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Productivity, Morphology and Chemical Composition of *Brachiaria* spp. Ecotypes, under Two Solar Illumination Intensities, in Yucatan, Mexico

Reyes B. Torres-Lugo [1,*], Francisco Javier Solorio-Sánchez [1], Luis Ramírez y Avilés [1], Juan Carlos Ku-Vera [1], Carlos Fernando Aguilar-Pérez [1] and Jesús Santillano-Cázares [2,*]

[1] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Yucatan, Merida 97300, Yucatan, Mexico
[2] Instituto de Ciencias Agricolas, Universidad Autonoma de Baja California, Ejido Nuevo Leon 21705, Baja California, Mexico
* Correspondence: a10013706@alumnos.uady.mx (R.B.T.-L.); jsantillano@uabc.edu.mx (J.S.-C.)

Abstract: There are mixed reports about the advantages and disadvantages of the presence of shade produced by trees over the understory-growing grasses; thus, it is urgent to test grass species and cultivars with potential to develop in shaded conditions associated with trees that occur in silvopastoral systems. The objective of the present study was to identify *Brachiaria* spp. ecotypes adapted for cultivation under tree shade, typical of silvopastoral systems. The study was conducted at Kampepen ranch, located 14 km south of Merida, Yucatan, Mexico. A factorial 6 (ecotypes) x 2 (sunlight intensities) treatment structure was used, arranged in split-plot design; with sunlight intensity levels being the plots and ecotypes the subplots, with three replications. The ecotypes were T1: *Brachiaria* hybrid cv. Mulato II, T2: *Brachiaria* hybrid cv. Cayman Blend, T3: *Brachiaria* hybrid cv. Talisman (BR05/1467), T4: *Brachiaria* hybrid cv. Camello Blend, T5: *Brachiaria brizantha* cv. Marandu and T6 (the control): *M. maximus* cv. Mombasa, while the two sunlight intensity levels were higher and lower sunlight reductions with respect to full sunlight (25% and 50% light transmission with respect to full sunlight, respectively). Variables were plant height, forage yield and chemical composition; leaf, stem and dead material fractions and leaf:stem ratio; plant canopy cover, plant maturity, and growth rate. Significant ($p < 0.050$) ecotype x sunlight intensity interactions were recorded for most of the studied variables. Independent of the shading levels, ecotype Talisman showed superior performance compared with the other assessed ecotypes, yielding up to 20 ton ha^{-1} under the highest light intensity, means across light intensities for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of 11.8%, 59.7%, and 34.7%, respectively, and more than 90% plant canopy cover. It is concluded that Talisman has promising characteristics for cultivation under silvopastoral systems, where tree shading is common, in the dry tropics of Yucatan, Mexico.

Keywords: growth rate; forage quality; forage yield; palisade grass; shading; silvopastoral systems

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Citation: Torres-Lugo, R.B.; Solorio-Sánchez, F.J.; Ramírez y Avilés, L.; Ku-Vera, J.C.; Aguilar-Pérez, C.F.; Santillano-Cázares, J. Productivity, Morphology and Chemical Composition of *Brachiaria* spp. Ecotypes, under Two Solar Illumination Intensities, in Yucatan, Mexico. *Agronomy* **2022**, *12*, 2634. https://doi.org/10.3390/agronomy12112634

Academic Editors: Massimiliano Probo and Pierre Mariotte

Received: 3 October 2022
Accepted: 23 October 2022
Published: 26 October 2022

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1. Introduction

Demand for animal products is projected to increase by 1.4 during the next decade [1] as a result of increasing population and economic growth. Food security still is of paramount importance in Latin America, where animal production plays a fundamental role in marginal and climate change vulnerable lands [2]. However, livestock feeding in tropical regions is managed under a few species of monoculture forage grasses with great dependence on external inputs and little efficiency. Increased productivity per unit area encourages pressure on natural resources. Conventional livestock activities seriously threaten natural resources. Currently, more than 60% of pastures are in a strong process of degradation, consequently reducing the potential for animal feeding. Silvopastoral systems can help reduce the seasonality of plants and animal production, and therefore contribute to mitigate and

adapt to the effects of climate change. Additionally, silvopastoral systems have improved animal performance under tropical condition. Trees and shrubs contribute to decreased body temperature and increased welfare and performance, including the improvement of reproductive performance [3].

The integration of trees, herbaceous vegetation (grasses mainly) and animals was originally called agroforestry, which was defined as "a sustainable land management system which increases the yield of the land, combines the production of crops (including tree crops) and forest plants and/or animals simultaneously or sequentially on the same unit of land, and applies management practices that are compatible with the cultural practices of the local population" [4]. A silvopastoral system is an agroforestry practice that combines trees, forage crops, and livestock into a structural system of mutually beneficial interactions [5]. The shade of the trees can mitigate the impact of solar radiation on the understory herbaceous forages. In an early study, Chen [6] found that, under moderate shading conditions, the nitrogen supply in soil and forages increased. Furthermore, he stated that forages increased their nutritive value under tree canopies of multiple species composition, resulting in higher animal productivity, compared with that in open pasture. However, he also found that under heavily shaded environments by trees, there were profound effects even in shade-tolerant species. Belsky, in another classic paper [7], reported that savanna trees competed more aggressively with understory vegetation at nonwater-limiting sites, where the tree roots limited growth to close to their crown zones, than at drier sites, where their roots stretched farther into the open grassland. An important benefit of trees is their litter falling onto the soil surface contributing to enrichment with nutrients for the benefit of understory productivity by increased soil fertility. Shade promoted more biomass regrowth after defoliation than under the absence of shade by trees. Paciullo et al. [8] compared two production systems under organic milk production operations: A diverse system composed of grass (*Brachiaria* sp.), several herbaceous legumes, and legume trees (*Acacia mangium* Willd., *Gliricidia sepium* (Jacq.) Kunth and *Leucaena leucocephala* (Lam.) de Wit)), versus one open pasture. They found that herbaceous forage mass produced similar yields in both treatments; dry matter intake was also similar across systems, and milk yield was higher in the diverse-rich system than in the open pasture on the first year, but similar in subsequent years. Lana et al. [9] reported that trees contributed to increasing crude protein and mineral levels in the forage as compared with treeless systems.

However, the beneficial role of trees within typical silvopastoral systems is not always reported as positive for herbaceous biomass production (grasses, mainly). Lima et al. [10] recorded a decrease in tiller population density and a reduction in forage production due to the shading effect of trees, as compared with an open pasture. Paciullo et al. [11] found that green forage and root biomass were decreased under the canopy of trees and it was greater as they warded away from the tree rows. The magnitude of the decrement due to the shading effect was 22 and 41% for aerial biomass and root biomass, respectively. Consequently, along with our interest in studying the effect of shade by trees over the subjacent grass stratus in a grass and tree system, grass species and cultivar themselves with potential to tolerate shading by trees are also of interest. For a long time, *Brachiaria* spp. have been extensively reported to have varying levels of shade tolerance [12–15], while *Megathyrsus maximus* (Jacq.) B.K. Simon et S.W.L. Jacobs is one of the most prevalent species currently used in Yucatán [16,17] and other surrounding tropical regions [18]. Identifying the correct grass species and cultivars in a silvopastoral system not only has environmental advantages (increase in soil organic carbon), it also has economic advantages, mainly when forage grasses are associated with legumes that are efficient in fixing atmospheric nitrogen. The quality of the forage is considerably increased and the need to use synthetic fertilizers is reduced. Additionally, some *Brachiaria* hybrids are tolerant to acid soils and flood plains, typical of tropical regions, and pastures based in *Brachiaria* spp. have positive impact on milk productivity and feed sufficiency [19].

