

Adaptive Responses of *Paspalum oteroi* Native Grass to Fertilization and Shading

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Abstract

The aim of this study was to evaluate the *Paspalum oteroi* native grass and its adaptive responses to shading and foliar fertilization in two pasture systems in the Cerrado-Pantanal transition region. A completely randomized block design with factorial arrangement was adopted to this study: three types of fertilizers [no fertilization - control (C); Foliar fertilizer for pasture (FF) and Soil fertilizer + Foliar fertilizer (SF)] x two pasture systems (WS - with shading, wooded P. oteroi pasture; NS - no shading, exclusive P. oteroi pasture) with four repetitions. P. oteroi seedlings were transplanted in November 2015. Data collection was from May 2016 to September 2016, during dry season. There were differences (p < 0.05) in the sward height variables (H), dry matter (DM), crude protein (CP) content, dry matter production (DMP) and shoot: root ratio (S:R). In the WS system increased of 8,61% of the CP content and fertilizer SF increased of 8.81% of the CP content. However, in the WS system was observed reduced DM, DMP and S:R and in the FF and SF fertilizers increased leaf area index (LAI), in the latter months of the application. Probably, P. oteroi grass developed adaptation strategies to deal with the environmental conditions to which it was submitted during this study, such as variations in its morphophysiological traits: low shoot / root ratio, height, chemical composition, leaf area index e light interception. In conclusion, P. oteroi showed adaptation to wooded pasture systems, during dry period, however, the use of fertilization depends on economic viability studies.

Keywords: leaf area index, light interception, native forage resource, silvopastoral systems, chemical composition

1. Introduction

In general, water shortage, and light and soil nutrient availability are considered resources that limit plant growth in grasslands (Craine e Dybzinski, 2013; Siebenkäs et al., 2015), affecting pasture productivity and nutrient supplies for the animals. On the other hand, integrated crop-livestock systems, such as the silvopastoral system, have been used to optimize the sustainability of pastures through the production of wood-based and forage. In addition to this, the silvopastoral system can result in accumulated soil C and reduced emissions of greenhouse gases, increasing economic and environmental outcome (Balbino et



al., 2012; Salton et al., 2014).

Integrated systems must be designed differently among the Brazil regions according to specific characteristics (Salton et al., 2014). Savannas cover very extensive areas of Central Brazil including Pantanal, highlighting the importance of the Cerrado-Pantanal interface. One of the most frequent demands of the savanna region has been shade tolerant grasses aiming at pasture-forest production systems, however, these forages are limited (Euclides et al., 2010). Thus, studies with different forages are fundamental to evaluate tolerance and development under conditions of restricted luminosity (Carvalho et al., 2002).

In Brazil, only a few genera and species of forages are being studied focusing mainly on exotic species (Euclides et al., 2010). Thus, the research for native forage has a great relevance for the region and the country. In Pantanal, several grasses with potential for management and cultivation have been identified, but studies involving characterization and responses to different environmental conditions are yet to be done (Santos et al., 2011). Physiological and morphological plasticity may have a different role in plant adaptation so that it can deal with the environmental factor variability (Gratani, 2014).

Another limiting factor in the savanna region refers to soil fertility which is considered to have low and intermediate fertility, which may adversely affect the pastures establishment (Euclides et al., 2010). Besides the fact that the interaction of light and soil fertility on forage trait expressions have been poorly studied (Siebenkäs et al., 2015).

The aim of this study was to evaluate the *Paspalum oteroi* native grass and its adaptive responses to shading and foliar fertilization in two pasture systems in the Cerrado-Pantanal transition region.

2. Method

This work was carried out in a transition region from Cerrado-Pantanal, Aquidauana, Mato Grosso do Sul, Brazil. The experimental area belongs to State University of Mato Grosso do Sul, located at 147 m Altitude, Latitude 20°28'16" S, and Longitude 55°47'14" W. The experiment was carried out from November 2015 (planting) to September 2016. The climate is AW (humid tropical), according to the climatic classification of Köppen. The climatic conditions during the study period are showed in the Figure 1.

