

Competitive and spreading abilities of forage peanut in tropical mixed pastures

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Abstract

Forage peanut (*Arachis pintoi*) is considered a legume ideotype for use in mixed pastures in humid tropical regions, but its adoption has been hindered by low availability and high prices of commercial seeds. However, there is a concern if breeding of high seed-yielding cultivars could result in loss of compatibility with grasses. This study compared the competitive ability and spreading ability of 12 forage peanut genotypes with contrasting seed-yielding potential in mixtures with *Brachiaria humidicola* in northern Brazil. These genotypes were previously selected for high performance under clipping in pure stands. Twenty-five rooted stolons of forage peanut were transplanted to the central area (1 × 1 m) of each plot (5 × 5 m) seeded 35 days before with *B. humidicola*. The experiment used a randomized complete block design with five replicates per genotype and was evaluated under grazing from 6 to 30 months after pasture establishment using the intermittent stocking method. All genotypes persisted and increased their proportion in botanical composition over time. Spread of forage peanut stolons into surrounding *B. humidicola* sward varied from 0.56 to 1.46 m/year. Cultivar BRS Mandobi had the greatest competitive ability and cultivar Belomonte the greatest spreading ability. Two genotypes (cv. BRS Mandobi and accession BRA-00064752-9) were selected combining high seed-yielding and high compatibility with *B. humidicola*. This is evidence that both traits (compatibility with grasses and seed yield) can be simultaneously improved in this legume. The results are discussed in terms of the ecology of grass-legume mixtures and breeding of forage peanut.

KEYWORDS

Arachis pintoi, *Brachiaria*, compatibility with grass, forage breeding, grass-legume mixtures, tropical pastures

1 | INTRODUCTION

Forage legumes offer important opportunities for the sustainable intensification of pasture-based livestock production. The use of grass-legume pastures can help to solve some of the main current challenges of this activity, through (a) increasing forage production and pasture

carrying capacity; (b) improving the forage nutritive value and the efficiency of forage conversion into animal protein; (c) the substitution of inorganic nitrogen fertilizers by symbiotic fixation of atmospheric nitrogen; (d) the contribution to mitigate and facilitate adaptation to climate change; and (e) the reduction of production costs and increased profitability of livestock activity (Luscher et al., 2014).

FIGURE 1 Average monthly rainfall and mean temperature (1981–2010) in Rio Branco, Acre, Brazil. Data from Diniz et al. (2018)

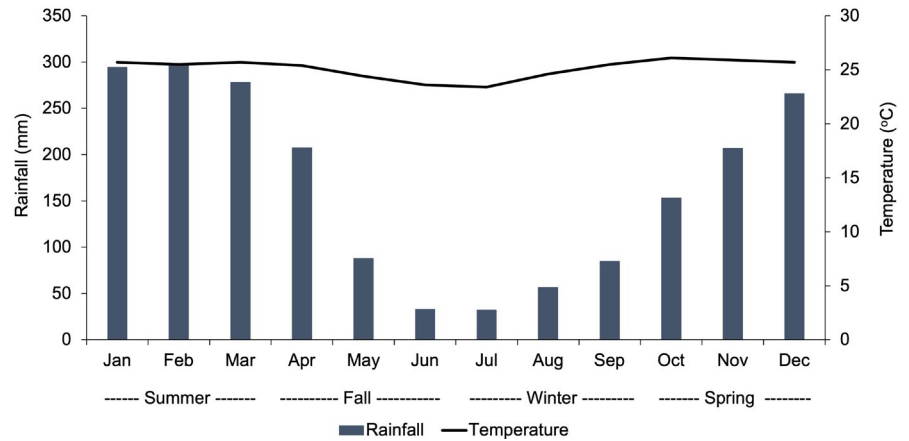


TABLE 1 Identification, origin and seed yield of forage peanut genotypes

Identification (BRA/cultivar/hybrid)	Previous identification	Origin	Seed yield class ^a
00066013-4	BRA-039799	São Paulo, Brazil (in cultivation)	Low
00066014-2	BRA-039985	São Paulo, Brazil (in cultivation)	Medium
00066036-5	BRA-040223	São Paulo, Brazil (in cultivation)	Low
00064831-1	BRA-030635	Minas Gerais, Brazil	High
00064752-9	BRA-030325	Minas Gerais, Brazil	High
00064728-9	BRA-014982	Minas Gerais, Brazil	Low
00064748-7	BRA-016357	Minas Gerais, Brazil	High
00190099-2	BRA-042242	Distrito Federal, Brazil (in cultivation)	Very low
Amarillo MG-100	BRA-013251	Bahia, Brazil	High
Belomonte	BRA-031828	Bahia, Brazil	Very low
BRS Mandobi	BRA-040550	Improved cultivar	High
Hybrid V1(59)	-	F1 Embrapa's Hybrid	Low

Note: (in cultivation) refers to accession collected outside its natural occurrence site.

Abbreviation: BRA, accession code from Embrapa, Brazil.

^aRelative seed yield potential based on experimental trials (Carvalho & Quesenberry, 2012; Miqueloni, 2018; Santos, 2018).

The success of this technology depends on the development of compatible grass and legume cultivars for each environment, in order to establish productive, harmonic and stable mixed pastures (de Andrade et al., 2015). Forage peanut (*Arachis pintoi* Krapov. & W.C. Greg.) has long been considered a legume ideotype for use in mixed pastures in humid tropical regions, similar to white clover (*Trifolium repens* L.) in temperate pastures (Fisher & Cruz, 1994). Literature evidence has increasingly supported this assumption by demonstrating an unusual combination of positive attributes in a forage plant. Forage peanut is palatable to cattle (Lascano, 2000), its forage quality is comparable to alfalfa (*Medicago sativa* L.; Carvalho & Quesenberry, 2012; Ladeira et al., 2002; Oliveira et al., 2011) and it offers no risk of bloat to cattle due to its close to ideal condensed tannins levels (Jackson et al., 1996; Min et al., 2003). It is highly productive in humid tropical climates, with best genotypes yielding around 20,000 kg DM ha⁻¹ year⁻¹ in pure stands in Brazil (Oliveira et al., 2011; Sales et al., 2002) and Venezuela (Urbano et al., 2010). Biological nitrogen fixation varies from 15 to 25 kg of N for each ton

of dry matter yielded, according to Thomas et al. (1997). Also, this clonal stoloniferous legume presents an outstanding regenerative capacity either from seeds, stolons or root fragments, all of which contributes to its high grazing and trampling tolerance and long persistence in mixed pastures (Fisher & Cruz, 1994).