Given the dilemma of the presence of shade produced by trees over the understory-growing grasses [20], which constitute a large component of the forage for grazing livestock, it is urgent to test new grass species to the Yucatan's peninsula environment, with potential to develop in shaded conditions associated with tree and shrub species that occur in silvopastoral systems. Searching for the correct combination of trees and grasses has the potential to provide benefits for society, environment and the economy of those who have successfully implemented sound silvopastoral systems in the world [21,22]. The general hypothesis of this study was that there are *Brachiaria* spp. ecotypes with desirable productive, nutritional and durability characteristics to be grown under shading conditions imposed by trees, in the Mexican dry tropics. The objective of the present research was to assess Brachiaria ecotype performance under two sunlight intensities in order to identify promising ecotypes with potential to be used in Yucatan silvopastoral systems.

2. Materials and Methods

The study was conducted at Kampepen ranch, San Jose Tzal, 14 km south of the city of Merida, Yucatan, Mexico; at 20°50′17″ N latitude and 89°39′23″ W longitude, at an altitude of 10 m above sea level. The soils in the area are very shallow and with high percentages of rock, classified as litosols or "tzek'el", in Mayan language [23]. This region has a warm subhumid climate, with regular rains in the summer (May to July). Mean minimum, maximum, and average annual temperatures are 30, 21, and 26 °C, respectively, precipitation 984 mm, and the relative humidity ranges from 66 to 89% [24]. The climatic conditions (precipitation and temperature) during the development of the current research are presented in Figure 1. The climatic data were recorded in a meteorological station located at the Facultad de Veterinaria y Zootecnia of UADY, located 4.5 km from the place where the present research was conducted.

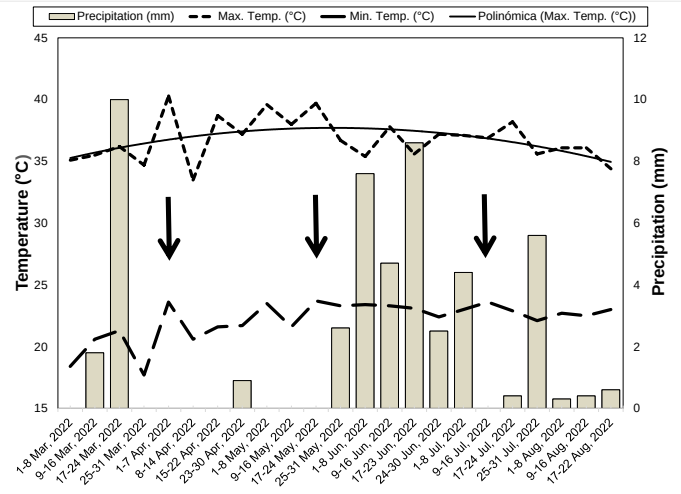


Figure 1. Climatic conditions at the experimental site, in Kampepen, Yucatan, Mexico. Solid line (polynomial trend line) represents the evolution of mean maximum temperatures. Arrows indicate dates of harvesting for each of three harvest dates.

A two-way factorial with six ecotypes and two sunlight intensity levels, arranged in a split-plot with main plots arranged in a randomized complete block design (RCBD), with sunlight intensity levels being the plots and ecotypes the subplots, with three replications, resulted in a total of 36 plots (six per each block). Each main plot had an area of 300 m^2 and each plot, containing one ecotype, measured 50 m^2. Thus, each ecotype was established in a total area of 300 m^2; three main plots were subjected to lower solar radiation intensity (heavily shaded) and three main plots were subjected to higher solar radiation intensity (slightly shaded). The three main plots with any given solar radiation intensity were randomly distributed across the entire experimental area. The experimental area has scattered trees of different species and numbers, as described in Table 1.

Table 1. Tree species and number across the experimental area in Kampepen, Yucatan, Mexico.

Block Number	Family	Scientific Name	Mayan Common Name	Number of Trees ha^{-1}
1	Fabaceae	*Caesalpinia gaumeri* (Greenm.)	Kitinché, Kitanché	720
	Boraginaceae	*Bourreria pulchra* (Millsp.)	Bakal che'	80
2	Fabaceae	*Piscidia piscipula* (L.) Sarg.	Habín	140
	Fabaceae	*Caesalpinia gaumeri* (Greenm.)	Kitinché, Kitanché	90
3	Fabaceae	*Caesalpinia gaumeri* (Greenm.)	Kitinché, Kitanché	100
	Fabaceae	*Piscidia piscipula* (L.) Sarg.	Habín	70
4	Fabaceae	*Caesalpinia gaumeri* (Greenm.)	Kitinché, Kitanché	100
	Fabaceae	*Piscidia piscipula* (L.) Sarg.	Habín	70
5	Fabaceae	*Piscidia piscipula* (L.) Sarg.	Habín	100
	Burseraceae	*Bursera simaruba* (L.) Rose	Chaká	25
	Fabaceae	*Havardia albicans* (Kunth.) Britton et Rose	Chukum	50
	Rhamnaceae	*Karwinskia humboldtiana* (Willd. ex Schult.) Zucc.	Lu'um chakte'	50
6	Fabaceae	*Piscidia piscipula* (L.) Sarg.	Habín	30

The treatments consisted of five *Brachiaria* ecotypes and *M. maximus* as control (the control belonging to a different species but is the most generalized species across the state's landscape, as pointed out earlier), and two levels of sunlight intensities. Photosynthetic photon flux density (PPDF) is defined as the amount of photosynthetically active photons, in the range 400 to 700 nm, which reach the surface of an object per second (the grass foliar canopy, in this case) [25–27]. Levels of PPDF, from now on, are simply called "solar radiation intensities". The grass ecotypes were T1: *Brachiaria* hybrid cv. Mulato II, T2: *Brachiaria* hybrid cv. Cayman blend, T3: *Brachiaria* hybrid cv. Talisman (BR05/1467), T4: *Brachiaria* hybrid cv. Camello blend, T5: *Brachiaria brizantha* cv. Marandú and T6 (control): *M. maximus* cv. Mombasa.