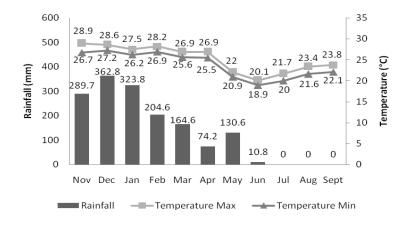


Figure 1. Accumulated rainfall, and minimum and maximum temperatures from November 2015 to September 2016, according to the National Meteorological Institute of the Municipality of Aquidauana - MS (Inmet, 2016)

Seedlings of the *Paspalum oteroi* native grass growing in their natural environment, in the region recognized as Nhecolandia's Pantanal (at Latitude 18° 59' S and Longitude 56° 39' W). These seedlings were collected, stored in wet newspaper, and finally transported to the experimental area, transplanted, and cultivated. Plant density was 10 plants m⁻¹, with 10 cm spacing between plants.

This study was conducted adopting a completely randomized block design (CRBD) with factorial arrangement 3 x 2: three types of fertilizers [No fertilization – control (C); Foliar fertilizer for pasture (FF) and Soil fertilizer + Foliar fertilizer (SF)] x two pasture systems (WS - with shading, wooded *P. oteroi* pasture; NS - no shading, exclusive *P. oteroi* pasture) with four repetitions each one. The experimental area had two hectares with one hectare for each pasture system. The shading of the first treatment was provided by trees of *Terminalia* spp, and *Paspalum oteroi* was cultivated between them. The area of the NS treatment had only *Paspalum oteroi* cultivated (with no natural or artificial shadow).

An inventory of all trees dispersed in WS system was conducted to characterize and describe richness, sociability, composition, and cover type characterization. In that area, just the *Terminalia* spp was identified, in an isolated grouping, totalling 19 trees distributed randomly among the plots. To characterize the treetop type and shadow projected by *Terminalia* spp, it was used the methodology cited by Da Silva et al. (2006). *Terminalia* spp. has inverted conic treetop and its shadows projections calculated for this type of cup. Average shade of the trees was 58.2 m^2 , and the total area of shade was $1,105\text{m}^2$.

Random soil samples were taken at 0-20 cm depth at the beginning of the experiment for chemical analysis. The samples were air-dried, processed, and sent to the chemical analysis laboratory. Based on the results (Table 1) it was not necessary to correct the pH and fertilize soil with potassium or other fertilizers than phosphorus.

Table 1. Results of soil sample's chemical analysis, and relationships between these chemical components, in two systems of pastures (with shading -WS; and without shading -NS)

Sample	р	Η	Р	MO	Κ	Ca	Mg	Ca+Mg	Al	Н	Al+H	S	Т	V
Systems ¹	$CaCl_2$	Water	mg/dm³	g/dm³				cm	nol/dm	3				%
WS	4.85	5.48	7.75	16.2	0.16	1.95	0.95	2.90	0.05	2.27	2.32	3.06	5.38	56.9
NS	4.69	5.32	3.96	15.8	0.15	1.90	1.00	2.90	0.10	2.58	2.68	3.05	5.73	53.2
	Relations Saturation %							6						
Systems	Ca	/Mg	Ca+M	lg/K	Ca	/K	N	/Ig/K		CA	Mg	K	М	Η
WS	2.	05	18.	1	12	2.2	:	5.94		36.2	17.6	3.00	1.61	42.2
NS	1	90	19.	2	12	7		6.67		33.2	174	2 70	3.20	45.0

Source: Laboratory of soil analysis and consulting LTDA. ¹WS - with shading -wooded *P. oteroi* pasture; NS - no shading - exclusive *P. oteroi* pasture.

Therefore, the experimental area was cleaned to cultivate the forage, and the native trees were preserved in the area determined as WS pasture system. Subsequently, the area was demarcated in 24 plots (experimental plots) of nine m². After the transplanting, the seedlings were irrigated until their establishment in the area.

In the FF treatment, the foliar fertilizer was Quimiorgen pasto[®] (six L ha⁻¹) plus Niphokam[®] (two L ha⁻¹). In the SF treatment, soil fertilizer was Simple Superphosphate – SS (390 kg ha⁻¹), and the foliar fertilizer was Quimiorgen pasto[®] (six L ha⁻¹) + Nipokam[®] (two L ha⁻¹). These doses have been recommended by the fertilizer manufacturer. In the Tables 2 and 3 are shown the chemical composition of foliar fertilizer registered by a Brazilian's commercial company with the names of Niphokam[®] and Quimiorgen pasto[®] (FÊNIX Agro, Tietê, São Paulo).

