JOURNAL OF THE NACAA

ISSN 2158-9429

VOLUME 16, ISSUE 2 – DECEMBER, 2023

Editor: Linda Chalker-Scott

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***Brachiaria* (*Urochloa*) Cultivar Response to Nitrogen Fertilizer as A Summer Forage Crop in the Southern USA**

Abstract

Brachiaria (*Urochloa*) is a tropical perennial warm-season grass that could be used as a summer forage, but little is known about its production and fertilization requirements in the southern USA. The objective of this study was to determine forage biomass production of *Brachiaria (Urochloa)* spp. under different fertilization regimes. The study was conducted at Mississippi State University on a Marietta sandy loam. The experimental design was a randomized complete block design with a split-plot arrangement replicated three times. The main plots were four nitrogen (N) rates applied at 0, 60, 90, and 120 lb N ac^{-1} in split applications using urea ammonium sulfate (33-0-0-18S). The subplots were two cultivars, ‘Basilisk’ (*Urochloa decumbens*) and ‘Mulato II’ (*Urochloa ruziziensis x U. brizantha x U. decumbens*). Cultivars were established on July 12, 2022, in a prepared seed bed at 10 lbs PLS (Pure Live Seed) ac^{-1}. Seasonal biomass response indicated that a N application of 60 to 90 lb N ac^{-1} yr^{-1} is optimal. There were no differences in nutritive value among cultivars except for CP, where MUII had an 11% greater concentration. Leaf area index was maximized at 70 lb N ac^{-1}. Green canopy cover and NDVI were 41 and 23% greater, respectively, during the second harvest.

Keywords: *Brachiaria*, *Urochloa*, nitrogen, biomass, nutritive value.

Abbreviations: PLS = pure live seed; N = nitrogen; DM = dry matter; LAI = leaf area index; GCC = green canopy cover; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVTDMD = *in vitro* dry matter digestibility; WSC = water-soluble carbohydrates; LSD = least significant difference

Introduction

The extended growing season in the southern USA and semitropical environment conditions might provide the opportunity to introduce tropical forages that are more climate resilient. These forages could provide higher biomass production than traditional warm-season annual grasses such as forage sorghum (*Sorghum bicolor* L. Moench), sorghum x sudan hybrids (*Sorghum bicolor)*, and pearl millet (*Pennisetum glaucum*). *Brachiaria* is a vigorous and fibrous tropical forage that is high-yielding and adapted to a wide range of soils (Heuzé et al., 2017). It is native to Africa (Fukumoto and Lee, 2003), and exhibits perennial growth characteristics in tropical regions. However, it could be utilized like an annual warm-season grass in the southern USA until the first killing frost. Boddey et al. (2004) reported that over 200 million acres of *Brachiaria* grasses are widely grown forages in South America. There could be a potential to use *Brachiaria* grasses in approximately 4 million acres in the southern USA.

The growing interest to maximize the utilization of warm-season grasses in the southern USA has prompted an urgent need to evaluate *Brachiaria* cultivars with outstanding agronomic characteristics, greater range of adaptation, greater nitrogen (N) efficiency, greater biomass production and nutritional quality, and resistance to Rhizoctonia (a fungal disease) and spittlebug species (*Homoptera: Cercopidae)* (damaging insect). *Urochloa decumbens* cv. Basilisk is adapted to medium to low fertility but is highly responsive to fertilizer applications. It is very resistant to drought due to its deep root system, and displays tolerance to lower temperatures and shade, but minimal adaptability to flooded areas (Conrado et al., 2021). Basilisk can have protein content ranging from 7 to 9% and 50 to 60% digestibility. *Urochloa ruziziensis* × *U. decumbens* × *U. brizantha* cv. Mulato II is a fast-growing hybrid that is adapted to well-drained soils and has relatively pubescent leaves. 'Mulato II' has deep, branched roots, which helps it tolerate droughty conditions. 'Mulato II' has excellent nutritional characteristics in terms of crude protein (CP) content and digestibility. Crude protein can range from 14 to 21% CP and in vitro digestibility is between 55 to 66%, respectively, when harvested between 25 and 35 days (Angel et al., 2007).

To attain high forage production continuous replenishment of nutrients is required. Nitrogen is especially important since it has the greatest impact on forage yield. The efficacy and efficiency of N fertilizer on forage grasses is strongly influenced by rates, sources, times, and methods of N application (Malhi et al., 2004). Nakamura et al. (2005) reported that *Urochloa brizanata* and *Urochloa decumbens* had higher productivity under high N conditions than *Urochloa humidicola*. Marsetyo and Syukur (2009) indicated that a urea application of 60 lb N ac^{-1} increased tiller number, dry matter production, and crude protein content of the *Brachiaria* while reducing the neutral detergent fiber content of the plant compared to a control. Hughes et al. (2022) indicated that the combination of herbage biomass, in-vitro organic matter digestibility, and crude protein was optimized with 50 lb N ac^{-1} and harvested every 4-weeks.

In the southern USA, forage-based livestock systems have great economic importance in regard to sustainability. The introduction of new grass species is one approach that may overcome the low nutritional contents and seasonal availability of warm-season grasses for beef cattle production systems. Sometimes this approach might require the best management practices such as fertilization and cultivar selection to ensure productivity. Information on N fertilization of *Brachiaria* cultivars in semitropical environments is limited. Application of fertilizers is essential to sustain the yield of forage crops, hence, the objective of this study was to evaluate yield and nutritive value of two *Brachiaria* cultivars by applying four fertilizer rates.