In Brazil, the Forage Peanut Breeding Program is coordinated by Embrapa (Brazilian Agricultural Research Corporation) since 2005. One of the main breeding objectives of this program is the release of high seed-yielding cultivars, as low commercial seed availability and its high costs have impaired forage peanut adoption in mixed pastures (Assis & Valentim, 2013). However, there is a concern if selection of high seed-yielding cultivars could result in loss of compatibility with grasses. In white clover (*T. repens* L.), the selection for seed yield is hindered because the negative genetic correlation with forage yield and persistence, as predicted by stolon density (Annicchiarico et al., 1999). Potential seed yield of some *A. pintoi* genotypes is very high, reaching 7,000 kg/ha in cultivar Amarillo (Ferguson, 1994) and 4,500 kg/ha in cultivar BRS Mandobi (Valentim

et al., 2009). Such high investment in reproduction could diverge resources necessary to compete with grasses. Cultivar Belomonte, a very low seed-yielding cultivar, is currently the most planted in Brazil and is claimed as the most compatible with grasses among the released cultivars. Thus, there is a need to better understand the mechanisms of *A. pinto*-grass compatibility and its relationship with seed production potential, in order to support the Forage Peanut Breeding Program in selecting better cultivars for use in mixed pastures. This study was conducted to compare the competitive ability and spreading ability of 12 forage peanut genotypes with contrasting seed-yielding potential in mixtures with koronivia grass [*Brachiaria humidicola* (Rendle) Schweick cv. Tully]. This grass was chosen because of its good compatibility with forage peanut (Cook et al., 2020; Valentim et al., 2017).

2 | MATERIALS AND METHODS

2.1 | Experimental site

This study was conducted at the Experimental Station of Embrapa Acre (10°02'S, 67°42'W, 175 m above sea level), in Rio Branco, Acre, northern Brazil, from October 2016 to May 2019. The local climate is Am according to Köppen-Geiger classification, with average annual rainfall of 1,998 mm, average temperature of 25.1°C and 85.2% mean relative humidity (Diniz et al., 2018). The rain distribution is seasonal, with a dry season from June to September (Figure 1). The soil is classified as Haplic Plinthosol, with average clay, silt and sand (0- to 20-cm depth) of 35.7%, 48.6% and 15.7%, respectively. Prior to experiment establishment, mean soil pH (in water) was 5.35, Mehlich-1 extractable P and K were 2.15 and 62.4 mg/dm³, and KCl exchangeable Ca and Mg were 2.29 and 1.02 cmol_c/dm³, respectively.

2.2 | Treatments and experimental design

Treatments were 12 *A. pinto* genotypes (Table 1) growing in mixture with *B. humidicola* cv. Tully in a randomized complete block design with five replicates. The blocks were distributed along a slight slope over the experimental paddock. All the genotypes were previously evaluated and selected for high forage yield in different Brazilian edaphoclimatic regions (Fernandes et al., 2017; Miqueloni, 2018; Oliveira et al., 2011; Santos, 2018; Simeão et al., 2017). The relative seed yield potential in pure stands was also accessed (Table 1) in some of these trials and contrasting genotypes for this trait were considered in the experiment.

2.3 | Plot establishment and management

The 0.55-ha experimental paddock was tilled by disc-harrowing and sown using 5 kg/ha of pure germinable seeds of koronivia grass

on 28 October 2016. Grass seeds were broadcast using a well calibrated pendulum-type spreader and immediately incorporated into the soil by a roller. We tried to keep the seeding rate as uniform as possible over the experimental paddock. Average initial grass stand at 20 days after sowing was 15.5 seedlings/m², within the intended range of 15–20 seedlings/m². *A. pinto* stolon pieces 10-cm long were planted into 128-cell trays filled with commercial substrate and kept irrigated twice daily for 3 weeks in a nursery. Twenty-five rooted stolons were transplanted to the central area (1 × 1 m) of each plot (5 × 5 m) on 2 December 2016, 35 days after sowing the grass. The distance between plots was 2 m. Plots were kept at least 5 m away from the fences.

Pasture was fertilized only at planting with 250 kg/ha of single superphosphate (180 g P₂O₅/kg, 120 g S/kg and 180 g Ca/kg) and 40 kg/ha of the micronutrient mixture FTE BR-10 (25 g B/kg, 10 g Cu/kg, 40 g Fe/kg, 40 g Mn/kg, 1 g Mo/kg and 70 g Zn/kg). Sedges and dicotyledonous weeds were controlled 30 days after seeding *B. humidicola* with the post-emergent herbicide Bentazon at 1.5 kg ai/ha plus 1 L/ha of mineral oil as adjuvant.

During the establishment period, the sward was mowed to a 20-cm stubble height at 50 and 130 days after transplanting. Thereafter the entire experimental pasture was grazed by a group of 10–12 beef heifers every 4 weeks until the end of the experiment using an intermittent stocking method. Grazing period varied from 5 to 7 days according to average forage mass and sward height in each grazing cycle. We determined the moment to interrupt each grazing period based on the daily visual inspection of the degree of koronivia grass defoliation. The animals were maintained in an adjacent pasture during the resting periods. Once the average body weight of heifers surpassed 300 kg they were replaced by another group of heifers weighting around 200 kg. A total of 23 grazing cycles occurred from May 2017 to May 2019.

2.4 | Measurements

Herbage mass was measured pre- and post-grazing by clipping all herbage to a 2-cm stubble height using a 0.25-m² quadrat and a garden shear (model HSA 25, Stihl). Ten samples were collected both before and after each grazing period, then dried at 60°C for 72 hr, and weighed. Samples were clipped only between plots to avoid interfering with the botanical composition dynamics within experimental plots. Herbage mass data were used only to adjust stocking density.

Pre- and post-grazing canopy heights were measured with a ruler in four points within each 5 × 5 m plot. Grazing depth (%) was calculated by dividing the difference between pre- and post-grazing canopy height by pre-grazing canopy height, then multiplying by 100. Percentage of bare ground was also estimated visually in each plot (pre- and post-grazing) in order to identify major trampling or lying damages to swards by grazing animals.

Forage peanut plants were visually scored in each pre-grazing condition for plant vigour, flowering intensity and intensity of

putative pests (arthropods) and biotic diseases. Plant vigour score was based on a 1–5 scale, with: 1 = very poor vigour; and 5 = excellent vigour. Flowering intensity score was based on the percentage of plants with flowers: 0 = no plants flowering; 1 = 1%–20% of plants flowering; 2 = 21%–40%; 3 = 41%–60%; 4 = 61%–80%; 5 = 81%–100% flowering (Assis et al., 2018). The intensity of biotic foliar diseases in forage peanut was assessed according to the scale: 0 = absence of injury; 1 = minor injury; 2 = moderate injury; 3 = severe injury without dead plants; 4 = severe injury with some dead plants; 5 = severe injury with all dead plants. When present, biotic diseases were attributed to virus and/or fungus according to leaf symptoms (Gonçalves et al., 2014; Sánchez et al., 2016) and the data was analysed as frequency of virus-like diseases and fungal diseases. Injury level caused by pests to forage peanut was scored based on a scale of 1–5, with: 1 = very light (0%–10% of leaves with injury); 2 = light (11%–25%); 3 = moderate (26%–50%); 4 = abundant (51%–75%); 5 = severe (76%–100% of leaves with injury).