Composition	Percentage	Concentration
Nitrogen	10 %	135.0 g L ⁻¹
Phosphorus (P ₂ O ₅)	8 %	108.0 g L ⁻¹
Potassium (K ₂ O)	8 %	108.0 g L ⁻¹
Calcium (Ca)	1 %	13.5 g L ⁻¹
Magnesium (Mg)	0.5%	6.75 g L ⁻¹
Boron (B)	0.5%	$6.75 \mathrm{g \ L^{-1}}$
Copper (Cu)	0.2%	2.70 g L ⁻¹
Manganese (Mn)	0.5%	6.75 g L ⁻¹
Zinc (Zn)	1 %	13.5 g L ⁻¹

Table 2. Main chemical composition of Niphokam®

Sources of data: (Fenix[®] Agro, 2016).



Composition	Percentage	Concentration
Phosphorus (P ₂ O ₅)	20 %	270.0 g L ⁻¹
Boron (B)	0.5 %	6.75 g L ⁻¹
Manganese (Mn)	3 %	40.5 g L ⁻¹
Zinc (Zn)	3 %	40.5 g L ⁻¹

Table 3. Main chemical composition of the Quimiorgen pasto®

Sources of data: (Fenix[®] Agro, 2016).

A pressure equipment was used to applicate the foliar fertilizer, with the following calibration: tip XR 11002; Spout thickness 0.50 m; Gout = Fine; Volume = 200 L ha⁻¹; Speed = 1 m s⁻¹ = 3.6 km h⁻¹ and pressure = 1.7 bar. With this equipment it was applied the fertilizers Quimiorgen Pasto[®] and Niphokam[®]. To applied SS, it was used 390 kg ha⁻¹. Thus, each plot was fertilized with 0.351 kg of SS, distributed along the planting lines, before transplanting the seedlings.

After transplanting *P. oteroi* seedlings in the experimental plots, their development was monitored monthly. As soon as the plants reached 100% of cover a uniform cut was made at five cm from the soil. Then, the corresponding foliar fertilizer was applied to each plot. Data collection was from May 2016 to September 2016, during dry season.

One sample of 0.25 m² per experimental plot was harvested, in each 28 days, to evaluate forage quality and biomass production. The dry matter production per hectare (DMP ha⁻¹) was estimated. From each sample collected was taken a representative subsample. Subsamples were oven dried at 65 °C/72 hours and finely ground for analysis content of crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) accordance with methods INCT-CA N-001/1, INCT-CA F-002/1, INCT-CA F-002/1 and INCT-CA F-004/1, respectively (Detmann et al., 2012).

For the measurements of light interception (LI) and leaf area index (LAI), the AccuPAR model device - LP 80 was used. Readings were performed between 11:00 am and 01:00 pm, making an average of two readings in each experimental plot, in both pasture systems (WS and NS). Two measurements were taken simultaneously: above the forage sward and below it. The LAI was estimated simultaneously to the monitoring of light interception by the average of the forage canopy, since the AccuPAR calculates the LAI based on PAR (Photosynthetically Active Radiation) measured above and below the canopy simultaneously with other variables related to the canopy architecture and position of the sun. Sward height (H) was measured simultaneously with the use of a graduated ruler. These measurements were made weekly throughout the experimental period. Light interception (LI, %) at a given position was calculated as $(I_0 - I/I_0) \times 100$, where (I₀) is the irradiance above sward and (I) is the irradiance under the same sward.

The monolith method was used to obtain root and soil to evaluate the ratio between shoot and root (S:R ratio) (Bohm, 1979) at the end of the experiment. Two soil cubes with 25 cm x 25



cm sides in dept of zero to 25 cm, were sampled in each experimental plot and the roots were washed and separated in a sieve. The shoot was cut. Both shoot and root were dried at 65 °C (pre-drying) to constant weight to determination dry matter (DM) in accordance with Detmann et al. (2012). The S:R ratio was calculated by dividing the yield in shoot DM by the root DM yield.

