DEFOLIATION FREQUENCY AND LOCATION EFFECTS ON ROOT-RHIZOME MASS, HERBAGE ACCUMULATION, AND CANOPY CHARACTERISTICS OF RHIZOMA PEANUT ENTRIES DIFFERING IN GROWTH HABIT

By

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To my child – may you be brave like me when I bring you into this world
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Grasslands occupy nearly 40% of USA land area and, in addition to supplying feed for livestock, provide valuable services to society including storing carbon in soil, minimizing soil erosion, purifying water by removing excess nutrients, and providing wildlife habitat. Most grasslands in the southeastern US are comprised of warm-climate grass species that are dependent on nitrogen fertilizer and have relatively low nutritional value for livestock. Inclusion of legumes in mixture with grasses can increase nutritive value and benefit nutrient cycling and soil quality. Rhizoma peanut (RP; *Arachis glabrata* Benth.) is a long-lived legume in the US Gulf Coast Region, providing an array of ecosystem services. Most current RP cultivars were selected for upright growth and hay production, but newer introductions and selections exist that are lower growing with potential for grazing or ornamental use. Data describing key forage responses are lacking for these entries, and evaluation of productivity, nutritive value, and persistence is needed in different soil environments and under different cutting regimes. Also needed is a better understanding of root-rhizome accumulation, which is a large contributor to
soil carbon storage. One study compared the response of 14 RP entries over three years to two defoliation frequency treatments, including one harvest per year at season end vs. two harvests per year occurring at mid-season and end of the growing season. A single defoliation event per year resulted in significant reductions in annual herbage accumulation (HA) and nutritive value for most RP entries relative to harvests at both the middle and end of the growing season. Herbage accumulation was not affected by defoliation frequency for the entry Quincy-Beta, perhaps because of its disease tolerance. Greater defoliation frequency decreased root-rhizome mass and non-structural carbohydrate pool, a response of particular importance to producers using RP fields both for forage and as a source of rhizomes for planting material. A second study compared the 14 RP entries growing for two years in a well-drained soil at Quincy, Florida and in a seasonally flooded soil at Hague, Florida. Well-drained soil at Quincy was more favorable to RP entries in general than seasonally-saturated soil at Hague, which led to many entries having greater annual HA at Quincy than Hague. The entry Ona 33 had nearly the same HA at both locations, and it is considerably better adapted to seasonally-saturated soils than all RP entries currently being used commercially. A third study reports on the design and use of a modified ingrowth core for measuring root accumulation rate. Increasing the mesh size of the fabric from 2 to 4 mm and using a wire frame cage to support the core allowed it to maintain its geometry over 100-d deployment periods and detect differences among RP entries. Finally, results of these studies suggest there is opportunity to select new RP cultivars that are adapted to wetter soils and are well suited for grazing and ornamental uses while maintaining high levels of forage production, nutritive value, and below-ground biomass.
CHAPTER 1
INTRODUCTION

Successful forage production is challenging and requires thoughtful planning that integrates environmental and economic variables including weather patterns, soil characteristics, minimizing cost of inputs, and selection of adapted species and cultivars. Increasing input costs and more erratic weather conditions associated with climate change can thwart planning, making achievement of production goals more difficult. One strategy to address these challenges is to increase plant species diversity in pastures and hayfields (Tracy et al., 2018). The presence of legume species in perennial grasslands may help to buffer environmental and economic fluxes through their contributions including N fixation, which decreases dependence on N fertilizer, greater forage nutritive value, and potentially greater animal production (Muir et al., 2011).

Legumes contribute significantly to ecosystem services provided by grasslands (Jensen et al., 2012). In addition to forage and animal products, important services from legumes include enhanced rates of soil C sequestration (Jensen et al., 2012) and N fixation (Dubeux et al., 2017). Carbon sequestration is a critical ecosystem service. Well-maintained grasslands occupy a large area and likely have a positive impact on the global C cycle (Scurlock and Hall, 1998). Although much is known about grass-dominated areas, more studies are needed on legume-based systems as C sinks in the Southeast US. Plants with large allocation of biomass to below-ground organs have significant potential to contribute to soil C (Rasse et al., 2005). Well-established stands of rhizoma peanut (RP; Arachis glabrata Benth.) may have root-rhizome mass of more than 20 Mg ha\(^{-1}\) (Shepard et al., 2018; Dubeux et al., 2017), so they are candidates to accumulate significant amounts of soil C. Variation among experimental lines in below-ground biomass and root
accumulation rate within a species may be important to consider because of their potential impact on soil C accumulation.

Nitrogen fixation is another critical ecosystem service provided by legumes, owing to associated reduction in inorganic N inputs that results in savings on fertilizer costs and reduced potential for environmental degradation. In Florida, limited legume participation in pastures minimizes N fixation, but even a relatively small proportion of legume in grass-based pastures may contribute significant amounts of N (Santos et al., 2018). Nitrogen fertilizer cost has ranged from approximately $0.65 to $0.90 kg\(^{-1}\) N in recent years (Sollenberger, personal communication), thus the savings from biologically-fixed N are significant to producers. Rhizoma peanut growing in monoculture can fix 120 to 280 kg N ha\(^{-1}\) yr\(^{-1}\) (Dubeux et al., 2017). Along with creating economic value for agriculture, lessening the need for fertilizer has an environmental benefit. It reduces the likelihood of over-application of fertilizer and subsequent eutrophication of waterbodies from nutrient runoff or leaching. In addition, large peaks in N\(_2\)O fluxes can occur from N-fertilized pastures following fertilization, and cumulative N\(_2\)O losses from heavily N-fertilized grasslands can be up to four-fold greater than from unfertilized legume–grass pastures (Soussana et al., 2010; Klumpp et al., 2011).

Of perennial forage legumes proposed for use in Florida, RP is perhaps the best option because it is well adapted to Florida’s climate, has documented persistence in association with grasses (Ortega-S. et al., 1992b; Mullenix et al., 2016a), and the herbage has excellent nutritive value (Mullenix et al., 2016b). ‘Florigraze’ RP is the most widely used cultivar in Florida (Quesenberry et al., 2010). The University of Florida has released several RP genotypes in the past decade (Prine et al., 2010; Quesenberry et al., 2010) that are superior to Florigraze. Efforts
continue to identify other superior RP lines, and currently there are several RP experimental lines being studied that have potential for use in Florida forage-livestock systems. Data describing key forage responses are lacking for these lines, and evaluation of productivity, nutritive value, and persistence are needed in different environments and under different defoliation practices.

Because of potential benefits to livestock production and delivery of regulating and supporting ecosystem services from using RP as a forage, additional work is needed to describe the various RP experimental lines in terms of their forage responses and potential contribution to ecosystem services. The research described in this thesis addresses the themes of herbage and below-ground biomass characteristics of numerous RP plant introductions growing at different locations under different harvest management practices. The objectives were to 1) quantify and compare the effects of defoliation frequency on herbage production, nutritive value, and root-rhizome traits of selected RP introductions with those of existing cultivars and germplasms (Chapter 3), 2) characterize herbage production, nutritive value, and root-rhizome traits of selected RP introductions with those of existing cultivars and germplasms when grown at two locations with markedly different soil characteristics (Chapter 4), and 3) develop and test a prototype root-ingrowth core device designed to quantify root accumulation rate of a group of RP entries (Chapter 5).
CHAPTER 2
LITERATURE REVIEW

Use of Rhizoma Peanut in Florida

Several factors have stimulated interest in rhizoma peanut (RP) and framed RP research efforts. Rhizoma peanut herbage in vitro digestibility is comparable to alfalfa (*Medicago sativa* L.) (Terrill et al., 1996), and RP is very persistent and can spread in grass pastures under good management (Dunavin, 1992; Ortega-S. et al., 1992b). Rhizoma peanut’s ability to fix atmospheric N, ranging from 120 to 280 kg N ha$^{-1}$ yr$^{-1}$ (Dubeux et al., 2017), provides cost-savings potential and reduction in environmental impact compared with N fertilizer use. Establishment is the biggest challenge to more widespread RP use because the plant produces few viable seeds and must be propagated vegetatively. With resultant high establishment costs and relatively low economic return per hectare when used for livestock grazing compared with hay production, producers are wary to use it as a pasture forage. Thus, RP is used primarily for hay production (Mullenix et al., 2014).

The factors limiting RP use have stimulated research; many studies have been conducted on improved establishment methods (Castillo et al., 2013a, 2013b, 2014; Mullenix et al., 2014). Another theme has been evaluation of germplasm under different management practices (Hernández-Garay et al., 2004; Mullenix et al., 2016a, 2016b). In addition, the interaction of growth habit with management practices, as it affects RP establishment, persistence, and herbage accumulation, has been a major topic in RP research (Mullenix et al., 2014, 2016a, 2016b; Shepard et al., 2018).
Rhizoma Peanut Introduction and Available Cultivars

Rhizoma peanut was first introduced into the US in 1936 when a germplasm of *A. glabrata* was brought to the USDA from a collection in Mato Grosso, Brazil (Quesenberry et al., 2010). Further collections continued through the 1960s and 1980s, prompting research on the forage potential of RP in Florida (Prine et al., 2010). As the culmination of that effort, the first RP cultivar released was Florigraze (PI 421707).

Currently, there are two germplasms (Ecoturf and Arblick; Prine et al., 2010) and five commercially-available cultivars (Florigraze, ‘Arbrook’, ‘UF Tito’, ‘UF Peace’, and ‘Latitude 34’) in the US. The most widely used cultivar is Florigraze, but Florigraze herbage accumulation is negatively affected by the peanut stunt virus (*Cucumovirus* spp.; Prine et al., 2010). As such, there is a clear need for a more diverse genetic base among RP cultivars (Blount et al., 2006). Arbroom (PI 262817) was released in 1986. It was developed from germplasm collections from Paraguay in 1960 and is well-adapted to the drought-prone sandy soils of Florida (Prine et al., 2010). Its upright habit is favorable for hay production, but it has a low tolerance to overgrazing (Hernández-Garay et al., 2004; Prine et al., 2010). Arblick and Ecoturf germplasms were selected from RP accessions in the 1950s. Both are low-growing and adapted to inland Florida and the southeastern US Coastal Plain. Ecoturf has shown rapid establishment (Prine et al., 2010) and tolerance to grazing (Mullenix et al., 2016a, Shepard et al., 2018). New RP cultivars UF Tito (PI 262826) and UF Peace (PI 658214) were plant introductions from Paraguay in the 1950s and were released in 2008. Both have a slightly more upright habit than Florigraze and high herbage accumulation (HA; Quesenberry et al., 2010). Except for Latitude 34, all of these RP cultivars and germplasms were released from the University of Florida. Latitude 34 was selected for early
spring production and persistence under dry, cool climatic conditions in Texas (Muir et al., 2011).

**Rhizoma Peanut Responses to Defoliation**

**Herbage Accumulation**

Herbage accumulation is described as the difference between post-grazing herbage mass and the pre-grazing herbage mass of the subsequent grazing event, or simply, it is the change in herbage mass from immediately following haying or grazing until immediately prior to the next defoliation. Rhizoma peanut HA was 8.3 to 12 Mg ha\(^{-1}\) yr\(^{-1}\) during a 4-yr clipping trial in north-central FL (Prine et al., 2010), 10.8 Mg ha\(^{-1}\) yr\(^{-1}\) across 4 yr under clipping in south Florida (Mislevy et al., 2007), up to 13 Mg ha\(^{-1}\) yr\(^{-1}\) in northwest Florida (Dubeux et al., 2017), and 6.0 to 9.2 Mg ha\(^{-1}\) yr\(^{-1}\) during 2 yr of grazing in Gainesville (Mullenix et al., 2016a). Mullenix et al. (2016a) also found that HA under grazing was similar across Florigraze, UF Peace, UF Tito, and Ecoturf.

Grazing intensity and frequency can affect HA. Ortega-S. et al. (1992b) found that Florigraze had 35% lower HA in Year 1 when grazed frequently to a short stubble compared with treatments including longer regrowth intervals and greater residual biomass after grazing. By Year 2, the decline in HA was 70% for frequently and closely grazed treatments. They concluded that “The inability of frequently and closely grazed rhizoma peanut canopies to attain high levels of light interception before subsequent defoliation explains in part the low rhizoma peanut herbage accumulation observed. (Ortega-S. et al. 1992b). Ecoturf responded differently to grazing (Shepard et al., 2018). When grazed frequently and closely, it assumed a prostrate growth habit with high herbage bulk density and post-grazing leaf mass. This allowed the plants
to reduce dependence on reserves for regrowth and maintain high levels of HA, even when grazed weekly to a 4-cm stubble.

While the effect of defoliation on HA varies, there is a point at which HA decreases with increasing defoliation frequency. In Florida, an experiment evaluated the effects of cutting frequency on Florigraze RP herbage accumulation during 24 wk each year for 2 yr (Beltranena et al., 1981). Plots were cut at 2-, 4-, 6-, 8-, 10-, and 12-wk intervals. Herbage accumulation increased with increasing interval between defoliation events through 6 wk in Year 1 and 8 wk in Year 2, after which HA remained the same or decreased. In Australia, 11 accessions of RP (some were *Arachis glabrata* and others *Arachis pintoi* Krapov. & W.C. Greg.) were harvested at two different frequencies (2 times yr\(^{-1}\) or 3-4 times yr\(^{-1}\)) for 2 yr (Bowman et al., 1998). They reported that the two harvests yr\(^{-1}\) treatment had greater herbage HA of approximately 1 Mg ha\(^{-1}\) yr\(^{-1}\) than the more frequent clipping treatment. Similar observations were made in an experiment evaluating HA and nutritive value of Florigraze RP harvested at 6, 9, or 12 wk during summer and fall in Florida (Romero et al., 1987). As interval between summer harvests increased from 6 to 12 wk, RP HA increased from 3.1 to 8.2 Mg ha\(^{-1}\). The authors suggested that RP accumulation was least in summer with the most frequent harvest interval because 6 wk did not allow time for the plant to recover from defoliation. However, in the fall, HA increased only through 9 wk, after which it decreased.

It has been suggested that excess herbage, as the result of either tall stubble height or low stocking rate can decrease HA (Adjei et al., 1980; Sanchez et al., 2018). In an experiment in South Florida, overseeding ‘Amarillo’ pinto peanut (*Arachis pintoi* Krapov. & W.C. Greg.) into Jiggs bermudagrass (*Cynodon dactylon* (L.) Pers.) was evaluated for pastures grazed at 15- and
25-cm stubble heights in 2 yr (Sanchez et al., 2018). In pastures grazed to the taller stubble height, there was 50% slower HA rate. The authors attributed this response to excess herbage, which can cause self-shading, reduced photosynthesis, and accumulation of senescent material. This is supported by a study evaluating the HA and nutritive value response of stargrass (Cynodon sp.), digitgrass (Digitaria eriantha Steud.), and bahiagrass (Paspalum notatum Flugge) to stocking rate (Adjei et al., 1980). There was a positive relationship between stocking rate, HA, and herbage in vitro digestible organic matter (IVDOM) concentration. The authors attributed this response to greater amounts of non-photosynthetic residue remaining in the low intensity treatment and the subsequent increase in self-shading and reduction in photosynthesis. Although these two experiments did not evaluate defoliation frequency, long defoliation intervals typically result in excess herbage, similar to what would occur with tall stubble heights and low stocking rates. So, it may be possible that the same physiological mechanisms (self-shading, reduced photosynthesis, and non-photosynthetic residue) affect the HA of pastures managed with long intervals between defoliation events, low stocking rate, and tall stubble heights.

**Below-ground Biomass, Persistence, and Vegetative Propagation**

Well-established stands of RP may have root-rhizome mass of more than 20 Mg ha\(^{-1}\) (Dubeux et al., 2017; Shepard et al., 2018), and this has been associated with excellent persistence (Mullenix et al., 2016b). Species persistence under different defoliation management practices is often associated with changes in storage organ mass (Sollenberger et al., 2012). Ortega-S. et al. (1992a) found that Florigraze RP proportion in pre-grazing herbage mass decreased from ~ 90 to 65% after one growing season of close, frequent grazing and to 30% after two growing seasons. Correspondingly, rhizome mass of Florigraze decreased from 4.0 to about
0.5 Mg ha\(^{-1}\) during the same time period (Ortega-S. et al., 1992b). In contrast to Florigraze, when Ecoturf RP was grazed weekly to 4 cm over two growing seasons, root-rhizome mass remained the same or increased and proportion of RP in pre-grazing herbage mass stayed the same or decreased no more than four percentage units (Shepard et al., 2018). In another grazing study, Ecoturf had greater root-rhizome mass than Florigraze under a range of grazing treatments (4450 and 3490 kg ha\(^{-1}\), respectively; Mullenix et al., 2016b), and the authors suggested that greater residual leaf area of Ecoturf after grazing allowed it to be less dependent on stored energy and to preserve root-rhizome mass. Thus, root-rhizome biomass and changes in biomass over time are useful indicators of the vigor and potential persistence of the sward.

After defoliation, root-rhizome carbohydrate reserves are mobilized to support above-ground growth, subsequently decreasing root-rhizome mass, total non-structural carbohydrate (TNC) concentration, and TNC pool (Saldivar et al., 1992a; 1992b). This relationship was described in a clipping study evaluating effects of defoliation frequency (undefoliated and defoliated at 1-, 4-, and 8-wk intervals) of Florigraze RP on above- and below-ground biomass (Williams, 1994). Starting in early June, root-rhizome mass of defoliated treatments decreased during the first 8 wk after defoliation, but they recovered to pre-defoliation levels by the latter part of the growing season. By the end of the season, below-ground biomass of the defoliated plots was 32% less than the non-defoliated plots. Similarly, as grazing intensity increased, Florigraze root-rhizome mass and TNC concentration decreased markedly during 2 yr (Rice et al., 1995).

