhizoma or seeded peanut in this study was above the average values for tropical legumes reported by Poppi and
McLennan (1995), further indicating the potential for use as forage species in North
Florida.

**Herbage δ^{15}N and N Responses**

Peanut herbage N concentration had a treatment by harvest date interaction ($P <
0.001$). TUFRunner 727 herbage exhibited greater N concentration than other
cultivars, except for the September 2016 harvest (Figure 2-5). During the first growing season
(2014), TUFRunner 727 N concentrations decreased from 33 g N kg$^{-1}$ in August, to 25 g
N kg$^{-1}$ in October. In 2016, TUFRunner 727 herbage N concentration also exhibited a
decline ($P = 0.002$) from 26 to 20 g N kg$^{-1}$ from May to September, respectively.
Florigraze exhibited the overall lowest herbage N concentration during all three growing
seasons, with an average of 21.9 g N kg$^{-1}$. Ecoturf and Amarillo were intermediate with
average herbage N concentrations of 25 and 24 g kg$^{-1}$. TUFRunner 727 presented
significantly greater ($P < 0.0001$) herbage N concentration (29 g kg$^{-1}$) than RP cultivars
(23 g kg$^{-1}$).

Grass herbage N concentration had a treatment ($P = 0.008$) and harvest date ($P
< 0.001$) effect (Table 2-3). Grass N concentration in peanut mixtures (12.7 g kg$^{-1}$) were
greater ($P = 0.024$) compared with unfertilized BG monocultures (12.2 g kg$^{-1}$), indicating
there is evidence of nutrient transfer from the peanut to the grass. Potential N transfer
pathways in mixed legume-grass systems exist mostly through root and nodule decay,
decomposition and mineralization of the organic components within the litter material,
and also through root exudates (Dubeux et al., 2007). Integrating peanut into BG
pastures will help reduce livestock supplementation costs since nutritive value of
pastures increases, while also helping to reach desired animal performance levels
(Hernández Garay et al., 2004).
Peanut aboveground N yield in grass-peanut mixtures had a treatment by harvest date interaction ($P < 0.001$). Ecoturf-BG mixtures showed the greatest magnitude in peanut aboveground N accumulation, ranging from 0.5 kg N ha$^{-1}$ harvest$^{-1}$ (August 2014) to 10.4 kg N ha$^{-1}$ harvest$^{-1}$ (August 2016; Figure 2-6), and yielded a total 25.1 kg N ha$^{-1}$ in 2016. TUFRunner 727-BG mixtures yielded the overall greatest N amounts in 2014, peaking in August 2014 (7.9 kg N ha$^{-1}$ harvest$^{-1}$), with yields tending to decrease thereafter, as the percentage of peanut in the botanical composition decreased. Legume N yields from Florigraze-BG mixtures tended to stay stable, except during August 2015 harvest where the greatest yield occurred (4.6 kg N ha$^{-1}$). On average, Florigraze-BG mixtures yielded 1.3 kg N ha$^{-1}$ harvest$^{-1}$. Lowest N yields were obtained from Amarillo-grass mixtures with an average of 0.7 kg ha$^{-1}$ harvest$^{-1}$, for all three growing seasons.

Peanut herbage $\delta^{15}$N had a treatment by harvest date interaction ($P = 0.003$). All values were negative for each of the four peanut cultivars during the three growing seasons (Figure 2-6), indicating presence of BNF throughout the experimental period. Negative $\delta^{15}$N values result from soils being more abundant in $^{15}$N than atmospheric N$_2$; N$_2$-fixing plants use N$_2$ derived from both soil and atmosphere, while non-N$_2$-fixing plants use soil N as their main N source, resulting in positive $\delta^{15}$N values for non-N$_2$-fixing species since they will be more abundant in $^{15}$N (Shearer and Kohl, 1986). Numerous factors such as environmental and nutrient stress can affect $\delta^{15}$N in plants through changes in soil and soil microorganism development (Craine et al., 2015).

Biological N$_2$-fixation (BNF) had a treatment by harvest date interaction ($P < 0.001$). Overall, Ecoturf-BG mixtures fixed the greatest amount of N$_2$, ranging from 0.3
to 6.7 kg N ha\(^{-1}\) harvest\(^{-1}\) (Figure 2-6). TUFRunner 727-BG mixtures BNF ranged from 0.03 to 7.9 kg N ha\(^{-1}\) harvest\(^{-1}\) during the three growing seasons and had an overall decreasing pattern to BNF. Florigraze-BG mixtures peaked on July 2015 with 2.5 kg N ha\(^{-1}\) harvest\(^{-1}\), and the values tended to remain constant until the end of the 2016 growing season. Average BNF for Florigraze-BG ranged from 0.2 to 2.5 kg N ha\(^{-1}\) harvest\(^{-1}\). The lowest BNF values were observed for Amarillo-BG mixtures, which ranged from 0 to 1.9 kg N ha\(^{-1}\) harvest\(^{-1}\). It is likely that the values for Amarillo are underestimated given that it is a prostrate-growing species and averaged only 10 cm height, limiting the amount of herbage present that was harvested.

Nitrogen transfer from peanut to BG was not reported in this study because evidence exists of bahiagrass having associative BNF capabilities (Dobereiner, 1966; Kass et al., 1971). The BNF reported is also believed to be under-estimated for this same reason. Dobereiner (1966) reported *Azotobacter paspali* sp. n. was found within the rhizosphere of various *Paspalum notatum* cultivars in southern Brazil and it proved to be capable of fixing atmospheric N\(_2\). Furthermore, Kass et al. (1971) estimated that the symbiosis between BG and *A. paspali* could potentially fix up to 20 kg N ha\(^{-1}\) yr\(^{-1}\).

**Root and Rhizome Responses**

Root and rhizome mass had a treatment by year interaction (\(P = 0.019\)). There was no difference between peanut-BG mixtures and unfertilized BG (\(P = 0.735\)). There was an overall increase in root and rhizome mass from 2015 to 2016 (Figure 2-7). Ecoturf-BG mixtures had the lowest (4 Mg OM ha\(^{-1}\)) root and rhizome mass for 2015. TUFRunner 727-BG mixtures had the greatest root and rhizome mass (7.5 Mg OM ha\(^{-1}\)) for 2015. This might be partially explained since TUFRunner 727, an annual species, was actively growing during the 2015 growing season and possibly transferring
N to bahiagrass via root and nodule decay and/or aboveground litter. As a result, bahiagrass root mass increased in the mixtures likely due to root and nodule turnover and decay from the annual legume, given that harvests took place every 5 weeks. In 2016, Amarillo-BG mixtures had the lowest root rhizome mass with 7.4 Mg OM ha\(^{-1}\). The other cultivars varied from 9.3 Mg OM ha\(^{-1}\) (Florigraze-BG mixtures) to 9.9 Mg OM ha\(^{-1}\) (Ecoturf-BG mixtures). The large total increase in root and rhizome mass for Ecoturf-BG mixtures over time is attributed to relatively slow establishment; by the third year Ecoturf was becoming much more fully established. In addition, the overall lack of N fertilization can also be a potential stressor for the plants, which leads to the allocation of carbohydrate resources to be stored in the rhizomes.

