



# Response of Bahiagrass hybrids to nitrogen fertilization or mixture with legumes

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**ABSTRACT** - The objective of this work was to determine *Paspalum notatum* genotypes appropriate for the establishment of mixtures with temperate legumes and compare the productivity of mixtures with mineral-fertilized systems along two years. Four hybrids of *P. notatum*, ecotype Bagual and cv. Pensacola, were either subjected to mixtures with white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*) or fertilized with 0, 60, 120, 240, and 480 kg N ha<sup>-1</sup> year<sup>-1</sup>. *P. notatum* leaf blades and stems and herbage accumulation denoted a management × year interaction. Herbage accumulation varied between the mixtures and fertilized systems in first year, while in the second year, herbage accumulation of mixed systems employing the B26, B43, C22, C9, and Bagual genotypes were similar to that of systems fertilized with 120 and 240 kg N ha<sup>-1</sup>. Hybrids B26 and C9 and ecotype Bagual, once made available to producers, could be indicated for mixtures with temperate legumes because they are superior to cv. Pensacola. Herbage accumulation in mixtures involving white clover plus birdsfoot trefoil and *P. notatum* genotypes is similar to that of mineral nitrogen-fertilized systems with 240 kg N ha<sup>-1</sup>, emphasizing the viability of mixed between these species.

**Keywords:** herbage accumulation, mixed management, *Paspalum notatum*, white clover

## 1. Introduction

Bahiagrass (*Paspalum notatum* Flüggé) is a perennial warm season grass, one of the major constituents of the native grasslands in the Americas (Chase, 1929), and has economical importance mainly because of its use as forage and utility turf (Blount and Acuña, 2009).

In several species of *Paspalum*, apomixis occurs mainly at polyploid levels, and sexual counterparts of the same ploidy are not available in nature (Ortiz et al., 2020). In Bahiagrass, sexual tetraploid genotypes were generated by chromosome doubling of sexual diploids using colchicine (Quarin et al., 2003), allowing sexual plants to form compatible crosses with apomictic plants.

The general idea of using hybridization in Bahiagrass relates to the possibility of releasing the natural diversity present in apomictic ecotypes and fixing superior F<sub>1</sub> hybrids that may result in new forage cultivars (Acuña et al., 2009). Weiler et al. (2018) artificially hybridized sexual plants with apomictic plants and evaluated novel Bahiagrass hybrids. The authors observed higher productivity than most productive parents and the most productive hybrids also showed the most vigorous regrowth following winter.

Nitrogen is an important agronomic input for warm-season grass production, and early research on Bahiagrass responses to N fertilization was focused on maximizing herbage accumulation and avoiding