Botanical composition was monitored four times per year, in February (summer), May (fall), August (winter) and November (spring). It was used a metallic frame 5-m long, containing five 0.25-m² quadrats strategically distributed, which was always positioned in the same location across the center of the plots (Figure 2). One quadrat occupied the central plot zone where forage peanut was transplanted. In this zone, we observed the persistence and competitive ability of the legume. Two quadrats were positioned in the midway plot zone, 125–175 cm distant from the borders of the plots, and another two quadrats (border plot zone) where 25–75 cm distant from the borders. Average of five quadrats was used to estimate plot botanical composition. Four components were considered: koronivia grass, forage peanut, other monocotyledonous (monocot) and other dicotyledonous (dicot) plants. Compatibility with grass was measured as average forage peanut percentage in the five quadrats. We used the method of direct estimation of percentage composition (Whalley & Hardy,

2000) in which the proportion of each component in relation to total herbage mass inside each quadrat was visually estimated, always in the pre-grazing condition. The same well-trained observer, with more than 20 years of experience in evaluating botanical composition of mixed grass-forage peanut pastures using several methods, estimated botanical composition throughout the experimental period.

Spreading ability was estimated as the width of strip colonized by forage peanut in May 2017 (initial) and May 2019 (final). The presence of the legume over the plot was observed and the distance between its extreme stolon tips was measured in two directions (north-south and east-west) to estimate the average width of the strip colonized by forage peanut. Clonal mobility (m/year) was calculated as the annual increment in spreading ability.

2.5 | Statistical analysis

All analyses were performed using SAS version 9.4 software (SAS Inst. Inc.). Percentage data were submitted to angular transformation and back transformed after analysis for presentation.

Data of canopy height, grazing depth and bare ground were pooled for each season (summer, fall, winter and spring) and analysed according to a randomized complete block design with repeated measures in time (season), using the PROC MIXED with the fixed effects of genotype, season and their interactions, and the random effect of block. The covariance structure was chosen based on the Schwarz's Bayesian information criteria. Least Square Means were compared using Fisher's protected LSD ($p < .05$).

All other data were analysed via one-way analysis of variance for a randomized block design with five replicates using the PROC GLM. These included initial (6 months) and final (30 months) spreading ability and competitive ability; clonal mobility (from 6 to 30 months); final (30 months) compatibility with grass, % koronivia

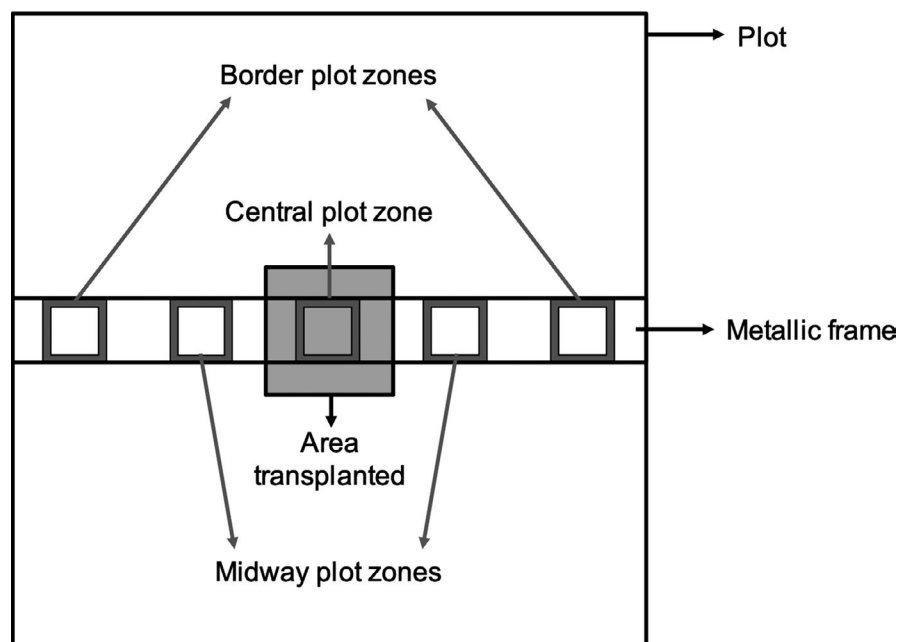


FIGURE 2 Schematic representation of the metallic frame with its five quadrats used to evaluate botanical composition in different plot zones

grass, % monocots and % dicots; and average plant vigour, pest and disease scores, and flowering intensity. Least Square Means were compared using the adjusted Scott-Knott test (Conrado et al., 2017) with $p < .10$ for data based on botanical composition or $p < .05$ for all other data.

Pearson correlation coefficients among compatibility with grass, spreading ability, competitive ability, plant vigour, flowering intensity, injury by pests and diseases, and frequency of virus and fungal diseases were determined using PROC CORR.

3 | RESULTS

Variables describing sward condition were affected only by season (Table 2). The p -values for the genotype and interaction effect for these variables were as follows: pre-grazing ($G = 0.125$ and $G*S = 0.745$) and post-grazing ($G = 0.139$ and $G*S = 0.690$) canopy height; grazing depth ($G = 0.977$ and $G*S = 0.794$); pre-grazing ($G = 0.477$ and $G*S = 0.190$) and post-grazing ($G = 0.654$ and $G*S = 0.110$) bare ground. Swards were taller in summer, intermediate in spring and fall, and shorter during winter. Greater grazing depths were observed in spring-summer, intermediate in fall and lower in winter. Pre-grazing percentage of bare ground (%BG) was greater during winter than in other seasons, although post-grazing %BG was greater in winter compared with summer and fall, and intermediate in spring.

Six months after transplanting to central plot zones (1×1 m) all forage peanut genotypes spread into the surrounding koronivia grass sward (Table 3). Cultivar Belomonte showed the highest initial and final spreading abilities and clonal mobility. On contrary, accessions BRA-00064748-7, BRA-00064831-1 and BRA-00190099-2 showed inferior spreading abilities and clonal mobility. Accessions BRA-00066014-2, BRA-00066013-4 and BRA-00066036-5 showed intermediate spreading abilities and clonal mobility. Cultivar BRS Mandobi and hybrid V1(59) showed intermediate initial and final spreading abilities, but inferior clonal mobility. On the other hand, cv. Amarillo, BRA-00064752-9 and BRA-00064728-9 had inferior initial spreading ability, but intermediate final spreading ability and clonal mobility.

Competitive ability was measured as percentage of forage peanut (%FP) in central plot zone where it was transplanted (Table 4). Cultivar BRS Mandobi, BRA-00066036-5 and BRA-00064752-9 showed superior initial competitive ability than other genotypes. However, differences among the genotypes were accentuated during the experiment so that we were able to discriminate four groups with respect to the final competitive ability. It was greater for cv. BRS Mandobi, followed by BRA-00064752-9, BRA-00066036-5, BRA-00190099-2, BRA-00066013-4 and BRA-00064728-9, then by hybrid V1(59), BRA-00064748-7, cv. Belomonte, BRA-00066014-2 and cv. Amarillo. Accession BRA-00064831-1 had the lowest final competitive ability.