Unfertilized BG increased in root and rhizome biomass from 5.5 (2015) to 9.9 (2016) Mg OM ha\(^{-1}\). Grise et al. (2006) supplied ‘Pensacola’ bahiagrass swards with 40 kg N ha\(^{-1}\) yr\(^{-1}\) and reported an average root and rhizome biomass of 18.0 Mg DM ha\(^{-1}\) in a grazing system. Interrante et al. (2009) reported a decrease, from 18.8 to 12.6 Mg DM ha\(^{-1}\), in root and rhizome biomass in ‘Pensacola’ bahiagrass fertilized with 200 kg N ha\(^{-1}\) yr\(^{-1}\) and harvested every 21 d at 8-cm stubble height. In this study, the increase in root and rhizome mass for all treatments can be potentially due to two reasons: I) a physiological response to drought stress, since plants tend to allocate resources towards root growth when experiencing drought stress (Loomis and Connor, 1998), and II) root-rhizome growth responses to greater N availability.

Metabolites related to energy production and growth are shifted from shoot to root for storage while under drought stress (Gargallo-Garriga et al., 2014), thus having a more extensive root system allows the plant to exploit water and nutrient resources.
further down in the soil profile. For the duration of the experiment, data suggest that in 2016 rainfall was below average in May, June, and July, October and November. Rainfall was only at the 30-yr average in April, August, and September. Root and rhizome samples were collected in November, and the lack of rainfall, added with the low water holding capacity sandy soils, throughout the growing season can potentially indicate drought and nutrient stress, especially N, for this specific treatment. Additionally, since grass-legume mixtures have, inherently, greater N availability, this promoted the root/rhizome growth since increases in aboveground biomass production also belowground root-rhizome growth.

Nitrogen concentration of root and rhizomes had a treatment by year interaction ($P = 0.044$). Both rhizoma peanut-grass mixtures (Ecoturf and Florigraze) tended to decrease in N concentration from 2015 to 2016 (Figure 2-7). Ecoturf-BG mixtures decreased from 7.9 to 6.6 g N kg$^{-1}$, and Florigraze-BG mixtures decreased from 7.2 to 6.2 g N kg$^{-1}$. TUFRunner 727-BG mixtures had the greatest N concentrations in 2015 (8.9 g kg$^{-1}$), and decreased to 6.0 g kg$^{-1}$ in 2016. A possible explanation for this decline is loss of stand, especially since TUFRunner 727 is an annual species. Likewise, N concentration levels in 2016 are similar to those in unfertilized BG, which remained at 5.8 g N kg$^{-1}$ during both years. The only mixture that increased in N concentration from 2015 to 2016 was Amarillo-BG mixtures, which increased from 7.1 to 8.5 g N kg$^{-1}$, each year respectively, which indicates Amarillo is storing a greater amount of N within its roots. Decreases in root and rhizome N concentrations (in Ecoturf, Florigraze, and TUFRunner 727) are attributed to the dilution effect, since an increase in root-rhizome OM mass was observed for all three species from 2015 to 2016.
Ecoturf-BG mixtures had the greatest overall increase in root and rhizome N pool ranging from 41 to 115 kg N ha\(^{-1}\), from 2015 to 2016 respectively (Figure 2-7). TUFRunner 727-BG mixtures had the greatest N pool in 2015, with 74 kg N ha\(^{-1}\). In 2016, N pool ranged from 74 kg N ha\(^{-1}\) (Florigraze-BG mixtures) to 115 kg N ha\(^{-1}\) (Ecoturf-BG mixtures). The increases across growing seasons can be due to the increase in root and rhizome biomass. As long as nutrient requirements are being met, increases in root and rhizome N pool are beneficial to the system since active N transfer is a significant source of N within a system through the decomposition of legume roots which are high in N concentration (Rouquette and Smith, 2010). The large increase noted in the Ecoturf-BG mixtures can be attributed to the root and rhizome turnover within the mixtures, since this phenomenon can add significant amounts of N to a system (Dubeux et al., 2017). The greatest increase (from 2015 to 2016) in N pool was observed in Ecoturf-BG mixtures, which also had increasing participation in the botanical composition from 2015 to 2016.

Root and rhizome δ\(^{15}\)N were affected by treatment (\(P = 0.02\)) and year (\(P = 0.04\)). Unfertilized BG monocultures were the only treatment that exhibited a positive value of 0.06 ‰ (Table 2.3). Peanut-BG mixtures varied from -0.70‰ (Ecoturf-BG mixtures) to -0.28‰ (Amarillo-BG mixtures).

Treatment differences approached significance (\(P = 0.10\)) for legume proportion in the root and rhizomes. The greatest percentage of peanut roots was observed for Ecoturf-BG mixtures (15.3%). Florigraze-BG mixtures had the lowest percentage, with 4.78%. Amarillo and TUFRunner 727-BG mixtures were intermediate, with 7.9 and 5.1% respectively.
Conclusion

Although rhizoma and seeded peanut treatments differed in their response levels, all mixtures (RP-seeded peanuts) proved to outperform unfertilized bahiagrass monocultures. Rhizoma peanut cultivars, as expected, took two entire growing seasons before becoming established and productive. Of the two rhizoma peanut entries, Ecoturf outperformed Florigraze in above and belowground responses, possibly indicating that long-term performance of Ecoturf may be greater than that of Florigraze mixture with bahiagrass. Future research should investigate strategies for decreasing the establishment period of rhizoma peanut species when into bahiagrass under low-input systems. Amarillo was largely under-represented in the responses presented in this study due to its prostrate growth habit. Further research is needed to determine appropriate stubble heights and harvesting frequencies to ensure a productive stand of this variety, though the limiting factor when it comes to implementation of this species is high seed costs. A. hypogaea can be a viable legume for incorporation into bahiagrass pastures in North Florida. The results indicate it can serve as a reseeding annual legume since there is evidence of some reseeding from one growing season to the next. This entry had the greatest N concentration among all treatments, indicating its benefit as a forage species. Further research is also needed to determine the best stubble height and harvest frequency when A. hypogaea is planted into bahiagrass pastures. Special attention to A. hypogaea as a forage species appears warranted since it is commonly planted in the southeastern USA and producers have access to planting equipment and knowledge about the crop. Overall, this study serves to further indicate that Arachis spp. are viable legumes for incorporation into bahiagrass pastures in North Florida, and their incorporation leads to increases in productivity of pasture systems.
Table 2-1. Harvest dates for three growing seasons.

