Production and nutrition of irrigated Tanzania guinea grass in response to nitrogen fertilization

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ABSTRACT - The objective of this study was to evaluate the effects of nitrogen (N) fertilization in the four seasons of the year on forage production, nitrate (NO_3) in the sap, total N in the forage and relative chlorophyll index (SPAD reading) in the leaves of irrigated *Panicum maximum* cv. Tanzania grass, establishing their critical ranges. In addition, we evaluated the ability to predict forage production based on NO_3 in the sap, total N in the forage and relative chlorophyll index. The soil in the experimental area was classified as an Oxisol (Red-Yellow Latosol) with a clayey texture. Annual rates of N (0, 200, 400 and 800 kg ha^{-1}) in the form of urea were the treatments tested. Irrigation was performed through a conventional spray system. The NO_3 content in the sap and the relative chlorophyll index were measured in leaves using a portable meter with NO_3 selective electrode and the SPAD-502 portable chlorophyll meter device, respectively. Tanzania guinea grass was very responsive to N fertilization, except in the winter. The critical ranges of the SPAD reading proved to be more adequate for monitoring the nutritional state of N of Tanzania guinea grass in the different seasons of the year than the NO_3 content in the sap and the total N content in the dry matter. Use of the chlorophyll meter is more advantageous than the use of the portable meter with an nitrate selective electrode for predicting the nutritional status of Tanzania guinea grass.

Key Words: chlorophyll, NO_3 in the sap, *Panicum maximum*, SPAD, total N content

Introduction

Nitrogen deficiency has been reported as the main cause of low dry matter production or delay of biochemical functions in plant metabolism, since N is part of the chlorophyll pigment structure and is involved in NO_3 reductase activation (Lavres Junior et al., 2010). In the search of technologies to correct management of N fertilization, rates of N have been quantified as based on results of N analysis in the soil and in the plant. According to Minnoti et al. (1989) and Tremblay et al. (1999), determinations of N in the dry matter may have questionable practical viability since, in addition to their high cost, they require a great deal of time from sample collection to obtaining results. These contents often do not reflect the nutritional state of the plant in relation to N. To get around this problem, determinations of chlorophyll contents by a chlorophyll meter and of NO_3 in the sap by means of a portable meter equipped with a specific microelectrode for NO_3 have been recommended as auxiliary tools for monitoring the nutritional state of N in the plants. Because they provide quick results, these technologies allow for corrections in the N fertilization program to be made during the crop cycle. The potential of NO_3 analysis in leaf sap has been shown in various studies with vegetable crops (Huett and White, 1992; Fontes et al., 2003). However, for forage grasses, studies are still very much in the initial stages.

The SPAD-502 portable chlorophyll meter is a useful device for indirect determination of the a+b chlorophyll content in plant leaf tissue (Yadava, 1986). This chlorophyll meter expresses the results in SPAD unit values and has the advantages of being portable, light weight, and easily handled. It may be used in field conditions without the need for destroying the sampled part of the plant. Its use for monitoring the nutritional state of N has been evaluated in forage grasses with satisfactory results (Premazzi et al., 2003; Erthal et al, 2010). However, calibration for forage grasses is necessary.

The aim of this study was to evaluate the effects of N fertilization in the four seasons of the year with respect to forage production, NO_3 content in the sap, total N content

in the forage, and the relative chlorophyll index (SPAD reading) in the leaves of irrigated *Panicum maximum* cv. Tanzania guinea grass as well as to establish its critical ranges and evaluate its predictive ability for forage production based on these variables.

Material and Methods

The experiment was set up at Santa Rita Experimental Farm/EPAMIG, Prudente de Morais, MG, Brazil, located at 19º27'15" latitude south, 44º09'11" longitude west, at 732 m altitude. The climate in the region is the Aw type, with two well-defined seasons: dry (May to October) and rainy (November to April). The soil of the experimental area was classified as an Oxisol (Red-Yellow Latosol) with a clayey texture, according to the Brazilian soil classification system (EMBRAPA, 2006) and it was chemically analyzed before setting up the experiment (EMBRAPA, 1999). The following results were observed in the 0-20 cm layer: pH in H_2O = 6.3; organic matter = 3.27 dag kg^{-1}; P = 16 mg dm^{-3} (Mehlich-1); K = 67 mg dm^{-3} (Mehlich-1); Ca^{2+} = 4.27 $cmol_c$ dm^{-3}; Mg^{2+} = 1.16 $cmol_c$ dm^{-3}; and Al^{3+} = 0.05 $cmol_c$ dm^{-3}. In the 20-40 cm layer the results were: pH in H_2O = 6.3; organic matter = 3.07 dag kg^{-1}; P = 8.6 mg dm^{-3}; K = 40 mg dm^{-3}; Ca^{2+} = 3.77 $cmol_c$ dm^{-3}; Mg^{2+} = 0.89 $cmol_c$ dm^{-3}; and Al^{3+} = 0.05 $cmol_c$ dm^{-3}.