Materials and Methods

The exploratory study was conducted during the summer of 2022 at the Henry H. Leveck Animal Research Farm on the campus of Mississippi State University located in Starkville, MS (33°25′18″ N, 88°47′32″ W). The soil type is a Marietta sandy loam (Fine-loamy, siliceous, active, thermic Fluvaquenti Eutrudepts). The experimental design was a randomized complete block design with a split-plot arrangement replicated three times. The main plots were represented by four N rates applied at 0, 60, 90, and 120 lb N ac^{-1} in a 50/50 split applications using urea ammonium sulfate (33-0-0-18S). The first N application was applied when plants reached a height of two inches after emergence and the second application was applied after the first harvest. Based on soil test recommendations, phosphorus (0-46-0) was applied at planting at a rate of 46 lb P ac^{-1}, and potassium (K) was applied twice (at planting and after the first cut) at a rate of 60 lb K ac^{-1}. The subplots were represented by two cultivars, *Urochloa decumbens* cv. Basilisk (BA) and *Urochloa ruziziensis x U. brizantha x U. decumbens* cv. Multo II (MU). Cultivars were established on July 12, 2022, in a prepared seed bed at 10 lbs PLS (Pure Live Seed) ac^{-1}. Green canopy cover (GCC, %) was measured using the Canopeo® app (Oklahoma State University, OK) installed in MatLab (The MathWorks, Inc., MA). Leaf Area Index (LAI) was measured using LI-2000 (Li-Cor, Lincoln, NE). Normalized Difference Vegetation Index (NDVI) measurements were taken using a hand-held GreenSeeker® crop sensing system (Trimble, CA). Plots were harvested on September 1 and October 3, 2022, to a three-inch stubble with a self-propelled Cub Cadet mower equipped with a bagging system when 50% of the plots reached 12 to 15 inches tall (vegetative to early boot stage). Plot biomass was weighed, and a forage subsample sample was dried at 140 °F in a forced-air oven for 72 h for dry matter biomass determination. Forage subsamples were ground to pass through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) for nutritive analysis. Samples were analyzed for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), *in vitro* dry matter digestibility at 48h (IVTDMD), and water-soluble carbohydrates (WSC) using a Foss DS2500 near infra-red reflectance spectroscopy (NIRS) instrument (Foss North America, Eden Prairie, MN). The analysis was conducted using the 2022 grass hay equation developed by the NIRS Forage and Feed Testing Consortium (Berea, KY).

Agronomic use efficiency (AUE) is used as a short-term indicator of the impact of applied nutrients on productivity (Geng et al., 2017; Prasad, 2020). It is the efficiency of applied nutrients in increasing biomass yield per unit of nutrient applied. Agronomic use efficiency was calculated by subtracting biomass production of the N-treated biomass from the control, dividing it by the total amount of N applied to the grass, and then multiplying by 100 to obtain a percent increase. Seasonal AUE was calculated by using the total seasonal biomass.

$$AUE\ (\%) = [(Y_x - Y_0)/N_x]*100 \text{ (Geng et al., 2017)}$$

Where, Y_x = biomass of the N applied treatments; Y_0 = biomass of the control; N_x = N rate of the applied treatments.

Nutrient removal is calculated by taking the concentration of each nutrient in harvested biomass and multiplying by the harvest yield. The seasonal nutrient removal was determined by adding the nutrient removal of the two harvests. There are estimates of nutrient removal per unit yield (lb ac^{-1}).

Data were analyzed using harvest frequency as a repeated measure for each of the dependent variables. Data were further analyzed in the PROC GLIMMIX of SAS and the least significant difference was used to determine treatment differences at $\alpha = 0.05$. A correlational analysis was conducted between mean harvest biomass and LAI using PROC CORR in SAS.

Results And Discussion

Weather conditions

Total precipitation during the duration of the study was very similar to the 30-year normal. Precipitation was 7 and 46% lower during August and October (Table 1). The mean temperature was consistent with the 30-year normal except for October which was cooler than the long-term normal. Total growing degree days (GDD) is slightly less than long-term data. There was a 3 and 14% decrease in GGD in September and October. This is related to a decline in temperature towards the end of the study.

Table 1**.** Weather conditions (precipitation, temperature, and growing degree days) during the 2022 growing season at Starkville, MS along with the 30-year normal for each parameter (NOAA, 2023).

	Month				
Weather Factor	**Jul**	**Aug**	**Sep**	**Oct**	**Total/Mean**
Precipitation (in)	6.6	4.1	4.4	2.1	17.2
30-yr	4.7	4.4	4.1	3.9	17.1
Deviation	1.9	-0.3	0.3	-1.8	--
Temperature (°C)	83.7	81.0	74.5	62.0	75.3
30-yr	81.6	81.0	75.1	64.0	75.4
Deviation	2.1	0.0	-0.6	-2.0	--
GGD*	1052	967	742	390	3151
30-yr	984	964	766	453	3167
Deviation	68	3	-24	-63	--

*Growing degree days (GDD) base 50.