<table>
<thead>
<tr>
<th>Year</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 August</td>
<td>11 May</td>
<td>4 May</td>
<td></td>
</tr>
<tr>
<td>16 September</td>
<td>17 June</td>
<td>9 June</td>
<td></td>
</tr>
<tr>
<td>21 October</td>
<td>28 July</td>
<td>15 July</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 September</td>
<td>17 August</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 October</td>
<td>22 September</td>
<td></td>
</tr>
</tbody>
</table>
Table 2-2. Herbage accumulation totals by year for peanut-bahiagrass (BG) mixtures and unfertilized bahiagrass.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year 2014</th>
<th>Year 2015</th>
<th>Year 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecoturf- BG</td>
<td>1800 aA‡</td>
<td>2275 aA</td>
<td>4157 aB</td>
</tr>
<tr>
<td>Florigraze- BG</td>
<td>1606 aA</td>
<td>2212 aAB</td>
<td>2991 abB</td>
</tr>
<tr>
<td>Amarillo- BG</td>
<td>1718 aA</td>
<td>2724 aAB</td>
<td>3362 abB</td>
</tr>
<tr>
<td>TUFRunner 727- BG</td>
<td>1875 aA</td>
<td>1881 aA</td>
<td>3351 bB</td>
</tr>
<tr>
<td>Unfertilized BG</td>
<td>1912 aA</td>
<td>2166 aA</td>
<td>2707 bA</td>
</tr>
<tr>
<td>SE</td>
<td>138</td>
<td>138</td>
<td>138</td>
</tr>
</tbody>
</table>

‡Least square means followed by the same letter, lowercase letters within a column and uppercase letters within a row, do not differ ($P > 0.05$) according to PDIFF procedure adjusted by Tukey.
Table 2-3. Bahiagrass N concentration (g kg\(^{-1}\)) and root-rhizome \(\delta^{15}N\) of mixtures with rhizoma or seeded peanut or in monoculture.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grass herbage N (g kg(^{-1}))</th>
<th>Root-rhizome (\delta^{15}N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecoturf-BG</td>
<td>13.2 a‡</td>
<td>-0.77 b</td>
</tr>
<tr>
<td>Florigraze-BG</td>
<td>12.6 ab</td>
<td>-0.29 ab</td>
</tr>
<tr>
<td>Amarillo-BG</td>
<td>12.5 b</td>
<td>-0.28 ab</td>
</tr>
<tr>
<td>TUFRunner 727- BG</td>
<td>12.4 b</td>
<td>-0.38 ab</td>
</tr>
<tr>
<td>Unfertilized BG</td>
<td>12.2 b</td>
<td>0.06 a</td>
</tr>
</tbody>
</table>

\(P\) 0.008 0.02

Standard Error 0.22 0.22

‡ Means followed by different letters differ (\(P > 0.05\)) according to PDIFF adjusted by Tukey.
Figure 2-1. Monthly rainfall at the North Florida Research and Education Center (NFREC), Marianna, FL, for 2014, 2015, 2016, and the 30-yr average.
Figure 2-2. Herbage accumulation (HA), in kg DM ha\(^{-1}\) harvest\(^{-1}\), during three growing seasons (2014, 2015, and 2016). Bars with different letters denote statistical differences ($P < 0.05$) among treatments within each evaluation according to PDIF adjusted by Tukey.
Figure 2-3. Percentage of rhizoma or seeded peanut participation in botanical composition (BC), mixed with bahiagrass (BG), across three growing seasons (2014, 2015, 2016). Treatment by harvest date interaction was observed ($P < 0.0001$).
Figure 2-4. In vitro organic matter digestibility (IVOMD) of grass (top) and peanut (bottom) components of bahiagrass (BG) mixed with rhizoma or seeded peanut for 2014 and 2015 growing seasons. Bars with different letters denote statistical differences ($P < 0.05$) among treatments within each evaluation according to PDiff adjusted by Tukey.
Figure 2-5. Peanut herbage N concentrations when mixed with bahiagrass (BG). Bars with different letters denote statistical differences ($P < 0.05$) among treatments within each evaluation according to PDIIFF adjusted by Tukey.
Figure 2-6. Rhizoma or seeded peanut component total aboveground N yield (in kg N ha\(^{-1}\); top), peanut herbage \(\delta^{15}N\) (middle), and biological N\(_2\)-fixation (BNF; bottom), for all mixtures with bahiagrass (BG) across three growing seasons (2014, 2015, 2016). Bars with different letters denote statistical differences (\(P \leq 0.05\)) among treatments within each evaluation according to PDIFF adjusted by Tukey.
Figure 2-7. Root and rhizome mass (top), N concentration (middle), and N pool (bottom) for each rhizome or seeded peanut-bahiagrass (BG) mixture and unfertilized BG treatment for 2015 and 2016 growing. * Indicates significant differences among years within each treatment (P ≤ 0.05) according to PDIF adjusted by Tukey.
CHAPTER 3
‘TIFTON 85’ BERMUDAGRASS (CYNODON SPP.) MIXED WITH ANNUAL OR PERENNIAL PEANUT FOR HAY PRODUCTION IN NORTH FLORIDA

Introduction

Hay production is an important enterprise in Florida’s agriculture commodity sector producing $130 million in sales per year and having over 117,000 hectares in production (USDA NASS, 2016). Nitrogen fertilizer is commonly used in hay production systems since it increases herbage yields, however, over-fertilization has led to instances of nitrate leaching to groundwater (Agyin-Birikoran et al., 2012). Carbon emissions from N fertilizer use typically range from 3.3 to 6.6 kg CO₂ equivalents per kg N produced (Lal, 2004). Agricultural fertilizer use can be diminished through increased adoption of grass-legume mixed stands, since legumes have the capacity of fixing atmospheric N₂ and potentially transferring to companion grasses.

In Florida, options are limited for producers when it comes to warm-season legumes. Aeschynomene (Aeschynomene americana L.), and carpon desmodium [Desmodium heterocarpon (L.) D.C.] are viable species most commonly planted in south Florida pastures, though Sollenberger and Kalmbacher (2005) reported decreasing adoption rates for these legumes among producers due to problems related to poor establishment or persistence. Other options available include rhizoma or seeded peanuts. Rhizoma peanut (Arachis glabrata Benth) has shown potential as a perennial, warm-season legume that is adapted to the Florida environment and persistent under a wide range of grazing management practices (Ortega-S. et al., 1992). Rhizoma peanut is reported to have average crude protein (CP) and in vitro organic matter digestibility (IVOMD) of 158 and 655 g kg⁻¹, respectively (Prine et al., 2010).
Potential seeded peanut species include *Arachis pintoi* Krap. & W.C. Greg and *A. hypogaea* L. *Arachis pintoi* is a perennial, stoloniferous, prostrate-growing species with high importance in tropical regions for its grazing tolerance (Cook et al., 1993; Fisher and Cruz, 1993). Several *A. pintoi* cultivars have been released for forage use, including ‘Amarillo’ in Australia, ‘Mani Forrajero’ in Colombia and Bolivia, ‘Mani Mejorador’ in Costa Rica, ‘Pico Bonito’ in Mexico and Honduras, and ‘Golden Glory’ in USA (Valentim et al., 2001). Accessions of *A. pintoi* evaluated in Florida were reported to have average herbage yields of 4.35 Mg ha⁻¹ year⁻¹, and CP and IVOMD of 180 and 670 g kg⁻¹ (Carvalho and Quesenberry, 2012).

*Arachis hypogaea* L. is not frequently planted as a forage species, but it is planted for its seed. This species is commonly affected by diseases such as leaf spot (*Cercospora*), tomato spotted wilt virus, and white mold disease (*Sclerotinia*), and thus researchers have developed a cultivar that has some resistance to these diseases (Tillman, 2013). The ‘TUFRunner’ line, developed by the University of Florida, is among the cultivars with the greatest oleic acid concentration, yield potential, and peanut quality grades (Tillman, 2013). Accessions of *A. hypogaea* grown in North Florida are reported to have CP and IVOMD of 157 and 646 g kg⁻¹, respectively (Gorbet et al., 1994).

