The development and production of leaves and tillers by Marandu palisadegrass fertilised with nitrogen and sulphur

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Abstract

The effects of combined nitrogen and sulphur fertilisation on the dynamics of leaf and tiller appearance in Marandu palisadegrass (*Brachiaria brizantha* cv. Marandu) and its impact on dry matter production were evaluated in a greenhouse study. Grass seedlings were grown in pots filled with a soil classified as an Entisol and were harvested after 43 days, a further 35 days and finally after 48 more days. Five rates of N (0, 100, 200, 300 and 400 mg/dm^3) and 5 rates of S (0, 10, 20, 30 and 40 mg/dm^3) were tested in an incomplete factorial design with 4 replications. Leaf and tiller development were monitored every 3 days by counting the appearance of recently expanded leaves and new basal tillers. The phyllochron and thermal time between appearance of tillers decreased as N and S fertiliser levels increased to about 300 and 25 mg/dm^3, respectively, then tended to increase. In contrast, leaf and tiller appearance rates increased with the supply of these nutrients to similar levels, then tended to decline. Leaf and tiller production and dry matter yields were affected by both N and S levels, with the role of S increasing as the growth phases increased.

Introduction

The development of pasture grasses is characterised by the emergence of tillers, which are basic production units from which the leaves that make

up the canopy grow (Moore and Moser 1995; McMaster 2005). Therefore, alterations in the rates of leaf and tiller appearance directly determine the quantity and seasonality of the dry matter production of grasses.

Plant development is influenced by many environmental factors, most notably air temperature (McMaster 2005; Gramig and Stoltenberg 2007). From the relationship between air temperature and plant development emerges the concept of thermal time or degree-days that consist of the accumulated values of mean daily air temperature exceeding the base temperature (Tb), the threshold below which plant development ceases or is negligible (Wilhelm and McMaster 1995). According to this model, the production of leaves per plant is commonly represented by the phyllochron, which is defined as the thermal time interval between the appearance of two successive leaves on the same stem and is represented by the unit degree-days per leaf (°C.day/leaf) (Wilhelm and McMaster 1995; Frank and Bauer 1995; McMaster *et al.* 2003). Similarly, tillering rate can be calculated based on thermal time (Chauvel *et al.* 2000).

Other environmental factors such as water, CO_2, light and nutrient availability can affect the phyllochron and tillering of plants (Wilhelm and McMaster 1995; McMaster *et al.* 2003; Martuscello *et al.* 2005). Few studies have examined the effect of nutrient availability on the phyllochron, and their results are equivocal. In the case of nitrogen, some authors have found a reduction in phyllochron values with an increase in available N (Longnecker *et al.* 1993; Martuscello *et al.* 2005), while others have found no change in this parameter with changing N levels (Cruz and Boval 2000; Stanford *et al.* 2005).

Characteristics such as high productivity and rapid regeneration of the canopy after cutting are highly desirable in forage grasses and are major goals for the nutritional management of pasture plants. Although several studies have demonstrated the positive effects of N use in increased

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production and N concentration in forage plant tissue (Lauriault *et al.* 2002; Reich *et al.* 2003; Premazzi *et al.* 2003), few studies have evaluated the influence of N availability on the rate of leaf or tiller appearance in these plants (Martuscello *et al.* 2005). In addition, we are unaware of any previous study that has evaluated the combined effects of N and sulphur (S) on the production dynamics of forage grasses, even though the close connection and interdependence of these nutrients in plant metabolism is known (Crawford *et al.* 2002). Physiological studies have shown that sulphur deficiency suppresses synthesis of sulphur-containing amino acids (Nikiforova *et al.* 2006), nitrate reductase activity (Migge *et al.* 2000) and nitrate and ammonium uptake (Clarkson *et al.* 1989). Therefore, the present study aimed to evaluate the effects of combined N and S fertilisation on the dynamics of leaf and tiller appearance in Marandu palisadegrass (*Brachiaria brizantha* cv. Marandu) and its impact on dry matter production.

Materials and methods

The study was carried out in a greenhouse in Piracicaba, São Paulo, Brazil (22° 43'S, 47° 38'W), from January to May of 2006, using the forage grass *Brachiaria brizantha* cv. Marandu. Pots with capacities of 3.6 L were filled with 5.6 kg of dry and sifted soil (mesh sieve = 4 mm) that had been collected from the 0–20 cm soil layer of a pasture area. The soil was classified as an Entisol.

The chemical characteristics of the soil sample before treatments were applied included: pH ($CaCl_2$) = 4.53; organic matter (OM) = 32.1 g/kg; phosphorus extracted by resin (P-resin), S-sulphate, total N, N-ammonium and N-nitrate = 5.8, 7.4, 919.0, 21.8 and 14.3 mg/dm^3, respectively; potassium, calcium, magnesium, hydrogen *plus* aluminium (H + Al), sum of bases (S) and cation exchange capacity (CEC) = 2.43, 13.0, 8.67, 40.0, 24.1 and 64.1 mmol$_c$/dm^3, respectively; and base saturation (V) and aluminium saturation (m) = 37.6 and 16.9%, respectively. Considering the nutritional requirements of *Brachiaria brizantha* cv. Marandu, lime was added to achieve 50% base saturation using 300 mg/dm^3 and 230 mg/dm^3 of $CaCO_3$ and $MgCO_3$ salts, respectively.