Biomass and agronomic use efficiency

Differences in biomass production were impacted by cultivar (P = 0.0006) and nitrogen application (P = 0.0037). Basilisk (BA) produced 58% greater biomass than ‘Mulato II’ (MU) (3891 vs. 2461 lb DM ac^{-1}). Biomass production increased with N application and plateaued at 90 lb N ac^{-1} (Figure 1). There was a quadratic relationship between N application and biomass production ($R^2 = 0.8728$). This suggests that *Brachiaria* could reach the metabolic crop N demand between 60 and 90 lb N/ac/yr. This could be identified as the quantity of total crop N uptake corresponding to the maximum biomass achievable in a given environmental condition without any limitation. Above that level we could see a unique negative allometric response. An increase of 22, 44, and 15% in biomass production was observed with N applications of 60, 90, and 120 lb N ac^{-1}, respectively, when compared to control. Argucino and Gorme (2020) indicated that the appropriate fertilization rate for optimum production of *Brachiaria* was 80 lbs N ac^{-1}. Our findings under semitropical conditions are within their recommended application rate. A cultivar x harvest interaction (P = 0.0091) was observed (Figure 2). Basilisk had a 58% greater mean harvest than MU (1946 vs. 1230 lb DM ac^{-1}). Basilisk had 147 and 18% greater biomass production than MU in both harvest dates. The second harvest had 41% greater biomass than the first harvest (1860 vs. 1316 lb DM ac^{-1}). The greater biomass response of Basilisk could be because BA is highly responsive to fertilizer applications and adapted to heavy clays (Conrado et al., 2021). This is corroborated by the significant interaction between cultivar and N rate when determining AUE (Figure 3). 'Mulato II' exhibited a greater AUE at 60 lb N ac^{-1} (44%) and then declined by 24 and 98% at application rates of 90 and 120 lb N ac^{-1}, respectively. Mutai et al. (2017) indicated that *Brachiaria* can host 84 different bacterial strains of which 41 had a minimum of three plant-beneficial properties. There could be a possibility that the ability of *Brachiaria* grasses to host genetically diverse bacteria might have contributed to high biomass production and increased NUE.

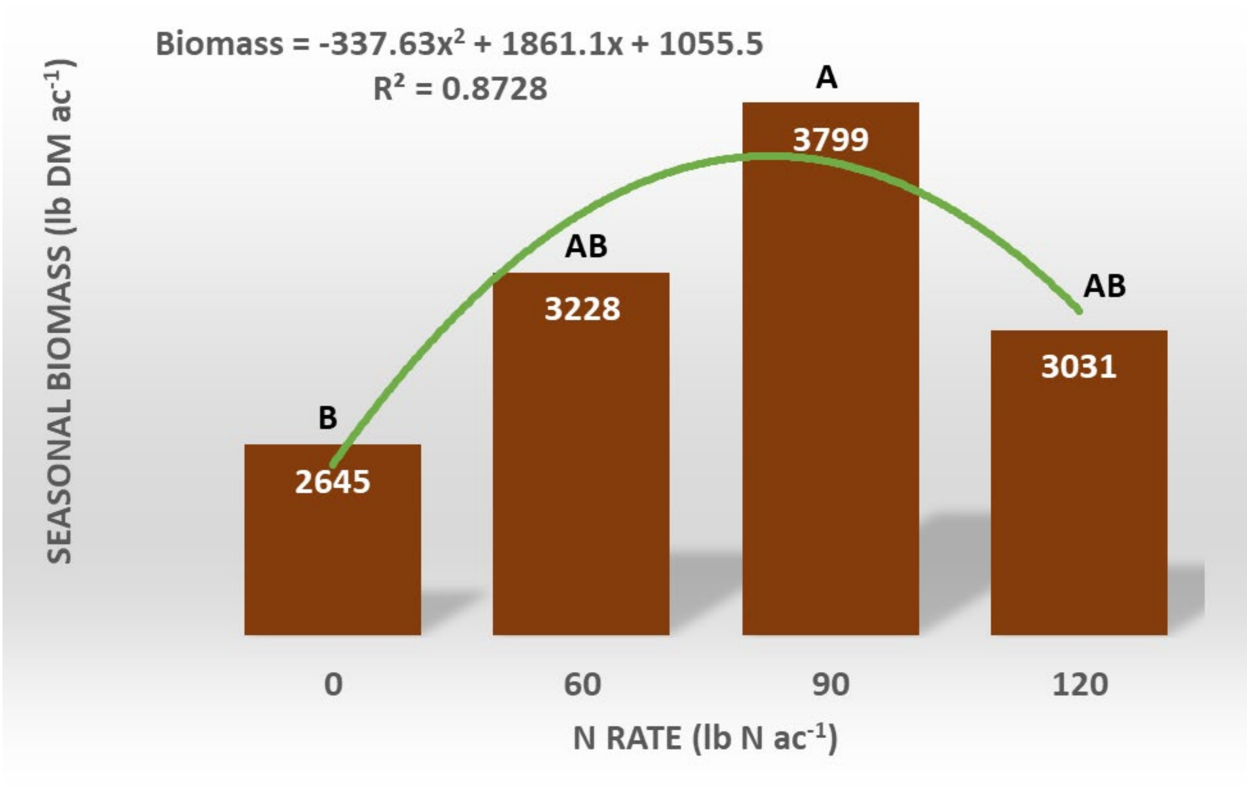

Figure 1. Effect of nitrogen rate application on *Brachiaria* biomass production pooled from across cultivars during the 2022 growing season at Starkville, MS.