Carbohydrate reserves of RP affect pasture establishment as well as stand persistence (Rice et al., 1996). Thus, the relationship between harvest frequency and root-rhizome
carbohydrate storage is critical to producers managing RP defoliation in order to produce rhizomes for use as planting material. In an experiment studying the effect of grazing intensity on establishment performance of Florigraze RP rhizomes, it was found that rhizomes from leniently grazed stands had more shoot production and a faster rate of herbage accumulation than pastures grazed more intensively (Rice et al., 1996). The authors suggested this was because severe grazing drains rhizome carbohydrate reserves.

**Nutritive Value**

Nutritive value is the digestibility, chemical composition, and nature of digested products of a forage. In general, legumes have greater nutritive value than warm-season grasses. Stobbs et al. (1975) found that when legumes were present in pastures mixed with C₄ grasses, ruminants increased intake, resulting in greater live weight gain. Legumes are valuable to ruminant nutrition because they have high levels of crude protein (CP) (Muir et al., 2011). Legumes may increase intake and animal performance given their overall greater digestibility and CP during the growing season, compared with warm-season grasses (Muir et al., 2011). If grass diets provide insufficient levels of N and energy to ruminants, legume addition to diets can increase N retention by livestock (Foster et al., 2009).

For Florigraze, UF Tito, and UF Peace cultivars and Ecoturf germplasm, the effect of defoliation management on herbage CP and IVDOM concentrations was relatively small (Mullenix et al., 2016a). They found all entries had CP ≥ 140 g kg⁻¹ and IVDOM ≥ 660 g kg⁻¹. In a separate study with Ecoturf RP, herbage CP and IVDOM varied little among grazing treatments, averaging 181 and 698 g kg⁻¹, respectively (Shepard et al., 2018). The authors attributed this lack of response in part to the relatively decumbent growth habit of Ecoturf and
suggested that this trait offered wide flexibility in timing of grazing or harvesting this forage. Saldivar et al. (1990) drew a similar conclusion, observing that the greater leaf:stem ratio of Florigraze RP minimized the typically negative effect of increasing maturity on nutritive value.

While the effect of harvest frequency on nutritive value of RP may not be as dramatic as for many other species, there is generally a negative relationship between defoliation frequency and nutritive value. The effects of cutting frequency on Florigraze nutritive value was evaluated by Beltranena et al. (1981). Nutritive value, as measured by CP and IVDOM, was greatest in the most frequently cut samples (2 wk) and decreased as cutting interval increased. In a multi-location study in Louisiana evaluating the effect of harvest frequency on nutritive value, Florigraze RP was harvested every 30 or 60 d (4 or 2 times yr⁻¹, respectively) (Redfearn et al., 2001). In this experiment, as in the others, the greatest herbage CP was observed in the most frequent defoliation treatment.

**Grasslands and Soil C Sequestration**

Increasing atmospheric CO₂ levels and their associated impacts on global climate have stimulated research in soil C sequestration of grasslands. Grassland soil can serve as a C sink by storing CO₂ that was removed from the atmosphere during photosynthesis. Fertilization, organic amendments, tillage, crop selection, and crop rotation affect the quality, quantity, and placement of C in the soil (Magdoff and Weill, 2004). The soil organic C (SOC) pool is vulnerable to disruption by agricultural practices, especially tillage. Organic matter that is exposed and oxidized will deplete the SOC pool, diminishing soil quality and subsequent plant biomass productivity (Follett, 2001). Soil texture, total soil nitrogen (TSN), plant species, and environmental factors can affect SOC.
Management practices that increase forage production also increase soil C (Conant et al., 2001; Allard et al., 2007; Ammon et al., 2007). Likewise, poor management of forages can inhibit C accumulation in grasslands. Although Florida’s sandy soils are inherently low in characteristics that build and protect SOC (Hassink, 1997), there is potential to increase SOC by improving management practices (Conant, 2001). The effect of management on rate of change of SOC and TSN at various soil depths of a ‘Coastal’ bermudagrass pasture was studied by Franzluebbers et al. (2009). Treatments included low (5.8 steers ha\(^{-1}\)) and high (8.7 steers ha\(^{-1}\)) stocking rates of grazed swards and unharvested and monthly-hayed bermudagrass. Significantly more C accumulated in the upper 15 cm of soil in grazed as compared with hayed pastures, with the greatest annual rate of change of SOC occurring in the low stocking rate treatment (1.17 Mg C ha\(^{-1}\) yr\(^{-1}\)). Stocking rates were also evaluated in a 26-yr study of their effects on soil C and N in bermudagrass pastures in Texas (Wright et al., 2004). The authors found that low stocking rate (2 to 2.5 cow-calf pairs ha\(^{-1}\)) increased C and N more than high stocking rate. The high stocking rate (5 to 7.4 cow calf pairs ha\(^{-1}\)) physically disturbed the soil to a greater extent and resulted in more rapid turnover of plant residues and greater amounts of nutrients cycling in animal excreta than the lower stocking rate.

The effects of defoliation and fertilizer input on C sequestration over 3 and 6 yr was evaluated in two studies by Ammann et al. (2007; 2009). In the 2007 study, the site was converted from crop rotation to grass-clover mixtures and included two management regimes; an intensive regime where grass was frequently cut and N fertilizer was applied at a rate of 200 kg ha\(^{-1}\) yr\(^{-1}\), and an extensive regime where there was no N input and cutting was less frequent. The extensive treatment lost a net of 57 g C m\(^{-2}\) yr\(^{-1}\), while the intensive treatment sequestered 147 g
C m$^{-2}$ yr$^{-1}$ over the course of the 3-yr study. In the six-yr study, the management treatments continued, and the results were largely similar with more net C sequestered with the intensive treatment due to greater plant productivity, as compared with the extensive treatment (Ammann et al., 2009).

Carbon sequestration potential of grasslands may also be affected by inclusion of legumes or by species richness or functional type. Legumes may enhance C sequestration in pastures because of their ability to increase soil N through N fixation. The decay-resistant, humic N polymers formed by N inhibit decomposition of humified soil C, increasing SOC (Fog, 1998; Resh et al., 2002). Reviewing several studies, Jensen et al. (2012) observed that incorporating perennial legumes into pastures increases SOC, yet there are few studies on this. When seeded in mixtures in Minnesota, Fornara and Tilman (2008) observed the N provided by legumes (four species tested) increased SOC by 100% over 12 yr and also increased root biomass of associated C$_4$ species (four species tested). Cong et al. (2014) observed that N fixation of legumes increased the rate of C sequestration in temperate grasslands where productivity was limited by low N. In a study on the effects of haying on soil C pools within plots of various species richness, only when the legumes birdsfoot trefoil (*Lotus corniculatus* L.) and white clover (*Trifolium repens* L.) were present did soil C and N accumulation increase relative to treatments including no legumes (DeDeyn et al., 2009). Neither the number of species nor functional group richness changed either C or N levels. In a continuation of that study, DeDeyn et al. (2011) further observed the presence of the legume red clover (*T. pratense* L.) improved soil C and N accumulation compared with treatments without red clover. The authors attributed this to the fact that high rates of C and N additions reduced soil respiration, improved soil structure and increased soil
organic matter (SOM) concentration. However, fine root production of legumes and its relationships with below-ground plant biomass accumulation and SOC content are not well understood.

Planted warm-season perennial grasses (C\textsubscript{4} photosynthetic pathway) may also enhance grassland C sequestration potential compared with native grasslands. Conant et al. (2001) observed that their extensive root system and permanent vegetative cover are conducive to SOC accumulation. The effects of stocking rates and N fertilization rates on SOM were evaluated in a bahiagrass pasture in a 3-yr study (Dubeux et al., 2006). The C and N concentrations in the SOM light density fraction were observed to increase as management intensity increased (Dubeux et al., 2006).

Cool-season grasses (C\textsubscript{3} photosynthetic pathway), such as small grains, can maintain or improve SOC when planted as cover crops in crop-rotation systems compared with the row crop alone without a cover crop. Franzluebbers and Stuedemann (2008) observed that total particulate organic matter remained relatively constant over time (2.3 Mg ha\textsuperscript{-1} yr\textsuperscript{-1}) with summer cropping of sorghum (\textit{Sorghum bicolor}) followed by winter grazing of rye (\textit{Secale cereale} L.). This integrated crop-livestock study was conducted over 3 yr in Georgia. Cover crop rotations incorporating winter forages could span up to 51 million ha in the US, potentially sequestering 100 to 300 kg C ha\textsuperscript{-1} yr\textsuperscript{-1} (Lal et al., 1999). With mild winters in Florida, incorporation of winter forages into year-round forage production systems may play a role in maintaining or increasing soil C in grasslands.
Relationship of Below-Ground Biomass and Root Growth with Soil C Accumulation in Grasslands

Plants with large allocation of biomass below-ground have significant potential to contribute to soil C (Rasse et al., 2005), but below-ground processes that drive SOC accumulation are not well-understood. Root dynamics is considered to be a critical component of the C cycle in grasslands, but it is difficult to measure or predict and can vary temporally and among and within species. Bradford et al. (2013) observed that stable SOC pools may be enhanced by fine root abundance which transfer C to the rhizosphere through exudation of amino acids, sugars, and polysaccharides. Phillips et al. (2011) showed that transfer of C through fine root exudates from live and decaying roots can dramatically impact nutrient cycling and SOC pools. Within a grassland context, fine root decay and root exudates are likely to regulate productivity and SOC accumulation and they are affected by defoliation (Augustine et al., 2011; Hafner et al., 2012). However, a deeper understanding of the relationship between below-ground biomass dynamics with soil C accumulation in grasslands is needed, but in order to establish these relationships better methods of measuring root mass accumulation are needed.

The objectives of the studies reported in this thesis were to 1) quantify and compare the effects of defoliation frequency on herbage production, nutritive value, and root-rhizome traits of selected RP introductions with those of existing cultivars and germplasms (Chapter 3), 2) characterize herbage production, nutritive value, and root-rhizome traits of selected RP introductions with those of existing cultivars and germplasms when grown at two locations with markedly different soil characteristics (Chapter 4), and 3) develop and test a prototype root-
ingrowth core device designed to quantify root mass accumulation rate of a group of RP entries (Chapter 5).
CHAPTER 3
RHIZOMA PEANUT HERBAGE ACCUMULATION, NUTRITIVE VALUE, AND ROOT-RHIZOME RESPONSES TO DEFOLIATION FREQUENCY

Overview

Perennial legumes can contribute significantly to forage systems. Their generally high nutritive value and ability to fix N are valuable characteristics to producers and can reduce the potential for negative impacts on the environment (Muir et al., 2011; Jensen et al., 2012). Of perennial legumes proposed for use in pastures in Florida, rhizoma peanut (RP; *Arachis glabrata* Benth.) is perhaps the best option because it is well adapted to Florida’s climate, has documented persistence under grazing (Ortega-S. et al., 1992b; Hernandez-Garay et al., 2004; Shepard et al., 2018), can spread in mixtures with grasses (Castillo et al., 2013a, 2013b; Mullenix et al., 2014), and has excellent nutritive value (Mullenix et al., 2016b; Shepard et al., 2018).

‘Florigraze’ is the most widely used RP cultivar in Florida, but it is negatively affected by the peanut stunt virus (Quesenberry et al., 2010). The University of Florida has released several forage RP genotypes in the past decade (Prine et al., 2010; Quesenberry et al., 2010) that are superior to Florigraze. Most released RP lines were selected for upright growth, favoring use in hay production, but there are germplasms and experimental lines that vary in growth habit, which may affect their optimal forage use. For example, tall-growing released lines, ‘UF Tito’ and ‘UF Peace’ have demonstrated disease tolerance, persistence, and herbage accumulation (HA) yield under hay management. Lower-growing germplasms Ecoturf and Arblick may be better suited to grazing or ornamental use (Prine et al., 2010). Ecoturf is productive and persistent under close, frequent grazing (weekly to 4 cm) due to its ability to increase sward canopy bulk density and maintain residual leaf area close to the soil surface (Shepard et al., 2018). Efforts continue to
identify superior RP lines, and currently there are numerous RP plant introductions and selections with potential for use in Florida forage-livestock systems. Data describing key forage responses are lacking for these entries, and evaluation is needed of forage productivity, nutritive value, and stand persistence.

Defoliation management is critical to the successful use of any forage, and RP is no exception. Although defoliation intensity is the most important determinant of plant and animal response (Sollenberger et al., 2012), in RP hay systems cutting height varies relatively little. Thus, response to defoliation frequency is important and may affect HA, nutritive value, and rhizome mass and chemical composition. Rhizoma peanut HA increased with increasing interval between grazing events up to 63 d for Florigraze (Ortega-S. et al., 1992b) and 42 d for Florigraze, UF Tito, UF Peace, and Ecoturf (Mullenix et al., 2016a). Nutritive value was minimally affected by defoliation frequency (Mullenix et al., 2016a; Shepard et al., 2018), but frequent, close defoliation reduced rhizome mass and total non-structural carbohydrate (TNC) concentration of Florigraze (Ortega-S. et al., 1992a; Rice et al., 1995) and rhizome mass of several RP entries (Mullenix et al., 2016b). Response of Ecoturf, a phenotypically plastic germplasm that adapts to close grazing by developing a short, dense canopy, was different than Florigraze, as it maintained or increased herbage HA and rhizome mass under frequent, close grazing (Shepard et al., 2018).

Previous research has shown that RP responses to defoliation frequency differ among entries of varying growth habit and that both above- and below-ground plant responses are affected (Mullenix et al., 2016a). Thus, both herbage characteristics, important to hay producers, and rhizome characteristics, important to those who vegetatively propagate RP and for long-term
stand persistence, are affected by defoliation frequency and growth characteristics of individual entries. The objective of this study was to quantify and compare the effects of defoliation frequency on herbage production, nutritive value, and root-rhizome traits of selected RP introductions with those of existing cultivars and germplasms. Relatively long intervals between defoliation events were evaluated in order to address two primary scenarios: i) the difficulty of timely hay harvest in Florida because of frequent rain events during the summer growing season; and ii) situations in which RP is harvested for hay during the growing season and also used as a source of rhizomes for vegetative propagation in the subsequent dormant season.

Materials and Methods

Experimental Sites

The 3-yr experiment yr (2015, 2016, and 2017) was conducted at the North Florida Research and Education Center (NFREC) in Quincy, FL (30.55° N, 84.60° W) on well-established plots that were planted in July 2009. Prior to planting the plots for the current experiment, the area was occupied by bahiagrass (Paspalum notatum Flugge). The soil at the site is a Norfolk loamy fine sand (fine-loamy, kaolinitic, thermic Typic Kandiudults), described as a well-drained soil. Soil samples were taken to a depth of 15 cm and were analyzed at the University of Florida Extension Soil Testing Laboratory. Soil pH was 6.0 and Mehlich-3 extractable P, K, and Mg were 38, 28, and 58 mg kg\(^{-1}\), respectively. In April 2017, K was applied at a rate of 56 kg ha\(^{-1}\) and P was applied at a rate of 15 kg ha\(^{-1}\). No fertilizer was applied in 2015 and 2016.
Treatments and Experimental Design

Rainfall data for the site during the experimental period is shown in Table 3-1. Treatments were the factorial combinations of two defoliation frequencies and 14 RP introductions/selections, germplasms, and cultivars (Table 3-2), here forward referred to as entries. The design was a split-plot arrangement of a randomized complete block design, with five replications. Main plots were 2.5-m wide x 3-m long (7.5 m²), with a 1.8-m alley between main plots. The entry main plots were split into two subplots (each 2.5-m x 1.5 m) to which defoliation frequency levels of one (1X; fall) or two (2X; summer and fall) harvests per season were allocated.

Response Variables

Peanut rust, herbage accumulation, nutritive value, and canopy characteristics

Harvests for the 2X treatment occurred on 26 July and 23 Nov. 2015, 14 July and 7 Nov. 2016, and 27 June and 20 Oct. 2017. The 1X treatment was cut only at the October or November date already mentioned for the 2X treatment. Harvest dates were chosen based on typical conditions in North Florida, where spring drought precludes significant growth of rain-fed RP before June, and in the absence of irrigation most hay producers get only two harvests per year. The 1X treatment reflects that summer harvests are challenging due to frequent and unpredictable rainfall events and poor drying conditions. Thus, some producers in some years fail to harvest RP in summer due to lack of sufficient consecutive days of satisfactory drying conditions. In addition, some hay producers also dig rhizomes from hay fields for use in planting new areas to RP. They are interested in knowing the impact of multiple vs. single harvests per
year on root-rhizome mass and TNC reserves, factors that affect the amount of area that can be planted and the likelihood of establishment success (Rice et al., 1995; 1996).

At each harvest date, herbage from one (2015 and 2016) or two (2017) 0.25-m² quadrats was harvested to a 6-cm stubble height from each experimental unit. The remainder of the subplot was clipped with a flail mower to the target stubble height and all herbage removed. Samples were dried at 60°C until constant weight, weighed, and HA was expressed as Mg DM ha⁻¹ yr⁻¹. These samples were ground to pass a 1-mm screen using a Wiley mill (Model 4 Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedeboro, NJ). Samples were then analyzed for N and in vitro digestible organic matter (IVDOM) concentrations. Nitrogen was measured using the aluminum block digestion technique (Gallaher et al., 1975), and IVDOM was determined using a modification of the two-stage technique (Moore and Mott, 1974). Crude protein (CP) was calculated as N concentration x 6.25. Considering the typically low coefficient of variation associated with measures of nutritive value and the cost of laboratory analyses, samples from only three of the five replicates were analyzed for CP and IVDOM.