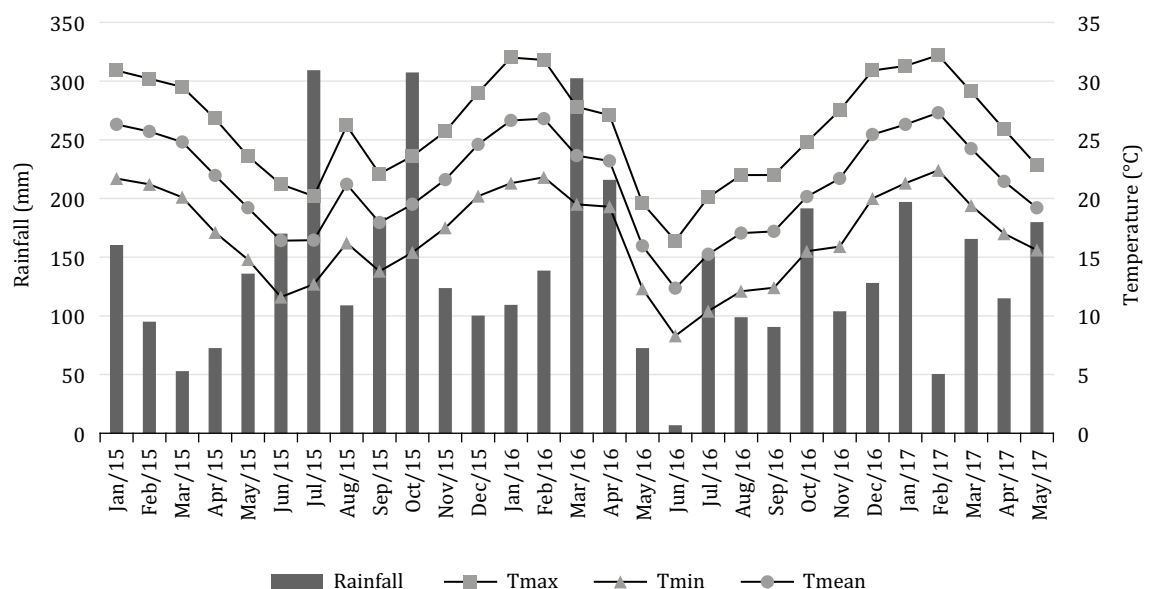
crop N deficiency applying high N fertilization levels (Silveira et al., 2015). Because of the increasing costs of commercial fertilizers and environmental problems, new fertilizer management strategies for Bahiagrass must aim at balancing agronomic requirements while reducing the risks of environmental contamination. Graminho et al. (2019) evaluated herbage accumulation, tiller density, and N use efficiency of four Bahiagrass hybrids (B26, B43, C9, and C22), ecotype Bagual and cv. Pensacola, in response to N fertilization (0, 60, 120, 240, and 480 kg N ha<sup>-1</sup>) for three agricultural years. The authors observed that hybrid C22 had high herbage accumulation and tiller density, and that level of 120 kg N ha<sup>-1</sup> provided better N use efficiency for Bahiagrass genotypes.

The grass-legume mixture can minimize the environmental impact, making it an efficient and economical alternative N supply for soil-plant-animal systems. This alternative can help increase herbage accumulation, reducing the use of synthetic N fertilizers, mitigating against climate change, and increasing the forage nutritional value (Lüscher et al., 2014). Recently, the use interspecific *Paspalum* hybrids mixed with temperate legumes has been highlighted for increasing herbage accumulation and improving the nutritional value of pasture (Motta et al., 2020a).

The strategy of mixing legumes with Bahiagrass provides an alternative to increasing N input in forage systems, as well as balancing and extending herbage accumulation during the year. Therefore, it is necessary to develop grass cultivars that have suitable establishment, productivity, and persistence, as well as ability to grow in mixture with legumes. This study was carried out to determine the genotypes of Bahiagrass most suitable for mixing with legumes and compare the productivity of mixed management with N supply management.

## 2. Material and Methods

The experiment was carried out in Eldorado do Sul, RS, Brazil (30°05' S, 51°39' W, and 34 m altitude). The climate in the region is Cfa, humid subtropical, according to the Köppen classification. The experiment was performed in agricultural years 2015/2016 (named year 1) and 2016/2017 (named year 2). The average monthly temperature and rainfall during the conduction of the experiment are presented in Figure 1. The soil of the experimental area was classified as typical dystrophic Red Argisol. Soil analyses were performed before implementation and during the evaluation years of the experiment. Soil characteristics are presented in Table 1.



**Figure 1** - Maximum (Tmax), minimum (Tmin), and mean (Tmean) monthly temperature (°C) and rainfall (mm) during the experimental period.

**Table 1** - Soil chemical characteristics in the evaluation years

| Year                      | pH               | OM  | Clay | SMP                 | P    | K     | S    | Ca                                 | Mg  | Al+H | CEC |
|---------------------------|------------------|-----|------|---------------------|------|-------|------|------------------------------------|-----|------|-----|
|                           | H <sub>2</sub> O | %   |      | mg dm <sup>-3</sup> |      |       |      | cmol <sub>c</sub> dm <sup>-3</sup> |     |      |     |
| Pre-implantation analysis | 5.5              | 1.5 | 22.0 | 6.5                 | 8.9  | 105.0 | 7.1  | 2.4                                | 1.0 | 2.5  | 6.2 |
| 2015/2016 analysis        | 5.5              | 1.4 | 24.0 | 6.5                 | 16.0 | 72.0  | 11.0 | 2.6                                | 1.1 | 2.5  | 6.4 |
| 2016/2017 analysis        | 5.8              | 1.5 | 17.0 | 6.6                 | 18.0 | 136.0 | -    | 3.0                                | 1.2 | 2.2  | 6.7 |

OM - organic matter; SMP - potential acidity estimated by SMP-pH (Shoemaker, McLean and Pratt); CEC - cation exchange capacity.