The data of sward height (H), light interception (LI), leaf area index (LAI), dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry matter production (DMP) per hectare was analyzed as repeated measures over time using the PROC MIXED of the SAS University (SAS Institute., Inc., Cari, CA). The S:R ratio was analyzed as a single measurement, taken at the end of the experiment. Furthermore, when a significant effect occurred, the means values were compared by t test. Pearson correlation coefficient was used to examine the linear relationship among variables, using PROC CORR of the same software. For all analyses, it was adopted a significance level of 5%.

3. Results and Discussion

There was no interaction between fertilizer type and the pasture systems. Thus, the effects of all variables were analyzed independently (Table 4).

		Fertilizer ^{2,3}		Pasture s	ystems ^{2,3}	P-	Value
Variables ¹	С	FF	SF	WS	NS	Fertilizer	Pasture systems
Н	10.2 ± 0.369	9.91 ± 0.369	9.89 ± 0.484	$9.35\pm0.348b$	$10.6\pm0.323a$	0.850	0.016
IL	38.8 ± 2.28	35.3 ± 2.28	42.6 ± 2.98	40.4 ± 2.14	37.4 ± 1.99	0.182	0.316
DM	$35.5\pm0.537^{\text{a}}$	$36.8\pm0.537a$	$32.7\pm0.705b$	$33.2\pm0.506b$	36.8 ±0.470a	0.001	<.0001
CP	$7.32 \pm 0.151b$	7.50 ±0.151b	$8.81 \pm 0.199a$	$8.61 \pm 0.143a$	$7.15\pm0.132b$	<.0001	<.0001
EE	1.61 ± 0.083	1.67 ± 0.083	1.83 ± 0.109	1.79 ± 0.078	1.61 ± 0.072	0.309	0.111
NDF	67.7 ± 0.430	67.6 ± 0.430	67.4 ± 0.563	67.4 ± 0.405	67.7 ± 0.375	0.936	0.599
ADF	37.2±0.384	36.7 ± 0.384	35.7 ± 0.503	36.2 ± 0.362	36.8 ± 0.335	0.102	0.222
DMP	$0.999 \pm 0.015a$	$1.03\pm0.015a$	$0.920 \pm 0.019b$	$0.935\pm0.014b$	$1.035 \pm 0.013a$	0.001	0.0001

Table 4. Least square means and standard errors of the variable analyzed

¹H (sward height, cm); LI (light interceptation, %); DM (dry matter, %); CP (crude protein, % DM); EE (ethereal extract, %DM); NDF (neutral detergent fiber, %DM); ADF (acid detergent fiber, %DM); DMP (Dry matter production ton ha⁻¹).² C - no fertilization; FF – foliar fertilizer for pasture and SF– soil fertilizer + foliar fertilizer. WS - with shading - wooded *P. oteroi* pasture -; NS - no shading - exclusive *P. oteroi* pasture -.³ Means followed by the same lower case letters on the lines do not differ significantly by the t test (p < 0.05).

There was no effect (p > 0.05) of type of fertilization on sward height. However, a pasture systems effect (p < 0.05) was observed on this characteristic. Sward height reached lower values under shaded conditions when compared to full sunlight, a result which was contrary to expectations because shading can reduce solar radiation with consequent taller and longer stems in their search for luminosity (Martuscello et al., 2009; Souza et al., 2007). However, some other authors have reported similar results to this study (Carvalho et al., 1995; Eriksen & Whitney, 1981) while Santos (2014) did not find significant differences in height under shading in *Brachiaria brizantha* cv. Marandu, *Brachiaria decumbens* cv. Basilisk, and *Panicum maximum* cv. Tanzania. According to Carvalho et al. (1995) the reduction of height under the effect of shading may be related to the tree components that prevent luminosity. In



this study, grasses were transplanted in an already established understory.

There were no significant differences (p>0.05) between C and FF for DM and DMP. The lowest DM and DMP contents were observed in SF fertilization. Nevertheless, Pietroski et al. (2015) observed higher dry matter using a dose of 35.24 kg ha⁻¹ of N in *Panicum maximum* cv. Mombaça.

