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**METHODS OF ESTABLISHMENT OF PERENNIAL PEANUT AS
MONOCULTURE OR PEANUT-WARM-SEASON GRASS MIXED
SWARDS**

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OR PEANUT-WARM-SEASON GRASS MIXED SWARDS**

Tese apresentada ao Programa de Pós-graduação em
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**Methods of Establishment of Perennial Peanut as Monoculture or Peanut-Warm-Season
Grass Mixed Swards**


Por

Nayara Martins Alencar

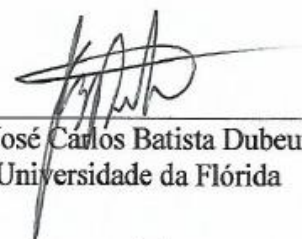
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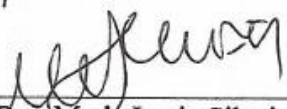
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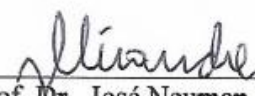
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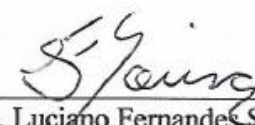
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To my parents, Cicero e Ana Alencar

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	4
TABLE OF CONTENTS	5
ABSTRACT	8
Methods of Establishment of Perennial Peanut as Monoculture or Peanut-Warm-Season Grass Mixed Swards	8
RESUMO.....	10
Métodos de Estabelecimento de Amendoins Perenes como Monocultura ou em Consórcio Amendoim-Gramíneas de Estação Quente.....	10
LIST OF TABLES.....	12
LIST OF FIGURES	13
LIST OF ABBREVIATIONS	14
INTRODUCTION	15
CHAPTER 1	17
LITERATURE REVIEW	17
General description of genus <i>Arachis</i>	17
Pintoi peanut description.....	17
Pintoi peanut agronomic characteristics	18
Pintoi peanut intercropping.....	19
Rhizoma peanut description.....	19
Rhizoma peanut agronomic characteristics	20
Rhizoma peanut in situ disappearance	21
REFERENCES	23
CHAPTER 2.....	28
Methods of Establishment of Pintoi Peanut as Monoculture or Pintoi Peanut-Palisadegrass Mixed Swards	28
ABSTRACT	28

INTRODUCTION	29
MATERIALS AND METHODS	30
Experimental site	30
Establishment experiment.....	30
Response variables.....	31
Pintoi peanut ground cover, density, and spread	31
Pintoi peanut HA.....	32
Palisadegrass herbage characteristics	32
Palisadegrass and pintoi peanut nutritive value	32
Overseeding experiment	32
Pintoi peanut biological N ₂ fixation.....	33
Statistical Analysis.....	34
RESULTS AND DISCUSSION.....	34
Establishment experiment.....	34
Overseeding experiment	35
SUMMARY AND CONCLUSIONS	37
REFERENCES	38
CHAPTER 3	44
Methods of establishment of rhizoma peanut into bahiagrass pastures.....	44
ABSTRACT	44
INTRODUCTION	45
MATERIALS AND METHODS	46
Experimental site	46
Treatments and design	46
Response variables.....	47
Soil cover and rhizoma peanut ground cover	47
Rhizoma peanut density and canopy height.....	47

Herbage accumulation, leaf:stem ratio and rhizome-root mass.....	47
Rhizoma peanut N ₂ fixation.....	47
Statistical Analysis.....	48
RESULTS AND DISCUSSION.....	49
CONCLUSIONS	51
REFERENCES	52
CHAPTER 4.....	57
Impact of different leaf:stem proportions in DM and CP in situ disappearance of different rhizoma peanut genotypes	57
ABSTRACT	57
INTRODUCTION.....	58
MATERIAL AND METHODS.....	59
Location, establishment, and treatments.....	59
In situ disappearance procedure.....	60
In situ dry matter and crude protein degradation.....	60
Statistical Analysis.....	61
RESULTS AND DISCUSSION.....	61
Dry matter and crude protein degradation parameters.....	61
Dry matter and CP fraction concentration	62
SUMMARY AND CONCLUSIONS	64
REFERENCES	65
FINAL REMARKS	72

ABSTRACT

Methods of Establishment of Perennial Peanut as Monoculture or Peanut-Warm-Season Grass Mixed Swards

Pintoi peanut (*Arachis pintoi* Krapovickas and Gregory) and rhizoma peanut (*Arachis glabrata* Benth.) are warm-season perennial legumes that have been extensively used in grass-legume mixtures. However, the establishment of grass-legume mixed pastures has been a major limiting factor for the widespread adoption of legumes. The objective of this thesis was to evaluate and develop management strategies for establishment of pintoi peanut-palisadegrass [*Urochloa brizantha* (A. Rich.) R.D. Webster] and rhizoma peanut-bahiagrass mixtures in tropical and subtropical regions. Two experiments were conducted in Brazil to evaluate different methods of establishment of pintoi peanut or pintoi peanut-palisadegrass mixed swards. The establishment experiment evaluated pintoi peanut and palisadegrass established as monocultures or in a pintoi peanut-palisadegrass mixture. In the overseeding experiment, treatments were pintoi peanut seeded into glyphosate treated rows followed by prepared seedbed, pintoi peanut seeded into glyphosate treated rows with no seedbed preparation (no-till), or undisturbed palisadegrass monoculture swards. Pintoi peanut ground cover and density was greater for pintoi peanut than pintoi peanut-palisadegrass mixed swards (29.4 vs. 7.8%, and 41.8 vs. 19.0 plants m⁻² for ground cover and density, respectively). Concomitant seeding of pintoi peanut and palisadegrass showed no negative effect on palisadegrass herbage accumulation (HA). Prepared seedbed and no-till treatments had similar pintoi ground cover (2.9%), density (8.7 plants m⁻²), spread (11.7 cm), and HA (170 kg ha⁻¹ yr⁻¹). Overseeding pintoi peanut reduced palisadegrass HA and nutritive value. In Florida, two experiments were conducted, and the first experiment evaluated the management practices to establish ‘Florigraze’ and ‘Ecoturf’ rhizoma peanut into bahiagrass (*Paspalum notatum* Flüggé) pastures, with two establishment methods (no-till and prepared seedbed). Ecoturf and Florigraze had similar ground cover (12.2%), canopy density (17.5 plants m⁻¹), height (3.9 cm), HA (558 kg ha⁻¹), and rhizome-root mass (9.4 Mg ha⁻¹). Prepared seedbed and no-till treatments had similar rhizoma peanut soil cover (49%), N concentration (22.0 g kg⁻¹), Atmospheric nitrogen fixation (Ndfa) (364 g kg⁻¹), biological N fixation (BNF) (5.8 kg N ha⁻¹). However, rhizoma peanut ground cover (14.9 vs. 9.4%) and HA (701 vs. 414 kg ha⁻¹) were greater for the prepared seedbed than no-till treatment. The preparing seedbed after glyphosate application had greater rhizoma peanut ground cover and HA. In the second experiment, we evaluated the effect of leaf:stem proportion (LS) on dry matter (DM) and crude protein (CP) disappearance of Ecoturf

and Florigraze rhizoma peanut, with three LS (100:0, 50:50 and 0:100). Florigraze had greater DM fraction A than Ecoturf (349 vs. 339 g kg⁻¹) but there was no difference in fractions B and C (mean = 427 and 230 g kg⁻¹, respectively). The LS ratio has a significant impact on DM and CP fractions and effective degradability. The LS is an important indicator of nutritive value of rhizoma peanut and the models generated by this study may allow managers to have a more accurate prediction of performance of ruminants consuming rhizoma peanut.

Keywords: *Arachis glabrata*. *Arachis pintoi*. Degradability. Management practices.

RESUMO

Métodos de Estabelecimento de Amendoins Perenes como Monocultura ou em Consórcio Amendoim-Gramíneas de Estação Quente

O amendoim pintoi (*Arachis pintoi* Krap. e Greg.) e o amendoim rizomatoso (*Arachis glabrata* Benth.) são leguminosas perenes de estação quente utilizadas extensivamente em consórcios de gramíneas-leguminosas. No entanto, o estabelecimento de pastagens consorciadas com gramíneas e leguminosas é um dos principais fatores limitantes na adoção de leguminosas. O objetivo desta tese foi desenvolver estratégias de manejo para o estabelecimento de amendoim pintoi-capim Marandu e amendoim rizomatoso-capim Bahia em consórcio em regiões tropicais e subtropicais. Foram realizados dois experimentos no Brasil, para avaliar diferentes métodos de estabelecimento de amendoim pintoi ou amendoim pintoi-capim Marandu [*Urochloa brizantha* (A. Rich.) R.D. Webster] em consórcio. O experimento de estabelecimento avaliou amendoim pintoi e capim Marandu estabelecidos como monoculturas ou em consórcio amendoim pintoi-capim Marandu. No experimento de sobressemeadura, os tratamentos foram estabelecidos com amendoim pintoi em linhas tratadas com glifosato, com preparo do solo, amendoim pintoi semeados em linhas tratadas com glifosato sem preparação de solo ou monocultura de capim Marandu. A cobertura e a densidade de amendoim pintoi foram maiores para o amendoim pintoi em monocultura do que em consórcio com amendoim pintoi-capim Marandu (29,4 vs. 7,8% e 41,8 vs. 19,0 plantas m⁻² para cobertura e densidade, respectivamente). A semeadura concomitante de amendoim pintoi e capim Marandu não mostrou efeito negativo sobre o acúmulo forragem (AF) do capim Marandu. Os tratamentos com semeadura com preparo do solo e sem preparo do solo apresentaram resultados para amendoim pintoi semelhantes para as variáveis cobertura (2,9%), densidade (8,7 plantas m⁻²), propagação (11,7 cm) e AF (170 kg ha⁻¹ ano⁻¹). A sobressemeadura do amendoim pintoi reduziu o AF e o valor nutritivo do capim Marandu. Na Flórida, realizou-se dois experimentos; o primeiro experimento avaliou as práticas de manejo para estabelecer amendoim rizomatoso Florigraze e Ecoturf em pastagens de capim Bahia (*Paspalum notatum* Flüggé), com dois métodos de estabelecimento (plantio direto e preparo do solo). Ecoturf e Florigraze tiveram cobertura semelhante (12,2%), densidade (17,5 plantas m⁻¹), altura (3,9 cm), AF (558 kg ha⁻¹) e massa da raiz (9,4 Mg ha⁻¹). Os tratamentos de plantio direto e de preparo do solo apresentaram cobertura similar de solo (49%), concentração de N (22,0 g kg⁻¹), N derivado da atmosfera (Ndfa) (364 g kg⁻¹), fixação biológica de N (FBN) (5,8 kg N ha⁻¹). No entanto, a cobertura por amendoim rizomatoso (14,9 vs. 9,4%) e AF (701 vs. 414 kg ha⁻¹) foi maior para

o plantio com preparo de solo do que o tratamento sem preparo do solo. A preparação do solo para o plantio após a aplicação de glifosato apresentou maior cobertura por amendoim rizomatoso e AF. No segundo experimento, foram avaliados a efeito da proporção folha:caule (FC) no desaparecimento da matéria seca (MS) e da proteína bruta (PB) do Ecoturf e do Florigraze, com três proporções FC (100:0, 50:50 e 0:100). O Florigraze apresentou maior fração A do que Ecoturf (349 vs. 339 g kg⁻¹), mas não houve diferença nas frações B e C (média = 427 e 230 g kg⁻¹, respectivamente). A relação FC tem um impacto significativo no desaparecimento das frações MS e PB, e na degradabilidade efetiva. A FC é um indicador importante do valor nutritivo do amendoim rizomatoso e os modelos gerados por este estudo podem permitir uma previsão mais precisa do desempenho de ruminantes que consomem amendoim rizomatoso.

Palavras-chave: *Arachis glabrata*. *Arachis pintoi*. Degradabilidade. Práticas de manejo.

LIST OF TABLES

Table 2-1. Recorded and 28-yr average total monthly rainfall and maximum and minimum temperatures for 2014, 2015 and 2016.	41
Table 2-2. Pintoï peanut ground cover, density and spread on pintoï peanut only and pintoï peanut-palisadegrass mixture plots (Establishment experiment).	41
Table 2-3. Seasonal variation in herbage characteristics of palisadegrass and pintoï peanut cultivated as grass monoculture or pintoï peanut-palisadegrass mixed swards (Establishment experiment).....	42
Table 2-4. Treatment × month interaction effects on herbage accumulation and leaf area index of palisadegrass (Overseeding experiment).....	42
Table 2-5. Herbage characteristics of palisadegrass as monoculture (control) or subjected to different methods of pintoï peanut overseeding (Overseeding experiment).	43
Table 2-6. Seasonal variation in herbage characteristics of palisadegrass as monoculture or subjected to different methods of pintoï peanut overseeding (Overseeding experiment).	43
Table 3-1. Recorded 18-yr average total monthly rainfall and maximum and minimum temperatures for 2014 and 2015 for the experimental location.....	54
Table 3-2. Soil cover (%) as affected by genotype and month.....	54
Table 3-3. Establishing method effects on rhizoma peanut ground cover and herbage accumulation.....	55
Table 3-4. Rhizoma peanut ground cover, density, and height as affected by month.....	55
Table 4-1. Effect of genotype on dry matter and crude protein degradation of rhizoma peanut.	67
Table 4-2. Effect of proportions of the leaf on dry matter and crude protein degradation of rhizoma peanut.	68

LIST OF FIGURES

Figure 3-1. Schematic of experimental units. RP: Rhizoma peanut.....	56
Figure 4-1. Genotype effects on the dry matter (I) and crude protein (II) disappearance of rhizoma peanut. †Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.10$) (SAS Institute Inc., 1996). There was effect on the dry matter disappearance for Fraction A ($P = 0.01$; SE = 2.8), there was no effect for Fraction B ($P = 0.19$; SE = 11.7) and C ($P = 0.32$; SE = 11.5). There was effect on the crude protein disappearance for Fraction A ($P = 0.098$; SE = 20.5) and B ($P = 0.037$; SE = 27.7), there was no effect for Fraction C ($P = 0.18$; SE = 10.0).	69
Figure 4-2. Dry matter degradation and estimative for genotypes of Fraction A, Fraction B, Fraction C. Leaf:stem ratio Ecoturf and Florigraze, 66 and 63%, respectively. The coefficient of determination (R^2) are for rhizoma peanut leaf:stem proportion.	70
Figure 4-3. Crude protein degradation and estimative for genotypes of Fraction A, Fraction B, Fraction C. Leaf:stem ratio Ecoturf and Florigraze, 66 and 63%, respectively. The coefficient of determination (R^2) are for rhizoma peanut leaf:stem proportion.	71

LIST OF ABBREVIATIONS

BNF	Biological N ₂ fixation
CP	Crude protein
DM	Dry matter
HA	Herbage accumulation
IVDOM	In vitro digestible organic matter
LAI	Leaf area index
LS	Leaf:stem proportion
Ndfa	Nitrogen derived from the atmosphere
OM	Organic matter

INTRODUCTION

Brazil has the largest commercial cattle herd in the world with 218.3 million of the animals, while Tocantins has the third largest herd of the Brazilian northern region with 8.7 million animals (IBGE, 2016). Most of the beef cattle production in Tocantins is pasture-based and the state has approximately 8.1 million ha of grasslands (IBGE, 2006). In 2016, 20% of the Tocantins beef production was exported to 20 countries and generated a revenue of US\$ 162.2 million (SEAGRO, 2017).

