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Nitrate reductase activity and corn hybrid yields intercropped with *Brachiaria brizantha* in three sowing arrangements

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Crop-livestock systems consists in the cultivation of agricultural and foraging species in the same area, in consortium, rotation or crops succession. Among several used crops, corn and brachiaria intercropping stands out, for its importance, cultivation tradition and adaptation of species to intercropping cultivation. This study aimed to evaluate the influence of *Brachiaria brizantha* (brachiaria) sowing arrangements on yield and nitrate reductase activity in different corn hybrids. A randomized block design was used with four replications, in a 4×3 factorial scheme. Treatments constituted of four corn hybrids DKB 747, BM2202, AGN30A00 and AG2060 and three forage sowing arrangements: a) corn crop at 0.9 m spacing, two rows of *B. brizantha* in the inter-row at 0.3 m from the corn row; b), corn crop at 0.9 m spacing, one brachiaria row in the corn sowing row, mixed with starter fertilizer, and another one in the inter-row center; c) corn crop at 0.45 m spacing, with brachiaria in the corn sowing row, mixed with starter fertilizer. The spacing reduction between corn rows promoted yield increase and reduced brachiaria growth. Lower nitrate reductase activity in corn plants was found in the corn crop at 0.9 m spacing, with two *B. brizantha* rows located in the corn interrow at 0.3 m.

Key words: Brachiaria, competition crop-livestock systems, *Zea mays*.

INTRODUCTION

The establishment of forage species intercropped with annual crops, named integrated crop-livestock systems, is an efficient and economically viable technique for formation, recovery and renovation of pastures (Jakelaitis et al., 2004; Freitas et al., 2005a, b). This integration allows the development of two activities, agricultural and livestock production, which, through simultaneous or sequential crop cultivation, contributes to yield and

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improvement of soil physical and chemical characteristics (Amaral Filho et al., 2005).

Several crops have been cultivated with *Brachiaria* species. Among them, corn stands out, mainly, due to its cultivation tradition, great number of commercial cultivars well-adapted to different ecological regions of Brazil, numerous uses in the rural property. In addition, corn has excellent adaptation when intercropped and can be used for production of sweet corn, grains or silage (Santos and Araújo, 2011).

However, when two species are simultaneously cultivated, they are submitted to mutual interferences, which may result in competition for environmental resources, leading to losses that may make intercropping unfeasible. Among the factors that affect corn yield and pasture establishment in intercropped cultivation, the sowing arrangement of species deserves special attention.

Planting of the agricultural crop and forage grass can be carried out simultaneously or sequentially, and it is necessary to favor the early development of the crop so that there is no significant grain yield loss. Some direct seeding machines have a forage seedling device. When this equipment is not available, forage seeds can be mixed to fertilizer or broadcast by hand. Thus, forage sowing arrangements can be carried out by broadcast seeding, in the same rows or in the inter-rows of the crop (Santos and Araujo, 2011). Planting forage in the same row as corn may promote corn yield reduction, when compared with planting only in the inter-rows (Borghi and Crusciol, 2007). Reports on inter-specific competition vary according to the adopted planting arrangement: some studies reported that the forage did not interfere with corn yield, while others showed the necessity of application of herbicide subdoses to reduce forage growth and allow full corn development (Jakelaitis et al., 2005). Another factor that can influence corn yield and pasture establishment is the use of corn hybrids with different leaf architecture and life cycle, as simple hybrids have modern leaf architecture, with smaller plants and erect leaves, allowing higher light penetration in the canopy and favoring forage development, when compared with double and triple hybrids, which are larger plants with flat leaves (Jakelaitis et al., 2005). Among other factors, the period in which the forage competes for environment resources with the crop may alter crop yield, making the use of control measures necessary, like the use of sub-doses of herbicides, to avoid this interference (Jakelaitis et al., 2004). When brachiaria is mixed to fertilizer and sown in the corn row, it is deposited at a higher depth, delaying its emergence, favoring the corn crop (Kluthcouski et al., 2000). Forage emergence subsequently to corn emergence favors competitive advantage to corn, due to reduction of the competition period for water and nutrients between the species. The purpose of the present study was to evaluate the influence of *Brachiaria brizantha* cv. Marandu in diferente planting arrangement son nitrate reductase activity and yield of four corn hybrids.