Before each harvest event in 2017, canopy height was measured at 10 locations in each experimental unit to aid in quantifying differences in growth habit among entries and to allow calculation of herbage bulk density. Herbage bulk density was calculated by dividing HA (kg ha⁻¹) at a given harvest by the depth of the harvested canopy and expressed in kg ha⁻¹ cm⁻¹. Thus, if canopy height was 20 cm and the stubble height was 6 cm, bulk density was calculated by dividing HA by 14 cm. Peanut rust (Puccinia arachidis) disease was assessed in each experimental unit on 27 June and 11 Oct. 2017. Percentage of leaflet area infected by the disease was estimated visually, and the average infection in each plot was converted to the Horsfall-
Barratt (H-B) scale of plant disease. The H-B scale is a visual assessment of plant disease and is based on a semi-quantitative scale (Horsfall and Barratt, 1945).

**Root-rhizome mass and total non-structural carbohydrate concentration and rhizome diameter**

Root-rhizome mass was measured by taking three cores from randomly selected locations in the center of each experimental unit on 13 Dec. 2016 and 12 Dec. 2017. Cores were 10 cm in diameter and 20-cm deep. The three samples per plot were composited and washed over a 2-mm mesh screen to remove soil. The sample was then dried at 60°C to a constant weight and weighed. Samples were ashed at 500°C for 4 h and mass was expressed on an organic matter basis to avoid potential effects of soil contamination. Mean rhizome diameter was quantified for each experimental unit. Four representative rhizomes were selected from each rhizome sample and diameter was measured at the mid-point of the length of the rhizome. Mean rhizome diameter was calculated as the average of the four measurements per plot.

Root-rhizome samples were ground in a Wiley mill to pass a 1-mm stainless steel screen prior to analysis for TNC concentration. The TNC concentration was determined using a modification of the procedure of Christiansen et al. (1988) that was described by Chaparro et al. (1996), and it was expressed as a proportion of root-rhizome organic matter. This procedure uses amylglucosidase and invertase to convert starch and oligosaccharides into monosaccharides and measures reducing sugars with a photometric copper reduction method (Nelson, 1994). As described for herbage nutritive value, root-rhizome samples from three of five replicates were analyzed for TNC concentration. The TNC pool was calculated as root-rhizome mass times TNC concentration.
Statistical Analyses

Data were analyzed using a mixed model with entry, defoliation frequency, and their interaction as fixed effects and block and year as random effects. Year was considered a repeated measure. Defoliation frequency means were separated using the F test, and RP entry means were separated using Tukey’s test. Treatment means were considered different when $P \leq 0.05$. Some data are presented graphically in the form of box plots. The structure of box plots is such that the upper and lower hinges (terminal ends of the box) relate to the first and third quartiles (or 25th and 75th percentiles), respectively. The thick solid line within the box and perpendicular to the sides of the box is the median. The upper and lower whiskers (solid lines extending above and below the box) extend from the hinge to the largest and smallest values (at most 1.5 interquartile range) of the hinge, respectively. Data points beyond the whiskers are considered outliers.

Results and Discussion

Peanut Rust, Herbage Accumulation, and Sward Canopy Characteristics

Peanut rust incidence

In the June sampling date, all lines except Ecoturf showed no disease (H-B rating of 1) (Table 3-3). Ecoturf 1X and 2X had a H-B score of 4. All replicates of each entry had the same rating in June, so it was not possible to analyze those data statistically. In October, there was no entry x defoliation frequency interaction for peanut rust disease ($P = 0.85$), nor was there a defoliation effect ($P = 0.84$). However, there was an effect of entry (Table 3-3; $P < 0.0001$). Ecoturf was moderately to severely affected (H-B rating of 7) as over half its leaflets were diseased. Apalachee and UF Tito were moderately diseased (H-B rating of 5) and Quincy-Alpha
was mildly diseased (H-B rating of 4). Entries Quincy and Waxy Leaf had less than 3% of their leaves diseased (H-B rating of 2), and their rating was not different than lines with no disease.

**Annual herbage accumulation**

There was an entry x defoliation frequency interaction for HA ($P < 0.0001$; Figure 3-1). The 2X treatment had greater HA than 1X for all entries ($P < 0.0001$) except for Quincy-Beta, for which there was no defoliation frequency effect. For many of the entries (Quincy-Beta being the primary exception), the 2X treatment had nearly twice the HA as 1X, as evidenced by defoliation treatment main effect means of 10.5 vs. 5.4 Mg ha$^{-1}$ yr$^{-1}$, respectively. Among interaction means, HA of the 2X treatments of Arbrook (13.9 Mg ha$^{-1}$ yr$^{-1}$), UF Tito (12.9 Mg ha$^{-1}$ yr$^{-1}$), and UF Peace (12.3 Mg ha$^{-1}$ yr$^{-1}$) were greater than the 2X treatments of Arblick (9.33 Mg ha$^{-1}$ yr$^{-1}$), Cowboy (9.11 Mg ha$^{-1}$ yr$^{-1}$), Florigraze (8.43 Mg ha$^{-1}$ yr$^{-1}$), and Apalachee (7.68 Mg ha$^{-1}$ yr$^{-1}$). Least herbage accumulation was ~4 Mg ha$^{-1}$ yr$^{-1}$ for numerous entries harvested once per year, including 3.86 Mg ha$^{-1}$ yr$^{-1}$ for industry standard Florigraze. Among the 1X treatments, Quincy-Beta had greater herbage accumulation (8.77 Mg ha$^{-1}$ yr$^{-1}$) than all entries except Arbrook (7.92 Mg ha$^{-1}$ yr$^{-1}$), Quincy (7.67 Mg ha$^{-1}$ yr$^{-1}$), UF Peace (6.53 Mg ha$^{-1}$ yr$^{-1}$), and Waxy Leaf (6.29 Mg ha$^{-1}$ yr$^{-1}$).

Lack of difference between Quincy-Beta with 2X and 1X frequencies (10.4 vs. 8.77 Mg ha$^{-1}$ yr$^{-1}$, respectively) may be due to greater disease tolerance of Quincy-Beta than other lines resulting in greater leaf retention for full-season growth (Blount, personal communication). Herbage accumulation of Quincy-Beta was greater than that reported by Santos et al. (2017) in a 2-yr experiment in which Quincy-Beta (5.37 Mg ha$^{-1}$ yr$^{-1}$) and the germplasm Ecoturf RP were harvested by clipping four times per year. In that study, annual HA of Ecoturf was 5.00 Mg ha$^{-1}$
compared with 3.91 Mg ha\(^{-1}\) (1X treatment) and 10.5 Mg ha\(^{-1}\) (2X treatment) in the current experiment. Lesser annual HA for 1X in the current study can be explained in part due to moderate to severe levels of peanut rust infestation for Ecoturf that is thought to reduce effective leaf area and HA.

Average annual HA for the 2X treatment falls within the range observed in previous experiments. Annual RP HA in the southeastern US was 7 to 11 Mg ha\(^{-1}\) (Terrill et al., 1996; Venuto et al., 1999). Reported RP HA in grazing or clipping trials in Florida have included 13 Mg ha\(^{-1}\) yr\(^{-1}\) (Dubeux et al., 2017), 6.0 to 9.2 Mg ha\(^{-1}\) yr\(^{-1}\) (Mullenix et al., 2016), and 8.3 to 12 Mg ha\(^{-1}\) yr\(^{-1}\) (Prine et al., 2010). However, annual HA for the 1X treatments was generally well below these means. The average of the two defoliation treatment means for Ecoturf, UF Tito, Quincy-Alpha, and Apalachee, the four lines with significant peanut rust damage, were at the lower end of reported HA means. However, 2X means of Ecoturf and UF Tito are well-within reported means.

Previous studies have evaluated defoliation frequency effects on various RP entries. Six defoliation frequencies ranging from 2 to 12 wk were compared for Florigraze RP during 2 yr (Beltranena et al., 1981). Herbage accumulation increased as interval between defoliation events increased up to 6 wk in the first year of study and up to 8 wk in the second year. The 12-wk interval treatment in that experiment was harvested twice per year, with an average annual HA of 10.1 Mg ha\(^{-1}\) yr\(^{-1}\), comparable to the response of the 2X treatment in the current study. When grazed, Florigraze HA increased as interval between grazing events increased if pastures were grazed to a residual herbage mass of 500 to 1500 kg ha\(^{-1}\) (Ortega-S. et al., 1992). When residual herbage mass was greater, there was little effect of defoliation frequency on HA. Averaged
across four RP entries (UF Tito, UF Peace, Florigraze, and Ecoturf), HA was greater when pastures were grazed every 6 than every 3 wk (Mullenix et al., 2016a). There is evidence in the literature, however, that more frequent defoliation may result in greater HA of some RP entries under some conditions. For example, in a grazing experiment with Ecoturf, weekly close defoliation (4- or 8-cm stubble) resulted in greater HA than defoliating every 4 or 7 wk (Shepard et al., 2018). Likewise, in a mowing experiment with Ecoturf, frequent, close mowing was associated with greater HA, more new shoots, and a dense canopy (Rouse et al., 2004). Thus, the HA response to defoliation frequency is not uniform across RP entries. It appears that those with shorter growth habit or greater phenotypic plasticity are favored by more frequent defoliation, while more upright types are favored by less frequent defoliation. The response of Quincy-Beta supports this conclusion, as it is among the shortest of the entries evaluated (described later in this chapter). However, in the current study both levels of defoliation frequency imposed are considered infrequent and the response to frequency was more likely related to greater disease incidence in the 1X treatment than to physiological mechanisms that are at play when plants are defoliated more frequently.

**Canopy height**

There was no entry x defoliation frequency interaction ($P = 0.071$) or defoliation frequency main effect ($P = 0.15$), but there was an effect of entry ($P < 0.001$) on canopy height (Figure 3-2). Arbrook was taller (29) than all other entries except UF Tito (26 cm), while Quincy-Beta was the shortest (20 cm). Intermediate entries included Ecoturf (22 cm), Ona 33 (23 cm), and UF Peace (24 cm), and they were not different from UF Tito or Quincy-Beta. This follows the observation by Quesenberry et al. (2010) that UF Tito is an upright variety, while UF
Peace is intermediate, and Ecoturf is more decumbent. When grazed every 3 or 6 wk, UF Tito was tallest, UF Peace and Florigraze were intermediate, and Ecoturf was shortest during early, mid-, and late season (Mullenix et al., 2016b). However, in the current study there were no differences in canopy height among these four entries. This may be due in part to Ecoturf’s phenotypic plasticity, whereby it assumes a shorter canopy with greater bulk density when frequently and closely defoliated (Mullenix et al., 2016b; Shepard et al., 2018). Under infrequent cutting like that utilized in this study, Ecoturf assumes a more intermediate growth habit.

**Herbage bulk density**

There was an entry x defoliation frequency interaction ($P = 0.003$) for herbage bulk density (Figure 3-3). Interaction occurred because bulk density was similar between defoliation frequencies for all entries except UF Peace, where the bulk density was 410 kg ha$^{-1}$ cm$^{-1}$ for 2X and 244 kg ha$^{-1}$ cm$^{-1}$ for 1X. The defoliation frequency effect also approached significance for UF Tito ($P = 0.66$; 360 and 251 kg ha$^{-1}$ cm$^{-1}$ for 2X and 1X, respectively). The treatment combinations with greatest bulk density were Quincy-Beta harvested once or twice per year (567 and 452 kg ha$^{-1}$ cm$^{-1}$) and UF Peace harvested twice per year (410 kg ha$^{-1}$).

The ability of a forage plant to change its bulk density under different defoliation methods can be an indication of phenotypic plasticity. In 2 growing seasons of grazing every 3 or 6 wk, Ecoturf always had greater herbage bulk density (223-260 kg ha$^{-1}$ cm$^{-1}$) than UF Tito (105-150 kg ha$^{-1}$ cm$^{-1}$), UF Peace (117-188 kg ha$^{-1}$ cm$^{-1}$), and Florigraze (130-152 kg ha$^{-1}$ cm$^{-1}$) (Mullenix et al., 2016b). Ecoturf was also the only one of the four entries in which herbage bulk density was greater in both years for the 3- vs. the 6-wk grazing interval (260 vs. 175 and 268 vs. 223 kg ha$^{-1}$ cm$^{-1}$ in Years 1 and 2, respectively). UF Peace had greater herbage bulk density for
the 3-wk treatment in the first year of that study only (156 vs. 117 kg ha\(^{-1}\) cm\(^{-1}\)). In other experiments investigating the effect of Ecoturf RP defoliation, close, frequent cutting or grazing resulted in greater herbage bulk density (Rouse et al., 2004; Shepard et al., 2018). When grazed weekly to 4 cm, Ecoturf bulk density reached nearly 500 kg ha\(^{-1}\) cm\(^{-1}\), and it declined when post-grazing height was 8 cm and when interval between grazing increased to 4 or 7 wk (Shepard et al., 2018). As observed previously with Ecoturf canopy height in the current experiment, defoliation only once or twice a year is likely not frequent enough to affect bulk density significantly.

**Nutritive Value Responses**

**Herbage crude protein**

There was no entry x defoliation frequency interaction (\(P = 0.146\)), however, there was an effect of entry on herbage CP (\(P < 0.0001\)) (Figure 3-4). Apalachee and Cowboy had greatest CP concentrations (153 and 154 g kg\(^{-1}\), respectively), and Arbrook, Quincy-Beta, and Florigraze had the lowest levels (106, 123, and 124 g kg\(^{-1}\), respectively). The latter three were lower in CP than all other entries except Quincy and Ona 33 (126 and 132 g kg\(^{-1}\), respectively). The average CP across entries was 138 g kg\(^{-1}\). Even entries with greatest CP concentration had values lower than observed in an experiment comparing Arbrook and Florigraze under continuous stocking (Hernandez-Garay et al., 2004). After three years in a moderately stocked pasture, average CP of Florigraze was greater than Arbrook (177 vs. 161 g kg\(^{-1}\), respectively). Those cultivars were ranked similarly in the current experiment (124 vs. 106 g kg\(^{-1}\), respectively).

There was also an effect of defoliation frequency (\(P < 0.0001\)) (Table 3-4) on herbage CP in the current study, with the 2X treatment having greater herbage CP concentration than 1X
(150 vs. 125 g kg\(^{-1}\)). Generally, herbage CP of RP has followed the expected pattern for most forages, i.e., it decreases with increasing interval between defoliation events. Crude protein concentration decreased from 219 to 147 g kg\(^{-1}\) as interval between defoliation events increased from 2 to 12 wk (Beltranena et al., 1981), and CP of several entries averaged 170 g kg\(^{-1}\) when harvested three times per year (Dubeux et al., 2017). When Ecoturf plots were grazed every 4 or 7 wk, CP was greater for the 4- than 7-wk defoliation frequency (187 vs. 175 g kg\(^{-1}\)) (Shepard et al., 2018). Ecoturf, UF Peace, UF Tito, and Florigraze nutritive value was compared at grazing frequencies of 3 and 6 wk (Mullenix et al., 2016a). Defoliation frequency did not affect CP concentration of Ecoturf or Florigraze, but the generally more upright-growing types, i.e., UF Peace and UF Tito, had greater CP when grazed at 3- instead of 6-wk frequencies. In that experiment, Florigraze CP concentration was less than all other entries at both regrowth intervals, while Ecoturf had the greatest CP concentration at 6 wk, but it was less than only UF Peace at 3 wk.

**Herbage in vitro digestibility**

There was an entry x defoliation frequency interaction for herbage IVDOM \((P < 0.0001)\) (Figure 3-5). When differences between defoliation frequencies occurred within an entry, the 2X treatment was always favored over the 1X treatment, but differences were limited to entries Ona 33 (642 vs. 577 g kg\(^{-1}\)), Cowboy (645 vs. 547 g kg\(^{-1}\)), Pointed Leaf (637 vs. 533 g kg\(^{-1}\)), and Florigraze (645 vs. 550 g kg\(^{-1}\)). The treatments with greatest IVDOM concentrations were Quincy-Alpha and Apalachee (658 and 651 g kg\(^{-1}\), respectively) when harvested twice per year, while the lowest concentrations were Pointed Leaf (533 g kg\(^{-1}\)) and Cowboy (547 g kg\(^{-1}\)) harvested once per year.
Arbrook, UF Peace, and UF Tito were among the most productive entries tested, but their IVDOM was relatively low and the range in IVDOM across defoliation frequencies was narrow. For the 2X and 1X treatments, respectively, Arbrook IVDOM was 578 and 562 g kg\(^{-1}\), UF Peace IVDOM was 595 and 579 g kg\(^{-1}\), and UF Tito IVDOM was 593 and 559 g kg\(^{-1}\). In contrast, greater IVDOM, especially for the 1X treatment was observed for more decumbent-growing types, with Waxy Leaf (21 cm), Arblick (21 cm), and Quincy-Beta (20 cm) having IVDOM of 631, 607, and 599 g kg\(^{-1}\) when harvested only once per year. In general, both 1X and 2X treatments can be considered infrequent defoliation, and IVDOM was often less than previously reported for RP in the literature. For example, Florigraze and Arbrook herbage from continuously stocked pastures had average IVDOM over 3 yr of 705 and 661 g kg\(^{-1}\), respectively (Hernández-Garay et al., 2004). Under rotational stocking with 6-wk intervals between grazing events in Florida (Sollenberger et al., 1989) and when harvested twice per season in Georgia (Terrill et al., 1996) Florigraze IVDOM was 650 g kg\(^{-1}\) or greater. When Florigraze, UF Tito, UF Peace, and Ecoturf were grazed at 3- or 6-wk frequencies, IVDOM ranged from 660 to 690 g kg\(^{-1}\) and there were no differences among entries and generally no difference between grazing frequencies. Ecoturf IVDOM was greater when grazed every 4 vs. 7 wk (718 g kg\(^{-1}\) vs. 687 g kg\(^{-1}\), respectively), but the range in IVDOM was relatively small (Shepard et al., 2018). Thus, those producers who are harvesting only once or twice per year will likely experience a significant reduction in IVDOM relative to more frequently harvested material, and the penalty may be most severe for the more upright-growing and productive entries.
Below-Ground Responses

Root-rhizome mass

For root-rhizome mass, there was no entry x defoliation interaction ($P = 0.347$), but there was an effect of entry ($P = 0.0002$) (Figure 3-6) and defoliation frequency ($P < 0.0001$) (Table 3-4). Arbrook had greater root-rhizome mass (9.01 Mg ha$^{-1}$) than Apalachee (5.63 Mg ha$^{-1}$), Pointed Leaf (5.92 Mg ha$^{-1}$), and Florigraze (5.22 Mg ha$^{-1}$). Other entries with relatively large below-ground mass included Quincy-Alpha (8.67 Mg ha$^{-1}$), UF Peace (8.17 Mg ha$^{-1}$), and UF Tito (7.14 Mg ha$^{-1}$). Plots cut once per year had 54% more root-rhizome mass than those cut twice per year (8.51 vs 5.52 Mg ha$^{-1}$, respectively).