Grasslands in tropical and subtropical regions are generally characterized by extensive grazing systems cultivated with warm-season perennial grasses. These grazing systems are characterized by limited use of commercial fertilizer; however, to maintain sustainable forage production and nutritive value, warm-season grass monocultures normally require some level of N fertilization (SOLLENBERGER et al., 2009). Extensive areas of planted pastures are degraded in tropical areas of the world, primarily due to inadequate soil N supply (BODDEY et al., 2004). The stand of the desirable forage species often declines, being replaced by a weed or leaving uncovered soil, resulting in erosion (MÜLLER et al., 2004; MUIR et al., 2011) and decreased forage and livestock production.

Introduction of legume into pure grass stands represents a viable alternative to increase N supply to warm-season grass pastures. Warm-season forage response to N addition via either fertilizer or presence of legume is typically positive because N is often the most limiting nutrient. Legumes can biologically fix N₂ due to symbiotic association with *Rhizobium* bacteria, thus contributing to the increase of forage grass production and persistence. In addition, the increase in N concentration in forage legumes may supply additional crude protein (CP) to livestock, by ingestion of legumes or improvement in the quality and quantity of the companion warm-season grass (SHELTON et al., 2005; MUIR et al., 2011). Nitrogen is transferred from legumes to grasses through exudation and leakage of N from roots and nodules, senescence and degradation of nodules and roots, direct transfer from legume roots to non-legume roots through connections made by arbuscular mycorrhizal fungal hyphae, movement of N from legume herbage to the soil by leaching or decomposition of surface litter, and re-deposition of consumed N by livestock (VENDRAMINI et al., 2014).

The adoption of tropical legumes worldwide has limited, with adoption successes in Asia and Australia, to a lesser extent in USA and Brazil. The most commonly used legumes are *Stylosanthes*, tree legumes and evergreen shrubs, and forage *Arachis* species (SHELTON; FRANZEL; PETERS, 2005). Legume characteristics contribute to its success in the grass-legumes mixes are persistence, vigor, and longevity in systems of cutting or grazing, ease of

establishment, high seed production and vegetative propagation easiness, making possible the sustainability of production systems (VALENTIM et al., 2003; SHELTON et al., 2005). According to Muir et al. (2014) and Shelton et al. (2005), pintoï peanut (*Arachis pintoï* Krap. and Greg.) and rhizoma peanut (*Arachis glabrata* Benth.) are legume species that have been successfully used in grazing, hay production, and ground cover in Brazil and in the southeastern USA, respectively.

Pintoï peanut and rhizoma peanut have been successfully used as forage for livestock due to superior nutritive value, persistent, and excellent adaptation to various soil types and locations (VALENTIM et al., 2003; BARCELLOS et al., 2008; MULLENIX et al., 2016a). However, establishment of grass-legume mixed pastures has been a major limiting factor in the adoption of legumes in tropical and subtropical regions. The difference in rate of establishment and growth of grass and legumes, primarily due to differences in carbon fixation pathways, usually promotes the dominance of the grass and poorly establishment of legumes.

In addition, it has been reported that rhizoma peanut hay may have decreased nutritive value due to improper management during harvest and bailing. If the plants stay for extended time in the field after harvest, the leaves may shed, which would result in decrease leaf:stem ratio and overall nutritive value. However, there are no reports in the literature addressing the effects of leaf:stem ratio on nutritive value of rhizoma peanut.

The general objective of this thesis was to develop management strategies for successful establishment of pintoï peanut-palisadegrass and rhizoma peanut-bahiagrass mixtures in tropical and subtropical regions. The specific objectives were to evaluate the effects on establishment success of: 1) Methods of establishment of pintoï peanut as monoculture or pintoï peanut-palisadegrass mixed swards (Chapter 2); 2) Methods of establishment of rhizoma peanut into bahiagrass pastures (Chapter 3); and 3) Impact of different leaf:stem proportions in dry matter (DM) and CP in situ disappearance of different rhizoma peanut genotypes (Chapter 4).

CHAPTER 1 LITERATURE REVIEW

General description of genus *Arachis*

The genus *Arachis* belongs to the Fabaceae family, Papilionoideae subfamily, Aeschynomeneae tribe, Stylosanthinae subtribe, with diploid ($2n = 2x = 20$) and tetraploid ($2n = 4x = 40$) species. It has perennial, biennial, or annual plants, erect, decumbent, or procumbent, can be rhizomatous or stoloniferous. Tretafoliolate or trifoliolate leaves, taproot with branches thickened or not, rhizomes, stolons, and branches produced from adventitious buds on the roots. Stipules partially fused to the petiole, petiole, and rachis canaliculate, leaflets from suborbicular to lanceolate, papilionaceous corolla; tubular hypanthium. Subterranean fruit, peg short and vertical or horizontal up to more than 1-m long, and adventitious roots. Seed smooth, seed coat whitish or yellow-brown in the wild species or various colors in the cultivated peanut (KRAPOVICKAS; GREGORY, 2007).

The genus *Arachis* is native to South America, the species distributed in South America east of the Andes, south of the Amazon, north of La Plata and from northwest Argentina to northeast Brazil. The genus has 80 species described, with nine taxonomic sections, *Arachis*, *Caulorrhizae*, *Erectoides*, *Extranervosae*, *Heteranthae*, *Procumbentes*, *Rhizomatosae* (Series *Prorhizomatosae* and *Rhizomatosae*), *Triectoides* and *Triseminatae* (VALLS; SIMPSON, 2005; KRAPOVICKAS; GREGORY, 2007). The two sections studies of genetic diversity with forage potential are *Caulorrhizae* (*Arachis pintoii*) and *Rhizomatosae* (*Arachis glabrata*), with the most represented in the world collection (VALLS et al., 1994).

Pintoii peanut description

Arachis pintoii is a perennial plant, with propagation by seed and vegetative, with stoloniferous growth habit, axonomorfus root, without enlargements, with dense amounts of branched stolons, the branches extended, rooting at the nodes, cylindrical, angular, and caducous bristles. The leaves are tetrafoliolate, with obovate, glabrous leaflets, but with silky hairs on the margins. Stipples with a fused portion to the petiole. Petiole up to 6 cm long, canaliculate, with some bristles on the back. The stems are branched, cylindrical, and slightly flattened, with short internodes and stolons that can reach 1.5 m in length. The hypanthium with long silky hair. Fruit subterranean, biarticulated, peg 5-32.5 cm long, with pericarp smooth (KRAPOVICKAS; GREGORY, 2007).

Pintoi peanut agronomic characteristics

The geographical distribution of *A. pintoi* comprises part of the states of Goiás, Bahia and Minas Gerais. *A. pintoi* has good growth in tropical humid and subtropical regions, with sea level from 0 to 1800 m, with an annual rainfall of 1500 to 3500 mm (PIZARRO; RINCON, 1994). The ideal temperature for growth is around 25-30°C with reduced growth at temperatures below 10°C. The limitations in subtropical climate are low temperatures and high humidity during the winter. It has been observed that plants exposed to frost generally recover at the beginning of the warm-season (NASCIMENTO, 2006). Pintoi peanut is better adapted to sandy loam soils and tolerate poorly drained soils and long periods without precipitation (PIZARRO; RINCON, 1994). The ideal pH for pintoi peanut growth is 6.0-6.5, but it can tolerate acidic soils with high Al concentration (RAO; KERRIDGE, 1994).

In Guápiles, Costa Rica, the accessions of *A. pintoi* presented average herbage accumulation of 4.1 Mg DM ha⁻¹ yr⁻¹ in a 2-yr study (ARGEL, 1994). In a mixed pasture in Colombia, herbage accumulation was from 5.2 to 9.6 Mg DM ha⁻¹ yr⁻¹ when harvested at 4-wk interval (GROF, 1985). In Brazil, the ground cover of pintoi peanut was 12.7% 43-d after transplanting and 54% 102-d after transplanting. The leaf:stem ratio of 54%, and CP for leaf and stem was 191 and 110 g kg⁻¹ DM, respectively (TEIXEIRA et al., 2010).

In Central Florida, Carvalho and Quesenberry (2012) reported that the average herbage accumulation of pintoi peanut accessions was 4.36 Mg DM ha⁻¹ yr⁻¹, with average of CP and in vitro organic matter digestibility (IVOMD) 180 g kg⁻¹ and 670 g kg⁻¹ of DM, respectively. In South Florida, Bryan et al. (2001) evaluated accessions of *Arachis* sp. and observed that Amarillo pintoi peanut had herbage accumulation of 4.38 Mg DM ha⁻¹ yr⁻¹, root-rhizome mass of 9.9 Mg DM ha⁻¹, leaf:stem ratio of 83% and spread of 53.8 cm. The authors concluded that Amarillo was a potential cultivar to be used as turf and forage in South Florida. Valentim et al. (2003) tested several accessions of pintoi peanut and reported that Amarillo had herbage accumulation of 2.6 Mg DM ha⁻¹, canopy height of 7.0 cm, ground cover 86%, spread of 92 cm, and CP of 198 g kg⁻¹ DM. Despite of significant spreading, it was observed that Amarillo has reduced herbage accumulation.

Atmospheric nitrogen fixation (Ndfa) is an important characteristics for selection and potential use of warm-season legumes in forage systems. Thomas et al. (1997) evaluated pintoi peanut and estimated that the proportion of the Ndfa from the total N was 80.3% (mean 3-yr). The authors suggest that is necessary to maintain %Ndfa above 80% to maintain a positive N balance without external inputs of fertilizer N. Miranda et al. (2003) observed that

the biological N fixation contribution from pintoï peanut in Brazil may range from 26 to 99 kg of N ha⁻¹ yr⁻¹.

Pintoï peanut intercropping

Andrade et al. (2006) reported that pintoï peanut in consortium with Massai grass [*Megathyrsus maximus* (Jacq.) Simon and S.W.L Jacobs x *P. infestus* (Peters) B.K. Simon and S.W.L Jacobs] had herbage mass from 360 to 790 kg DM ha⁻¹ in different seasons of the year. In pintoï peanut-palisadegrass [*Urochloa brizantha* (A. Rich.) R.D. Webster] mixed pastures, Andrade et al. (2010) observed that pintoï peanut was 6.7% and reported that the limited herbage accumulation of pintoï peanut was not enough to supply significant amounts of Ndfa to the system. The superior growth of the palisadegrass resulted in unfavorable competition and development of the pintoï peanut.

Skonieski et al. (2011) overseeded annual ryegrass (*Lolium multiflorum* Lam.) into pintoï peanut pastures and observed decreased herbage accumulation of pintoï peanut during the winter months, which was expected due to seasonal production of warm-season legumes and limited growth with decreased temperature, day length, and rainfall. According to Nyfeler et al. (2011), greater herbage accumulation in grass-legume mixed pastures are reached at low to moderate levels of N fertilization (50 to 150 kg N ha⁻¹), with legume proportions in the pastures of 40-60%.

Hernandez et al. (1995) evaluated pintoï peanut-palisadegrass mixed pastures with two stocking rates (Low: 1.3 AU ha⁻¹ and High: 2.6 AU ha⁻¹) in Costa Rica. There was no difference in animal performance on pastures with low stocking rate; however, high stocking rates had greater gain ha⁻¹ than low stocking rates (937 vs. 716 kg ha⁻¹). González et al. (1996) compared milk production of dairy cows grazing stargrass (*Cynodon nlemfuensis* Vanderyst) or stargrass-pintoï peanut mixed pastures in Costa Rica and observed that cows grazing mixed pastures increased milk production by 1.2 kg cow⁻¹ d⁻¹ (mean 2-yr), which was the equivalent of 12% increase.

Rhizoma peanut description

Arachis glabrata is a perennial plant, rhizomatous with robust roots. The rhizomes may reach the depth of 5-20 cm. The aerial stems are decumbent, pubescent, with several bristles. The stems are extremely short, almost subterranean, with leaves fully pressed on the ground. Leaves are tetrafoliolate, leaflets elongated, elliptical or obovate, with slightly marked margin on the underside. The upper leaf surface is usually glabrous, but the younger leaves may exhibit some shorter and dispersed hairs. The lower surface of the leaf having hairs to subglabrous, and hairs a little longer in the middle region. The hypanthium is well developed

with villous. The flower color is orange and rarely yellow. The fruit is subterranean, bi-articulated, with short isthmus, and smooth pericarp (KRAPOVICKAS; GREGORY, 2007).

Rhizoma peanut agronomic characteristics

Rhizoma peanut is native to Brazil, Paraguay, and Argentina, with widespread distribution in the states of Mato Grosso and Mato Grosso do Sul. It was introduced in Florida in 1936 from Mato Grosso, Brazil, in cooperation with the United State Department of Agriculture Soil Conservation Service (USDA), with the release of Florigraze (1978) and Arbrook (1986) cultivars. It is a warm climate legume whose production extending approximately 32° N latitude, with deep rooting, adapted to well-drained soils in areas further south and the Gulf Coast of the United States, being of vegetative propagation by rhizomes is one of the limiting factors for adoption in other areas (FRENCH et al., 1994). Rhizoma peanut is adapted to humid tropical, subtropical, or mild temperate climates with long wet, warm-season, and where cool-season temperatures rarely reach or fall below -10° C. It grows on well-drained or moderately drained soils, but not on soils with poor drainage. Rhizoma peanuts are slow to establish and provide little usable forage the first season. Usually, the normal production of forage is obtained in the third growing season (PRINE et al., 1986).

Carvalho and Quesenberry (2012) reported herbage accumulation of Arbrook and Florigraze of 9.67 and 4.98 Mg DM ha⁻¹, respectively. The CP and IVDOM were lesser for Arbrook than Florigraze (153 vs. 172 g kg⁻¹ DM and 690 vs. 740 g kg⁻¹ DM, respectively). Mislevy et al. (2007) compared different rhizome peanut cultivars and entries (Arbrook Select, Arbrook, PI 262839, PI 262826, Florigraze, Ecoturf, and PI 262833) in South Florida during four years. They observed that the herbage accumulation is most variable in the drought year, ranging from 3.4 Mg DM ha⁻¹ for Arbrook and Arbrook Select to 1.3 Mg DM ha⁻¹ for PI 262833. Herbage accumulation was less variable during years of extremely wet conditions. All genotypes declined, according to the authors, it occurred due the flooding stress, with the plots experiencing extended periods of water above the soil surface. The Arbrook and Arbrook Select showed high declines, likely because these two selections are the least flooding tolerant. Ecoturf, PI 262833, and Florigraze had increases of herbage accumulation between the first and fourth year of the study of 89, 54, and 26%, respectively.

