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ABSTRACT

Soil hydric availability and nitrogen fertilization are important environmental factors that influence sugarcane production. In the present study, the physiological attributes SPAD index, maximum photochemical efficiency of photosystem II (F_v/F_m), leaf area index (LAI), chlorophyll and carotenoid content, and sugarcane productivity were assessed under different nitrogen doses (0, 50, 100, 150, and 200 kg N ha^{-1}) applied in the form of urea via subsurface drip fertigation. The physiological attributes were determined 38, 121, 208, 291, and 381 days after the third harvest (DAH), and stalk and sugar productivity at 381 DAH. The 100 kg N ha^{-1} dose has produced better results for the sugarcane physiological attributes. Increasing doses of nitrogen applied via subsurface drip fertigation increased the productivity of stalks and sugar considerably.

Introduction

Sugarcane, *Saccharum* spp., is distinctively one of the best sources of renewable energy, making the case for it to be an important crop in Brazil and in the world. Remarkably, it presents a wide adaption scale, being cultivated in tropical and subtropical regions, including more than 90 countries between parallels 35°N and 30°S (Alfonsi et al., 1987). Sugarcane is cultivated in different soil types, under the influence of several abiotic factors in each region, showing different levels of production (Silva et al., 2008). Among the environmental factors that reduce the plant growth and development, water deficiency is considered one of the most important limitations to productivity and distribution of crops, having a negative influence on 10% of the agricultural areas in the globe (Bartels and Sunkar, 2005), particularly in crops of great economic importance, such as rice, corn, wheat, and sugarcane. Such limitations are due to the fact that water deficiency causes severe damages to plant growth, mainly through the reduction of cellular expansion (Taiz and Zeiger, 2010). Taking this aspect into account, sugarcane producers are heavily dependent on irrigation in order to reach production targets (Silva et al., 2007). Besides leading to an increase in productivity, reaching amounts larger than 100%, irrigation also allows for a longer time of plant exploration as well as a larger number of harvests (Dalri et al., 2008). However, water for irrigation is a limited and contentious resource, which means an effective management is essential, not only to reduce prodigal use, but also to reduce production costs, and maintain productivity (Silva et al., 2007).

Nitrogen fertilization is also one of the main abiotic factors that determine the sugarcane production, particularly regarding the longevity of ratoons (Muchow et al., 1996; Vale et al., 2013). Nitrogen is the fertilizer that is most frequently applied through water irrigation, given that, compared to other nutrients such as phosphorus, potassium, calcium, and magnesium, it presents high mobility in the soil, although it has a high loss potential,

mainly due to nitrate leaching (Roberts, 2008). Fertigation allows for wider nutrient lateral and vertical movements, which is considerably important in perennial and semi-perennial crops (Phogat et al., 2013), given the difficulty to incorporate side-dressing without damaging the root system (Vieira et al., 2010).

Although it constitutes exclusively 1% of the total sugarcane dry matter, nitrogen is directly involved in the synthesis of amino acids, in the composition of the chlorophyll molecule, and the production of carbohydrates, and, therefore, plays a determinant role in crop development (Vitti et al., 2010). Studies reveal that chlorophyll concentration or greening of leaves is positively correlated to the leaf nitrogen concentration, given that most of the N contained in leaves is in the chloroplasts, participating in the synthesis and structure of chlorophyll molecules (Torres Netto et al., 2005). Moreover, the function of such element in the metabolism of carbohydrates and in forming the chlorophyll molecule contributes positively to the growth and quality of stalks in the milling process, as well as to the vigor of the plant (Shekinah et al., 2012). Nevertheless, nitrogen fertilization is distinctively one of the management practices with the highest research demand for sugarcane, because studies on nitrogen present very varied and even contradictory results, as well as the response mechanisms have not been completely elucidated (Vale et al., 2013).

For the reasons explained above, the objective of the present research was to characterize sugarcane physiological attributes and productivity under nitrogen doses applied via subsurface drip fertigation.

Materials and methods

The experimental area was installed in November 2006, in a location close to the town of Jaú, São Paulo state, Brazil (22°17′S and 48°34′W, 580 m of altitude). The climate in the region is Aw (Köppen), tropical rainy with dry winter, annual averages of 22.7°C for temperature and 1,344 mm for precipitation. Soil has been classified as Eutrophic Argisoil. Before the experiment was installed, soil samples were collected from layers at 0–20, 20–40, and 40–80 cm for chemical and granulometric analysis (Table 1).

The experimental plots were made of five double furrow lines (0.40 × 1.40 m) with 8 m in length. Between the double furrows, dripping tubes of the irrigation system (22135 FL, DripNet PC, Adana, Turkey) were buried at 20 cm of depth, with drippers every 0.5 cm and flow rate of 1.0 L h^{-1}.

The present study was carried out between September 2009 and October 2010, after the third cut of sugarcane SP80–3280. The soil hydric condition was monitored by tensiometers and the frequency of irrigation was calculated based on the available water capacity (AWC) in the soil of 70 mm, precipitation (P), atmospheric demand, and sugarcane evapotranspiration (ETC). Total rainfall during the experiment period was 1,435 mm. The amount of water applied by the irrigation system was 390 mm, distributed throughout the sugarcane development cycle, in order to supply 100% of the crop's

Table 1. Chemical and particle size analysis of soil Eutrophic Argisoil the experimental area.

