Article

Leaf tissue replacement in *Brachiaria humidicola* CIAT 6133 with different defoliation management

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Abstract

The defoliation in the tissue replacement of *Brachiaria humidicola* CIAT 6133 was evaluated by cuts at 9, 12 and 15 cm in height, harvested at 3, 4 and 5 and 5, 6 and 7 weeks in rains and norths, respectively. Every seven days the leaf appearance rate (LAR), leaf elongation rate (FER), leaf senescence rate (LSR) and net leaf growth (NFG) and stem density (DS) were measured every month. In rains, the highest LAR was 0.097 leaves stem^{-1} day^{-1} with cuts at 15 cm, every three weeks, in north 0.044 leaves stem^{-1} day^{-1} with cuts at 9 cm and 0.047 leaves stem^{-1} day^{-1} every seven weeks. The highest FER and NFG occurred with cuts at 15 cm; in rains of 1.85 and 1.81, and in norths of 0.53 and 0.45 cm stem^{-1} day^{-1}, respectively. In rains the highest FER occurred in week two of the regrowth (1.8 cm stem^{-1} day^{-1}), while in the north it was in week three of regrowth (0.45, 0.5 and 0.46 cm stem^{-1} day^{-1}). The LSR increased from week four onwards in both seasons. The DS was maximum at three and four weeks in rains and norths, at five and six weeks. The greatest leaf growth occurred in sections 15 cm high three weeks after regrowth, regardless of the interval between cuts and time of year, and the highest stem density at three and four weeks in rains and five and six weeks in norths.

Keywords: *Brachiaria humidicola* CIAT 6133 cv. Isleño, defoliation management, leaf tissue replacement.

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Introduction

The genus *Brachiaria* (Trin.) Griseb. (syn. *Urochloa* P. Beauv.), has expanded rapidly in the tropical areas of Mexico, replacing the traditionally used 'Guinea' [*Megathyrsus maximus* (Jacq.) BK Simon & SWL Jacobs], 'Pangola' (*Digitaria decumbens* Stent.) and 'Estrella Africana' (*Cynodon nlemfuensis* Vanderyst) (Cruz *et al*., 2011). One of the species of this genus with the greatest acceptance is the Isleño grass (*B. humidicola* CIAT 6133), due to its high forage and seed production, good adaptation to flooded soils and acid soils, tolerance to the pint fly and resistance to grazing (Enríquez *et al*., 2011).

Most of the grasslands in Mexico are used for grazing cattle, which causes defoliation of the pastures, which later undergo a change of tissue when developing new leaves and stems. Tissue turnover, determined by analysis of leaf and stem growth, associated with stem population, is useful for understanding the response of plants to defoliation management (Menezes *et al*., 2003).

The basic unit of forage production in a meadow is the stem, which is made up of a chain of phytomers (knot, internode, leaf and axillary bud units) at different stages of development. Each phytomer, after formation in the apical meristem, has a life cycle, ages, dies, and is replaced by new phytomers.

The axillary buds of the phytomer can develop a new stem, called a child stem, that replaces the dead stems, due to the continuous replacement, the population of stems of a prairie can remain stable (Carlassare and Karsten, 2003). The rate of stem replacement and senescence depends on environmental conditions, grassland management, and stem density (Hirata and Pakiding, 2004).

The leaf growth cycle consists of a period of active extension followed by maturation, senescence and death unless the leaves are cut or grazed (Hodgson, 1990). The relationship between leaf elongation, aging and senescence is essential to maintain the productivity of a meadow (Bircham and Hodgson, 1983; Lemaire *et al*., 2009). The element of the climate that most influences the rate of appearance and elongation of leaves is the temperature.

When it increases, the rate of leaf emergence and elongation increases (Berone *et al*., 2007), resulting in faster leaf growth during spring and summer than in autumn and winter (McKenzie *et al*., 1999). The optimum growth temperature of tropical grasses is around 30 °C and at temperatures below 15 to 17 °C, growth is restricted and can be stopped (Da Silva *et al*., 2008).

The frequency and intensity of defoliation affect the rate of production of new tissue (Lemaire *et al*., 2009) and the morphology of the plant (Lemaire, 2001), since it has to readjust its metabolism for the formation of new leaves (Da Silva *et al*., 2015).

