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Post-emergence nicosulfuron application enhanced leaf-stem ratio in maize-intercropped with *Urochloa* species after shading and sunlight re-exposure

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Abstract

Low rates of nicosulfuron used in post-emergence provide an advantage to maize during the coexistence of the crops, when maize is intercropped with *Urochloa* grasses. Nonetheless, the effect of this practice on the morphology of these grasses is not known, neither how it affects forage growth after the coexistence period nor when the grass is re-exposed to full sunlight. The aim of this study was to determine biomass and leaf-stem ratio of *Urochloa* hybrid cv. Mulatto II (CIAT 36087), *Urochloa brizantha* (Hochst. ex A. Rich.) and *Urochloa ruziziensis* (Germ. & Evrard) after the development in a shade simulated environment promoted by maize in intercrop systems. The treatments consisted of three brachiaria species with two herbicides managements. Evaluations occurred at 0, 30 and 60 days after the sunlight re-exposure (DASR). Nicosulfuron application and *Urochloa* species did not affect biomass yield. However, herbicide increased leaf-stem ratio of the species between 25% at 0 DARS to 62% at 30 DARS on old tillers (tillers that grew under shade). The biomass yield, as well the leaf-stem ratio of the new tillers was not affected. Among the species, the greater ratio of leaves in the plant biomass was recorded for *Urochloa brizantha* (Hochst. ex A. Rich.) and *Urochloa hybrid* cv. Mulatto II (CIAT 36087). Nicosulfuron use in post-emergence enhanced the leaf-stem ratio of *Urochloa* grasses after sunlight re-exposure and a reliable alternative to improve forage quality in intercrop systems.

Keywords: Crop-livestock systems; Forage quality; Intercrop; Low rate Herbicides.
Abbreviations: DAS_days after sowing, DASR_days after sunlight re-exposure, ICLS_integrated crop-livestock system, New tillers_tillers that grew under full sunlight, Old tillers_tillers that grew under shade, New tillers_tillers that grew under sunlight.

Introduction

The intercropping of maize with perennial tropical forages provides an effective mean of deploying Integrated Crop-Livestock System (ICLS) in tropical agriculture. In this intercrop, the forage and maize are sown at the same time, coexisting through all maize cycle. After harvesting the maize, forage can be used for grazing. If well-managed, this intercrop results in the same yield as sole maize production and an additional 2 to 5 Mg ha^{-1} of *Urochloa spp.* biomass in dry season, the most used forage genus for this purpose (Pariz et al., 2011; Almeida et al., 2018).

During coexistence period, the forage can compete with maize for resources, such as light, water, nutrients and space. Ensuring lower competition for maize growth resources is a key factor for the success of the intercrop. For this matter, some measures have been used, such as spraying low herbicides rates on the forage. Using lower herbicides rates than the recommended aims at hindering forage growth during coexistence period (Jakelaitis et al., 2006). The most used herbicide to suppress the forage growth in ICLS is nicosulfuron (Almeida et al., 2018; Oliveira et al., 2018; Pariz et al., 2017). Despite the fact that the recommended rate of nicosulfuron is lethal to *Urochloa spp*., the low rate may cause suppression on forage, decreasing the competition between the two species (Anésio et al., 2017). It provides maize competitive advantage over *Urochloa* spp, resulting in reduced light availability to the forage. As maize intercepts more sunlight, it grows faster and uses resources quicker. By doing so, it becomes the dominant species of the intercrop and *Urochloa.*, the subordinate one.

Urochloa remains shaded during maize cycle and most of the times it results in etiolation process of the forage (Paciullo et al., 2011). Etiolation alters the chemical composition and morphology of the forage, affecting fibrous accumulation on stem (Pariz et al., 2016). Etiolation effects on *Urochloa* could be reduced using post-emergence herbicides during the period of shadow, which would be desirable because etiolation enhances the lignin content in the plant tissues, decreasing its digestion by ruminants (Gallego-Giraldo et al., 2016). Overall, stretched out plants have less leaf/stem ratio and enhanced lignin content, limiting factors for selection and forage intake by grazing animals (Glienke et al., 2016). However, the effect of the herbicide applied post-emergence on intercropped grasses morphology is unknown, or its effects after the end of coexistence period of the two species, when the forage is used for animal grazing.