The experimental design consisted of a randomised complete block design with 4 replications. Combinations of 5 rates of N (0, 100, 200, 300 and 400 mg/dm^3) with 5 rates of S (0, 10, 20, 30 and 40 mg/dm^3) were evaluated in a surface response study in an incomplete factorial with 13 treatments according to Littell and Mott (1975). The 13 combinations of N and S rates (in mg/dm^3) were: 0–0, 0–20, 0–40, 100–10, 100–30, 200–0, 200–20, 200–40, 300–10, 300–30, 400–0, 400–20 and 400–40. The N and S were provided as NH_4NO_3 and $CaSO_4.2H_2O$, respectively, and the quantity of calcium was balanced in the experimental units by the use of $CaCl_2$. Basal fertilisation with macronutrients for the establishment of Marandu palisadegrass was as follows: phosphorus = 200 mg/dm^3, potassium = 150 mg/dm^3 and magnesium = 50 mg/dm^3, provided through CaH_2PO_4, KH_2PO_4, KCl and $MgCl_2.6H_2O$. After each harvest (see below), N, S and K fertilisation was repeated as above, while only 20 mg/dm^3 of additional Mg was applied. No P was applied after the first harvest, which reduced the quantity of calcium to be balanced. Basal micronutrient fertilisation with boron, copper, zinc and molybdenum was performed, together with macronutrient fertilisation, using the following sources and amounts: H_3BO_3 = 1.5 mg/dm^3; $CuCl_2.2H_2O$ = 2.5 mg/dm^3; $ZnCl_2$ = 2.0 mg/dm^3; and $Na_2MoO_4.2H_2O$ = 0.25 mg/dm^3.

During the experiment, soil moisture was maintained at 80% of field capacity by an automatic subsurface irrigation system, as described in Bonfim-Silva *et al.* (2007), which replaced the water according to the evapotranspiration of the soil-plant system.

Five *Brachiaria brizantha* cv. Marandu seedlings, grown in washed sand and deionised water, were transplanted into each pot when they reached a height of approximately 5 cm (3 developed leaves). Every 3 days, the recently expanded leaves (visible ligules) and new basal tillers produced by the plant were counted. Three harvests of the plants were performed at the following times: 43 days after transplantation, 35 days after the first harvest and 48 days after the second harvest (total growing time of 126 days). Each plant was cut at 4 cm above the soil surface, and the plant material was dried in an oven with forced ventilation at 65°C until a constant mass was reached.

Air temperatures inside the greenhouse were measured and registered by micro data loggers (NOVUS® series Pingüim). Measurements were performed every half hour for 24 hours each

day. The mean maximum, minimum and average daily air temperatures during the experiment are presented in Figure 1.

The daily thermal time (TTd, °C.day) was determined according to Arnold (1960):

$$TTd = (Tave – Tb).1 \text{ day}$$

where Tave is the average air temperature obtained from the arithmetic mean of the 48 air temperature readings performed during the day and Tb (base temperature) is the temperature threshold for the development of *Brachiaria brizantha* cv. Marandu, defined as 15°C by Mendonça and Rassini (2006). The accumulated thermal time (TTa, °C.day) was calculated by summing the TTd values of the assessment period.

The leaf appearance rate (LAR, leaves/tiller/°C.day) was determined by the formula:

$$LAR = NL / TTP.TTa$$

where NL is the number of new recently expanded leaves, TTP is the total number of tillers per plant and TTa is the accumulated thermal time for the assessment period (3 days).

In turn, the tiller appearance rate (TAR, tillers/°C.day) was calculated as:

$$TAR = NNT / TTa$$

where NNT is the number of new tillers produced by the plant and TTa is the accumulated thermal time for the assessment period (3 days).

The phyllochron (°C.day/leaf) and the thermal time between appearances of tillers (TBAT, °C.day/tiller) were obtained from the inverses of the LAR (1/LAR) and TAR (1/TAR), respectively.

The variables were evaluated by response surface analysis using the SAS software (SAS Institute, Cary, NC, USA). Each period of plant growth and development was considered independently for the statistical analysis because it is believed that the regrowth after grazing or harvest changes according to plant age (Muldoon and Pearson 1979; Caloin *et al.* 1990). Initially, an analysis of variance was performed for the combinations of N and S rates. In cases where the F-test indicated a significant interaction effect of N and S, a polynomial regression (response surface) study was conducted by using the Response Surface Regression (RSREG) procedure. In cases where the interaction of N and S was not significant, first- and second-degree regressions were performed using the General Linear Model (GLM) procedure. A level of significance of 5% was used for all statistical tests. The symbols (*)
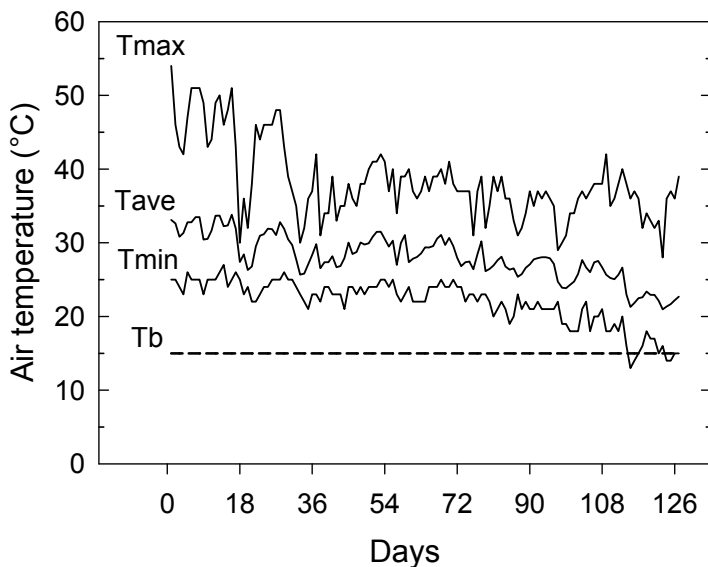

Figure 1. Mean maximum (Tmax), minimum (Tmin) and average (Tave) daily air temperatures during the experimental period and the defined temperature threshold (base temperature, Tb = 15°C) for the development of Marandu palisadegrass (Mendonça and Rassini 2006).

and (**) in the regression and polynomial equations indicate significance at 5% or 1%, respectively, for the corresponding coefficients.