Compatibility with koronivia grass was measured as average plot %FP at the end of experiment (Table 4). Cultivars BRS Mandobi

and Belomonte, together with accessions BRA-00064752-9, BRA-00066036-5, BRA-00066013-4 and BRA-00064728-9 showed superior compatibility than cv. Amarillo, hybrid V1(59), BRA-00190099-2, BRA-00066014-2, BRA-00064748-7 and BRA-00064831-1.

There was an effect of forage peanut genotype on proportion of koronivia grass (%KG) but not on proportions of other dicotyledonous (%Dicot) or monocotyledonous plants (%Mono) (Table 5). Higher %KG was observed when mixed with cv. Amarillo, BRA-00064831-1, hybrid V1(59), BRA-00190099-2 and BRA-00064748-7 than with other genotypes. Average %Dicot was 8.0% and average %Mono was 8.3%. Tropical kudzu [*Pueraria phaseoloides* (Roxb.) Benth.], recruited from soil seed bank, was the main component classified as other dicotyledonous while sedges and some native grasses were the other monocotyledonous plants.

Evolution of %FP (average of 12 genotypes) in different plot zones over time are shown in Figure 3. The legume progressively colonized and increased its participation in botanical composition in the central plot zone where it was originally transplanted, reaching a plateau of 56% of herbage mass at the end of experiment. At the same time, subsequent plot zones were gradually colonized by clonal growth. Evolution of mean plot botanical composition from 6 to 30 months after establishment is shown in Figure 4. While %FP increased from 4.8% to 28.1%, %KG decreased from 66.3% to 54.7%, and %Dicot decreased from 19.8% to 9.1%, over the same period. Less variation was observed for %Mono (from 9.2% to 8.1%).

Forage peanut genotypes differed in plant vigour and injury by pests and diseases (Table 6). Plant vigour was superior in cv. Belomonte, cv. BRS Mandobi, BRA-00066036-5, BRA-00064752-9, BRA-00064728-9, BRA-00066013-4 and hybrid V1(59) than in other genotypes. Plant vigour was positively correlated with compatibility with grass, spreading and competitive abilities, but negatively correlated with injury by diseases (Table 7). Injury by pests was generally scored as very light (0%–10% of leaves injured) or light (11%–25%) throughout the experiment. Greater injury by pests was observed in cv. BRS Mandobi, BRA-00064831-1 and BRA-00064728-9, while cv. Belomonte and BRA-00064752-9 were less injured. Cultivar Amarillo and other six accessions were intermediate (Table 6). Injury by pests was positively correlated with injury by diseases and negatively with spreading ability (Table 7). No genotype was severely injured by biotic foliar diseases throughout the study. Injury by diseases was lower in cv. Belomonte, BRA-00066014-2, and hybrid V1(59), intermediate in cv. BRS Mandobi, BRA-00066036-5, BRA-00064752-9, and BRA-00190099-2, and higher in cv. Amarillo, BRA-00064831-1, BRA-00066013-4, BRA-00064748-7 and BRA-00064728-9. Intensity of foliar diseases was also negatively correlated with spreading ability (Table 7). We discriminate four groups in relation to frequency of virus-like symptoms. Cultivar Belomonte, BRA-00066036-5 and BRA-00066014-2 presented low frequency of symptoms, followed by cv. BRS Mandobi, hybrid V1(59) and BRA-00064752-9. Genotypes apparently most infected by virus were cv. Amarillo, BRA-00066013-4 and BRA-00064728-9. However, it is important to highlight that, especially for the symptoms of viruses,

TABLE 2 Seasonal variation in pre- and post-grazing canopy height, grazing depth and percentage of bare ground in a mixed koronivia grass and forage peanut pasture, in Rio Branco, AC, Brazil

Season	Canopy height (cm)			Bare ground (%)	
	Pre	Post	Grazing depth (%)	Pre	Post
Spring	27.5 ^C	18.9 ^C	30.8 ^A	1.21 ^B	3.24 ^{AB}
Summer	33.9 ^A	23.3 ^A	31.0 ^A	1.35 ^B	2.97 ^{BC}
Fall	28.7 ^B	20.9 ^B	26.7 ^B	1.49 ^B	2.63 ^C
Winter	20.4 ^D	16.0 ^D	21.6 ^C	2.31 ^A	3.55 ^A
SEM	0.32	0.18	0.79	0.14	0.18
<i>p</i> -value	<.001	<.001	<.001	<.001	<.001

Note: ^{A-D}Means with different superscripts within columns are different ($p < .05$).

Abbreviation: SEM, standard error of mean.

TABLE 3 Spreading ability and clonal mobility of forage peanut in mixtures with koronivia grass in Rio Branco, AC, Brazil

Genotype	Spreading ability		Clonal mobility From 6 to 30 months
	Initial (6 months)	Final (30 months)	
cv. Belomonte	3.23 ^A	6.15 ^A	1.46 ^A
BRA-00066014-2	2.73 ^B	5.09 ^B	1.18 ^B
BRA-00066013-4	2.66 ^B	4.69 ^B	1.02 ^B
BRA-00066036-5	2.61 ^B	4.52 ^B	0.96 ^B
cv. BRS Mandobi	2.74 ^B	4.36 ^B	0.82 ^C
Hybrid V1(59)	2.71 ^B	4.30 ^B	0.8 ^C
BRA-00064752-9	2.23 ^C	4.27 ^B	1.02 ^B
BRA-00064728-9	2.1 ^C	4.24 ^B	1.07 ^B
cv. Amarillo	2.19 ^C	4.08 ^B	0.95 ^B
BRA-00064748-7	2.15 ^C	3.59 ^C	0.72 ^C
BRA-00064831-1	1.93 ^C	3.04 ^C	0.56 ^C
BRA-00190099-2	1.72 ^C	3.03 ^C	0.66 ^C
SEM	0.16	0.26	0.11
<i>p</i> -value	<.001	<.001	<.001

Note: Spreading ability = width of strip colonized by forage peanut (m). Clonal mobility = annual increment in spreading ability (m/year)

^{A-C}Means followed by the same letter within columns belong to the same group ($p < .05$) by Scott-Knott test.

Abbreviation: SEM, standard error of mean.

the infection was not confirmed by specific methods, and there may be different causes for virus-like symptoms, even of abiotic origin (Jeong et al., 2014). Frequency of foliar fungal diseases was higher in cv. BRS Mandobi, BRA-00066036-5 and BRA-00066014-2 than in the other genotypes. Intensity of foliar diseases was positively correlated with frequency of virus-like symptoms but not with frequency of fungus symptoms, and frequency of virus-like symptoms was negatively correlated with frequency of fungus symptoms and spreading ability (Table 7).