Dubeux et al. (2017) conducted a study in North Florida comparing seven genotypes (Arblick, Arbrook, Ecoturf, Florigraze, Latitude 34, UF Peace, and UF Tito) of rhizoma peanut. Herbage accumulation ranged from 6.8 to 11.6 Mg DM ha⁻¹ yr⁻¹ (mean 2-yr), for Florigraze and Arbrook, respectively. The authors reported that Arbrook, UF Peace, and UF Tito are upper ranking with an average of herbage accumulation across years >10 Mg DM ha⁻¹

yr⁻¹. Arblick and Ecoturf are intermediate, with Florigraze and Latitude 34 in the lower ranking. Rhizome-root mass was greater for Ecoturf and Latitude 34 (26.9 and 27.8 Mg OM ha⁻¹, respectively), indicating their potential for greater tolerance to grazing. Florigraze (10.6 Mg OM ha⁻¹) was similar to other genotypes, ranking low for below- and aboveground biomass. Rhizoma peanut N concentration ranged from 19.2 to 36.3 g kg⁻¹ DM in 2014 and 2015, and the average Ndfa and BNF was 86% and 202 kg N ha⁻¹ yr⁻¹ respectively.

Jaramillo (2017) measured the Ndfa of annual and perennial peanut mixed with bermudagrass [*Cynodon dactylon* (L.) Pers.] for hay production and the proportions ranged from 20 to 87% for Ecoturf and from 16 to 90% for Florigraze. The BNF for Ecoturf and Florigraze were 4.8 and 2.7 kg N ha⁻¹ harvest⁻¹, respectively. Santos (2017) in the study with two genotypes of rhizoma peanut (Ecoturf and Q6B) in monoculture or mixed with bahiagrass, found Ndfa values ranging from 59 to 83% in 2016, and BNF of 32 and 31 kg N ha⁻¹ harvest⁻¹, for Ecoturf and Q6B, respectively. The mean BNF for mixed stands was 13 kg N ha⁻¹ harvest⁻¹.

Mullenix et al. (2016a,b) compared four rhizoma peanut genotypes (Ecoturf, Florigraze, UF Peace and UF Tito), under four rotational stocking strategies, and reported average herbage accumulation of 7.5 Mg DM ha⁻¹ yr⁻¹, leaf:stem ratio of 57%, ground cover of 87.4%, canopy height 15.7 cm, CP of 186 g kg⁻¹ DM, and IVMOD of 677 g kg⁻¹ OM. They concluded that there were no differences among genotypes in total herbage accumulation, but responses to grazing management are favored with grazing intensity of 50% removal with the 6-wk regrowth interval.

Castillo et al. (2013) in study with strip planting rhizoma peanut Florigraze into bahiagrass and four practices of defoliation (No defoliation, hay production, simulated continuous, and rotational) reported a range of rhizoma peanut ground cover from 4 to 32% and frequency of plants from 21 to 67% in the year of establishment. The authors concluded that defoliation management is critical during the year of establishment when strip-planting rhizoma peanut into bahiagrass pastures.

Rhizoma peanut in situ disappearance

The in situ procedure can be used to quantify the protein fractions, the instantly degradable Fraction A; the undegradable fraction after an extended rumen incubation, Fraction C; and Fraction B, the slowly degradable fraction, obtained by difference (% Fraction B= 100 - % Fraction A - % Fraction C). Although the technique allows the quantification of the different N fractions of the forage as well as the rate of digestion of B, it cannot be used to quantify the rate at which A is degraded. Nocek (1985) demonstrated that a certain portion of the test feed

escapes the bag prior to ruminal degradation. This fraction is generally assumed to be readily available to rumen microbes and digested at a rapid rate. Potentially degradable nutrient fractions have been described by first-order kinetic rate constants (MERTENS, 1973). Primary assumptions are that the pools in question are homogeneous and that the substrate remaining will be degraded as a linear function of time in the rumen. Ørskov and McDonald (1979) and other researchers have developed mathematical models to fit the estimated ruminal degradability of feedstuffs. The most common approach included the use of non-linear regression. Mertens and Loften (1980) proposed the inclusion of lag time for protein degradation in the models used for fitting N disappearance. If a delay appears at the beginning of the disappearance of Fraction B, a lag component should be included in the model (MCDONALD, 1981).

Rhizoma peanut has greater nutritive value than most warm-season grasses and greater proportion of bypass protein, which results in efficient N utilization by ruminants (ROMERO et al., 1987). Foster et al. (2011) evaluated the in situ disappearance kinetics of annual peanut [*Arachis hypogaea* (L.) FL MDR 98] and Florigraze rhizoma peanut silage. The pre-ensiled DM disappearance for annual and rhizoma peanut readily degradable fraction plus potentially degradable values were 809 and 799 g kg⁻¹ DM. After ensiling, DM disappearance readily degradable fraction plus potentially degradable value were 777 and 816 g kg⁻¹ DM. There were no differences between annual and perennial peanut and pre- and post-ensiling.

Romero et al. (1987) tested the Florigraze in situ DM disappearance at different regrowth intervals. There was a significant interaction between plant part and regrowth interval. The leaf and stem disappearance at 6-wk regrowth interval were 653 and 516 g kg⁻¹ DM, and 561 and 487 g kg⁻¹ DM at 12-wk regrowth interval. The leaves had a greater DM disappearance in the early stage of maturity and the leaves had greater DM disappearance. Teixeira et al. (2010) evaluated the chemical composition of leaf and stem of pinto peanut and reported that stem had greater cellulose and lignin concentration than leaf.

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CHAPTER 2

Methods of Establishment of Pinto Peanut as Monoculture or Pinto Peanut-Palisadegrass Mixed Swards

ABSTRACT

Pinto peanut (*Arachis pinto* Krapovickas and Gregory) is a warm-season perennial legume that has been extensively used in grass-legume mixtures; however, management practices to successfully establish pinto into warm-season grass swards have not been fully examined. Two experiments were conducted to evaluate different methods of establishment of pinto peanut or pinto peanut-palisadegrass [*Urochloa brizantha* (A. Rich.) R.D. Webster] mixed swards. The establishment experiment evaluated pinto peanut and palisadegrass established as monocultures or in a pinto peanut-palisadegrass mixture. Pinto peanut ground cover and density was greater for pinto peanut than pinto peanut-palisadegrass mixed swards (29.4 vs. 7.8%, and 41.8 vs. 19.0 plants m⁻² for ground cover and density, respectively). Concomitant seeding of pinto peanut and palisadegrass showed no negative effect on palisadegrass herbage accumulation (HA). In the overseeding experiment, treatments were pinto peanut seeded into glyphosate treated rows followed by prepared seedbed, pinto peanut seeded into glyphosate treated rows with no seedbed preparation (no-till), or undisturbed palisadegrass monoculture swards. Prepared seedbed and no-till treatments had similar pinto ground cover (2.9%), density (8.7 plants m⁻²), spread (11.7 cm), and HA (170 kg ha⁻¹ yr⁻¹). Overseeding pinto peanut reduced palisadegrass HA and nutritive value. Limited pinto peanut contribution during the early stages after establishment should be considered when making decisions regarding warm-season legume species selection.

INTRODUCTION

Brazil has the largest commercial cattle inventory in world, with an ~ 218.3 million animals (IBGE, 2016). The majority of beef cattle operations in Brazil relies on warm-season perennial grasses as the main source of nutrients for animals. Marandu palisadegrass [*Urochloa brizantha* (A. Rich.) R.D. Webster], one of the predominant warm-season grass species in Brazil, is primarily used in extensive grazing systems that are subjected to relatively low levels of inputs such as commercial fertilizer and liming (MILES et al., 2004). Although most warm-season grass species can thrive under limited nutrient inputs, continued lack of fertilization and proper soil fertility management often results in poor forage production and subsequent pasture degradation (BODDEY et al., 2004). Adequate N fertilization is particularly important to sustain adequate biomass growth and nutritive value to ruminants (SOLLENBERGER et al., 2009). Nitrogen is also responsible for maintaining the integrity and longevity of warm-season grass swards in tropical regions.

Incorporation of warm-season perennial legumes into warm-season grass swards has been suggested as an effective management strategy to minimize farmers dependence on commercial N fertilizer (SALES et al., 2010) and also an effective agronomic practice to improve forage production, nutritive value, and persistence (SANTOS et al., 2002; SHELTON et al., 2005; MUIR et al., 2011).

Symbiotically fixed atmospheric N₂ by the association between specific bacteria (*Rhizobium*) and legumes can be transferred to forage grass species through different pathways such as exudation and leakage of N from roots and nodules, senescence and degradation of nodules and roots, direct transfer of N from legume to non-legume roots, contributions by arbuscular mycorrhizal fungal hyphae, incorporation of legume derived N into the soil by leaching or decomposition of surface litter, and re-deposition of consumed N by livestock (VENDRAMINI et al., 2014).

Despite the vast body of literature indicating the benefits associated with legumes in mixed swards, widespread utilization of tropical legumes worldwide is still scarce. Globally, most success of legumes in mixed pastures have been achieved in Asia and Australia, and, to a lesser extent, the USA and Brazil (SHELTON et al., 2005). Factors contributing to the relative poor adoption of tropical legumes are generally associated with the lack of persistence, vigor, and longevity in response to cutting or grazing systems, lack of establishment and propagation, and high seed production (MUIR et al., 2011).

Because of its superior nutritive value, persistence, soil cover, tolerance to shading (BARCELLOS et al., 2008), and fast establishment (VALENTIM et al., 2003), pintoi peanut

represents a potential viable candidate to be used in mixed stands in tropical regions. Research conducted in northern Brazil demonstrated that Amarillo pinto peanut exhibited satisfactory HA ($2639 \text{ kg DM ha}^{-1} \text{ yr}^{-1}$) and crude protein (CP) concentrations (198 g kg^{-1}) 120 d after establishment (VALENTIM et al., 2003). However, inter-species competition between C_4 grasses and C_3 legumes during the establishing period can negatively impact the success of grass-legume mixed stands (DUNAVIN, 1992). Research has demonstrated that strip-planting rhizoma peanut (*Arachis glabrata* Benth.) into grass swards has been shown to be an effective management practice to establish legume into warm-season grass swards in subtropical conditions (CASTILLO et al., 2013; MULLENIX et al., 2014). However, limited information is available relative to the most appropriate method to establish pinto peanut into grass pastures. While rhizoma peanut does not produce viable seeds and it is mainly propagated by rhizomes, pinto peanut is propagated by seeds and it is expected that it would have a faster germination and establishment.

The hypothesis was that palisadegrass has faster establishment and growth than pinto peanut and would decrease pinto peanut frequency, cover, and HA due to competition. Therefore, the objectives of this study were: 1) to evaluate the performance of pinto peanut and palisadegrass established either as monocultures or as grass-legume mixed sward; and 2) to investigate how different methods of establishment into palisadegrass pastures impacts pinto peanut performance in northern Brazil.

MATERIALS AND METHODS

Experimental site

Two experiments (herein referred as Establishment and Overseeding) were conducted at the Federal University of Tocantins, Araguaina, Brazil ($07^\circ 5'S$; $48^\circ 12'W$) from December 2014 to May 2015 and December 2015 to May 2016. Predominant soil order was Entisol (psamments, Quartzipsamments) (USDA-NRCS, 2014). Initial soil characterization (0 to 15 cm depth) indicated that mean soil pH in distilled H_2O was 5.7 and Mehlich-1 extractable P, K, Mg, and Ca concentrations were 2, 4, 125, and 260 mg kg^{-1} , respectively. Monthly rainfall during the 2014, 2015 and 2016 calendar years and the 28-yr average for this location are shown (Table 2-1).

Establishment experiment

Treatments consisted of Marandu palisadegrass or Amarillo pinto peanut seeded as monoculture (seeding rate of 8 and 10 kg ha^{-1} , respectively), or pinto peanut-palisadegrass mixed swards with the same seeding rates. Treatments were arranged in a randomized complete

block design with four replications, totaling 12 experimental units. Plots were 5 x 4 m with a 1-m alley. In November 2014 and 2015, existent signalgrass [*Urochloa decumbens* (Stapf) R. D. Webster] was sprayed with glyphosate [N- (phosphomethyl) glycine] at a level of 1.44 kg ha⁻¹, and the seedbed disked until there was no remaining vegetation on the soil surface. Dolomitic lime (1 Mg ha⁻¹) was applied after seedbed preparation to raise the soil pH to the desirable level of 6.0. Twenty eight days after lime application, palisadegrass and pinto peanut were seeded either alone or in a mixture. Palisadegrass seeds were manually broadcasted in the soil surface and incorporated with a rake to ensure proper seed contact with the soil. Pinto peanut seeds were manually placed on soil rows (2-cm deep, rows spaced 50-cm apart). Because limited information is available on the specific inoculant for pinto peanut, seeds were not inoculated (MIRANDA et al., 2016). At seeding, all plots received 26 kg of P ha⁻¹, followed by an application of 30 kg N ha⁻¹ and 50 kg K ha⁻¹ 2-wk after germination (ANDRADE et al., 2014).

In both experiments, the pinto peanut did not persist after the dry season. The experimental areas described above were established in different areas in 2014 and 2015.

Response variables

Response variables were evaluated every 28-d (02 February 2015 to 25 May 2015 and 02 March 2016 and 09 June 2016), with four evaluations in 2015 and three in 2016. Lack of rainfall negatively impacted establishment in 2015-2016 and, consequently, the first evaluation in 2016 was delayed because the palisadegrass did not reach the 20-cm target height for initial staging until March 2016.

Pinto peanut ground cover, density, and spread

Pinto peanut ground cover was estimated visually every 28-d. A 1-m² (1 x 1 m) quadrat was placed in the center of the pinto peanut rows at two permanent marked locations in each plot. The quadrat was divided into 100, 10 by 10 cm squares and the proportion of area covered by pinto peanut estimated by two observers and the average of two observers and two locations per evaluation event was reported.

Density was defined as the number of pinto peanut plants per unit of area. The density was determined on the same quadrats used for ground cover estimates. Pinto peanut density was estimated 28-d after seeding and pinto peanut spread at the termination of the trials in 2015 and 2016. Spread was estimated as the distance from the center of the pinto peanut planted strip to the farthest location where pinto aboveground biomass was identified. Mean value across six measurements of spreading per experimental unit was recorded.

Pintoï peanut HA

At the end of the experimental period, May 2015 and June 2016, pintoï peanut HA was estimated by clipping two random 1-m² quadrats in each experimental unit at ground level. Above-ground biomass was dried at 55°C until constant weight.

Palisadegrass herbage characteristics

Mean undisturbed palisadegrass sward height was estimated from five measurements per plot before the harvest with a calibrated yardstick. Herbage accumulation of palisadegrass was estimated by clipping 10 random 1 x 0.5 m (0.5 m²) quadrats at 0.2-m stubble height every 28-d. A subsample was manually separated in leaf and stem and the leaf:stem proportion calculated. An additional subsample was used for leaf area index (LAI) determination using a destructive method. A portion of the leaf was cut in 10-cm segments and the sum of the average width of all segments multiplied by 10 cm. A total of 100 segments were measured for determination of specific leaf area and the leaf total weight was used to estimate the leaf area referent to the quadrat area (0.5 m²). The LAI (m² m⁻²) was obtained by dividing the estimated leaf area by 0.5 m² (ALEXANDRINO et al., 2005).