Results

Development patterns of leaves and tillers during successive grass growth cycles

Analysis of the dynamics of leaf appearance of Marandu palisadegrass demonstrated that the phyllochron increased according to the accumulated degree-days during each of the 3 periods of plant growth and development (Figure 2). In contrast, the development of leaves (LAR) by the grass was more pronounced during initial growth or regrowth, decreasing as the plant advanced to phenological stages closer to maturation. The general pattern of variation in TBAT and in TAR was similar to that seen for the leaf variables,

phyllochron and LAR, respectively (Figure 2). It is interesting to observe that the average phyllochron also increased with successive growth cycles in Marandu palisadegrass in the following order: 99.0 < 145.8 < 201.9°C.day/leaf (Figure 2).

Phyllochron and leaf appearance

The amount of N supplied to the plants affected the phyllochron of Marandu palisadegrass during all 3 growth periods. Phyllochron values generally decreased as a function of N rate (Figures 3a, 3b and 3c). Considering the 3 growth and development cycles of this forage plant, the application of 300 mg/dm^3 N reduced the accumulated temperature demand for the appearance of one leaf (expressed in °C.day) by approximately 40%.

The effects of N and S fertilisation on the LAR per tiller of Marandu palisadegrass was opposite
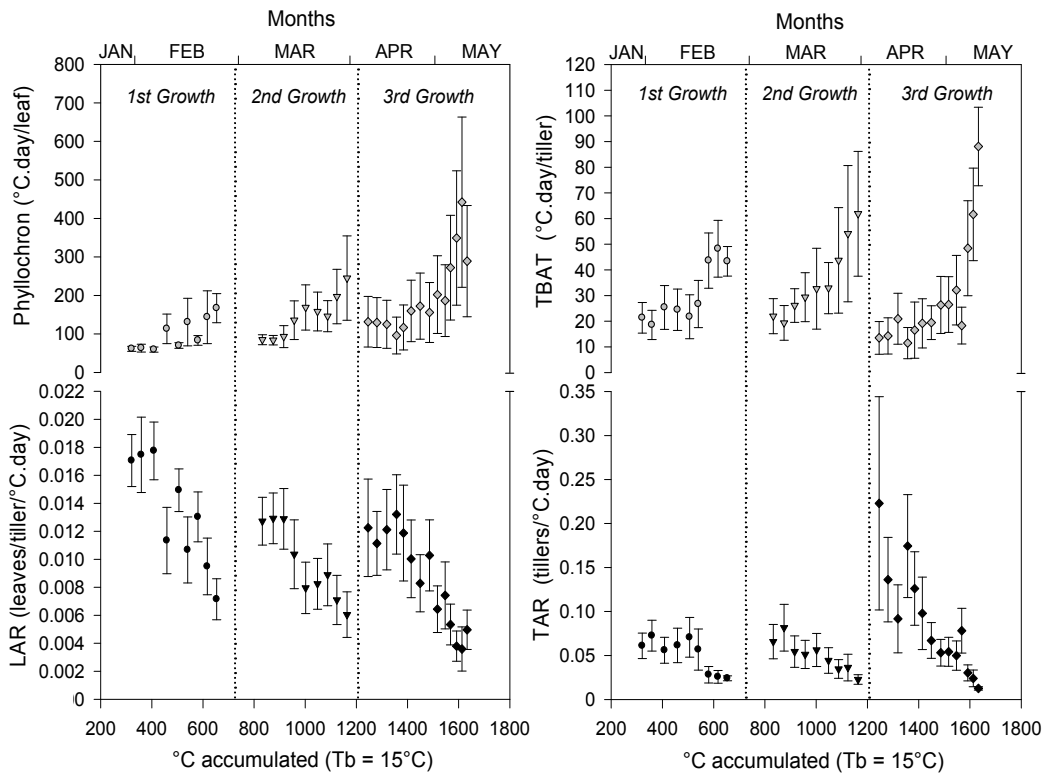

Figure 2. Dynamics of phyllochron, leaf appearance rate (LAR), thermal time between appearance of tillers (TBAT) and tiller appearance rate (TAR) in Marandu palisadegrass according to the degree-days accumulated (base temperature of 15°C) and plant growth periods.