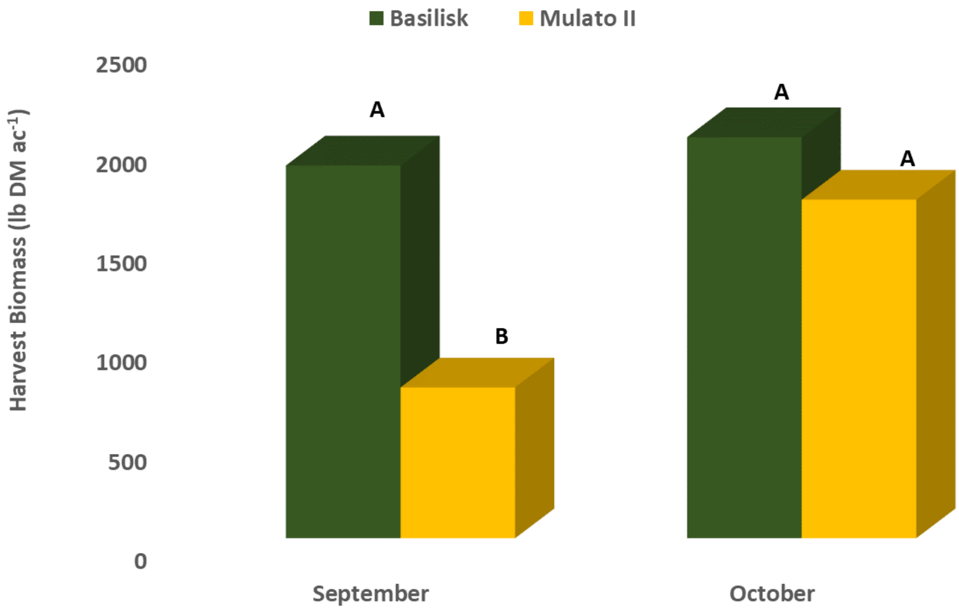

Figure 2. Impact of harvest date and *Brachiaria* cultivar on harvest biomass production during the 2022 growing season at Starkville, MS.

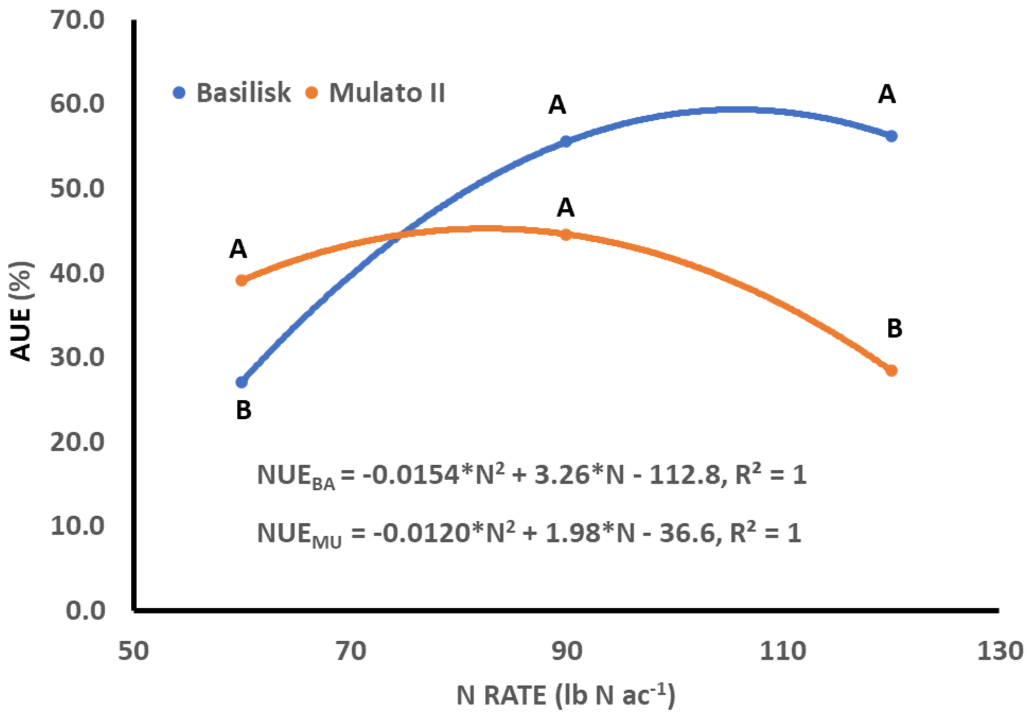

Figure 3. Impact of nitrogen rate and *Brachiaria* cultivar on agronomic use efficiency during the 2002 growing season at Starkville, MS.

Leaf area index (LAI), green canopy cover (GGC), normalized difference vegetation index (NDVI)

Leaf area index (LAI) was significantly influenced by N fertilization ($P = 0.0282$) and by a cultivar x harvest interaction ($P < 0.0001$). Leaf area index has an exponential increase with N fertilization ($R^2 = 0.9966$, Figure 4). The optimum increase in LAI occurred at N rates between 60 and 90 lb N ac^{-1}. A polynomial relationship between LAI and biomass production was observed [Biomass = $(3054.7*LAI^2) – (23858*LAI) + 49186$, $R^2 = 0.8826$]. As LAI value increased, there was an increase in biomass production. Bosque and Herrero (2001) indicated that leaf area was responsive both to temperature and nitrogen, but did not change with the level of insertion of the leaf in the tiller.