				Chemical analysis[1]					
Depth	pH	O. M.	P resin	S-SO$_4$	K	Ca	Mg	H+Al	V
cm	CaCl$_2$	g dm^{-3}	mg dm^{-3}		mmol$_c$ dm^{-3}				%
0–20	5.2	19	19	3	0.9	27	14	22	66
20–40	5.3	14	11	2	0.4	21	12	20	64
40–80	5.3	7	3	6	0.2	21	7	16	66

	Particle size analysis			
Depth	Sand	Silt	Clay	
cm	g kg^{-1}			Textural class
0–20	660	70	270	Medium
20–40	600	60	340	
40–80	530	70	400	

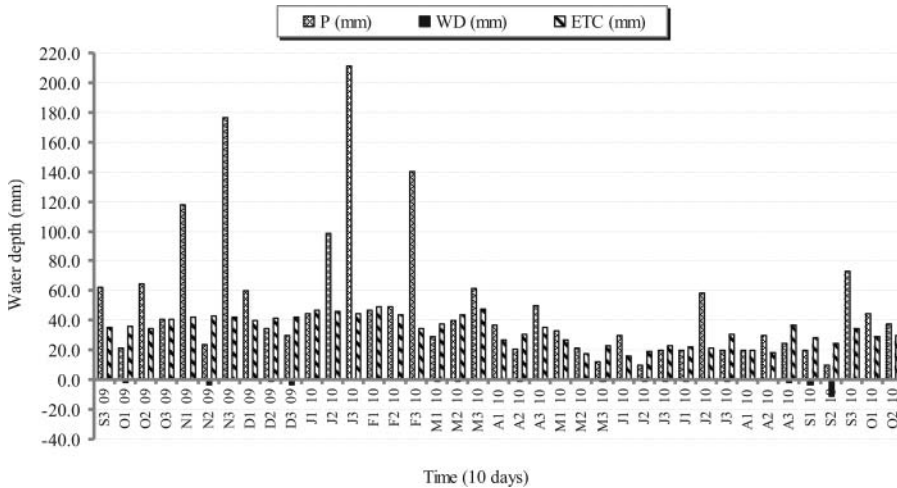[1]O.M.: organic matter; V: base saturation.

Figure 1. Decendial water balance in subsurface drip irrigation management from September 2009 to October 2010. P = precipitation + water depth; WD = water deficit; ETC = crop evapotranspiration.

evapotranspiration, in accordance with the Penman-Monteith method (Howell and Evett, 2004), which had an accumulation of 1,320 mm (accumulated ETC). Thereby, the ten-day water balance was estimated and water deficit (WD) was calculated, reaching only 28 mm (Figure 1).

Different nitrogen doses (0, 50, 100, 150, and 200 kg N ha^{-1}), in the form of urea (45% N), were applied via full subsurface fertigation twice a week, distributed between the harvest of the previous cycle up to four months before the next harvest. 150 kg ha^{-1} dipotassium oxide (K$_2$O) in the form of potassium chloride (KCl) was also added to the treatments and distributed throughout the cycle, with such application being interrupted three months prior to the harvest. Physiological attributes were determined in five periods of plant development: 38, 121, 208, 291, and 381 days after third cut (DAC), in a central meter of the double furrow of each plot. The chlorophyll content estimation (SPAD index) was determined by a chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA). Contents of chlorophyll a, b, total ($a+b$), chlorophyll ratio a/b and carotenoids were determined in ten discs removed from leaves +1 (Top Visible Dewlap) from each plot and immersed in dimethylformamide for 24 hours, and the extract was read in spectrophotometer in different wave lengths according to Lichtenthaler (1987). The maximum photochemical efficiency of photosystem II (F_V/F_M) was obtained using a portable fluorometer (OS-30p, Opti-Sciences, Inc., Hudson, NH, USA). The leaf area index (LAI) was determined between 1 and 2 pm using a ceptometer (Accupar LP-80, Decagon Devices, Inc., Pullman, WA, USA).

The stalks productivity (TSH), t stalk ha^{-1}, was determined by the weight of stalks in each plot at 381 DAC. The sugar productivity (TPH), t pol ha^{-1}, was obtained by the product between TSH and the apparent sugar percentage in the stalks (PCC). Thereby, the experimental design used for the physiological attributes was randomized blocks, in a 5 × 5 factorial scheme, with the following factors: nitrogen doses (N) and evaluation period (E), in four repetitions. For TSH and TPH, randomized blocks experimental design was also used, according to nitrogen doses, in four repetitions. Data was submitted for variance analysis by test F followed by the application of Turkey test (5%) for comparing the averages. The analysis of polynomial regression was used to decompose the degrees of freedom of the quantitative factor nitrogen doses applied via subsurface drip fertigation.