MATERIALS AND METHODS

The experiment was carried out in the growing season of 2008 to 2009 at the Experimental Unit at Coimbra, of the Federal University of Viçosa, situated in the Coimbra region, Zona da Mata, MG, at 715 m altitude. Climatic data from the period of the test are shown in Figure 1. A randomized blocks design, 4 × 3 factorial arrangements, with four replications was employed. The treatments were composed of the combination of four corn hybrids and three *B. brizantha cv*. Marandu (brachiaria) sowing arrangements: a) arrangement 1, corn crop at 0.9 m spacing, with two brachiaria rows in the inter-row at 0.3 m from the corn row; b) arrangement 2, corn crop at 0.9 m spacing, with one brachiaria row in the corn sowing row, mixed to fertilizer, and another one in the interrow center; c) arrangement 3, corn crop at 0.45 m spacing, with brachiaria in the corn sowing row, mixed with fertilizer.

The used corn hybrids were DKB 747 (double hybrid, medium/large size, precocious), BM2202 (double hybrid, medium/large size, flat leaves, precocious), AGN30A00 (simple hybrid, small size, erect leaf architecture, extra-precocious) and AG2060 (double hybrid, high, flat normal leaves). Prior to experiment, soil samples were collected at 0 to 0.2 m depth, for chemical and physical analysis. The results revealed the following characteristics: 70% clay; water pH of 4.9; 8.2 mg dm^{-3} of P (Mehlich-1); 55 mg dm^{-3} of K; 0.2 $cmol_c dm^{-3}$ of Al; 1.1 $cmol_c dm^{-3}$ of Ca; 0.6 $cmol_c dm^{-3}$ of Mg; and 1.8 g dm^{-3} of MO. Nearly 30 days before sowing, desiccation was carried out in the area, using 1.8 kg ha^{-1} of glyphosate, with syrup volume of 250 L ha^{-1}

Corn and brachiaria sowings were carried out on November 21 and 22nd, by a seeding-fertilizing machine. Corn seeds were deposited at 5 cm depth. The corn population was 70.000 plants ha^{-1} for hybrid AGNA30A00 and 55.000 plants ha^{-1} for the others, according to planting recommendations of the seed producing companies. It was used 3.8 kg ha^{-1} of viable pure brachiaria seeds. In sowing arrangements where the forage was planted in the corn inter-rows, the seeds were conditioned in the machine seed deposit and planted at 3 cm depth. In arrangements in which the forage was sown in the corn row, seeds were mixed to fertilizer and conditioned in the machine fertilizer compartment, distributed at 8 cm depth, below the corn seed. The starter fertilizer application consisted of 28 kg ha^{-1} of N, 98 kg ha^{-1} of P_2O_5 and 56 kg ha^{-1} of K_2O, corresponding to 350 kg ha^{-1} of the formulated fertilizer NPK (8-28-16). When the corn crop reached the stage of four completely expanded leaves, the top dressing was carried out with 210 kg ha^{-1} of urea. The weeds were controlled at 25 days after sowing (DAS) by application of 1.5 kg ha^{-1} of atrazine herbicide, using a backpack sprayer, pressurized with CO_2, constant pressure of 200 kPa, equipped with two pulverization tips (TT 11002), and calibrated to apply 100 L it ha^{-1} of syrup. At application, weed and brachiaria plants presented an average of two leaves. The climatic conditions at application were clear sky, moist soil, wind speed inferior to 5 km h^{-1}, air temperature around 25°C and relative moisture superior to 65%. For control of corn armyworm, 129 g ha^{-1} of Metomil insecticide was used.

Each experimental unit constituted of six rows of 6 m length, totalizing an area of 32.4 m^2. The data corn and brachiaria yields were collected in the two central rows, non-considering 1.0 m in the edges. The sampling for determination of nutrient levels in the leaves was carried out when at least 50% of the corn plants exhibited tassels and style-stigma presence. In this case, the leaf opposite to the superior ear was collected, according to Malavolta et al. (1997), in 10 plants per experimental unit. At corn harvest, mean ear weigh, mean per thousand grain weight and yield, corrected to 13% moisture were evaluated. For determination of