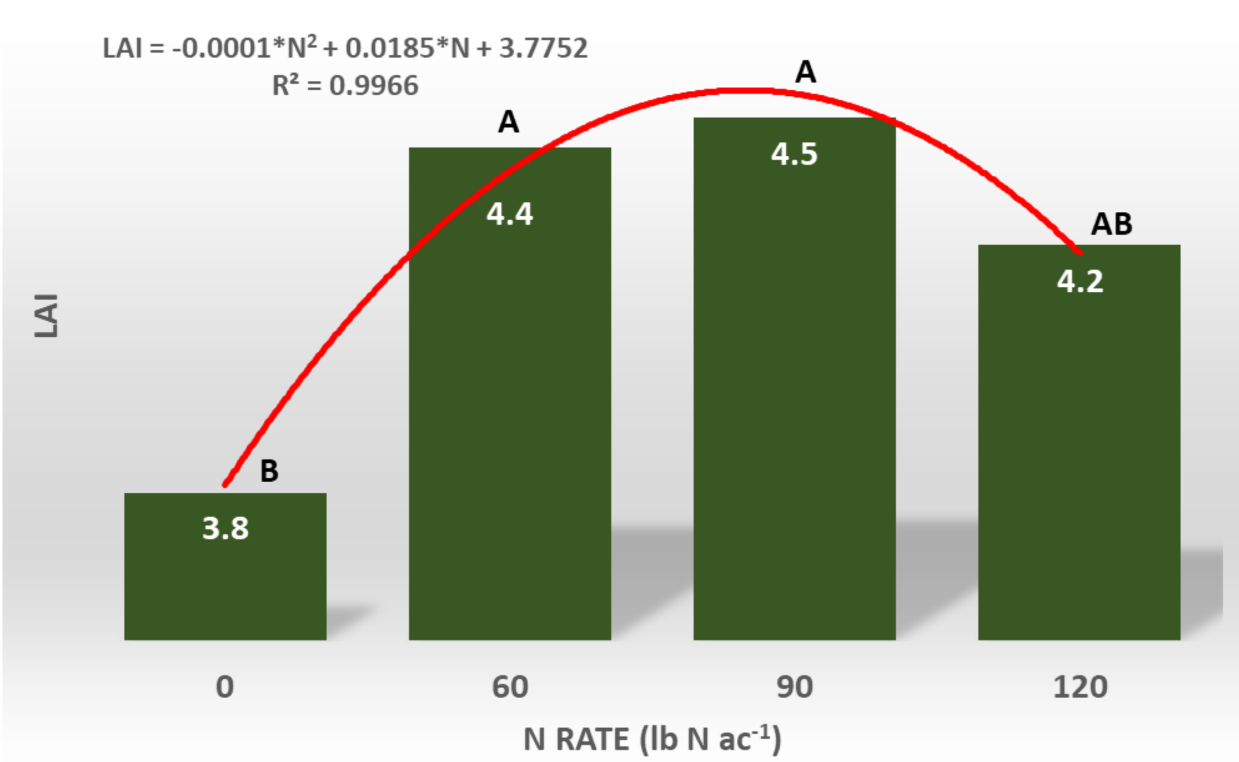

Figure 4. Impact of nitrogen fertilization rates on leaf area index (LAI) of *Brachiaria* grasses during the 2022 growing season at Starkville, MS.

Green canopy cover (GCC) is the percent canopy cover of live green vegetation that can be measured. The GCC is an index that measures from 0.0 to 100. The lower the index, the more dead material might be present in the plot. Green canopy cover (GCC) was affected by harvest ($P < 0.0001$) where the October harvest had 41% greater GCC than September (93.3 vs. 65.6). The increase in GCC could be related to an increase in tiller production after harvest. Vendramini et al. (2014) reported that ground cover increased linearly as the regrowth interval increased from 2 to 6 wk, and 'Mulato II' had greater ground cover than 'Cayman'.

Normalized difference vegetation index (NDVI) quantifies vegetation by measuring the difference between two wavelengths: near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs). It is expressed as an index of 0.0 to 1.0. An NDVI value close to +1 indicates a high possibility of dense green leaves (strong red light by the chlorophyll in the leaves). No differences in normalized difference vegetation index (NDVI) were detected among cultivars. However, polynomial relationships were observed between NDVI and N removal and NDVI and LAI (Figure 5). The correlation shows an exponential increase in the N content of the plant (Figure 5a) and the leaf area (Figure 5b) with increase in NDVI index. These regression models indicate that LAI and N content could be used as predictive tools to confirm the direct relationship between leaf area and spectrodiometric readings. It is expected that greener leaves will have greater chlorophyll content and increase red light absorption and therefore, greater number of leaves per area reducing any noise interference compared to bare ground. Cabrera-Bosquet et al. (2011) reported that strong correlations were observed between NDVI measurements and dry aboveground biomass, total green area, green area and aboveground N content in wheat without spikes.

