### RESEARCH

### Herbage Accumulation, Nutritive Value, and Organic Reserves of Continuously Stocked 'Ipyporã' and 'Mulato II' Brachiariagrasses

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#### ABSTRACT

Although Brachiaria spp. grasses are important components of sustainable forage-livestock systems in the Amazon biome, cultivar diversification is needed to reduce risk from pests and diseases. Brachiaria hybrid 'BRS RB331 Ipyporã' [B. ruziziensis Germ. & Evrard × B. brizantha (Hochst. ex A. Rich.) Stapf] was released in 2017 as an alternative for intensively managed forage-livestock systems. Our objective was to compare herbage accumulation (HA), nutritive value, and organic reserves of lpyporã and standard hybrid 'Mulato II' (B. ruziziensis  $\times$  *B. brizantha*  $\times$  *B. decumbens* Stapf) under continuous stocking during 2 yr in the Amazon biome. Treatments were the two cultivars replicated four times in a randomized complete block design, and each experimental unit was 1.5 ha. Pastures of Mulato II presented  $\sim$ 15% greater HA than Ipyporã (17,360 vs. 14,930 kg dry matter ha<sup>-1</sup> yr<sup>-1</sup>) across the 2 yr, and Mulato Il leaf mass was greater than Ipyporã (1440 vs. 1900 kg dry matter ha<sup>-1</sup>) in the dry season. Both cultivars had greater herbage mass, HA, and herbage bulk density during the rainy season of 2016-2017 compared with 2017-2018 due to a shorter period of water deficit (30 d) and greater rainfall (2147 vs. 1762 mm) in the first than second year. Mulato II herbage crude protein was 10 g kg<sup>-1</sup> greater than Ipyporã. In this severe risk region for spittlebug, Mulato II required spittlebug monitoring and control due to occurrence of foliar damage. Although Ipyporã had lesser HA, no spittlebug damage was evident. Thus, Ipyporã is an attractive alternative for diversification of forage-based livestock systems in the Amazon biome.

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**Abbreviations:** ADF, acid detergent fiber; CP, crude protein; HA, herbage accumulation; HAR, herbage accumulation rate; HBD, herbage bulk density; HM, herbage mass; LAI, leaf area index; NDF, neutral detergent fiber; TNC, total nonstructural carbohydrate.

**B**<sub>RAZIL</sub> is one of the primary beef-producing and -exporting countries in the world. The production system is mostly forage based, and the largest livestock herd in the world (215 million head) relies on  $\sim$ 163 million ha of grasslands (ABIEC, 2019). In addition, only 12.6% of the slaughtered animals come from feedlots (ABIEC, 2019). The forage-based system reduces the production cost and brings competitive advantages to Brazil compared with countries where beef production is highly dependent on feedlots that are labor intensive and depend heavily on expensive equipment, concentrate feeds, and fossil fuel (Dias-Filho, 2014).

Despite the importance of grazed pastures,  $\sim$ 70% of Brazilian planted pastures are in some stage of degradation (Macedo et al., 2013), which negatively affects system sustainability (Pequeno et al., 2015). Among the main causes of pasture degradation are the incompatibility of the grass with the local environment and management practices used (Dias-Filho, 2014). Forage breeding programs are important tools for generating new cultivars (i.e., greater productivity, quality, and pest and disease resistance), supporting the release of plants that are well adapted to specific

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edaphic and climatic conditions (Valle et al., 2009). Despite the efforts to release new grasses adapted to the different production systems, forage cultivar diversity is still relatively low in Brazilian pasture-based systems (Euclides et al., 2018).

The Brachiaria (syn. Urochloa) grasses are the most used in pasture-based systems in Brazil (Silva et al., 2016b) because they are more productive and present greater nutritive value, contributing to enhanced animal productivity (Silva et al., 2016a). In this scenario, diversification within brachiariagrasses could contribute to development of more sustainable systems, providing animal products while contributing a wide array of ecosystem services to society (Sollenberger et al., 2019). In 2017, the Brachiaria hybrid 'BRS RB331 Ipyporã' was released for use in intensively managed production systems (i.e., those characterized by practices to significantly enhance forage production) where spittlebug [Deois flavopicta, Notozulia entreriana, and Mahanarva spp. (Hemiptera: Cercopidae)] is a challenge, due to its resistance through an antibiosis mechanism (Valério et al., 2012). Moreover, Ipyporã has a greater leaf percentage, leaf/stem proportion, and nutritive value than 'Marandu' palisadegrass [Brachiaria brizantha (Hochst. Ex A. Rich.) Stapf.], and it supports similar animal performance as Marandu, the most widely planted cultivar in Brazil (Euclides et al., 2018).

Although Ipyporã is considered to be an excellent alternative for pasture diversification (Euclides et al., 2018), there are few existing comparisons with cultivars already known by producers. One cultivar that has been used in the region is 'Mulato II' brachiariagrass, a hybrid released in 2005 by the International Center for Tropical Agriculture (CIAT) (Argel et al., 2007). Mulato II provides excellent herbage accumulation (HA) and nutritive value at least as great as that of warm-season annual grasses (Vendramini et al., 2012). In addition, it possesses a physiological mechanism that reduces water loss during the dry season, increasing the opportunity to provide green herbage in areas with longer dry periods (Cardoso et al., 2015). Although Mulato II is resistant to several spittlebug species (Argel et al., 2007), it is susceptible to damage by Mahanarva spp., a species common in the Amazon biome. If Mulato II is to be used in this environment, spittlebug control is generally required.