The main effect of defoliation is the decrease in the length of the leaf blades (Duru and Ducrocq, 2000a; Duru and Ducrocq 2000b) when the leaf area index is less than 1, the regrowth depends on the carbohydrate reserves and proteins that the plant assigns to the stem meristems for the formation of new leaf area (Richards, 1993; Lemaire, 2001). The rate of leaf appearance is negatively affected by the intensity of defoliation; when all the leaves are removed from a stem, the leaf emergence rate is reduced from 15 to 20% (Davies, 1974).

On the other hand, the stem size and the leaf elongation rate increase with light defoliation (Lemaire, 2001; Berone *et al*., 2007) and the senescence in the leaves increases as the interval between defoliation increases (Menezes *et al*., 2003). In the coastal regions of the Gulf of Mexico, temperature fluctuations, rainfall and solar radiation occur throughout the year, elements of the climate that are strongly associated with the growth of pastures. These variations give rise to three seasons in the year: rain, north and dry.

In rains (June to November), the temperature and rainfall are high; in norths (October to February), there is high cloudiness and temperatures are low, and in the dry season (February to May), temperatures are high and there is little rain. The aforementioned causes a marked seasonality in forage production, corresponding from 65 to 70% in the rainy season, from 13 to 21% in the norths season and from 8 to 14% in the dry season (Martínez *et al*., 2008).

There is no information on the seasonal effect of different management practices on the replacement of grass tissue *B. humidicola* CIAT 6133, in the tropical region of the Gulf of Mexico slope, therefore, the objective was to evaluate the effect of three intensities and three cut frequencies in the tissue replacement of this grass, during the rainy and norths seasons, in Isla, Veracruz.

Materials and methods

Location and description of the experimental site

The experiment was conducted in a meadow of *B. humidicola* CIAT 6133 cv. Isleño, established in 1988 and subjected to different operations, at the INIFAP Papaloapan Experimental Site, located at km 66 of the Cd. Aleman-Sayula highway, in the municipality of Isla, Veracruz, at 18º 06’ north latitude, 95º 32’ west longitude and 65 m altitude.

The climate of the region is Aw_0 (García, 1988), the driest of the warm sub-humid, with rains in summer, average annual precipitation of 1 000 mm and average annual temperature of 25.7 °C. The soil is Arctic Acrisol with a sandy loam texture, with a pH of 4.0 to 4.7, poor in organic matter, nitrogen, calcium and potassium, and with a medium to high content of phosphorus and magnesium (Enríquez and Romero, 1999).

Treatments and experimental design

Nine treatments were evaluated, resulting from the combination of three cutting heights: 9, 12 and 15 cm with respect to the ground level, and three cutting intervals: three, four and five weeks in the rainy season and five, six and seven weeks in the norths season. The treatments were distributed randomly, using a randomized block design with a 3 x 3 factorial arrangement, with three replications. The experimental units measured 2 m long x 1.5 m wide (3 m^2). The blocks were located perpendicular to the slope of the terrain.

The cuts were made manually with a knife. The experiment was conducted from May 30, 2004 to March 15, 2005, during the rainy and norths seasons. During the conduction period of the experiment, no fertilizers or pesticides were applied to the meadow and its development was exclusively under temporary conditions. The monthly temperature and rainfall data for the evaluation period were obtained from the INIFAP meteorological station of the Papaloapan Experimental Site.

Variables evaluated

Leaf emergence rate (LAR)

In each experimental plot, a 1 m transect was drawn in which five stems were randomly selected, approximately every 20 cm, the second mature leaf present on each stem was marked with white paint, after making the corresponding cut. The day before the next cut, the new leaves present on each stem were counted. The LAR (leaves stem^{-1} day^{-1}) was obtained by dividing the number of leaves that appeared by the number of days in each cut interval.

Leaf growth

Elongation, senescence and net leaf growth were determined according to the Bircham and Hodgson (1983) methodology. In the middle of the rainy and norths seasons, a 1 m transect was drawn, in which five stems were randomly selected every 20 cm, which were identified with plastic rings the day after cutting. The length of the green lamina of each leaf was measured, from the ligule to the apex in green leaves and to the base of the chlorotic tissue in leaves in process of senescence, in the case of immature leaves, the length was from the apex of the leaf to the base of the previous leaf.