to their effect on the phyllochron (Figures 3*d*, 3*e* and 3*f*). It seems that providing N and S up to rates of approximately 300 and 25 mg/dm^3, respectively, increased LAR, with a decrease in the values of this variable as these rates were exceeded.
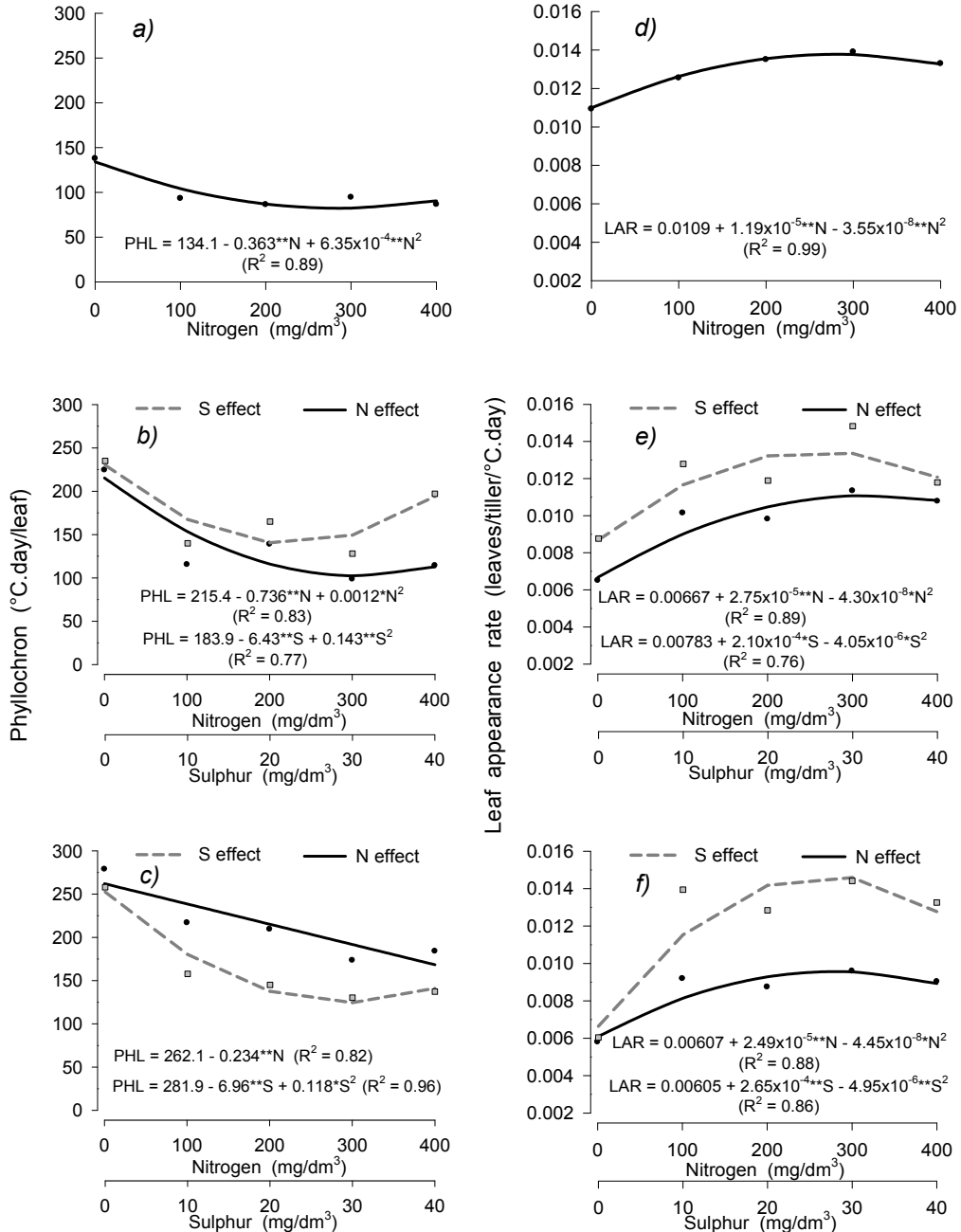

Figure 3. Phyllochron and leaf appearance rate (base temperature of 15°C) during the first (*a*; *d*), second (*b*; *e*) and third (*c*; *f*) growth periods of Marandu palisadegrass fertilised with nitrogen and sulphur.

Tiller development and emergence

The tillering dynamics of Marandu palisadegrass (measured by TBAT and TAR) was strongly influenced by N fertilisation (Figure 4). It is worth mentioning that, during the third cycle of grass growth, the interaction between N and S was significant in the production of tillers (Figure 4*f*). Plants supplied with high N rates (300 mg/dm^3) achieved the maximal TAR (0.16 tillers/°C.day) only when an adequate S rate was also applied.
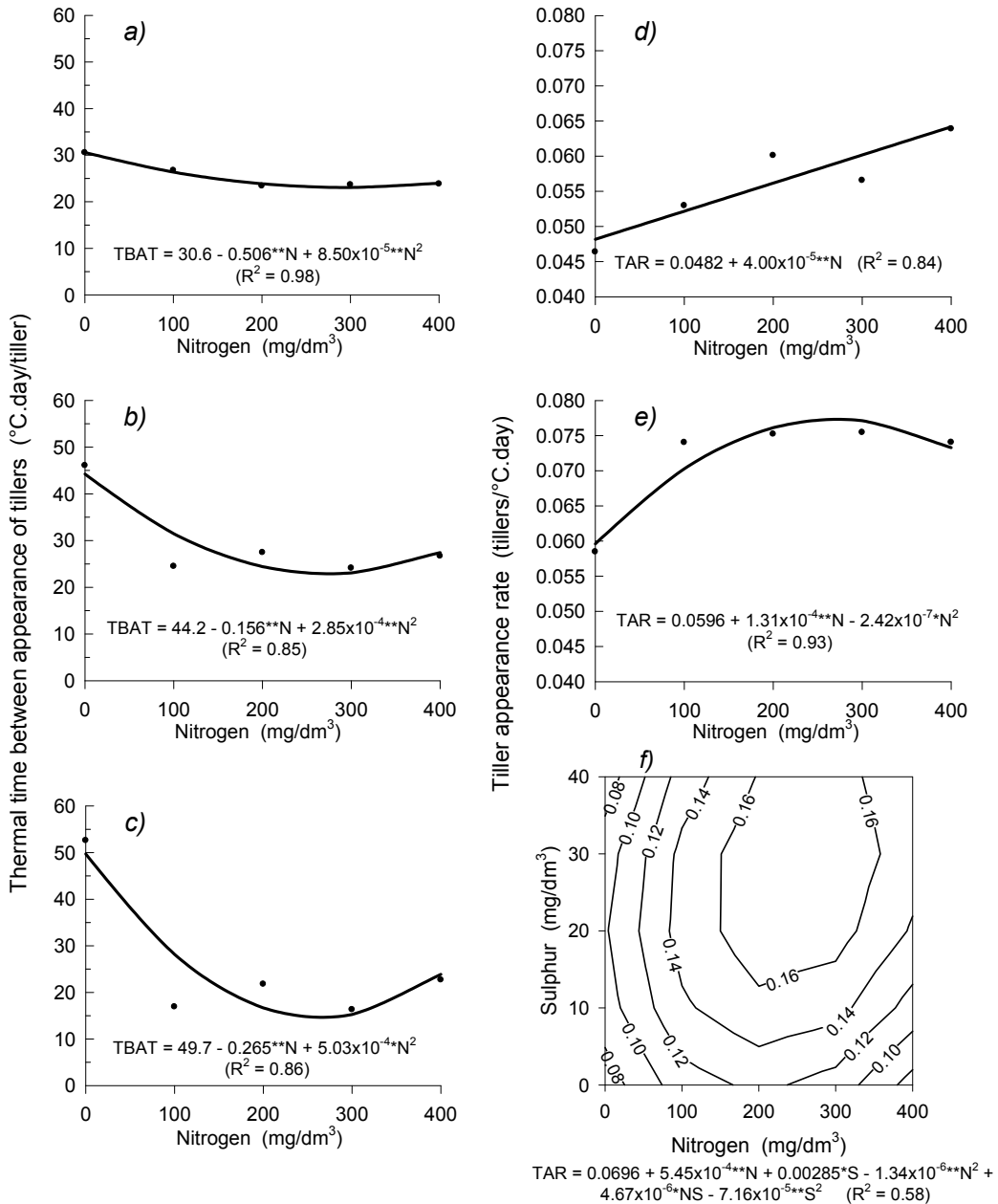