The procedure for determining the levels of solar radiation intensities consisted in measuring PPDF [28–30] in three subsamples (sites located at random) within each plot for each block. The software, Photone—Grow Light Meter for Plants on Windows PC (Lightray Innovation GmbH), was installed in a mobile phone (Samsung, Model Galaxy A71). The measurements were taken during three consecutive days, three times a day, at 9:00, 12:00, and 15:00 h. At the same time, using an infrared thermometer gun (Spectrum Technologies, Plainfield, IL, USA), the soil and grass canopy temperatures were collected. The average of the three daily measurements, averaged over the three days of measurements, was used as the criterion to discriminate between the three most illuminated (or less shaded) and the least illuminated (or more shaded) blocks. The mean PPDF value among the group of three blocks assigned to the lowest sunlight intensity was 1572 µmol m^2 seg^{-1}, while the mean PPDF of the three blocks assigned to the highest sunlight intensity was 2257 µmol m^2 seg^{-1}. Thus, considering that a PPDF of ≈3000 µmol m^2 seg^{-1} was recorded for points within the experimental area, free of shade by trees, under clear sky conditions, and with the sun in the zenith, the lowest sunlight intensity treatment represented a reduction of approximately 50%, while in the highest illuminated blocks the sun was blocked approximately 25%, as compared with the full sunlight incidence (100% sunlight intensity).

Bearing in mind that in these kinds of soils, due to their rocky nature, agricultural mechanization is impossible; thus, driving machinery around the scattered trees would be difficult and the seeding of grasses was therefore done by hand; i.e., cleaning for old fallen tree branches and removing herbaceous vegetation by hoeing, marking rows with plastic cord and wooden sticks, making rows on the soil surface to about 1 cm depth with a coa (a pointed wooden stick), seeding by hand and covering with soil, from November 8th to the 10th, 2021 (Figures A2–A14 show aspects from soil clearing and seeding up to fully grown grass stages). Plots consisted of 5 rows with a separation of 0.5 m between rows. A seeding density of 8 kg ha^{-1} was used, following the company's recommendations that

provided the seed (Semillas Papalotla S.A. de C. V.). No synthetic fertilizers were applied or other kind of synthetic chemical products to control pests. Weed control was done by hand and supplementary irrigation was used (by sprinklers) for both the establishment and the growing phases.

The response variables in the study were plant height, forage yield and chemical characteristics; morphologic components (leaves, stems, leaf:stem ratio, and dead material); plant canopy coverage and growth rate. Plant height was measured 77, 106, and 138 days after seeding (DAS) and 48 days after the first harvest. A metal ruler was used to measuring plant height, from the soil level to the tip of the plants. The first two measurements were collected in five randomly selected points from the three central rows on each plot, while the last two measurements were collected from three points only.

Three forage harvests were done by hand. The first harvest was done 148 DAS, the second one was done 55 days after the first harvest, and the third harvest occurred after 48 days from the previous harvest. Forage samples were collected by clipping three subsamples of 1 m row-length in each of the three central rows, at a residual forage height of 15–18 cm; subsamples were weighed fresh individually and discarded about half of the total harvested herbage. The remaining herbage was in turn divided into two parts. One of these parts was used to determine the dry matter concentration of forage, which was estimated by drying a sample of herbage of between 200 to 300 g in a forced air oven for 72 h at a temperature of 50 °C. The dry material was weighed and then, knowing the green weight prior to drying in the oven, the percentage of dry matter was calculated (% dry matter = (dry weight/fresh weight) × 100). Forage yield was calculated by multiplying the original fresh weight by the dry mater concentration, to obtain yield in dry basis.

From the other half of the herbage, an aliquot of approximately 200 g was separated into three components; leaves (blades only), stems, and dead material. These morphologic components were also dried, following the same procedure described to determine the dry matter concentration. Once having the fraction of leaves, stems and dead material in dry basis, the leaf:stem ratio was estimated by dividing the weight of leaves by the weight of stems. The grass growth rate was assessed by dividing the yield by the number of days from planting to the first harvest or by the number of days between harvests (for the second and third harvests). Starting in the second harvest, the phenological stages (plant maturity) of the ecotypes were determined, just prior to harvests. If joints (or nodes) were visible above the clipping height, plant maturity was determined by counting their numbers. If no joints were visible or if grass showed a visible inflorescence, these characteristics were recorded. A scale from 1 to 4 was used as follows: 1 = visible inflorescence; 2 = 4 to 5 visible joints; 3 = 2 to 3 visible joints; or 4 = 0 to 1 visible joints (tillering or leafy stage), as described first by Moore et al. and later by Moore and Moser [31,32]. Finally, immediately before the third harvest, a visual estimation of plant canopy cover was performed [33,34]. This was achieved by estimating the percent of plant canopy cover for the three central grass rows along the entire row lengths by three trained individuals, who assigned a qualification of 4, 3, 2 or 1 corresponding to 80–100, 60–80, 40–60 or <40% of plant canopy cover, respectively.

The chemical forage composition was determined for the second harvest only. The forage samples used to determine the dry matter concentration were ground to pass a sieve of 1 mm and determinations of dry matter content, crude protein content (CP %), neutral detergent fiber (NDF %) and acid detergent fiber (ADF %) were carried out. These analyses were performed at the bromatology laboratory located at the Campus of Biological and Animal Science of the University of Yucatan. Dry matter (DM) was determined through methodology proposed by AOAC [35]. Total nitrogen concentration (N %) was determined by micro-Kjeldahl and CP content was estimated by multiplying 6.25 × N % [36]. The fractions of NDF and ADF were assessed with the procedure developed by Goering and Van Soest [37].

Analyses of variance (ANOVA) were performed for each of the variables and, when significance was found ($p < 0.050$) among means, means comparisons were done using the

least significant difference (LSD) method. For all statistical analyses, PROC GLM from the statistical package SAS (version 9.0) was used [38].

3. Results

3.1. Plant Height

Mean plant heights that the ecotypes reached during the different measurement dates were 40, 73, 96 and 65 cm at 77, 106 and 138 DAS, and 48 days after first harvest, respectively. Significant ($p < 0.050$) ecotype x sunlight intensity interactions were recorded for the first and second measurement dates (Figure 2a,b). In the third measurement date, only the ecotypes factor was highly significant ($p < 0.0001$); which measured, from the tallest to the shortest, Mombasa, Marandu, Cayman, Talisman, Camello and Mulato II, with 120, 109, 91, 89, 84 and 82 cm, respectively. At the fourth measuring date (48 days after the first harvest), both ecotype and sunlight intensity were highly significant ($p < 0.0001$, in both cases), reaching heights of 92, 70, 66, 58, 56 and 50 cm for Mombasa, Marandu, Camello, Cayman, Talisman and Mulato II, respectively, while for sunlight intensities, the tallest ecotypes (73 cm) were found under a low sunlight intensity (heavier shading), and the shortest (58 cm) were found under the highest sunlight intensity (lightest shading).