The apomictic intraspecific hybrids evaluated in this study were derived from artificial hybridizations and were named B26, B43, C22, and C9 (Weiler et al., 2018). The main plots were composed by hybrids B26, B43, C9, and C22, *P. notatum* ecotype Bagual and cv. Pensacola, described in detail in Graminho et al. (2019). The subplots were composed of five N fertilization levels (0, 60, 120, 240, and 480 kg<sup>-1</sup> N ha<sup>-1</sup>) and mixtures of Bahiagrass genotypes with white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*).

The preparation of clones began in September 2014, from seedlings obtained from the mother plant of each genotype. Clones were kept in a greenhouse in plastic bags with a commercial substrate. Planting (60 clones with a plant spacing of 20 cm) was carried out on 15 December 2014, as described in Graminho et al. (2019).

In the experimental area, 1.5 tons ha<sup>-1</sup> of limestone, 200 kg ha<sup>-1</sup> of triple superphosphate, and 100 kg ha<sup>-1</sup> of potassium chloride were applied for soil correction following the recommendations of the Comissão de Química e Fertilidade do Solo (CQFS) - RS/SC (2004). In the second year, 150 kg ha<sup>-1</sup> of triple superphosphate and 100 kg ha<sup>-1</sup> of potassium chloride (replenishment dose) were applied (CQFS - RS/SC, 2004). The legumes were overseeded on 04/10/15 and reseeded on 04/11/16. Seeds of white clover (*T. repens* cv. BRSURS Entreveiro) and birdsfoot trefoil (*L. corniculatus* cv. URSBRS Posteiro) were inoculated with specific rhizobia before sowing via the pelleting process. A seeding density of 8 and 20 kg ha<sup>-1</sup> was used for white clover and birdsfoot trefoil, respectively.

Nitrogen fertilizer (as ammonium sulfate) was applied in four equal amounts annually. Nitrogen was applied on 11/04/2015, 12/14/2015, 01/06/2016, and 02/26/2016 (year 1), and on 10/29/2016, 12/22/2016, 1/19/2017, and 02/17/2017 (year 2). Subplots where the hybrids were mixed with legumes did not receive mineral N.

The yield of genotypes was determined by harvesting in two areas of each subplot (placed randomly), delimited by a 0.25-m<sup>2</sup> frame, when average canopy height reached around 20 cm (variation = 17-23 cm). After each harvest, canopy height was monitored throughout regrowth, and a new harvest was conducted when the level of 480 kg N ha<sup>-1</sup> or the mixture with legumes reached 20 cm. A stubble height of 5 and 8 cm was maintained for N fertilization and mixture with legumes, respectively.

Dry matter (DM) content of samples was determined by drying the samples in an oven at 60 °C until constant weight. The accumulation of leaf blades, stems, and inflorescences of Bahiagrass, dead material, white clover, and birdsfoot trefoil were determined by separating the botanical and structural components. Herbage accumulation (grass DM + nitrogen or grass DM + legumes DM) and botanical (white clover and birdsfoot trefoil) and structural (leaf, stem, and inflorescence) components were determined from the sum of the DM produced in the harvests made in each evaluation year.

In year 1, the evaluations (harvest of plots) began on 08/14/2015 and ended on 04/28/2016. In year 2, the evaluations began on 08/22/2016 and ended on 04/24/2017. Cold tolerance was evaluated after the occurrence of frosts, on 06/24 and 07/19/2015 (year 1) and on 06/14 and 07/22/2016 (year 2). Visual scores were given for cold tolerance, using a scale of 1 to 5; 1 for lower and 5 for higher tolerance, according to Acuña et al. (2009).