Figure 4. Thermal time between appearance of tillers and tiller appearance rate (base temperature of 15°C) during the first (*a*; *d*), second (*b*; *e*) and third (*c*; *f*) growth periods of Marandu palisadegrass fertilised with nitrogen and sulphur.

Leaf, tiller and dry matter production

In addition to the effects on the phyllochron, LAR, TBAT and TAR (Figures 3 and 4), fertilisation with N and S positively affected the total production of Marandu palisadegrass leaves and tillers (Figure 5). There was a significant interaction between the N and S doses for both the number of observed leaves at the second and third harvests (Figures 5*b* and 5*c*) and the number of tillers produced by the grass during the third growth and development period (Figure 5*f*). By affecting the number of leaves and tillers produced by the plants, the interaction of N rate x S rate significantly altered the forage dry matter of Marandu palisadegrass during the 3 growth periods (Figure 6).

Discussion

As has been shown with other grasses (Nemoto *et al.* 1995; Moore and Moser 1995; Chauvel *et al.* 2000; McMaster *et al.* 2003), the development pattern of Marandu palisadegrass showed a strong relationship between leaf appearance and tillering events (Figure 2). The rates of leaf and tiller production slowed down as plants matured (Frank *et al.* 1985; Skinner and Nelson 1992; Frank and Bauer 1995). As long as nutritional requirements are met (as in this experiment) and in the absence of environmental stresses (water deficit, diseases, pests, etc.), this process is attributed to the changes in vegetative development of adult plants with shifts in available resources (carbohydrates, nutrients) to the formation of reproductive structures at the expense of leaves and tillers (Obeso 2002). In addition, the reduction of incident solar energy in the lower canopy as a result of shading by the upper leaves of plants in advanced phenological stages can also reduce TAR and LAR (Frank and Hofmann 1994).

Although the interaction of N and S was not significant, the addition of S alone changed the phyllochron of Marandu palisadegrass during the second and third growth periods (Figures 3*b* and 3*c*), in a similar way to N fertiliser. Applying 25 mg/dm^3 S in the form of calcium sulphate to Marandu palisadegrass reduced the accumulated degree-days for the appearance of a new leaf by 37.5%, clearly accelerating forage production. Actually, the supply of N or S efficiently enhanced the rates of leaf appearance (Figures 3*d*,

3*e* and 3*f*) in the grass plants up to a critical point that probably coincided with the optimal leaf area index (Hodgson *et al.* 1981; Lemaire and Chapman 1996). Beyond this point, increasing rates of these nutrients did not result in positive effects, possibly because the energy losses caused by the self-shading of the canopy leaves in well developed plants (Hodgson *et al.* 1981; Lemaire and Chapman 1996) outweighed the gains generated by N and S fertilisation.

Nitrogen and S act together in plant protein synthesis (Crawford *et al.* 2002) and the metabolism of grasses is affected by their availability in the soil (De Bona 2008). Therefore, applying N and/or S to Marandu palisadegrass plants probably increased the synthesis of proteins and, as a result, enhanced the growth and production of leaves (Figures 3 and 5). This resulted in a 'snowball effect'. With accelerated leaf growth, both leaf area and photosynthetic capacity increased accordingly (Parsons *et al.* 1983; Akmal and Janssens 2004), further favouring the process of development of new leaves and reducing the phyllochron (Figure 3). According to McMaster *et al.* (2003), while cellular division is predominantly controlled by temperature, cellular expansion may be affected considerably by other factors, including nutritional factors.

Besides leaf development and growth, tillering is one of the determining factors in the forage production (dry matter) of grasses (McKenzie *et al.* 2002; Premazzi *et al.* 2003) and changes according to soil fertility. Thus, comparing growth of control plants with those receiving 200 mg/dm^3 N, it can be seen that nitrogen limitation in controls gradually slowed the development of tillers as the plants matured. As a result, the amount of heat required for the appearance of one tiller (°C.day, Tb = 15°C) in controls was 22, 45 and 77% greater than in the N-fertilised plants during the first, second and third growing periods, respectively (Figures 4*a*, 4*b* and 4*c*). This clearly demonstrates the importance of N fertilisation in promoting tillering in plants. These effects were due not only to nutritional factors (protein synthesis), but also to the secondary function of N in promoting the synthesis and translocation of phytohormones (Wang and Below 1996; Walch-Liu *et al.* 2000) involved in the breakdown of dormancy of lateral buds (Doust 2007; Ongaro and Leyser 2008).