Root-rhizome mass data vary widely in the literature. Means from the current study are within the range reported for Florigraze under grazing (Ortega-S. et al. 1992b; Rice et al., 1995), less than those measured under clipping for several entries by Dubeux et al. (2017) and under grazing for Ecoturf by Shepard et al. (2018), and greater than those found under grazing of four entries by Mullenix et al. (2016b). Arbrook, UF Peace, and Florigraze root-rhizome mass were 17.3, 21.5, and 10.5 Mg ha$^{-1}$, respectively, when clipped three times per year for 2 yr (Dubeux et al., 2017). Ecoturf root-rhizome mass ranged from 16 to 20 Mg ha$^{-1}$ for long-established pastures that were defoliated by grazing every 1, 4, or 7 wk for 2 yr (Shepard et al., 2018), and it ranged from 3.2 to 4.5 Mg ha$^{-1}$ for young stands that had been grazed every 3 or 6 wk for 2 yr (Mullenix et al., 2016b). In that study, Ecoturf and UF Tito had greater root-rhizome mass than Florigraze and UF Peace.

It is well established that defoliation affects root-rhizome mass of Florigraze RP. After 3 yr of grazing, average root-rhizome mass was greatest (17.0 Mg ha$^{-1}$) for an ungrazed control
compared with pastures that were grazed every 63 d to a residual herbage mass of 2500 kg ha\(^{-1}\) (9.4 Mg ha\(^{-1}\)) or every 21 d to a residual herbage mass of 500 kg ha\(^{-1}\) (2.3 Mg ha\(^{-1}\)) (Rice et al., 1995). Similarly, after 2 yr of grazing, root-rhizome mass was least when Florigraze pastures were grazed frequently and closely; it increased with increasing interval between grazing events when grazed closely, but it changed only slightly due to different intervals between grazing events when post-grazing residual herbage mass was above 1700 kg ha\(^{-1}\) (Ortega-S. et al., 1992a). For Ecoturf pastures, grazing to a 4-cm stubble during 2 yr resulted in lesser root-rhizome mass than grazing to 8-cm stubble (16.2 vs. 19.7 Mg ha\(^{-1}\)), but there was no effect of regrowth intervals of 1, 4, and 7 wk.

Data from the current and previous studies have implications for producers hoping to utilize their RP fields for both grazing or haying and as a source of planting material in the subsequent dormant period. Increasing the frequency of defoliation, even only from one to two cuts per year, in conjunction with short stubble heights is likely to reduce root-rhizome mass that will decrease the amount of rhizomes that can be harvested from that area for use to plant new areas.

**Rhizome diameter**

There was no entry x defoliation interaction \((P = 0.109)\), but there was an effect of entry \((P < 0.0001; \text{Fig. 3.7})\) and defoliation frequency approached significance \((P = 0.055)\). The 1X treatment rhizome diameter was 3.23 mm vs. 3.07 mm for the 2X treatment, leading to the nearly significant response. Arbrook root-rhizome diameter (4.07 mm) was greater than that of any other entry, and it was followed by UF Peace (3.70 mm) that was not different from Quincy-Alpha (3.20 mm), Arblick (3.20 mm), Waxy Leaf (3.15 mm), Ona 33 (3.08 mm), and Ecoturf
Cowboy (2.68 mm), Quincy-Beta (2.80 mm), Pointed Leaf (2.80 mm), Florigraze (2.83 mm), Quincy (2.90 mm), and Apalachee (2.95 mm) rhizomes had lesser diameters than UF Peace. There are very limited data regarding rhizome diameter, but Prine et al. (1986) noted that Arbrook produced thicker rhizomes than all known RP lines at that time. The importance of rhizome diameter has not been studied by others, but it is likely to reduce the number of viable bud sites per unit mass of rhizome and thus large-diameter rhizomes should likely be associated with greater planting rates.

**Root-rhizome total non-structural carbohydrate concentration**

There was no entry x defoliation interaction ($P = 0.297$) or defoliation frequency effect ($P = 0.305$; Table 3-4) on root-rhizome TNC concentration, but there was an effect of entry ($P < 0.0001$; Figure 3-8). UF Peace had greater TNC concentration (312 g kg$^{-1}$) than Cowboy (234 g kg$^{-1}$), Apalachee (232 g kg$^{-1}$), Pointed Leaf (232 g kg$^{-1}$), Arblick (228 g kg$^{-1}$), Waxy Leaf (227 g kg$^{-1}$), and Ecoturf (202 g kg$^{-1}$). Interestingly, the lowest TNC concentrations in this experiment are similar to the greatest concentrations reported for Florigraze by Ortega-S et al. (1992a). At the end of a 2-yr experiment that evaluated a range of grazing treatments on Florigraze, they found TNC concentrations from 58 g kg$^{-1}$ under frequent, close grazing to 210 g kg$^{-1}$ when residual herbage mass was large (> 1500 kg ha$^{-1}$) and regardless of grazing frequency. The least frequent grazing treatment in that study was three events per year, thus it was somewhat more intense than in the current study where Florigraze TNC was 241 g kg$^{-1}$.

There are several studies that report a reduction in RP root-rhizome TNC concentration with more frequent defoliation. These include Ortega-S et al. (1992a) and Rice et al. (1995) with Florigraze. Mullenix et al. (2016b) found no effect of 3- or 6-wk grazing frequencies on UF Tito,
UF Peace, Ecoturf, or Florigraze, while Shepard et al. (2018) reported a relatively small linear increase in Ecoturf root-rhizome TNC (198 to 214 g kg\(^{-1}\)) as regrowth interval between grazing events increased from 1 to 7 wk. Perhaps the lack of response to defoliation frequency in this study was due to the narrow range of defoliation frequencies evaluated and all levels could be considered infrequent. These data suggest that although grazing twice a year reduces root-rhizome mass it is not frequent enough to diminish storage organ TNC concentration. Thus, 2X defoliation results in fewer rhizomes produced for subsequent planting activities, but the rhizome quality should still be good.

**Root-rhizome total non-structural carbohydrate pool**

There was no entry x defoliation frequency interaction \((P = 0.458)\). Both RP entry \((P = 0.0001)\) (Figure 3-9) and defoliation frequency effects were significant \((P < 0.0001)\) (Table 3-4). The 1X treatment resulted in 45% greater TNC pool than 2X (2010 vs. 1390 kg TNC ha\(^{-1}\)) due to an average 54% greater root-rhizome mass for the 1X than the 2X treatment. Among entries, Arblick and Arbrook had the least and greatest TNC pools, respectively (1200 kg ha\(^{-1}\) vs 2720 kg ha\(^{-1}\)). Arbrook TNC pool was also greater than Apalachee (1240 kg ha\(^{-1}\)), Waxy Leaf (1360 kg ha\(^{-1}\)), Pointed Leaf (1400 kg ha\(^{-1}\)), Florigraze (1410 kg ha\(^{-1}\)), Ecoturf (1430 kg ha\(^{-1}\)), and Cowboy (1500 kg ha\(^{-1}\)), but not different than the other entries.

**Implications of the Research**

A single defoliation event near the end of the growing season resulted in significant reductions in annual HA, herbage CP, and herbage IVDOM for most RP entries relative to harvests at both the middle and end of the growing season. Greater disease pressure for the one harvest per year treatment likely caused leaf drop that decreased photosynthesis and HA of some
entries. Herbage accumulation was not affected by defoliation frequency for only the entry Quincy-Beta, perhaps because of Quincy-Beta’s greater tolerance to disease that has been observed previously (Blount, personal communication). It is also likely that a single harvest was associated with long periods of self-shading that reduced HA rate. Defoliation either once or twice a year was not frequent enough to affect morphological mechanisms that would significantly alter canopy height or bulk density (i.e., phenotypic plasticity). The reduction in herbage IVDOM with a single vs. two harvests was more severe for the most upright-growing and productive entries. Greater defoliation frequency (2X vs. 1X) in conjunction with short stubble heights decreased harvestable root-rhizome mass and root-rhizome TNC pool, a response of particular importance to producers hoping to use RP fields both for grazing or haying and as a source of planting material the subsequent winter season. However, planting material quality, evidenced by storage organ TNC, was similar in both 1X and 2X defoliation treatments.

In conclusion, rhizoma peanut hay producers should prioritize at minimum a summer and a fall harvest from their hay fields, even if summer weather conditions are not optimal for drying. Preservation as haylage or balage, options that require less field drying, should be explored. Those producers wishing to dig rhizomes from hay fields should keep in mind that defoliation, especially more than one defoliation event per year will reduce the amount of rhizome mass available for digging the following winter or spring. Finally, the impact of harvest frequency varies among entries of RP, with Quincy-Beta being particularly tolerant of infrequent harvests in terms of both HA and herbage IVDOM. More upright-growing entries (e.g., Arbrook and UF Tito) and those most susceptible to leaf diseases (e.g., Ecoturf) are likely to show some of the greatest negative impacts associated with a single vs. multiple harvests per year.
Table 3-1. Monthly rainfall at the experimental site in Quincy, FL and the 30-yr average rainfall for Quincy. Data obtained from the Florida Automated Weather Network (FAWN) which has a recording station on site.

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>30-yr average</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>138</td>
<td>150</td>
<td>237</td>
<td>122</td>
</tr>
<tr>
<td>February</td>
<td>88</td>
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<td>November</td>
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<td>11</td>
<td>89</td>
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<tr>
<td>December</td>
<td>175</td>
<td>134</td>
<td>81</td>
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<tr>
<td>Total</td>
<td>1380</td>
<td>1486</td>
<td>1330</td>
<td>1516</td>
</tr>
</tbody>
</table>
Table 3-2. Rhizoma peanut cultivars, germplasms, introductions, and selections (referred to as entries) evaluated at the North Florida Research and Education Center (NFREC) at Quincy, FL.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Abbreviation in figures</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ona 33</td>
<td>33</td>
<td>An introduction from Paraguay. PI 262833. Notable for tolerance to short (2 d) water conditions at Ona. <em>Arachis glabrata var. hagenbeckii</em>.</td>
</tr>
<tr>
<td>QS6W/Quincy-</td>
<td>Alpha</td>
<td>Origin has been lost. Surviving plants from an introduction nursery planted at the NFREC in the early 1970s. Survived and spread over the past 50 yr. Selected for its ability to spread into bahiagrass pasture.</td>
</tr>
<tr>
<td>Alpha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apalachee</td>
<td>Apala</td>
<td>Collected from a planting in Blountstown, FL.</td>
</tr>
<tr>
<td>Arblick</td>
<td>Arbli</td>
<td>Collected near Bela Vista, Brazil. PI 658528. Released as a germplasm in 2008. Low-growing “groundcover” type, slow to establish.</td>
</tr>
<tr>
<td>Arbrook</td>
<td>Brook</td>
<td>Developed from germplasm collections from Paraguay. PI 262817. Released in 1985. Recommended for droughty soils with warm winter temperature, not tolerant of poorly-drained soils. Erect growth, thick stems, and distinctive larger rhizomes.</td>
</tr>
<tr>
<td>QS6W/Quincy-Beta</td>
<td>Beta</td>
<td>Origin has been lost. Surviving plants from an introduction nursery planted at the NFREC in the early 1970s. Survived and spread over the past 50 yr. Selected for its aggressive growth habit and competitiveness, compared with Alpha and QS5W when planted with bahiagrass.</td>
</tr>
<tr>
<td>Cowboy</td>
<td>CowB</td>
<td>Collected from volunteer clone in Tifton, GA. PP-1. Originated from either a superior genetic recombination or outcross from an <em>Arachis glabrata</em> introduction. Most related to Florigraze.</td>
</tr>
<tr>
<td>Ecoturf</td>
<td>Eco</td>
<td>Collected near Bela Vista, Brazil. PI 658529. Released as a germplasm in 2008. Low growing, quick to establish and tolerant of grazing. Susceptible to peanut stunt virus, peanut rust, and powdery mildew.</td>
</tr>
<tr>
<td>Florigraze</td>
<td>Flor</td>
<td>Possible outcrossing between two plant introductions or a vigorous seedling from Arb. PI 421707. Released as a cultivar in 1978. Intermediate growth habit, susceptible to peanut stunt virus and powdery mildew.</td>
</tr>
</tbody>
</table>
Table 3-2. Continued

<table>
<thead>
<tr>
<th>Entry</th>
<th>Abbreviation in figures</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pointed Leaf</td>
<td>Point</td>
<td>From Brazil. Introduced in 2002. NRCS#9056068. Low-growing, produces many flowers. Also called “Brooksville 68 germplasm”.</td>
</tr>
<tr>
<td>QS5W/Quincy</td>
<td>Qncy</td>
<td>Origin has been lost. Surviving plants from an introduction nursery planted at the NFREC in the early 1970s. Survived and spread over the past 50 yr. Selected for its ability to spread into bahiagrass pasture.</td>
</tr>
<tr>
<td>UF Tito</td>
<td>Tito</td>
<td>An introduction from Paraguay. PI 262826. Released as a cultivar in 2008. Upright habit, high DM yield. Named in honor of Dr. Edwin C. “Tito” French, the late Associate Professor of Agronomy at UF.</td>
</tr>
<tr>
<td>Waxy Leaf</td>
<td>Waxy</td>
<td>Collected from Corrientes, Argentina. Introduced in 2002. PI 262801. Low-growing, produces few flowers and seeds. Also called “Brooksville 67 germplasm”.</td>
</tr>
</tbody>
</table>

Table 3-3. Peanut rust (*Puccinia arachidis*) disease incidence observed on leaflets of 14 rhizoma peanut entries at June and October 2017 harvest dates at Quincy, FL. Data presented are based on the Horsfall-Barratt scale (1 to 12) of plant disease (Horsfall and Barratt., 1945).

<table>
<thead>
<tr>
<th>Entry</th>
<th>27 June 2017</th>
<th>11 October 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ona 33</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Quincy-Alpha</td>
<td>1</td>
<td>4 bc</td>
</tr>
<tr>
<td>Apalachee</td>
<td>1</td>
<td>5 b</td>
</tr>
<tr>
<td>Arblick</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Quincy-Beta</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Arbrook</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Cowboy</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Ecoturf</td>
<td>4</td>
<td>7 a</td>
</tr>
<tr>
<td>Florigraze</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>UF Peace</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Pointed Leaf</td>
<td>1</td>
<td>1 c</td>
</tr>
<tr>
<td>Quincy</td>
<td>1</td>
<td>2 c</td>
</tr>
<tr>
<td>UF Tito</td>
<td>1</td>
<td>5 b</td>
</tr>
<tr>
<td>Waxy Leaf</td>
<td>1</td>
<td>2 c</td>
</tr>
</tbody>
</table>

† Data for 27 June 2017 could not be analyzed statistically because there was no variation among replicates for any treatment.
Figure 3-1. Annual herbage accumulation from 14 rhizoma peanut entries cut once (1X) or twice (2X) per year in 2015, 2016, and 2017 at Quincy, FL. Data are entry x defoliation frequency means across years. * indicates defoliation treatments within an entry are different ($P \leq 0.05$) NS; $P > 0.05$. SE = 0.67.
Figure 3-2. Mean canopy height at harvest of 14 rhizoma peanut entries cut once or twice per year at Quincy, FL. Data are entry means across defoliation frequencies from the 2017 harvest year. Mean canopy height was not measured in 2015 and 2016, thus it was not considered in this analysis. Entry means that have the same letter above the boxplot are not different ($P > 0.05$). SE = 0.95.
Figure 3-3. Herbage bulk density of 14 rhizoma peanut entries harvested once (1X) or twice (2X) per year at Quincy, FL. Data are entry x defoliation frequency means from the 2017 harvest year. * indicates defoliation treatments within an entry are different ($P \leq 0.05$); NS, $P > 0.05$. SE = 38.2.