These determinations were made every week until one day before the next cut, with a ruler graduated in millimeters. Because only the green leaf was recorded, senescence was obtained indirectly by the difference in the lengths of the senescent leaves in successive measurements. The leaf elongation rate (FER) was determined by the difference between the final length (LF) and the initial length (LI) of the expanding leaves, divided by the number of days between measurements (ND): TE = (LF - LI)/ND.

The senescence rate (LSR) was determined by the difference between the initial length (LIHM) and the final length (LFHM) of the mature leaves, divided by the number of days between measurements (ND): LSR = (LIHM - LFHM)/ND. Net leaf growth (NFG) was calculated as the difference between the leaf elongation rate (FER) and the leaf senescence rate (LSR): NFG = FER- LSR.

Stem density

At the beginning of the study (May 30), a 20 cm diameter and 5 cm high PVC cylinder was placed in each plot. All stems within the area of each cylinder were marked and counted immediately after treatments were assigned to the plots; For this, blue plastic rings 0.5 cm in diameter and 1 cm long were used. Every four weeks counts were made of the number of living and dead stems, the new stems were marked with a different color in each evaluation. With the individual data of each circle, the number of stems per square meter was calculated.

Statistical analysis

An analysis of variance was applied to each variable under study using PROC GLM from SAS, and Tukey's mean test (Steel *et al*., 1997; SAS Institute, 2002). The leaf appearance data was analyzed by time of year. The analysis of the leaf growth data was done by season in an arrangement of divided plots, where the large plot was the treatments and the small plot the weeks. The stem density variable was analyzed per month.

Results and discussion

Climatic characteristics during the experimental period

In the rainy season the rainfall was 1 134 mm and the average temperature was 27.7 °C, while in the norths season the rainfall was 201 mm and the average temperature was 23 °C (Figure 1).

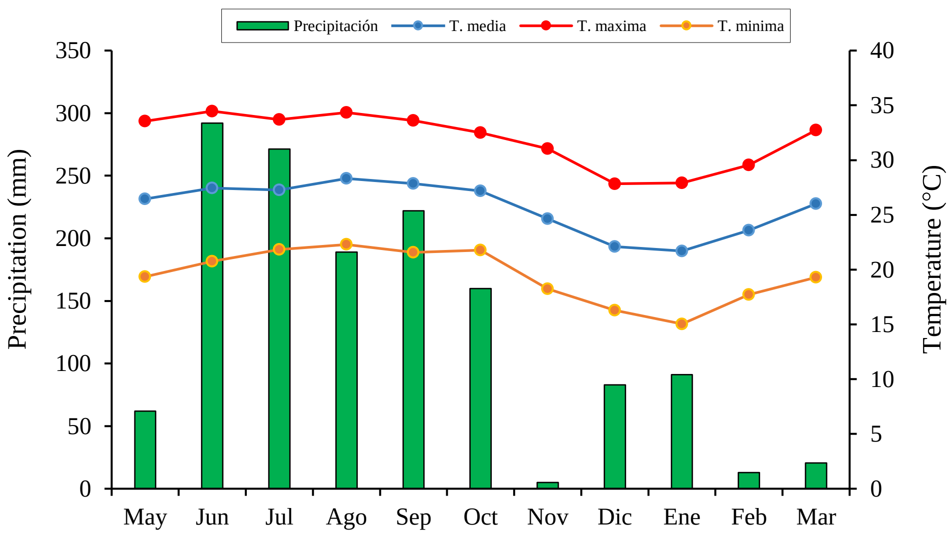

Figure 1. Distribution of rainfall and temperature during the study period (May 2004 to March 2005) at the Papaloapan Experimental Site, in Isla, Veracruz.

Leaf appearance rate

The LAR was affected by the season of the year, as well as by the height and the cut interval ($p \leq 0.05$). The LAR was 2.3 times higher in the rainy season than in the norths season (Table 1), this difference is similar to those reported in the Bahia grass (*Paspalum notatum*) (Hirata and Pakiding, 2004) between summer and winter.