Results and discussion

Physiological attributes

The SPAD index decreased significantly throughout the evaluation seasons, independently of the 0, 50, 100, 150, and 200 kg N ha^{-1} doses applied via subsurface drip fertigation (Figures 2A, 2B, 2C, 2D, and
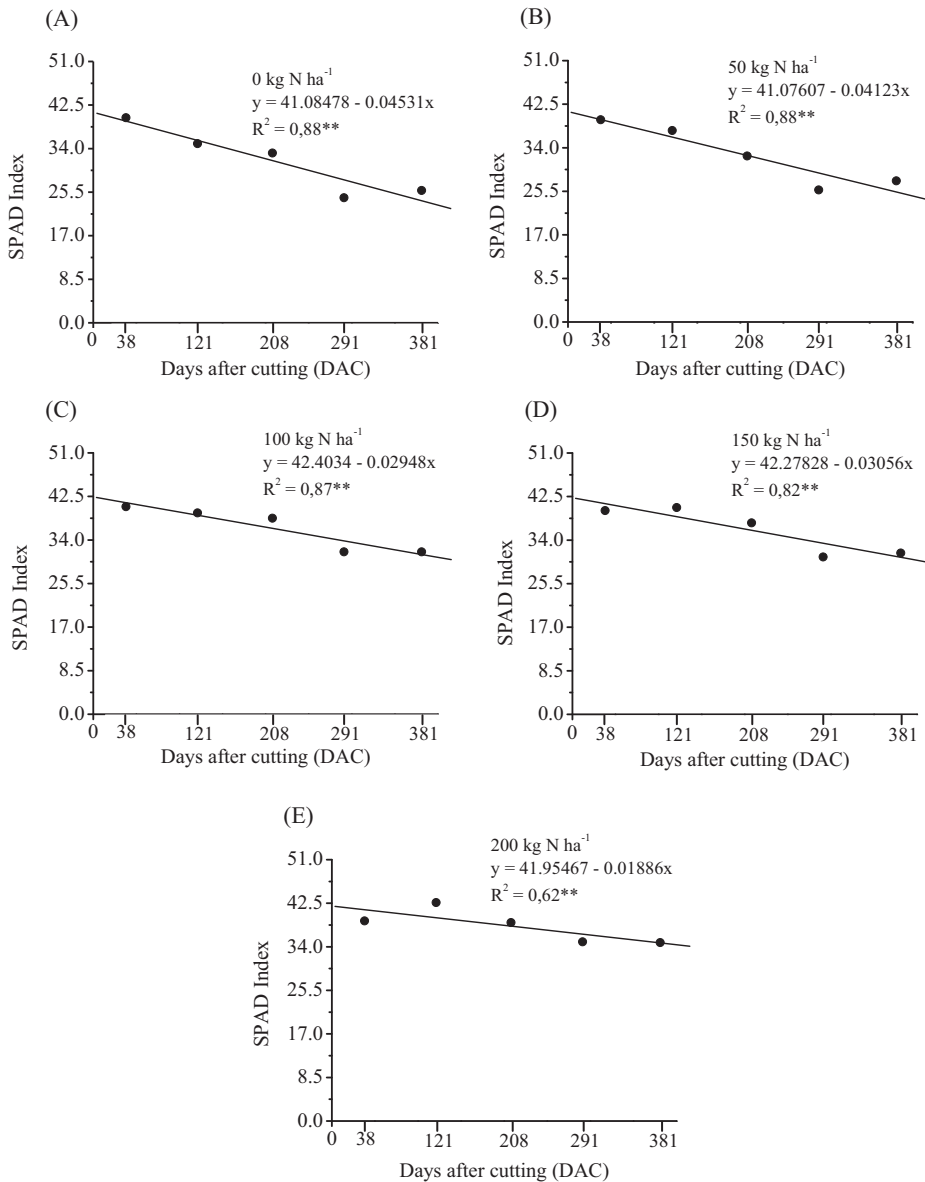
Figure 2. Mean values of SPAD index for sugarcane cv. SP80-3280, using five evaluation periods (38, 121, 208, 291, and 381 DAC) in doses of 0, 50, 100, 150, and 200 kg N ha^{-1} applied via subsurface drip fertigation represented in graphs A, B, C, D, and E, respectively. Jaú, SP, Brazil. 2009–2010.

2E, respectively). Such decrease, particularly from 208 days after cut onwards, is due to the vigorous vegetative growth of the crop induced by the N-fertilizer, causing the dry matter production rate to exceed the nitrogen accumulation rate in plant issues (Vitti et al., 2010), since the plant develops in other organs, such as roots and stalks, which act as drains and reduce nitrogen concentration in the leaves (Oliveira et al., 2005).