DM and DMP were affected significantly by pasture systems. The dry matter content of *P. oteroi* was reduced by shade at about 10%. According to Gobbi et al. (2011) the shaded system can modify leaf anatomy reducing sclerenchymatous tissue and thickness of the palisade parenchyma, thus, reducing the dry matter content. On the other hand, this decrease in thickness of the palisade parenchyma, which contains most chloroplasts, reduces net photosynthetic rate (Boardman, 1977; Paciullo et al., 2007). These same factors may also have influenced forage quality. There were significant differences (p<0.05) among fertilizers type as well as between the pasture systems in CP content. Higher (p<0.05) values of CP content were observed for the treatment SF and WS (Table 5).

According to Eriksen and Whitney (1981), the dry matter content decreased with shading, but increased the N content. Several studies demonstrated that shading increased crude protein in grasses. Santos (2014) evaluated four shade levels (0, 30, 50 and 70 %) and observed the highest CP content with the level of 70% in *Brachiaria decumbens, Brachiaria brizantha* cv. Marandu, and *Panicum maximum* cv. Tanzânia (18%, 18% e 16% respectively). Barro et al. (2008) found an average increase of 2% of the CP content in *Lolium multiflorum, Avena strigosa.* and *Avena sativa* submitted to the shading of *Pinus elliottii.*

The SF fertilization (Soil fertilizer + Foliar fertilizer) also contributed to a higher CP content. In general, the nutrients offered by SF may have acted directly on the photosynthetic process and on the enzymatic metabolism, resulting in larger photoassimilates (Rosolem, 2002).

The CP mean content for *P. oteroi* in the control treatment was 7.32% a satisfactory value considering the dry period and the fact that the CP levels are influenced by growth stage and soil fertility. According to Taylor and Allison (1982) another factor which seems to have affected the CP level in three warmed-season grasses involved the leaf: stem ratio.

As a result, just shading reduced significantly (p < 0.05) the shoot/root ratio of *P. oteroi* that reflected in an increase of root mass in relation to the shoot, probably an adaptive characteristic of the species that allows greater survival (Table 5).



Table 5. Least squares means and standard errors of the shoot:root ratio of the *P. oteroi* submitted to fertilization and shading

	Fertilizer ²			Pasture	systems ³		P-Value		
Variables ¹	С	FF	SF	WS	NS	Fertilizer	Pasture systems		
S:R	1.16 ± 0.063	1.23 ± 0.063	1.07 ± 0.082	$0.958 \pm 0.06b$	$1.35\pm0.054a$	0.365	0.0002		

 ${}^{1}S:R$ – shoot:root ratio.^{2,3} Means followed by the same lower case letters on the lines do not differ significantly by the t test (p < 0.05). C - no fertilization; FF – foliar fertilizer for pasture and SF– soil fertilizer + foliar fertilizer. WS - with shading - wooded *P. oteroi* pasture -; NS - no shading - exclusive *P. oteroi* pasture.

An increase in shoot: root ratio of grass species under shading has been reported by several authors. Santos et al. (2011) obtained opposite results with *Brachiaria brizantha* and *Brachiaria humidicola*, under conditions of 70% shading. According to Durr and Rangel (2000) the increase of the aerial part is a strategy of the plant to increase the ability to intercept larger amounts of radiation. For Comas et al. (2013) this adaptive response of the shoot: root ratio to environmental conditions should be associated with the evaluation of root morphology or architecture traits.

Significant correlations among variables were reported (Table 6). There was a high and a negative correlation between S:R ratio and CP. This result can be explained by lower (p<0.05) leaf: stem ratio when S:R increases as well as the positive relationship between DM, DMP, H and CP.

Table 6. Pearson's coefficient among analyzed variables including different pasture systems the in Cerrado-Pantanal transition region

Variables ¹	LI	LAI	DM	СР	EE	NDF	ADF	DMP
Н	0.116	-0.196*	0.006	-0.186	-0.072	0.165	0.255**	0.042
LI	1	0.086	0.295**	0.012	0.043	-0.263**	-0.313**	0.279**
LAI		1	-0.052	0.345**	0.233*	-0.143	-0.232*	-0.091
DM			1	-0.557***	-0.046	-0.186	-0.422***	0.991***
CP				1	0.087	-0.297**	-0.131	-0.623***
EE					1	-0.088	-0.114	-0.049
NDF						1	0.504***	-0.095
ADF							1	-0.362***

¹H (plant height, cm); LI (light interceptation, %); LAI (leaf area index); DM (dry matter, %); CP (crude protein, % DM); EE (ethereal extract, %DM); NDF (neutral detergent fiber, %DM); ADF (acid detergente fiber, %MS); DMP (dry matter production ton ha⁻¹).* p<0.05; ** p<0.01, ***p<0.001.