The experiment used a randomized block design, with a subdivided plot structure, with genotypes as the main plots (14.4 m<sup>2</sup>), N fertilization levels or the presence of legumes as subplots (2.4 m<sup>2</sup>),

and year as sub-subplots, with three replicates of each. Following the analysis of a normality test, an analysis of variance was performed using SAS (Statistical Analysis System, version 9.2).

A mixed model was used, with genotypes, levels of N fertilization, or presence of legumes and their interactions as fixed effects, and residue, plots, and block-nested-within-genotypes as random effects. The model used was:

$$Y = \mu + a + \beta + \varepsilon + b + (ab) + e,$$

in which Y is the value observed in the genotype and fertilization,  $\mu$  is a constant, a is the effect of the genotype factor,  $\beta$  is the effect of the block,  $\varepsilon$  is the residual (a) of the plot, b is the effect of the fertilization factor, (ab) is the interaction between the genotype and fertilization factors, and e is the residual (b) of the subplot.

A structural selection test was performed using Bayesian information criterion (BIC) to determine the model that best fit the data. The interactions between genotypes and N fertilization levels or mixture with legumes (named as management) were further examined at significant level of 5%. Means were compared using the *LSmeans* procedure. Dunnett's test was used to compare means between presence of legume and fertilization levels.

### 3. Results

Herbage, leaf, and stem accumulation of Bahiagrass genotypes mixed with legumes showed a significant management  $\times$  year interaction ( $P < 0.05$ ; Table 2). There was no management  $\times$  year interaction for legume accumulation in mixed genotypes with white clover and birdsfoot trefoil

**Table 2** - Herbage accumulation and botanical and structural components production in the management of Bahiagrass genotypes in mixture with white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*), Rio Grande do Sul, Brazil

| Genotype  | Year 1               | Year 2       | Year 1            | Year 2       | Year 1            | Year 2      |
|-----------|----------------------|--------------|-------------------|--------------|-------------------|-------------|
|           | Herbage <sup>1</sup> |              | Leaf <sup>2</sup> |              | Stem <sup>3</sup> |             |
| B26       | 10558a (1079)        | 10570a (908) | 4853a (300)       | 5010a (211)  | 377c (140)        | 520b (146)  |
| B43       | 9317b (670)          | 11513a (519) | 2106b (312)       | 3981ab (444) | 718ab (40)        | 1811a (146) |
| C22       | 12046a (979)         | 9156b (183)  | 5615a (461)       | 3469b (321)  | 675b (110)        | 402b (112)  |
| C9        | 11540a (436)         | 10743a (763) | 5054a (625)       | 4110a (390)  | 652b (95)         | 290b (190)  |
| Bagual    | 12595a (661)         | 11643a (783) | 5450a (491)       | 5303a (440)  | 973a (20)         | 1248a (18)  |
| Pensacola | 10045ab (867)        | 9294b (537)  | 3078b (496)       | 3227b (394)  | 527c (50)         | 100b (29)   |
| CV (%)    | 7.4                  | 6.9          | 10.6              | 15.1         | 13.3              | 15.0        |
| Genotype  | Legumes <sup>4</sup> |              | WC <sup>5</sup>   |              | BT <sup>6</sup>   |             |
|           |                      |              |                   |              |                   |             |
| B26       | 4969 (497)           | 4344 (455)   | 3505 (352)        | 4090 (657)   | 1464 (394)        | 254 (131)   |
| B43       | 5475 (710)           | 3716 (650)   | 3874 (630)        | 3393 (657)   | 1601 (557)        | 323 (83)    |
| C22       | 5339 (720)           | 3790 (96)    | 3801 (612)        | 3517 (87)    | 1538 (118)        | 273 (13)    |
| C9        | 5440 (273)           | 4713 (623)   | 3720 (448)        | 4462 (631)   | 1720 (422)        | 251 (90)    |
| Bagual    | 5660 (174)           | 3910 (153)   | 3216 (287)        | 3582 (178)   | 2444 (674)        | 328 (35)    |
| Pensacola | 5761 (178)           | 4300 (186)   | 4047 (281)        | 3896 (170)   | 1714 (519)        | 404 (64)    |
| CV (%)    | 8.9                  | 10.5         | 8.4               | 11.6         | 16.3              | 15.9        |

CV - coefficient of variation; DM - dry matter.