On the other hand, the SPAD index has increased significantly according to the increase in nitrogen doses applied via subsurface drip fertigation 121, 208, 291, and 381 DAC (Figures 3A, 3B, 3C, and 3D, respectively). In the first evaluation, 38 DAC, no difference was verified in the reading of the SPAD index, which may have occurred due to the reduced time for the absorption of nitrogen applied via subsurface dripping. Moreover, at the beginning of its development, sugarcane presents a reduced demand for this nutrient, given its slow initial growth (Vitti et al., 2010).
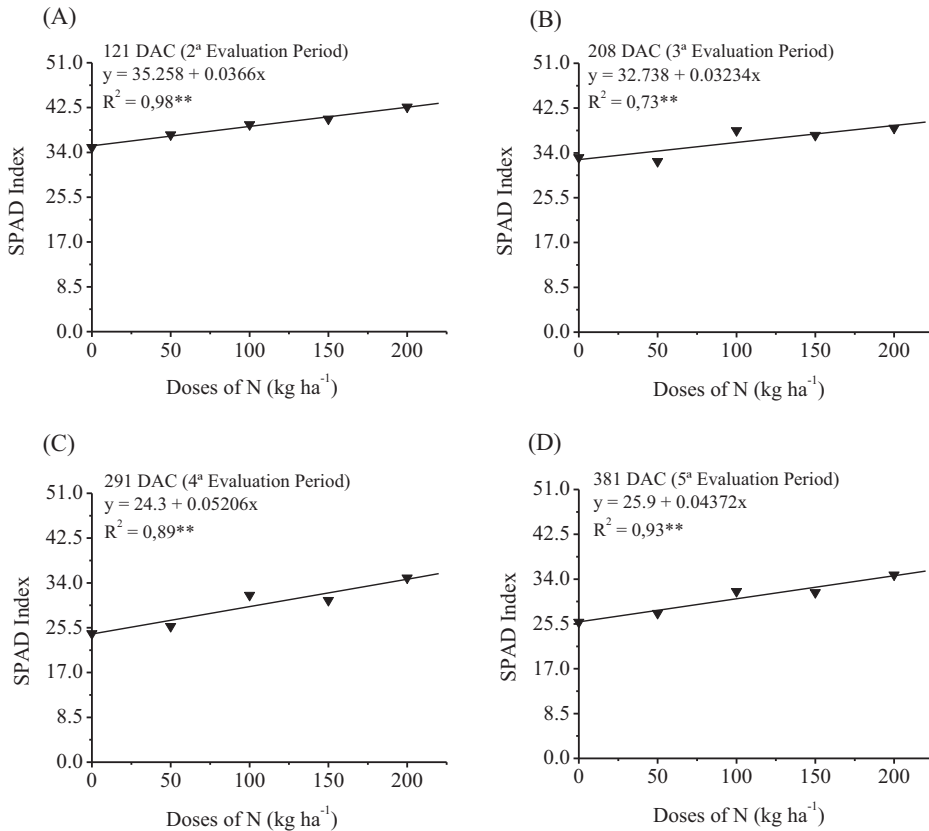
Figure 3. Mean values of SPAD index for sugarcane cv. SP80-3280, obtained under doses of nitrogen (0, 50, 100, 150, and 200 kg N ha^{-1}) applied via subsurface drip fertigation in four evaluation periods (121, 208, 291, and 381 DAC), represented in graphs A, B, C, and D, respectively. Jaú, SP, Brazil. 2009–2010.

Other studies have revealed that sugarcane plants cultivated under different nitrogen concentrations have presented a considerable increase in SPAD index values (Kawamitsu et al., 1999). Glaz et al. (2008) have verified the significant increase in SPAD index in sugarcane under flood periods associated to nitrogen fertilization with 84 and 113 kg N ha^{-1}.

The chlorophyll *a*, *b*, total (*a*+*b*), and carotenoids contents increased due to the nitrogen doses, reaching the highest value under the application of 200 kg N ha^{-1} via subsurface dripping, however, the chlorophyll ratio *a*/*b* did not demonstrate change according to such doses (Table 2). The increments verified due to the increase in nitrogen doses under full irrigation was evidence by the fact that such nutrient is part of the constitution of chlorophyll molecules, which captures luminous energy through photosystems I e II of the photochemical phase of photosynthesis, used for the conversion into the chemical energy necessary to incorporate and form carbonated skeletons (Pedó et al., 2012).

Studies have reported that the accumulation of carotenoid pigments in plants may be influenced by physiological and biochemical attributes, as well as abiotic factors, such as light, temperature, and fertility, particularly nitrogen fertilization (Kopsell et al., 2007). Studies with basil (Politycka and Golcz, 2004) and spinach (Lefsrud et al., 2007) have recorded an increase in carotenoid pigments contents proportional to higher nitrogen concentration.

Results have also shown that chlorophyll *a*, *b*, total (*a*+*b*), and carotenoids contents decreased throughout the evaluation periods, particularly from 208 DAC onwards (Table 3), such as observed for the SPAD index (Figure 2). The highest concentration of photosynthetic pigments observed until this period with reduction afterwards matches with the period of more vigorous crop development, characterized by intense tillering and leaf expansion, and from this period on, such pigment start to decrease

Table 2. Tukey test for contents of chlorophyll *a* (CC*a*), b (CC*b*), Total (CC (*a*+*b*)), ratio chlorophyll *a/b* (CC (*a/b*)), and the carotenoid pigments contents (CAROT) for sugarcane cv. SP80-3280, obtained for doses of nitrogen (0, 50, 100, 150, and 200 kg ha⁻¹) applied via subsurface drip fertigation. Jaú, SP, Brazil, 2009–2010.