This presents opportunities for increased adoption of Ipyporã, but more information is needed on year-round patterns of forage accumulation and nutritive value to assess its potential to improve efficiency of forage-based livestock systems under favorable soil and climatic conditions of the Brazilian midwest (Pequeno et al., 2015). We hypothesized that Ipyporã will enhance or maintain pasture HA and nutritive value when compared with Mulato II pastures in the Amazon biome, where *Mahanarva* spp. spittlebug is a highly prevalent pest. To test this hypothesis, we compared HA, nutritive value, and organic reserves of Ipyporã and Mulato II pastures for 2 yr under continuous stocking.

### MATERIALS AND METHODS Site Characteristics and Fertilization Management

The research was performed at Embrapa Agrossilvipastoril, Sinop, Mato Grosso, Brazil (11°51′ S, 55°35′ W; 370 m asl), in the Amazon biome, according to ethical standards and approved by the Ethics Committee on Animal Use (Protocol no. 008/2016). The climate was classified according to Köppen criteria as a monsoon that alternates between rainy and dry seasons (Alvares et al., 2013). The annual average temperature was 25°C, with an average minimum of 20°C and an average maximum of 33°C (Embrapa, 2018). The average annual rainfall is 1974 mm (1971– 2010) (Souza et al., 2013). Weather data during the experimental period were obtained from a recording station located 500 m from the experiment site (Fig. 1).

The soil was classified as a Rhodic Hapludox (Soil Science Division Staff, 2017) with soil texture characterized by 358 g sand kg<sup>-1</sup>, 574 g clay kg<sup>-1</sup>, and 68 g silt kg<sup>-1</sup>. Soil chemical analysis was performed in September 2015, August 2016, September 2017, and May 2018 (Table 1).

On 3 Sept. 2015, dolomitic lime was incorporated (2.0 Mg  $ha^{-1}$ ). On 6 Jan. 2016, during planting, 20 kg P  $ha^{-1}$  was incorporated into the soil. For both cultivars, 5 kg  $ha^{-1}$  of pure live seed was sown using a no-tillage drill (Stara Prima 4590). Row spacing was 45 cm, but to assure rapid establishment and uniform cover, two passes of the seeder (2.5 kg  $ha^{-1}$  of pure live seed with each pass) were made over the entire pasture area, once across the length and the other across the width of each experimental unit. To improve pasture establishment, 50 kg N  $ha^{-1}$  and 40 kg K  $ha^{-1}$  were surface applied on 15 Mar. 2016.

In the Amazon biome, tropical grasses establish rapidly. The first grazing should occur 45 to 80 d after planting (Dias-Filho, 2012), because thereafter, grazing management is challenging due to excessive herbage mass (HM) and stem and dead material accumulation (Pedreira et al., 2017). The pastures in this study were considered fully established by 2 Apr. 2016 (87 d after planting). Grazing began to achieve the target canopy heights and to maintain the swards in a steady state condition before initiating data collection on 24 May (139 d after planting).

Throughout the experimental period, 1.5 Mg ha<sup>-1</sup> of dolomitic lime (10 Oct. 2016), 20 kg P ha<sup>-1</sup>, 50 kg N ha<sup>-1</sup>, and 40 kg K ha<sup>-1</sup> (26 Jan. 2017) were surface applied in the first year, and 20 kg P ha<sup>-1</sup> (19 Oct. 2017), 50 kg N ha<sup>-1</sup>, and 40 kg K ha<sup>-1</sup> (1 Feb. 2018) were surface applied in the second year. Before and during the experiment, N, P, and K were applied using urea, single superphosphate, and potassium chloride, respectively.

### **Treatment Description**

The experimental period was 24 May 2016 to 25 May 2018, comprising rainy (1 Oct. 2016–31 Mar. 2017 and 1 Oct. 2017–31 Mar. 2018) and dry seasons (26 May–30 Sept. 2016, 1 Apr.–30 Sept. 2017, and 1 Apr.–25 May 2018). The experimental design was a randomized complete block with two cultivars (treatments)—Ipyporã [*B. ruziziensis* Germ. & Evrard × *B. brizantha* (Hochst. ex A. Rich.) Stapf hybrid] Mulato II (*B. ruziziensis* × *B. brizantha* × *B. decumbens* Stapf hybrid)—and four replicates, totaling eight experimental units. Each experimental unit was 1.5 ha (150 × 100 m), for a total of 12 ha of experimental area.



Fig. 1. Water balance and weather data throughout the experimental period.

The pastures were monitored for spittlebug presence. The number of nymphs was counted at three 1- by 0.5-m sites per experimental unit at sites representing average pasture condition (Fig. 2). On 16 Jan. 2017, due to severe spittlebug damage in Mulato II, Engeo  $(0.3 \text{ L ha}^{-1})$  was applied to all experimental units.

### Canopy Height, Livestock Management, and Herbage Evaluation

Starting on 2 Apr. 2016, Ipyporã and Mulato II pastures were continuously stocked with a variable stocking rate. The canopy height was monitored weekly at 75 points per experimental unit to maintain an annual average canopy height of  $30 \pm 5.0$  cm (Silva et al., 2016a). Canopy height was maintained at  $29 \pm 3.0$  cm for Ipyporã and  $29 \pm 4.2$  cm for Mulato II pastures throughout the experimental period. Within a year and season, heights for the two cultivars were virtually identical.