The difference is due to the fact that the temperature is lower in the winter than in the summer, being one of the elements of the climate that most affects the LAR, although the photoperiod and the solar radiation can also have an effect (Duru and Ducrocq, 2000a; Duru and Ducrocq, 2000b).

Table 1. Rate of appearance of leaves (leaves stem^{-1} day^{-1}) in *Brachiaria humidicola* CIAT 6133 at different cutting heights in the rainy and norths seasons.

Season	Cutting height (cm)			
	9	12	15	Average
Rains	0.09 b	0.09 b	0.097 a	0.092 A
Norths	0.044 a	0.04 ab	0.037 b	0.04 B

Lowercase literals compare means in a row, uppercase literals compare means in the same column, different literal indicates significant differences ($p \leq 0.05$) by Tukey's test.

The LAR had a different response to the height of cut between times of the year, in rains a higher LAR was observed when harvesting at 15 cm height, and in the north, with cuts at 9 cm. The effect of the cut interval on the LAR was also different between seasons (Table 2) in rains, the LAR was higher in cuts at three and four weeks, while, in North, the highest LAR occurred in the cut interval at seven weeks, which were 25 and 27% higher at the five and six week intervals.

Table 2. Rate of appearance of leaves (leaves stem^{-1} day^{-1}) in the rainy and norths seasons at different cutting intervals (CI) in *Brachiaria humidicola* CIAT 6133.

CI (weeks)	Rains	CI (weeks)	Norths
3	0.097 a	5	0.037 b
4	0.095 a	6	0.037 b
5	0.084 b	7	0.047 a
Average	0.092	Average	0.04

Literals compare means in the same column, different literal indicates significant differences ($p \leq 0.05$) by Tukey's test.

The results in the LAR found in this study are different from those reported in Guinea grass, by Montagner *et al*. (2012), who found no effect of the height of defoliation on the rate of leaf appearance, but of the season of the year, mention that this is because there was no intense competition for light between stems, because the defoliation was done at 95% light interception. In addition to the quantity and quality of light, the LAR is favored with the availability of photosynthates in the plant (Davies, 1974), which are related to the leaf area index, the highest LAR was achieved with cuts at 15 cm in the rainy season, which probably favors the formation of the leaf area and a greater synthesis of photosynthates in the plant (Table 3).

Table 3. Tissue replacement of grass *Brachiaria humidicola* CIAT 6133 in the rainy and norths seasons at different cutting heights.

Season	Cutting height (cm)			
	9	12	15	Average
	Elongation rate (cm stem^{-1} day^{-1})			
Rains	1.34 b	1.35 b	1.85 a	1.51 A
Norths	0.37 b	0.35 b	0.53 a	0.42 B
	Senescence rate (cm stem^{-1} day^{-1})			
Rains	0.036 a	0.036 a	0.042 a	0.038 A
Norths	0.064 a	0.048 a	0.073 a	0.062 B

Season	Cutting height (cm)			
	9	12	15	Average
	Net growth (cm stem^{-1} day^{-1})			
Rains	1.3 b	1.32 b	1.81 a	1.47 A
Norths	0.31 b	0.31 b	0.46 a	0.36 B

Lowercase literals compare means in rows, uppercase literals compare means in the same column, different literal indicates significant differences ($p\leq 0.05$) by Tukey's test.

In the norths season, the LAR increased as the cutting height decreased, contrary to what happened in the rainy season; this different response from the plant is due to the fact that in the norths season, the growth of the grass was very slow due to the decrease in temperature (Figure 1) and the competition for light does not occur, being the length of the pseudo-stem (tube formed by the leaf sheaths), which influences the LAR: the longer the pseudo-stem, the leaves take longer to emerge above the previous leaf (Duru and Ducrocq, 2000a). The decrease in LAR as the cutting interval increases, in the rainy season in *B. humidicola* CIAT 6133 is explained by the fact that as the plant grows, the quantity and quality of light that reaches its lower part, causing elongation of the pseudo-stem, since the leaf in formation has to travel a greater distance, and take longer to emerge, resulting in an increase in the growth time of each leaf (Duru and Ducrocq, 2000a; Duru and Ducrocq, 2000b; Lemaire, 2001; Anasenko *et al*., 2006).