While the variation in TAR across the three growth periods changed with nutritional levels,
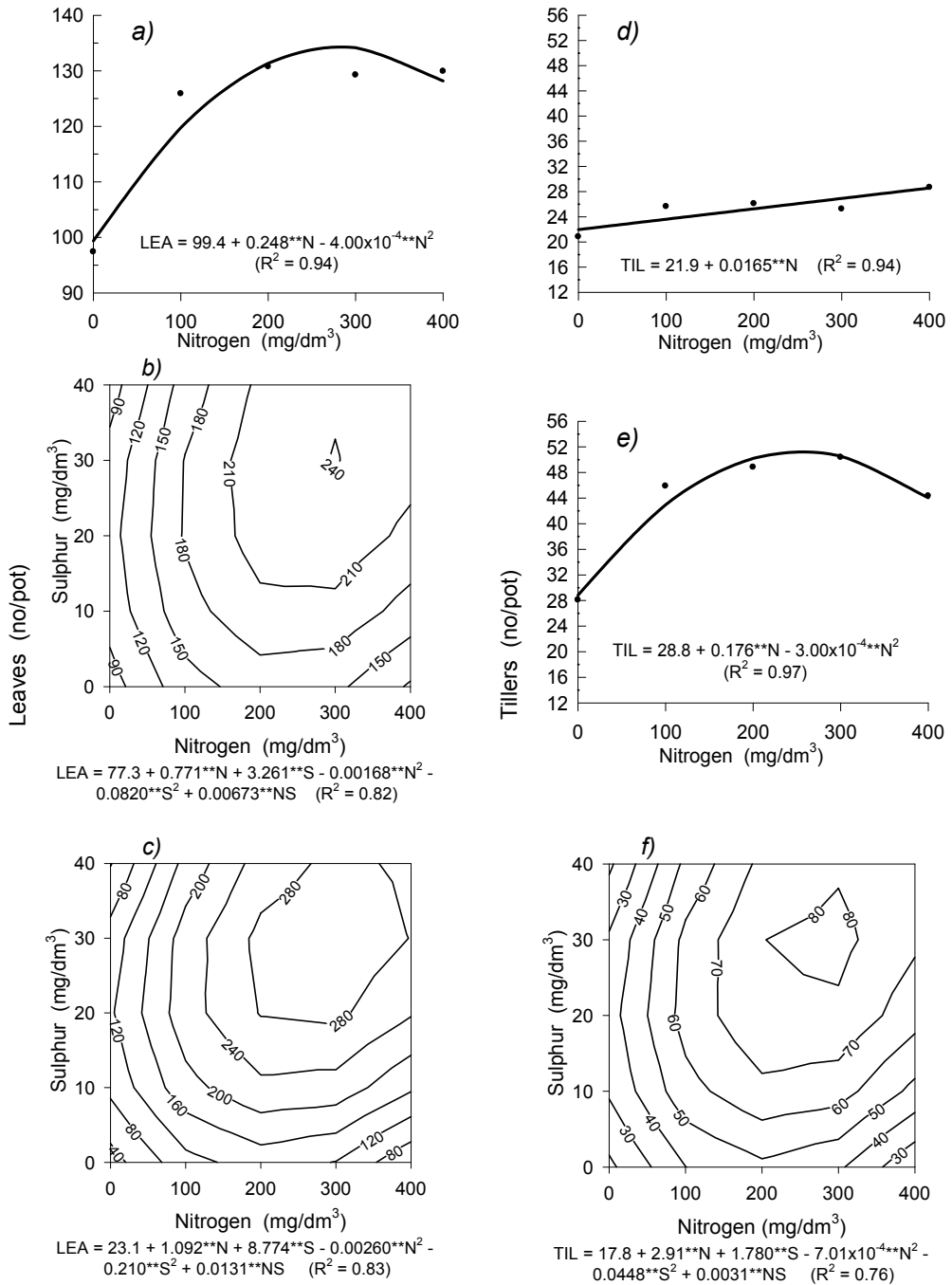
a)

LEA = 99.4 + 0.248**N - 4.00x10^{-4}**N^2
(R^2 = 0.94)

d)

TIL = 21.9 + 0.0165**N   (R^2 = 0.94)

b)

Leaves (no/pot)

Sulphur (mg/dm^3)

Nitrogen (mg/dm^3)

LEA = 77.3 + 0.771**N + 3.261**S - 0.00168**N^2 -
0.0820**S^2 + 0.00673**NS   (R^2 = 0.82)

e)

Tillers (no/pot)

TIL = 28.8 + 0.176**N - 3.00x10^{-4}**N^2
(R^2 = 0.97)

Nitrogen (mg/dm^3)

c)

Sulphur (mg/dm^3)

Nitrogen (mg/dm^3)

LEA = 23.1 + 1.092**N + 8.774**S - 0.00260**N^2 -
0.210**S^2 + 0.0131**NS   (R^2 = 0.83)

f)

Sulphur (mg/dm^3)

Nitrogen (mg/dm^3)

TIL = 17.8 + 2.91**N + 1.780**S - 7.01x10^{-4}**N^2 -
0.0448**S^2 + 0.0031**NS   (R^2 = 0.76)

Figure 5. Leaf and tiller production in the first (*a*; *d*), second (*b*; *e*) and third (*c*; *f*) growth periods of Marandu palisadegrass fertilised with nitrogen and sulphur.