	CC*a*	CC*b*	CC(*a*+*b*)	CC(*a/b*)	CAROT
Doses of N (kg ha^{-1})			(μg cm^{-2})		
0	17.75c[2]	5.00c	22.75c	3.77	1029.14c
50	22.50b	6.38b	28.88b	3.71	1261.11ab
100	22.94ab	6.65ab	29.59ab	3.71	1388.40ab
150	25.13ab	7.12ab	32.25ab	3.61	1257.13b
200	26.79a	7.67a	34.47a	3.61	1449.32a
LSD[1]	4.02	1.27	5.25	NS[3]	189,43

[1]LSD represents least significant difference.
[2]Means within a column followed by the same letter are not significant at $P \leq 0.05$.
[3]NS represents not significant at $P \leq 0.05$.

with leaf senescence, process through which leaves slowly loose such pigments, RNA, lipids, and proteins, even under humidity maintenance and nutrients provision (Taiz and Zeiger, 2010). The similarity in the response between photosynthetic pigments and the SPAD index is explained by the positive and significant correlation between both variables (Silveira et al., 2003).

Two of the main pigments related to photosynthetic efficiency and, as a consequence, to plant growth and adaptation to different environment are chlorophyll *a* and carotenoids (Force, 2003). Due to their unstable chemical structure, chlorophylls are easily degraded, leading to decomposition product that modifies the perception and quality of plants. There is also variation in the carotenoid content according to different cultivars, stages of maturation, climate conditions, or geographical location, as well as to the part of the plant that was used and the agricultural production technique (Meschede et al., 2011).

Moreover, the chlorophyll ratio *a/b* has recorded a significant increase throughout the experimental evaluations (Table 3). Such behavior is evidenced by the greater degradation of chlorophyll *b* compared to chlorophyll *a.* As a matter of fact, in the leaf senescence stage, the first step of degradation of chlorophyll *b* involves its conversion into chlorophyll *a* (Santos et al., 2001).

The maximum photochemical efficiency of photosystem II (F_V/F_M) has also increased with the increment of nitrogen doses, particularly under the application of 100 and 150 kg N ha⁻¹ via subsurface dripping. Nevertheless, it decreased depending on the evaluation periods, particularly 291 days after cut and beyond (Table 4).

Photosynthesis occupies a central position in metabolism and provides the connection between the plant's internal processes and the external environment. The use of chlorophyll fluorescence parameters has been widespread, particularly in studies on the photosynthetic capacity of plants, because it is a non-destructive method that allows the qualitative and quantitative analysis of the absorption and

Table 3. Tukey test for contents of chlorophyll *a* (CC*a*), *b* (CC*b*), Total [CC (*a*+*b*)], ratio chlorophyll *a/b* (CC (*a/b*)], and carotenoids pigments contents (CAROT) for sugarcane cv. SP80-3280, obtained in five evaluation periods (38, 121, 208, 291, and 381 DAC) applied via subsurface drip fertigation. Jaú, SP, Brazil, 2009–2010.

	CC*a*	CC*b*	CC(*a*+*b*)	CC (*a/b*)	CAROT
Evaluation period			(μg cm^{-2})		
1st (038 DAC)	30.26a[2]	9.51a	39.77a	3.95c	1563.99a
2nd (121 DAC)	30.91a	9.05a	39.96a	3.43bc	1538.73a
3rd (208 DAC)	23.03b	6.51b	29.54b	3.57b	1287.42b
4th (291 DAC)	14.90c	4.13c	19.02c	3.68b	938.02c
5th (381 DAC)	16.02c	3.63c	19.66c	4.59a	1056.93c
LSD[1]	4.02	1.27	5.25	0.31	189.43

[1]LSD represents least significant difference.
[2]Means within a column followed by the same letter are not significant at $P \leq 0.05$.

Table 4. Tukey test of maximum photochemical efficiency of photosystem II (F_V/F_M) for sugarcane cv. SP80-3280, obtained under doses of nitrogen (0, 50, 100, 150, and 200 kg ha^{-1}) applied via subsurface drip fertigation in five evaluation periods (38, 121, 208, 291, and 381 DAC). Jaú, SP, Brazil, 2009–2010.

Treatment doses of N (kg ha^{-1})	Tukey Test[2] F_V/F_M	Treatment evaluation periods	Tukey test[2] F_V/F_M
0	0.76ab	1ª (038 DAC)	0.77ab
50	0.74b	2ª (121 DAC)	0.78a
100	0.77a	3ª (208 DAC)	0.78a
150	0.77a	4ª (291 DAC)	0.75bc
200	0.76ab	5ª (381 DAC)	0.74c
LSD[1]	0.02		0.02

[1]LSD represents least significant difference.
[2]Means within a column followed by the same letter are not significant at $P \leq 0.05$.

the use of light energy by the photosynthetic apparatus (Krause and Weis, 1991; Torres Netto et al., 2005).

Additionally, the photosynthetic capacity of plants depends on the nitrogen supply (Fontes et al., 2008). A considerable fraction of such element is found in leaves, allocated in the proteins involved in the photosynthetic process (Onoda et al., 2004). Also, photosynthesis depends on various nitrogenous compounds, such as enzymes and photosynthetic pigments, for the production of carbon compounds, which comprise the aerial part (Taiz and Zeiger, 2010). The metabolism of nitrogen and carbon are interdependent, given that the energy needed for assimilating nitrogen derives directly or indirectly from photosynthesis, whereas the later depends on nitrogen supply (Fontes et al., 2008).