Figure 3-4. Mean herbage crude protein concentration of 14 rhizoma peanut entries cut once or twice per year for three years at Quincy, FL. Data are entry averages across defoliation frequency treatments from harvests in 2015, 2016, and 2017. Entry means with the same letter above the boxplot are not different ($P > 0.05$). SE = 6.01.
Table 3-4. Main effects of defoliation frequency on 14 rhizoma peanut entries during 3 yr of defoliation at Quincy, FL. Data are presented for all variables for which there was no entry x defoliation frequency interaction.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Defoliation Frequency (# yr⁻¹)</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Canopy height (cm)</td>
<td>23.3</td>
<td>23.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Herbage crude protein (g kg⁻¹)</td>
<td>125</td>
<td>150</td>
<td>5.59</td>
</tr>
<tr>
<td>Root-rhizome mass (Mg ha⁻¹)</td>
<td>8.5</td>
<td>5.5</td>
<td>0.61</td>
</tr>
<tr>
<td>Rhizome diameter (mm)</td>
<td>3.2</td>
<td>3.1</td>
<td>0.09</td>
</tr>
<tr>
<td>TNC concentration (g kg⁻¹)</td>
<td>247</td>
<td>255</td>
<td>8.39</td>
</tr>
<tr>
<td>TNC pool (kg ha⁻¹)</td>
<td>2010</td>
<td>1390</td>
<td>158</td>
</tr>
</tbody>
</table>

Figure 3-5. Mean in vitro digestible organic matter concentration (IVDOM) of herbage from 14 rhizoma peanut entries cut once (1X) or twice (2X) per year for three years at Quincy, FL. Data are means from herbage harvested in 2015, 2016, and 2017. * Indicates defoliation treatments within an entry are different (P ≤ 0.05) NS; P > 0.05. SE = 18.8.
Figure 3-6. Root-rhizome mass of 14 rhizoma peanut entries cut once or twice per year for three years at Quincy, FL. Data are entry means across defoliation frequency treatments and years from annual sampling events that occurred at the end of the 2016 and 2017 (Years 2 and 3) growing seasons. Entry means that have the same letter above the boxplot are not different \((P > 0.05)\). \(SE = 0.83\).

Figure 3-7. Mean rhizome diameter of 14 rhizoma peanut entries cut once or twice per year for three years at Quincy, FL. Data are entry means across defoliation frequency treatments and years from annual sampling events that occurred at the end of the 2016 and 2017 (Years 2 and 3) growing seasons. Entry means with the same letter above the boxplot are not different \((P > 0.05)\). \(SE = 0.17\).
Figure 3-8. Mean root-rhizome total nonstructural carbohydrate concentration (TNC) for 14 rhizoma peanut entries cut once or twice per year for three years at Quincy, FL. Data are entry means across defoliation frequency treatments and years from annual sampling events that occurred at the end of the 2016 and 2017 (Years 2 and 3) growing seasons. Entry means that have the same letter above the boxplot are not different \((P > 0.05)\). SE = 15.7.

Figure 3-9. Mean root-rhizome total nonstructural carbohydrate (TNC) pool for 14 rhizoma peanut entries cut once or twice per year for three years at Quincy, FL. Data are entry means across defoliation frequency treatments and years from annual sampling events that occurred at the end of the 2016 and 2017 (Years 2 and 3) growing seasons. Entry means that have the same letter above the boxplot are not different \((P > 0.05)\). SE = 256.
Overview

The development of legume-based, grassland-livestock systems is considered a key component for more sustainable ruminant production in the future (Lüscher et al., 2014; Schultze-Kraft et al., 2018). Including perennial legumes in warm-climate grasslands has numerous potential benefits. Legumes can increase forage nutritive value and animal performance (Rusland et al., 1988; Sollenberger et al., 1989), fix large amounts of atmospheric N (Dubeux et al., 2017), increase the amount of N mineralized from plant litter (Kohmann et al., 2018), increase soil C accumulation (De Deyn et al., 2009, 2011; Jensen et al., 2012), reduce methane (Archimède et al., 2011) and nitrous oxide (Soussana et al., 2010; Klumpp et al., 2011) emissions, and decrease the potential for other negative impacts on the environment (Jensen et al., 2012).

Of perennial legumes proposed for use in pastures in the US Gulf Coast region, rhizoma peanut (RP; *Arachis glabrata* Benth.) is perhaps the best option because it is well adapted to sandy soils, persists under grazing (Ortega-S. et al., 1992b; Shepard et al., 2018), can spread in mixtures with grasses (Mullenix et al., 2014), and has excellent nutritive value (Mullenix et al., 2016b; Shepard et al., 2018). ‘Florigraze’ is the most widely used RP cultivar in Florida. It is negatively affected by the peanut stunt virus (Quesenberry et al., 2010) and is recommended for use only on moderately-well to extremely well-drained soils (Prine et al., 1981). ‘Arbrook’ RP, the second cultivar released from University of Florida, is specifically recommended for droughty, sandy soils (Prine et al., 1986). The University of Florida has released additional
forage RP genotypes in the past decade (Prine et al., 2010; Quesenberry et al., 2010) that are superior to Florigraze and Arbrook, but they too are adapted to well-drained soils. Large areas of the US Gulf Coast region are occupied by seasonally-saturated soils, and there are very few legumes adapted to these environments. Efforts continue to identify superior RP lines, including those adapted to less well-drained sites. Currently there are numerous RP plant introductions and selections with potential for use in Florida forage-livestock systems. Data comparing key forage responses on well-drained and poorly drained soils are lacking for these entries. The objective of this study was to quantify and compare the effects of location, and particularly soil drainage, on herbage production, nutritive value, and root-rhizome traits of selected RP introductions with those of existing cultivars.

**Materials and Methods**

**Experimental Sites**

The experiment was conducted on well-established RP plots at two locations: the North Florida Research and Education Center (NFREC) in Quincy, FL (30.55° N, 84.60° W) and the Agronomy Forage Research Unit in Hague, FL (29.80° N, 82.41° W). Plots were planted at 2009 in Quincy and 2010 in Hague. The soil at Quincy is a Norfolk loamy fine sand (fine-loamy, kaolinitic, thermic Typic Kandiudults). The Norfolk soil is characterized as well drained. At Hague, the soil is a Chipley sand (thermic, coated Aquic Quartzipsamments). The Chipley series consists of very deep, somewhat poorly drained soils that formed in thick deposits of sandy marine sediments. At Hague, the depth to the highest seasonal water table is 76 cm and occurs from June to September. At Quincy, the highest seasonal water table occurs from January to May and is 155 cm. Given that the seasonal water table at Hague is only one-half as deep as at
Quincy, an important difference between the two locations was soil drainage. Likelihood of saturated soils during the summer rainy season was much greater at Hague. Prior to planting the current experiment, the Hague site was occupied by spaced plantings of tall fescue (*Festuca arundinacea* Schreb.) and various clovers (*Trifolium* sp.) and the Quincy site was occupied by bahiagrass (*Paspalum notatum* Flügge).

Soil samples were taken from both locations to a depth of 15 cm and were analyzed at the University of Florida Soil Testing Laboratory. At Hague, soil pH was 5.7 and Mehlich-3 extractable P, K, and Mg were 108, 13, and 18 mg kg\(^{-1}\), respectively. Based on these results, dolomitic lime was applied at a rate of 1100 kg ha\(^{-1}\) on 21 Feb. 2017. Potassium was applied at a rate of 50 kg ha\(^{-1}\) on 27 May 2017. At Quincy, soil pH was 6.0 and Mehlich-3 extractable P, K, and Mg were 38, 28, and 58 mg kg\(^{-1}\), respectively. In April 2017, K was applied at a rate of 56 kg ha\(^{-1}\) and P was applied at a rate of 15 kg ha\(^{-1}\). No fertilizer was applied at either site in 2016.

**Treatments and Experimental Design**

The experiment was conducted for 2 yr at both locations (2016 and 2017). Rainfall data for the sites during the experimental period is shown in Table 4-1. Treatments at each location were introductions/selections, germplasms, and cultivars of RP (Table 3-1), here forward referred to as entries. There were 14 entries evaluated at Quincy and 15 at Hague. All entries were the same at the two locations except for the experimental line Chico, which appeared only at Hague. Data from Chico are not included in the results presented in this chapter, but they are summarized in Table A-1. The design at both locations was a randomized complete block, with four replications at Hague and five replications at Quincy. Plots were 2.5-m wide x 3-m long (7.5
m²) with a 1.8-m alley between plots at Hague, and 2.5-m wide x 1.5-m long (3.75 m²) at Quincy.

Response Variables

Herbage accumulation, nutritive value, and canopy characteristics

All plots were harvested twice per growing season, once each in summer and fall. Harvest dates at Hague were 18 Aug. and 16 Oct. 2016 and 13 July and 19 Oct. 2017. At Quincy, harvests occurred on 14 July and 7 Nov. 2016 and 27 June and 20 Oct. 2017. Harvest dates were chosen to represent typical hay harvest management in North Florida, where spring drought precludes significant growth of rain-fed RP before June, and most hay producers operating without irrigation harvest only twice per year.

At each harvest date, herbage was harvested to a 6-cm stubble height as described in Chapter 3. Remaining herbage was removed from the plot using a flail chopper. The forage samples were processed and analyzed for crude protein (CP) and in vitro digestible organic matter (IVDOM) concentrations using the methods described in Chapter 3. Before each harvest event in 2017, canopy height was measured at 10 locations in each experimental unit to aid in quantifying differences in growth habit among entries. Herbage bulk density was calculated by dividing herbage accumulation (HA; kg ha⁻¹) at a given harvest by the depth of the harvested canopy and expressed in kg ha⁻¹ cm⁻¹. Thus, if canopy height was 20 cm and the stubble height was 6 cm, bulk density was calculated by dividing HA by 14 cm. Values of bulk density reported are the averages of the two harvests per year.
Root-rhizome mass and total non-structural carbohydrate concentration and rhizome diameter

Root-rhizome mass was measured by sampling three circular cores per experimental unit on 6 Dec. 2016 and 14 Dec. 2017 at Hague and 13 Dec. 2016 and 12 Dec. 2017 at Quincy. Cores were 10 cm in diameter and 20-cm deep. Sample processing, rhizome diameter measurement, and total non-structural carbohydrate (TNC) concentration analyses were conducted using the methods described in Chapter 3.

Statistical Analyses

Data were analyzed using a mixed model with entry, location, and their interaction as fixed effects and block and year as random effects. Year was considered a repeated measure. Location means were separated using the F test, and RP entry means were separated using Tukey’s test. Treatments were considered different when $P \leq 0.05$. Some data are presented graphically in the form of box plots. The structure of box plots is such that the upper and lower hinges (terminal ends of the box) relate to the first and third quartiles (or 25$^{th}$ and 75$^{th}$ percentiles), respectively. The thick solid line within and perpendicular to the sides of the box is the median. The upper and lower whiskers (solid lines extending above and below the box) extend from the hinge to the largest and smallest values (at most 1.5 interquartile range) of the hinge, respectively. Data points beyond the whiskers are considered outliers.
Results and Discussion

Herbage Accumulation and Sward Canopy Characteristics

Annual herbage accumulation

There was an entry x location interaction for HA ($P < 0.0001$) (Figure 4-1). Seven of the entries had greater HA at Quincy than at Hague. Conversely, none of the entries had greater HA at Hague than at Quincy (Figure 4-1). While acknowledging the presence of entry x location interaction, it is informative to note that location main effect means were 10.5 and 7.28 Mg ha$^{-1}$ yr$^{-1}$ for Quincy and Hague, respectively. The generally superior performance at Quincy reflects soil drainage characteristics that were more favorable to RP. Among entries, notable exceptions to greater HA at the Quincy location were Ona 33, with HA of 11.0 and 10.5 Mg ha$^{-1}$ yr$^{-1}$ at Quincy and Hague, respectively, and Waxy Leaf, which had HA of 9.8 and 8.6 Mg ha$^{-1}$ yr$^{-1}$ at the two locations, respectively. At Hague, where soils experience seasonal water logging in summer, Ona 33 (10.5 Mg ha$^{-1}$ yr$^{-1}$) had the same HA as the average of all entries at Quincy. Ona 33 at Hague had greater HA than Florigraze (4.8 Mg ha$^{-1}$ yr$^{-1}$), Arbrook (5.8 Mg ha$^{-1}$ yr$^{-1}$), Pointed Leaf (5.9 Mg ha$^{-1}$ yr$^{-1}$), Apalachee (6.7 Mg ha$^{-1}$ yr$^{-1}$), Ecoturf (6.8 Mg ha$^{-1}$ yr$^{-1}$), and Quincy-Alpha (6.9 Mg ha$^{-1}$ yr$^{-1}$) at that location. Those that had the greatest advantage in HA at Quincy vs. Hague included Arbrook (13.7 vs. 5.8 Mg ha$^{-1}$ yr$^{-1}$), Florigraze (8.4 vs. 4.8 Mg ha$^{-1}$ yr$^{-1}$) and Pointed Leaf (10.5 vs. 5.9 Mg ha$^{-1}$ yr$^{-1}$).

During 4 yr of defoliation on a poorly drained Spodosol, annual HA of Ona 33 increased 54% from Year 1 to Year 4, while annual HA of Arbrook decreased 41% over the same time period (Mislevy et al., 2007). Thus, these results support a conclusion that Ona 33 is much more tolerant of seasonal water logging than Arbrook. The mechanism for this response has not yet
been elucidated. In the current study, the most recently released cultivars, UF Peace and UF Tito, performed relatively well at both locations (> 7.8 Mg ha\(^{-1}\)), but all of the currently released cultivars (Arbrook, Florigraze, UF Peace, and UF Tito) and the released germplasm Ecoturf had greater HA at Quincy. On the Spodosol previously referenced, UF Peace and UF Tito had greater HA than Ona 33 during the first year, but there was no difference in HA during Years 2 through 4 (Mislevy et al., 2007). At that location, annual HA of Ona 33 increased 54% from Year 1 to Year 4 compared with a decline of 5 and 6% for UF Peace and UF Tito, respectively, during the same period. Thus, it is unlikely that Ona 33 will have greater HA than UF Peace or UF Tito early in stand life or on well-drained soils, but Ona 33 does appear to have greater tolerance of seasonal waterlogging that can occur in many Florida locations during summer.

Annual RP HA in the southeastern US was in the range of 7 to 11 Mg ha\(^{-1}\) (Terrill et al., 1996; Venuto et al., 1999; Prine et al., 2010). In the current experiment, HA at the Quincy location was within or exceeded this range, but except for Ona 33 the HA at Hague was in the lower part of this range or even below. In a previous experiment conducted at Hague, Florigraze, Arbrook, UF Peace, and UF Tito were harvested for 6 yr under a management regime similar to that of the current experiment (Freire et al., 2000). For 3 yr, samples were clipped twice per year and the annual HA values were as follows: Arbrook (11.0 Mg ha\(^{-1}\)); UF Tito (10.4 Mg ha\(^{-1}\)); UF Peace (9.5 Mg ha\(^{-1}\)); and Florigraze (9.2 Mg ha\(^{-1}\)). These means for Arbrook and Florigraze were considerably above those reported at Hague in the current experiment. For Florigraze, this can be explained based on the emergence of peanut stunt virus as a major disease concern in recent years, but it is not clear if rainfall or other factors allowed Arbrook to perform better at this location in the study of Freire et al. (2000) than in the current study.
Canopy height

There was an interaction of entry x location ($P = 0.0005$) (Figure 4-2). Eight of 14 entries were taller at Quincy than at Hague, and this is reflected in location main effect means of 24 and 18 cm, respectively. Arbrook (29 cm) and UF Tito (27 cm) were among the tallest entries at Quincy, while at Hague UF Tito (24 cm) and Ona 33 were among the tallest (23 cm). This supports the observation by Quesenberry et al. (2010) that UF Tito is an upright-growing cultivar. At Hague, UF Peace (18 cm) and Ecoturf (15 cm) were shorter than UF Tito (24 cm) and Florigraze was not different (19 cm), but at Quincy, neither UF Peace, Ecoturf, nor Florigraze (22, 21, and 25 cm, respectively) differed in height from UF Tito (27 cm). Although Ecoturf is considered to be a lower growing “ground cover” plant (Prine et al., 2010), these results show it is not different in height from intermediate types like UF Peace and Florigraze when intervals are long between defoliation events. Ecoturf was found previously to be phenotypically plastic, assuming a shorter canopy height under frequent, close defoliation (Mullenix et al., 2016; Shepard et al., 2018), but the interval between defoliation events in the current study was not sufficiently short to elicit this type of response.

Herbage bulk density

There was an entry x location interaction ($P < 0.0001$) for herbage bulk density (Figure 4-3). Interaction occurred because bulk density was different between sites only for Apalachee, where the bulk density was 443 kg ha$^{-1}$ cm$^{-1}$ at Hague and 261 kg ha$^{-1}$ cm$^{-1}$ at Quincy. The site effect also approached significance for Quincy-Alpha ($P = 0.09$; 416 and 258 kg ha$^{-1}$ cm$^{-1}$ for Hague and Quincy, respectively). Ona 33, Ecoturf, and Florigraze had nearly the same herbage bulk density at both sites. At the Quincy location, entries Quincy-Beta (453 kg ha$^{-1}$ cm$^{-1}$) and UF
Peace (450 kg ha\(^{-1}\) cm\(^{-1}\)) had greater herbage bulk density than Apalachee, Quincy-Alpha, and Florigraze (259-269 kg ha\(^{-1}\) cm\(^{-1}\)), while at Hague, Waxy Leaf, Apalachee, and Quincy-Alpha had greater bulk density (404-443 kg ha\(^{-1}\) cm\(^{-1}\)) than UF Tito (225 kg ha\(^{-1}\) cm\(^{-1}\)), Ecoturf (203 kg ha\(^{-1}\) cm\(^{-1}\)), and Arbrook (187 kg ha\(^{-1}\) cm\(^{-1}\)). Quincy-Beta bulk density was among the greatest numerically at both locations, ranging from 355 kg ha\(^{-1}\) cm\(^{-1}\) at Hague to 453 kg ha\(^{-1}\) cm\(^{-1}\) at Quincy. It was also consistently among the shorter entries with heights ranging from 16.4 to 19.3 cm at the two locations.