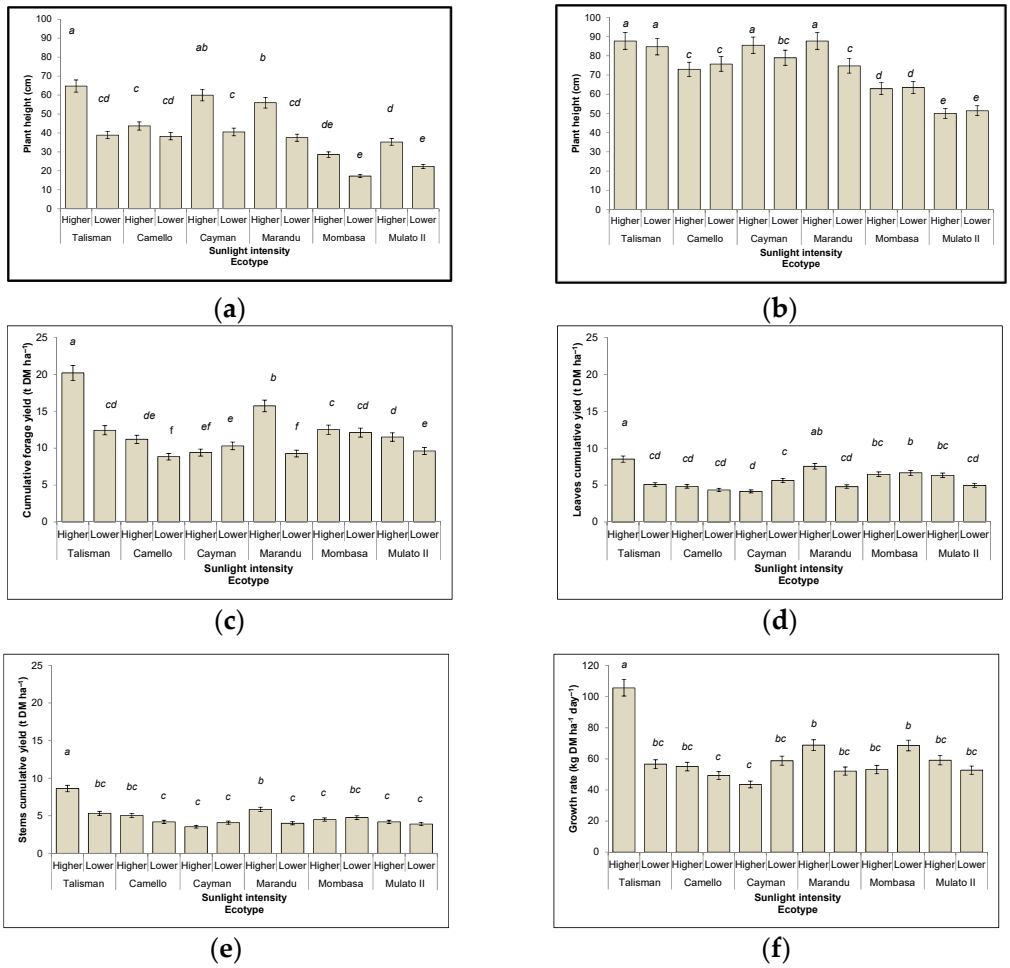


Figure 2. Effect of the interaction between ecotypes of *Brachiaria* spp. and *M. maximus* with sunlight intensity on (**a**) plant height at 77 DAS and (**b**) at 107 DAS; (**c**) cumulative forge; (**d**) leaf; (**e**) stem yields, and (**f**) on plant growth rate. Lines above each mean value (gray bars) represent 5% errors. Different letters across means are different ($p < 0.050$).

3.2. Cumulative Forage Yield

Mean cumulative forage yield from three harvests was 12.0 t DM ha^{-1}. A significant ecotype x sunlight intensity interaction ($p = 0.003$) was found. This interaction is associated with the fact that ecotypes Cayman and Mombasa produced similar ($p > 0.050$) and showed the lowest yields, independent of the sunlight intensity, while ecotypes Talisman, Camello, Marandu and Mulato II resulted in higher forage yields ($p < 0.050$) when grown under a higher rather than lower sunlight intensity (Figure 2c). Furthermore, the mean forage yield of all ecotypes was 46% lower than the ecotype Talisman (11.5 vs. 16.7 ton ha^{-1}, respectively).

3.3. Morphologic Components

3.3.1. Leaf

Cumulative leaf yield was highly significantly ($p = 0.001$) affected by the ecotype x sunlight intensity interaction. The interaction is illustrated in Figure 2d. It can be noticed that performance of ecotypes Camello, Mombasa and Mulato II were not significantly ($p > 0.050$) affected by growing in either sunlight intensity condition. Talisman and Marandu significantly ($p < 0.050$) yielded a greater leaf biomass when grown under the highest sunlight intensity, while Cayman was the only ecotype that significantly ($p < 0.050$) yielded more leaves under lower light intensity.

3.3.2. Stems

Stem yield, similar to the leaves component, resulted in a significant ($p = 0.021$) ecotype x sunlight intensity interaction (Figure 2e). Ecotypes Talisman and Marandu yielded greater stem biomass under the highest rather than the lowest sunlight intensity; whereas the rest of the ecotypes, Camello, Cayman, Mombasa and Mulato II, yielded similarly ($p > 0.050$), regardless of the sunlight intensity regime to which they were subjected.

3.3.3. Leaf:Stem Ratio

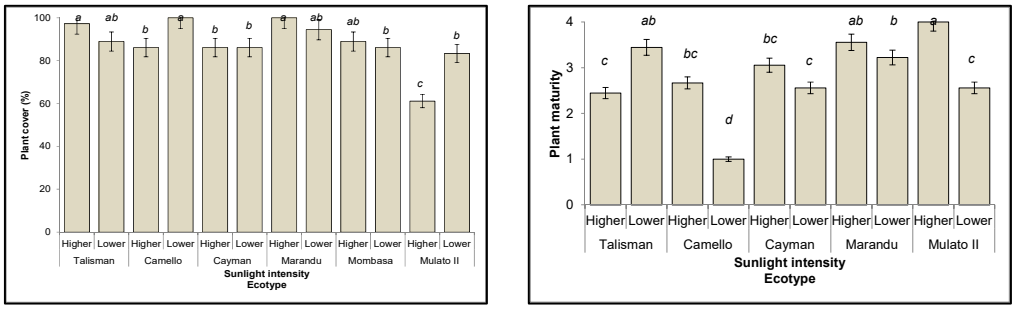
Leaf:stem ratio was highly significantly ($p < 0.0001$) affected by the ecotypes per se; with a general average of 1.3. Ecotypes with the greatest leaf:stem ratio were Mombasa and Mulato II, followed by Marandu and Cayman, whilst the ecotypes with the lowest leaf:stem ratio were Camello and Talisman, with 1.6, 1.4, 1.3, 1.3, 1.0 and 1.0, respectively.

3.3.4. Dead Material

Cumulative dead material production in forage biomass was highly significantly ($p < 0.0001$) affected by both ecotypes and sunlight intensities, but not for their interaction ($p = 0.174$). The greatest dead material biomass yield accumulation was found for ecotype Talisman, followed by a single group composed of the other ecotypes, with 2.7, 1.6, 1.3, 1.3, 1.0 and 1.0 ton ha^{-1} for Talisman, Marandu, Mombasa, Cayman, Mulato II and Camello, respectively. The average dead material yield was found at the high sunlight intensity (1.8 ton ha^{-1}) and was higher than that found at low sunlight intensity (1.1 ton ha^{-1}).