At the beginning of the experimental period, Nellore steers (Bos taurus indicus, initial body weight of  $250 \pm 11$  kg and age of  $11 \pm 2$  mo) were allocated to experimental units according to weight and age. In May 2017, the first group was slaughtered and new steers were allocated ( $276 \pm 12$  kg of body weight and  $14 \pm 2$  mo). Throughout the evaluation period, mineral salt was offered to the animals ad libitum. The animals were allocated to two groups: testers and regulators. They were weighed every 28 d after a 16-h feed and water fast for performance evaluation and stocking adjustment (data not shown). Three tester animals remained in the pasture throughout the entire

experimental period, and the regulating animals were used to adjust the stocking rate and maintain the target canopy height.

Herbage mass and HA were assessed every 28 d. Herbage bulk density (HBD) was calculated as the quotient of HM and the canopy height at the time of measurement. The HA was determined using the paired-cage method (Klingman et al., 1943). Specifically, circular exclusion cages (0.64 m<sup>2</sup> and 1.1 m in height) were placed at four representative sites per pasture. At the same time, the HM-to-soil level was measured in a circular 0.64-m<sup>2</sup> quadrat just outside each caged area. Twenty-eight days later, the HM to soil level was determined inside the cage. Pasture HA was calculated as the difference between the HM in the cage 28 d after placement, and the HM measured in the pasture on the day that cages were allocated. New sites for cage placement were chosen every 28 d. At two sampling dates in each rainy (11 Oct. 2016, 2 Feb. 2017, 10 Oct. 2017, and 2 Feb. 2018) and dry season (19 July 2016, 27 Apr. 2017, 20 July 2017, and 27 Apr. 2018), four 0.64-m<sup>2</sup> circular quadrats per pasture were clipped to soil level at representative sites to characterize plant-part composition (leaf blade, sheath + pseudostem, and dead material) of the HM. After separation, the herbage was dried at 55°C in a forced-air dryer until constant weight and weighed.

Nutritive value was assessed on the same dates as plantpart composition. At 30 sites per experimental unit, forage was hand plucked to represent the portion of the canopy grazed by animals. The forage from the 30 sites was composited and a subsample was separated into leaf blade, sheath + pseudostem,

Table 1. Results of soil analysis (0- to 20-cm layer) at the experimental area.

Year	pH (CaCl <sub>2</sub> )	OM†	P (Mehl‡)	К	Ca	Mg	H + Al	CEC§	BS¶
		g dm <sup>-3</sup>	mg d	m <sup>-3</sup> ——		cmol	dm-3		%
2015	4.78	28.1	8.63	77.0	2.07	0.93	6.4	9.6	33.4
2016	4.88	24.7	8.03	120.0	2.13	0.96	5.1	8.5	39.4
2017	5.28	29.8	7.92	31.2	1.23	0.73	5.6	7.6	26.5
2018	5.05	30.7	8.32	25.8	0.85	0.71	5.8	7.8	24.6

+ OM, organic matter.

‡ Mehl, Mehlich 1.

§ CEC, cation exchange capacity.

¶ BS, base saturation.



Fig. 2. Number of nymphs in Ipyporã and Mulato II pastures.

and dead material. Components were dried separately at 55°C in a forced-air oven to constant weight and weighed (Nave et al., 2010). Dried samples were ground in a Wiley mill to pass a 1-mm screen and used for determining crude protein (CP) (AOAC, 1990), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (Van Soest et al., 1991).

### Tussock Number, Root Mass, and Organic Reserves

In April 2017 and May 2018, the number of tussocks ("an erect and clumped growth form"; Allen et al., 2011) inside a 1- by 0.5-m quadrat was counted at 10 sites per experimental unit that represented average pasture conditions. To characterize organic reserve status, root and aboveground stubble samples of three representative tussocks were taken in each year. The aboveground herbage of the three tussocks was cut at soil level, combined into a single sample, and dried at 105°C for 1 h and then at 60°C to constant weight. Soil core samples were collected to a 0.20-m depth at the base of the three representative tussocks so as to remove all root material to that depth. The soil core samples were washed on 2.1-mm sieves, and the roots of the three tussocks were combined and dried following the same drying procedure. All dried samples were later ground in a Wiley mill to pass a 1-mm screen (Pedreira et al., 2017).

Nitrogen concentration in the shoot and root samples was determined using the AOAC method (AOAC, 1990). Total nonstructural carbohydrate (TNC) concentration was determined by a modification of the procedure of Smith (1981), as described by Christiansen (1982), which combines an enzymatic digestion phase for conversion of starch and oligosaccharides into monosaccharides with a photometric Cu reduction method for reducing sugars. The TNC and N concentrations were multiplied by the respective fraction dry mass to calculate the TNC and N pools.

### **Statistical Analyses**

The cultivars were compared annually and seasonally (rainy and dry). For the latter, the analyses were performed using a split-plot in time, with cultivars as the main plot treatment and seasons as the subplot. Cultivar, season, and year were considered fixed effects, and block was considered as a random effect. The data were analyzed using a mixed models

method with parametric structure in the covariance matrix, through the MIXED procedure of SAS 9.4 (Littell et al., 2006) with repeated measurements and using the maximum likelihood restricted method (REML). Linear predictor and quantile-quantile plots of the residues were used to verify homogeneity of variance and error normality. The covariance matrix structure was selected considering the number of parameters, the interpretation of the structure, and the fixed effects results. The command "TYPE" specifies the covariance matrix structure (e.g., Toeplitz [TOEP], autoregressive [AR(1)], heterogeneous autoregressive [ARH(1)], compound symmetric [CS], heterogeneous compound symmetric [CSH], Huynh-Feldt [HF], first-order autoregressive [ARMA(1,1)], unstructured [UN], etc.]. The Akaike information criterion (AIC) was used to choose the covariance matrix (Wolfinger, 1993), and the denominator degrees of freedom was corrected using the method of Satterthwaite (1941). The least squares means (LSMEANS) statement was used to compute the means of the fixed effects and comparison was performed using the probability of the difference (PDIFF) of the *t* test (P < 0.05).