The study was carried out from July/2010 to June/2011 in a Tanzania guinea grass (*Panicum maximum*) pasture established in November 2009 which was fertilized at planting with 50 kg ha^{-1} of P_2O_5 (simple superphosphate), 30 kg ha^{-1} of K_2O (potassium chloride) and 50 kg ha^{-1} of FTE BR12 (B = 1.8%; Cu = 0.8%; Mn = 2.0%; Mo = 0.1%; and Zn = 9.0%) according to Cantarutti et al. (1999). A randomized block experimental design was used with four replications. Annual rates of N (0, 200, 400 and 800 kg ha^{-1}) in the form of urea (45% of N) were the treatments tested. The N rates were divided into nine equal broadcast applications: the first soon after plot-leveling grazing and the others after each grazing cycle.

Grazing was carried out with a group of dry cows, but no measurement of animal performance was made. The animals were used only as grazers. The grazing cycle was established on the height of Tanzania guinea grass on a rotational grazing management. Sward height was monitored using a ruler graduated in centimeters through systematic readings in each paddock. Readings of sward height were taken from ground level considering the leaf horizon on the top of the sward as a reference. The animals were placed in the paddocks simultaneously when any treatment reached a height of 70 cm. In turn, the animals

were removed as the grass reached a height of 30 cm. Then, all treatments were grazed nine times.

The experimental plot measured 720 m² (24 × 30 m). A conventional spray irrigation system was used with buried PVC pipes, and the water level was calculated based on reference values for evapotranspiration, precipitation and the Kc for Tanzania guinea grass. Based on crop coefficients found in the literature for Tanzania guinea grass (Lourenço et al., 2001; Rodrigues et al., 2011), an adjustment was made to the region of Sete Lagoas, MG. The chlorophyll index (SPAD reading) and NO_3 content were measured in ten totally open newly mature leaves before animals entered each paddock. A portable meter with a selective electrode for NO_3 (C-141 Cardy Nitrate Meter – Horiba, Inc.) was used to determine the NO_3 content in the sap. For sap extraction, 20 leaves were sampled using 2 cm-long sections of the base of the midrib. These sections were macerated in a garlic press along with a piece of tape that goes with the aforementioned meter so as to maintain the sap released, for purposes of analysis, based on modified methodology from Guimarães (1998). The portable SPAD-502 Chlorophyll Meter (Minolta) was used to determine the SPAD reading.

To evaluate dry matter production, before each grazing period, three areas of 3 m² were sampled from each Tanzania guinea grass pasture plot, cutting the grass at 30 cm from the ground. Total N content in the dry matter was analyzed after the collected material was dried in a forced air-circulation oven at 60 ºC for 72 hours, weighed and ground up, followed by determination of total N content by the micro Kjeldahl method.

Accumulated forage production in the various samplings within each season of the year (Winter: 07/5/2010, 08/25/2010; Spring: 10/04/2010, 11/16/2010, Summer: 12/27/2010, 01/24/2011, 02/15/2011; Fall: 03/25/2011, 05/04/2011) was used for statistical analyses. Samples from intermediate grazing cycle representative of each season (based on climatic conditions) were chosen for NO_3 contents in the sap, SPAD readings and the total N contents in the forage.