Supporting organs, such as roots and stems, have a higher lignin contents in xylem and sclerenchyma tissues (Valente et al., 2016) and as a result, there are several studies correlating the nutritive quality and lignin content of the forages through the relation between leaves and stems (Brito et al., 2004; Dumont et al., 2015). On the other hand, spraying herbicides could reduce forage yield, which would affect the carrying capacity of the ILCS area. This biomass reduction is unwanted, in view of the fact that *Urochloa* is an important source of forage in winter dry season, as in the Brazilian Cerrado, which has average rainfall around 60 mm ha^{-1} during these seasons (Oliveira et al., 2015).

The objective of this study was to investigate the effect of low nicosulfuron rate on morphology of the genus *Urochloa* spp. sprayed post-emergence at shadow conditions, similar to the conditions of maize and forage intercropping. Also, we investigated three species of *Urochloa*, since significant differences among these species had been reported, when shaded by maize in intercropping (Paciullo et al., 2011; Maia et al., 2014; Simão et al., 2018).

Results and Discussion

Biomass

The spraying of low herbicide rate and the *Urochloa* species did not affect the biomass of the tillers growing under shadow conditions, named old tillers (Table 1). A positive correlation between biomass and sampling data was recorded, from 32.5 g pot-1 to 62.5 g pot^{-1}. Tillers that grew under full sunlight (new tillers) - after the removal of the shading net - were also not affected by the low herbicide rate spray or *Urochloa* species. The new tillers biomass average was 21.1 g pot^{-1}. There was no difference in the total biomass (new + old) among the species. Total biomass increased throughout time sampling, 54, 67.7 and 84.4 g pot^{-1} for 0, 30 and 60 DARS, respectively (Table 1).

Biomass yield of *Urochloa* growing under shadow conditions was not affected by low herbicide rate. Alvarenga et al. (2011) also recorded no differences in *Urochloa brizantha* biomass between control (no herbicide) and plants that received low nicosulfuron rates at maize harvesting. The authors also concluded that low nicosulfuron rates were an important management to avoid interspecific competition in the intercrop.

Leaf stem ratio

The leaf stem ratio of the old tillers presented significant interactions for sampling date vs species and sampling date vs low herbicides rate (Table 2). *U. brizantha* and *U. mullato II* reached the greater leaf proportion resulting in larger leaf-stem ratio (Figure 1A). Throughout the sampling dates, the leaf-stem ratio decreased in all *Urochloa* species (Figure 1A). Low herbicide rate increases leaf-stem ratio for all species at 0 and 30 DARS in comparison to the control with no spraying (Figure 1B). For new tillers, species and herbicide spray did not affect the leaf stem ratio (Table 1). Overall, the leaf-stem ratio among new tillers was 1.32. Among the species, *U. brizantha* and *U. mullato II* obtained the greater total leaf-stem ratio through the sampling date, 1.25 and 1.27 respectively (Table 1). Significant interaction of sampling date and graminicide were recorded to total leaf-stem ratio. Low rate herbicide spraying increased leaf-stem ratio for all *Urochloa* species at 0 and 30 DARS collection period (Figure 1C).