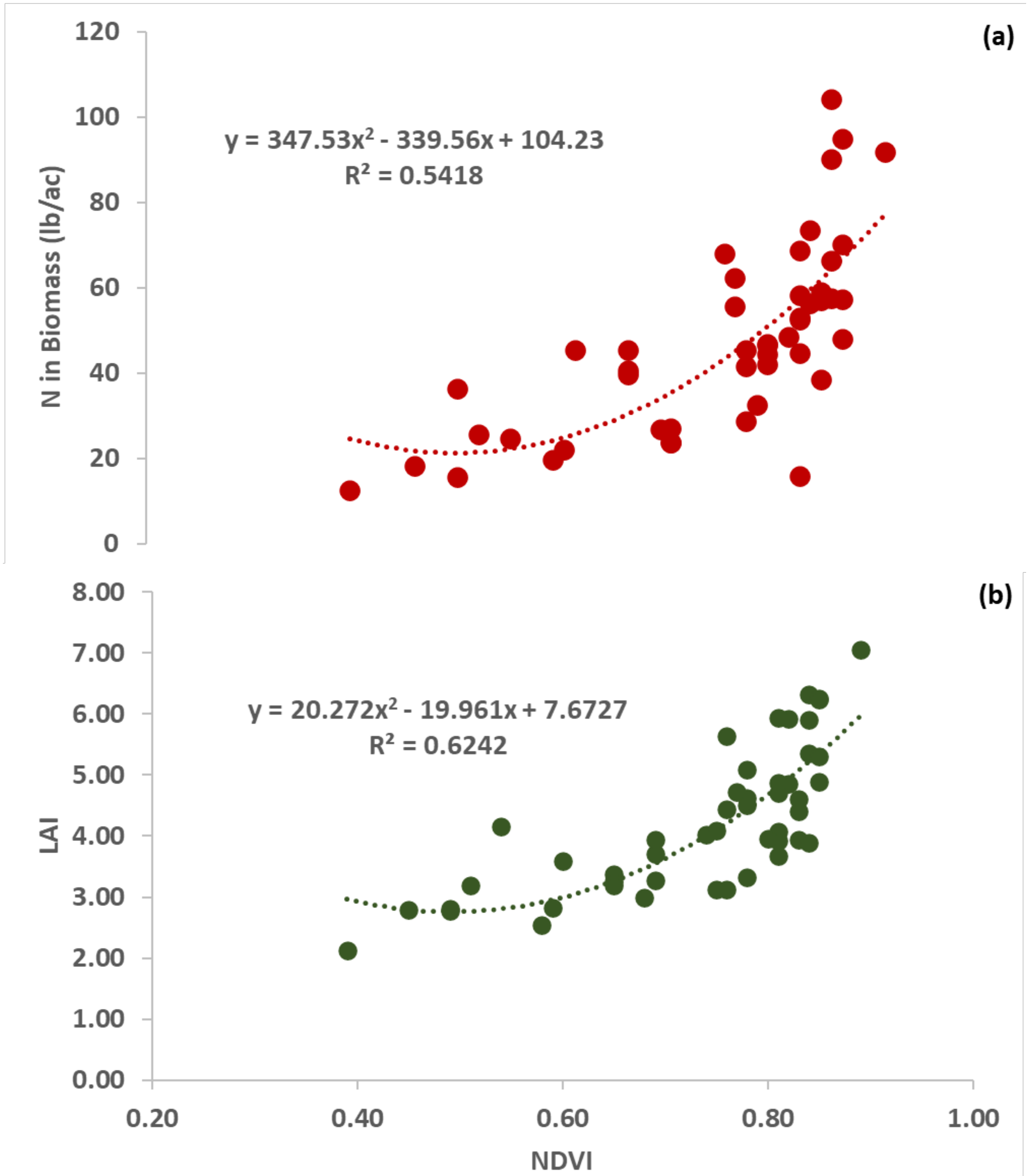

Figure 5. Seasonal correlation of normalized difference vegetation index (NDVI) to the nitrogen content (a) in the biomass and to leaf area index (b).

Nutrient removal

Cultivar interaction significantly affected the total nutrient removal for nitrogen (N, P = 0.0023), phosphorus (P, P = 0.0006), potassium (K, P = 0.0015), calcium (Ca, P = 0.010), and magnesium (Mg, P = 0.0003). ‘Basilisk’ had 42, 52, 56, 57, and 54% greater N, P, K, Ca, and Mg total removal than ‘Mulato II’, respectively (Figure 6). There was a significant effect of N rates on harvest nutrient accumulation for nitrogen (P = 0.0153) and magnesium (P = 0.0093) removal. The harvest removal of N and Mg increased with N application up to 90 lb N ac^{-1} and then declined (Figure 7). There was a cultivar x harvest interaction for N (P = 0.0112), P (P = 0.0108), K (P = 0.0154), Ca (P = 0.0334), and Mg (P = 0.0214). Most of the differences among cultivars occurred during the September harvest (Table 2). ‘Basislik’ had a greater removal of nutrients compared to Mulato II across harvest. Such differences in nutrient removal among cultivars were not observed during the October harvest. N and K were the most extracted nutrients in biomass. Overall, the plant nutrient balance between September and October harvests indicated greater differences during the September harvest. This indicates that a possible increase in root mass after harvest could help to extract soil-available nutrients.

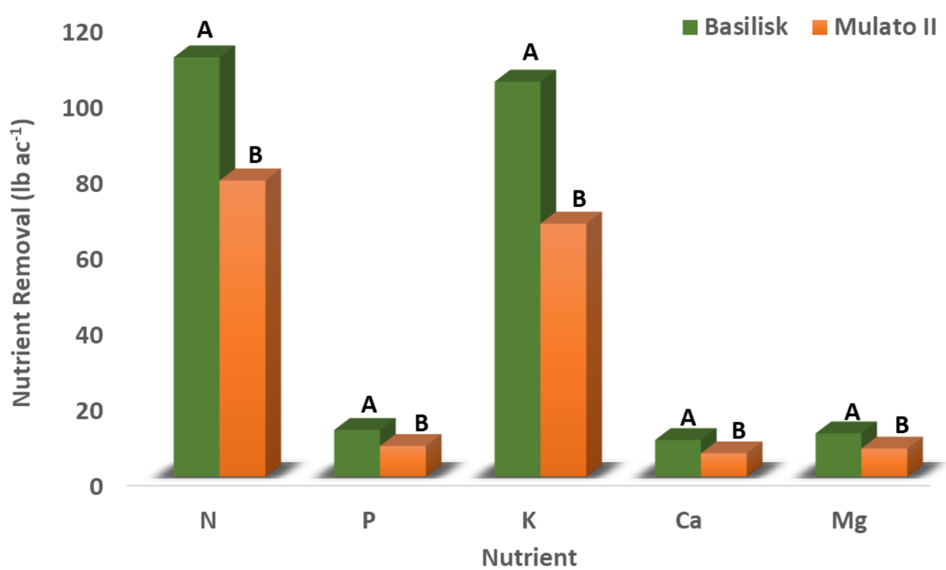

Figure 6. Impact of *Brachiaria* cultivar on total nutrient removal for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium during the 2022 growing season at Starkville, MS. Letters are for cultivar comparison within a nutritional parameter.