Flowering intensity varied greatly among forage peanut genotypes (Table 8). Cultivar Amarillo, followed by cv. BRS Mandobi, hybrid V1(59) and BRA-00064831-1 showed the highest flowering intensity. Conversely, it was lower in BRA-00190099-2 followed by cv. Belomonte and BRA-00064728-9. Accessions BRA-00064752-9,

BRA-00066036-5, BRA-00066014-2 and BRA-00064748-7 were intermediate. Flowering intensity was positively correlated with injury by pests and diseases, and frequency of fungus symptoms (Table 7).

4 | DISCUSSION

4.1 | Spreading ability

Spreading ability varies greatly in clonal plants. Lovett-Doust (1981) recognized a broad spectrum of growth forms in clonal species, varying from guerrilla species with high spreading ability in one extreme to phalanx species, characteristic of tussock grasses, in another

Genotype	Competitive ability		Compatibility with koronivia grass
	Initial (6 months)	Final (30 months)	Final (30 months)
cv. BRS Mandobi	21.4 ^A	77.1 ^A	34.6 ^A
BRA-00064752-9	17.2 ^A	66.4 ^B	30.6 ^A
BRA-00066036-5	18.7 ^A	63.0 ^B	29.9 ^A
BRA-00190099-2	15.0 ^B	62.6 ^B	21.1 ^B
BRA-00066013-4	13.0 ^B	61.6 ^B	28.8 ^A
BRA-00064728-9	15.7 ^B	58.6 ^B	28.5 ^A
Hybrid V1(59)	13.7 ^B	55.7 ^C	25.7 ^B
BRA-00064748-7	14.7 ^B	53.5 ^C	22.7 ^B
cv. Belomonte	11.6 ^B	50.9 ^C	31.6 ^A
BRA-00066014-2	12.3 ^B	48.2 ^C	25.5 ^B
cv. Amarillo	12.8 ^B	46.4 ^C	22.3 ^B
BRA-00064831-1	13.5 ^B	37.7 ^D	15.9 ^B
SEM	2.11	4.10	2.91
p-value	.026	<.001	.015

Note: Competitive ability = % forage peanut (%FP) in central plot zone. Compatibility with koronivia grass = average plot %FP.

^{A-D}Means followed by the same letter within columns belong to the same group ($p < .10$) by Scott-Knott test.

Abbreviation: SEM, standard error of mean.

Genotype	Koronivia grass (%)	Dicotyledonous (%)	Monocotyledonous (%)
cv. Amarillo	63.5 ^A	7.8	6.4
BRA-00064831-1	63.4 ^A	9.1	11.6
Hybrid V1(59)	61.5 ^A	7.1	5.6
BRA-00190099-2	60.5 ^A	9.9	8.5
BRA-00064748-7	60.5 ^A	9.2	7.5
BRA-00066014-2	56.8 ^B	8.6	9.1
BRA-00064752-9	55.6 ^B	7.7	9.2
BRA-00064728-9	55.3 ^B	8.3	7.9
BRA-00066013-4	55.0 ^B	8.2	8.1
cv. Belomonte	54.1 ^B	7.4	6.9
BRA-00066036-5	51.0 ^B	6.1	13.0
cv. BRS Mandobi	50.3 ^B	6.8	8.3
SEM	2.74	1.31	1.64
p-value	.019	.821	.181

Note: Botanical composition 30 months after establishment.

Abbreviation: SEM, standard error of mean.

^{A,B}Means followed by the same letter within columns belong to the same group ($p < .10$) by Scott-Knott test.

extreme. Although forage peanut has a typical guerrilla growth form, spreading ability varied greatly among genotypes. Cultivar Belomonte showed the greatest spreading ability (Table 3). Its clonal mobility (1.46 m/year) was 2.6 times greater than that of accession

BRA-00064831-1. We did not find in literature such high clonal mobility for any other clonal legume in mixed pastures. In a previous study in Acre, clonal mobility of cv. Belomonte was 0.85 m/year when strip-planted in koronivia grass and signal grass (*B. decumbens*

TABLE 4 Competitive ability of forage peanut and its compatibility with koronivia grass in Rio Branco, AC, Brazil

TABLE 5 Proportions of koronivia grass and other botanical components in mixed pastures with different forage peanut genotypes in Rio Branco, AC, Brazil

FIGURE 3 Variation in percentage of forage peanut in three plot zones from 6 to 30 months after establishment in mixtures with koronivia grass under grazing in Rio Branco, AC, Brazil. Data pooled from 12 forage peanut genotypes

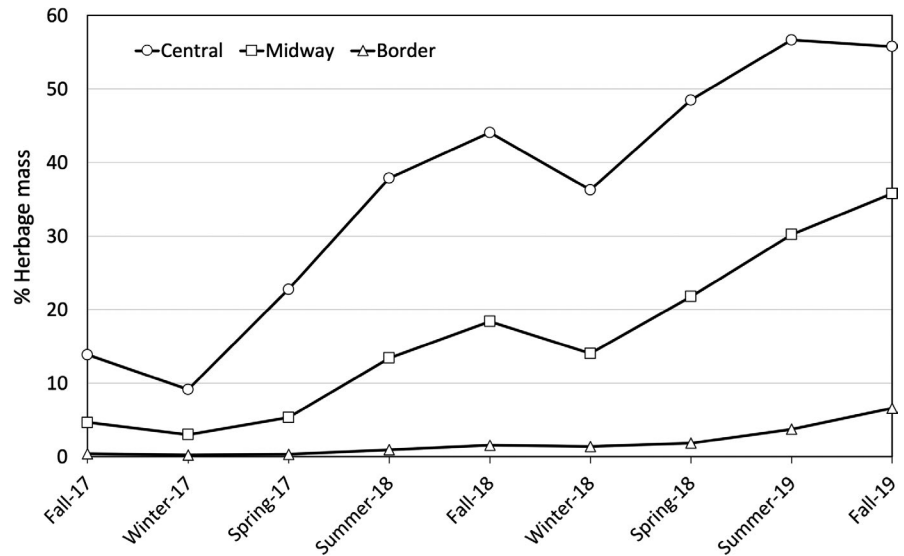
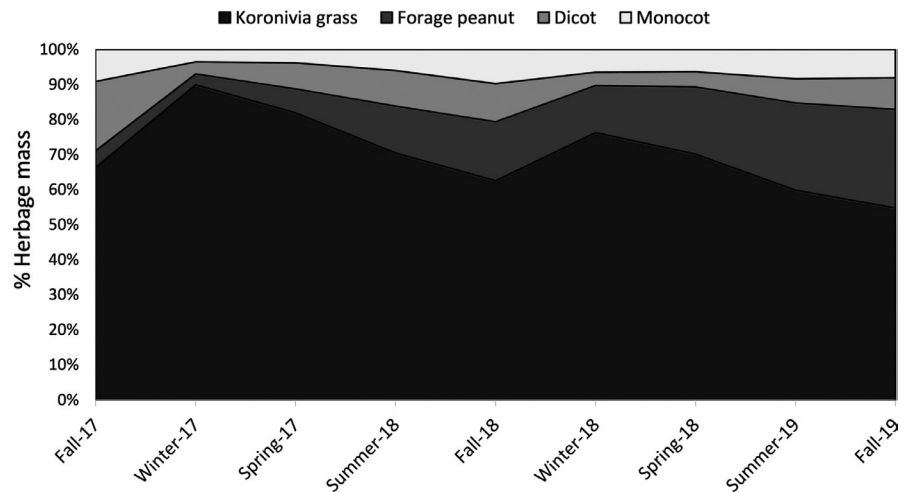