Year 1 = 2015/2016; year 2 = 2016/2017.

<sup>1</sup> Herbage accumulation (kg DM ha<sup>-1</sup> year<sup>-1</sup>; Bahiagrass + white clover + birdsfoot trefoil).

<sup>2</sup> Leaf accumulation (kg DM ha<sup>-1</sup>; Bahiagrass).

<sup>3</sup> Stem accumulation (kg DM ha<sup>-1</sup>; Bahiagrass).

<sup>4</sup> Legume accumulation (kg DM ha<sup>-1</sup>; white clover + birdsfoot trefoil).

<sup>5</sup> White clover accumulation (kg DM ha<sup>-1</sup>).

<sup>6</sup> Birdsfoot trefoil accumulation (kg DM ha<sup>-1</sup>).

Averages followed by the same letters in the columns (mean standard error are shown in parentheses) did not differ according to the *LSmeans* test at 5% probability.

( $P > 0.05$ ; Table 2). However, there was a difference between the years for legume accumulation and birdsfoot trefoil accumulation ( $P < 0.05$ ; Table 2), which was not observed for white clover accumulation.

The rainfall observed during the first year of evaluation was 1658 mm, in the second year, it was 1157 mm (Figure 1). Average monthly temperature varied between 8.3 °C in June 2016 (coldest month) and 32.2 °C in February 2017 (hottest month; Figure 1).

### 3.1. Herbage accumulation

In year 1, herbage accumulation was higher where ecotype Bagual and hybrids C22, C9, and B26 were mixed with legumes; however, they were not statistically different from cv. Pensacola (Table 2). In year 2, herbage accumulation was higher where ecotype Bagual and hybrids B43, C9, and B26 were mixed with legumes and were statistically different than those of cv. Pensacola and hybrid C22 (Table 2).

In year 1, legume mixture with hybrids B26, C22, and C9 and ecotype Bagual had higher leaf accumulation when compared with hybrid B43 and cv. Pensacola (Table 2). In year 2, when ecotype Bagual and hybrids B26, B43, and C9 were mixed with legumes, they showed higher leaf accumulation than that of hybrid C22 and cv. Pensacola (Table 2).

In both years, ecotype Bagual and hybrid B43 showed the greatest stem accumulation. In year 1, the lowest stem accumulation was observed for hybrid B26 and cv. Pensacola, which were similar to each other; however, there was no difference from hybrids C9 and C22 in year 2 (Table 2).

### 3.2. Legume forage accumulation and cold tolerance

In year 1, legume proportion in mixed management ranged from 44% (hybrid C22) to 59% (hybrid B43) of herbage accumulation. In year 2, legume proportion ranged from 32% (hybrid B43) to 46% (Pensacola) of herbage accumulation.

In the evaluation of cold tolerance (data not shown) in Bahiagrass genotypes, a genotype  $\times$  year interaction ( $P < 0.05$ ) was verified. In year 1, ecotype Bagual showed greater cold tolerance with a score of 4.8. Hybrids B26 and C9 had an intermediate cold tolerance score (4.5) and were similar to each other. Hybrid B43 and cv. Pensacola had similar scores with a mean of 3.4. Finally, hybrid C22 presented a cold tolerance score (4.0) higher than hybrid B43 and cv. Pensacola and a lower score than the other genotypes. In year 2, hybrid C22 did not repeat the cold tolerance observed in the first year and had a score of 3.5, which was higher only than the cv. Pensacola (3.1) and lower than those observed for ecotype Bagual (4.7) and hybrids B26 (4.5), C9 (4.5), and B43 (4.4).

### 3.3. Nitrogen fertilization $\times$ legume intercropping

In year 1, ten and eight harvests were carried out in the management Bahiagrass mixed with legumes and Bahiagrass fertilized with nitrogen, respectively. In year 2, out eight harvests were carried out in the management Bahiagrass mixed with legumes and seven harvests in the management Bahiagrass fertilized with N.