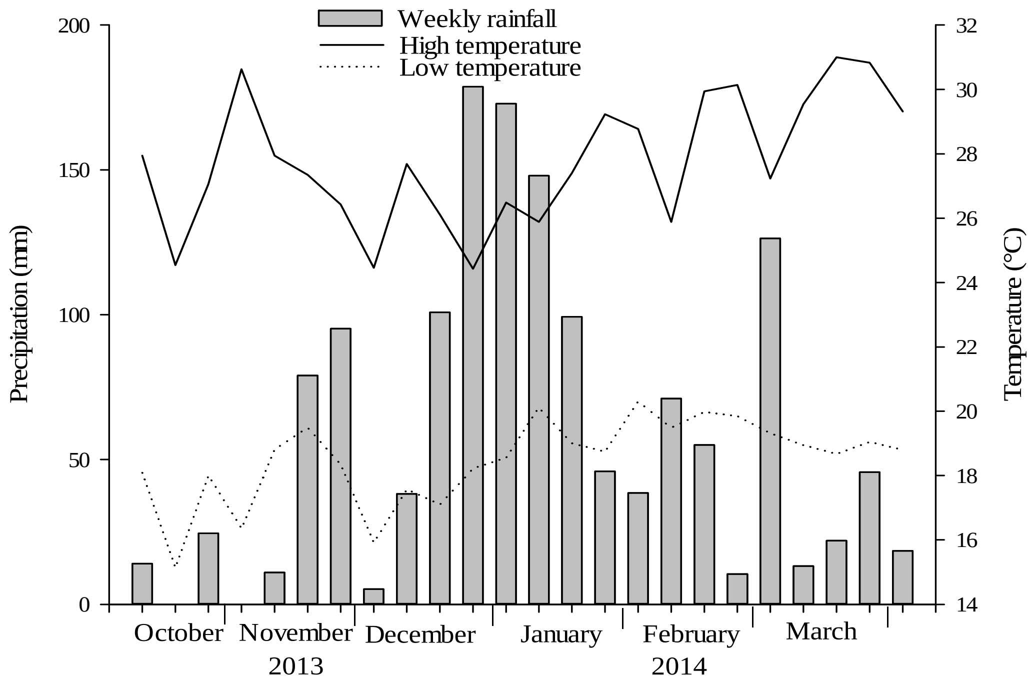

Figure 1. Rainfall and weekly average temperature during the conduct of the experiment.

forage species biomass all brachiaria plants were collected at the soil level from the two central rows of each experimental unit. This sampling was carried out at corn harvest. All collected material was dried in stove with forced air circulation, approximately 60°C, for 72 h and weighed subsequently.

The nitrate reductase (NR) activity was determined in the leaf opposite to the superior ear. Leaves were collected at 55 days after emergence, between 7 and 8 a.m. Samples were conditioned in thermal box with ice. At the laboratory, 1 cm diameter leaf discs were used, which were incubated in 10 ml phosphate buffer (KH_2PO_4 and K_2HPO_4) 0.2 M (pH 7.5), 0.25 M potassium nitrate, propanol and Triton X-100 10%. After immersion, samples were transferred to a dissecator and submitted to vacuum infiltration for one minute, three times, aiming at increasing solution penetration in the tissues. Next, incubation bottles wrapped in foil, were taken to water bath at 30°C. At previously set times of 0.5 to 1 h, 1 ml samples were withdrawn, in which 0.3 ml of 1% sulfanilamide, 0.3 ml of 0.02% naphthalenediamine and 2.4 ml of water were added. The reading was done in spectrophotometer at 540 nm. The NR activity was determined by the amount of nitrite produced, comparing the obtained values with the standard curve for this ion. The activity was expressed in µmoles of nitrite per hour per gram of fresh matter (µmoles of NW^{-2} hour/g fresh matter). The data were submitted to analysis of variance, in case of significance, means were compared by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

There was significant difference in the thousand grains weight and mean ear weight between the evaluated hybrids; however, this difference did not result in statistical difference between yields (Table 1). The AGNA30A00 hybrid, though has presented lower ear weight, obtained satisfactory yield value due to higher population of plants.

The AGNA30A00 presented lower leaf area index (LAI) than the other corn hybrids (Table 1). The leaf area index that determines maximum growth rate is known as critical leaf area index, and varies according to the environment where the plant is. The critical LAI for the corn crop oscillates between values from 3 to 5, according to the region, hybrids and production system considered (Fancelli and Dourado-Neto, 2004). The simple hybrid AGN30A00 presented LAI lower than the critical value (Table 1), compromising its productive potential.

On average, the nitrate reductase activity values were 20 to 30% higher for BM2202 and AGN30A00 hybrids, in relation to the others. Despite not differing significantly, these hybrids were also the most productive (Table 1). The genotypic differences as for grain production can be attributed to the soil nitrate absorption capacity (Purcino et al., 1998) and consequently elevation of the nitrate reductase activity, since the enzyme activity varies according to the substrate concentration (Redinbaugh and Campbell, 1991).