Palisadegrass and pintoï peanut nutritive value

Pintoï peanut was only harvested at the termination of the experimental periods (May 2015 and June 2016) and palisadegrass harvested every 28-d from February to May, 2015 and March to June 2016. Samples were ground in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ) to pass a 1-mm stainless steel screen. Palisadegrass samples were analyzed for in vitro digestible organic matter (IVDOM) using the two-stage technique described by Tilley and Terry (1963) and modified by Moore and Mott (1974). Pintoï peanut and palisadegrass total N was determined by dry combustion using a Flash EA 1112-NC elemental analyzer (CE Elantech, Lakewood, NJ). Crude protein was calculated by multiplying N concentration by 6.25.

Overseeding experiment

The experimental period was from November 2014 to May 2015, and from November 2015 to May 2016. Treatments consisted of different methods of pintoï peanut establishment into established Marandu palisadegrass plots. Treatments were: 1) establishment of Amarillo pintoï peanut into glyphosate-treated rows followed by prepared seedbed, 2) establishment of pintoï peanut in glyphosate treated rows with no seedbed preparation (no-till), or 3) intact plots of palisadegrass with no pintoï peanut establishment (control). Treatments were distributed in a randomized complete block design with four replicates.

Plots were 5 x 4 m and rows with 0.4-m width and 5-m length with 2-m between rows were sprayed with glyphosate at the level of 1.44 kg ha⁻¹ in the plots subjected to the overseeding treatments in November 2014 and 2015. Three weeks later, glyphosate treated rows subjected to the prepared seedbed treatment were disked until there was no remaining vegetation on the soil surface. Palisadegrass was staged at 0.2 m stubble height and the prepared seedbed and no-till treatments were overseeded with pintoi peanut with a seeding rate of 10 kg ha⁻¹. Seeds were manually placed at 2-cm soil depth and covered with soil. Similarly to experiment 1, seeds were not inoculated. A fertilization of 26 kg of P ha⁻¹ was applied at the time of seeding and 30 kg N of ha⁻¹ and 50 kg of K ha⁻¹ 2 weeks after germination.

Response variables

Response variables were evaluated every 28-d (from 16 January 2015 to 08 May 2015 and 16 January 2016 to 02 May 2016), with four evaluations per year. Response variables evaluated in the overseeding experiment were pintoi peanut ground cover, density, spread, HA, and nutritive value, as described for the establishment experiment. In addition biological N fixation (BNF) was analyzed in the overseeding experiment.

Pintoi peanut biological N₂ fixation

Pintoi peanut above-ground biomass and reference plants of Mombaça guineagrass [*Megathyrsus maximus* (Jacq.) B.K. Simon and S.W.L. Jacobs] were simultaneously collected from the same area and analyzed for total N and $\delta^{15}\text{N}$ concentrations using an isotopic ratio mass spectrometer (Isoprime 100TM, Isoprime, UK) interfaced in continuous flow with an elemental analyzer (vario MICRO cubeTM, Elementar). Isotope ratio N were reported conventionally in per mil (‰) using standard delta (δ) natural abundance. The contribution of BNF to pintoi peanut is calculated from the ¹⁵N abundance and a companion non-N₂-fixing reference plant as indicated by the following equation by Shearer and Kohl (1986):

$$\%N_{dfa} = \left(\frac{\delta^{15}\text{N}_{ref} - \delta^{15}\text{N}_{pintoi\ peanut}}{\delta^{15}\text{N}_{ref} - B} \right) \times 100$$

Where $\delta^{15}\text{N}_{ref}$ represents the level of $\delta^{15}\text{N}$ detected in a reference plant growing in the same soil at the same time as the pintoi peanut; $\delta^{15}\text{N}_{pintoi\ peaunt}$ is the $\delta^{15}\text{N}$ abundance of the legume, and B : is the $\delta^{15}\text{N}$ of the pintoi peanut obtained from N₂ fixation. The B value of *Arachis hypogaea* uninoculated shoot of -2.27 was utilized (OKITO et al., 2004).

Biological N fixation was calculated by multiplying total N and the proportion derived from N₂ fixation (N_{dfa}) (UNKOVICH et al., 2008).

Statistical Analysis

Responses variable were pintoï peanut ground cover, density, spread, HA, CP; and palisadegrass HA, LAI, herbage height, leaf:stem ratio, tiller density, CP, and IVDOM. The BNF was analyzed only for the overseeding experiment. The data were analyzed using PROC MIXED of SAS (SAS Institute, 1996), with treatments and months as fixed effects and blocks and years as random effects. Months were analyzed as repeated measurements using the covariant structure that resulted in the least Akaike value. Means reported statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996).

RESULTS AND DISCUSSION

Establishment experiment

Pintoï peanut established as monoculture resulted in greater ground cover and density than pintoï peanut-palisadegrass mixed treatment (Table 2-2). There were no treatment \times month interaction effects on pintoï peanut ground cover; however, averaged across all treatments, pintoï peanut ground cover increased from February to May, but did not differ from March and April, and April and May (Table 2-3). Results demonstrated that the competition with the palisadegrass decreased the ground cover of pintoï peanut, primarily due to the more efficient C fixation pathway of palisadegrass (C_4) than pintoï peanut (C_3). Euclides et al. (1998) observed a decrease in the proportion of calopo (*Calopogonium mucunoides* Desv.) in pastures overseeded with palisadegrass and signalgrass due to the greater HA and ground cover of warm-season grasses than the legume.

Overseeding pintoï peanut into grass-legume mixed swards at seeding rates evaluated in the current study showed no effect on palisadegrass HA (1,144 kg DM ha⁻¹; $P = 0.65$; SE = 132.7), LAI (1.94 m² m⁻²; $P = 0.59$; SE = 0.23), tiller density (579 tillers m⁻²; $P = 0.72$; SE = 38.8), leaf:stem ratio (0.91; $P = 0.61$; SE = 0.015), CP (110 g kg⁻¹; $P = 0.95$; SE = 3.0) and IVDOM (634 g kg⁻¹; $P = 0.87$; SE = 6.3) compared to palisegrass monoculture swards. Relatively greater tiller density and faster growth and development of palisadegrass than pintoï peanut, likely due to the more efficient carbon fixation pathway (C_4), resulted in more competitive plants that were not negatively affected by the presence of pintoï peanut in mixed stands. In addition, reduced HA and short growth habit of pintoï peanut likely decreased the ability of the peanut to compete with palisadegrass. This hypothesis is further supported by Cecato et al. (2011) who observed similar Coastcross bermudagrass [*Cynodon dactylon* (L.) Pers] HA as monoculture or in mixed pintoï peanut-bermudagrass swards mainly due to the predominance of bermudagrass in the mixture. Results from previous studies indicated that

differences in grass and legume morphology impacted the HA and tiller density of both species in grass-legume mixed swards. Vendramini et al. (2013) observed that bahiagrass (*Paspalum notatum* Flugge) pastures overseeded with stylo [*Stylosanthes guianensis* (Aublet.) Sw] had similar total (bahiagrass + stylo) herbage mass of non-overseeded pastures (bahiagrass only); however, the stylo was 17% of the total herbage mass, indicating that bahiagrass herbage mass decreased when legume was present in the mix. However, in the current study, pinto peanut showed no effect on HA and nutritive value of palisadegrass.

Previous research has demonstrated that warm-season grass-legume mixed swards may have greater CP concentration than warm-season grass monocultures due to legume BNF (ZIECH et al., 2015); however, this response is often associated to grazed systems where animal excreta plays an important role on nutrient recycling (VENDRAMINI et al., 2014). Results from the current study demonstrated no impact of pinto peanut on palisadegrass CP concentrations, primarily due to the absence of grazing and, consequently, limited nutrient recycling. In addition, limited pinto peanut growth and BNF also contributed to the lack of response of warm-season grass under mixed swards.

There was a month effect on palisadegrass HA, LAI, and tiller density and these response variables increased from February to May in both treatments (Table 2-3). Conversely, leaf:stem ratio decreased from 0.95 to 0.89 from February to May, but did not differ from March to May (Table 2-4). Herbage accumulation, LAI and tiller density was expected to increase as the establishment period progressed. In addition, favorable climatic conditions likely promoted HA, and LAI from February to April, while no difference were observed in April and May due to limited rainfall. Leaf:stem ratio decreased from February to March but remained similar from March to May. Crude protein decreased from February to March and did not change in March, April, and May. Conversely, IVDOM remained relatively constant during both growing seasons. The nutritive value of pinto peanut reported in the current study was sufficient to meet the energy nutritional requirements of most beef cattle categories (NRC, 1996).

Overseeding experiment

There was no effect of methods of establishment on pinto ground cover (2.9%; $P = 0.59$; SE = 0.79), density (8.7 plants m^{-2} ; $P = 0.81$; SE = 2.04), spread (11.7 cm; $P = 0.55$; SE = 2.07), HA (170 kg ha^{-1} ; $P = 0.79$; SE = 63.5), and pinto:palisadegrass proportion (14%; $P = 0.57$; SE = 5.01). Results demonstrated that pinto peanut establishment was similar between no-till and prepared seedbed treatments. Glyphosate treatment followed by no-till was an effective method to suppress palisadegrass competition with pinto peanut. Previous studies also indicated that this approach can also increase soil moisture (COSTA et al., 2003; SALES

et al., 2010), and, consequently, promote pintoï peanut HA, ground cover, and spreading compared to other methods of establishment.

Although ground cover and density were expected to increase with time, no temporal changes in pintoï peanut ground cover and plant density were observed during the 4-mo establishment period (2.9%; $P = 0.27$; SE = 0.79 and 8.7 plants m^{-2} ; $P = 0.28$; SE = 2.18). These data contradicted previous work with *Arachis glabrata* Benth. in Florida, that reported significant increases in ground cover and frequency as the experiment progressed (CASTILLO et al., 2013; MULLENIX et al., 2014). The apparent discrepancy in our data may be partially due to the shading effect of palisadegrass, which may have decreased the growth and development of pintoï peanut.

Pintoï peanut overseeded into prepared seedbed had greater CP concentration than no-till treatments (149 $g\ kg^{-1}$ vs. 136 $g\ kg^{-1}$, respectively; $P = 0.005$; SE = 4.7). Soil physical disturbance associated with seedbed cultivation may have resulted in greater N mineralization from the plant residues, thus resulting in greater N availability for pintoï peanut (SILVA et al., 2006).

There was no effect of methods of establishment on pintoï peanut Ndfa (801 $g\ kg^{-1}$; $P = 0.55$; SE = 13.9) and BNF (3.4 $kg\ N\ ha^{-1}$; $P = 0.65$; SE = 1.2). The Ndfa and BNF reported in this study indicated that atmospheric N fixation represented a significant proportion (~ 80%) of the N used for the pintoï peanut growth. Miranda et al. (2003) evaluated the N fixation of pintoï peanut and observed an average of 718 $g\ kg^{-1}$ of Ndfa. However, the overall amount of N fixation in this study was relatively small (3.4 $kg\ ha^{-1}$) due to the limited growth of the pintoï peanut during the establishment period.

There was a treatment \times month effect on palisadegrass HA and LAI (Table 2-4). The interaction occurred because the undisturbed palisadegrass treatment had greater HA and LAI in January and February but there was no difference among treatments in March and April. The suppression of palisadegrass in the rows of the overseeded treatments decreased palisadegrass HA in January and February. Limited palisadegrass HA in March and April was due to unfavorable climate conditions.

Palisadegrass plots overseeded with pintoï peanut had lesser canopy height and leaf:stem ratio and greater CP concentrations than palisadegrass monoculture (Table 2-5). Relatively smaller CP concentrations associated with control treatments was due to greater palisadegrass growth, which resulted in a dilution effect (VENDRAMINI et al., 2013).

Palisadegrass decreased canopy height, CP, and IVDOM from January to April; while leaf:stem ratio was greater in February and April, and decreased in January and March

(Table 2-6). Tiller density did not vary during the experimental period (Table 2-6). Greater CP and IVDOM concentrations in January may be due to the fertilization and greater rainfall during this period (Table 2-1).

SUMMARY AND CONCLUSIONS

Establishment of pinto peanut and palisadegrass as mixed swards decreased pinto peanut plant density and ground cover and resulted in a negative impact on subsequent productivity of pinto peanut-palisadegrass mixed stands. Adjusting seeding rates, fertilization levels, and clipping schedule may be feasible management practices to decrease palisadegrass competition in early stages post-establishment, however further research is needed to validate these management practices.

Any method of suppression of the palisadegrass growth will reduce palisadegrass HA, which, in turn, may also decrease stocking rates and animal productivity. Other methods to suppress competition between palisadegrass and pinto peanut, such as mechanical, warrant further evaluation. Limited pinto peanut HA and BFN during the early stages after establishment should be considered when making decisions regarding warm-season legume species selection.

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Table 2-1. Recorded and 28-yr average total monthly rainfall and maximum and minimum temperatures for 2014, 2015 and 2016.

Month	2014			2015			2016			28-yr average Rainfall (mm)
	Rainfall (mm)	Temp. Max. °C	Temp. Min. °C	Rainfall (mm)	Temp. Max. °C	Temp. Min. °C	Rainfall (mm)	Temp. Max. °C	Temp. Min. °C	
Jan	199	30.3	20.9	145	30.6	19.7	361	29.5	22.9	252
Feb	371	30.2	21.3	266	30.4	20.3	125	33.5	22.3	262
Mar	280	30.1	21.4	199	31.0	21.9	256	32.0	23.3	281
Apr	230	31.3	21.3	101	31.4	22.6	108	31.8	22.9	212
May	95	31.9	20.7	141	32.1	21.6	56	32.6	21.6	104
Jun	0	33.3	18.3	0	33.3	19.4	89	33.0	19.8	20
Jul	12	33.9	17.6	27	33.4	18.9	0	34.8	17.6	11
Ago	15	35.1	16.7	4	34.8	17.8	4	35.5	18.1	15
Set	26	34.5	19.1	13	35.7	20.6	102	34.8	20.8	53
Oct	162	32.5	19.8	35	35.1	21.9	81	33.7	21.4	125
Nov	317	32.0	20.7	59	33.3	22.6	291	32.3	22.0	213
Dec	286	31.0	20.7	48	32.5	22.1	98	31.6	22.1	206
Total	1992			1039			1570			1754

Table 2-2. Pintoï peanut ground cover, density and spread on pintoï peanut only and pintoï peanut-palisadegrass mixture plots (Establishment experiment).

Response variables	Treatments		SE	<i>P</i> value
	Pintoï peanut	Pintoï peanut-Palisadegrass		
Ground cover, %	29 a [†]	8 b	3.6	0.001
Density, plants m ⁻²	42 a	19 b	5.4	0.002
Spread, cm	32.4	24.0	4.3	0.08

[†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Data are means across four replicates, two years, and four harvests ($n = 32$).