### RESULTS

### **Annual Herbage Responses**

There were no cultivar × year interactions for total annual HA (P = 0.8272) and average annual herbage accumulation rate (HAR, P = 0.4769); however, these responses differed among cultivars (P = 0.0013 and P = 0.0053, respectively) and years (P < 0.0001 and P < 0.0001) (Table 2). The HA and HAR were 15% greater for Mulato II than Ipyporã and 65% greater in the first year than in the second.

### **Seasonal Canopy Characteristics**

Herbage mass was affected by cultivar  $\times$  year (P = 0.0079) and year  $\times$  season (P = 0.0399) interactions (Table 3). The greatest HM was measured in the Ipyporã pastures during the first year; however, in the second year, Mulato II had greater HM than Ipyporã. The HM did not differ across seasons in the first year. However, in the second year, HM was lowest in the rainy season, 25% lower than in the rainy season of the first year. The HM for cultivars and seasons was greater in the first year compared with the second year.

There were cultivar  $\times$  year (P = 0.0001) and year  $\times$  season (P = 0.0032) interactions for HBD (Table 3). Ipyporã had greater HBD in the first year than in the second year and Mulato II did not differ across years. In both years, the HBD was greater during the dry season. In the rainy season, the HBD was 10% lower in the second year than in the first year.

There was cultivar × year × season interaction for the HA (P = 0.0143) and HAR (P = 0.0003) (Table 4). In the rainy season of the first year, Mulato II presented greater HA and HAR than Ipyporã (19 and 18%, respectively). In the second year, there was no difference between cultivars in the rainy season, when the greatest HA typically occurs. Also in the second year, Mulato II presented greater HA and HAR than Ipyporã during the dry season. From the first to the second year, during the rainy season, HA decreased 44 and 49% for Ipyporã and Mulato II, respectively.

There were cultivar  $\times$  year (P = 0.0041), cultivar  $\times$  season (P = 0.0003), and year  $\times$  season (P = 0.0478) interactions for leaf mass (Table 5). The greatest leaf mass was measured in the first year for both cultivars and in the rainy vs. the dry season. In the dry season, Ipyporã presented less leaf mass than Mulato.

The stem mass was affected by cultivar  $\times$  year  $\times$  season interaction (P = 0.0008), with greater values in the first than in the second year for both cultivars and seasons (Table 6). In the first year, both cultivars presented greater values during the dry season; however, during the second year, Ipyporã had similar stem mass across seasons.

Dead material mass differed across years (P = 0.0003) and seasons (P = 0.0004), but there was no difference between cultivars (P = 0.7437). In the second year, the dead material mass was greater (3510 kg ha<sup>-1</sup>) than in the first year (2400 kg ha<sup>-1</sup>), as well in the dry season (3480 kg ha<sup>-1</sup>) compared with the rainy season (2430 kg ha<sup>-1</sup>).

There were cultivar  $\times$  year (P < 0.0493), cultivar  $\times$  season (P = 0.0001), and year  $\times$  season (P = 0.0301) interactions for leaf proportion (Table 7). For both cultivars, the greatest leaf proportion occurred in the first year and in the rainy season. The leaf proportion was less in the second year than the first year in both seasons.

There was cultivar  $\times$  year  $\times$  season interaction for leaf area index (LAI, P = 0.0118, Table 8). In the dry season, Ipyporã presented greater LAI in the first year than in the second year, and Ipyporã LAI in the dry season was 36% less than Mulato II in the second year.

Table 2. Average and SE for herbage accumulation (HA) and herbage accumulation rate (HAR) of Ipyporã and Mulato II pasture	s
under continuous stocking during dry and rainy seasons of 2 yr.	

		Cult	ivars		
Response	Year	Ipyporã	Mulato II	Avg.	SE
HA (kg dry matter ha <sup>-1</sup> yr <sup>-1</sup> )	1	18,960	21,300	20,130A†	450
	2	10,900	13,450	12,180B	
	Avg.	14,930b	17,360a		
HAR (kg ha <sup>-1</sup> d <sup>-1</sup> )	1	60	70	65A	1.5
	2	35	40	38B	
	Avg.	48b	55a		

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

Table 3. Average and SE for herbage mass	; (HM) and herbage b	oulk density (HBD) o	of Ipyporã and Mulato	o II pastures under
continuous stocking during dry and rainy se	asons of 2 yr.			