In terms of relative bulk density between Ecoturf, Florigraze, UF Peace, and UF Tito, the results of this experiment were different than those previously reported (Mullenix et al., 2016b). They observed bulk density of Ecoturf (range of 175 to 268 kg ha\(^{-1}\) cm\(^{-1}\)) to be greater than UF Peace (range of 117 to 188 kg ha\(^{-1}\) cm\(^{-1}\)), Florigraze (range of 130 to 152 kg ha\(^{-1}\) cm\(^{-1}\)), and UF Tito (range of 105 to 150 kg ha\(^{-1}\) cm\(^{-1}\)) in each of 2 yr when grazed every 3 or 6 wk. When Ecoturf was grazed weekly to a 4-cm stubble, herbage bulk density was greater than when grazed every 4 or 7 wk (Shepard et al., 2018). With long regrowth intervals in the current study, Ecoturf bulk density (328-375 kg ha\(^{-1}\) cm\(^{-1}\)) was not different than UF Peace, UF Tito, or Florigraze at either location. In contrast, when defoliated frequently and closely, it assumed a short, compact canopy structure with greater herbage bulk density (Mullenix et al., 2016a; Shepard et al., 2018).

**Nutritive Value Responses**

**Herbage crude protein**

There was no entry x location interaction \((P = 0.20)\) for herbage CP, however, there were effects of location \((P < 0.0001)\) (Table 4-2) and entry \((P < 0.0001)\) (Figure 4-4). Herbage CP at Hague was greater than at Quincy (169 and 151 g kg\(^{-1}\), respectively), most likely because of
greater HA at Quincy and associated dilution of N. Arbrook CP (129 g kg\(^{-1}\)) was less than all entries except Quincy-Beta (143 g kg\(^{-1}\)) and Florigraze (141 g kg\(^{-1}\)). Waxy Leaf CP (182 g kg\(^{-1}\)) was greater than all other entries except Pointed Leaf (176 g kg\(^{-1}\)), Cowboy (175 g kg\(^{-1}\)), Arblick (171 g kg\(^{-1}\)), Apalachee (169 g kg\(^{-1}\)), and Ecoturf (167 g kg\(^{-1}\)). Ona 33 (162 g kg\(^{-1}\)), UF Tito (158 g kg\(^{-1}\)), and UF Peace (158 g kg\(^{-1}\)) were intermediate in herbage CP concentration.

These CP concentrations are generally within, or slightly lower than the CP reported in other defoliation studies in North Florida, where CP of a number of different entries ranged from 145 to 210 g kg\(^{-1}\) (Hernandez-Garay et al., 2004; Mullenix et al., 2016a; Prine et al., 2010; Shepard et al., 2018). Length of regrowth period likely played a role in the somewhat lower CP observed for some entries in the current vs. previous studies. In previous experiments, RP swards were continuously stocked (Hernandez-Garay et al. 2004), grazed every 3 to 6 wk (Mullenix et al. 2016a), or grazed every 1, 4, or 7 wk (Shepard et al., 2018), compared with the two harvests per year frequency employed in the current study. Crude protein of RP lines was evaluated when harvested three or four times per year in a 3-yr clipping study at the Range Cattle Research and Education Center in South Florida (Mislevy et al., 2007). Average CP was greatest for Ecoturf (191 g kg\(^{-1}\)) followed by UF Peace (188 g kg\(^{-1}\)), Ona 33 (178 g kg\(^{-1}\)), UF Tito (176 g kg\(^{-1}\)), Florigraze (166 g kg\(^{-1}\)), and Arbrook (146 g kg\(^{-1}\)). Herbage CP of most lines followed similar patterns of response to that observed in the current study. An exception was Ecoturf, for which long regrowth intervals increase occurrence of peanut rust leaf disease (Chapter 3), negatively affecting nutritive value. In contrast, when harvested more frequently, disease presence is reduced or not observed and nutritive value is greater.
**Herbage in vitro digestibility**

There was an entry x location interaction for herbage IVDOM ($P = 0.0002$). Interaction occurred because there were differences between locations only for Florigraze and Pointed Leaf (Figure 4-5); in both cases IVDOM was greater at Quincy than Hague. While acknowledging the presence of entry x location interaction, location main effect ($P < 0.0001$) means were 639 and 612 g kg$^{-1}$, for Quincy and Hague, respectively. Herbage IVDOM of UF Peace (617 vs. 614 g kg$^{-1}$ at Hague and Quincy, respectively) and UF Tito (608 vs. 604 g kg$^{-1}$ at Hague and Quincy, respectively) was not different between locations. Ecoturf (577 g kg$^{-1}$) and Arbrook (588 g kg$^{-1}$) IVDOM at the Quincy location were less than all other entries except UF Tito (604 g kg$^{-1}$), UF Peace (614 g kg$^{-1}$), and the entry called Quincy (634 g kg$^{-1}$). Greater leaf disease incidence may have lowered Ecoturf IVDOM at Quincy compared with Hague (577 vs. 602 g kg$^{-1}$). At Hague, there was no difference among entries and IVDOM ranged from 592 g kg$^{-1}$ for Arbrook to 638 g kg$^{-1}$ for Waxy Leaf.

Previous studies have reported a range in RP entry IVDOM from 650 to 705 g kg$^{-1}$ (Sollenberger et al., 1989; Terrill et al., 1996; Hernandez-Garay et al., 2004; Shepard et al., 2018). In a previous experiment conducted near the research site in Quincy, average IVDOM was 712 g kg$^{-1}$ for Arblick, Arbrook, Ecoturf, Florigraze, UF Peace, and UF Tito when harvested three times per year (Dubeux et al., 2017). In an experiment in south Florida, several RP entries were harvested three or four times per year and average IVDOM was greater (688 g kg$^{-1}$) than means reported in the current experiment. As already described for herbage CP, the primary reason for lower IVDOM in the current study was long intervals between harvest events. In south Florida, Ona 33 IVDOM was greater than Arbrook and UF Tito in 2 of 3 yr and greater than UF
Peace and Ecoturf in 1 of 3 yr. In the current study, Ona 33 IVDOM (653 g kg\(^{-1}\)) was greater than Arbrook and Ecoturf at Quincy but not different (605 g kg\(^{-1}\)) than any other entry at Hague.

**Below-Ground Responses**

**Root-rhizome mass**

There was no entry x location interaction \((P = 0.158)\) for root-rhizome mass, but there was an effect of location \((P = 0.001)\) (Table 4-2) and entry \((P = 0.0001)\) (Figure 4-6). Below-ground biomass was greater at Hague compared with Quincy (6.48 vs 5.64 Mg ha\(^{-1}\), respectively). Arbrook (7.47 Mg ha\(^{-1}\)) and Quincy-Alpha (7.44 Mg ha\(^{-1}\)) had greater root-rhizome mass than Apalachee and Florigraze (4.72 and 4.27 Mg ha\(^{-1}\), respectively). The entries Quincy and Waxy Leaf (6.82 and 7.08 Mg ha\(^{-1}\), respectively) also had greater root-rhizome mass than Florigraze.

In a clipping study near Quincy, root-rhizome mass of UF Peace, Arbrook, and Florigraze was 21.5, 17.3, and 10.6 Mg ha\(^{-1}\), respectively, values well above those observed in the current study. Near the Hague site in an experiment evaluating a Florigraze RP pasture under a range of grazing treatments (from severe to lax grazing), Rice et al. (1995) reported mean Florigraze root-rhizome mass of 5.75 Mg ha\(^{-1}\), similar to the average across locations and entries of 6.06 Mg ha\(^{-1}\) in the current study. When 2-yr-old plots of four entries were grazed every 3 or 6 wk (Mullenix et al., 2016b), root-rhizome mass of Ecoturf, UF Tito, Florigraze, and UF Peace was 4.45, 4.11, 3.49, and 3.17 Mg ha\(^{-1}\), respectively. In the current experiment, the same four entries had root-rhizome mass of 6.02, 5.73, 4.27, and 6.59 Mg ha\(^{-1}\), respectively. Greater values than observed in the Mullenix et al. (2016b) study was likely due to a combination of factors including less
frequent defoliation and greater pasture age at time of initiation of defoliation in the current
study.

**Rhizome diameter**

There was no entry x location interaction ($P = 0.613$) for rhizome diameter, but there
were effects of location ($P < 0.0001$; Table 4-2) and entry ($P < 0.0001$) (Figure 4-7). Rhizome
diameter was greater at Quincy than Hague (3.08 vs. 2.63 mm, respectively), in spite of lesser
root-rhizome mass at Quincy. The effects of the growing environment or defoliation
management on rhizome diameter are not known. Arbrook rhizome diameter was greater (4.8
mm) than all other entries. UF Peace (3.6 mm) rhizome diameter was less than Arbrook but
greater than all entries except Florigraze (2.9 mm), Waxy Leaf (2.9 mm), and Quincy-Beta (2.8
mm). Other entries ranged from a low of 2.2 mm for Cowboy to 2.78 mm for Ona 33. While
there are limited data regarding rhizome diameter, Prine et al. (1986) noted that Arbrook
produced thicker rhizomes than those of any known RP line at that time.

**Total non-structural carbohydrate concentration**

There was no entry x location interaction for root-rhizome TNC ($P = 0.532$), but there
were effects of entry ($P < 0.0001$) (Figure 4-8) and location ($P = 0.05$) (Table 4-2). Root-
rhizome TNC concentration was marginally greater at Hague (269 g kg$^{-1}$) than at Quincy (255 g
kg$^{-1}$), corresponding with greater root-rhizome mass at Hague. Arbrook (343 g kg$^{-1}$) had greater
TNC concentrations than Florigraze (260 g kg$^{-1}$) and Arblick (251 g kg$^{-1}$), while both Arbrook
and UF Peace (308 g kg$^{-1}$) had greater root-rhizome TNC concentrations than Ona 33 (235 g kg$^{-1}$),
Waxy Leaf (235 g kg$^{-1}$), Pointed Leaf (234 g kg$^{-1}$), UF Tito (232 g kg$^{-1}$), Cowboy (231 g kg$^{-1}$),
Apalachee (230 g kg$^{-1}$), and Ecoturf (224 g kg$^{-1}$).
These TNC concentrations are generally within the ranges or greater than those observed in previous experiments. At the end of a 2-yr experiment evaluating an array of grazing treatments on Florigraze RP pastures in Gainesville, FL, TNC concentrations ranged from 58 for closely and frequently grazed treatments to 210 g kg\(^{-1}\) for leniently defoliated treatments (Ortega-S. et al., 1992a). In another grazing experiment at the same location, Shepard et al. (2018) reported Ecoturf RP pastures with a range of 198 to 314 g kg\(^{-1}\) for grazing frequencies from 1 to 7 wk in combination with residual stubble heights of 4 to 8 cm. When 2-yr-old stands of Ecoturf, Florigraze, UF Peace, and UF Tito were defoliated every 3 or 6 wk, root-rhizome TNC ranged from 115 to 142 g TNC kg\(^{-1}\) (Mullenix et al., 2016b). A TNC concentration above 130 g kg\(^{-1}\) was suggested for Florigraze rhizomes used as planting material (Rice et al., 1995), as those with lesser concentrations resulted in stand failure. All entry means for TNC concentration in the current experiment are well above that level, but it is not known what concentrations are optimal for entries other than Florigraze. These data clearly indicate that Arbrook maintains a large amount of below-ground biomass with very thick rhizomes and high concentrations of TNC. In contrast, entries like Apalachee and Cowboy have lesser rhizome mass, rhizome diameter, and TNC concentration.

**Total non-structural carbohydrate pool**

There was no entry x location interaction (\(P = 0.651\)) for TNC pool, but both RP entry (\(P < 0.0001\)) (Figure 4-9) and location effects were significant (\(P < 0.0001\)) (Table 4-2). Hague TNC pool was 26% greater than Quincy (1760 vs. 1390 kg ha\(^{-1}\); Table 4-2) due to an average 15% greater root-rhizome mass and 5.5% greater TNC concentration at Hague than Quincy. Among entries, Arbrook had greater TNC pool (2350 kg ha\(^{-1}\)) than Arblick (1450 kg ha\(^{-1}\)) and
UF Tito (1300 kg ha\(^{-1}\)), and both Arbrook and Quincy-Beta (2090 kg ha\(^{-1}\)) were greater than Pointed Leaf (1260 kg ha\(^{-1}\)), Ona 33 (1250 kg ha\(^{-1}\)), Ecoturf (1250 kg ha\(^{-1}\)), Cowboy (1200 kg ha\(^{-1}\)), Florigraze (1130 kg ha\(^{-1}\)), and Apalachee (1080 kg ha\(^{-1}\)). Entries that were not different than Arbrook included Quincy-Beta (2090 kg ha\(^{-1}\)), UF Peace (2020 kg ha\(^{-1}\)), Quincy (2010 kg ha\(^{-1}\)), Quincy-Alpha (1970 kg ha\(^{-1}\)), and Waxy Leaf (1740 kg ha\(^{-1}\)). These values are less than those previously reported for a more than 10-yr-old stand of Ecoturf defoliated at a range of grazing intervals and stubble heights (3040 to 4060 kg ha\(^{-1}\)) (Shepard et al., 2018), but greater than 2- or 3-yr-old stands of Ecoturf, Florigraze, UF Tito, and UF Peace grazed every 3 to 6 wk to remove 50 to 75\% of herbage mass (< 1000 kg ha\(^{-1}\); Mullenix et al., 2016b). These data support the previously-mentioned characterization of Arbrook as an entry with a large root-rhizome mass and supply of reserves. In contrast, the TNC pool of Apalachee and Cowboy is only 46 and 51\% as great as that of Arbrook.

**Implications of the Research**

Soil drainage characteristics at Quincy were more favorable to RP entries in general than those at Hague, which led to many entries having greater HA at Quincy than Hague. Ona 33 had nearly the same HA at both locations, and its HA in poorly-drained soils at Hague was nearly identical to the average HA of all other entries at the well-drained Quincy location. These data support a conclusion that Ona 33 is considerably better adapted to seasonally saturated soils than nearly all other RP entries currently being used commercially or being evaluated for potential cultivar release.

Previous studies have characterized Ecoturf as a low-growing plant with greater herbage bulk density than current cultivars, however, this experiment showed no difference in height or
bulk density between Ecoturf and intermediate-growth-habit types like UF Peace and Florigraze. The most likely explanation for these differences is that Ecoturf is phenotypically plastic, assuming a shorter canopy height with greater herbage bulk density under frequent, close defoliation, but not differing in growth characteristics from other intermediate types when intervals between defoliation events are long, like those in the current study.

Greater HA and associated dilution of N at Quincy resulted in greater herbage CP at Hague. In general, the longer regrowth periods in this study than most previous studies resulted in lesser herbage CP and IVDOM concentrations in the current experiment. The longer defoliation interval likely had the greatest negative effect on nutritive value of Ecoturf, which had the greatest incidence of leaf rust.

Unlike HA, root-rhizome mass and TNC pool were greater at Hague, but rhizome diameter was larger at Quincy. Arbrook and Quincy-Alpha had greater root-rhizome mass than Apalachee and Florigraze. In general, Arbrook maintained a large amount of below-ground biomass with very thick rhizomes and high concentrations of TNC. In contrast, entries like Apalachee and Cowboy have lesser rhizome mass, rhizome diameter, and TNC concentration.

These data suggest that there may be potential to expand the current zone of adaptation of RP into wetter-soil environments by evaluating additional entries and releasing new cultivars. Additionally, there are a wide range of above- and below-ground growth characteristics available among entries being tested, providing opportunity for targeting entries to specific intended uses or production goals.
Table 4-1. Monthly rainfall at the experimental sites near Quincy and Hague, FL and the 30-year average rainfall for Quincy and Hague, FL. Data obtained from the Florida Automated Weather Network (FAWN) which has recording stations at each site.

<table>
<thead>
<tr>
<th></th>
<th>Quincy</th>
<th></th>
<th>Hague</th>
<th></th>
<th>30-yr average</th>
<th>30-yr average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2016</td>
<td>2017</td>
<td>mm</td>
<td>2016</td>
<td>2017</td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>150</td>
<td>237</td>
<td>122</td>
<td>72</td>
<td>73</td>
<td>85</td>
</tr>
<tr>
<td>February</td>
<td>75</td>
<td>75</td>
<td>121</td>
<td>118</td>
<td>34</td>
<td>81</td>
</tr>
<tr>
<td>March</td>
<td>73</td>
<td>31</td>
<td>149</td>
<td>72</td>
<td>21</td>
<td>110</td>
</tr>
<tr>
<td>April</td>
<td>480</td>
<td>87</td>
<td>93</td>
<td>96</td>
<td>102</td>
<td>68</td>
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<tr>
<td>May</td>
<td>50</td>
<td>151</td>
<td>128</td>
<td>64</td>
<td>84</td>
<td>63</td>
</tr>
<tr>
<td>June</td>
<td>157</td>
<td>246</td>
<td>150</td>
<td>248</td>
<td>477</td>
<td>181</td>
</tr>
<tr>
<td>July</td>
<td>112</td>
<td>103</td>
<td>187</td>
<td>122</td>
<td>150</td>
<td>154</td>
</tr>
<tr>
<td>August</td>
<td>117</td>
<td>168</td>
<td>172</td>
<td>124</td>
<td>166</td>
<td>162</td>
</tr>
<tr>
<td>September</td>
<td>119</td>
<td>91</td>
<td>105</td>
<td>192</td>
<td>344</td>
<td>112</td>
</tr>
<tr>
<td>October</td>
<td>9</td>
<td>49</td>
<td>104</td>
<td>28</td>
<td>51</td>
<td>73</td>
</tr>
<tr>
<td>November</td>
<td>10</td>
<td>11</td>
<td>89</td>
<td>2</td>
<td>87</td>
<td>52</td>
</tr>
<tr>
<td>December</td>
<td>134</td>
<td>81</td>
<td>96</td>
<td>54</td>
<td>79</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>1486</td>
<td>1330</td>
<td>1516</td>
<td>1192</td>
<td>1668</td>
<td>1201</td>
</tr>
</tbody>
</table>
Figure 4-1. Annual herbage accumulation of 14 rhizoma peanut entries harvested twice per year in Quincy and Hague, FL during 2016 and 2017. Data are entry x location means across two years. * indicates locations within an entry are different (P ≤ 0.05); NS, P > 0.05. SE = 0.97.
Figure 4-2. Canopy height of 14 rhizoma peanut entries harvested twice per year in Quincy and Hague, FL. Data are entry x location means across harvests for 2017. * indicates locations within an entry are different ($P \leq 0.05$); NS, $P > 0.05$. SE = 1.25.