The late phenological stage of plants and the increase in leaf senescence have interfered negatively in chlorophyll content (Table 3) and, as a consequence, contributed for the decrease in F_V/F_M (Baker and Rosenqvst, 2004) verified at the final evaluation period (Table 4), result that was also observed for the SPAD index (Figure 2).

The IAF presented a significant increase up until 208 DAC due to doses 0, 50, 100, 150, and 200 kg N ha^{-1}, applied via subsurface dripping (Figures 4A, 4B, 4C, 4D and 4E, respectively). This period is characterized by high tillering and emergence of intense amount of leaves. Later, the emission of new leaves and tillers was stabilized, corresponding to the period between 200 and 300 DAC (Ramesh, 2000), when there was a decrease in IAF, associated to the sugarcane maturation phase, that is 360 DAC, in which there is more leaf senescence.

Regarding the nitrogen doses, there was a significant increase in IAF according to the highest concentration of nutrients (Figure 5). However, between 208 and 381 DAC there was a decrease and stabilization of the IAF values, respectively, from 100 kg N ha^{-1} (Figures 5C and 5E). Wiedenfeld and Enciso (2008) have also recorded an increase in IAF in sugarcane under doses of 0, 60, 120, and 180 kg N ha^{-1} applied via dripping.

IAF is a non-dimensional measure, which characterized the plant canopy in an ecosystem (Sandhu et al., 2012) and is related to liquid photosynthesis (accumulation of dry matter mass) obtained by the crops (Wiedenfeld and Enciso, 2008). Such relation is established due to the fact that the IAF is directly associated to the amount of light absorbed and, as a consequence, to the photosynthesis carried out by plants (Scarpari and Beauclair, 2009).

The increase in IAF observed throughout the evaluation periods is probably due to the increase in the number of leaves per stalk and the increase in the leaf area by individual. Such fact may also promote a significant association between crop productivity and the total photosynthetically active surface (Sandhu et al., 2012). Thus, the solar radiation intercepted by leaves and the chemical energy transformations during the photosynthetic process are directly related to determining sugarcane productivity (Teruel et al., 1997). However, the increase in such physiological parameters does not guarantee the same effect on productivity for the entire crop growth period, since after a more advanced development phase a self-shading effect is verified, generating a reduction in photosynthetic rates (Ramesh, 2000). Thus, at the end of the cycle, the reduction of the emission of new leaves, as well as in leaf senescence,
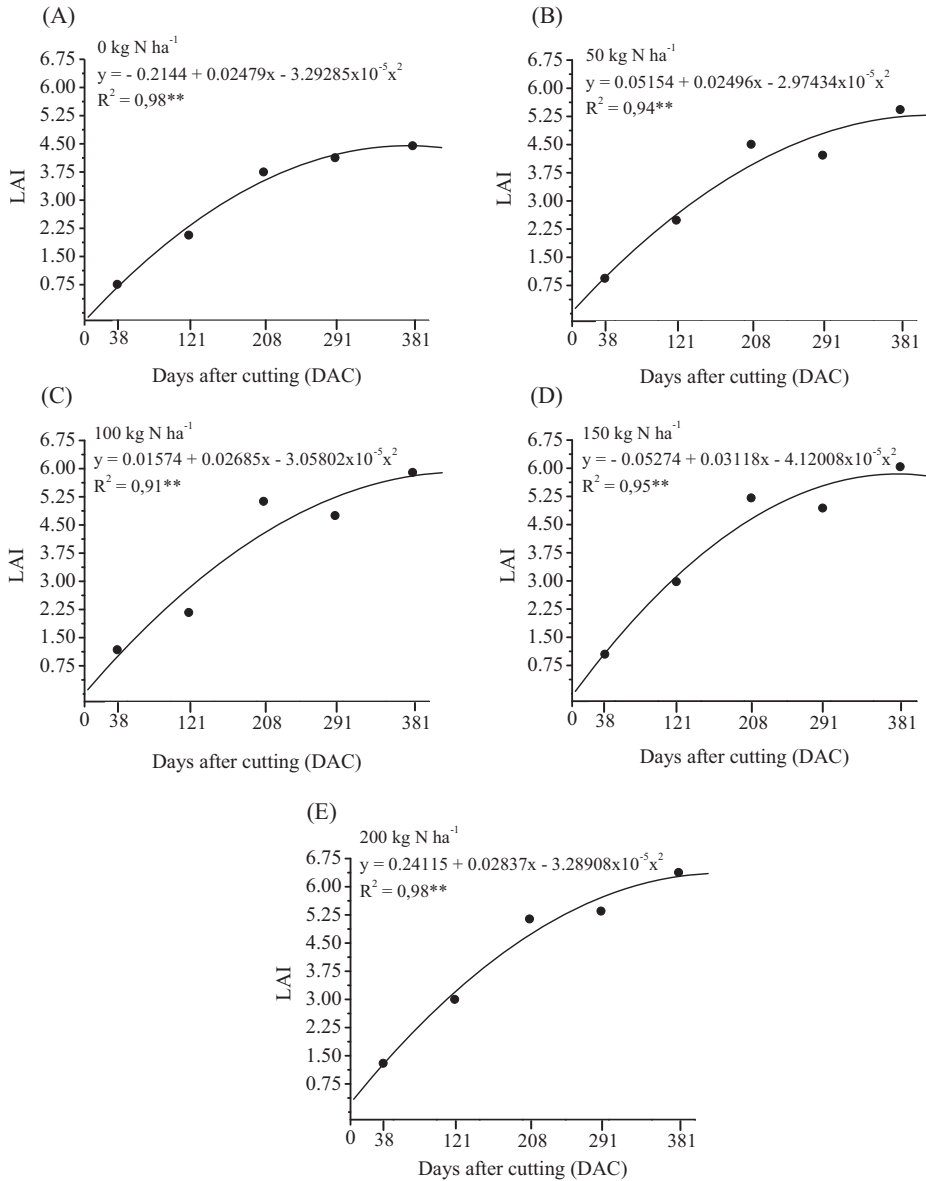
Figure 4. Mean values of leaf area index (LAI) for sugarcane cv. SP80-3280, obtained in five evaluation periods (38, 121, 208, 291, and 381 DAC) in doses of 0, 50, 150, and 200 kg N ha^{-1} applied via subsurface drip fertigation, represented in graphs A, B, C, D, and E, respectively. Jaú, SP, Brazil. 2009–2010.