Variable	Year	Ipyporã	Mulato II	Dry	Rainy	SE
HM (kg dry matter ha <sup>-1</sup> )	1	6905Aa†	6300Ab	6640Aa	6560Aa	135
	2	5315Bb	5820Ba	6235Ba	4900Bb	
HBD (kg dry matter ha <sup>-1</sup> cm <sup>-1</sup> )	1	230Aa	210Ab	230Aa	205Ab	5
	2	195Bb	215Aa	225Aa	185Bb	
	2					

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

### Table 4. Average and SE for herbage accumulation (HA) and herbage accumulation rate (HAR) of Ipyporã and Mulato II pastures under continuous stocking during dry and rainy seasons of 2 yr.

			orã	Mulato II			
Variable	Year	Dry	Rainy	Dry	Rainy	SE	
HA (kg dry matter ha <sup>-1</sup> )	1	5.550Ac†	14.880Ab	6.720Ac	17.770Aa	460	
	2	4.500Ac	8.360Ba	6.500Ab	9.000Ba		
HAR (kg dry matter ha <sup>-1</sup> d <sup>-1</sup> )	1	32Ad	77Ab	40Ac	91Aa	2	
	2	26Ac	44Ba	37Ab	45Ba		

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

The stem proportion was affected by cultivar  $\times$  year  $\times$  season interaction (P = 0.0005, Table 8). Across seasons and among cultivars, the stem proportion was greater in the first year compared with the second year. In the first year, Ipyporã had the greatest stem proportion during the dry season and, in the second year, there was no difference between seasons or cultivars.

There were cultivar  $\times$  year (P = 0.0006) and cultivar  $\times$  season (P = 0.0001) interactions for leaf/stem ratio. Both cultivars presented greater leaf/stem ratio in the second year (on average, 1.3) compared with the first year (on average, 0.9) and in the rainy season compared with the dry season. Mulato II had a greater ratio (1.2) than Ipyporã (0.75) during the dry season, but in the rainy season, Ipyporã presented a greater leaf/stem ratio than Mulato (1.5 vs. 1.3, respectively). The dead material proportion was affected by cultivar  $\times$  year  $\times$  season interaction (P = 0.0001, Table 8). For both cultivars, the greatest dead material proportion occurred in the dry season and increased from the first to the second year. Ipyporã had greater dead material than Mulato II in the dry season of the second year.

## Hand-Plucked Plant-Part Composition and Nutritive Value

There was cultivar  $\times$  year  $\times$  season interaction for leaf (P < 0.0057) and stem proportion (P = 0.0006) (Table 9). The hand-plucked leaf proportion was less in the second than in the first year for both cultivars during the dry season. The hand-plucked leaf proportion was twofold greater than the HM leaf proportion. Both cultivars had the greatest hand-plucked stem proportion during the

Table 5. Average and SE for leaf mass in Ipyporã and Mulato II pastures under continuous stocking during dry and rainy seasons of 2 yr.

Year or season	lpyporã	Mulato II	SE	Dry	Rainy	SE
	kg dry ma	atter ha-1		– kg dry m	atter ha <sup>-1</sup>	
Year 1	2400Aa†	2205Aa	85	2030Ab	2575Aa	77
Year 2	1210Bb	1645Ba		1315Bb	1545Ba	
Dry	1435Bb	1900Aa	85			
Rainy	2180Aa	1940Ab				

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

Table 6. Average and SE for stem mass in Ipyporã and Mulato II pastures under continuous stocking during dry and rainy seasons of 2 yr.

lpyp	orã	Mula	ato II	
Dry	Rainy	Dry	Rainy	SE
	kg	ha-1	· · · · · · · · · · · · · · · · · · ·	
2920Aa†	1720Ac	2080Ab	1780Ac	105
920Bab	740Bb	1170Ba	840Bb	
	<b>Dry</b> 2920Aa† 920Bab	Ipyporã           Dry         Rainy           2920Aa†         1720Ac           920Bab         740Bb	Ipyporã         Mula           Dry         Rainy         Dry           2920Aa†         1720Ac         2080Ab           920Bab         740Bb         1170Ba	Ipyporã         Mulato II           Dry         Rainy         Dry         Rainy           2920Aa†         1720Ac         2080Ab         1780Ac           920Bab         740Bb         1170Ba         840Bb

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

Table 7. Average and SE for leaf proportion of tot	al herbage mass of	Ipyporã and Mulato II	l pastures under	continuous stocking
during the dry and rainy seasons of 2 yr.				

Year or season	lpyporã	Mulato II	SE	Dry	Rainy	SE
	g k	(g <sup>-1</sup>		g k	(g <sup>-1</sup>	
Year 1	370Aa†	375Aa	7	315Ab	430Aa	7
Year 2	305Bb	345Ba		250Bb	400Ba	
Dry	240Bb	325Ba	7			
Rainy	430Aa	395Ab				

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

### Table 8. Average and SE for leaf area index (LAI) and stem and dead material proportion in Ipyporã and Mulato II pastures under continuous stocking during dry and rainy seasons of 2 yr.

		Ipyporã		Mula		
Response	Year	Dry	Rainy	Dry	Rainy	SE
LAI	1	5.1Aa†	5.7Aa	4.9Aa	5.9Aa	0.4
	2	3.4Bb	6.2Aa	5.3Aa	5.4Aa	
Stem (g kg <sup>-1</sup> )	1	445Aa	360Ac	375Ac	405Ab	11
	2	235Ba	225Ba	255Ba	250Ba	
Dead material (g kg <sup>-1</sup> )	1	270Ba	190Bb	280Ba	190Bb	14
	2	570Aa	360Ac	440Ab	370Ac	

† Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

first year, and Ipyporã presented greatest values in the dry period for both years. Among cultivars and seasons, the hand-plucked stem proportion was 48 and 72% less in the first and in the second year, respectively, when compared with the HM stem proportion.

There were cultivar  $\times$  year (P = 0.0352) and year  $\times$  season (P = 0.0014) interactions for hand-plucked leaf/stem ratio (Table 10). The greatest leaf/stem ratio occurred in the second year for both cultivars, in which Mulato II was 42% greater than Ipyporã. In both years, the hand-plucked leaf/stem ratio was greater in the rainy than in the dry season. However, in the second year, the rainy season values were 3.4-fold greater than the dry season.