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is the most commonly planted species in Florida hay production systems, given it is highly productive and dries faster than other similar species (Chambliss et al., 2006). ‘Tifton 85’ (*Cynodon spp.*) is among the most popular bermudagrass cultivars and has average CP concentrations ranging between 160 and 180 g kg⁻¹ (Michelangeli et al., 2010; Agyin-Birikoran et al., 2012).
Tifton 85 is very responsive to N fertilization, which is required for high productivity. Agyin-Birikoran et al. (2012) reported herbage yields for fertilized (300 kg N ha\(^{-1}\) year\(^{-1}\)) and unfertilized Tifton 85 swards to be 18 and 1.5 Mg DM ha\(^{-1}\) year\(^{-1}\), highlighting the importance for fertilization in Tifton 85 swards.

Since hay production systems in Florida are largely based on grass monocultures with a high rate of N fertilization, we assessed the potential use of *Arachis* spp. for incorporation into Tifton 85 bermudagrass swards in aims to reduce the overall N fertilizer use. In this study, we evaluated the potential use of *A. hypogaea* L. and its re-seeding potential in subsequent growing seasons. We also evaluated the performance of *A. pintoi* and two *A. glabrata* entries in mixture with Tifton 85. The overall objectives were to i) determine viability of *A. hypogaea* for forage use in mixed swards with bermudagrass ii) evaluate the performance of *A. pintoi* and *A. glabrata* in North Florida when mixed with Tifton 85 and iii) to make recommendations on which peanut variety is best suited for incorporation into Tifton 85 pastures in low-input systems in order to decrease the demand for N fertilization in North Florida.

**Materials and Methods**

**Experimental Site**

The study was carried out during the 2014, 2015, and 2016 growing seasons at the North Florida Research and Education Center of the University of Florida, located in Marianna, Florida (30°52’ N, 85°11’ W, 35 m altitude). The soil is classified as an Orangeburg loamy sand (fine-loamy- kaolinitic, thermic Typic Kandiudults). Initial soil surface samples (0-15 cm depth), collected on May 2014, indicated 6.3 soil pH, CEC was estimated at 3.7 meq 100g\(^{-1}\), and 7.7 g kg\(^{-1}\) soil organic matter (SOM). Mehlich-1 extractable P, K, Mg, and Ca concentrations of 25, 58, 53, and 453 mg kg\(^{-1}\),
respectively. Total rainfall for 2014, 2015, and 2016 was 1573, 1403, and 1378 mm, respectively (Figure 3-b1).

Treatments, Plot Establishment, and Design

Treatments consisted of unfertilized Tifton 85 bermudagrass, two rhizoma peanut entries (Arachis glabrata Benth): the germplasm Ecoturf, and ‘Florigraze’; Arachis pintoi Krap. & W.C. Greg cv. ‘Amarillo’, and Arachis hypogaea L. cv. TUFRunner ‘727’. Glyphosate [Glyphosate-4 Plus, Alligare, LLC. (N-(phosphonomethyl) glycine)] was applied on 25 Mar. 2014 at 12 L ha⁻¹ prior to preparing the study site. The site was disked and harrowed on 15 Apr. 2014. Tifton 85 rhizomes were dug on 17 Apr. 2014 and planted the same day at 1700 kg ha⁻¹. Rhizoma peanut entries were also dug and planted on 17 Apr. 2014 using 1300 kg rhizomes ha⁻¹, and a Holden Sodmaster No-till Bermudagrass Sprigger (Holden-Sodmaker. Southwest City, MO) with four rows spaced 71 cm apart. Seeded peanut species were planted on 6 May 2014 using a twin-row planter (Burch Farm Implements Inc.; Evansville, IN) with rows 71 cm apart and eight rows per plot, placing ten seeds per linear meter. The plots were stripped-tilled prior to planting. Seeding rates for A. hypogaea and A. pintoi were 110 and 12 kg seed ha⁻¹, respectively. The entire study area was cultipacked using a Brillion cultipacker (Landoll Corp.; Marysville, KS) promptly after planting. A. hypogaea and A. pintoi seeds were treated with Dynasty PD [Syngenta Crop Production, a.i. Azoxystrobin (C₂₂H₁₇N₃O₅), Fludioxonil (C₁₂H₆F₂N₂O₂), Mefenoxam (C₁₅H₂₁NΟ₄)], at 2.6 mL kg⁻¹. A. hypogaea seeds were inoculated with Graph-EX SA for Peanuts (ABM: Advanced Biological Marketing) (5 x 10⁹ Rhizobia spp. + 1 x 10⁸ cfu gm⁻¹ Trichoderma spp.) at 15 mL per 11 kg seed (1.36 mL kg⁻¹), and A. pintoi seeds were inoculated with CIAT 3103 A. pintoi inoculant [Centro de Investigacion de Agricultura Tropical (1 x 10⁹ Bradyrhizobium spp.)]
at 25 g of inoculant per 25 kg of seed. Irrigation was provided (29 mm) on 13 May 2014. Each experimental unit measured 6 x 6 m, with no adjacent alleys and the treatments were arranged in a randomized complete block design with four replications per treatment.

All experimental units were supplied with 7 kg P ha\(^{-1}\) and 56 kg K ha\(^{-1}\) following each sampling period (336 kg ha\(^{-1}\) of the commercial formula 0-5-20), except for 24 September 2015, 20 May 2015, and 6 May 2016, where 336 kg ha\(^{-1}\) of Kmag (183 g K kg\(^{-1}\), 108 g Mg kg\(^{-1}\), 220 g S kg\(^{-1}\)) was applied, which is equivalent to 61 kg K ha\(^{-1}\), 36 kg Mg ha\(^{-1}\), 74 kg S ha\(^{-1}\). Harvests took place every 5 wk (Table 3-1) at a 10-cm stubble height. Prowl H\(_2\)O [BASF Corporation, pendimethalin (C\(_{13}H_{19}N_3O_4\))] was applied 24 Mar. 2015 and 7 Apr. 2016, at 2.33 L ha\(^{-1}\), in order to control pre-emergent weeds early in each growing season.

**Sampling Techniques**

A 3-m\(^2\) (3 x 1 m) strip was cut through the center of each plot, at 10-cm stubble height, using a flail style mower (Carter Mfg. Co. Brookston, IN), to evaluate herbage accumulation. Botanical composition was determined on a dry matter basis by harvesting aboveground forage at the 10-cm stubble height using a 0.25-m\(^2\) quadrat and hand-separating the components (grass, peanut, or weed). Peanut plant height was measured by averaging 10 random measurements of plant sprouts within each plot. Peanut stand per linear meter was determined by averaging three measurements using a 1-m ruler parallel to the planted peanut rows and counting the number of plants within that length. After each sampling period, the entire area was staged at the 10-cm stubble height using a rotary chopper (John Deere 972, Deere Company, Moline, IL).
All samples were dried at 55°C for 72 h and dry weights recorded. Herbage accumulation samples, as well as weeds (from hand-separated samples) were discarded after weights were recorded and grass and peanut components were ground to pass through a 1-mm screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific; Swedesboro, NJ). Samples were ball milled using a Mixer Mill MN400 (Retsch, Newton, PA) at 25 Hz for 9 min. Total N, total C, δ\(^{13}\)C, and δ\(^{15}\)N were determined using a CHNS analyzer by the Dumas dry combustion method (Vario Micro Cube; Elementar, Hanau, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK). In-vitro organic matter digestibility (IVOMD) was determined using the two-stage technique described by Moore and Mott (1974).