exceed the beneficial effects of nitrogen fertilization on IAF, as already concluded in other studies (Ng Kee Kwong et al., 1999).

Productivity

There was a considerable increase in sugarcane stalk productivity (TSH) after the third cut with the increase on nitrogen doses applied via subsurface dripping (Figure 6). The 150 and 200 kg N ha^{-1} doses have generated 100 and 120 t ha^{-1} TSH, respectively. Dalri and Cruz (2008) also reported an increase of productivity of stalks for ratoon cane cycle under subsurface drip fertigation. Wiedenfeld and Enciso (2008) obtained 108 and 115 t ha^{-1} TSH in second cut sugarcane under 70 and 140 kg N ha^{-1} doses applied via subsurface dripping, respectively. Such results may indicate that the demand for
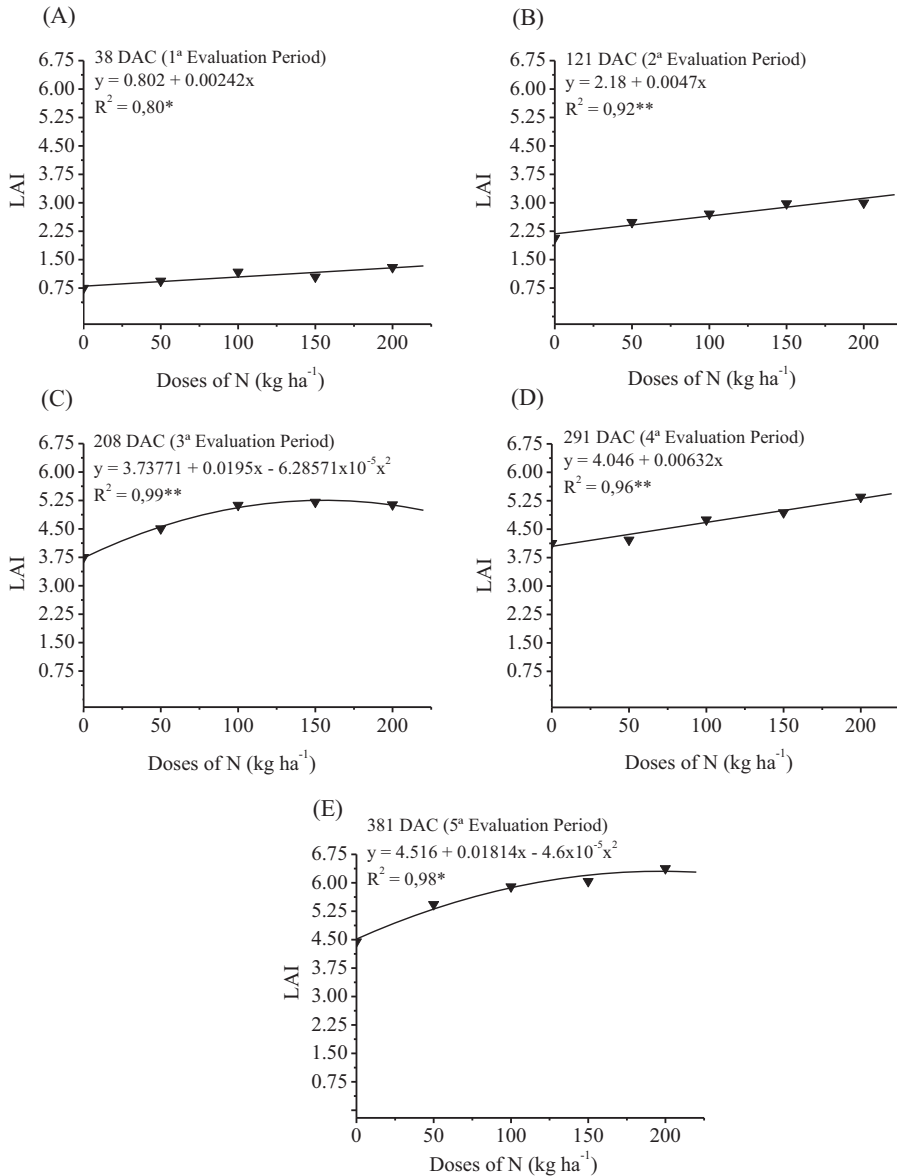
Figure 5. Mean values of leaf area index (LAI) for sugarcane cv. SP80-3280, obtained under doses of nitrogen (0, 50, 100, 150, and 200 kg N ha^{-1}) applied via subsurface drip fertigation in five evaluation periods (38, 121, 208, 291, and 381 DAC), represented in graphs A, B, C, D, and E, respectively. Jaú, SP, Brazil. 2009–2010.

nitrogen in irrigated sugarcane ratoon can be higher than the currently recommended dose for dry farming management, which varies between 100 and 120 kg N ha^{-1} for productivities expected to be above 100 t stalks ha^{-1} (Van Raij et al., 1997).