$$DM = 8.137 + 0.165^{**}N + 1.236^{**}S - 3.61 \times 10^{-4**}N^2 - 0.0299^{**}S^2 + 0.0019^{**}NS \quad (R^2 = 0.85)$$
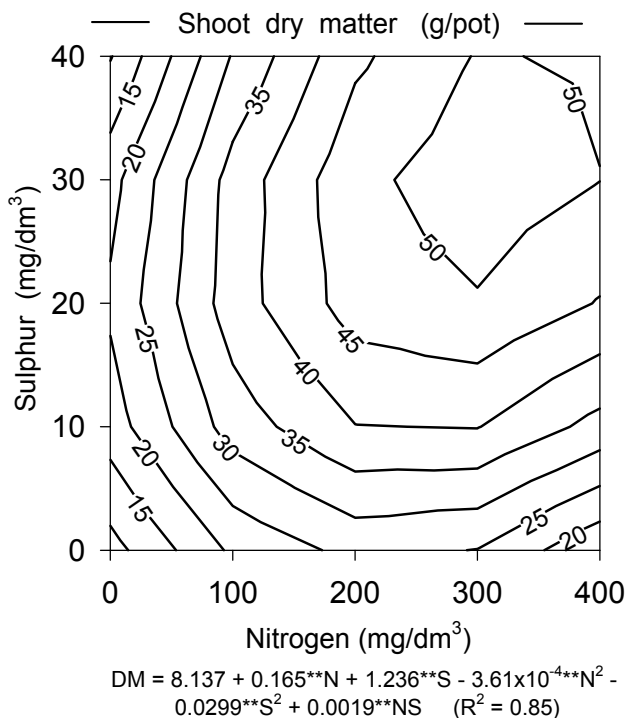
Figure 6. Average shoot dry matter production in the 3 growth periods of Marandu palisadegrass fertilised with nitrogen and sulphur.

environmental factors possibly also played a part (Figures 4*d*, 4*e* and 4*f*). During the initial growth of the grass and the formation of the canopy (Figure 4*d*), N supply had a positive and linear effect on TAR. During the second growth period (Figure 4*e*), when the plants were already established, competition for light (Hodgson *et al.* 1981; Lemaire and Chapman 1996) would have impacted negatively on this relationship in plants supplied with high nutrient rates (dense canopy). The increase in nutritional requirements with the complete establishment of Marandu palisadegrass, associated with increasing intra- and inter-plant competition for solar energy (light), emphasised the importance of supplying extra S in addition to N fertiliser (Figure 4*f*).

Our results indicate that applying N and S at optimal rates and proportions to a grass pasture under non-adverse environmental conditions and subjected to adequate management enhanced the development (phyllochron, TBAT) and production of leaves and tillers. This explains why fertilised grass pastures recover more quickly after harvest or grazing and produce more forage for livestock than unfertilised ones.

References

Akmal, M. and Janssens, M.J.J. (2004) Productivity and light use efficiency of perennial ryegrass with contrasting water and nitrogen supplies. *Field Crops Research,* **88**, 143–155.

Arnold, C.Y. (1960) Maximum-minimum temperature as a basis for computing heat units. *Proceedings of the American Society for Horticultural Science,* **76**, 682–692.

Bonfim-Silva, E.M., Monteiro, F.A. and Silva, T.J.A. (2007) Nitrogênio e enxofre na produção e no uso de água pelo capim-Braquiária em degradação. *Revista Brasileira de Ciência do Solo,* **31**, 309–317.

CALOIN, M., CLEMENT, B. and HERRMANN, S. (1990) Regrowth kinetics of *Dactylis glomerata* following defoliation. *Annals of Botany,* **66**, 397–405.

CHAUVEL, B., MUNIER-JOLAIN, N., LETOUZÉ, A. and GRANDGIRARD, D. (2000) Developmental patterns of leaves and tillers in a black-grass population (*Alopecurus myosuroides* Huds.). *Agronomie,* **20**, 247–257.

CLARKSON, D.T., SAKER, L.R. and PURVES, J.V. (1989) Depression of nitrate and ammonium transport in barley plants with diminished sulphur status. Evidence of co-regulation of nitrogen and sulphate intake. *Journal of Experimental Botany,* **40**, 953–963.

CRAWFORD, N.M., KAHN, M.L., LEUSTEK, T. and LONG, S.R. (2002) Nitrogen and sulphur. In: Buchanan, B.B., Gruissem, W. and Jones, R.L. (eds) *Biochemistry and Molecular Biology of Plants*. pp. 786–849. (American Society of Plant Physiologists: Rockville-MA).

CRUZ, P. and BOVAL, M. (2000) Effect of nitrogen on some morphogenetic traits of temperate and tropical perennial forage grasses. In: Lemaire, G., Hogdson, J., Moraes, A., Nabinger, C. and Carvalho, P.C.F. (eds) *Grassland Ecophysiology and Grazing Ecology*. pp. 151–168. (CAB International Publishing: Wallingford).

DE BONA, F.D. (2008) *Nitrogen and sulphur for gramineaceous forage plants: soil attributes and metabolic, nutritional and productive aspects of plants*. Ph.D. Thesis. University of São Paulo, Brazil.

DOUST, A.N. (2007) Grass architecture: genetic and environmental control of branching. *Current Opinion in Plant Biology,* **10**, 21–25.

FRANK, A.B., BERDAHL, J.D. and BARKER, R.E. (1985) Morphological development and water use in clonal lines of four forage grasses. *Crop Science,* **25**, 339–344.

FRANK, A.B. and HOFMANN, L. (1994) Light quality and stem numbers in cool-season forage grasses. *Crop Science,* **34**, 468–473.

FRANK, A.B. and BAUER, A. (1995) Phyllochron differences in wheat, barley and forage grasses. *Crop Science,* **35**, 19–23.

GRAMIG, G.G. and STOLTENBERG, D.E. (2007) Leaf appearance base temperature and phyllochron for common grass and broadleaf weed species. *Weed Technology,* **21**, 249–254.