3.4. Plant Canopy Cover

Plant canopy cover (measured only after the last harvest) was significantly ($p = 0.012$) influenced by the ecotype x sunlight intensity interaction (Figure 3a). This interaction is explained on the basis that the ecotypes showed all types of responses to sunlight intensity levels. Talisman, Cayman, and Marandu did not vary significantly ($p > 0.050$) under either of the two sunlight intensities; Mombasa showed a greater plant canopy cover when grown under the highest solar intensity regime than under the lowest sunlight intensity; however, Camello and Mulato II showed a significantly ($p < 0.050$) lower plant canopy cover when subjected to the highest sunlight intensity treatment.



(**a**) (**b**)

Figure 3. Effect of the interaction between *Brachiaria* spp. ecotypes and *M. maximus* with sunlight intensity on (**a**) percent plant canopy cover; and (**b**) plant maturity just prior to second harvest (excepting *M. maximus*)—numbers 1 to 4 on the *y*-axis in (**b**) represent degree of maturity: 1 = visible inflorescence, 2 = 4 to 5 visible joints, 3 = 2 to 3 visible joints and 4 = 0 to 1 visible joints (tillering or leafy stage). Lines above each mean value (gray bars) represent 5% errors. Different letters across means are different ($p < 0.050$).

3.5. Plant Maturity

Ecotype *M. maximus* was not included in the plant maturity analyses because number of visible joints was used to relate to plant maturity and since the morphology of *M. maximus* was markedly different from *Brachiaria* spp. ecotypes (*M. maximus* having longer and fewer internodes than *Brachiaria* spp.); therefore, the comparison was not appropriate.

Ecotype maturity at the moment of the second harvest was highly significantly ($p < 0.0001$), affected by the ecotype x sunlight interaction (Figure 3b). The interaction is explained due to ecotype Talisman being more mature ($p = 0.003$) under the lowest rather than under the highest sunlight intensity; contrary to this, Camello and Mulato were more mature ($p < 0.050$) under the highest rather than under the lowest sunlight intensity. However, neither Cayman nor Marandú were not significantly ($p > 0.050$) affected by either sunlight intensity regime, although, numerically, these latter ecotypes always tended to be more mature under the highest rather than under the lowest sunlight intensity. In Figure 3b, note that the lower the score, the more mature were the ecotypes and vice versa.

By the last harvest, ecotypes was the only significant ($p < 0.0001$) source of variation. Mulato and Marandu were similar among them but the least mature of all ecotypes ($p < 0.050$), followed Cayman with intermediate maturity. The second most mature ecotype was Talisman, while the most mature of all ecotypes was Camello ($p < 0.050$). The mean values for these ecotypes were 3.1, 2.9, 2.5, 1.9 and 1.2, respectively.

3.6. Growth Rate

Mean growth rate, averaged over all three harvests, with reasonably mature grass plants (late harvests), was highly significantly ($p < 0.0001$) and affected by the ecotype x sunlight intensity interaction (Figure 2f). This interaction occurred because Talisman had a growth rate 53% greater ($p < 0.0001$) under the highest sunlight intensity than under the lowest one (106 vs. 57 kg ha^{-1} day^{-1}), while the other ecotypes did not significantly ($p > 0.050$) vary their growth rates, regardless of the sunlight intensity under which they were exposed. Furthermore, the lowest growth rate of Talisman (under the lowest sunlight intensity) was at least similar or higher ($p < 0.050$) than the other ecotypes growing in any sunlight intensity regime.

3.7. Forage Chemical Composition

Means with standard errors for each of the chemical variables of the forages (only the second harvest) are presented in Table 2. Forage dry matter was not significantly ($p > 0.050$) different for any of the main factors or the ecotype x sunlight intensity interaction. The general mean DM was 22%. The CP content was not significantly ($p > 0.050$) affected by any of the sources of variation. However, sunlight intensity showed a trend to significantly

(p = 0.078) influence PC concentration, with ecotypes grown under the exposure of the highest sunlight intensity showing greater PC concentrations than ecotypes grown under the lowest sunlight intensity regime, 12.1 and 10.6%, respectively. NDF was significantly (p = 0.012) influenced only by the ecotypes. NDF concentrations were 66.5, 66.0, 62.7, 60.4, 60.3 and 59.7%, for Mombasa, Camello, Marandu, Cayman, Mulato II and Talisman, respectively. Mombasa and Camello had the highest and similar, as opposed to Cayman, Mulato II and Talisman, which were the lowest; Marandu was intermediate between these two groups. ADF, similar to CP, was significantly affected by none of the sources of variation in the model. However, similar to that reported for CP content, ADF also showed a trend (p = 0.052) to be influenced by sunlight intensity. Ecotypes exposed to the lower sunlight intensity accumulated an average of 37.6%, while the same ecotypes under higher sunlight intensity recorded an average of 35.8%.

Table 2. Mean forage dry matter (DM %), crude protein content (CP %), neutral detergent fiber (NDF %) and acid detergent fiber (ADF %) for *Brachiaria* spp. and *M. maximus* ecotypes, grown under two sunlight intensities.

n	Variable	Mean	S.E.	*n*	Variable	Mean	S.E.
	Higher Sunlight Intensity				**Lower Sunlight Intensity**		
			Talisman				
3	DM	27	4.0	3	DM	20	0.7
	CP	11	0.3		CP	12	2.6
	NDF	61	0.5		NDF	59	2.6
	ADF	35	1.1		ADF	34	2.7
			Camello				
3	DM	25	1.3	3	DM	23	1.9
	CP	12	2.2		CP	9	1.8
	NDF	65	2.7		NDF	67	2.7
	ADF	37	2.8		ADF	40	2.2
			Cayman				
3	DM	23	3.6	3	DM	17	1.1
	CP	12	1.5		CP	10	2.9
	NDF	60	2.9		NDF	61	2.9
	ADF	35	2.2		ADF	36	2.9
			Marandu				
3	DM	23	2.3	3	DM	19	1.6
	CP	10	2.5		CP	11	2.3
	NDF	63	3.9		NDF	62	3.3
	ADF	38	4.1		ADF	37	2.7
			Mombasa				
3	DM	21	0.4	3	DM	21	1.2
	CP	12	1.1		CP	9	2.9
	NDF	66	1.8		NDF	67	2.4
	ADF	39	2.7		ADF	40	2.7
			Mulato II				
3	DM	22	0.8	3	DM	20	1.5
	CP	15	0.8		CP	11	2.1
	NDF	57	1.5		NDF	63	2.1
	ADF	32	0.6		ADF	38	2.4

4. Discussion

4.1. Forage Yields and Chemical Composition

General mean dry yield was 12.3 ton ha^{-1}, which was reached under shading by trees, without the application of any synthetic fertilizer, and remaining for two months (at least)

for one more harvest in the establishment year. Our tested ecotypes could easily reach at least similar or higher yields than most of the reported studies [39–43]. It is estimated that forage yields from *Brachiaria* spp. are in the order of 9 to 13 ton ha^{-1} year^{-1} during the establishment year as supported by previous reports in the literature [44]. Furthermore, if the *Brachiaria* crop remains healthy, it would be expected a yield increment for the second year, as observed in other studies [44,45].