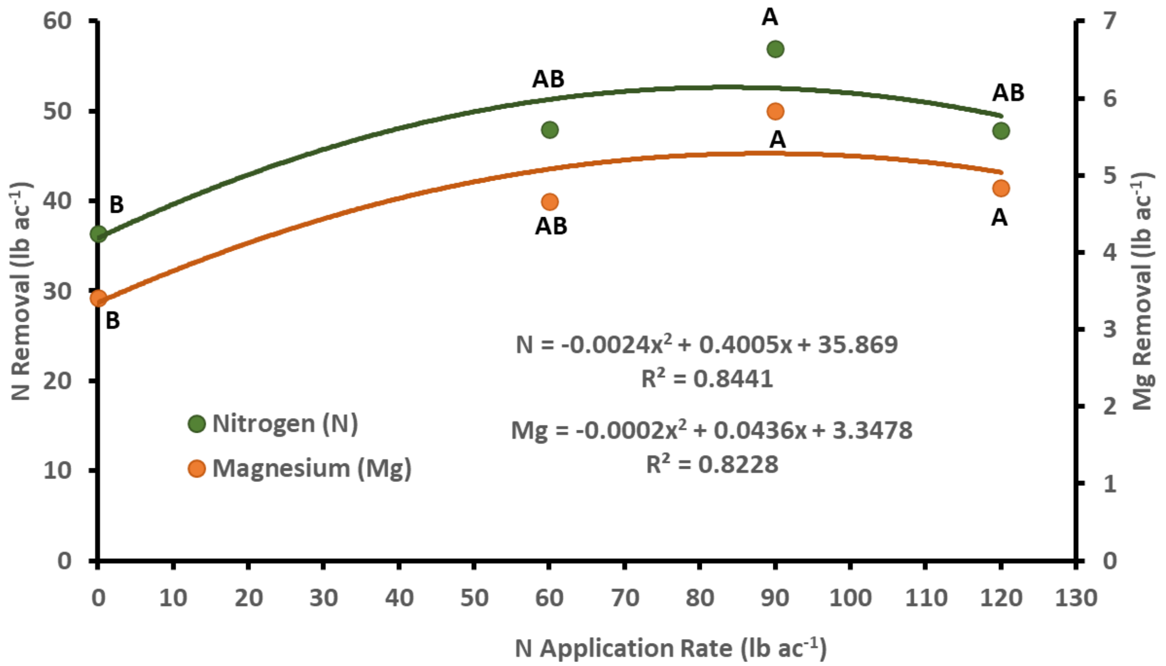

Figure 7. Impact of nitrogen fertilization on total nutrient removal for nitrogen (N) and magnesium (Mg) during the 2022 growing season at Starkville, MS. Letters are for cultivar comparison within a nutrient parameter.

Table 2. Influence of *Brachiaria* cultivar and harvest date on biomass removal of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) during the 2022 growing season at Starkville, MS. Letters are for comparison of cultivars within a harvest for each nutrient.

	Nutrient Removal (lb ac^{-1})				
Cultivar	**N**	**P**	**K**	**Ca**	**Mg**
	September Harvest				
‘Basilisk’	53 A	6 A	51 A	5 A	6 A
‘Mulato II’	25 B	2 B	20 B	2 B	2 B
Mean	39	4	35	4	4
	October Harvest				
‘Basilisk’	57 A	6 A	54 A	4 A	5 A
‘Mulato II’	53 A	6 A	47 A	4 A	5 A
Mean	56	6	50	4	5

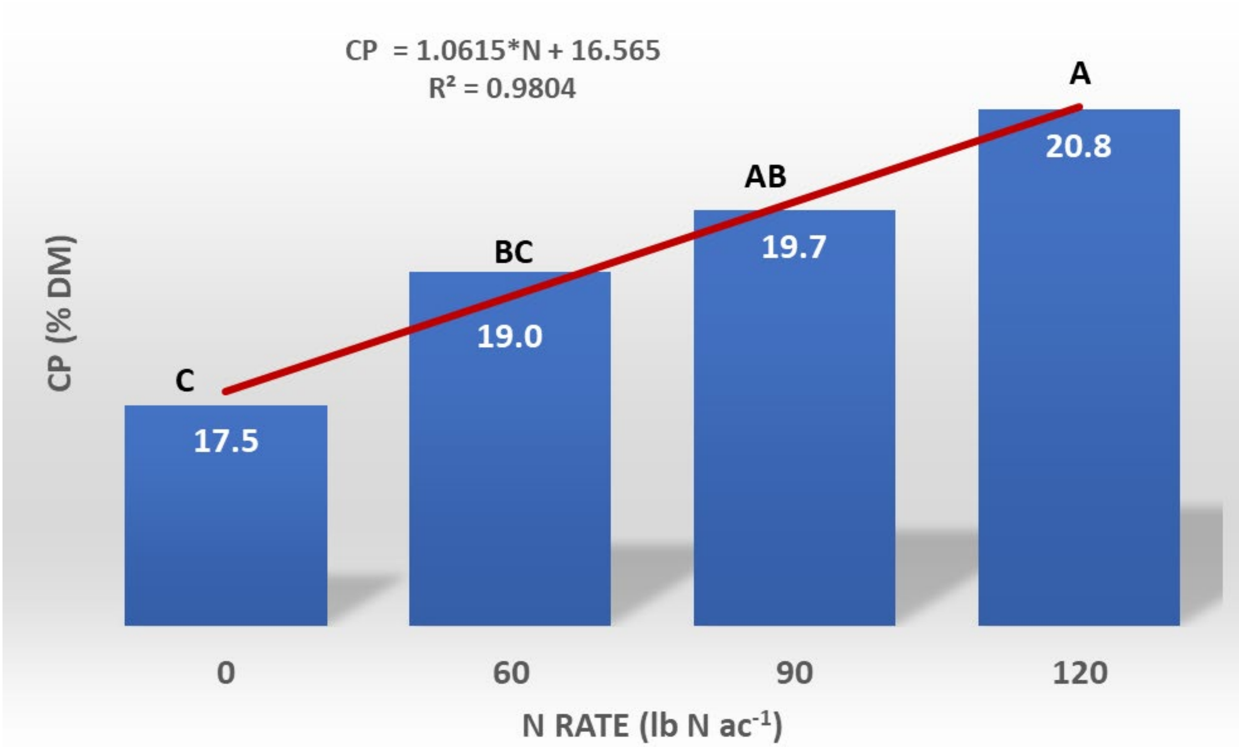

Figure 8*.* Impact of nitrogen fertilization on crude protein (CP) of *Brachiaria* grasses during the 2022 growing season at Starkville, MS.