The dry mass of the brachiaria plants aerial part did not differ statistically from the evaluated corn hybrids (Table 1). Although the AGN30A00 hybrid has presented lower plant height and smaller leaf area comparing with the others (Table 1), providing less shade in the inter-row and allowing, thus, higher brachiaria plant growth. The biomass production of this forage was not significantly higher possibly due to the inter-specific competition caused by higher population of this corn hybrid plants.

The reduction of space between corn rows increased

Table 1. Yield (YD, kg ha^{-1}), thousand grain weight (TGW, g), mean ear weight (MEW, g), leaf area index (LAI) and nitrate reductase activity (NR, µmoles of NO^{2-}/hour/g of fresh mass) of corn hybrids and dry mass of *Brachiaria brizantha cv.* Marandu (DMB, $kg.ha^{-1}$).

Variables	TGW (g)	MEW (g)	YD (kg^{-1})	LAI	NR (µmoles of NO_2-/hour / g M.F).	DMB (kg^{-1})
BM2202	292 ^{b}	153 ^{a}	6.918 ^{a}	3.42 ^{a}	4.0 ^{ab}	3.156 ^{a}
AG2060	260 ^{c}	140 ^{ab}	5.533 ^{a}	3.22 ^{a}	3.4 ^{b}	4.852 ^{a}
DKB747	326 ^{a}	152 ^{a}	6.206 ^{a}	3.24 ^{a}	3.3 ^{b}	3.751 ^{a}
AGN30A00	298 ^{b}	118 ^{b}	6.626 ^{a}	2.89 ^{b}	4.4 to	4.690 ^{a}
CV %	7.8	17.0	21.2	8.5	19.1	38.1

^{ns} Not significant at 5% probability. Means followed by same letter in column do not differ at 5% probability by Tukey's test. CV: Coefficient of variation.

Table 2. Corn yield (YD, $kg.ha^{-1}$), corn leaf area index (LAI), nitrate reductase enzyme activity (NR, µmoles of NO^{2-}/hour/g of fresh mass) and dry mass of aerial part of *Brachiaria brizantha cv*. Marandu (DMB, $kg.ha^{-1}$) according to sowing arrangements.

Arrangements*	YD	LAI	NR	DMB
1	5.510 ^{b}	3.0 ^{b}	3.1 ^{b}	6.284 ^{a}
2	5.568 ^{b}	3.1 ^{b}	4.2 ^{a}	3.735 ^{b}
3	8.033 ^{a}	3.5 ^{a}	4.1 ^{a}	2.318 ^{c}
CV %	21.2	8.5	19.6	38.0

Means followed by same letter in column do not differ at 5% by Tukey's test. CV: Coefficient of variation , * 1, corn cultivation at 0.9 m spacing, with two rows of *Brachiaria brizantha* (brachiaria) located in interrow at 0.3 m from corn row; 2, corn cultivation at 0.9 m spacing, with a row of brachiaria in corn sowing row, mixed to starter fertilizer, and another one in interrow center; 3, corn cultivation at 0.45 m spacing, with brachiaria in corn sowing row, mixed with starter fertilizer.

yield in 45% (Table 2). Similar results were reported by Silva et al. (2006) with reduction in space between rows from 0.8-0.1 m to 0.4-0.5 m, increasing yield in approximately 10%, in subtropical regions in the South of Brazil. In the Middle West region, space reduction from 0.9 to 0.45 m promoted growth even higher than those in the South of the country, reaching values 10 to 40% higher. The sowing space reduction between rows favors better space distribution of corn plants (Sangoi et al., 2002). This reduces intra-specific competition for the surrounding resources, stimulates crop growth at the beginning of its cycle and develops light inter-capitation, increasing its use efficiency (Andrade et al., 2002). All hybrids presented yield reduction when the spacing of 0.9 m was adopted between rows (Table 2). However, planting two rows of brachiaria in the inter-row or brachiaria in the same row and in the inter-row did not interfere with corn yield.

The spacing reduction provided higher shade for the inter-row due to the space arrangement of plants, which reduced brachiaria growth and consequently of the produced biomass (Table 2). At 0.45 m spacing, all corn hybrids presented greater leaf area, intensifying the shade effects on the brachiaria plants (Table 2). Similar results were obtained by Borghi and Crusciol. (2007).