The hand-plucked dead material proportion was affected by year  $\times$  season interaction (P = 0.0001, Table 10). For both seasons, the greatest dead material proportions occurred in the second year. Across years, dead material was greater during the dry than rainy season.

The CP concentration was greater for Mulato II (125 g kg<sup>-1</sup>) than Ipyporã (115 g kg<sup>-1</sup>, P = 0.0474). However, NDF was similar for both cultivars (P > 0.1519), with an average of 585 g kg<sup>-1</sup>. Crude protein and NDF were affected by season × year interaction (P < 0.001 and P = 0.0297, respectively). The greatest CP and the least NDF concentrations occurred in the rainy season of the first year (Table 11).

There was a cultivar  $\times$  year  $\times$  season interaction for ADF concentration (P = 0.0286, Table 12). The greatest ADF was measured during the dry season in the first year for both cultivars. In the dry season of the second year, Ipyporã presented the greatest ADF concentration, and both cultivars increased in ADF concentration from the first to the second year during the rainy season.

## Tussock Number, Root and Stubble Mass, and Organic Reserves

There was cultivar  $\times$  year interaction for tussock number (P = 0.0001). In the first year, Mulato II had greater tussock number (12.8) than Ipyporã (9.0); however, in the second year, tussock number was similar between cultivars. Also in the second year, the tussock number increased for Ipyporã (11.6) while it decreased for Mulato II (10.7).

The root mass was affected by cultivar (P = 0.0037) and year (P < 0.0001). Ipyporã had less root mass (3220 kg ha<sup>-1</sup>) than Mulato II (3700 kg ha<sup>-1</sup>), and first-year root mass was greater (4500 kg ha<sup>-1</sup>) compared with second-year root mass (2420 kg ha<sup>-1</sup>).

The first-year stubble N concentration was greater (5.0 g kg<sup>-1</sup>) than the second-year concentration (4.3 g kg<sup>-1</sup>). Root N concentration was affected by cultivar × year interaction (P = 0.0073). Mulato II had greater root N concentration than Ipyporã in both years. However, across years, Ipyporã increased root N concentration from 6.8 to 7.5 g kg<sup>-1</sup>, whereas Mulato II decreased root N from 9.7 to 9.0 g kg<sup>-1</sup>.

Stubble mass TNC concentration was affected only by year (P = 0.017), with first-year concentration greater than the second-year concentration (27.3 vs. 15.0 g kg<sup>-1</sup>). The root TNC concentration was not affected by cultivar, year, or any interaction (P > 0.05), averaging 11.7 g kg<sup>-1</sup>.

The root and stubble mass N pools were affected by cultivar × year interaction (P = 0.0028 and P = 0.0209, respectively). In the first year, Mulato II root N pool was greater than Ipyporã, but they were not different in Year 2. Root and stubble mass N pools were greater in the first year than in the second year (Table 13). On the other hand, root and stubble mass TNC pools did not differ between cultivars (P > 0.05) and were affected only by year (P = 0.0034

		Ірур	orã	Mula	ato II	
Variables	Year	Dry	Rainy	Dry	Rainy	SE
			g k			
Leaf	1	480Ac†	875Aa	635Ab	835Ba	14
	2	425Bc	870Aa	520Bb	895Aa	
Stem	1	365Aa	95Ad	220Ab	140Ac	11
	2	120Ba	50Bb	65Bb	40Bb	

Table 9. Average and SE for hand-plucked leaf and stem proportion in Ipyporã and Mulato II pastures under continuous stocking during dry and rainy seasons of 2 yr.

+ Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

Table 10. Average and SE for hand-plucked leaf/stem ratio and hand-plucked dead material proportion in Ipyporã and M	ulato
II pastures under continuous stocking during the dry and rainy seasons of 2 yr.	

Variable	Cultivar or season	Year 1	Year 2	SE
Hand-plucked leaf/stem ratio	lpyporã	5.2Ab†	10.8Ba	1
	Mulato II	4.5Ab	15.3Aa	
	Dry	2.1Bb	5.9Ba	1
	Rainy	7.5Ab	20.2Aa	
Hand-plucked dead material proportion (g kg <sup>-1</sup> )	Dry	150Ab	430Aa	7
	Rainy	26Bb	70Ba	

+ Least squares means followed by a common uppercase letter in a column and lowercase letter in a row are not different by t test (P > 0.05).

and P = 0.0024, respectively). First-year root and HM TNC pools were greater (62 and 180 g m<sup>-2</sup>, respectively) than in the second year (23 and 83 g m<sup>-2</sup>, respectively).

### DISCUSSION

In forage-based livestock systems, diversification of forage species is fundamental to reduce environmental risks. Forage productivity is driven by favorable environmental conditions, which allow cultivars to express their genetic potential (Souza Sobrinho et al., 2011). Although base temperatures are not likely to be similar among cultivars (Moreno et al., 2014), if 11.1°C is considered the base temperature for *Brachiaria* (Pequeno et al., 2014), there was no temperature limitation to forage growth in the current study (Gomes et al., 2019). During the dry season, growth was mainly limited by reduced soil moisture (Euclides et al., 2014) (Fig. 1).