Management and environmental conditions affected the leaf-stem ratio for *Urochloa spp*. In monoculture, the leaf-stem ratio ranged from 0.71 to 2.5 (Fagundes et al., 2006; da Silveira et al., 2016) and from 0.4 to 2.0, when growing in intercrop arrangement (Portes et al., 2000; Martuscello et al., 2017). Our results are in accordance with what was previously reported in the literature, but when low herbicide rates were used, significant differences among species and collected periods were recorded (Figure 1B and C). Herbicides from the sulfonylurea group, such as nicosulfuron, are absorbed and transported to apical points (Durner et al., 2008; Shergill et al., 2018), to restrict amino acids production. Low nicosulfuron rate did not kill *Urochloa* plants. However, they certainly impaired the stems elongation and redirected plant growing to leaves, enhancing leaf-stem ratio. According to Jakelaitis et al. (2006) even doses ten times lower (2 g a.i. ha^{-1}) than used at the present work can promote the biomass reduction in *Urochloa* plants, showing that the hormesis effect can be probably discarded once even lower doses can be toxic to this gender. After sunlight re-exposure at 150 DAS, *Urochloa* growth was enhanced and the leaf-stem ratio of the plants that received low herbicide rates reached similar values of the plants with no herbicide spraying. Portes et al. (2000) suggested that there is a rearrangement of the morphology of the intercropped *Urochloa* after the re-exposure to sunlight. The authors reported no differences between intercropped and monoculture *Urochloa brizantha* in biomass, number of tillers and leaf area index at 70 DASR, when oldest leaves were re-exposed to full sunlight. Almeida et al. (2017) evaluated methods of establishment of the intercrop between maize and forage with cultivars of *Panicum* spp. and concluded that the late implantation of the forage also increased the leaf-stem ratio. In this study, forage stress caused high levels of shade, instead of herbicide application, but biomass yield was lower. In the present study, the simulation was done for simultaneous planting of maize and forage and the application of low herbicide rate increased the proportion of leaves with no biomass yield impairment. Among the *Urochloa* species, *U. brizantha* and *U. mullato II* presented a greater proportion of leaves comparing to *U. ruziziensis*. Investigating six *Urochloa* species intercropped with maize, Maia et al. (2014) reported similar results for leaf-stem ratio. After the maize grain harvest, values of cellulose, hemicellulose, and N-lignified were lower for *U. brizantha* cultivars. For the same parameters, Pariz et al. (2010) reported similar variation among *Urochloa* species due to shadow effects caused by intercrop arrangement. These results are consistent with findings reported herein and show that *Urochloa* species is an important factor to provide greater forage quality under shadow conditions. Through our results, it is possible to affirm that in the short term, the low herbicide rates in post-emergence in the intercropping of maize with *Urochloa* will not affect the biomass production but will improve the leaf-stem ratio. This information is useful for obtaining high pasture quality after maize harvesting, or for maize silage

Table 1. Combined analysis of variance among date sampling, species and low herbicide rate use for biomass.

Treatments		Biomass "Old"		Biomass "New"		Biomass Total	
DASR[1]	*Urochloa spp.*	Without herbicide g. pot^{-1}	Nicosulfuron	Without herbicide g.pot^{-1}	Nicosulfuron	Without herbicide g.pot^{-1}	Nicosulfuron
	U. brizantha	36.4	30.7	21.5	20.6	57.9	51.2
0	*U. mullato II*	31.8	27.2	20.7	21.8	52.5	49.0
	U. ruziziensis	34.7	34.1	21.2	23.7	55.8	57.7
Mean 0 DASR		32.5 C		21.6 A		54.0 C	
	U. brizantha	48.2	35.0	19.5	19.3	67.7	54.4
30	*U. mullato II*	48.2	49.3	19.5	19.3	67.7	68.7
	U. ruziziensis	54.0	53.1	19.9	19.0	73.9	72.2
Mean 30 DASR		48.0 B		19.4 B		67.4 B	
	U. brizantha	87.2	85.4	24.0	23.7	87.2	85.4
60	*U. mullato II*	93.2	86.1	20.9	22.7	93.2	86.1
	U. ruziziensis	79.5	77.4	20.5	21.7	79.5	77.4
Mean 60 DASR		62.5 A		22.3 A		84.4 A	
[1] DASR = days after sunlight re-exposure; ANOVA (Pr> F)							
	Colleted Period (C)	***		ns		***	
	Urochloa spp. (U)	ns		ns		ns	
	Graminicide (G)	ns		ns		ns	
	C*U	ns		ns		ns	
	C*G	ns		ns		ns	
	U*G	ns		ns		ns	
	C*U*G	ns		ns		ns	
	CV %	25.3		12.3		19.1	

ns = non-significant; * = significative at 5%; ** = significative at 1%; *** = significative at <1%.