In the norths season, due to little growth, stem elongation does not appear with increasing cutting interval, being another factor the one that modified the LAR, most probably the reserve of structural carbohydrates; to better understand this response and determine the most appropriate cutting interval, further studies are required on this pasture.

Leaf growth

The time of year and the height of cut affected ($p\leq 0.05$) the leaf elongation rate (LER) and the net leaf growth (NFG) (Table 3). The leaf senescence rate (LSR) was only affected ($p\leq 0.05$) by the cutting interval (Table 4). In the rainy season, the LER and NFG were 1.51 and 1.47 cm stem^{-1} day^{-1} and in the north, 0.42 and 0.36 cm stem^{-1} day^{-1}, respectively. On the other hand, the highest LER and NFG were obtained with cuts at a height of 15 cm at both times of the year.

In the rainy season, the LER with cuts at 15 cm of residual forage height was higher than the LER with cuts at 9 and 12 cm, 8 and 37%, respectively. In the NFG, the differences were similar (Table 3). In the norths season, the LER with cuts at 15 cm in height was 43 and 50% greater than those obtained at 9 and 12 cm, in turn, the NFG of 15 cm was 50% higher than the NFG obtained at heights of 9 and 12 cm. The LSR increased with increasing cutoff interval (Table 4).

Table 4. Tissue replacement of the *Brachiaria humidicola* CIAT 6133 grass in the rainy and norths seasons at different cutting intervals (CI).

CI (Weeks)	Rains	CI (Weeks)	Norths
	Elongation rate (cm stem^{-1} day^{-1})		
3	1.66 a	5	0.41 a
4	1.61 a	6	0.44 a
5	1.35 a	7	0.41 a

CI (Weeks)	Rains	CI (Weeks)	Norths
	Senescence rate (cm $stem^{-1}$ day^{-1})		
3	0.009 b	5	0.048 a
4	0.044 ab	6	0.05 a
5	0.051 a	7	0.082 b
	Net growth (cm $stem^{-1}$ day^{-1})		
3	1.65 a	5	0.37 a
4	1.57 a	6	0.39 a
5	1.29 a	7	0.33 a

Literals compare means in the same column, different literal indicates significant differences ($p \leq 0.05$) by Tukey's test.

The highest LER and NFG occurred in the rainy season, they exceeded by 360 and 410% those obtained in the norths season, similar results were reported by Ramírez *et al*. (2010), who indicate that the net leaf growth rate was 144% higher in the rainy season compared to the dry season. These differences are due to the fact that in the rainy season favorable conditions of humidity, photoperiod and temperature for the growth of plants occur, increasing the rates of appearance and elongation of leaves (Lemaire, 2001; Anasenko *et al*., 2006).

The LSR of the norths season was 63% higher than that of the rains, meanwhile, the greatest senescence in the norths season was probably due to the effect of low temperatures and wind, conditions that prevail during this time. In both seasons, the highest LER and NFG with the cutting height at 15 cm, are due to the fact that by increasing the cutting height, there is a greater residual leaf area and soluble carbohydrates (Kim *et al*., 2001), which favors greater growth of the plant, manifesting itself in a higher rate of leaf elongation.

The increase in LER with the highest cutting height is also explained by the morphology of the leaves: those with a greater length of leaf blade have a faster LER than those of shorter length (Durand *et al*., 1999; Brandão de Carvalho *et al*., 2013). Various studies indicate that the length of the leaf blade increases in plants with pseudo-long stems (Duru and Ducrocq, 2000a; Duru and Ducrocq, 2000b; Lemaire, 2001), which happened in the cuts at 15 cm of height at both times of the year, the difference in the size of leaves being more noticeable between cut heights, in the rainy season.

The increase in LSR with increasing cutoff interval at both times of the year is consistent with that reported by Velasco-Zebadua *et al*. (2005) in perennial ryegrass grass and the results obtained in Mombaza grass (Ramírez *et al*., 2009). This is because, as the cutting interval increases, the leaves mature and senescence increases.