Both cultivars (Mulato II and Ipyporã) had lesser HM, HA, HAR, and HBD values in the rainy season of the second year (2017–2018) of grazing than in the first year (2016–2017). A shorter period of water deficit (30 d less)

Table 11. Average and SE for hand-plucked herbage crude protein (CP) and neutral detergent fiber (NDF) concentrations in continuously stocked Ipyporã and Mulato II pastures during dry and rainy seasons of 2 yr.

		Sea				
Variable	Year	Dry	Rainy	SE		
		g kg <sup>-1</sup>				
CP	1	110Ab†	160Aa	4		
	2	100Ab	115Ba			
NDF	1	575Ba	485Bb	7		
	2	665Aa	610Ab			

† Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

# Table 12. Average and SE for hand-plucked herbage acid detergent fiber concentration in continuously stocked Ipyporã and Mulato II pastures during rainy and dry seasons of 2 yr.

	lpyp	lpyporã		Mulato II	
Year	Dry	Rainy	Dry	Rainy	SE
		g k	g <sup>-1</sup>		
1	305Ba†	250Bb	310Aa	235Bb	20
2	355Aa	285Ab	305Ab	290Ab	

† Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

# Table 13. Average and SE for root and stubble N pools of continuously stocked lpyporã and Mulato II pastures across two seasons during 2 yr.

Variables	Year	Ipyporã	Mulato II	SE
	g m <sup>-2</sup>			
Root N pool	1	27.6Ab†	47.6Aa	2.0
	2	17.8Ba	22.5Ba	
Stubble N pool	1	33.2Aa	32.1Aa	1.3
	2	22.5Ba	26.0Ba	

† Least squares means followed by a common uppercase letter in the column and lowercase letter in the row are not different by t test (P > 0.05).

and greater total rainfall (2147 mm) in the first compared with the second year (1762 mm) explains some of this response. In addition, the first sampling year represented the year of pasture establishment. This is not considered to be greatly significant in this case, however, because in this environment, full pasture establishment can be achieved within 60 d of planting, and measurements for this study did not begin until nearly 150 d after planting. Perhaps of greater importance than the extent of plant establishment during the year of planting is the proximity in time to soil tillage operations that occurred during land preparation leading to planting. Tillage has been shown to shift the relationships between soil-C substrates and microbial diversity, contributing to additional nutrient mineralization (Lienhard et al., 2013). Soil organic matter mineralization and turnover are important components of soil fertility (Craswell and Lefroy, 2001), and greater nutrient mineralization in the first year may have enhanced the forage growth of Ipyporã and Mulato II.

Pastures of Mulato II presented  $\sim$ 15% greater HA and HAR than Ipyporã. The lesser HA of Ipyporã may be related to the relative productivity of the *B. ruziziensis* parent vs. other *Brachiaria* spp., which could somewhat limit its genetic potential for growth (Rodrigues et al., 2015; Euclides et al., 2018). Although both cultivars are apomictic tetraploid hybrids, Mulato II has alleles from *B. ruziziensis*, *B. decumbens*, and *B. brizantha* compared with only *B. ruziziensis* and *B. brizantha* for Ipyporã (Argel et al., 2007).

The greatest leaf mass also occurred during the rainy season for both cultivars and was associated with the greatest HA. However, Mulato II had lesser leaf mass than Ipyporã during the rainy season, which may be attributed to spittlebug damage (Fig. 2). Ipyporã is resistant to spittlebug attack due to the antibiosis mechanism, which affects the reproductive potential of the insect (Valle et al., 2017). The spittlebug is abundant during the rainy season in the Amazon biome (Fig. 2) as a result of greater temperature and precipitation (Holmann and Peck, 2002). In this study, foliar damage occurred only in Mulato II pastures. It is important to highlight that Mulato II was released as a spittlebug tolerant grass (Argel et al., 2007); however, it is not tolerant of Mahanarva spp., the most common species present in the Amazon biome. As this species has spread, it has had a huge impact on forage-livestock systems.

In the dry season, Mulato II presented greater leaf mass and proportion than Ipyporã. Mutimura and Everson (2012) studied Mulato II in a lower-precipitation (750 mm) tropical environment in Africa and verified maintenance of green leaves even under water stress conditions. In Colombia, Mulato II reduced transpiration via stomatal control, which allowed a longer growth period than other *Brachiaria* spp. cultivars under water deficit (Cardoso et al., 2015). Pequeno et al. (2015) reported that the superiority

of Mulato II is related to greater leaf mass in the stubble, mainly during the dry season, which resulted in faster regrowth compared with other grasses.

Greater leaf/stem ratios in the dry season for Mulato II and in the rainy season for Ipyporã were associated with greater leaf proportion or mass, respectively. Lesser leaf/stem ratio occurred in the first year due to greater stem proportion associated with greater HA and HAR. Environmental conditions and management strategies that enhance tropical forage growth also may increase stem and dead material accumulation (Silva et al., 2016b). Leaf/stem ratio of Mulato II pastures decreased from 2.85 to 2.31 and 1.38 for canopies defoliated at 50-, 65- and 80-cm heights, respectively, with the progressive reduction in leaf/stem ratio attributed to greater stem elongation driven by light competition (Cabral et al., 2017).

In the current study, the first-year LAI was 6% greater than in the second year and 24% greater in the rainy than in the dry season. Greater rainfall and possibly greater soil nutrient levels in the first experimental year resulted in the greatest leaf proportion and LAI. Under continuous stocking, where leaves are constantly being removed, new foliar tissue and its photosynthetic rate are responsible for continued forage production (Parsons et al., 1983) and persistence (Vendramini et al., 2012).