The data were subjected to variance and regression analyses. Models were chosen based on the significance of the regression coefficients, using the t test. To determine the critical ranges, mathematical models were used to estimate the N rates relative to the point of maximum forage dry matter production. This point of maximum production was obtained by carrying out the first derivative of the quadratic equation equal to zero. The equivalent to 90% of maximum production was determined. In turn, two new N rates were established by entering the value relating to maximum forage production in the same equation used in the calculation of

point of maximum production. With these new N rates and equations that explain the response of SPAD reading, NO_3 content in the sap and total N content in the forage to N rates applied, the critical ranges for these variables were calculated. Correlation analyses were performed to evaluate the predictive ability for forage production based on SPAD readings, NO_3 content in the sap and total N contents in the dry matter. The SISVAR statistical program (Ferreira, 2011) was used and the regression and correlation coefficients were tested up to 10% of probability.

Results and Discussion

There were significant effects of treatments for all variables evaluated. The interactions between N rates and season were significant. Thus, statistical analyses were performed into each season. Accumulated dry matter production was affected by the rates of N applied in the seasons of the year (Tables 1 and 2). The greatest dry matter production occurred in the summer, while, in the winter, the lowest production was observed due to less favorable climatic conditions. In addition to providing an immediate effect on forage production, it may be inferred that N supply is strengthened by the greater quantity of rainfall and by higher temperatures observed throughout the summer months. Under tropical conditions, the temperature, moisture and luminosity during the winter are inadequate for good development of tropical C4 forage (Maranhão et al., 2010). In contrast, in the summer, these climatic variables are adequate and, depending on forage management conditions, high biomass production may be obtained. Maranhão et al. (2009) evaluated the productive

characteristics of *B. decumbens* and reported that N fertilization increased dry matter production by 97% in the summer and 10% in the winter, without differences in the fall. According to Vitor et al. (2009) the greater forage production can be attributed mainly to the effects of N, which promotes significant increase in the rates of enzymatic reactions in plant metabolism.

The maximum dry matter production values estimated corresponded to N rates of 540, 698 and 661 kg ha^{-1} $year^{-1}$ in the winter, summer and fall, respectively. These rates are higher than the greatest rates recommended in the literature (Cantarutti et al., 1999; Freitas et al., 2005). It was not possible to estimate the point of maximum forage production in the spring since the response to N rates was linear. Maximum forage yields were obtained by entering these dosages in the equations: 2,445 kg ha^{-1} in the winter, 6,939 kg ha^{-1} in the summer and 5,000 kg ha^{-1} in the fall. Despite the use of irrigation and N fertilization, the low temperature conditions associated with decreasing photoperiod in the winter promotes lower responses of forage dry matter production compared with the summer.

The values of the NO_3 contents in the sap were affected by the N rates applied in the different seasons of the year (Tables 3 and 4). In the summer, an increase in N rates led to a linear increase in the NO_3 contents. However, in the winter there was a quadratic increase in the NO_3 content, and in fall and spring, this content decreased quadratically. Although not explained biologically, the quadratic model with negative linear coefficient for spring and fall suggested some resistance to increase NO_3 content in the sap with application of lower rates. This behavior can be possibly attributed to the fact that spring and fall differ from the

Table 1 - Accumulated dry matter production of Tanzania guinea grass in each season of the year according to N rates

Annual rates of N (kg ha^{-1})	Accumulated dry matter production (kg ha^{-1})			
	Summer	Fall	Winter	Spring
0	2,427	1,631	1,129	1,346
200	3,654	3,381	1,934	2,895
400	6,687	4,465	2,345	3,087
800	6,747	4,857	2,126	4,386

Table 2 - Equations that relate accumulated dry matter production (kg ha^{-1}) of Tanzania guinea grass in each season of the year to N rates (kg ha^{-1} yr^{-1})

Season of the year	Equation	R^2
Summer	$\hat{y} = 2116 + 13.820**x - 0.0099*x^2$	0.91
Fall	$\hat{y} = 1637 + 10.178**x - 0.0077**x^2$	0.99
Winter	$\hat{y} = 1132 + 4.862**x - 0.0045**x^2$	0.99
Spring	$\hat{y} = 1706 + 3.494**x$	0.91

** and * - Significant at 1 and 5% probability by the t test, respectively.

Table 3 - Nitrate (NO_3) contents in the sap of Tanzania guinea grass in each season of the year according to N rates

Annual rates of N (kg ha^{-1})	NO_3 content in the sap (g L^{-1})			
	Summer	Fall	Winter	Spring
0	1.29	2.00	1.60	1.12
200	1.53	1.00	2.43	1.30
400	2.82	2.00	3.23	1.25
800	3.62	4.33	3.60	3.38