Corn cultivation at 0.45 m spacing, with brachiaria in the corn sowing row, mixed to planting fertilizer, allowed better corn plants arrangement, as well as quick growth and yield. Besides, it provided greater shade for brachiaria plants, delaying biomass accumulation by the forage during the inter-specific competition period (Table 2).

Corn cultivation was also adopted at 0.9 m spacing, with a brachiaria row in the corn sowing row, mixed to planting fertilizer, and another one in the center of the row space, brachiaria seeds were mixed to fertilizer and deposited at 8 cm depth in the corn planting row. Under this condition, brachiaria plants were intensely shaded by corn plants and had a delayed emergence, which favored the corn development. Portes et al. (2000), while evaluating the same intercropped species used in this experiment, reported that the deposition of brachiaria seeds at 10 cm depth, with fertilizer, delayed the emergence of forage plantlets in approximately five days and weakened them. Owing to the shade provided by the corn during the intercropping period, the forage presented slow growth, mainly because both species have C4 carbon fixation.

Higher growth of brachiaria plants in the corn inter-row may cause competition for water and nutrients, reducing nitrogen availability for corn plants. Nitrogen moves in the soil by mass flow, and the exploration of the same soil

Table 3. Effect of planting arrangements on yield (YD, kg^{-1}), thousand grain weight (TGW, g), mean ear weight (MEW, g), leaf area index (LAI), and nitrate reductase (NR activity, µmoles of NO^{2-}/hour/g of fresh mass) of corn hybrids and dry mass of aerial part of *Brachiaria brizantha* cv. Marandu (DMB, kg ha^{-1}), in intercropped cultivation.

Planting arrangements*	YD	LAI	NR	DMB
		AGN30A00		
1	4.094 ^{c}	2.65 ^{b}	2.61 ^{b}	6.492 ^{a}
2	6.081 ^{b}	2.72 ^{b}	5.66 ^{a}	4.499 ^{b}
3	9704 ^{a}	3.33 ^{a}	4.93 ^{a}	3.079 ^{c}
		DKB 747		
1	5.605 ^{b}	2.98 ^{b}	3.04 ^{a}	5.017 ^{a}
2	5.763 ^{b}	3.26 ^{ab}	3.76 ^{a}	3.832 ^{b}
3	7.252 ^{a}	3.51 ^{ab}	3.28 ^{a}	1.795 ^{c}
		AG 2060		
1	4.856 ^{b}	3.42 ^{ab}	3.46 ^{a}	8.100 ^{a}
2	4.601 ^{b}	2.97 ^{ab}	2.94 ^{a}	3.868 ^{b}
3	7.142 ^{a}	3.42 ^{a}	3.89 ^{a}	2.588 ^{c}
		BM 2202		
1	6.888 ^{b}	3.15 ^{b}	3.28 ^{a}	5.527 ^{a}
2	5.829 ^{b}	3.43 ^{ab}	4.36 ^{a}	2.741 ^{b}
3	8.036 ^{a}	3.69 ^{ab}	4.49 ^{a}	1.200 ^{c}
CV %	21.0	8.5	19.1	30

In each hybrid, means followed by same letter in column do not differ at 5% by Tukey's test. CV: Coefficient of variation
* 1, corn cultivation at 0.9 m spacing, with two rows of *Brachiaria brizantha* (brachiaria) in interrow at 0.3 m from corn row; 2, corn cultivation at 0.9 m spacing, with a brachiaria row in corn sowing row, mixed with starter fertilizer, and another one in interrow center; 3, corn cultivation at 0.45 m spacing, with brachiaria in corn sowing row, mixed with starter fertilizer. VC: variation coefficient.

area by the radicular system of the two crops can reduce the influx of this nutrient for the roots, causing reduction in the nitrate supply through the xylem, reducing, thus the nitrate reductase activity (Kawachi et al., 2002). Lower nitrogen availability for the plant can cause reduction in the photosynthetic rate, reduction of chlorophyll levels (Ciompi et al., 1996; Gouy et al., 2001) and reduction in leaf expansion and in the activity of some enzymes of the nitrogen reducing cycle, as nitrate reductase. The nitrate reductase enzyme activity was statistically lower in the corn crop at 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row (Table 2). This result can be attributed to greater forage development, caused by higher light availability and quick emergence. The nitrate reductase activity is positively correlated with nitrate availability in the environment, corn plants, when evaluated in soils with higher nitrogen availability, present higher nitrate reductase activity, when compared with those cultivated at below N (Miranda et al., 2005; Majerowics et al., 2002; Oliveira, 2009).