Herbage accumulation in grazed pastures is also affected by the grazing strategy, which affects plant morphology (Pedreira et al., 2017), physiology (Nascimento et al., 2019), and nutritive value (Tesk et al., 2018). Herbage bulk density is an important variable that influences accessibility of forage for animal intake (Hodgson, 1990). In this study, canopy height was similar among cultivars and years; however, HM differed, resulting in minor differences in HBD. This highlights the importance of a well-defined grazing strategy in sustaining desirable canopy characteristics.

In the first year, greater HA was due to the greater leaf, stem, and dead material mass; however, the greatest stem and dead material proportions were measured in the second year. This is consistent with previous work that showed despite maintaining a near constant canopy height under continuous stocking, dead material percentage increased and leaf percentage decreased over time (Euclides et al., 2019). In addition, dead material proportion increased during the dry season, as a consequence of the leaf senescence and water deficit (Euclides et al., 2008).

Improving pasture productivity (i.e., greater HA) is only a first step toward enhancing forage-based livestock systems, as animals must also consume the forage produced and convert it into animal products (Hodgson, 1990). Consumption and conversion are influenced by canopy structure, plant-part composition, and nutritive value of herbage selected by the animals (Tesk et al., 2018). The hand-plucked leaf proportion was twofold greater, and the stem proportion was 50% less than the proportions in total HM, highlighting the importance of characterizing composition of the grazed portion of the canopy (Trindade et al., 2012). Mulato II presented greater leaf proportion during the dry season than Ipyporã, which also resulted in, on average, 9% greater CP than in Ipyporã. Mulato II CP concentrations were similar to those reported by Vendramini et al. (2012), which varied from 100 to 131 g kg<sup>-1</sup> under continuous stocking to the same 30-cm canopy height used in the current study.

The CP concentration in the rainy season of the first year was 40% greater than in the second year, even with the same fertilization practices. This was likely a consequence of increased organic matter mineralization (Lienhard et al., 2013) in the first year, the year of planting, making more nutrients available to the plant during that year and increasing CP and HA. Overall, herbage CP was 30% greater (32 g kg<sup>-1</sup>) in the rainy than in the dry season. The N fertilization was applied during the rainy season to ensure greater N use efficiency (Bourscheidt et al., 2019). Moreover, in the rainy season, the plant produces the greatest amount of new tissue, and most of this is leaf, where the majority of CP is concentrated (Vargas et al., 2013). The greatest proportion of the photosynthetic enzymes is located in the leaves, and these enzymes are responsible for the greater N concentration (Irving, 2015).

Although hand-plucked stem proportion was greatest in first year, NDF and ADF concentrations were greater in the second year due to greater dead material proportion compared with the first year. Likewise, dry-season NDF and ADF were greater as a result of increased dead material. During the dry season, the production of new leaves is reduced, and tissue senescence causes reduction in cell contents and an increase in structural carbohydrates and lignin, which decrease nutritive value (Echeverria et al., 2016).

In grazed systems, it is important to understand how grazing management affects the regrowth of each cultivar to assure system longevity and sustainability. Grazing management is a determinant of pasture persistence (Sollenberger and Newman, 2007), and each forage species presents adaptive features to address persistence (Sollenberger et al., 2012). Under grazing, assimilate partitioning to root and shoot is affected by leaf removal (intensity and frequency of grazing), which affects root growth (Silva et al., 2016b). In our study, first-year HM and root mass were  $\sim 15$  and 85% greater than in the second year, regardless of cultivar. Those results contributed to greater TNC concentrations and pools in root and stubble mass in the first year than in the second year. The TNC pool was greatly affected by the total organ mass; thus, pastures with lesser stubble or root mass also presented smaller TNC pools (Silva et al., 2016b). The N concentrations, as well as the N pools, in the stubble mass and root were greatest in the first year, except for the Ipyporã that had a greater root N concentration in the first year compared with the second year, probably due to their greatest tussock number.

The large decrease in HA and organic reserves from the first to the second year for both cultivars provides evidence of the need for nutrient replenishment under grazing (Bourscheidt et al., 2019). In a forage-livestock system, efficiency of conversion of light energy into biomass affected most by light level (Sheehy and Cooper, 1973), although it is also affected by nutrient availability. In our study, declining second-year soil nutrient levels relative to those in Year 1, especially K (71%) and to a lesser extent Ca (50%) and Mg (23%), likely contributed to lesser HA and reserve storage in the second than first year (Table 1). The forage-livestock system sustainability, when based on high producing cultivars, depends on both soil fertility and grazing management to enhance and maintain pasture productivity (Pedreira et al., 2018) and levels of soil organic C (Lal, 2006).

### SUMMARY AND CONCLUSIONS

In general, Mulato II and Ipyporã were characterized by excellent HA, leaf/stem ratio, and nutritive value, and both persisted under continuous stocking when canopy height was maintained at 30 cm. Forage-livestock operations should generally use several cultivars, and for that reason, our data indicate that if greater productivity is the primary objective, Mulato II is indicated due to its greater HA than Ipyporã. However, careful pest monitoring is required and insecticide applications will likely be needed for spittlebug control. The susceptibility of Mulato II to spittlebug contributes to a more vulnerable system in the Amazon biome, requiring greater attention to management and likely greater inputs if stands are to persist. On the other hand, although Ipyporã had lower HA, there were no concerns about spittlebugs, even in a severerisk region, which supports consideration of Ipyporã as an alternative for diversification of forage-based livestock systems in the Amazon biome.

### **Conflict of Interest**

The authors declare that there is no conflict of interest.

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