Nutritive value

Crude protein was affected by cultivar (P = 0.0001) and N application rate (P = 0.0015). ‘Mulato II’ had a 13% greater CP concentration than BA (20.3 vs. 18% CP). The CP content obtained for both cultivars was higher than that reported in other studies, using ‘Mulato II’. Gonzales Muñoz et al. (2020) reported CP values of 15%. There was a linear increase in CP with N application (Figure 8). The N application of 120 lb ac^{-1} had 19 and 9% greater CP concentration than the control and the other two N applications, respectively. Jimenez et al. (2010) reported that any type of fertilization of *Brachiaria* grasses influences CP, which was confirmed in this study. Neutral detergent fiber was affected by cultivar (P <.0001) in which ‘Mulato II’ had 8% greater concentration compared to BA (52.2 vs. 48.5% NDF). *In vitro* dry matter digestibility was significantly different among cultivars (P = 0.0024). ‘Mulato II’ exhibited a 2% increase in IVTDMD compared to BA (84.4 vs. 82.6% IVTDMD). Harvest date had a significant effect on ADF

(P <0.0001), NDF (P = 0.0427), and WSC (P < 0.0001). The September harvest had 34% greater ADF concentration than the October harvest (29.3 vs. 21.9% ADF) and 3% greater NDF (51.2 vs. 49.5 % NDF). The October harvest had a 35% greater WSC concentration than September (8.1 vs. 6% WSC). The higher CP and IVTDMD in September and October could be related to lower biomass accumulation and resulting in less dilution of the nutritive value parameters. Newman et al. (2005) indicated that reduced growth temperatures in the autumn have been associated with greater digestibility. Inyang et al. (2010) reported that 'Mulato II' in November had lesser biomass accumulation and greater nutritive value than in October. These data indicate that *Brachiaria* could provide a higher nutritive value than some of the traditional warm-season grasses planted in the southern USA such as crabgrass (*Digitaria sanguinalis*), forage sorghum (*Sorghum bicolor*), sorghum-sudan hybrid (*Sorghum bicolor x S. bicolor*) and pearl millet (*Pennisetum glaucum*) (Lemus, 2015).

Vendramini et al. (2021) indicated that 'Mulato II' *Brachiaria* had similar production to bermudagrass (*Cynodon dactylon*), stargrass (*Cynodon nlemfuensis*), and limpograss (*Hemarthria altissima*), but greater forage digestibility comparable to Tifton 85 bermudagrass (67%).

Conclusion

This preliminary study concluded that the overall biomass yield and nutritive value of *Brachiaria* are optimized with annual applications of 60 to 90 lb N ac^{-1} in split applications. It was observed that nutrient removal depends on forage plant production and it increased with greater doses of N because of the greater dry matter production. This study has shown that 'Basilisk' produced greater biomass production than 'Mulato II' in north Mississippi. Cumulative biomass accumulation was 3,176 lb DM ac^{-1}. The biomass accumulation observed in this study is greater than the 2,857 lb DM ac^{-1} recorded by Vendramini et al. (2010) for 'Mulato II' with a 6-week regrowth interval in summer in south Florida. Reduced biomass accumulation in our study was possibly due to later planting. Overall, the data suggest significant differences in nutritive value concentrations between cultivars but greater IVTDMD in ‘Mulato II’. Preliminary results could provide more useful information about how to manage the nitrogen fertility of *Brachiaria* grasses in the southern USA and how they can provide beneficial pin-point grazing to livestock producers when determining best management practices and future economic benefits.

Brachiaria could provide an opportunity for pinpoint grazing systems in the southern USA due to their potential biomass and nutritive value. They can also be plated with a traditional cultipacker-type seeder or drilled in a prepared seedbed to ensure seed-to-soil contact. Seed should be planted at 10 lb ac^{-1} and placed at half-inch depth. However, there is still a need to do further work on the best planting time since despite commercial cultivars having good seed vigor and germinating from 5 to 15 days, they could have some dormant seeds that could cause irregular and prolonged germination (20 to 35 days). Although there are several cultivars available for purchase in the USA, there is a seed cost that needs to be addressed to determine the cost of establishing, the cost of biomass production, and the cost of gain per animal when implementing this system. Commercially available seed in the USA can range from $11.00/lb to $13.00lb. This means that seed cost per acre can range from $110 to $130. The average seed cost for pearl millet, forage sorghum, and sorghum-sudan is $2.00, $2.50, and $1.50 per pound, respectively. This means that the cost of establishment of *Brachiaria* could be up to 60% higher but also could increase production justifying the cost of seed in biomass and nutritive value return. Another issue that needs to be addressed is the productivity and persistence of the *Brachiaria* since they are of tropical origin.

Further studies should be conducted at the regional scale involving more cultivars across different soil types, temperatures, and rainfall patterns to define a region of adaptation and survivability. These data could help justify the economic investment and producer’s adoption of more climate-smart forage systems. There is also the need for further studies that compare *Brachiaria* species' performance under grazing with traditional warm-season annual grasses to determine gain per acre and cost of gain to develop an economic model.

Conflict of Interest

The author declares that there is no conflict of interest.

Acknowledgement

This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station (MAFES) and the Mississippi State University Extension Service (MSU-ES). This material is based upon work that is also supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession number 1016223, Project Number MIS-169140. Thank you to Carson Dewberry, Jon Albritton, and Jay Mundy for assisting with data collection, sample processing, and analysis.

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