As noted earlier, with the exception of NDF being affected ($p = 0.012$) by ecotypes, none of the forage chemical composition variables (from the second harvest only) in the present study were affected ($p > 0.050$) by any source of variation. However, the case of CP content is interesting since it tended to be greater under the highest sunlight intensity. This result is not in agreement with the majority of the published literature, which instead reported an increment in PC under shade produced by trees in silvopastoral systems [10,46–48]. A possible explanation for our results could be related to a confounding effect of soil fertility with shading levels, as pointed out by Cruz et al. [20].

The mean yield in the first harvest from blocks under higher sunlight intensity (less shaded) was 6.2 ton ha^{-1}, while the mean yield in blocks under low sunlight intensity (more shaded) was 4.5 ton ha^{-1}; a difference of 1.7 ton ha^{-1} was found (Figure A1a). Assuming a similar distribution of nutrients across all plots at the beginning of the experiment, the plots under the highest sunlight intensity, because these supported substantially higher yields than the plots under lowest sunlight intensity, soil fertility was likely lower in the former than in the latter. Therefore, we suggest that grasses that regrew in the lowest sunlight intensity had higher mineral nutrients availability for the following harvest cycle (the second harvest). If a modest PC concentration is assumed, for example, 8% in the first harvest (not measured), that would represent a N percentage in forage of 1.28%. In the first harvest, the plots under the highest sunlight intensity yielded 1700 kg ha^{-1} more than plots under the lowest sunlight intensity. This yield difference represents removing about 22 kg N ha^{-1} in excess in the highest intensity plots, compared with the lowest sunlight intensity plots. This exceeding N (equivalent to an application of 50 kg ha^{-1} of urea) in the plots under the lowest sunlight intensity probably caused the opposite in the second harvest, i.e., higher forage yields (3.2 and 2.2 ton ha^{-1}, respectively) than in the highest sunlight intensity plots (Figure A1b). Such an effect, higher forage yields now in the lowest sunlight intensity plots, likely caused the second harvest forage to come out lower in CP content than in the lowest intensity compared to the highest intensity plots. It is important to note that the forage that was chemically analyzed in this study was from the second harvest only.

A negative relation between forage quality and yield has long been recognized by several authors and, in general, occurs due to the N dilution in a greater biomass production [49–52]. In the second harvest, as additional evidence for the occurrence of this general quality–yield relationship (not statistically analyzed), the PC level in block one (heavily shaded) was substantially higher than in the other blocks (although the group of more heavily shaded blocks in average were slightly lower in PC than less shaded blocks), while this same block (one) recorded the lowest yields in the second harvest (despite the group of more heavily shaded blocks in average yielding more than the less shaded blocks).

The mean PC value in our study was 11.3%, 62.6% for NDF and 36.7% for ADF. Ali et al. [52] reported PC values of 11 and 8% in two growing cycles of *Brachiaria* spp. in a silvopastoral system and open pasture, respectively: 35 and 33% ADF and 66 and 68% NDF; de Castro Santos et al. [53] reported an average of 10.3% for PC, 65.1% for NDF and 31.9% for ADF, in three silvopastoral systems and two growing seasons. Thus, our mean values for PC, NDF and ADF were in agreement with similar previous research results.

4.2. Leaves, Stems and Dead Material Production

Leaf production in the present study, in the first harvest, having clipped very mature forage (five months after seeding), the fraction of leaves to the total forage was 45%. To the second harvest (after almost two months from the first harvest), the leaf fraction increased

to 63%. The results from the second harvest (data not shown) are similar to those reported by Pizarro et al. [43], who tested *Brachiaria* spp. ecotypes in two wet seasons in Thailand. In the first assay (of two reported), the mean leaf production of 16 *Brachiaria* spp. hybrids accounted for 67 and 83%. In the second assay, the mean leaf production was 64 and 80%. In de Góis Fontes et al. [45], leaf fractions of 53, 51, 47 and 44% were reported for clipping heights of 10, 20, 30 and 40 cm, respectively. Pedreira et al. [42] reported leaf proportions of 59%, in average, for two clipping heights. The leaf production in the study conducted by Cruz Hernández et al. [40] was 85 and 84%. The difference between the results for our research and those reported by Cruz Hernández et al. [40], regarding leaf fraction, while a general coincidence with results from Pizarro et al., Pedreira et al. and de Góis Fontes et al. [42,43,45], may find justification in the differences in maturity at the time of harvests. This is in view of the long periods between harvests (150 DAS, 60 days after first harvest, and 48 days after second harvest); the Cruz Hernández et al. [40] study's grazing was done every 14, 21 and 28 days. In contrast, from the de Góis Fontes et al. [45] report, it can be deduced that the maturity of their forages were also very advanced, as these were harvested every 2.2 months in average (5 clippings in 11 months).

According with Moore et al. [54] and Mitchell et al. [55], growth of perennial grasses involves the continuous production of leaves and stems. Once an environmental signal is sensed, the apical meristem switches from producing leaves to reproductive structures, the time at which the apical meristem turns to determinate. Ecotypes of *Brachiaria* spp., in the present study at the first harvest, surely had already easily overpassed their leaf production and growth phase, and the new carbon sink organs became likely reproductive organs. By the second and third harvests, although to a lesser degree, leaf production rates had likely already declined production and growth and started the reproductive phases, as shown in Figure 3b.

The hypothesis that suggests that leaf production in our study was less than most of that reported in published literature due to advanced maturity of our forages is further supported by the fraction of dead biomass and stems production. The general average of stems fraction was 40% and dead material was 12%. In Brazil, de Góis Fontes et al. [45] reported fractions of dead material and stems of 15, 16, 17 and 16%, and 38, 35, 30 and 29%, respectively. From Pedreira et al. [42], we estimate that they had 12% dead material. However, as noted earlier, these two studies harvested fairly mature forages, as it was in the present study. To this regard, Cruz Hernández et al. [40] stated that as the grazing interval increases in tropical grasses, the forage yield increases but with a lower leaf fraction proportion and greater stems and dead material quota.

4.3. Particularities of Ecotypes

In the present study, the precocity of ecotypes Talisman and Camello was identified. As far as we know today, there are no reports that document the precocity of Talisman. In fact, Hanson et al. [56] very recently stated that Talisman, is in a pre-commercialization stage, in the process of being released for commercialization in the next five years. Concerning ecotype Camello, on the other hand, it was advertised as a very early ecotype [57,58]. According to Menendez and Miranda, [59], it was recorded that maximum forage accumulation in Camello decreased after six weeks following regrowth, and leaf production declined after four weeks following regrowth; thus, these authors recommended clipping or grazing before six weeks after regrowth. In the present study, in every harvest, we exceeded the optimum timing for harvesting (six weeks). Therefore, we recommend closely observing the maturity of Camello, particularly when growing under shaded conditions (Figure 3b) because we confirmed its tremendous earliness for maturation. A fast-growing ecotype can be useful under water supply uncertainties (without irrigation access, under dryland).