Table 2-3. Seasonal variation in herbage characteristics of palisadegrass and pintoï peanut cultivated as grass monoculture or pintoï peanut-palisadegrass mixed swards (Establishment experiment).

Response variables	Month				SE	P value
	February	March	April	May		
<i>Palisadegrass</i>						
Herbage Accumulation, kg ha ⁻¹	839 b [†]	981 b	1421 a	1337 a	149	0.001
Herbage height, cm	25 ab	28 a	27 a	23 b	1.7	0.03
Leaf:stem ratio	0.95 a	0.90 b	0.89 b	0.89 b	0.02	0.001
Leaf area index, m ² m ⁻²	1.5 b	1.7 b	2.5 a	2.1 a	0.3	0.001
Tiller density, tiller m ⁻²	501 b	515 b	626 a	674 a	45	0.002
CP, g kg ⁻¹	95 b	111 a	116 a	118 a	4	0.001
IVOMD, g kg ⁻¹	625	638	643	630	11	0.67
<i>Pintoï Peanut</i>						
Ground cover, %	14 c [†]	17 bc	20 ab	23 a	3	0.005

[†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Data are means across two years, two treatments, and four replicates (n = 16).

Table 2-4. Treatment × month interaction effects on herbage accumulation and leaf area index of palisadegrass (Overseeding experiment).

Month	Treatments			SE	P value
	Undisturbed Sward	Prepared seedbed	No-till		
Herbage Accumulation, kg ha ⁻¹					
January	2710 Aa [†]	1749 Ba	1912 Ba		
February	2184 Ab	1791 Ba	1797 Ba	187	0.04
March	1421 Ac	1210 Ab	1185 Ab		
April	1113 Ac	1182 Ab	1178 Ab		
Total	7428	5932	6072		
Leaf area index, m ² m ⁻²					
January	4.7 Aa	3.0 Ba	3.2 Ba		
February	3.4 Ab	2.8 Aba	2.5 Ba	0.03	0.001
March	1.9 Ac	1.8 Ab	1.7 Ab		
April	1.6 Ac	1.8 Ab	1.7 Ab		

[†]Means within row followed by the different letter uppercase and within column by the different letter lowercase are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Data are means across two years and four replicates (n = 8).

Table 2-5. Herbage characteristics of palisadegrass as monoculture (control) or subjected to different methods of pintoï peanut overseeding (Overseeding experiment).

Response variables	Treatments			SE	P value
	Undisturbed Sward	Prepared seedbed	No-till		
Canopy height, cm	32 a [†]	31 b	31 b	0.57	0.03
Leaf:stem ratio	0.84 a	0.81 b	0.80 b	0.015	0.003
Tiller density, tiller m ⁻²	826	715	718	71.3	0.11
CP, g kg ⁻¹	97 b	102 a	103 a	1.96	0.04
IVDOM, g kg ⁻¹	597	607	607	5.49	0.19

[†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Data are means across two years, four replicates, and four harvests (n=32).

Table 2-6. Seasonal variation in herbage characteristics of palisadegrass as monoculture or subjected to different methods of pintoï peanut overseeding (Overseeding experiment).

Response variables	Month				SE	P value
	January	February	March	April		
Canopy height, cm	38 a [†]	35 b	27 c	25 c	0.9	0.001
Leaf:stem ratio	0.79 b	0.84 a	0.81 b	0.84 a	0.01	0.001
Tiller density, tiller m ⁻²	842	774	676	719	84.1	0.25
CP, g kg ⁻¹	117 a	92 c	92 c	101 b	4	0.001
IVDOM, g kg ⁻¹	637 a	585 b	584 b	608 b	8	0.001

[†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Data are means across two years, three treatments, and four replicates (n = 24).

CHAPTER 3

Methods of establishment of rhizoma peanut into bahiagrass pastures

ABSTRACT

Rhizoma peanut (*Arachis glabrata* Benth.) is a warm-season legume adapted to the southern USA; however, methods of establishment of rhizoma peanut have not been fully examined in this region. Florigraze is the most commonly cultivated rhizoma peanut genotype but recent studies have demonstrated that Ecoturf has superior forage characteristics. The objectives of this study were to evaluate the impacts of different establishment methods of Florigraze and Ecoturf rhizoma peanut into bahiagrass (*Paspalum notatum* Flüggé) swards. Treatments were two genotypes of rhizoma peanut (Florigraze or Ecoturf) and two establishment methods (no-till and prepared seedbed) distributed in a randomized complete design with four replicates. Ecoturf and Florigraze had similar ground cover (12.2%), canopy density (17.5 plants m⁻¹), height (3.9 cm), herbage accumulation (HA) (558 kg ha⁻¹), and rhizome-root mass (9.4 Mg ha⁻¹) at the end of the 120-d period. Prepared seedbed and no-till treatments had similar rhizoma peanut soil cover (49%), N concentration (22.0 g kg⁻¹), Atmospheric nitrogen fixation (Ndfa) (364 g kg⁻¹), BNF (5.8 kg N ha⁻¹). However, rhizoma peanut ground cover (14.9 vs. 9.4%) and HA (701 vs. 414 kg ha⁻¹) was greater for the prepared seedbed than no-till treatment. Ecoturf and Florigraze did not differ for ground cover and HA. The additional cost of preparing seedbed after glyphosate application may be justified by rhizoma peanut greater ground cover and herbage accumulation.

INTRODUCTION

Grasslands in the southeastern USA are generally characterized by extensive grazing systems with warm-season perennial grasses. These grazing systems are characterized by the reduced use of commercial fertilizer; however, to maintain sustainable forage production and nutritive value, warm-season grass monocultures normally require some level of N fertilization (SOLLENBERGER et al., 2009). Intercropping warm-season perennial legumes into warm-season perennial grass pastures is a management practice to supply N to warm-season perennial grass systems. Legumes provide greater soil coverage and supply of biologically fixed N. Legumes can biologically fix N due to symbiosis with specific bacteria, which can increase forage production, nutritive value, and persistence (SHELTON et al., 2005; SALES et al., 2010; MUIR et al., 2011).

Rhizoma peanut (*Arachis glabrata* Benth.) is a warm-season legume adapted to the southern USA and has been an attractive forage due to superior herbage accumulation, ground cover, and persistence (PRINE et al., 2010; QUESENBERRY et al., 2010; MULLENIX et al., 2016a). Florigraze was released in 1978 and it is still the most cultivated rhizoma peanut cultivar in the southern USA (PRINE et al., 1986; QUESENBERRY et al., 2010). Florigraze has a genetic vulnerability to peanut stunt virus in commercial fields, which may negatively affect forage production and stand longevity (BLOUNT et al., 2006). Ecoturf is a rhizoma peanut genotype that has shown potential to be used as alternative Florigraze monocultures. It was observed that Ecoturf can have similar herbage accumulation, and greater crude protein and leaf mass than Florigraze (PRINE et al., 2010; MULLENIX et al., 2016a,b).

Mullenix et al. (2016a,b) compared four rhizoma peanut genotypes and four rotational stocking strategies and reported average herbage accumulation of 7.5 Mg DM ha⁻¹ yr⁻¹, and reported that there were no differences among genotypes in total herbage accumulation, but responses to grazing management are favored with grazing intensity of 50% removal with the 6-wk regrowth interval.

Castillo et al. (2013) in study with strip planting rhizoma peanut Florigraze into bahiagrass and four practices of defoliation (No defoliation, hay production, simulated continuous, and rotational) reported a range of rhizoma peanut ground cover from 4 to 32% and frequency of plants from 21 to 67% in the year of establishment. The authors concluded that defoliation management is critical during the year of establishment when strip-planting rhizoma peanut into bahiagrass pastures.

Technology for the establishment of strip planting has been developed recently for increasing the contribution of rhizoma peanut into bahiagrass pastures (CASTILLO et al., 2013;

MULLENIX et al., 2014). Strip planting reduces the competition of the warm-season grass with the warm-season legume and increases the chances of obtaining a successful rhizoma peanut establishment. However, there is limited information in the literature about cultural practices to plant rhizoma peanut in strips into established bahiagrass pastures.

The hypothesis was that rhizoma peanut establishment into strips in existent bahiagrass pastures increases the chances of a successful rhizoma peanut establishment. Ecoturf and Florigraze have similar productivity and ground cover. The objective of this research was to evaluate establishment methods of rhizoma peanut into established bahiagrass (*Paspalum notatum* Flügge) pastures in Florida.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in Wauchula, FL (27° 29N latitude, 81° 49W longitude) from May to November of 2014 and 2015. Soil at the research site was a Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod) (USDA-NRCS, 2014). Before initiation of the experiment, mean soil pH in distilled H₂O was 5.1, and Mehlich-1 extractable P, K, Mg, and Ca concentration in the 0-15 cm depth were 51, 34, 72, and 756 mg kg⁻¹, respectively. Monthly rainfall during the 2014 and 2015 calendar years and the 18-yr average for this location are shown (Table 3-1).

Treatments and design

Treatments were the factorial combination of two rhizoma peanut genotypes (Florigraze or Ecoturf) and two establishment methods (prepared seedbed or no-till), distributed in a randomized complete block design with four replicates.

The experiment was established in an existent Pensacola bahiagrass pasture. The rhizoma peanut was planted in 2.5-m strips. Plots (experimental units) had 3 strips of peanut and 3.5 strips of bahiagrass between plots, resulting in an area of 130.5 m² (14.5 m width x 9 m long) (Figure 3-1).

In early May 2014, the strips were sprayed with 11.7 L ha⁻¹ of glyphosate (N-phosphonomethyl glycine) and 1 Mg ha⁻¹ of dolomitic lime and 66 kg of K ha⁻¹ were broadcasted on the soil surface. In July 2014, plots in a prepared seedbed treatment were disked with a tandem disk until there was no remaining grass on the soil surface and planted with a sprig planter (Bermuda King, Ringwood, OK). Plots in the no-till treatment were planted on the same day with no disturbance of the soil. Rhizomes were planted at a rate of 1200 kg ha⁻¹ to an approximately 5-cm depth. The strips were 2.5-m wide and accommodated four rows of

rhizoma peanut, with spacing between rows of 0.5 m. The planted strips were bounded on both sides by a strip of undisturbed bahiagrass sod.

Response variables were evaluated every 28-d (from 20 August 2014 to 11 November 2014 and 11 June 2015 to 02 November 2015), with three evaluations in the first year and five evaluations in the second year.

Response variables

Soil cover and rhizoma peanut ground cover

Soil cover and rhizoma peanut ground cover was estimated visually every 28-d. A 1 m² (1 x 1 m) quadrat was placed in the center of the rhizoma peanut rows at three permanent marked locations in each plot. The quadrat was divided into 100, 10 by 10 cm squares and the proportion of area covered and covered by rhizoma peanut estimated by two observers. The average of two observers and three locations per evaluation event was reported.

Rhizoma peanut density and canopy height

Rhizoma peanut density was defined as the number of rhizoma peanut plants per unit of area. An average of six measurements of density per experimental unit was reported. Canopy height was measured in five rhizoma peanut heights per row and repeated in three rows, with 15 measurements per strip.

Herbage accumulation, leaf:stem ratio and rhizome-root mass

The rhizoma peanut harvest occurred on November 2014 and 2015 with 4 mo regrowth interval. Three 0.25-m² rings were harvest per plot at 3-cm stubble height. The samples were dried at 55°C until constant weight for determination of the dry matter. After dried, samples were separated into leaf (leaflet) and stem (stem + sheath + petiole) for determination of leaf:stem ratio.

Rhizoma peanut root mass was collected on the same dates of harvested, using auger with an area of 0.0017 m³. The auger was placed in the center of the rhizoma peanut rows, and three samples per plot were collected, at 0-20 cm depth. Roots were washed in water, sieved (2-mm mesh), and oven dried at 55°C until constant weight for determination of the dry matter.

Rhizoma peanut N₂ fixation

Rhizoma peanut and common bermudagrass [*Cynodon dactylon* (L.) Pers] (reference plant) samples were collected from the same experimental area. Samples were dried at 55°C until constant weight, and ground thereafter using a Wiley Mill (Thomas-Wiley Laboratory Mill, Thomas Scientific) to pass through a 1-mm stainless steel screen and subsequently pulverized using ball milled (Mixer Mill MM 400 – Retsch) at 25 Hz for 9 min. Samples were analyzed for total N and $\delta^{15}\text{N}$ concentration using an isotopic ratio mass

spectrometer (Isoprime 100™, Isoprime, UK) interfaced in continuous flow with an elemental analyzer (vario MICRO cube™, Elementar). Nitrogen elemental composition was reported as the proportion of the DM of the sample and isotope ratio N were reported conventionally in per mil (‰) using standard delta (δ) natural abundance. The contribution of biological N fixation (BNF) to rhizoma peanut (%Ndfa) was calculated from the ^{15}N abundance and a companion non- N_2 -fixing reference plant as indicated by the following equation by Shearer and Kohl (1986):

$$\%Ndfa = \left(\frac{\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{rhizoma peanut}}}{\delta^{15}\text{N}_{\text{reference}} - B} \right) \times 100$$

Where $\delta^{15}\text{N}_{\text{ref}}$ represents the level of $\delta^{15}\text{N}$ detected in a reference plant growing in the same soil at the same time as the rhizoma peanut; $\delta^{15}\text{N}_{\text{rhizoma peanut}}$ is the $\delta^{15}\text{N}$ abundance of the legume, and B : is the $\delta^{15}\text{N}$ of the plant grown obtained from N_2 fixation with absence of inorganic N. The B value of *Arachis hypogaea* of -1.41 was utilized (OKITO et al., 2004). The BNF was calculated by multiplying total plant N (N from the total on dry matter basis) by the proportion derived from N_2 fixation (Ndfa) (UNKOVICH et al., 2008).

Statistical Analysis

Data of model residuals were used to check normality, and in the case of non-normal distributions, data transformations were used. Square root transformation was used for rhizoma peanut ground cover data. Logarithmic transformation was used for rhizoma peanut cover and height data. The non-transformed data was presented in the tables, with significance obtained from transformed data.

Reponses variable were rhizoma peanut ground cover, density, HA, canopy height, N, Ndfa, BFN and root mass. The data were analyzed using PROC MIXED of SAS (SAS Institute, 1996), with treatments and months as fixed effects and blocks and years as random effects. Year was evaluated as fixed effect for the root mass response variable only. Months were analyzed as repeated measurements using the covariant structure that resulted in the least Akaike value. Interactions not discussed in the Results and Discussion section were not significant ($P > 0.05$). Means reported statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Treatments were considered different when $P \leq 0.05$.

RESULTS AND DISCUSSION

There was a genotype \times month effect on ground cover ($P = 0.012$) (Table 3-2). This interaction occurred because Ecoturf had greater soil cover in September than Florigraze, but there was no difference among treatments in June, July, August, and October. Soil cover was lesser in June than other months, which may be related to the establishment in 2014 and recover from the cool-season in 2015. Ecoturf was selected as turf with infrequent cutting requirement (PRINE et al., 2010) and it is less sensitive to daylength than Florigraze (WILLIAMS et al., 2008), which may have resulted in greater ground cover in September.