In year 1, herbage accumulation in the management with hybrid B26 mixed with legumes was similar to the observed in the management that received N fertilization (Table 3). In the management where hybrid C9 and ecotype Bagual were mixed, herbage accumulation was similar to management receiving 240 and 480 kg N ha<sup>-1</sup> year<sup>-1</sup> (Table 3), respectively. In the management where cv. Pensacola was mixed, herbage accumulation was similar to the management that received 120, 240, and 480 kg N ha<sup>-1</sup> year<sup>-1</sup> (Table 3). In the management where hybrids B43 and C22 were mixed, herbage accumulation was similar to the management that received 120 and 240 kg N ha<sup>-1</sup> year<sup>-1</sup> (Table 3).

In year 2, herbage accumulation in the management where hybrids B26, B43, and C9 and ecotype Bagual were mixed with legumes was similar to the management that received 120 and 240 kg N ha<sup>-1</sup> year<sup>-1</sup>

**Table 3** - Herbage accumulation of Bahiagrass genotypes in mixture with white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*) or fertilized with nitrogen, Rio Grande do Sul, Brazil

| Genotype | Treatment        | Herbage accumulation (kg DM ha <sup>-1</sup> ) |        |        | Genotype  | Year 1 | Year 2 | Σyears |
|----------|------------------|--|--------|--------|-----------|--------|--------|--------|
|          |                  | Year 1   | Year 2 | Σyears |           |        |        |        |
| B26      | Leg <sup>1</sup> | 10558  | 10570  | 21128  | C9        | 11540  | 10743  | 22283  |
|          | N0               | 6732*  | 4487*  | 11219  |           | 7219*  | 5394*  | 12613  |
|          | N60              | 7694   | 6044*  | 13738  |           | 8334*  | 6519*  | 14853  |
|          | N120             | 8793   | 9328   | 18121  |           | 9558*  | 8440   | 17998  |
|          | N240             | 11209  | 11941  | 23151  |           | 11328  | 11160  | 22488  |
|          | N480             | 13036  | 15371* | 28407  |           | 13217  | 15813* | 29030  |
| B43      | Leg              | 9317   | 11513  | 20830  | Bagual    | 12595  | 11643  | 24238  |
|          | N0               | 5299*  | 4153*  | 9452   |           | 7339*  | 4897*  | 12236  |
|          | N60              | 6248*  | 6259*  | 12507  |           | 8788*  | 7032*  | 15820  |
|          | N120             | 7546   | 9131   | 16677  |           | 10130* | 9492   | 19622  |
|          | N240             | 9620   | 12775  | 22394  |           | 13211  | 12745  | 25956  |
|          | N480             | 13411*   | 15330* | 28741  |           | 14650  | 15141* | 29791  |
| C22      | Leg              | 12046  | 9156   | 21202  | Pensacola | 10045  | 9294   | 19339  |
|          | N0               | 7904*  | 5245*  | 13149  |           | 4649*  | 6197*  | 10846  |
|          | N60              | 9096*  | 6344   | 15440  |           | 6234*  | 6396   | 12630  |
|          | N120             | 11214  | 8307   | 19520  |           | 7927   | 8339   | 16266  |
|          | N240             | 14401  | 12648* | 27049  |           | 7949   | 9928   | 17877  |
|          | N480             | 15481*   | 15436* | 30917  |           | 11290  | 11837  | 23127  |

DM - dry matter.

Year 1 = 2015/2016; year 2 = 2016/2017; Σyears = year 1 + year 2.

<sup>1</sup> Mixed management = Bahiagrass genotypes + white clover (*Trifolium repens*) + birdsfoot trefoil (*Lotus corniculatus*).

\* Means differ significantly from the treatment of legumes by Dunnett's test at 5% probability.

(Table 3). Herbage accumulation of hybrid C22 in the management with legumes was similar to the management that received 60 and 120 kg N ha<sup>-1</sup> year<sup>-1</sup>, while cv. Pensacola had herbage accumulation similar to all managements that received N fertilization (Table 3).