Because high evapotranspiration rates begin in the Yucatan peninsula around the beginning of April–May [60,61], our ecotypes around the second harvest were probably more water stressed than at the first harvest (Figure 1). Ecotypes Cayman, Marandú and Mulato II yielded more under the lowest sunlight intensities (under a more shaded

conditions and likely lower evapotranspiration). Concerning this, Guenni et al. [14] stated that at reduced sunlight intensity, due to tree shading, positive plant responses have been observed in forage production and quality in tropical and subtropical C-4 grasses when water and nutrients are in short supply. Thus, Cayman, Marandú and Mulato II are ecotypes that in our study have shown signs to be susceptible to water stress conditions. Cayman, in fact, was selected for having good capacity to grow in soils susceptible to flooding conditions [58]. This characteristic (yielding more under higher shading conditions) can be explained by a lower evaporative rate when growing under shade.

The effect of trees over microclimatic conditions and evapotranspiration has been amply studied [62–66]. Benegas et al. [67] studied the main processes by which trees influence the soil water dynamics in a tropical grassland, with dispersed trees in Honduras. It was found that trees reduced water losses through evapotranspiration on the soil surface. The findings of this study provide evidence that trees can have positive effects on the local hydric balance. Because it was identified that a large fraction of the irrigation water in crops in central California was lost due to evapotranspiration, Yu et al. [63] recommended the use of shade to cover field crops, which resulted in a 37% reduction in evapotranspiration during the hottest months.

In contrast with hydric-stress-susceptible ecotypes, toward the second harvest (Cayman, Marandú and Mulato II), other ecotypes expressed some tolerance to this condition. Such cases were Camello and Mombasa (Figure A1b), which maintained similar yields, independent of the sunlight intensity under which they grew. Talisman is worth mentioning because it was the only ecotype tested in the present study that, at the time of the second harvest, under apparent water stress conditions (Figure 1), yielded substantially more under the higher sunlight intensity (and also possibly higher evapotranspiration) (Figure A1b). A relationship between hydric stress tolerance and precocity in grasses was found by González et al. [68,69], who reported that barley (*Hordeum vulgare* L.), under terminal hydric stress (from flag leaf on), the yield negatively correlated with the number of days to spike emergence. Thus, it was concluded that the early appearance of the inflorescence, a good osmotic adjusting capacity, and high values of relative water content, all contributed to increasing barley grain yields, even under terminal hydric stress.

In contrast to the conditions that may be faced by our tested ecotypes in the second harvest, in the first, when water was likely less limited for all ecotypes (since the crop was under irrigation but, more importantly, with yet moderate evapotranspiration rates), the yields, in absolute terms, were always greater under the highest sunlight condition when other factors such as sunlight may have been more limiting to the plants, as has been reported [53,70,71]. In the end, when considering the cumulative yields, compensation effects apparently occurred among the different ecotypes interacting with contrasting environmental conditions at different times during their lifecycles, yields were always greater ($p < 0.050$) under the highest sunlight intensities, except for Cayman and Mombasa, which were insensible to our two tested sunlight intensities, but yielding similar or lower than the lowest yield of Talisman and, obviously, much less than the highest yield of Talisman.

Concerning plant canopy cover, it is important to notice that the apparent decrease in Mulato II (61% plant canopy cover) under the higher sunlight intensity should be taken with caution, since this figure was surely affected by a problem with one irrigation sprinkler that caused a large portion of one plot to wilt.

4.4. Ecotype X Sunlight Intensity Interaction

The significant ecotype x sunlight intensity interaction, affecting most of our variables, coincides with other studies. Martuscello et al. [72] evaluated one ecotype of *Brachiaria decumbens* (Stapf) (or *Urochloa decumbens* (Stapf)) and two of *Brachiaria brizantha* (Hochst. ex A. Rich.) or (*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster) under contrasting shading levels; reporting significant interactions for most of their response variables such as forage cumulative yield, leaf fraction proportion, and plant height. The interaction in their work [72], also observed in the present study, showed that the greatest

shading (70%) substantially decreased their cumulative yields, as compared with the treatment of 0 and 50% shading levels, which resulted in similar yields, although this pattern was not proportional across ecotypes.

Additionally, similar to what was found in the present study, Paciullo et al. [73] found that the growth rate (mm stem^{-1} day^{-1}) was affected differently (significant interaction), which depended on their ecotypes and shading levels. The stem elongation rate decreased drastically as the shading level increased, although this response occurred only for *Brachiaria decumbens* (Stapf) (or *Urochloa decumbens* (Stapf)) and *Brachiaria ruziziensis* (Germ. et C.M. Evrard) (or *Urochloa ruziziensis* (R. Germ. et C.M. Evrard) Crins), but at levels of 0 and 36% shading the yields were similar for *Brachiaria brizantha* (Hochst. ex A. Rich.) (or *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster), cv. Marandú and *B. brizantha*, cv. Xaraés. They concluded that *Brachiaria* spp. ecotypes experienced phenotypic adjustments to manage moderate shading levels, as suggested earlier for cumulative yields.

As a result from the present study, the need for testing the same ecotypes in the future was identified, also under monocropping conditions (without shading), making it possible to obtain information about the degree of yield decrements, persistency and chemical composition, if they exist, as compared with the shading scenarios tested in the present study. Another aspect that is necessary to take into account in the future is the phenological stage at the time of harvesting, with the purpose of reaching several objectives, such as the greatest possible biomass accumulation, forage quality and stand conservation, instead of harvesting all the ecotypes at the same time. Additionally, we need to introduce the animal component to graze our *Brachiaria* spp. ecotypes, so we then could properly refer to silvopastoral systems.

4.5. Limitations of Our Study

It is worthwhile to address a few limitations of the results reported in the present experiment. Firstly, the duration of our study was short, although the grass stands (and trees) in the system were still very healthy and producing forage vigorously at the time of writing this manuscript. It was previously reported that some grass species may produce well in the initial months after establishment, although some grasses may completely disappear due to competition with trees after some time [20]. Our study is not in the position for concluding about this possibility. The reason for the short duration of the study relates to administrative reasons: this paper derives from a master's degree program of a student who graduated and left, and no replacement students are currently available to carry on with the research. Another aspect that we have missed was measuring evapotranspiration. If we had measured and presented evapotranspiration data, we would not have to rely just on literature reports. Another important aspect that would have tremendously enriched the robustness of our results would have been the inclusion of a zero light interference treatment (a full sunlight control). That would have provided information on the yield potential of non-shaded *Brachiaria* spp. ecotypes, which would have been useful for farmers who are convinced that trees in their pastures reduce their yields and therefore, are not ready to switch from grass monoculture to silvopastoral systems in the short term. The ranch where this experiment was conducted is privately owned and it was not possible to open a treeless area to test our grass ecotypes (clearing an area for grasses without trees would have implied tree-slashing on the property).