There was no effect of genotypes on rhizoma peanut canopy density (17 plants m^{-2} ; $P = 0.55$; SE = 1.6), height (3.9 cm; $P = 0.25$; SE = 1.0), HA (558 kg DM ha^{-1} ; $P = 0.66$; SE = 126.3), and rhizome-root mass (9.4 Mg DM ha^{-1} ; $P = 0.34$; SE = 0.86). Previous studies reported similar results and observed that Ecoturf had similar HA to Florigraze; however, it was expected that Florigraze would have greater canopy height (PRINE et al., 2010; MULLENIX et al., 2016a, b). The lack of difference in canopy height observed in this study was likely due to slow establishment and reduced HA. Rhizoma peanut is slow to establish and the potential HA is only obtained in the third growing season (PRINE et al., 1986). In addition, most previous studies with rhizome peanut were conducted in North Florida, where the environmental conditions and soil types are different than the southern part of the state. The extreme sandy nature of the soils in south Florida, associated with limited cation-exchange capacity (CEC) and poor drainage during the periods of greater rainfall, likely resulted in limited productivity of rhizoma peanut. In North Florida, Dubeux et al. (2017) reported Florigraze HA of 6835 kg DM ha^{-1} (mean 2-yr). Mullenix et al. (2016a) compared four rhizoma peanut genotypes (Ecoturf, Florigraze, UF Peace, and UF Tito) under four rotational stocking strategies and observed no differences in HA (7498 kg DM ha^{-1}), ground cover (87%) and canopy height (15.7 cm) among cultivars.

Ecoturf had greater leaf to stem ratio (LS, 66 vs. 62%, SE = 1.9, $P = 0.02$) than Florigraze. Mullenix et al. (2016a) reported similar results and also concluded that Ecoturf had greater LS than Florigraze (66 vs 63%) in North Florida. This result was consistent with previous reports that indicated that Ecoturf plants have greater leaf density and smaller stems than other rhizoma peanut cultivars (QUESENBERRY et al., 2010).

There was no effect of genotypes on rhizoma peanut above-ground N concentration (22.0 g kg^{-1} , $P = 0.36$; SE = 0.8), Ndfa (364 g kg^{-1} , $P = 0.24$; SE = 110), BNF (5.8 kg N ha^{-1} , $P = 0.09$; SE = 2.2). Mullenix et al. (2016a) evaluating four rhizoma peanut genotypes reported N concentrations ranging from 26.4 to 33.6 g kg^{-1} . Dubeux et al. (2017) evaluating N fixation

of rhizoma peanut genotypes reported N concentrations of 19.2 to 36.3 g kg⁻¹, with an average of 86% and 202 kg N ha⁻¹ yr⁻¹ of Ndfa and BNF, respectively. The overall N fixation observed in the current study was limited mainly because of the relatively poor growth of the rhizoma peanut during the establishment phase.

Rhizoma peanut ground cover and HA were greater for the prepared seedbed than no-till treatment (Table 3-3). This difference may be attributed to lesser competition between the existent vegetation with the newly established rhizoma peanut and potential nutrients released by the faster decomposition of the sod in the prepared seedbed treatment (SILVA et al., 2006). However, there was no effect of establishment methods on soil cover (49%; $P = 10.49$; SE = 3.9), rhizoma peanut density (17 plants m⁻¹; $P = 0.15$; SE = 1.6), and canopy height (3.9 cm; $P = 0.34$; SE = 1.1). Vendramini et al. (2012) observed that preparing seedbed resulted in better establishment and greater HA of annual ryegrass (*Lolium multiflorum* Lam.) than no-till bahiagrass sod. Castillo et al. (2013) studying the impacts of strip planting Florigraze into bahiagrass sod and different defoliation regimens (no defoliation, hay production, simulated continuous, and rotational) on rhizoma peanut responses reported rhizoma peanut ground cover ranging from 4 to 32%, while and frequency of plants from 21 to 67% during the year of establishment.

There was no effect of establishment methods on rhizoma peanut N concentration (22.0 g kg⁻¹, $P = 0.42$; SE = 0.8), Ndfa (364 g kg⁻¹, $P = 0.20$; SE = 110), and BNF (5.8 kg N ha⁻¹, $P = 0.07$; SE = 2.2). Despite the lack of treatment differences, N concentration, Ndfa, and BNF values indicated that the atmospheric N fixation accounted for a significant proportion of the N present in rhizoma peanut above-ground tissue, especially in the prepared seedbed treatment that resulted in greater rhizoma peanut ground cover and HA.

There was no effect of establishment methods on rhizoma peanut rhizome-root mass in 2014 (12.2 Mg DM ha⁻¹; $P = 0.46$; SE = 1.16) and 2015 (6.6 Mg DM ha⁻¹; $P = 0.55$; SE = 1.14). Greater rhizome-root mass observed in the year of establishment was due to the presence of bahiagrass roots from existent vegetation. Mullenix et al. (2016b) evaluated different genotypes of rhizoma peanut subjected to defoliation intensities of 50 or 75% of the herbage mass and observed a decrease in root mass from 4.3 to 3.4 Mg DM ha⁻¹ in the first and second year after establishment, respectively. The differences in rhizome-root mass observed in the current study and the previous reports were likely due to the methods and year of establishment and defoliation intensity and frequency. In the year of the establishment, the BNF and soil N may not be sufficient for meeting the N demand (CATHEY et al., 2013), it is

necessary the utilized belowground reserves to regenerated leaf area in frequently defoliated swards (MULLENIX et al., 2016b), that can be resulting in the decrease the root mass.

Rhizoma peanut ground cover increased from June to October, while plant density decreased from June to October (Table 3-4). Plant density in the rows decreased due to increased propagation and distribution of rhizoma peanut in the area, which resulted in increased rhizoma peanut ground cover. These data was similar previous work, that reported significant increases in ground cover and frequency as the experiment progressed (CASTILLO et al., 2013; MULLENIX et al., 2014).

CONCLUSIONS

Rhizoma peanut genotype Ecoturf and Florigraze had similar ground cover and HA. Ecoturf has potential to be used as an alternative to Florigraze monoculture due to similar forage characteristics.

Prepared seedbed and no-till resulted in similar rhizome-root mass, N concentration, % of N derived from atmosphere and biological N₂ fixation; however, prepared seedbed had greater ground cover and HA. Despite the relatively greater cost associated with glyphosate application and tillage, prepared seedbed represents a viable option for fast establishment and production of Ecoturf and Florigraze rhizoma peanut in south Florida.

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Table 3-1. Recorded 18-yr average total monthly rainfall and maximum and minimum temperatures for 2014 and 2015 for the experimental location

Month	2014			2015			18-yr average rainfall (mm)
	Rainfall (mm)	Temp. Max. °C	Temp. Min. °C	Rainfall (mm)	Temp. Max. °C	Temp. Min. °C	
Jan	94	28.4	-2.7	41	29.0	4.3	46
Feb	33	30.4	0.8	87	28.7	-2.2	50
Mar	79	29.1	4.3	28	31.3	5.5	65
Apr	28	33.9	8.3	98	32.7	13.2	58
May	149	34.5	10.9	43	35.5	12.9	82
Jun	166	35.1	17.9	228	35.5	18.7	205
Jul	213	34.6	20.4	205	35.1	20.2	177
Aug	95	36.1	20.4	380	36.3	21.3	233
Sep	296	34.5	20.1	114	35.5	21.4	182
Oct	21	33.4	11.1	43	35.9	16.2	53
Nov	106	30.3	4.2	29	34.1	9.5	40
Dec	6	28.5	2.7	53	31.2	6.7	47
Total	1288			1348			1236

Table 3-2. Soil cover (%) as affected by genotype and month.

Month	Genotype		SE	P value
	Ecoturf	Florigraze		
June	18 Ab [†]	21 Ab		
July	48 Aa	49 Aa		
August	55 Aa	56 Aa	12.3	0.012
September	60 Aa	51 Ba		
October	54 Aa	56 Aa		

[†]Means within row followed by the different letter uppercase and within column by the different letter lowercase are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). Data are means across two years, four treatments, and four harvests ($n = 32$).

Table 3-3. Establishing method effects on rhizoma peanut ground cover and herbage accumulation.

Response variables	Treatments		SE	P value
	No-till	Prepared seedbed		
Ground cover, % †	9 b†	15 a	3.9	0.04
Herbage accumulation, kg DM ha ⁻¹	414 b	702 a	126	0.007

†Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). ‡Square root transformation. Non-transformed data reported, with significance obtained from transformed data. Data are means across two years, four replicates, and four harvests (n=32).

Table 3-4. Rhizoma peanut ground cover, density, and height as affected by month.

Response variables	Month					SE	P value
	June	July	August	September	October		
Ground cover, % †	4 c†	13 b	12 b	13 b	19 a	3.9	0.001
Density, plants m ⁻¹ §	26 a	29 a	12 b	12 b	8 c	1.8	0.001
Height, cm §	0.7 c	4.2 ab	4.9 a	5.2 a	4.3 b	1.1	0.001

†Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.05$) (SAS Institute Inc., 1996). ‡Square root transformation; §Logarithmic transformation. Non-transformed data reported, with significance obtained from transformed data. Data are means across two years, four treatments, and four replicates (n = 32).

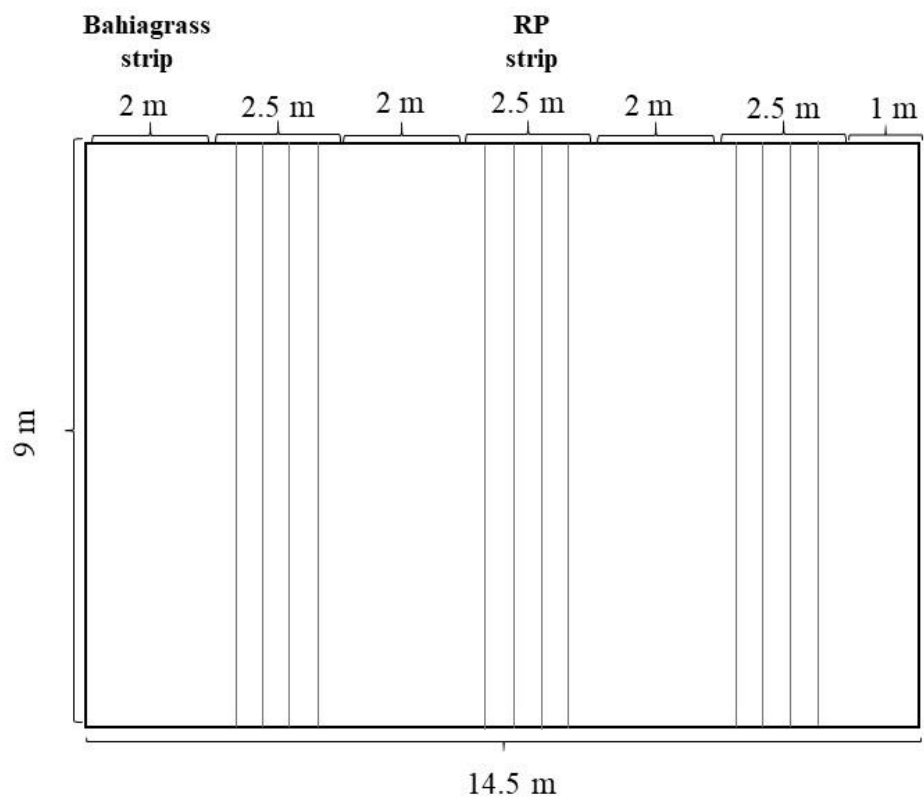


Figure 3-1. Schematic of experimental units. RP: Rhizoma peanut.

CHAPTER 4
Impact of different leaf:stem proportions in DM and CP in situ
disappearance of different rhizoma peanut genotypes

ABSTRACT

Rhizoma peanut (*Arachis glabrata* Benth.) is a warm-season legume adapted to the southern USA. Florigraze is the most cultivated rhizoma peanut; however, recent studies have demonstrated that the genotype Ecoturf has attractive forage characteristics. The objectives of this study were to evaluate the effect of leaf:stem proportion (LS) on dry matter (DM) and crude protein (CP) disappearance of Ecoturf and Florigraze rhizoma peanut. Plots were harvested on November 2014 and 2015 with 4 mo regrowth interval. Treatments were two genotypes of rhizoma peanut (Florigraze or Ecoturf) and three LS (100:0, 50:50 and 0:100) distributed in a randomized complete design with four replicates. Crude protein and DM disappearances were calculated according to Ørskov and McDonald (1979). DM and CP fractions were described as A, rapidly degradable; B, potentially degradable; and C, undegradable. Effective disappearance was estimated by passage rate from 0.02, 0.03, 0.04 and 0.05 h⁻¹. Florigraze had greater DM fraction A than Ecoturf (349 vs. 339 g kg⁻¹) but there was no difference in fractions B and C (mean = 427 and 230 g kg⁻¹, respectively). The LS ratio has a significant impact on DM and CP fractions and effective degradability. There was a linear decrease in DM fractions A and B and decrease in fraction C with increasing LS. Conversely, CP Fraction A and C increased with increasing LS, while fraction B decreased. The LS is an important indicator of nutritive value of rhizoma peanut and the models generated by this study may allow managers to have a more accurate prediction of performance of ruminants consuming rhizoma peanut.

INTRODUCTION

Rhizoma peanut (*Arachis glabrata* Benth.) is a warm-season legume adapted to the southern USA. Rhizoma peanut has been an attractive forage due to its superior herbage accumulation, ground cover, and persistence (PRINE et al., 2010; QUESENBERRY et al., 2010; MULLENIX et al., 2016a). In addition, rhizoma peanut has greater nutritive value than most warm-season grasses and greater proportion of bypass protein, which results in efficient N utilization by ruminants (ROMERO et al., 1987).

Florigraze was released in 1978 and it is still the most cultivated rhizoma peanut cultivar in the southern USA (PRINE et al., 1986; QUESENBERRY et al., 2010). However, recent studies demonstrate that the genotype Ecoturf can have similar herbage accumulation, and greater crude protein and leaf mass than Florigraze (Prine et al., 2010; Mullenix et al., 2016a;b).

Rhizoma peanut has greater nutritive value than most warm-season grasses and greater proportion of bypass protein, which results in efficient N utilization by ruminants (ROMERO et al., 1987). Foster et al. (2011) evaluated the in situ disappearance kinetics of Florigraze rhizoma peanut silage. The pre-ensiled DM disappearance rhizoma peanut readily degradable fraction plus potentially degradable value was 799 g kg⁻¹ DM. After ensiling, DM disappearance readily degradable fraction plus potentially degradable value was 816 g kg⁻¹ DM.