Sugar productivity (TPH) increased from 10 to 18 t ha^{-1} under the 150 and 200 kg N ha^{-1} doses (Figure 7). Muchow et al. (1996) also reported an increase in TPH in sugarcane under growing doses of nitrogen. Wiedenfeld (2000) obtained a 10 t ha^{-1} TPH of second ratoon sugarcane fertigated with 140 kg N ha^{-1}.

The adequate management of dripping irrigation can increase the water and nutrient use efficiency, such as nitrogen, leading to an increase in stalk and sugar productivity (Ng Kee Kwong et al., 1999).

The response potential to nitrogen fertilization in ratoon cane has proven to be superior, more frequent, and requiring higher doses of nitrogen compared to plant cane. The nitrogen doses currently recommended underestimates the potential of economic response to nitrogen in sugarcane, whose
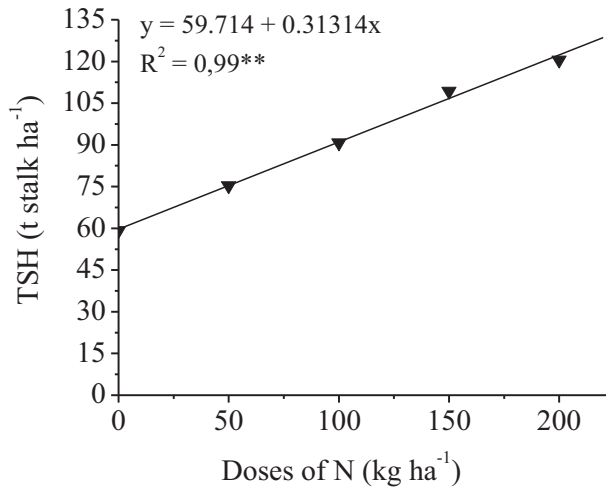
$y = 59.714 + 0.31314x$
$R^2 = 0,99**$

Figure 6. Stalk productivity (TSH), t stalk ha^{-1}, mean values for sugarcane cv. SP80-3280, obtained under doses of nitrogen (0, 50, 100, 150, and 200 kg N ha^{-1}) applied via drip fertigation subsurface, to 381 DAC. Jaú, SP, Brazil, 2009–2010.

$y = 10.2592 + 0.04804x$
$R^2 = 0,91**$

Figure 7. Sugar productivity (TPH), t pol ha^{-1}, mean values for sugarcane cv. SP80-3280, obtained under doses of nitrogen (0, 50, 100, 150, and 200 kg N ha^{-1}) applied via drip fertigation subsurface, to 381 DAC. Jaú, SP, Brazil, 2009–2010.
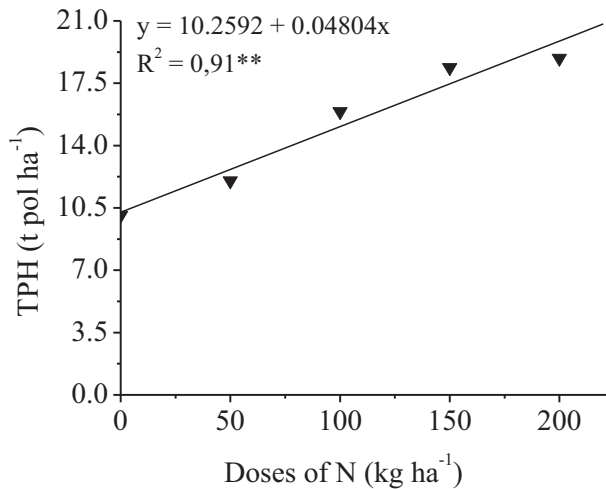
genetic material is more productive, grown in environments of higher production potential and with high amounts of waste (straw) with high ratio C:N (Vitti et al., 2010), as verified in the present study. Additionally, the application of N-fertilizers in ratoons is necessary and presents great variations, depending on management and type of soil (Gava et al., 2001). Thereby, fertigation with nitrogen has become relevant and compensatory for obtaining gains in productivity.

Conclusions

The physiological attributes SPAD index, chlorophyll and carotenoids contents, maximum photochemical efficiency of photosystem II (F_V/F_M), and leaf area index increase with the application of increasing doses of nitrogen via subsurface drip fertigation, but the best results are obtained in doses of 100 and 150 kg N ha^{-1} and in the period of great plant growth.

It was also concluded that the stalk and sugar productivity increases considerably with the increase of nitrogen doses applied to sugarcane via subsurface drip fertigation.

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