Biological N\(_2\)-fixation (BNF) was determined using the natural abundance technique described by Freitas et al. (2010). Unfertilized Tifton 85 herbage was used as the reference plant. The proportion of plant N derived from the atmosphere (\(\%N_{\text{dfa}}\)) was estimated using the equation described by Shearer and Kohl (1986):

\[
%N_{\text{dfa}} = \frac{\delta^{15}N_{\text{reference}} - \delta^{15}N_{\text{N\textsubscript{2}-fixing legume}}}{\delta^{15}N_{\text{reference}} - B} * 100
\]

In which \(\delta^{15}N_{\text{reference}}\) is the \(\delta^{15}\)N value for the non N\(_2\)-fixing reference plant, \(\delta^{15}N_{\text{N\textsubscript{2}-fixing legume}}\) is the \(\delta^{15}\)N value for the N\(_2\)-fixing rhizoma or seeded peanut cultivar in this study, and \(B\) is the \(\delta^{15}\)N value for the N\(_2\)-fixing plant grown absence of inorganic N. \(B\) value used in this study was reported by Okiko et al. (2004) for \(Arachis hypogaea\) L. (\(\delta^{15}\)N - 1.35‰). Reference plant \(\delta^{15}\)N ranged from -0.15 to 1.44‰ with confidential interval \((P < 0.05)\) of 0.82 ± 0.37, with values changing with sampling date. Shoot N yield was estimated by multiplying aboveground biomass by total N concentration. Shoot BNF was estimated by multiplying shoot N yield by \(\%N_{\text{dfa}}\).
Percent N transfer from rhizoma or seeded peanut to Tifton 85 bermudagrass was estimated using the equation described by Snoeck et al. (2000):

\[
\% N_{\text{trans}} = \frac{\delta_{15}N_{\text{non-fixing sp. in pure stand}} - \delta_{15}N_{\text{non-fixing sp. in mixed stand}}}{\delta_{15}N_{\text{non-fixing sp. in pure stand}} - B} \times 100
\]

Where \( \delta_{15}N_{\text{non-fixing sp. in pure stand}} \) is the \( \delta^{15}N \) value of the unfertilized Tifton 85 in monoculture, \( \delta_{15}N_{\text{non-fixing sp. in mixed stand}} \) is the \( \delta^{15}N \) of Tifton 85 within the rhizoma or seeded peanut mixtures. The \( B \) value (-1.35‰) used was reported by Okiko et al. (2004) for Arachis hypogaea L.

**Statistical Analyses**

Data were analyzed using the PROC MIXED procedure in SAS (SAS for Windows V 9.4, SAS Institute, 2009, Cary, NC, USA). Blocks were considered random effect. Fixed effects included peanut cultivar and harvest date. Repeated measures procedure was used for herbage responses and harvest date was the repeated measure. The LSMEANS were compared with the PDIFF procedure adjusted for Tukey’s test. Differences were declared significant at \( P \leq 0.05 \). Herbage accumulation was initially analyzed including year as a fixed effect and summing up all evaluations. Subsequently, herbage accumulation data was analyzed using harvesting date as repeated measure, as previously described.

**Results and Discussion**

**Herbage Responses**

Herbage accumulation was analyzed by year and showed a treatment (\( P = 0.03 \)) and year effect (Table 3-2; \( P < 0.0001 \)). The peanut-T85 mixtures produced greater herbage accumulation (\( P = 0.02 \)) when compared with the unfertilized Tifton 85 monoculture (2730 vs 2077 kg DM ha\(^{-1}\)). Herbage accumulation was affected by a
treatment by harvest date interaction ($P = 0.0058$; Figure 3-2). The total herbage accumulation by year are shown in Table 3-2. Herbage accumulation ranged from 392 (TUFRunner 727-Tifton 85 mixtures, on September 2014) to 2650 kg DM ha$^{-1}$ harvest$^{-1}$ (Ecoturf-Tifton 85 mixtures, August 2016). On average, Ecoturf-Tifton 85 mixtures had the greatest (900 kg DM ha$^{-1}$ harvest$^{-1}$) and TUFRunner 727-Tifton 85 mixtures had the lowest herbage accumulation (690 kg DM ha$^{-1}$ harvest$^{-1}$). There was no difference observed in herbage accumulation between the mixtures or unfertilized Tifton 85 ($P = 0.421$). Weed encroachment was problematic throughout the experimental period. Herbage accumulation observed in this study is lower than what has been reported in the literature. Agyin-Birikoran et al. (2012) reported average herbage accumulation for unfertilized, rainfed Tifton 85 of 2.3 Mg DM ha$^{-1}$ harvest$^{-1}$ across a two-year study in north central Florida on pre-established sod. Low yields observed in this study are largely attributed to the lack of N fertilizer applied during establishment, which led to increased competition from weeds, thus decreasing overall herbage accumulation.

Participation of rhizoma or seeded peanut in the botanical composition (BC) showed a treatment by harvest date interaction ($P = 0.002$; Figure 3-3). TUFRunner 727 showed the greatest initial participation in BC (24% on August 2014) in the first growing season, and declined thereafter to an average participation of 4 and 1.2%, in 2015 and 2016 respectively, showing there was minimal reseeding form this species following the first growing season. Amarillo had the lowest participation in BC throughout the experimental period, having 0% participation during all harvest dates in 2014 and having only 3% participation during one harvest date on July 2015. In 2016, Amarillo BC participation ranged from 0% (May and June) to 11% (September). Mixtures with
Amarillo were largely under-represented in the BC sample since the average plant height was 9 cm, the lowest among treatments ($P = 0.03$), and the experiment was harvested at 10-cm stubble height.

Rhizoma peanut showed overall greater participation (25%) in BC ($P < 0.0001$) than seeded peanut (2%). There was no difference in BC participation between Ecoturf and Florigraze ($P = 0.31$). Both entries averaged 8 and 19% participation in 2014 and 2015, respectively. In 2016, Ecoturf participation in BC ranged from 25 to 73% and Florigraze ranged from 15 to 38%. The increase in participation in BC observed in RP entries indicates the prolonged establishment period of this species since the RP mixtures increased in participation in BC in each growing season. These results are in accordance with previous literature stating RP establishment takes multiple growing seasons before becoming productive (Prine et al., 1986, 2010).

In vitro organic matter digestibility (IVOMD) was analyzed for each component of the treatment mixtures (Figure 3-4). There was a treatment by harvest date interaction among peanut components in the mixtures ($P = 0.005$; Figure 3-4). Amarillo was not evaluated for IVOMD concentration due to a lack of sufficient samples for analysis throughout the experiment period. The average peanut IVOMD in the mixtures was 705, 702, and 727 g kg$^{-1}$ for Ecoturf, Florigraze, and TUFRunner 727, respectively. There was no treatment effect ($P = 0.16$) for IVOMD in the grass components across all treatments, however, the grass components differed between the peanut-Tifton 85 mixtures and the Tifton 85 monoculture ($P = 0.029$). On average, IVOMD for the peanut-Tifton 85 mixtures and the Tifton 85 monoculture was 620 and 588 g kg$^{-1}$, respectively. Agyin-Birikoran et al. (2012) reported IVOMD of 410 g kg$^{-1}$ for unfertilized Tifton 85, and
Hill et al. (1993) reported IVOMD of 603 for Tifton 85 receiving 193 kg N ha\(^{-1}\) year\(^{-1}\). The increased IVOMD from the mixtures indicates a benefit through increases in production of new tissue, though it is important to note that the effects of N fertilization on forage digestibility are not consistent in all species (Coleman et al., 2004).