FIGURE 4 Variation in percentage of pasture components from 6 to 30 months after establishment in mixed pastures with forage peanut and koronivia grass under grazing in Rio Branco, AC, Brazil. Dicot = other dicotyledonous plants. Monocot = other monocotyledonous plants. Data pooled from 12 forage peanut genotypes



Stapf cv. Basilisk) pastures (de Andrade et al., 2007). In this same study, mobility of *Indigofera hendecaphylla* Jacq. and *Desmodium heterocarpon* subsp. *ovalifolium* (Prain) H. Ohashi cv. Itabela was 0.43 and 0.19 m/year, respectively. Average clonal mobility of white clover in temperate mixed pastures is 0.27 m/year (Duchoslavová & Herben, 2020).

Evolution of %FP (average of 12 genotypes) in different plot zones over time (Figure 3) describes the guerrilla growth strategy of forage peanut in this mixed pasture. Subsequent plot zones were gradually colonized by its clonal growth. In our equatorial climate, colonization of new spaces was interrupted only during winter due to drought stress, because this legume has lower drought tolerance than koronivia grass (Fischer & Cruz, 1994). If the experiment had continued for a longer time, probably similar %FP would be observed in different plot zones, although with time variation among genotypes due to spreading ability differences. The experiment was planned to last 36 months, but it was interrupted 6 months earlier because some genotypes started to invade adjacent plots, even with a distance of 2 m between plots.

Forage peanut was occasionally attacked by leaf-feeding beetles (*Cerotoma arcuata*), thrips (*Enneothrips flavens*) and peanut red

spider mite (*Tetranychus ogmophallos*), but only light injury occurred throughout the study. The same was observed in relation to foliar fungal diseases. Intensity of foliar diseases was positively correlated with frequency of virus-like symptoms, showing that viruses must have been the main shoot diseases in forage peanut during the experiment. This legume is host of several viruses (Pantoja et al., 2020; Sánchez et al., 2016). Gonçalves et al. (2016) reported 59.6% incidence of virus-like symptoms in the Active Germplasm Bank of Forage Peanut at Embrapa Acre. The impact of viruses on growth of forage peanut was not found in literature. In New Zealand, the white clover mosaic virus reduced in 36.5% the dry-matter yield of white clover, with major effect on stolon elongation (Dudas et al., 1998), a trait related with spreading ability of stoloniferous legumes. This agrees with our finding of negative correlation between spreading ability and frequency of virus-like symptoms and reinforces the need to investigate the impact of viruses on forage peanut growth and compatibility with grasses. The low incidence of pests and diseases, especially viruses, in cultivar Belomonte in this work (Table 6) corroborates field observations made on commercial pastures in State of Acre, Brazil, over the last 20 years (C.M.S. de Andrade, personal communication, 2021). This cultivar should be investigated as a

Genotype	Vigour	Pests	Diseases	Viruses	Fungus
BRA-00066036-5	3.56 ^A	1.53 ^B	0.93 ^B	0.11 ^D	0.78 ^A
BRA-00064752-9	3.55 ^A	1.41 ^C	0.98 ^B	0.44 ^C	0.51 ^B
cv. Belomonte	3.53 ^A	1.34 ^C	0.51 ^C	0.07 ^D	0.47 ^B
cv. BRS Mandobi	3.49 ^A	1.67 ^A	1.00 ^B	0.33 ^C	0.69 ^A
BRA-00064728-9	3.48 ^A	1.58 ^A	1.27 ^A	0.78 ^A	0.40 ^B
Hybrid V1(59)	3.45 ^A	1.48 ^B	0.80 ^C	0.33 ^C	0.46 ^B
BRA-00066013-4	3.43 ^A	1.50 ^B	1.44 ^A	0.89 ^A	0.29 ^B
BRA-00066014-2	3.35 ^B	1.51 ^B	0.71 ^C	0.15 ^D	0.60 ^A
cv. Amarillo	3.35 ^B	1.53 ^B	1.51 ^A	0.80 ^A	0.49 ^B
BRA-00190099-2	3.33 ^B	1.45 ^B	1.04 ^B	0.63 ^B	0.33 ^B
BRA-00064748-7	3.23 ^B	1.51 ^B	1.40 ^A	0.62 ^B	0.53 ^B
BRA-00064831-1	3.09 ^B	1.64 ^A	1.51 ^A	0.67 ^B	0.40 ^B
SEM	0.083	0.041	0.095	0.054	0.060
p-value	.009	<.001	<.001	<.001	<.001

Note: Data pooled across 23 sampling dates in 30 months. Plant vigour: 1 = very poor vigour; and 5 = excellent vigour. Injury by pests: 1 = very light (0%–10% of leaves injured); 2 = light (11%–25%); 3 = moderate (26%–50%); 4 = abundant (51%–75%); 5 = severe (76%–100%). Injury by foliar diseases: 0 = absence of injury; 1 = minor; 2 = moderate; 3 = severe injury without dead plants; 4 = severe injury with some dead plants; 5 = severe injury with all dead plants.

^{A–D}Means followed by the same letter within columns belong to the same group ($p < .05$) by Scott–Knott test.

Abbreviation: SEM, standard error of mean.

TABLE 7 Pearson correlation coefficients between compatibility with grass, competitive and spreading ability and other variables describing plant vigour, pests and diseases, and flowering intensity in forage peanut

Variable	Spreading ability	Competitive ability	Vigour	Pests	Diseases	Virus	Fungus	Flowering
Compatibility	0.62***	0.68***	0.73***	ns	ns	ns	ns	ns
Spreading ability		ns	0.51***	–0.39**	–0.48***	–0.45***	ns	ns
Competitive ability			0.63***	ns	ns	ns	ns	ns
Vigour				ns	–0.29*	ns	ns	ns
Pests					0.30*	ns	ns	0.26*
Diseases						0.75***	ns	0.27*
Virus							–0.53***	ns
Fungus								0.32*

Note: ns, *, ** and ***—not significant, significant at 5%, 1% and 0.1% of probability by t test, respectively.

source of resistance to viruses. The negative correlation between frequency of virus-like symptoms and frequency of fungus symptoms is evidence that both groups of pathogens interact with each other and with host plant, which also deserves further investigation.