Table 4-2. Main effects of location on responses for which there was no entry x location interaction.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Location</th>
<th>SE</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hague</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbage crude protein (g kg$^{-1}$)</td>
<td>169</td>
<td>5.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Root-rhizome mass (Mg ha$^{-1}$)</td>
<td>6.5</td>
<td>0.61</td>
<td>0.001</td>
</tr>
<tr>
<td>Rhizome diameter (mm)</td>
<td>2.6</td>
<td>0.14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TNC concentration (g kg$^{-1}$)</td>
<td>269</td>
<td>8.65</td>
<td>0.050</td>
</tr>
<tr>
<td>TNC pool (kg ha$^{-1}$)</td>
<td>1760</td>
<td>203</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 4-3. Herbage bulk density of 14 rhizoma peanut entries harvested twice per year in Quincy and Hague, FL. Data are entry x location interaction means across two harvests in 2017. * indicates location means within an entry are different ($P \leq 0.05$); NS, $P > 0.05$. SE = 36.1.
Figure 4.4. Herbage crude protein concentration of 14 rhizoma peanut entries harvested twice per year in Quincy and Hague, FL. Data are entry means across locations and harvests in 2016 and 2017. Entry means that have the same letter above the boxplot are not different ($P > 0.05$). SE = 7.01.
Figure 4-5. Herbage in vitro digestible organic matter (IVDOM) concentration for 14 rhizoma peanut entries harvested two times per year in Quincy and Hague, FL. Data are entry x location interaction means across harvests in 2016 and 2017. * indicates defoliation treatments within an entry are different \((P \leq 0.05)\); NS, \(P > 0.05\). SE = 18.0.
Figure 4-6. Root-rhizome mass of 14 rhizoma peanut entries harvested two times per year in Quincy and Hague, FL. Data are entry means across locations and years for annual mass measurements taken at the end of the 2016 and 2017 growing seasons. Entry means that have the same letter above the boxplot are not different ($P > 0.05$). SE = 0.2.
Figure 4-7. Rhizome diameter of 14 rhizoma peanut entries harvested by clipping two times per year in Quincy and Hague, FL. Data are entry means across locations and years for annual measurements taken at the end of the 2016 and 2017 growing seasons. Entry means that have the same letter above the boxplot are not different ($P > 0.05$). SE = 0.2.
Figure 4-8. Root-rhizome total nonstructural carbohydrate (TNC) concentration for 14 rhizoma peanut entries harvested for hay twice per year in Quincy and Hague, FL. Data are entry means across locations and years for annual measurements taken at the end of the 2016 and 2017 growing seasons. Entry means that have the same letter above the boxplot are not different ($P > 0.05$). SE = 16.3.
Figure 4-9. Root-rhizome total nonstructural carbohydrate (TNC) pool for 14 rhizoma peanut entries harvested twice per year for hay in Quincy and Hague, FL. Data are entry means across locations and harvests in 2016 and 2017. Entry means that have the same letter above the boxplot are not different ($P > 0.05$). SE = 256.
CHAPTER 5
A MODIFIED ROOT-INGROWTH CORE DEVICE TO MEASURE ROOT ACCUMULATION RATE OF PERENNIAL FORAGE SPECIES

Overview

Agricultural grassland soils can mitigate climate change by sequestering a significant amount of atmospheric C. Management practices, such as defoliation and fertilization, that promote forage production also increase soil C storage (Conant et al., 2001; Allard et al., 2007; Ammon et al., 2007). Of particular importance to the C cycle in grasslands is root production, as roots may contribute more SOC than above-ground biomass (Rasse et al., 2005). However, root production in grasslands is difficult to measure or predict. It is known that fine root exudates transfer C to stable SOC pools (Bradford et al., 2013), which can impact nutrient cycling and SOC storage (Phillips et al., 2011).

In grasslands that are frequently grazed or cut for hay, removal of above-ground biomass may render roots the most significant contributor of C to soil. There is little information available on root accumulation rates under perennial forage crops and more is needed to advance methods of studying root production in grasslands, as existing methods are limited. While indirect techniques of measurement, like rhizotron technologies, are desirable because they are non-destructive, the cost and time required for data processing may preclude their use. Also, damage sustained from hay harvesting machinery or grazing livestock makes rhizotron use challenging in defoliation studies.

Ingrowth cores are another option for studying root accumulation. They are more affordable than rhizotrons, as they are made from commercially-available window screen. However, the screen may not be ideal because the holes are small (≤ 0.84 mm) and may serve as
a barrier to small insects, rhizomes, or thick roots. A study was conducted to quantify the effect of mesh sizes (0.25, 0.5, 1.0, 1.5 and 2.0 mm) for measuring fine root growth of Poplar (*Populus nigra* L.) (Montagnoli et al., 2014). A considerable amount of fine root biomass was found outside bags that had a mesh size smaller than 1.5 mm, demonstrating that small mesh openings restrict root growth into the core. Another reason to improve this method is that window screen may not be strong enough to withstand the impact of grazing livestock. Livestock treading and subsequent soil compaction may distort the core’s geometry resulting in inaccurate calculations. Given the constraints of current methods to study root production, more techniques are needed to better measure root accumulation rate of agricultural grasslands. The objective of this study was to develop and test a prototype root-ingrowth core device designed to quantify root accumulation rate of a group of rhizoma peanut (RP; *Arachis glabrata* Benth.) entries.

**Materials and Methods**

**Design and Construction of the Ingrowth Core**

The ingrowth cores were constructed of 4-mm polyester mesh wrapped around 8-cm diameter cage wire mesh (Figure 5-1). The purpose of the cage wire is to reinforce the sides of the core without blocking root and rhizome ingrowth. In previous below-ground studies, it was observed that pressure from root and rhizome growth and insect movement disturbed polyester mesh bags used to measure below-ground litter decomposition (Sollenberger, personal communication). It was suspected that similar activity might distort the geometry of the ingrowth core and that such distortion could lead to miscalculation of soil volume and root-rhizome biomass accumulation. Thus, the cage wire served to maintain the form of the ingrowth core.
To prepare the cage wire for the cores, wire was cut into 26- x 31-cm pieces with pliers (Figure 5-2). The individual wires that form the cage wire are spaced 2.54-cm apart. The wire was then bent into a cylinder on a hard surface that can withstand scratches from exposed wire ends. To secure the cylindrical shape, the exposed wire was folded from one side of the core over the other side of the core. To save time and avoid snagging the mesh fabric when it was pulled over the core in later steps, the exposed wire was always folded toward the interior of the core. If the core is to be installed in the soil at a 45° angle, the top of the ingrowth core should be tapered to allow it to lie flush with the surface of the soil. To do so, 2.54 cm should be cut from 5 units (12.7 cm) of wire from the top of the core (Figure 5-3).

One 70-cm x 16-cm piece of polyester netting utility fabric and one 35 cm x 16 cm piece of architectural drafting paper should be cut per ingrowth core (Figure 5-4). Based on the dimensions in Figure 5-5, guidelines should be drawn to serve as a template for sewing of the mesh bags. The mesh fabric should be folded in half so that the template is inside the fabric, and the template can be secured to the fabric with tape. The top of the fabric can be removed (Figure 5-5). The sides of the mesh fabric should be sown with a zig-zag stitch and exterior-grade thread. The drafting paper should then be removed from the mesh and recycled. The mesh can then be pulled onto the core (Figure 5-6). The exposed wire ends should be pulled through the top of the fabric and the wire ends folded into the inside of the core. This will secure the fabric to the core for the core’s lifespan.
Use of the Ingrowth Core in a Field Experiment

A 2-yr experiment was conducted in 2017 and 2018 at the Agronomy Forage Evaluation Unit in Hague, FL (29.80° N, 82.41° W). The experiment site is the same Hague location as described in Chapter 4, and detailed site characteristics are presented in that chapter.

Treatments and experimental design

The treatments were six RP entries, including existing cultivars as well as selections under evaluation for potential cultivar release. Treatments were arranged in four replicates of a randomized complete block design. The six entries represent a subset of the 15 entries used at the Hague site that are described in Chapter 4. They included experimental lines Apalachee, Chico, and Ona 33, the released germplasm Ecoturf, and released cultivars ‘UF Peace’ and ‘UF Tito’. These entries were chosen to include a wide range in herbage accumulation, canopy height, and root-rhizome mass, based on data collected at this site in 2016 and 2017 (Table 5-1). Limiting the entries to six was necessary because of the time associated with construction, installation, and removal of the root-ingrowth cores.

Placement of the ingrowth cores

The ingrowth cores were constructed as described earlier in this chapter and were used to measure accumulation of root-rhizome mass based on methodology described by Makkonen and Helmisaari (1999). Three ingrowth cores were placed in each of four replicates of the six entries for a total of 72 cores. The ingrowth cores were installed at a 45° angle and measured to a depth of 23 cm. Based on measurements of RP rhizome diameter taken in 2016, it was determined that mesh with a 4-mm opening would be large enough to allow root and rhizome growth of each of these entries into the core.
In order to install each core, a 7.5-cm diameter x 28-cm deep core of soil, rhizomes, and roots was excavated with an auger angled at 45° at three locations per plot (Figure 5-7). The soil and plant material from each excavation was screened through a 250-um screen and plant material discarded. When high soil moisture prevented sieving plant material with a 250-um screen, material was passed through a 0.635-cm screen (Figure 5-8). Any remaining plant material was then removed by hand. The sieved soil was placed inside the constructed ingrowth cores, and they were inserted into the excavated cores such that the upper-most edge of the ingrowth core was just below the soil surface (Figure 5-9).

**Response Variables**

Roots in perennial pastures have a lifespan of approximately 100 d (14 wk) (Van der Krift & Berendse, 2002). Since it is important that the time period the ingrowth cores are left below-ground is shorter than the lifespan of the roots, the current experiment was conducted for approximately 100 d. During each year, all plots were harvested once during the period when cores were in place. Forage was cut to a 6-cm stubble on 13 July 2017 and 20 July 2018. Cores remained in place during 102 d from 9 June through 19 Sept. 2017 and 104 d from 13 June through 26 Sept. 2018. Prior to excavation, cores were located with a metal detector. At the end of the root-growth period, the ingrowth cores were excavated and any portion of the root protruding from the core was cut and discarded (Figure 5-10). All soil was washed from the roots on an 840-um screen, and the three cores per plot were composit (Figure 5-11). Root samples were oven dried at 60°C to a constant mass. Prior to weighing, root samples were screened again to remove any soil and small stones. Root-rhizome biomass was multiplied by a constant 

\[
100,000 / (\pi * 3.75^2)
\]

(to convert the measurements to kg ha⁻¹. Root-rhizome mass net
accumulation rate was calculated by dividing root-rhizome mass by number of days the cores were in place. Accumulation rate data are presented in kg ha\(^{-1}\) d\(^{-1}\).

**Statistical Analysis**

Root-rhizome mass accumulation rate data were analyzed using a mixed model with entry as a fixed effect and block as a random effect. The entry means were separated using Tukey’s test. Treatments were considered different when \(P \leq 0.05\). Data are presented graphically in the form of box plots. The structure of box plots is such that the upper and lower hinges (terminal ends of the box) relate to the first and third quartiles (or 25\(^{th}\) and 75\(^{th}\) percentiles), respectively. The thick solid line within the box and perpendicular to the sides of the box is the median. The upper and lower whiskers (solid lines extending above and below the box) extend from the hinge to the largest and smallest values (at most 1.5 interquartile range) of the hinge, respectively. Data points beyond the whiskers are considered outliers.

**Results and Discussion**

There was a significant effect of entry on root accumulation rate \( (P = 0.01) \) (Table 5-2). Root accumulation rate was greater in decumbent Chico (32.8 kg ha\(^{-1}\) day\(^{-1}\)) than either Ona 33 or UF Peace (10.0 and 14.2 kg ha\(^{-1}\) d\(^{-1}\), respectively). Apalachee, Ecoturf, and UF Tito root accumulation rates (22.8, 22.4, and 23.0 kg ha\(^{-1}\) day\(^{-1}\), respectively) were intermediate and not different than any other entry. That the ingrowth core was able to detect differences among entries of the same species with similar below-ground architecture suggests potential for the technology.

In a study using mesh ingrowth core technology in Alaska’s North Slope, fiberglass mesh tubes (2-mm mesh, 5 cm diameter tubes, 20-30 cm long) were used to estimate fine root
production during 1 yr in organic soils of moist tussock tundra and wet sedge ecosystems (Nadelhoffer et al., 2002). The authors found fine root production was greater in wet sedge than moist tundra (2.06 kg ha$^{-1}$ d$^{-1}$ vs 1.53 kg ha$^{-1}$ d$^{-1}$). Root accumulation rate during summer in the current experiment was on average 20 times greater than over 1 yr in the Alaska environment.

A key assumption Nadelhoffer et al. (2002) made was that root growth rates in the core were similar to those in the adjacent soil. They also assumed that because the dominant species in the grassland was annual (Eriophorum vaginatum L.) and non-mycorrhizal, senesced roots were easily detectible because they were flat and grey. Upon excavating the core, they found few senesced roots. Roots in perennial pastures have a lifespan of approximately 100 d (14 wk) (Van der Krift & Berendse, 2002). Since it is important that the time period the ingrowth cores are left below-ground is shorter than the lifespan of the roots, the current experiment was conducted for approximately 100 d.

A study comparing the ingrowth core technique with sequential soil cores for four consecutive growing seasons in a Scots pine (Pinus sylvestris L.) stand in Ferric Podzol soils found that root accumulation rate varied from 6.14 to 13.4 kg ha$^{-1}$ d$^{-1}$ for ingrowth cores and 9.59 to 37.8 kg ha$^{-1}$ d$^{-1}$ for soil cores (Makonnen and Helmisaari, 1999). The range reported for the ingrowth core includes the lower part of the range in the current experiment. The authors outlined a number of problems with the ingrowth core. One is that the ingrowth growing environment is different than natural soil because there are no channels created from decomposing roots. New rhizomes and roots might grow through the channels created by old, decomposing roots. Another challenge to ingrowth core use is that soil horizons in naturalized landscapes such as forests and unmanaged grasslands cannot be reconstructed after sieving. To
compensate, the authors put a humus clod on top of the installed core. In the current experiment, soil is likely not as stratified because it was tilled prior to planting in 2009. Due to management, using the ingrowth core on agricultural soils might not present the same challenges indicated by Makonnen and Helmisaari (1999).

In a recent study, sequential soil cores were compared with ingrowth cores on pastures mainly composed of perennial ryegrass (*Lolium perene* L.) with nearly equal parts smooth meadowgrass (*Poa pratensis* L.), timothy (*Phleum pratense* L.) and white clover (*Trifolium repens* L.) in Northern Germany (Chen, et al., 2015). Ingrowth cores were left in Eutric Luvisols for three periods: short-term (4 wk), medium-term (9 wk), and long-term (27 wk). Sequential soil cores were sampled by root auger at the same interval as medium-term ingrowth cores. Because the ingrowth cores are a direct and more accurate representation of root accumulation, the soil cores were compared with the medium-term ingrowth cores to evaluate their accuracy. Root accumulation rate in the sequential soil cores was underestimated by approximately half that of the medium-term ingrowth cores (56 vs. 118 kg ha$^{-1}$ d$^{-1}$).

An ingrowth core experiment in agricultural soils in Bavaria, Germany studied gross root growth in potato (*Solanum tuberosum* L.), spring wheat (*Triticum aestivum* L.), winter wheat (*Triticum aestivum* L.), and winter barley (*Hordeum vulgare* L.) for approximately 2 wk at a time (Steingrobe et al., 2001). Although the study measured root production as a function of root length density, not root accumulation rate, it addressed two common concerns with ingrowth cores. One is whether root growth can be affected by disparate soil conditions inside and outside the ingrowth core. In an experiment altering soil density, N, P, K and soil moisture, it was reported that only very high N (treatments fertilized with 80 kg N ha$^{-1}$) and high soil density
(1.64 g cm\(^{-3}\)) increased and decreased root growth, respectively, of rapeseed (*Brassica napus* L.) (Steingrobe et al., 2000). A second concern the authors addressed is whether root growth is reduced by compaction in soil walls during ingrowth core installation. The experiment concluded that installation did not alter the root growth pattern in and around the core. Other experiments evaluating the effect of rhizotrons on root growth patterns found that technology did not affect root growth patterns either (Ostonen et al., 2007; Rytter and Rytter, 2012).