HODGSON, J., BIRCHAM, J.S., GRANT, S.A. and KING, J. (1981) The influence of cutting and grazing management on herbage growth and utilization. In: Wright, C.E. (ed.) *Plant Physiology and Herbage Production*. pp. 51–62. (British Grassland Society: Hurley).

LAURIAULT, L.M., KIRKSEY, R.E. and DONART, G.B. (2002) Irrigation and nitrogen effects on tall wheatgrass yield in the southern high plains. *Agronomy Journal,* **94**, 792–797.

LEMAIRE, G. and CHAPMAN, D. (1996) Tissue flows in grazed plant communities. In: Hodgson, J. and Illius, A.W. (eds) *The Ecology and Management of Grazing Systems*. pp. 3–36. (CAB International Publishing: London).

LITTELL, R.C. and MOTT, G.O. (1975) Computer assisted design and analysis of response surface experiments in agronomy. *Soil Crop Society of Florida Proceedings,* **34**, 94–97.

LONGNECKER, N., KIRBY, E.J.M. and ROBSON, A. (1993) Leaf emergence, tiller growth, and apical development of nitrogen-deficient spring wheat. *Crop Science,* **33**, 154–160.

MARTUSCELLO, J.A., FONSECA, D.M., NASCIMENTO JÚNIOR, D., SANTOS, P.M., RIBEIRO JÚNIOR, J.I., CUNHA, D.N.F.V. and MOREIRA, L.M. (2005) Características morfogênicas e estruturais do capim-Xaraés submetido à adubação nitrogenada e desfolhação. *Revista Brasileira de Zootecnia,* **34**, 1475–1482.

MCKENZIE, F.R., JACOBS, J.L. and KEARNEY, G. (2002) The long-term impact of nitrogen fertiliser on perennial ryegrass tiller and white clover growing point densities in grazed dairy pastures in south-western Victoria. *Australian Journal of Agricultural Research,* **53**, 1203–1209.

MCMASTER, G.S. (2005) Phytomers, phyllochrons, phenology and temperate cereal development. *Journal of Agricultural Science,* **143**, 137–150.

MCMASTER, G.S., WILHELM, W.W., PALIC, D.B., PORTER, J.R. and JAMIESON, P.D. (2003) Spring wheat appearance and temperature: extending the paradigm? *Annals of Botany,* **91**, 697–705.

MENDONÇA, F.C. and RASSINI, J.B. (2006) Temperatura-base inferior e estacionalidade de produção de gramíneas forrageiras tropicais. http: www.cppse.embrapa.br/080servicos/070publicacaogratuita/circular-tecnica/circular45.pdf

MIGGE, A., BORK, C., HELL, R. and BECKER, T.W. (2000) Negative regulation of nitrate reductase gene expression by glutamine or asparagines accumulating in leaves of sulfur-deprived tobacco. *Planta*, **211**, 587–595.

MOORE, K.J. and MOSER, L.E. (1995) Quantifying developmental morphology of perennial grasses. *Crop Science,* **35**, 37–43.

MULDOON, D.K. and PEARSON, C.J. (1979) Primary growth and re-growth of the tropical tallgrass hybrid *Pennisetum* at different temperatures. *Annals of Botany,* **43**, 709–717.

NEMOTO, K., MORITA, S. and BABA, T. (1995) Shoot and root development in rice related to the phyllochron. *Crop Science,* **35**, 24–29.

NIKIFOROVA, V.J., BIELECKA, M., GAKIÈRE, B., KRUEGER, S., RINDER, J., KEMPA, S., MORCUENDE, R., SCHEIBLE, W.-R., HESSE, H. and HOEFGEN, R. (2006) Effect of sulfur availability on the integrity of amino acid biosynthesis in plants. *Amino Acids*, **30**, 173–183.

OBESO, J.R. (2002) The costs of reproduction in plants. *New Phytologist,* **155**, 321–348.

ONGARO, V. and LEYSER, O. (2008) Hormonal control of shoot branching. *Journal of Experimental Botany,* **59**, 67–74.

PARSONS, A.J., LEAFE, E.L., COLLET, B. and STILLES, W. (1983) The physiology of grass production under grazing. I. Characteristics of leaf and canopy photosynthesis of continuously-grazed swards. *Journal of Applied Ecology,* **20**, 117–126.

PREMAZZI, L.M., MONTEIRO, F.A. and CORRENTE, J.E. (2003) Tillering of Tifton 85 Bermudagrass in response to nitrogen rates and time of application after cutting. *Scientia Agricola,* **60**, 565–571.

REICH, P.B., BUSCHENA, C., TJOELKER, M.G., WRAGE, K., KNOPS, J., TILMAN, D. and MACHADO, J.L. (2003) Variation in growth rate and ecophysiology among 34 grasslands and savanna species under contrasting N supply: a test of functional group differences. *New Phytologist,* **157**, 617–631.

SKINNER, R.H. and NELSON, C.J. (1992) Estimation of potential tiller production and site usage during tall fescue canopy development. *Annals of Botany,* **70**, 493–499.

STANFORD, R.L., WHITE, R.H., KRAUSZ, J.P., THOMAS, J.C., COLBAUGH, P. and ABERNATHY, S.D. (2005) Temperature, nitrogen and light effects on hybrid bermudagrass growth and development. *Crop Science,* **45**, 2491–2496.

WALCH-LIU, P., NEUMANN, G., BANGERTH, F. and ENGELS, C. (2000) Rapid effects of nitrogen form on leaf morphogenesis in tobacco. *Journal of Experimental Botany,* **51**, 227–237.

WANG, X. and BELOW, F.E. (1996) Cytokinins in enhanced growth and tillering of wheat induced by mixed nitrogen source. *Crop Science,* **36**, 121–126.

WILHELM, W.W. and MCMASTER, G.S. (1995) Importance of the phyllochron in studying development and growth in grasses. *Crop Science,* **35**, 1–3.