It is known that forage morphology (leaf:stem ratio) has important implications on forage nutritive value (ROMERO et al., 1987; TEIXEIRA et al., 2010). In general, leaves have greater metabolic functions and stem structural functions in the plant, resulting in differences in N concentrations in different plant parts (LEMAIRE et al., 2005). Mullenix et al. (2014 and 2016a;b) observed that there are differences in rhizoma peanut morphology and nutritive value among rhizoma peanut genotypes, while Ecoturf had a decumbent growth habit and shorter canopy height than Florigraze. Although the genotypes had similar leaf:stem ratio (LS), Ecoturf had greater CP concentration than Florigraze (193 vs. 167 g kg⁻¹).

Romero et al. (1987) tested the Florigraze in situ DM disappearance at different regrowth intervals. And reported that the leaves had a greater DM disappearance in the early stage of maturity and the leaves had greater DM disappearance, with leaf and stem disappearance at 6-wk regrowth interval were 653 and 516 g kg⁻¹ DM, respectively. Teixeira et al. (2010) evaluated the chemical composition of leaf and stem of pinto peanut and reported that stem had greater cellulose and lignin concentration than leaf.

During the production of rhizoma peanut hay, the drying for a long time, can be cause the leaves loss, reducing the nutritive value of the hay. Although the differences in

rhizoma peanut ecotypes morphology and CP concentrations have been reported, the impacts of these differences in the ruminal DM and CP degradability are not known. The hypothesis was that differences in canopy architecture and leaf:stem proportion (LS) between Ecoturf and Florigraze has a significant impact on DM and CP disappearance. The objectives of this study were to evaluate the effect of leaf: stem proportion in DM and CP disappearance of Ecoturf and Florigraze rhizoma peanut genotypes and create models for CP and DM disappearance of rhizoma peanut models with different LS proportion.

MATERIAL AND METHODS

Location, establishment, and treatments

The study was conducted in Wauchula, FL (27° 29'N, 81° 49'W) from May to November of 2014 and 2015. Soil at the research site was a Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod) (USDA-NRCS, 2014). Before initiation of the experiment, mean soil pH in distilled H₂O was 5.1, and Mehlich-1 extractable P, K, Mg, and Ca concentration in the 0-15 cm depth were 51, 34, 72, and 756 mg kg⁻¹, respectively.

Treatments were the factorial combination of two rhizoma peanut genotypes (Florigraze and Ecoturf) and three leaf:stem proportion (100, 50, and 0%), distributed in a randomized complete block design with four replicates.

The experiment was established in an existent Pensacola bahiagrass pasture. The rhizoma peanut was planted in 2.5-m strips. Plots (experimental units) had 3 strips of peanut and 3.5 strips of bahiagrass between plots, resulting in an area of 130.5 m² (14.5 m width x 9 m long).

In early May 2014, the area was sprayed with 11.7 L ha⁻¹ of glyphosate (N-phosphonomethyl glycine) and 1 Mg ha⁻¹ of dolomitic lime and 66 kg of K ha⁻¹ were broadcasted on the soil surface. In July 2014, plots were established in a prepared seedbed with a spring planter (Bermuda King, Ringwood, OK). Rhizomes were planted at a level of 1200 kg ha⁻¹ to an approximately 5-cm depth. The strips were 2.5-m wide and accommodated four rows of rhizoma peanut, with spacing between rows of 0.5 m. The planted strips were bounded on both sides by a strip of undisturbed bahiagrass sod.

Rhizoma peanut harvest occurred on November 2014 and 2015 with 4 months regrowth interval. Three 0.25-m² rings were harvest per plot at 3-cm stubble height. The samples were dried at 60°C until constant weight for determination of the dry matter. After dried, samples were separated into leaf (leaflet) and stem (stem + sheath + petiole) for determination of leaf:stem ratio. Leaves and stems were ground separately in a Wiley mill, to

pass a 4-mm screen (VENDRAMINI et al., 2008), and the leaf:stem proportions created in a dry weight basis. The N concentration of initial samples and those from the in situ disappearance procedures were determined by dry combustion using a Flash EA 1112-NC elemental analyzer (CE Elantech, Lakewood, NJ). Crude protein was determined by multiplying N concentration by 6.25.

In situ disappearance procedure

In-situ disappearance procedure was conducted at the UF/IFAS Range Cattle, Ona, FL (27° 23'N, 81° 57'W). Dried and ground forage samples were weighed to 0.5 g and placed into Ankom F57 filter bags (Ankom Technology, Macedon, NY) with pore size of 25 µm and heat sealed using an impulse sealer (model H-190; Uline, Pleasant Prairie, WI). The proportion of weight to the surface area was 20 mg cm⁻². Samples were incubated for 0, 3, 6, 12, 24, 48, and 72 h (VENDRAMINI et al., 2008). The bags were soaked in water, and placed in the micro-mesh wash bag of polyester with 15.5 x 10 cm. The bags were soaked in water, attached to a rope, and placed into a ruminally-fistulated steer (*Bos spp.*) with 480 kg BW. The steer was housed in an individual stall with covered barn and fed rhizoma peanut (Florigraze) hay *ad libitum* for 20 d (12 d for animal adaptation, and 8 d for period of incubation).

Samples from all experimental units (48 bags; two genotypes of rhizoma peanut, three proportions of leaf, two years, and four replications) within and incubation time were placed in one fistulated steer and withdrawn at the same time. This procedure guaranteed identical rumen conditions among treatments at each incubation time. After removal of the bags from the rumen, bags were rinsed with water until the rinse was colorless. The 0-h bags were not placed in the rumen but had same rinsing procedures used for the ruminally incubated bags. Subsequently, bags were frozen (-20°C), and after all incubations were completed, they were washed together in a washing machine, using the high level of water, and run for one cycle. Bags were oven dried at 55°C until constant weight. Nitrogen concentration and crude protein in the samples post-incubation were determined using the combustion procedure described previously.

In situ dry matter and crude protein degradation

Dry matter (DM) and crude protein (CP) fractions were estimated using an in situ ruminal degradation. Fraction A was a soluble fraction washed out of the bag at 0 h, fraction C was the undegradable fraction after 72 h of incubation in the rumen, and fraction B was a fraction potentially degradable, calculated by difference [B = 100 - (A + C)] (VENDRAMINI et al., 2008).

In situ rumen DM and CP degradation were estimated using non-linear model described by Ørskov and McDonald (1979):

$$P = A + B[1 - e^{-c(t)}]$$

Where P is DM or CP degraded at time (g kg^{-1}), A was wash loss (g kg^{-1}), B is potentially degradable fraction (g kg^{-1}), c was rate at which B is degraded ($\text{g kg}^{-1} \text{ h}^{-1}$), and t = time (h) incubated in the rumen. The A , B , and c were estimated using non-linear regression procedures (SAS INSTITUTE INC., 1996).

Effective DM and CP degradability were calculated the ruminal passage rate (k) from 0.02, 0.03, 0.04 and 0.05 h^{-1} . The model used was proposed by Ørskov and McDonald (1979):

$$ED = a + \frac{bc}{c + k}$$

Statistical Analysis

Data were analyzed using PROC MIXED procedure of SAS (SAS INSTITUTE INC., 1996). Genotypes and leaf proportions were considered fixed effects and replicates and years were random effects. Means were considered different when $P < 0.10$. Interactions not mentioned in the text were not significant ($P > 0.10$). The means reported are least square means and were separated using the PDIF procedure of SAS (SAS INSTITUTE INC., 1996). The relation between DM and CP disappearance of A, B, and C fractions and LS proportion was analyzed using the PROC REG procedure of SAS (SAS Institute Inc., 1996) and the equations used to estimate the DM and CP disappearance of A, B, and C fractions based on the LS proportion evaluated in the original samples.

RESULTS AND DISCUSSION

Dry matter and crude protein degradation parameters

There was an initial leaf:stem ratio and CP concentration differences between genotypes. Ecoturf had greater LS (66 vs. 63%, $P < 0.05$, $SE = 2.1$) and CP concentration (151 vs. 140 g kg^{-1} , $P < 0.05$, $SE = 3.5$) than Florigraze. As Ecoturf was originally selected for turf, the usual shorter height of the canopy may have resulted in decreased elongation of the stem, which resulted in greater LS. Consequently, greater number of leaves may have resulted in greater CP concentration. The leaves have greater concentration of cell contents, which includes N compounds (LEMAIRE et al., 2005).

There were initial differences in CP concentration treatment, the CP concentration decreased linearly from 100 to 0 LS (from 180 to 109 g kg⁻¹, respectively, $P < 0.05$, SE = 3.9). These results were consistent with Romero et al. (1987) who also reported greater CP concentration in leaf than stem.

There was no difference in genotypes for the DM and CP disappearance rate and CP effective degradability; however, Florigraze had greater DM effective degradability than Ecoturf (Table 4-1). It is important to note that the DM effective degradability was calculated from the analyses of the treatment proportions of LS (100:0, 50:50, and 0:100), and therefore the initial greater proportion of leaves in the Ecoturf plants did not interfere in the results. As Ecoturf plants have larger leaves (PRINE et al., 2010), it may be hypothesized that Ecoturf leaves will have greater concentrations of vascular tissue, which may have resulted in lesser digestibility.

DM effective degradability and rate of disappearance decreased with decreased LS. Stems have a greater proportion of cell wall, which are less digestible than leaves. Romero et al. (1987) evaluating leaf and stem of Florigraze also and reported lower DM disappearance in the stems than leaves. Similar results were also observed by Teixeira et al. (2010), these authors evaluated the leaf and stem chemical composition of pinto peanut and reported that stem had greater cellulose and lignin concentration than leaf.

There was difference in CP effective degradability effective with the 0.02 and 0.03 h⁻¹ passage rate; however, there was no difference with 0.04 and 0.05 h⁻¹ passage rate (Table 4-2). It was expected that the CP would follow the same trend observed in the DM response variables; however, CP in forage legumes are rapidly and extensively degraded in the rumen (CHEN et al., 2009; KIRCHHOF et al., 2010), and the total CP have greater proportions of soluble CP and lesser proportion of cell wall-associated CP fraction (KIRCHHOF et al., 2010).

Dry matter and CP fraction concentration

Florigraze had greater DM fraction A than Ecoturf but there was no difference in fractions B and C (Figure 4-1-I). The greater DM fraction A of Florigraze may have contributed to the greater DM effective degradability of Florigraze. Foster et al. (2011) evaluated in situ DM disappearance of Florigraze and observed 799 g kg⁻¹ DM for A+B fractions and 202 g kg⁻¹ DM for C fraction, which are similar to the values observed in this study.

Conversely, Ecoturf had greater CP fraction A and less CP fraction B than Florigraze (Figure 4-1 1-II). Ecoturf had greater initial CP concentrations, which may have led to greater CP fraction A than Florigraze. The CP fraction A may be rapidly released in the rumen, not captured as microbial protein, and absorbed in the rumen epithelium. The greater

CP fraction B found in Florigraze may be desirable to synchronize the supply of CP and energy in the rumen, which can potentially optimize ruminal microbial production and enhance animal performance (MINSON, 1990).

There was a linear decrease in DM fractions A, and B and a linear increase in DM fraction C with decreasing LS proportions from 100:0 to 0:100 (Figure 4-2). As the stem has structural functions, it has decreased concentrations of nonstructural carbohydrate as sugars, starch and pectin (SNIFFEN et al., 1992; JOHNSON et al., 2002), which resulted in decreased fractions A and B. The reduction of digestibility in the stem is associated with greater cell wall and lignin found in structural tissues (TEIXEIRA et al., 2010). There were differences in the estimated DM fractions A, B, and C for the different LS observed for Ecoturf and Florigraze (Figure 4-2). According to the linear models, the difference (66 vs. 63%) in LS between Ecoturf and Florigraze resulted in greater fraction A and B for Ecoturf and lesser fraction C than Florigraze.

Crude protein fractions A and C decreased and fraction B increased with decreasing LS. It has been observed in some forage legumes stem that the CP concentration is usually lesser than leaves; however, a significant portion may be readily soluble in the rumen. The greater CP fraction A can be related to transport function of non-protein N as ammonia, peptides, and amino acid in stems (SNIFFEN et al., 1992). Hakl et al. (2016) compared the in situ ruminal disappearance of leaf and stem of alfalfa and observed that stems had greater concentrations of fractions A and C and lesser fraction B than leaves.

The leaves had the greatest CP fraction B likely because non-protein N compounds in young leaves are immediately converted into proteins, increasing CP fraction B and reducing fraction A (KRAWUTSCHKE et al., 2012). The greater CP fraction C with increasing the proportion of stems was expected due to a greater proportion of the N being linked to cell wall (SNIFFEN et al., 1992). As CP fractions were estimated by the linear models (Figure 4-3), Ecoturf had greater CP fraction B and lesser fraction A due to greater LS and initial CP concentration. Florigraze had greater CP fraction C among genotypes, due to greater proportion of stem.

It is important to note that LS proportion of 0:100 still had relatively high DM and CP fractions A and B, indicating that the superior overall digestibility of rhizoma peanut may be related to highly digestible stem fraction. Terrill et al. (1996) evaluated the in vitro organic matter digestibility of Florigraze and reported greater values of digestibility in the leaf than stem with average of 719 and 669 g kg⁻¹, respectively.

SUMMARY AND CONCLUSIONS

It is expected that Ecoturf and Florigraze have differences in canopy architecture and LS. Florigraze may have greater DM effective degradability in the rumen than Ecoturf, indicating there may be some anatomical and/or chemical differences between cultivars. This difference was detected with the greater DM fraction A for Florigraze than Ecoturf. Ecoturf had greater initial CP concentrations but there was no difference in CP effective degradability. The greater CP concentration may be related to readily rumen degradable protein due to greater CP fraction A found in Ecoturf.

The LS ratio has a significant impact on DM and CP fractions and effective degradability. It was observed that the differences in LS between Ecoturf and Florigraze found in this research had greater influence on CP fractions than DM fractions. The rhizoma peanut LS is an important indicator of nutritive value and the models provided in this study may allow managers to have a more accurate prediction of performance for ruminants consuming rhizoma peanut.

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Table 4-1. Effect of genotype on dry matter and crude protein degradation of rhizoma peanut.

Degradation parameters	Genotype		SE	P value
	Ecoturf	Florigraze		
	Dry matter			
Effective degradability				
0.02 h, g kg ⁻¹	654 b [†]	665 a	9.27	0.001
0.03 h, g kg ⁻¹	614 b	627 a	8.65	0.001
0.04 h, g kg ⁻¹	584 b	597 a	8.19	0.001
0.05 h, g kg ⁻¹	559 b	573 a	7.78	0.001
Rate of disappearance, h ⁻¹	0.054	0.050	0.003	0.14
	Crude protein			
Effective degradability				
0.02 h, g kg ⁻¹	715	717	6.72	0.77
0.03 h, g kg ⁻¹	656	657	7.33	0.96
0.04 h, g kg ⁻¹	611	610	8.09	0.89
0.05 h, g kg ⁻¹	575	572	8.81	0.77
Rate of disappearance, h ⁻¹	0.050	0.053	0.003	0.48

[†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.10$) (SAS Institute Inc., 1996). Data are means across two years, six treatments, and four replicates (n = 48).