There was an interaction among the pasture systems, fertilization and month of fertilization on the LAI variable (Table 7).

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			P-Value	
Month	С	FF	SF	Fertilizer
		WS^2		
May	0.045 ± 0.016	0.035 ± 0.016	0.060 ± 0.023	0.705
June	$0.032\pm0.008a$	$0.015\pm0.008b$	$0.060 \pm 0.011a$	0.048
July	0.070 ± 0.023	0.092 ± 0.023	0.080 ± 0.033	0.802
August	0.260 ± 0.080	0.115 ± 0.080	0.410 ± 0.112	0.162
September	$0.230\pm0.054b$	$0.032\pm0.054c$	$0.495 \pm 0.077a$	0.005
		$\underline{NS^2}$		
Total period	0.053 ± 0.009	0.062 ± 0.009	0.049 ± 0.011	0.656

Table 7. Least squares means and standard errors of the leaf area index (LAI) according to two pasture systems (WS and NS) and month of fertilization in WS

¹ C - no fertilization; FF – foliar fertilizer for pasture and SF– soil fertilizer + foliar fertilizer. WS - with shading - wooded *P. oteroi* pasture -; NS - no shading - exclusive *P. oteroi* pasture.^{1,2}L Means followed by the same lower case letters on the lines do not differ significantly by the t test (p < 0.05).

Higher values (p<0.05) of LAI occurred in the months of August and September in the fertilizer treatment with higher levels of SF, the effect of fertilization occurred at the end of the drought season. This fact can be explained by higher tillering because it was observed that *P. oteroi* kept its height during the drought. The lower levels of LAI observed in the wooded pasture with shading may be due to factors such as the reduction of tillers (Souza et al., 2007). For Paciullo et al. (2007), the lower the incidence of light that enters the wood, the lower the LAI and the tiller density in the understory.

There was no significant correlation between LAI and LI as well as H and LI as expected (Table 6). LI values were very slow (bellow 50%) for *P. oteroi* in both pasture systems (Figure 2 and 3).

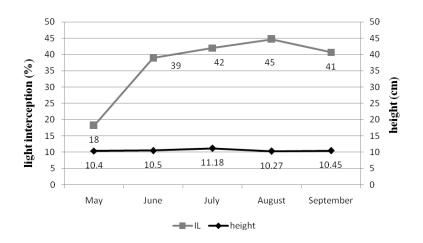
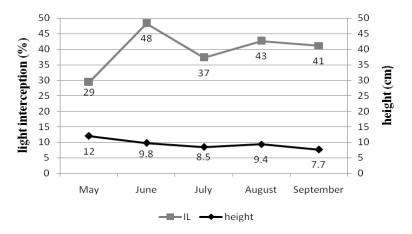
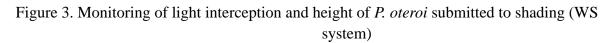


Figure 2. Monitoring of light interception and height of *P. oteroi* not submitted to shading (NS system)







Considering that environmental limitations during the hydrological year probably affected the total establishment of *P. oteroi*, mainly during the time of transplantation of seedlings, the results indicated that sward architecture (e.g., leaf shape and size) and tiller density may have resulted in lower LAI and LI. Further studies are necessary for a more profound knowledge of the management of the species in different environmental conditions to be obtained.

4. Conclusion

The shading condition in the wooded pasture system and the use of fertilization (foliar and soil) improved the crude protein level of *P. oteroi* during the drought. Thus, economic viability studies are necessary for decisions to be made.

Fertilization (foliar and soil) of *P. oteroi* provided higher LAI values at end of the drought, which requires long-term studies for monitoring this variable.

P. oteroi probably developed adaptation strategies to cope with the environmental conditions such as variations in the morpho-physiological traits, shown in this study by low shoot/root ratio, sward height, nutritional value, LAI and LI.

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