Spreading ability is, therefore, an important component of forage peanut compatibility with grasses and should be taken in account in breeding programs. Selecting genotypes with greater clonal mobility can improve their fitness in heterogeneous tropical pastures. Such genotypes are better able to locate resource-rich patches in these pastures (Hutchings & de Kroon, 1994), and can also better cope with grass competition locally. This trait has also practical importance in establishing mixed pastures with expensive seeds. Farmers

could use reduced seeding rates (Cook et al., 1994) or plant the legume in intercalated strips (Valentim et al., 2017) and wait for some time until ideal legume proportion is reached via stolon colonization. Genotypes with higher spreading ability are better suited for both practices.

4.2 | Competitive ability

Although spreading ability of clonal legumes is important for persistence and stability in mixed pastures, they should be competitive enough to reach a productivity level that guarantees their benefits.

TABLE 6 Visual scores of plant vigour, injury by pests and diseases and frequency of fungus and virus-like symptoms in forage peanut in mixtures with koronivia grass in Rio Branco, AC, Brazil

TABLE 8 Flowering intensity in forage peanut in mixtures with koronivia grass in Rio Branco, AC, Brazil

Genotype	Seed yield class	Flowering
cv. Amarillo	High	2.00 ^A
cv. BRS Mandobi	High	1.46 ^B
Hybrid V1(59)	Low	1.45 ^B
BRA-00064831-1	High	1.27 ^B
BRA-00064752-9	High	1.16 ^C
BRA-00066036-5	Low	1.14 ^C
BRA-00066014-2	Medium	1.12 ^C
BRA-00064748-7	High	1.10 ^C
BRA-00066013-4	Low	0.90 ^C
BRA-00064728-9	Low	0.54 ^D
cv. Belomonte	Very low	0.44 ^D
BRA-00190099-2	Very low	0.16 ^E
SEM		0.09
<i>p</i> -value		<.001

Note: Flowering intensity: 0 = no flowering; 1 = 1%–20% of plants flowering; 2 = 21%–40%; 3 = 41%–60%; 4 = 61%–80%; 5 = 81%–100%. Data pooled across spring, summer and fall sampling dates in 30 months.

^{A–E}Means followed by the same letter within columns belong to the same group ($p < .05$) by Scott–Knott test.

Abbreviation: SEM, standard error of mean.

Forage peanut was initially less competitive with koronivia grass because of its known slow establishment (Cook et al., 1994; Valentim et al., 2003). At six months after transplanting, %FP in central plot zones averaged 15% of herbage mass (Figure 3), in contrast with %KG averaging 63% and 22% for other pasture components (data not shown). This occurred despite the high planting density of 25 rooted stolons in the central plot zones (1 × 1 m). Koronivia grass seedling density at 20 days after planting was 15.5 m⁻². However, competitive ability increased thereafter and average %FP in central plot zones attained 56.0% after 24 months under grazing (Figure 3). In addition, mean plot %FP increased from 4.8% to 28.1% over the same period (Figure 4) as a result of the combined effect of its competitive and spreading abilities.

Cultivar BRS Mandobi showed the greatest competitive ability at the end of experiment (Table 4). At this time, dominance of forage peanut (over 50% of herbage mass) in central plot zones was shown even for genotypes with smaller competitive ability such as cv. Belomonte, hybrid V1(59) and BRA-00064748-7. This finding raises question about a possible excessive competitive ability in some forage peanut genotypes.

The experience with most non-clonal tropical legumes is that, in most cases, the grass suppresses the legume, particularly when pastures are heavily grazed (Lascano, 2000). Conversely, competitive ability of perennial *Arachis* species was highlighted at the beginning of research on their use in mixed pastures. Prine (1964) mentions the possibility of these legumes to dominate the grasses in mixtures as one of its disadvantages. Current experience with released cultivars

(Amarillo, Belomonte and BRS Mandobi) has shown that high grazing intensities (de Andrade et al., 2005; Homem et al., 2019; Ibrahim & Mannetje, 1998) and biotic (e.g. spittlebug attacks; Grof, 1985) or abiotic stresses (e.g. waterlogging; Valentim & de Andrade, 2015) affecting companion grasses are the main causes of forage peanut dominance.

Spittlebugs were monitored throughout the current study and controlled with a systemic insecticide when necessary (February 2018) in order to avoid damage to koronivia grass and affect the grass–legume balance. Waterlogging occurred every summer because the soil in the experimental area is poorly drained, but both forage peanut and koronivia grass are well-adapted to waterlogging (Cook et al., 2020; Dias-Filho & Carvalho, 2000).

A moderate intensity intermittent stocking was practiced in the current study. Swards were managed with pre-grazing canopy heights varying from 27.5 to 33.9 cm during the rainy season (spring to fall) and with 20.4 cm during the dry winter months (Table 2). Such pre-grazing heights are close to the target of 30 cm defined by Vilela (2011) for koronivia grass pastures, according with the criteria of 95% light interception. However, in heterogeneous pastures, forage plants respond to sward structure prevailing in the patch where they are growing and not to average pasture condition (Lemaire, 2001).

Canopy height differences among plot zones were not measured, but it was visually observed throughout the study that canopies were shorter in central plot zones, suggesting a greater grazing intensity on these sites than on average pasture. This assumption is supported by literature. Hess et al. (2002) studied legume selection by cattle grazing mixtures of forage peanut and koronivia grass in Colombia. Cattle preferentially selected the legume in pastures with low or medium legume mass, but showed no preference or selected against the legume in pastures with high legume mass. Similarly, when forage peanut is planted in strips in grass pastures cattle prefers grazing on strips with legumes (Valentim et al., 2017). Consequently, strips are grazed more intensively than whole pasture and this benefits the legume in these sites. Therefore, the dominance of most forage peanut genotypes in central plot zones was probably favoured by cattle preference due to low forage peanut content in this experimental mixed pasture as a whole.

In addition, there is enough evidence in recent literature suggesting that even highly competitive genotypes such as cv. BRS Mandobi can form balanced and stable mixed pastures when properly managed. For example, in pastures maintained for long term (45 months) at 10-cm height there was a predominance of cv. Belomonte (71%–85% of herbage mass) over *Brachiaria brizantha* (Homem et al., 2019). However, when managed at 20-cm height a balanced botanical composition was reached (Tamele et al., 2018). Similar responses were observed when cv. BRS Mandobi was managed under rotational stocking in association with *B. brizantha* (de Andrade et al., 2012; Gomes et al., 2018). Sward targets recommended for this mixture were a pre-grazing height of 24–30 cm and a stubble height of 15 cm (Gomes et al., 2018).