Table 4-2. Effect of proportions of the leaf on dry matter and crude protein degradation of rhizoma peanut.

Degradation parameters	Proportion (%)			SE	P value
	100	50	0		
Dry matter					
Effective degradability					
0.02 h, g kg ⁻¹	713 a [†]	660 b	606 c	9.37	<0.0001
0.03 h, g kg ⁻¹	674 a	621 b	567 c	8.82	<0.0001
0.04 h, g kg ⁻¹	643 a	591 b	538 c	8.41	<0.0001
0.05 h, g kg ⁻¹	618 a	567 b	514 c	8.03	<0.0001
Rate of disappearance, h ⁻¹	0.057 a	0.051 ab	0.049 b	0.003	0.08
Crude protein					
Effective degradability					
0.02 h, g kg ⁻¹	739 a	718 b	690 c	7.75	<0.0001
0.03 h, g kg ⁻¹	673 a	658 a	638 b	8.75	0.02
0.04 h, g kg ⁻¹	621	612	599	9.73	0.27
0.05 h, g kg ⁻¹	579	574	568	10.58	0.75
Rate of disappearance, h ⁻¹	0.055	0.053	0.047	0.003	0.15

[†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.10$) (SAS Institute Inc., 1996). Data are means across two years, six treatments, and four replicates (n = 48).

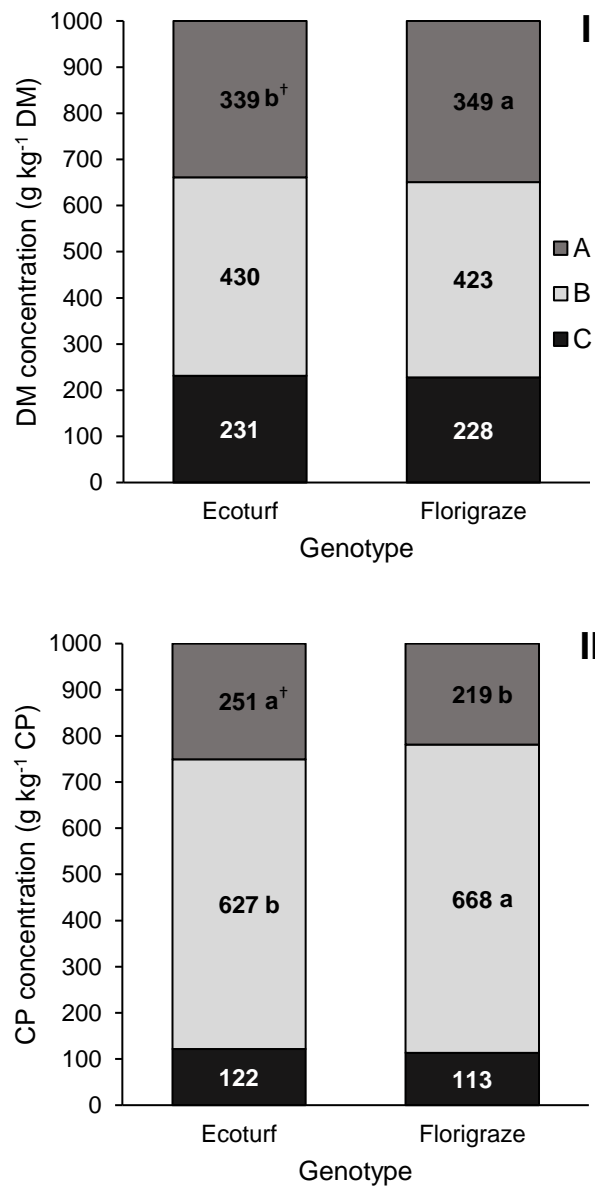


Figure 4-1. Genotype effects on the dry matter (I) and crude protein (II) disappearance of rhizoma peanut. [†]Means within row followed by the different letter are statistically different using the LSMEANS/PDIFF procedure ($P \leq 0.10$) (SAS Institute Inc., 1996). There was effect on the dry matter disappearance for Fraction A ($P = 0.01$; SE = 2.8), there was no effect for Fraction B ($P = 0.19$; SE = 11.7) and C ($P = 0.32$; SE = 11.5). There was effect on the crude protein disappearance for Fraction A ($P = 0.098$; SE = 20.5) and B ($P = 0.037$; SE = 27.7), there was no effect for Fraction C ($P = 0.18$; SE = 10.0).

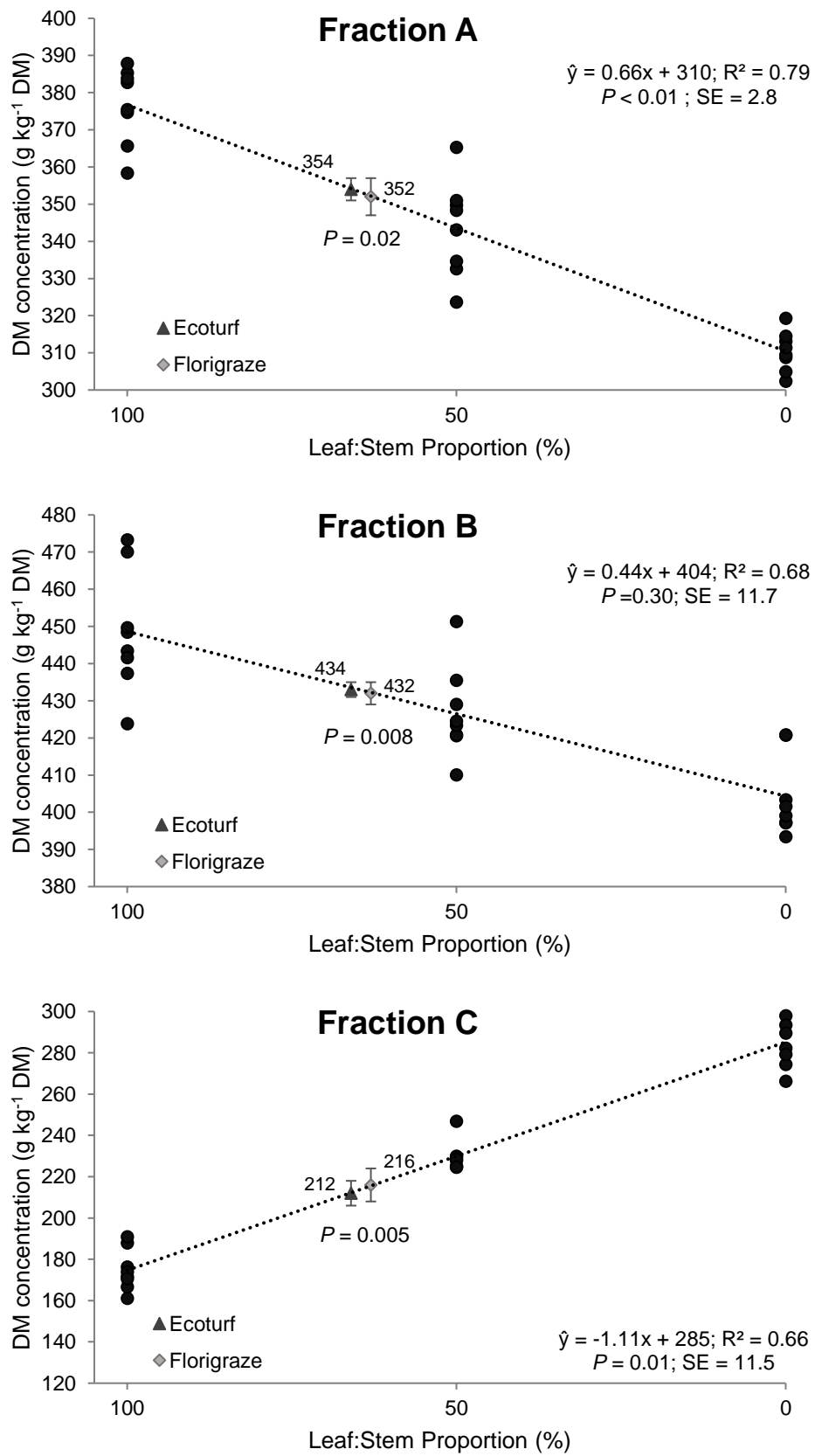


Figure 4-2. Dry matter degradation and estimative for genotypes of Fraction A, Fraction B, Fraction C. Leaf:stem ratio Ecoturf and Florigraze, 66 and 63%, respectively. The coefficient of determination (R^2) are for rhizoma peanut leaf:stem proportion.

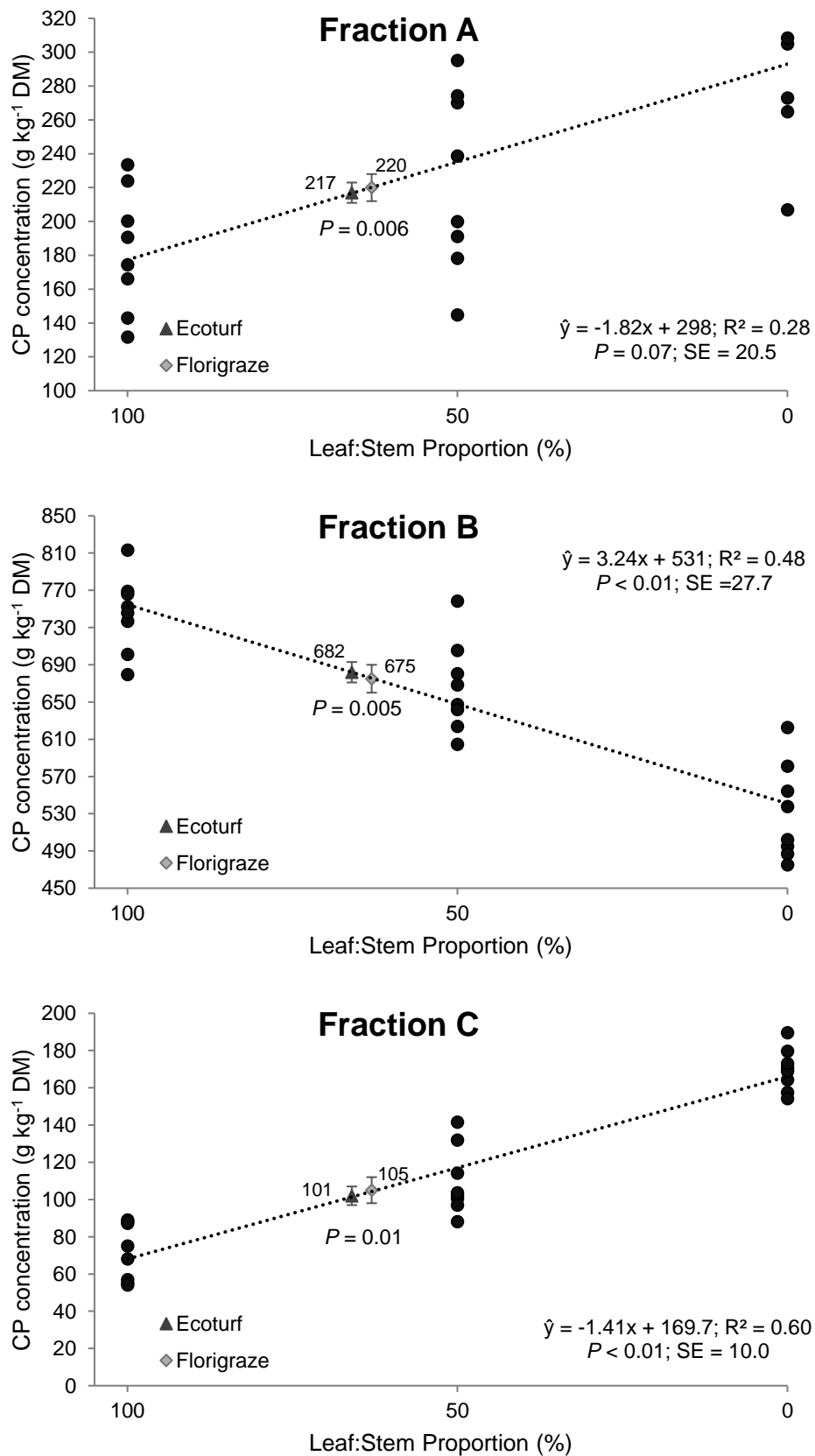


Figure 4-3. Crude protein degradation and estimative for genotypes of Fraction A, Fraction B, Fraction C. Leaf:stem ratio Ecoturf and Florigraze, 66 and 63%, respectively. The coefficient of determination (R^2) are for rhizoma peanut leaf:stem proportion.

FINAL REMARKS

The widespread adoption of tropical legumes worldwide has been relatively limited due to constraints associated with establishment of legumes into grass-legume mixed. *Arachis pintoii* and *Arachis glabrata* have the potential to be use as forage for livestock due to their superior nutritive value and persistence.

This research project evaluated management practices evaluated to establish pintoii peanut and palisadegrass mixed swards. Grass-legume-mixed swards decreased pintoii peanut plant density and ground cover, which decreased productivity of pintoii peanut-palisadegrass mixed stands. Adjusting seeding rates, fertilization levels, and harvest regimes may be feasible management practices to decrease palisadegrass competition in early stages post-establishment; however further research is needed to validate these management practices.

Overseeding pintoii peanut into established grass swards versus seedbed preparation represented an effective method to establish pintoii peanut into palisadegrass swards. Chemical treatment with glyphosate was effective to suppress the existing vegetation. Conversely, seedbed preparation after herbicide application did not enhance pintoii peanut establishment. Any method of suppression (no-till and seedbed preparation) of the palisadegrass growth will reduce palisadegrass HA, and stocking rates and animal productivity may be impacted negatively. Limited pintoii peanut HA and BFN during the early stages after establishment should be considered when making decisions regarding warm-season legume species selection.

The current study also evaluated establishment methods of rhizoma peanut (Ecoturf and Florigraze) into bahiagrass pastures in Florida. Similar forage characteristics between Ecoturf and Florigraze implies that Ecoturf has potential to be used as an alternative for Florigraze monoculture in Florida. Prepared seedbed had greater ground cover and HA. The greater cost associated with preparing seedbed after glyphosate application may be compensated by greater ground cover and HA of Ecoturf and Florigraze establishment.

The effect of leaf:stem proportion on DM and CP disappearance differed between cultivars. Florigraze may have greater DM effective degradability in the rumen than Ecoturf, indicating there may be some morphological and/or chemical differences between cultivars. It was observed that the LS differences between Ecoturf and Florigraze had greater influence on CP than DM disappearance fractions. Rhizoma peanut LS is an important indicator of nutritive value and the models provided in this study may allow managers to have a more accurate prediction of performance for ruminants consuming rhizoma peanut.

Collectively, these research projects provided valuable information to optimize management practices in grass-legume mixtures in tropical and subtropical regions. In addition,

the data generated by these research projects can be used as a platform for future research projects. Forage species, fertilization levels, and harvest regimes should be investigated regionally due to variable responses of these factors in different edaphic-climatic regions.