Table 4 - Equations of NO_3 content in the sap of Tanzania guinea grass in each season of the year related to N rates

Season of the year	Equation	R^2
Summer	$\hat{y} = 1.23 + 0.00311**x$	0.93
Fall	$\hat{y} = 1.88 - 0.00429**x + 0.000009**x^2$	0.99
Winter	$\hat{y} = 1.57 + 0.00546**x - 0.000004**x^2$	0.99
Spring	$\hat{y} = 1.20 - 0.00167**x + 0.000005**x^2$	0.97

** Significant at 1% probability by the t test.

other seasons in terms of temperature and radiation. According to Corrêa et al. (2007), NO_3 is the only inorganic form of N that is accumulated in the plant when the supply of this element exceeds that required for growth. There are diverse factors that affect NO_3 reduction and accumulation in plants, and they may be both genetic and environmental. Among the environmental factors, sunlight intensity is the most important because when it is low, there may be reduction in NO_3 accumulation in the leaves (Faquim and Andrade, 2004).

Young plants have a greater NO_3 concentration than mature plants. Nevertheless, mature plants may still have excessive concentrations of NO_3 if the environmental, soil, and meteorological conditions are favorable to its accumulation in the plants. These observations are in agreement with Viana et al. (2008), who evaluated the NO_3 in the sap, total N in the leaf, and green corn in the cob yield subjected to N rates and observed that the younger plants had higher free NO_3 contents in the sap than the older plants, implying that greater uptake occurred in the initial development phases of the plant.

According to the adopted procedures, the critical ranges of the NO_3 contents in the sap were 2.87 to 3.40 g L^{-1} in the winter, 2.57 to 4.21g L^{-1} in the summer, and 1.62 to 5.49 g L^{-1} in the fall.

A significant effect of the N rates was observed on the total N concentration in the dry matter of Tanzania guinea grass. A quadratic response to the N doses was observed in the spring (Tables 5 and 6). The greatest concentration of N occurred at the rate of 593 kg ha^{-1} yr^{-1} in the spring. In the summer, there was a linear increase in the total N content in the forage as a result of the increase of the N

rates applied. In the fall, there was a quadratic reduction in the total N contents, a biologically unexplained behavior. These results can be explained by dilution effect due to a greater increase in forage production on intermediate N rates (Table 1). There was no response to the N applied in the winter. Temperature and sunlight affect plant growth and ability to extract the soil nutrient. According to Prado (2008), N deficiency restricts tillering of forage plants and, more importantly, limits the growth of individual leaves and their photosynthetic ability. For Marandu grass, Primavesi et al. (2006) observed that as the N rates in the form of urea and ammonium nitrate increased the N content in the plant in a linear and quadratic response, respectively.

The critical ranges of total N in the dry matter were 16.44 to 16.95 g kg^{-1} in the summer and 15.08 to 36.20 g kg^{-1} in the fall. Due to a bad adjustment of the regression equation for fall (Table 6), the critical range calculated for this season did not show a biological explanation.

An effect of the N rates on the SPAD reading was seen in all seasons, with quadratic responses (Tables 7 and 8). Similar results of the SPAD reading were observed by Costa et al. (2009) for *Brachiaria brizantha* cultivars. These authors found that the increase in N doses led to a quadratic increase in biomass production. In the present study, the lowest values of the SPAD reading were observed in the winter period. In this season, the greatest SPAD reading was obtained with the annual rate of 747 kg ha^{-1} N, whereas in the summer, the estimated annual rate was 633 kg ha^{-1} N. This behavior may be attributed to the lower metabolic activity of the grass in the winter, reflecting more restrictive temperature and sunlight conditions in this period.

Table 5 - Total N content in the dry matter of Tanzania guinea grass in each season of the year according to N rates

Annual rates of N (kg ha^{-1})	Total N content in the dry matter (g kg^{-1})			
	Summer	Fall	Winter	Spring
0	16.60	21.26	18.63	21.33
200	19.59	15.81	23.47	29.04
400	16.20	14.96	22.07	29.39
800	25.04	28.68	22.99	30.87

Table 6 - Equations that relate total N content in the dry matter of Tanzania guinea grass in each season of the year to N rates