**Herbage δ\(^{15}\)N and N Responses**

A harvest date interaction \((P < 0.001)\) was observed for N concentrations of grass components across all treatments (Figure 3-5). Grass N concentrations ranged from 9.5 (July 2016) to 24 (October 2015) g N kg\(^{-1}\). There was an overall decreasing trend in N concentrations during 2016, which can be attributed to RP encroachment, especially from Ecoturf, as noted in the BC. There was an overall decrease in participation of the grass component in Tifton 85 mixtures with both Ecoturf and Florigraze (RP), indicating poor competitiveness of Tifton 85 under limited N supply. Seeded peanut entries (Amarillo and TUFRunner 727) had minimal participation in BC. The overall average grass N concentration observed was 14.6 g N kg\(^{-1}\), which is lower than N concentrations reported by Vendramini et al. (2012) of 18 g N kg\(^{-1}\) of rainfed, fertilized, Tifton 85.

Peanut components within the mixtures had a treatment by harvest date interaction for N concentrations \((P < 0.001)\) for N concentration (Figure 3-5). The overall lowest N concentrations were observed for both Ecoturf and Florigraze on August 2014 (11 g N kg\(^{-1}\)), and the greatest N concentration was observed for TUFRunner 727 on July 2015 (32 g N kg\(^{-1}\)). During the 2016 growing season, both RP entries tended to remain stable in their N concentrations, 24 g N kg\(^{-1}\), which is in accordance with Prine et al. (2010) who reported average N concentrations for Ecoturf and Florigraze to both be 24 g N kg\(^{-1}\). Average N concentration for Amarillo was 20 g N kg\(^{-1}\), ranging from 14
(August 2014) to 24 g N kg\(^{-1}\) (August 2015). TUFRunner 727 had an overall average N concentration of 23 g kg\(^{-1}\), confirming a high nutritive value for this species. During 2014, peanut N values were overall lower than in subsequent years and this can be attributed to lower levels of BNF observed during the same year. The increases in N concentrations observed for all \textit{Arachis} spp. in 2015 and 2016 exceeded the protein requirements for beef cows and replacement heifers (NRC- National Research Council, 2000). Incorporating annual or perennial peanut into hay systems can increase the nutritive value of the hay product. Foster et al. (2009) reported increases in dry matter intake and digestibility when supplementing sheep with annual and perennial peanut hays because legumes are degraded more easily and rapidly by ruminal microbes when compared with grasses. Increases in nutritive value of hay can therefore enhance the diet provided to an animal, while also enhancing the marketability of the product for the producer.

Percentage of N transfer from peanut to grass components in the mixtures was significant among treatments \((P = 0.05)\) and harvest dates \((P < 0.0001)\). The overall percentages of N transferred from peanut to grass ranged from 5 (Amarillo) to 17\% (TUFRunner 727; Figure 3-6). The lowest value across all treatments was observed in September 2016 (2\% N transfer) and the greatest was observed in June 2015 (26\%). In grass-legume mixtures, the majority of N transfer (from grass to legume) occurs through nodule decay, root-rhizome turnover, above-ground litter decomposition, and, for grazing systems, through excreta deposition and decomposition (Dubeux et al., 2007, 2017). In addition, it is important to note that overall nutrient transfer occurs at a greater extent in grazing systems given there is greater availability of soil nutrients since the net
nutrient export occurs to a lesser extent compared to hay production systems (Dubeux et al., 2006).

Peanut herbage N yield had a treatment by harvest date interaction ($P < 0.0001$; Figure 3-7). Amarillo was largely under-estimated given its low participation in the botanical composition due its prostrate growing habit, which was below harvesting stubble height. Herbage N yields from Amarillo tended to remain at 0 kg N ha$^{-1}$ harvest$^{-1}$, except for September 2016 (1.7 kg N ha$^{-1}$ harvest$^{-1}$). TUFRunner 727 had its greatest N yield (13 kg N ha$^{-1}$ harvest$^{-1}$) on August 2014, the first harvest of the experimental period, and decreased thereafter to 0 kg N ha$^{-1}$ harvest$^{-1}$. The decrease was observed since TUFRunner 727 is an annual species and its participation in BC decreased during each growing season. Increased N yields were observed for RP entries during 2016, which followed similar pattern to participation in BC. Florigraze had its lowest N yield in October 2014 (0.2 kg N ha$^{-1}$ harvest$^{-1}$) and the greatest on August 2016 (19.3 kg N ha$^{-1}$ harvest$^{-1}$), with 37 kg N cumulative N yield observed for 2016. Ecoturf had greater ($P = 0.03$) herbage N yield than Florigraze. Ecoturf ranged from 0.5 (October 2014) to 33 kg N ha$^{-1}$ harvest$^{-1}$ (August 2016), and yielded a total of 71 kg N during 2016. Roots and rhizomes are major N reservoirs, especially in rhizoma peanut species (Mullenix et al., 2016; Dubeux et al., 2017), therefore it is important to mention that the BNF estimates reported in this paper only take into account aboveground tissue. Dubeux et al. (2017) reported average root and rhizome N pool of 574 and 228 kg N ha$^{-1}$ for Ecoturf and Florigraze, respectively. Weed competition and encroachment was problematic throughout the experimental period, especially in the first year, and perhaps it is one reason why N yields were low in 2014 and 2015. Increased N yield in 2016 might be
attributed to RP becoming more productive, and to an extent, overtaking the grass components within those mixtures through greater herbage accumulation, while the seeded peanut species had minimal presence in the botanical composition, resulting in lower N yields.

Peanut herbage $\delta^{15}$N had a harvest date effect ($P < 0.0001$; Figure 3-8). The values were positive during all harvests in 2014, and decreased from 2.4 to 1.5‰ in the same year. Positive $\delta^{15}$N values in 2014 might indicate minimal BNF was occurring through this period, and these values are attributed to the interactions of land preparation practices and herbicide applications prior to planting. The seedbed was disked and harrowed prior to planting, which aided in the mineralization of soil organic matter, thus making soil mineral N more available to the plant (Brady and Well, 2002). Positive $\delta^{15}$N indicate a greater proportion of N was derived from the soil, compared to the atmosphere. Peanut herbage $\delta^{15}$N values became more negative as the experimental period progressed; indicating peanut plants were fixing atmospheric N$_2$.