The AGNA30A00 hybrid presented lower yield for corn cultivation at 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row, when compared to the other arrangements (Table 3). That may have been caused by higher competition of the brachiaria plants in this arrangement. In planting arrangements in which the brachiaria was sowed in the corn row space, brachiaria was deposited at a lower depth, promoting quicker emergence and being able to compete, with the corn. The above-mentioned hybrid was shorter and presented less leaf area regarding the rest (Table 3), promoting less shade in the inter-row and allowing, thus, higher brachiaria growth. Among the evaluated corn hybrids, the AGN30A00 presented higher nitrate reductase activity (Table 1). Due to its modulating role of reduced N availability for the plants metabolism, it has been suggested that the nitrate reductase activity is associated with corn yield and/or, with its capacity of responding to nitrogen fertilizing (Araujo et al., 2009; Borges et al., 2006). This tendency was confirmed, since in planting arrangements in which the AGN30A00 hybrid did not suffer brachiaria interference it presented yield and nitrate reductase activity values superior to the others (Table 3).

The AGNA30A00 hybrid presented lower nitrate

Table 4. Effect of corn planting arrangements intercropped with brachiaria on nitrate reductase (NR) enzyme activity (µmoles of NO^-_2 g^{-1} h^{-1} of fresh mass).

Planting arrangements*	AGNA30A00	BM 2202	AG2060	DKB747
1	2.61^{Ba}	3.28^{Aa}	3.46^{Aa}	3.04^{Aa}
2	5.66^{Aa}	4.36^{Aab}	2.94^{Ab}	3.76^{Ab}
3	4.93^{Aa}	4.49^{Aab}	3.89^{Aab}	3.28^{Ab}
CV %	19.6	19.6	19.6	19.6

Means followed by same capital letter in column, and lowercase letter in row do not differ at 5% probability Tukey's test. CV: Coefficient of variation, * 1, corn cultivation at 0.9 m spacing, with two rows of *Brachiaria brizantha* (brachiaria) in interrow at 0.3 m from corn row; 2 – corn cultivation at 0.9 m spacing, with a row of brachiaria in corn sowing row, mixed with starter fertilizer, and another one in interrow center; 3, corn cultivation at 0.45 m spacing, with brachiaria in corn sowing row, mixed with starter fertilizer.

reductase activity at 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row (Table 4). Lower enzyme activity may have been caused by higher competition of brachiaria plants in this arrangement. The AGN30A00 hybrid presented lower leaf rate area in this arrangement, when compared with the others (Table 3), allowing higher growth of brachiaria plants, causing greater competition for nutrients, reducing nitrogen availability, and consequently, the nitrate reductase activity. When studying the genetic and biochemical bases of nitrogen use efficiency in corn, Hirel et al. (2001) found significant correlations between physiologic characteristics (activities of NR and glutamine synthetase-GS enzymes and nitrate level in the aerial part of plants) and agronomic characteristics (yield and grain weight). Gallais and Hirel (2004) emphasized that these correlations were dependent of the quantity of nitrogenated fecundation. The activity of NR and GS enzymes in corn plants of appropriately supplied with nitrogen was superior comparing with plants submitted to stresses by nitrogen deficiency (Majerowics et al., 2002). There was no significant difference in leaves nitrogen level, regarding hybrids or adopted planting arrangements. The N level in leaves is below the ideal level for all evaluated hybrids, which is from 2.7 to 4.0 dag kg^{-1} (Ferreira et al., 2001). The lowest leaf nitrogen level was found in hybrid AGNA30A00, using 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row, where lower nitrate reductase activity was also verified. For the other hybrids and spacings, the nitrogen level in leaves varied between 2.13 and 2.64 dag kg^{-1}.

Conclusions

The spacing reduction from 0.90 m to 0.45 m between corn rows increased yield and reduced growth of *B. brizantha cv*. Marandu and corn rows increased corn leaf area. Smaller corn hybrids with erect leaves were more prone to competition with brachiaria, mainly when sowing the forage in the inter-row. Lower nitrate reductase activity in corn plants occured with higher brachiaria plants growth, in the arrangement of two forage rows in the corn inter-row.

Conflict of Interest

The authors have not declared any conflict of interest.

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