LER, LSR and NFG varied significantly ($p \leq 0.05$) with the week of regrowth (Table 5). In rains, the highest LER and CN occurred in week 2 (1.8 and 1.78 cm $stem^{-1}$ day^{-1}), the senescence rate increased in weeks 4 and 5. In the norths season, the highest LER and NFG were recorded in the first three weeks (0.453, 0.496 and 0.463 cm $stem^{-1}$ day^{-1}) and decreased with increasing age of the regrowth, the LSR was higher in weeks 6 and 7, which coincides with what was indicated by Dias -Filho (2000) in the same grass.

Table 5. Change of tissue in *Brachiaria humidicola* CIAT 6133 per week in the rainy and norths seasons.

Week	Elongation rate	Senescence rate (cm stem^{-1} day^{-1})	Net growth
		Rains	
1	1.55 b	0.01 c	1.54 b
2	1.8 a	0.02 c	1.78 a
3	1.55 b	0.02 c	1.53 b
4	1.2 c	0.09 b	1.11 c
5	1.04 c	0.13 a	0.91 d
		Norths	
1	0.45 ab	0.01 c	0.44 a
2	0.5 a	0.02 c	0.48 a
3	0.46 a	0.02 c	0.44 a
4	0.39 bc	0.07 c	0.32 b
5	0.39 bc	0.08 bc	0.31 b
6	0.33 cd	0.14 ab	0.19 c
7	0.29 d	0.19 a	0.1 c

Different literals in the same column are statistically different ($p\leq 0.05$).

Stem density

Stem density did not show significant changes due to the effect of cutting height, nor due to the interaction of cutting interval with height ($p\geq 0.05$), but due to the cutting frequency ($p\geq 0.05$) (Table 6). In the rainy season (May to October), the stem population increased, reaching its maximum in October. In the norths season, stem density decreased in November and increased in the following months; the highest density was obtained in March, regardless of the frequency and height of cut.

Table 6. Stem density (number m^{-2}) monthly in *Brachiaria humidicola* CIAT 6133 at different cutting intervals.

CI (weeks)	Rain 2004					
	May	June	July	August	September	October
3	2 923 ab	3 166 b	3 323 a	3 326 a	3 436 a	4 274 a
4	3 557 a	3 778 a	3 675 a	3 547 a	3 213 a	4 071 a
5	2 279 b	2 392 c	2 851 b	2 665 b	2 991 a	3 340 b
EE	306	228	182	256	259	261
	Norths 2004-2005					
	November	December	January	February	March	
5	3 833 a	4 185 a	4 617 a	5 339 a	5 695 a	
6	4 004 a	4 143 a	4 666 a	5 405 a	5 749 a	
7	3 059 b	3 255 b	3 573 b	4 134 b	4 452 b	
EE	266	298	356	432	479	

CI= cut interval. Different literals in the same column are statistically different ($p\leq 0.05$).

Regarding the cutting interval, with the exception of September, there were differences between cutting intervals, the lowest density occurred when harvesting every 5 weeks in the rainy season and every 7 weeks in the norths season, and there were no differences between the cuts of 3 and 4 weeks in rains and 5 and 6 weeks in norths. In grass *Panicum maximun* cv. Mombaza, Ramírez *et al*. (2011) reported that, during the dry season, the density of stems in cuts at intervals of 3 weeks was higher in 26 and 42% than in cuts at 5 and 7 weeks.

These differences are explained by the fact that, at longer cutting intervals, the height of the plants is greater and the structure of the meadow is modified, the competition for light increases during the regrowth period and the shading limits the light that it reaches the buds inhibiting their activity and the formation of new stems is reduced. In turn, frequent cuts favor the quantity and quality of light that penetrates the base of the canopy, conditions that promote the activation of the axillary buds located at the base of the remaining stems, which will give rise to new stems (Matthew *et al*., 2000; Lemaire, 2001).

Conclusions

The rate of leaf appearance and net leaf growth is higher in the rainy season than in the norths season. The greatest net leaf growth occurs when harvesting at 15 cm height, regardless of the time of year and cutting interval. The highest leaf elongation rate occurs in the first three weeks of regrowth and the senescence rate increases in regrowths of more than four weeks in the rainy and norths seasons. The leaf senescence rate increases with increasing cutting interval. The highest senescence rate occurs at cut-off intervals of 5 weeks in the rainy season and 7 weeks in the norths season. The stem population is lower when the forage is harvested at intervals of 5 weeks in the rainy season and 7 weeks in the norths season.

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