Nitrogen derived from the atmosphere (%Ndfa) had a treatment by harvest date interaction ($P < 0.0001$; Figure 3-8). TUFRunner 727 had variable proportions of Ndfa. Average for all harvests was 22%, ranging from 0 to 91%, and there was 0% Ndfa observed during all harvests in 2016. Although not tested in this study, it is possible that disease affected this species since there are instances of several diseases affecting A. hypogaea crop fields within the North Florida region, and thus producers take caution with multiple fungicide applications (up to 8 applications) throughout the growing season (Myer et al., 2010a; Tillman, 2013). Disease occurrence would likely cause shutdown of N$_2$-fixation pathways since these are energy demanding for the plant (Phillips, 1980).
Mokgehle et al. (2014) reported ranges of Ndfa proportion for *Arachis hypogaea* L. to be between 33 and 65%. Amarillo Ndfa proportions were 0% for all harvest dates except for July 2015 (19%) and July 2016 (23%), indicating an issue related to the rhizobium used to inoculate the seeds, in which it is likely that residues from herbicides negatively affected the bacteria and they were never quite able to recuperate. Thomas et al. (1997) evaluated *A. pintoi* and estimated Ndfa proportion greater than 80% for these species, which serve as a benchmark for future studies with *A. pintoi*, and also serve to show the BNF potential of the species. Both Ecoturf and Florigraze had the greatest proportion of Ndfa compared with Amarillo and TUFRunner 727 \( P < 0.0001 \), and both had similar proportion of Ndfa \( P = 0.9841 \). Ecoturf Ndfa proportions ranged from 20 to 87%, while Florigraze ranged from 16 to 90%. Dubeux et al. (2017) reported proportions of Ndfa of Ecoturf and Florigraze grown in pure stand, to range between 70 and 90%. The proportions observed in this experiment can be comparable to those reported by Dubeux et al. (2017) since the greatest proportions of Ndfa were observed in 2016, once Ecoturf and Florigraze were established and became productive. It is important to note that Ndfa within itself does not signify good levels of biological N\(_2\)-fixation since the calculations for BNF are linked with total biomass production and the N concentration of the species.

Biological N\(_2\)-fixation (BNF) had a treatment by harvest date interaction \( P < 0.0001 \); Figure 3-8), which had similar patterns to peanut herbage N yield. There was a difference between seeded and rhizoma peanut \( P < 0.0001 \). The difference was due to the low participation from both seeded peanut entries since Amarillo had low participation in BC, and TUFRunner 727 was an annual species with decreasing stand
throughout the experimental period. Ecoturf BNF was greater than Florigraze ($P = 0.039$), and averaged 4.8 and 2.7 kg N ha$^{-1}$ harvest$^{-1}$ each, respectively. Roots and rhizomes are also major N sinks in grass and legume species, and BNF estimated in this experiment only accounts for aboveground herbage; it is also important to consider the N being stored in belowground root and rhizome structures (Mullenix et al., 2016).

**Conclusions**

All *Arachis* spp. used in this study differed in their responses within establishing Tifton 85 bermudagrass. In the long-term, *A. glabrata* cultivars, Ecoturf and Florigraze, tended to outperform the other peanut species (*A. hypogaea* cv. TUFRunner 727 and *A. pintoi* cv. Amarillo), though careful management practices, such as harvesting frequencies and choosing appropriate stubble heights, must be taken into account in order to avoid complete encroachment by the peanut species and losing grass stand. *A. hypogaea* cv. TUFRunner 727 can be a viable annual legume for incorporation on to Tifton 85 swards in North Florida, especially if a producer has ready access to planting equipment for this species. Future research should be directed toward the grazing tolerance of this species, especially since it is such an important crop within the southeastern USA. Seed costs of *A. pintoi* cultivars will be the main deterent towards the adoption of this legume by producers. In all, results indicate that *Arachis* spp. are not completely capable of replacing fertilizers in Tifton 85 hay production systems however, they can decrease the N fertilizer demand. The low herbage accumulation was attributed to poor establishment, therefore resulting in poor competition with weeds. *Arachis* spp. can be viable warm-season legumes for incorporating onto Tifton 85 swards and future research should focus on implementing these on pre-established Tifton-85 swards.
Table 3-1. Harvest dates from 2014 to 2016.

<table>
<thead>
<tr>
<th>Year</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 August</td>
<td>11 May</td>
<td>4 May</td>
<td></td>
</tr>
<tr>
<td>16 September</td>
<td>17 June</td>
<td>9 June</td>
<td></td>
</tr>
<tr>
<td>21 October</td>
<td>28 July</td>
<td>15 July</td>
<td></td>
</tr>
<tr>
<td>3 September</td>
<td></td>
<td>17 August</td>
<td></td>
</tr>
<tr>
<td>6 October</td>
<td></td>
<td>22 September</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-2. Herbage accumulation totals by treatment and by year for peanut-Tifton 85 (T85) mixtures and unfertilized Tifton 85 in monoculture.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Herbage Accumulation kg DM ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecoturf- T85</td>
<td>3212 a⁺</td>
</tr>
<tr>
<td>Florigraze- T85</td>
<td>2869 a</td>
</tr>
<tr>
<td>Amarillo- T85</td>
<td>2290 ab</td>
</tr>
<tr>
<td>TUFRunner 727- T85</td>
<td>2546 a</td>
</tr>
<tr>
<td>Unfertilized T85</td>
<td>2077 b</td>
</tr>
<tr>
<td>SE</td>
<td>268</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Herbage Accumulation kg DM ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>2000 a</td>
</tr>
<tr>
<td>2015</td>
<td>1846 a</td>
</tr>
<tr>
<td>2016</td>
<td>3950 b</td>
</tr>
<tr>
<td>SE</td>
<td>208</td>
</tr>
</tbody>
</table>

⁺Least square means followed by the same letter do not differ (P > 0.05) according to PDIFF procedure adjusted by Tukey.
Figure 3-1. Monthly rainfall for 2014, 2015, 2016, and the 30-yr average at North Florida Research and Education Center (NFREC), Marianna, FL.
Figure 3-2. Herbage accumulation (HA), in kg DM ha\(^{-1}\) harvest\(^{-1}\), for all peanut-Tifton 85 (T85) mixtures, and unfertilized Tifton 85. Bars with different letters denote statistical differences \((P < 0.05)\) among treatments within each harvest date according to PDIF adjusted by Tukey.
Figure 3-3. Percentage of annual or perennial peanut participation in botanical composition (BC) mixed with Tifton 85 (T85) Bermudagrass (*Cynodon* spp.) from 2014-2016. Bars with different letters denote statistical differences (*P* < 0.05) among treatments within each harvest date according to PDIF adjusted by Tukey.
Figure 3-4. In vitro organic matter digestibility (IVOMD) of peanut components in Tifton 85 (T85) bermudagrass (*Cynodon* spp.) in mixtures with *Arachis glabrata* germplasm Ecoturf, *A. glabrata* cv. ‘Florigraze’, or *A. hypogaea* cv. TUFRunner ‘727’. Bars with different letters denote statistical differences (*P* < 0.05) among treatments within each harvest date according to PDIF adjusted by Tukey.
Figure 3-5. Top: Tifton 85 bermudagrass N concentrations in 2014, 2015, and 2016, when mixed with annual and perennial peanuts. Means followed by different letters differ (P < 0.05) according to PDIF adjusted by Tukey. Bottom: Peanut component N concentration when mixed with Tifton 85 bermudagrass in 2014, 2015, and 2016. Bars with different letters denote statistical differences (P < 0.05) among treatments within each harvest date according to PDIF adjusted by Tukey.
Figure 3-6. Proportion of N transfer to Tifton 85 bermudagrass from seeded or rhizoma peanut to grass in peanut-grass mixtures. Bars with different letters denote statistical differences ($P < 0.05$) among treatments according to PDIIFF adjusted by Tukey.
Figure 3-7. Herbage N yield from annual or seeded peanut mixed with Tifton 85 (T85) bermudagrass. Bars with different letters denote statistical differences ($P < 0.05$) among treatments within each harvest date according to PDIF adjusted by Tukey.
Figure 3-8. Peanut herbage δ\(^{15}\)N (top), proportion of N\(_2\) derived from atmosphere (Ndfa) from peanut herbage (middle), and biological N\(_2\)-fixation (BNF; bottom), for all annual or seeded peanut-Tifton 85 (T85) bermudagrass in 2014, 2015, and 2016. Bars with different letters denote statistical differences (\(P < 0.05\)) among treatments within each harvest date according to PD\(\text{DIFF}^\) adjusted by Tukey.
Reducing the C footprint of livestock production systems will become an increasingly important topic as human population grows. Reducing N-fertilizer demand is a major way in which C footprint of livestock production can be reduced. A wide range of studies in the literature indicate that legumes can successfully replace N fertilizers within livestock production systems. In this study, we evaluated the potential use of four peanut species (*Arachis* spp.) when planted into ‘Pensacola’ bahiagrass or ‘Tifton 85’ bermudagrass, two of the most common warm-season grass species in Florida pastures.