Season of the year	Equation	R^2
Summer	$\hat{y} = 16.03 + 0.00095{**}x$	0.63
Fall	$\hat{y} = 21.30 - 0.0405{**}x + 0.000062{**}x^2$	0.99
Winter	$\hat{y} = \bar{y} = 21.79$	
Spring	$\hat{y} = 21.95 + 0.032{**}x - 0.000027{**}x^2$	0.91

** Significant at 1% probability by the t test.

Table 7 - Relative chlorophyll index (SPAD reading) of Tanzania guinea grass in each season of the year according to N rates

Annual rates of N (kg ha^{-1})	SPAD reading			
	Summer	Fall	Winter	Spring
0	35.53	38.29	33.85	38.36
200	41.11	41.30	38.42	40.38
400	43.97	45.99	40.85	45.14
800	47.85	48.95	43.24	44.49

Table 8 - Equations relating the relative chlorophyll index (SPAD reading) in each season of the year to N rates

Season of the year	Equation	R^2
Summer	$\hat{y} = 35.70 + 0.0279{**}x - 0.000016{**}x^2$	0.99
Fall	$\hat{y} = 37.98 + 0.0231{**}x - 0.000012{**}x^2$	0.98
Winter	$\hat{y} = 33.96 + 0.0239{**}x - 0.000016{**}x^2$	0.99
Spring	$\hat{y} = 37.86 + 0.0228{**}x - 0.000018{**}x^2$	0.90

** Significant at 1% probability by the t test.

Table 9 - Correlation between dry matter production and SPAD reading, NO_3 content in the sap and total N in the dry matter in each season of the year

Characteristics	Linear correlation coefficients (r)			
	Summer	Fall	Winter	Spring
Dry matter production				
SPAD reading	0.927°	0.959*	0.900°	0.816ns
NO3 in the sap	0.949*	0.518ns	0.904°	0.818ns
Total N	0.426ns	0.183ns	0.831ns	0.930°

* and ° - Significant at 5 and 10% probability by the t test, respectively; ns - not significant.

In regard to the critical ranges of the SPAD reading determined for the seasons, the following values were found: 47.48 to 61.07 SPAD units in the summer, 45.38 to 49.07 SPAD units in the fall and 39.79 to 42.87 SPAD units in the winter. These critical ranges of SPAD reading compared with those reported for the NO_3 content in the sap and total N content in the dry matter, considering their amplitudes and ease of determination in practical terms, proved to be more adequate for monitoring the nutritional state of Tanzania guinea grass in regard to N in the seasons of the year.

For prediction of forage production, the relative chlorophyll index (SPAD reading) proved to be more efficient than the NO_3 content in the sap (Table 9). In the four seasons of the year, correlation coefficients (n = 16) of 0.77^{**} (significant at 1% by the t test), $0.47^°$ (significant at 10% probability by the t test), and 0.060 (non-significant) were obtained for the variables of SPAD reading, NO_3 content in the sap, and total N in the dry matter, respectively. The SPAD reading had good predictive ability for forage production in the winter, fall and summer, while the NO_3 content in the sap proved to be appropriate only in the winter and summer. The lower predictive ability of the NO_3 content in the sap may be attributed to a higher variability of the results. In part, this can be explained by the difficulty of sap extracting from the midrib of the leaf, which impaired the determination of its content by the portable meter with the NO_3 selective electrode. Operational aspects may often create difficulties for use of analytical methods.

In terms of comparison, both the chlorophyll meter and the portable meter with NO_3 selective electrode may be used as tools for monitoring the nutritional state of Tanzania guinea grass in regard to N content. In regard to the practicality of their use, the chlorophyll meter, in addition to having greater predictive ability for forage production, may be more advantageous due to its easy handling and possibility for use in field conditions without the need for destruction of the sampled part of the plant.

Conclusions

Tanzania guinea grass is very responsive to nitrogen fertilization, reflecting the climatic conditions more favorable to its development in summer, fall and spring. The critical ranges of SPAD reading are more adequate for monitoring the nutritional state of Tanzania guinea grass than the nitrate content in the sap and the total nitrogen content in the dry matter of the forage. Nitrate content in the sap of Tanzania guinea grass, and the SPAD reading demonstrate good ability for predicting dry matter production, constituting good indicators of the nutrition of this plant. Therefore, the use of the chlorophyll meter proves to be more advantageous than the use of the portable meter with nitrate selective electrode.

Acknowledgments

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