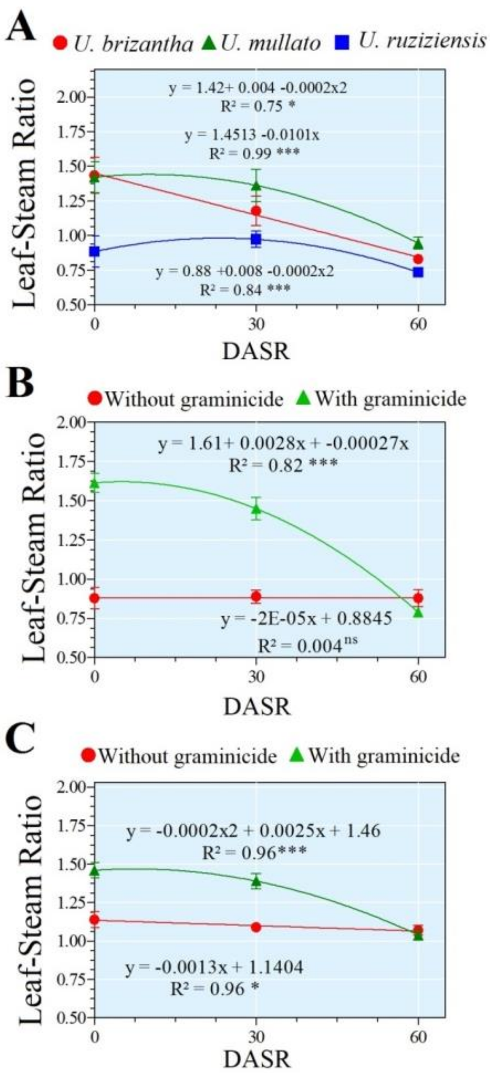

Fig 1. *Urochloa spp.* leaf-stem ratio of the old tillers; (A) low rate herbicide effects on the leaf-stem ratio of the old tillers (B) and total tillers (C) (mean of the species). Vertical bars indicate standard error.

Table 2. Combined analysis of variance among date sampling, species and herbicide use for leaf-stem ratio.

Treatments		Leaf-Steam Ratio [1] "Old"		Leaf-Steam Ratio "New"		Leaf-Steam Ratio Total	
DASR[2]	*Urochloa spp.*	Without herbicide	Nicosulfuron	Without herbicide	Nicosulfuron	Without herbicide	Nicosulfuron
0	*U. brizantha*	1.04	1.83	1.47	1.43	1.25	1.63
	U. mullato II	1.07	1.77	1.31	1.22	1.19	1.50
	U. ruziziensis	0.53	1.24	1.45	1.28	0.99	1.26
Means 0 DASR		0.88	1.61	1.36		1.14	1.46
30	*U. brizantha*	0.85	1.51	1.32	1.33	1.08	1.42
	U. mullato II	0.99	1.73	1.29	1.39	1.14	1.56
	U. ruziziensis	0.84	1.11	1.27	1.28	1.06	1.19
Means 30 DASR		0.89	1.45	1.31		1.09	1.39
60	*U. brizantha*	0.90	0.75	1.24	1.28	1.07	1.02
	U. mullato II	0.95	0.94	1.36	1.26	1.16	1.10
	U. ruziziensis	0.78	0.69	1.16	1.30	0.97	0.99
Means 60 DASR		0.88	0.79	1.27		1.07	1.04
				Forage means in DASR			
U. brizantha		0.93	1.36	1.35		1,25 A	
U. mullato II		1.00	1.48	1.31		1,27 A	
U. ruziziensis		0.72	1.01	1.29		1,08 B	
				ANOVA (Pr>F)			
	Colleted Period (C)	***		ns		***	
	Urochloa spp. (U)	***		ns		***	
	Graminicide (G)	***		ns		***	
	C*U	*		ns		ns	
	C*G	***		ns		***	
	U*G	ns		ns		ns	
	C*U*G	*		ns		ns	
	CV %	9.5		14.92		2.87	

[1] L/S = ratio; [2] DASR = days after sunlight re-exposure; ns = non-significant; * = significative at 5%; ** = significative at 1%; *** = significative at <1%.

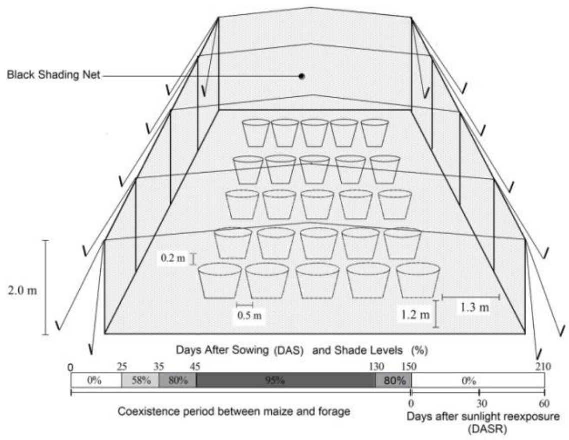

Fig 2. The greenhouse structure to simulate shade levels. All treatments received equal shade levels and at the same time.

and forage production (Freitas et al., 2005; Mendonça et al., 2014).