The two rhizoma peanut (*Arachis glabrata* Benth) entries evaluated in this study (the germplasm Ecoturf and cv. Florigraze) proved to be the best, long-term (3+ growing seasons) option for implementing into existing bahiagrass pastures since their stand was steadily increasing following each growing season, as each species reached establishment. More work is still needed to develop best management practices for establishing these species into existing bahiagrass pastures and decreasing the establishment period of these species, and also how to manage weed encroachment issues.

In the short-term (one or two growing seasons), however, the use of annual, seeded peanuts was a viable option. Disease resistant cultivars of *A. hypogaea*, as TUFRunner 727, are species producers should have in mind, since it requires less fungicide applications, and our results showed evidence of reseeding and can potentially establish seed banks. Seeds and planting equipment should be widely available to producers, given these species are commonly planted in the North Florida
region. Therefore, further studies should be conducted to study the grazing, or harvesting, tolerance of these species in order to make recommendations on the best ways in which these species should be used.

As this study indicated, establishing Tifton 85-peanut mixtures was less productive than expected. The overall low productivity serves to indicate the criticality of N-fertilization on Tifton 85 pastures, especially during establishment. For this reason, Tifton 85 may not be the species of choice to use under low-input pasture production systems. Further work should be conducted with reduced N-fertilizer rates in Tifton 85-legume mixtures in aims to examine the productivity in mixtures and reduced N-fertilizers.

*Arachis pintoi* cv. Amarillo appeared to be more compatible with bahiagrass than Tifton 85 bermudagrass. Adoption rates for this species will be limited to its high seed costs ($100 kg\(^{-1}\)). This species shows promising results, however, there needs to be more selection on *A. pintoi* for cultivars that are better adapted to the cold temperatures in North Florida. If the seed costs can be decreased, this species can be an attractive warm-season perennial legume for producers in Florida.

The results from both experiments highlight the importance of establishing mixed grass-legume pastures; especially since there was evidence of nutrient transfer, BNF, and increases in herbage accumulations in the mixtures in both experiments. Special emphasis should be placed on educating producers about the risks and benefits that can arise from implementing these legume types into their operations. Since *A. hypogaea* types cost ~ $100 ha\(^{-1}\) to plant and *A. glabrata* ~ $200-$500 ha\(^{-1}\) (depending on the cultivar) the cost-benefit can be broken down into short or long term options for
producers. In the short term (one-two growing seasons), *A. hypogaea* types are good options given their lower initial investment and relatively short establishment period. In the long-run (3+ growing seasons) however, *A. glabrata* types are better suited since with proper management techniques, stands can last numerous years, while maintaining high nutritive values.
### APPENDIX

#### SUMMARY STATISTICS

Table 5-1. Summary statistics of herbage responses for peanut-bahiagrass mixtures and unfertilized bahiagrass

<table>
<thead>
<tr>
<th>Herbage Responses</th>
<th>Herbage Accumulation</th>
<th>Botanical Composition</th>
<th>Peanut Stand</th>
<th>Peanut Height</th>
<th>IVOMD Peanut</th>
<th>IVOMD Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Harvest date</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Treatment*harvest date</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*indicates significant difference among least square means ($P > 0.05$) according to PDIFF procedure adjusted by Tukey, and NS indicated no significant difference.

Table 5-2. Summary statistics of herbage herbage δ15N and N responses for peanut-bahiagrass and unfertilized bahiagrass

<table>
<thead>
<tr>
<th>Herbage δ15N and N Responses</th>
<th>Peanut N Conc.</th>
<th>Grass N Conc.</th>
<th>Peanut N Yield</th>
<th>δ15N Peanut</th>
<th>BNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Harvest date</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Treatment*harvest date</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*indicates significant difference among least square means ($P > 0.05$) according to PDIFF procedure adjusted by Tukey, and NS indicated no significant difference.


<table>
<thead>
<tr>
<th>Root and Rhizome Responses</th>
<th>Root-rhizome mass</th>
<th>Root-rhizome N Conc.</th>
<th>Root-rhizome N Pool</th>
<th>Root-rhizome δ15N</th>
<th>Legume proportion in Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Year</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment*year</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*indicates significant difference among least square means ($P > 0.05$) according to PDIFF procedure adjusted by Tukey, and NS indicated no significant difference.
Table 5-4. Summary statistics of herbage responses for peanut-Tifton 85 mixtures and unfertilized Tifton 85

<table>
<thead>
<tr>
<th>Herbage Responses for Peanut-T85 mixtures and Unfertilized T85</th>
<th>Herbage Accumulation</th>
<th>Botanical Composition</th>
<th>IVOMD Peanut</th>
<th>IVOMD Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Harvest date</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Treatment*harvest date</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

*indicates significant difference among least square means ($P > 0.05$) according to PDIF procedure adjusted by Tukey, and NS indicated no significant difference.

Table 5-5. Summary statistics of herbage $\delta^{15}$N and N responses for peanut-Tifton 85 mixtures and Unfertilized Tifton 85

<table>
<thead>
<tr>
<th>Herbage $\delta^{15}$N and N Responses for Peanut-T85 mixtures and Unfertilized T85</th>
<th>Peanut N Conc.</th>
<th>Grass N Conc.</th>
<th>%N transfer</th>
<th>Peanut N Yield</th>
<th>$\delta^{15}$N Peanut</th>
<th>Ndfa</th>
<th>BNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Harvest date</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Treatment*harvest date</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*indicates significant difference among least square means ($P > 0.05$) according to PDIFF procedure adjusted by Tukey, and NS indicated no significant difference.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Originally from Medellin, Colombia, David M. Jaramillo and his family immigrated to the United States when he was 10-yr old. David earned a BS in animal science at North Carolina State University in 2015. Having a family background in beef cattle production in Colombia, David was interested in pursuing an MS degree related to livestock systems production. He started an MS at the University of Florida in agronomy with a concentration in agroecology, in January 2016, working under the guidance of Dr. Jose Dubeux. David’s research focused on establishing *Arachis* spp. into warm-season grasses in aims of decreasing the use of fertilizers. In Dr. Dubeux’s lab, David has assisted with other studies aimed at developing a 365-d grazing season in North Florida. Upon completion of his MS degree, David will continue working under the guidance of Dr. Jose Dubeux toward a PhD degree.