5. Conclusions

From the results obtained in the present research, it is concluded that there is sufficient genetic variability within *Brachiaria* spp. ecotypes because they significantly interacted with sunlight intensity levels for most of our variables. Thus, there is evidence that there are ecotypes that could be used for the benefit of producers in the dry tropical climate of the Yucatan peninsula. Given the observed characteristics, all of the tested *Brachiaria* spp. ecotypes could be recommended for different farmers' needs or interests. Since they were the earliest ecotypes of our tested ecotypes, Talisman and Camello are recommended for

zones where precipitation levels or distribution are limited or highly variable, since they can produce forage biomass within small-time windows, as compared to later-maturing ecotypes. Because Cayman, Marandú and Mulato II showed signs of being sensible to water shortages during the most acute evapotranspiration period (the second harvest), these ecotypes are the least recommended for regions prone to frequent droughts. Cayman and Mombasa, however, are recommended for highly shaded environments, since these ecotypes yielded similarly, independent of the sunlight regime. However, if we take into consideration all the variables indicative of greater adaptability and forage quality characteristics, it was found that the ecotype that clearly out-performed the other tested ecotypes in this research was Talisman. Talisman has shown that it can tolerate moderate shading levels (higher sunlight intensity) very well, and yields at least as much as the other ecotypes when growing under severely shaded conditions (lower sunlight intensity). Talisman ended with a plant canopy cover greater than 90% and a substantially faster growth rate, particularly under moderate shading. Furthermore, numerically, Talisman recorded the second highest CP content and the one that accumulated the least NDF or ADF fractions. It is also appropriate to note that Talisman was the ecotype that produced the greatest dead material and also recorded the lowest in leaf:stem ratio, which suggest that it is a very early ecotype, along with Camello, at least under the conditions of the present study. Thus, close monitoring of their phenology is recommended to adecuately grazing/clipping of this ecotype, allowing lower dead material to develop and achieving greater leaf:stem ratios, to obtain the best forage quality.

Author Contributions: Conceptualization, F.J.S.-S.; data curation, R.B.T.-L. and J.S.-C.; formal analysis, J.S.-C.; funding acquisition, F.J.S.-S.; investigation, R.B.T.-L., F.J.S.-S. and J.S.-C.; methodology, F.J.S.-S. and J.S.-C.; project administration, F.J.S.-S.; resources, F.J.S.-S.; supervision, F.J.S.-S., J.C.K.-V. and C.F.A.-P.; visualization, J.S.-C.; writing—original draft, J.S.-C.; writing—review and editing, R.B.T.-L., F.J.S.-S., L.R.y.A., C.F.A.-P. and J.S.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: All data reported in the present study can be shared upon request by Jesús Santillano-Cázares at: jsantillano@uabc.edu.mx.

Acknowledgments: The authors express their gratitude to CONACyT for the financial support to Reyes B. Torres-Lugo, through a scholarship, for the completion of his master's degree. Additionally, the authors appreciate the support to Semillas Papalotla, S.A. de C.V., for the donation of *Brachiaria* spp. seeds. Finally, we want to express our appreciation to Samuel Jeannerot, from the L'Institut Agro Dijon, le nouveau nom d'AgroSup Dijon, France, for his invaluable help in field work and friendship during the long hours that the study demanded.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

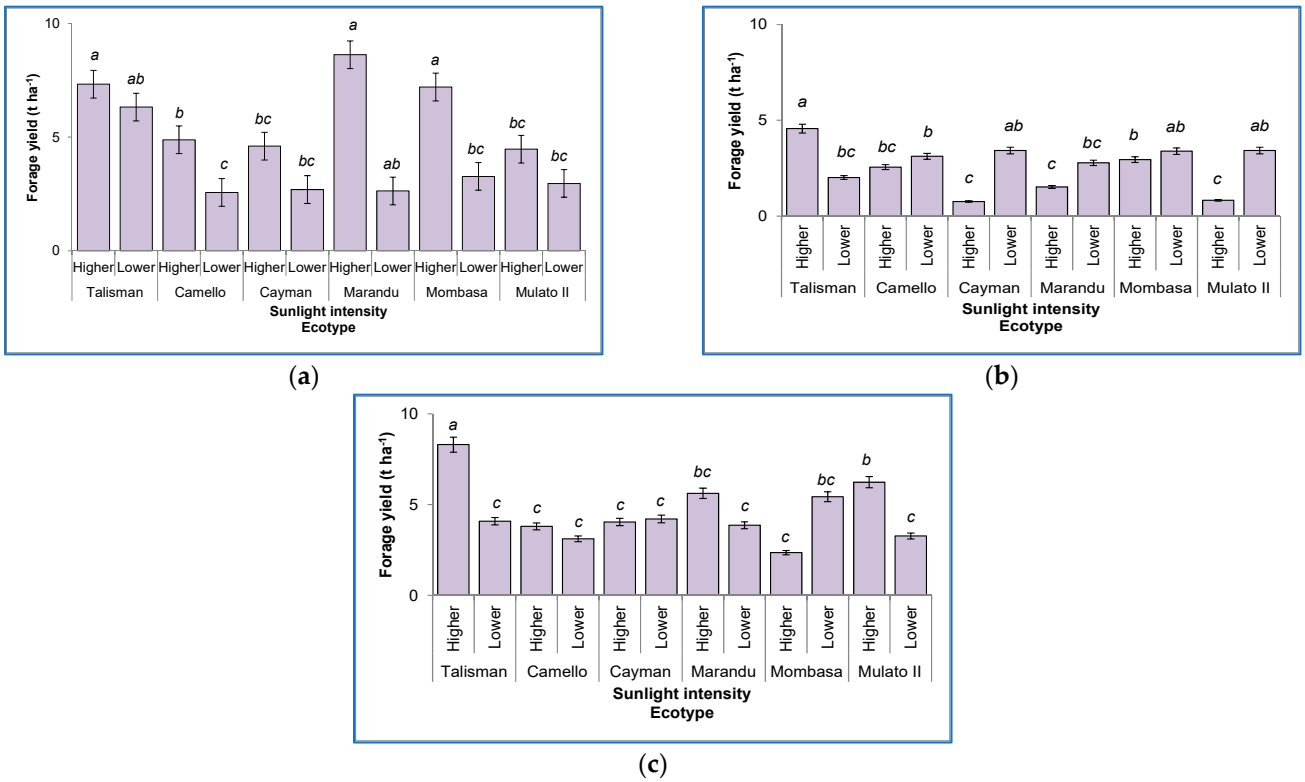


Figure A1. Effect of the interaction between ecotypes of *Brachiaria* spp. and *M. maximus* with sunlight intensities on forage yield in the (**a**) first, (**b**) second and (**c**) third harvest. Lines above each mean value (gray bars) represent 5% errors. Different letters across means are different ($p < 0.050$).

Figure A2. Brachiaria hybrids seed arrival for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A3. Original aspect of the experimental area for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A4. Soil preparation by hand for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A5. Plot measurement for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A6. Stakes nailed to tie cords for delimiting plots for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A7. Marking of plot limits with plastic cord for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A8. Small furrows ready for seed deposit for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A9. Seed deposited in soil by hand for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A10. Germination irrigation for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A11. Brachiaria emergence for the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A12. Brachiaria at tillering stage in the establishment of an experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A13. Growing Brachiaria spp. ecotypes in experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

Figure A14. Fully grown Brachiaria ecotypes in the experiment to identify the best ecotypes under two sunlight intensities in Kampepen, Yucatan, Mexico.

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