## 4. Discussion

As previously reported, Bahiagrass genotypes described in this study were also tested for herbage accumulation and N use efficiency (Graminho et al., 2019). In turn, in this study, we compared herbage accumulation of genotypes when mixed with legumes or subjected to different N levels. In addition, we evaluated which genotypes are best suited for mixing with legumes.

### 4.1. Herbage accumulation

Hybrids B26 and C9 and ecotype Bagual remained among the most productive in year 2, whereas cv. Pensacola and hybrid C22 did not (Table 2). Hybrid B26 had herbage accumulation of 14% and leaf accumulation of 63% greater than cv. Pensacola in year 2. Hybrid C9 showed herbage accumulation of 16% and leaf accumulation of 34% greater than cv. Pensacola in year 2 (Table 2). The lower temperatures during winter (June and July) and lower rainfall in year 2 (Figure 1) may have influenced the yield of cv. Pensacola and hybrid C22, indicating that these genotypes may be less tolerant to these types of abiotic stresses. Pensacola is one of the few warm-season cultivars commercialized as seeds and adapted to the climatic conditions of southern Brazil. Therefore, hybridization provided a new genetic resource with greater herbage accumulation and resistance to some abiotic stresses than the current cultivar of *P. notatum*.



In year 1, leaf accumulation represented between 23% (hybrid B43) and 47% (hybrid C22) of herbage accumulation, while in year 2, leaf accumulation represented between 35% (hybrid B43) and 47% (hybrid B26) of herbage accumulation. It is important to highlight that hybrid B26 presented approximately half of herbage accumulation with leaves in the 2 years. Genotype selection with high leaf accumulation is important, as leaf blades are the organs with best nutritional quality and are the structures preferentially consumed by animals (Bratti et al., 2009).

In year 1, stem accumulation represented between 6% (hybrid B26) and 17% (hybrid B43) of herbage accumulation. In year 2, stem accumulation represented between 2% (Pensacola) and 23% (hybrid B43) of herbage accumulation (Table 2). In general, ecotype Bagual and hybrid B43 showed the highest proportion of DM composed of stems, which is not desirable for forage species selected for improved forage. On the other hand, hybrids B26, C22, and C9 had stem accumulation 42% lower than ecotype Bagual, indicating that hybridization improved the structural characteristics of these plants, resulting in reduction of components less desirable for grazing animals.

#### 4.2. Legume forage accumulation and cold tolerance

Considering that there was no difference between legume accumulation in the mixed management in year 1, the variation in herbage accumulation in this management might have occurred due to the herbage accumulation of Bahiagrass genotypes. Hybrid B43 and cv. Pensacola failed to maintain a high presence in the mixture, which resulted in lower herbage accumulation, even though it had a high proportion of legumes. In year 2, there was a 25% reduction in legume accumulation (Table 2). This change might have occurred in part from the 83% reduction in birdsfoot trefoil accumulation in the composition of legume accumulation (Table 2), which indicates that even with reseeding in year 2, the proportion of birdsfoot trefoil was not maintained from year 1. According to Gierus et al. (2012), birdsfoot trefoil usually has low perennation and variation in maintaining its proportion when mixed. White clover accumulation remained stable over the years, and higher accumulation was usually observed in the fall-winter (data not shown). These findings suggest that mixtures with legumes can be an alternative to supply the reduction in Bahiagrass herbage accumulation in the fall-winter period, mainly with white clover species.

In the sum of two years, all mixed managements showed proportion of legumes between 30 and 50%, which according to Lüscher et al. (2014), provides many advantages, such as increased herbage accumulation in the system, increased N transfer from fixing plants to non-fixing plants, and reduction of greenhouse gas emissions and energy costs, as N from biological fixation replaced that from mineral N. In addition, they also contributed to the increase in nutritional value and voluntary consumption, with less quality lost as the grass phenological stage advanced, resulting in higher productive performance.