Materials and Methods

Plant materials

In this study, three Urochloa species, commonly cultivated as forage in Brazilian fields, were utilized. They were *U. brizantha* cv. Marandu, *U. ruziziensis*, and a *Urochloa* hybrid between *U. brizantha*, *U. decumbens* and *U. ruziziensis* called *Urochloa* spp. hybrid cv. Mulatto II (CIAT 36087).

Experimental conditions

A greenhouse experiment was carried out from September 2015 to May 2016 in the Crop Science Department of University of São Paulo in Piracicaba, Brazil. Throughout the experimental period, the average air temperature ranged from 23.6° to 27° C. Irrigation was managed to ensure 70% of the soil field capacity and to avoid water stress.

The experimental design was of complete randomized blocks with 18 treatments in factorial design 3x3x2 and five repetitions. The treatments were composed by three *Urochloa* sampling dates: 0 days after sunlight re-exposure (DASR), 30 DARS and 60 DASR; (2) three species of the genus *Urochloa* spp.: *Urochloa* spp. hybrid cv. Mulatto II (CIAT 36087), described as *U. mulatto II*; *Urochloa brizantha* cv. Marandu; and *Urochloa ruziziensis*; (3) with and without the application of herbicide nicosulfuron sub dose. All treatments aimed to simulate the shading condition that brachiaria species develop, when intercropped with maize in field.

Establishment of the treatments

The *Urochloa* species were sown in pots of 25 kg filled with typical Dystrophic Red Latosol (Oxisols) and spaced 0.5 m to each other, similar to the maize grass intercrop. The base saturation of the soil was adjusted to 70% with limestone application 120 days prior to the implementation of the experiment. General *Urochloa* fertility management was adjusted according to the recommendations of Raij et al. (1997).

The herbicide nicosulfuron (Sanson 40SC ®, 40 g ai L^{-1}, ISK Biosciences Brasil, 448 Av. Fábio Ferraz Bicudo, Indaiatuba, São Paulo, Brasil) was applied at a rate of 25 g a.i. ha^{-1}, 20 days after sowing, when all species started tillering. Previous studies report similar rate and time to *Urochloa* intercropped with maize on field (Garcia et al., 2013; Pariz et al., 2016). The application of the herbicide was performed with a backpack CO_2 pressurized sprayer that applied a constant pressure of 196 MPa and that was fitted with an application wand equipped with a 2 meters long bar and four Teejet XR 110.2 flat spray tip spaced 0.5 meter to each other and calibrated for a spray volume of 200 L ha^{-1}. The application was performed late in the late afternoon, for there was mild temperature and higher relative air humidity during the time of application, to increase its efficacy (Ramos et al., 2010). In order to simulate the light availability to *Urochloa* during intercropping, all pots were gradually shaded during the experiment according to shade levels and respective dates previously obtained in a maize plantation with 150-day cycle (unpublished data). Therefore, shading levels were 58%, 80%, 95% and 80% at 25, 35, 45, and 130 days after sowing (DAS), respectively. At 150 DAS, the plants were re-exposed to full sunlight, simulating post-harvest condition in the maize-*Urochloa* intercrop.

Shading levels were adjusted with a luximeter model LD 200. Full luminosity (100%) was measured at full sun, at 12:00 hours and with a clear sky. Thereafter, shadow levels were adjusted overlapping black shading nets. Light level was measured at ten random points 0.5 m above the pots. Details are presented in Figure 2.

Data collection

At the end of the shading period, at 150 DAS, all the tillers were marked in each pot and named "old". The evaluations of the old tillers were performed at 0, 30 and 60 DASR. All the tillers that emerged after the 150 DAS were called "new" and their evaluations were also performed. Total biomass and total leaf-stem ratio was obtained from new and old tillers.

Immediately after the samples harvest, a subsample was taken to determine the leaf-stem ratio. The leaf blade with the sheaths was considered as the leaf. The remaining material was named stem. The plant material collected from the pots was dried for 72 h in a forced-air oven at 60 °C to measure dry mass.

Conclusion

The application of low nicosulfuron rates and the different species of *Urochloa spp*. did not affect the forage biomass production under shading conditions. However, the leaf-stem ratio among the *Urochloa* species was increased by up to 100%, when the herbicide was sprayed over forage leaves. The greater leaf-stem ratio was recorded for *U. brizantha* and *U. mullato II*. These results suggest that low nicosulfuron rates in post-emergence enhanced the forage quality and can be a useful tool for intercrop arrangements aiming for animal grazing post-harvest of maize.

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