Six genotypes were identified with superior compatibility with koronivia grass in the current study (Table 4), including cultivars BRS Mandobi (greatest competitive ability) and Belomonte (greatest

spreading ability). The former presented inferior clonal mobility and the latter inferior competitive ability. Selection for competitive ability against associated grasses has proved to be a valuable strategy to obtain compatible white clover-grass mixtures in temperate pastures (Annicchiarico et al., 2015; Annicchiarico & Proietti, 2010). Our study confirms the importance of both competitive and spreading ability in breeding forage peanut with high compatibility with grasses. The other genotypes with superior compatibility with koronivia grass (BRA-00064752-9, BRA-00066036-5, BRA-00066013-4 and BRA-00064728-9) combined intermediate spreading and competitive abilities. Although we did not find any genotype combining superior competitive and spreading abilities, these traits were not correlated (Table 7), circumstance more suitable to breed forage peanut to combine superior abilities when compared to unfavourably correlated characteristics.

It is interesting to note that cv. Amarillo, native from Brazil but selected in Australia, showed inferior compatibility with koronivia grass than cultivars BRS Mandobi and Belomonte, both selected in Brazil. Amarillo presented intermediate spreading ability combined with inferior competitive ability, less vigorous plants and higher frequency of virus-like symptoms. A previous study in Minas Gerais, Brazil, also demonstrated the superior compatibility with tangola grass (natural hybrid of *Brachiaria arrecta* × *B. mutica*) of cv. Belomonte when compared with cv. Amarillo (Viana et al., 2004). Therefore, it is important to highlight the importance to evaluate and select genotypes in the region where the cultivar will be used.

4.3 | Implications for breeding forage peanut

Compatibility with grasses and seed yield are important agronomic traits for the Embrapa's Forage Peanut Breeding Program. Seed yield is a vital trait for the commercial success of a forage cultivar (Annicchiarico et al., 2015) and compatibility with grasses is fundamental for the long-term success of a forage legume for mixed pastures. Results of the current study do not support the concern about losing compatibility with grasses when selecting high seed-yielding forage peanut cultivars. Flowering intensity was not correlated with compatibility with grass ($r = 0.04$; $p = .751$), competitive ($r = -0.06$; $p = .649$) or spreading ability ($r = -0.05$; $p = .729$). Although the highest flowering intensity is not always associated with higher seed yields in *A. pintoi* and *A. repens* (Carvalho et al., 2009), good seed-yielding genotypes tend to flower more abundantly (Table 8).

In addition, high seed-yielding genotypes (cv. BRS Mandobi and BRA-00064752-9) presented superior compatibility with grass and competitive ability. Also, very low seed-yielding genotypes (cv. Belomonte and BRA-00190099-2) showed highly contrasting spreading abilities.

In fact, there is a huge difference in seed yield between a pure stand of forage peanut managed as a seed crop and a grazed mixed pasture. In Planaltina, Federal District, Brazil, a seed crop of *A. pintoi* cv. Itacambira (BRA-00065334-5) yielded 1,936–2,782 kg/ha at 20 months after establishment (de Andrade & Karia, 1997)

in contrast with a seed bank of 300 kg/ha at the second year of a mixed pasture with *Paspalum atratum* Swallen (Barcellos et al., 1999). Therefore, even high seed-yielding genotypes reduce the investment in seeds when growing in association with grasses, suggesting a trade-off between sexual reproduction and clonal growth induced by both interspecific competition and grazing. Because both modes of reproduction require resources, a trade-off between them is inevitable (Prati & Schmid, 2000; Zhang & Zhang, 2007).

Seed crop yields are higher when forage peanut is established by seeds than by stolons (Adjolohoun et al., 2013; Balzon et al., 2005; Ferguson, 1994; Miqueloni, 2018). This shows that seedlings have superior sexual reproductive ability than clonal offspring of forage peanut. In the current study, all genotypes were established vegetatively. Thus, it is unclear if different responses could result if established by seeds. However, clonal reproduction is predominant in stable perennial pastures because clonal integration enhances successful establishment of clonal offspring when compared to seedlings (Lovett-Doust, 1981; Winkler & Fischer, 2002). Therefore, even when seed planted, forage peanut will probably reduce investment in seeds once original plants are replaced by clonal reproduction in mixed pastures. The half-life of original plants of cv. Amarillo in Australia was 25 months (Jones, 1993).

Altogether, there is enough evidence from our findings and literature reviewed suggesting the possibility of improving both traits (compatibility with grasses and seed yield) simultaneously in *A. pintoi*. However, it is still necessary to estimate genetic parameters such as genotypic variance, heritability, repeatability and genetic correlations to define appropriate strategies of breeding.

We confirmed the high compatibility with grasses of four forage peanut ecotypes previously selected based on its performance in pure stands under clipping (Fernandes et al., 2017; Miqueloni, 2018; Santos, 2018; Simeão et al., 2017). Three of them are low seed-yielding (BRA-00066036-5, BRA-00066013-4 and BRA-00064728-9) and have potential to be released as vegetatively propagated cultivars in alternative to cv. Belomonte. However, BRA-00066013-4 and BRA-00064728-9 showed higher frequency of virus-like symptoms and BRA-00066036-5 higher frequency of foliar fungal disease symptoms when compared to cv. Belomonte. Conversely, the ecotype BRA-00064752-9 is high seed-yielding and showed less virus-like symptoms than cv. Amarillo and less foliar fungal disease symptoms than cv. BRS Mandobi. However, even though we scored for pests and diseases, specific resistance tests should be conducted to select for these traits. Different virus species were identified and isolated from accessions of the Germplasm Bank of Forage Peanut, including a new species of family Potyviridae (Pantoja et al., 2020) and resistance tests were applied for some genotypes. The ecotype BRA-00066013-4 proved to be resistant for seven virus species (data not shown) and probably the observed symptoms should not have viruses as a cause, or the virus has not yet been appropriately identified.

The result of this study reinforces that selected genotypes in pure stands for high forage yield (Fernandes et al., 2017; Miqueloni, 2018; Santos, 2018; Simeão et al., 2017) have genetic variability for spreading and competitive abilities, and it is essential to consider

these characteristics during the initial stages of selection. However, the measurement of both traits in hundreds or thousands of genotypes is of hard execution. In this sense, additional studies should be carried out looking for indirect selection (Gallais, 1984). To apply this method, it is necessary to identify secondary characters in pure stands that have greater heritability and are highly correlated with spreading and competitive abilities in mixed pastures.

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AUTHOR CONTRIBUTION

Carlos Mauricio Soares de Andrade: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Supervision (lead); Writing—original draft (lead). **Giselle Mariano Lessa de Assis:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Methodology (equal); Writing—original draft (equal). **Rivalvalve Coelho Gonçalves:** Investigation (supporting); Methodology (equal); Supervision (equal); Writing—original draft (equal). **Aliedson Sampaio Ferreira:** Investigation (equal); Project administration (equal); Supervision (equal). **Yves Matheus Ferreira de Oliveira:** Data curation (supporting); Investigation (supporting); Project administration (supporting).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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