There is some concern in the literature that sieving, as it affects N mineralization of moist soil could affect growing conditions (Hassink, 1994). However, the soils in the current experimental site do not have many aggregates that could be broken up and are low in organic matter and N. So, significant mineralization from sieving soil is less likely to occur.

In a study using the mesh-screen technology to measure fine root accumulation in Norway spruce (*Picea abies* L.), average root accumulation was 7.9 kg ha\(^{-1}\) d\(^{-1}\) (Jentschke et al., 2001). In mesh-screen studies, a blade is inserted into the soil and removed so a mesh screen can be installed in its place. It is excavated after a period of time and the roots that grow through the mesh are counted and weighed. Disadvantages to this technology are that soil can drop into the slit the blade makes or the mesh can be difficult to install as it can fold easily when pushed into the hole (Hirano et al., 2009). They attempted to improve this method by using two thin stainless-steel sheets to accompany the metal blade. The blade is removed, but the sheets stay in place until the mesh is installed, blocking any soil particles that might interfere with the mesh insertion. In all mesh-screen methods, miscalculation in root accumulation may occur (Hirano et al., 2009). Because roots are cut prior to installation, advantageous root growth may be
encouraged or cut roots may be stunted. Also, mesh screens or ingrowth cores cannot measure temporal information on root growth, unlike minirhizotron technology (Majdi et al., 2005).

The current experiment attempts to overcome many of the challenges presented in the aforementioned studies. In all of the ingrowth studies mentioned above, the cores were installed at a 45° angle to capture maximum root growth, as compared with vertically-installed cores. Soil compaction and high N is less of a concern in the current experiment’s soils as they are low in nutrients and composed mainly of fine sand. However, care was taken during installation to approximate soil conditions inside the ingrowth core to those in the surrounding soil. It was ensured that the soil inserted into the core was the same as that excavated from the hole and the soil was compacted to approximate the same bulk density.

**Implications**

Ona 33 and UF Peace had the slowest root accumulation rate, while decumbent Chico had the fastest. Apalachee, Ecoturf, and UF Tito were in the mid-range of root accumulation rates. Significant differences were detected among treatments, suggesting the modifications made to the ingrowth core are likely suitable to be used in subsequent studies. Choosing a mesh fabric with larger holes (4 mm) likely permitted a more accurate estimation of root-rhizome accumulation, as the holes were large enough to allow roots and rhizomes to grow into the core. Addition of the cage wire superstructure helped maintained the core’s geometry over 100-d deployment periods in the soil.
install at 45° from soil surface

4 mm polyester mesh fabric

2.54 cm spacing between wires
cage wire

Figure 5-1. A completed root-rhizome ingrowth core. Photo courtesy of the author.
Figure 5-2. Cutting the cage wire material to size and shaping it into a cylinder for the ingrowth core. Photo courtesy of the author.
Figure 5-3. Bottom and top of the ingrowth core before the polyester mesh is placed on it. Photo courtesy of the author.

If the core is to be installed at a 45° angle, cut 5 units off the top of the cagewire. This will allow it to lie flush with the soil surface.
Figure 5-4. Preparing the mesh covering of the ingrowth core. White material is polyester cargo netting utility fabric. Yellow material is a template made from transparent architectural drafting paper. Photo courtesy of the author.
Figure 5-5. Sewing the mesh covering of the ingrowth core with exterior-grade thread. (Not pictured: pull drafting paper from mesh to remove). Photo courtesy of the author.
Figure 5-6. Finished ingrowth core. Fabric is secured to cage wire by being pulled through exposed ends. Exposed ends are then folded into the core. Photo courtesy of the author.
Figure 5-7. Excavating soil for ingrowth core installation in a grass pasture. Photo courtesy of the author.
Figure 5-8. Sieving and screening plant material (leaves, roots, rhizomes) from excavations. Photo courtesy of the author.

Figure 5-9. Replacing ingrowth core with soil screened of roots and rhizomes into plots. Photo courtesy of the author.

Figure 5-10. Cutting roots and rhizomes protruding from the ingrowth core. Photo courtesy of the author.
Figure 5-11. Washing soil from ingrowth core to obtain root-rhizome mass. Photo courtesy of the author.
Table 5-1. Herbage accumulation and canopy height at harvest for six rhizoma peanut entries harvested twice per year in each of 2 yr, and root-rhizome mass of the same entries measured at season end of 2 yr. Data are entry means across 2 yr. Entry means that are followed by the same letter are not different ($P > 0.05$).

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Entry</th>
<th>Ona 33</th>
<th>Apalachee</th>
<th>Chico</th>
<th>Ecoturf</th>
<th>UF Peace</th>
<th>UF Tito</th>
<th>$P$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbage accumulation (Mg ha$^{-1}$)</td>
<td></td>
<td>10.5 a</td>
<td>6.7 cdef</td>
<td>9.6 ab</td>
<td>6.8 cdef</td>
<td>7.9 bcde</td>
<td>7.8 bcde</td>
<td>&lt; 0.0001</td>
<td>0.47</td>
</tr>
<tr>
<td>Canopy height (cm)</td>
<td></td>
<td>22.9 ab</td>
<td>13.0 d</td>
<td>13.7 d</td>
<td>15.2 cd</td>
<td>17.5 bcd</td>
<td>23.9 a</td>
<td>&lt; 0.0001</td>
<td>1.21</td>
</tr>
<tr>
<td>Root-rhizome mass (Mg ha$^{-1}$)</td>
<td></td>
<td>6.4 abc</td>
<td>5.7 bc</td>
<td>8.6 a</td>
<td>6.9 abc</td>
<td>6.9 abc</td>
<td>5.5 ab</td>
<td>&lt; 0.0001</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Table 5-2. Ingrowth rate of root-rhizome mass accumulation for six rhizoma peanut entries measured during 102 to 104 d during each of 2 yr in Hague, FL. Entry means that are followed by the same letter are not different ($P > 0.05$).

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Entry</th>
<th>Ona 33</th>
<th>Apalachee</th>
<th>Chico</th>
<th>Ecoturf</th>
<th>UF Peace</th>
<th>UF Tito</th>
<th>$P$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingrowth rate of accumulation (kg ha$^{-1}$ d$^{-1}$)</td>
<td></td>
<td>10.0 b</td>
<td>22.8 ab</td>
<td>32.8 a</td>
<td>22.4 ab</td>
<td>14.2 ab</td>
<td>23.0 ab</td>
<td>0.01</td>
<td>8.07</td>
</tr>
</tbody>
</table>
CHAPTER 6
CONCLUSIONS

Rhizoma peanut (RP; *Arachis glabrata* Benth.) is a perennial forage legume that has excellent nutritive value and demonstrated persistence when planted in monoculture or with grass species in the US Gulf Coast region. ‘Florigraze’ is the dominant cultivar in use, but it has limitations including susceptibility to peanut stunt virus (*Cucumovirus* spp.; Prine et al., 2010). The University of Florida has released several additional RP germplasms and cultivars in the past decade (Prine et al., 2010; Quesenberry et al., 2010) that are superior to Florigraze. Most RP cultivars were selected for upright growth, favoring use for hay production, but there are germplasms and experimental lines that vary in growth habit and perhaps in tolerance to a greater range of soil drainage conditions. These lines may provide opportunity for greater use of RP under grazing or as an ornamental plant, and perhaps increase the range of soil environments in which it can be grown. Data describing key forage responses are lacking for these entries, and evaluation is needed of forage productivity, nutritive value, and stand persistence responses under different defoliation practices and in different soil environments.

The goals of the three projects described in this thesis are to evaluate forage responses of 14 RP introductions/selections, germplasms, and cultivars for use in the southeast US and to assess the utility of a root-ingrowth core device to measure root accumulation rate within a pasture context. The objective of the first project was to quantify and compare the effects of defoliation frequency on herbage production, nutritive value, and root-rhizome characteristics of selected RP introductions with existing germplasms and cultivars. The second project was designed to characterize herbage production, nutritive value, and root-rhizome traits of selected
RP introductions with those of existing cultivars and germplasms when grown at two locations with different soil characteristics, one with well-drained and another with seasonally-saturated soil. The objective of the third project was to develop and test a prototype root-ingrowth core device designed to measure root-rhizome accumulation rate of perennial forage species.

**Rhizoma Peanut Herbage Accumulation, Nutritive Value, and Root-Rhizome Responses to Defoliation Frequency – Chapter 3**

The experiment was conducted at the North Florida Research and Education Center in Quincy, FL from 2015 to 2017. Two defoliation frequencies were imposed on split-plots of 14 RP entries and measures were made of above-ground and below-ground biomass, herbage nutritive value, and storage organ characteristics. The defoliation frequencies evaluated were one (1X; fall) or two (2X; summer and fall) harvests per season. Relatively long intervals between defoliation events were chosen in order to address two primary scenarios: i) the difficulty of timely hay harvest in Florida because of frequent rain events during the growing season; and ii) situations in which RP is harvested for hay during the growing season and also used as a source of rhizomes for vegetative propagation in the subsequent dormant season.

Overall, the results suggested that greater self-shading and disease pressure in the 1X treatment likely affected many of the response variables. Herbage accumulation was nearly twice as great in the 2X treatment for many entries. However, herbage accumulation of the Quincy-Beta entry was not affected by defoliation frequency, probably because it has greater disease tolerance than other entries. Canopy height was not affected by defoliation frequency. While previous experiments suggest that Ecoturf has a decumbent growth habit with high herbage bulk density, this was not observed in the current study. Since all defoliation treatments in the current
experiment were relatively infrequent, Ecoturf canopy height and bulk density were not affected by defoliation frequency, and it was generally intermediate in these traits. This supports the conclusion of Shepard et al. (2018) that Ecoturf is a phenotypically plastic RP line that adopts a short dense canopy under frequent, close defoliation, but when defoliated infrequently it does not demonstrate these characteristics.

The 1X treatment was associated with lesser crude protein and in vitro digestibility, most likely due to disease-induced senescence and leaf drop in the 1X plots and the effects of greater maturity. The 2X treatment caused root-rhizome mass to decrease when compared with 1X, thus multiple defoliation events are not recommended for fields that will subsequently be used as source fields for RP rhizomes. Defoliation frequency did not affect rhizome diameter, although among entries ‘Arbrook’ and ‘UF Peace’ produced the thickest rhizomes. Defoliation frequency did not affect root-rhizome non-structural carbohydrate concentration. However, given its effect on root-rhizome mass, the non-structural carbohydrate pool was almost twice as high in the 1X treatment as in the 2X.

Based on this study it is concluded that RP hay producers should prioritize obtaining at least a summer and a fall harvest from their hay fields, even if summer weather conditions are not optimal for drying. Preservation as haylage or balage, options that require less field drying, should be explored as an alternative when drying conditions are challenging. Those producers wishing to dig rhizomes from hay fields should keep in mind that defoliation, especially more than one defoliation event per year will reduce the amount of rhizome mass available for digging the following winter or spring. Finally, the impact of harvest frequency varies among entries of RP, with Quincy-Beta being particularly tolerant of infrequent harvests while more upright-
growing entries (e.g., Arbrook and ‘UF Tito’) and those susceptible to leaf diseases (e.g., Ecoturf) are likely to show some of the greatest negative impacts associated with a single vs. multiple harvests per year.

**Above- and Below-ground responses of Rhizoma Peanut Experimental Lines and Cultivars when Grown at Two Locations Differing in Soil Characteristics – Chapter 4**

The experiment was conducted at two locations: the North Florida Research and Education Center in Quincy, FL and the Agronomy Forage Research Unit in Hague, FL. The Quincy location is characterized by well-drained soils and the Hague location by seasonally-saturated soils. Above- and below-ground biomass and chemical composition responses were measured on 14 introductions/selections, germplasms, and cultivars of RP.

More favorable soil drainage at Quincy resulted in generally greater herbage accumulation than at Hague. However, herbage accumulation of the entry Ona 33 did not differ between sites, suggesting that it may be better suited to poorly-drained soils than most RP lines. Greater HA and associated dilution of N at Quincy resulted in greater herbage crude protein at Hague. In general, the longer regrowth periods in this study than most previous studies resulted in lesser herbage crude protein and in vitro digestible organic matter concentrations in the current experiment. The longer defoliation interval likely had the greatest negative effect on nutritive value of Ecoturf, which had the greatest incidence of leaf rust. Below-ground characteristics varied among entries, with Arbrook maintaining a large amount of below-ground biomass with very thick rhizomes and high concentrations of TNC. In contrast, entries like Apalachee and Cowboy had lesser rhizome mass, rhizome diameter, and TNC concentration.
These data suggest that there may be potential to expand the current zone of adaptation of RP into wetter-soil environments by evaluating additional entries and releasing new cultivars. Additionally, there are a wide range of above- and below-ground growth characteristics available among entries being tested, providing opportunity for targeting entries to specific intended uses or production goals.

**Design and Use of a Root-Ingrowth Core Device to Measure Root Accumulation Rate of Perennial Forage Species – Chapter 5**

A modified design for a root-ingrowth core was developed and tested in this study. The major differences in this ingrowth core design included a larger mesh size of the polyester fabric lining the ingrowth core and a wire mesh support structure to enhance the ability of the core to sustain its shape and volume throughout the ingrowth period. The cores were then tested in an experiment measuring root mass accumulation of six RP entries at the Hague location during 2 yr. Root accumulation rates varied among treatments, with low-growing Chico having more rapid root accumulation rate than Ona 33 and UF Peace. Entries Apalachee, Ecoturf, and UF Tito were intermediate in root mass accumulation rate. Enlarging the fabric holes and reinforcing the walls with wire allowed rhizomes and roots to grow into the core, while maintaining the geometry of the core. Significant differences were detected among treatments, suggesting this ingrowth core design may contribute to subsequent root-rhizome mass accumulation studies, including those comparing single species with similar root architecture.

**Implications of Research**

Twice a year defoliation is not frequent enough to reduce the quality of rhizomes for planting material, but it can reduce root-rhizome mass. Although once a year harvest may be
beneficial to root-rhizome mass for nursery production, it lowers forage nutritive value and herbage accumulation of most RP entries, especially in upright-growing lines. Thus, RP hay producers should prioritize at minimum a summer and a fall harvest from their hay fields. Compared with other RP entries currently being used commercially or being evaluated for potential release, Ona 33 is better adapted to seasonally saturated soils. Ecoturf is likely phenotypically plastic, assuming a shorter canopy height with greater herbage bulk density when frequently defoliated, but not differing in growth characteristics from other intermediate types when intervals between defoliation events are long.

**Future Research Needs**

Given that the recommended minimum of twice per year harvest for RP hay production is complicated by summer conditions, options that require less field drying, like haylage or balage, should be explored. As a range of above- and below-ground characteristics have been identified in RP entries, choice of RP entry to be planted can be tailored to management goals and desired production outcomes. Furthermore, additional entries and new cultivars may be tested and released that expand the current zone of RP adaptation into wetter soil environments.

The root ingrowth core developed in this thesis likely can be used to study root-rhizome mass accumulation in a wide range of perennial pasture species and under numerous management practices. As mild winters in Florida allow for year-round forage production, the ingrowth core should be used to investigate the relationship between defoliation, root accumulation, and seasonality.
APPENDIX

ABOVE- AND BELOW-GROUND RESPONSE VARIABLES FOR THE RHIZOMA PEANUT ENTRY CHICO

Herbage accumulation, canopy height, herbage bulk density, herbage crude protein, and herbage in vitro digestible organic matter at harvest for rhizoma peanut entry Chico and the average of all entries harvested twice per year in each of 2 yr at the Hague location. Root-rhizome mass, root-rhizome diameter, TNC concentration, and TNC pool of the same entries were measured at season end of 2 yr. Data are entry means across 2 yr.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Mean for Chico</th>
<th>Overall Hague location mean†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbage accumulation (Mg ha⁻¹)</td>
<td>9.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Canopy height (cm)</td>
<td>13.7</td>
<td>17.3</td>
</tr>
<tr>
<td>Herbage bulk density (kg ha⁻¹ cm⁻¹)</td>
<td>572</td>
<td>333</td>
</tr>
<tr>
<td>Herbage crude protein (g kg⁻¹)</td>
<td>196</td>
<td>170</td>
</tr>
<tr>
<td>Herbage in vitro digestible organic matter (g kg⁻¹)</td>
<td>661</td>
<td>615</td>
</tr>
<tr>
<td>Root-rhizome mass (Mg ha⁻¹)</td>
<td>8.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Root-rhizome total nonstructural carbohydrates (g kg⁻¹)</td>
<td>222</td>
<td>266</td>
</tr>
<tr>
<td>Root-rhizome pool (kg ha⁻¹ cm⁻¹)</td>
<td>1970</td>
<td>1780</td>
</tr>
<tr>
<td>Rhizome diameter (mm)</td>
<td>2.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

† RP entries at Hague included Ona 33, Quincy-Alpha, Apalachee, Arblick, Arbrook, Quincy-Beta, Chico, Cowboy, Ecoturf, Florigraze, UF Peace, Pointed Leaf, Quincy, UF Tito, and Waxy Leaf.
WORKS CITED


BIOGRAPHICAL SKETCH

Katie Cooley was born and raised in New England. She received her Bachelor of Arts in environmental analysis from Scripps College in Claremont, CA in May 2006 and her Master in Landscape Architecture from the Graduate School of Design at Harvard University in May 2013. Her interest in plant and soil science and passion for environmental stewardship motivated her to earn an additional graduate degree in agricultural science. In December 2018, she received her Master of Science in agronomy with a minor in soil and water science.