During the evaluation period, it was observed that Bahiagrass herbage accumulation was concentrated in the summer and was drastically reduced during the winter. Bahiagrass grows vigorously under high temperature and long days, and more than 85% of the accumulation occurs during the warmest months (Newman et al., 2014). Our results showed that the most productive hybrids also had a higher cold tolerance, being superior to cv. Pensacola in both evaluated traits. According to Saraiva et al. (2021), the cold tolerance trait is essential in subtropical regions, because more tolerant genotypes may have greater persistence and a greater supply of forage for animals grazing during the winter, along with faster regrowth in the spring.

#### 4.3. Nitrogen fertilization × legume intercropping

The management Bahiagrass fertilized with nitrogen showed forage supply from September and October during the first and second year of cultivation, respectively. These results are in agreement with Sinclair et al. (2003), who reported that Bahiagrass have dormancy induced by a reduced photoperiod, which is the factor that negatively influences seasonal yield, besides the low temperatures that occur during the fall-winter period. On the other hand, in both years, the

management Bahiagrass mixed with legumes provided a greater number of harvests, as well as a supply of forage during the winter period (August). These findings indicate that mixed management with legumes can increase productivity and improve the distribution of forage throughout the year, benefiting livestock production in subtropical regions.

Our results showed that the genotypes responded differently in the two years. Hybrids B26 and C9 and ecotype Bagual had similar herbage accumulation in the mixed management and in the management with 480 kg N ha<sup>-1</sup> in year 1 and different in year 2. Hybrids B43 and C22, in both years, showed difference between the mixed management and the management with 480 kg N ha<sup>-1</sup>, which was not observed for cv. Pensacola. Nitrogen has an effect on crop growth and physiological processes and determines plant productivity (Seepaul et al., 2016). Therefore, different responses suggest that genotypes showed differences in the ability to acquire and use N for herbage accumulation. In addition, the greater herbage accumulation in the management with 480 kg N ha<sup>-1</sup> compared with the mixed management may be due to the cumulative effect of high N levels for two years, which provided favorable growth conditions, reducing the competition between plants for nutrient and increasing their capacity for growing (Motta et al., 2020b).

On the other hand, results observed in this study showed that the mixed management had a productive potential similar to the system with 240 kg N ha<sup>-1</sup>. This result was superior to that observed by Motta et al. (2020b), who evaluated interspecific *Paspalum* hybrids in mixed management with legumes and management that received N fertilization. The authors reported that mixture with legumes can replace N levels between 60 and 120 kg N ha<sup>-1</sup>. Therefore, our findings indicate that mixture with legumes is a viable alternative to replace the application of synthetic N in pasture-based production systems.

In the literature, there are no studies evaluating herbage accumulation of mixed management with legumes and management fertilized with high N levels for Bahiagrass species. Based on the results of the present study, we believe that mixtures of Bahiagrass and legumes in subtropical regions is viable and could be a management technique to balance production over the agricultural year. Thus, the proposed mixed management can contribute to the formation of efficient pastures, attend to various levels of exploitation intensity, increase quality and diversification of the diet consumed by animals, and work collectively to reduce the direct expenses of fertilizers, bringing greater sustainability to forage systems by reducing production costs and environmental problems.

## 5. Conclusions

Considering the two years of study, hybrids B26 and C9 and ecotype Bagual were identified as potential materials to be used in mixed management with temperate legumes due to their herbage and leaf accumulation and cold tolerance. In addition, these genotypes are superior to cv. Pensacola in these evaluated variables.

Birdsfoot trefoil is not recommended for a mixture with Bahiagrass and white clover due to its low persistence and proportion in herbage accumulation.

Herbage accumulation in the management Bahiagrass mixed with legumes is similar to the management Bahiagrass fertilized with up to 240 kg N ha<sup>-1</sup>, showing the viability of mixture with legumes to balance herbage accumulation over the year in an economical and sustainable way.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: E.A.M. Motta, M. Dall'Agnol, C. Nabinger, C.H.L. Souza and R.L. Weiler. Data curation: L. Pötter. Formal analysis: L.A. Graminho and L. Pötter. Funding acquisition: M. Dall'Agnol. Investigation:



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