AUSTRALIAN JOURNAL OF CROP SCIENCE | SUBMITTED: 17-MAR-2017 | REVISED: 01-JUN-2017 | ACCEPTED: 07-JUN-2017

Australian Journal of Crop Science AJCS

AJCS 11(08):1051-1057 (2017) ISSN:1835-2707
doi: 10.21475/ajcs.17.11.08.pne621

Stalk yield and nitrogen (^{15}N) recovery of irrigated sugarcane during the plant-cane cycle using urea

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Abstract

Sugarcane is the most promising among crops that produce renewable biofuels. An adequate availability of water and nutrients, especially nitrogen (N), is of utmost importance. The objective of this study was to evaluate stalk yield and the recovery of N-urea by sugarcane (plant-cane) subjected to different levels of water replacements and nitrogen fertilization doses using the ^{15}N isotopic dilution technique. The experiment was conducted in the southwest region of the state of Goiás, Brazil, using plastic pots. The experimental design was complete randomized blocks analyzed in 3 × 3 split plots with three replications. The treatments consisted of three levels of water replacements (75, 50 and 25%) and three N doses (60, 120 and 180 kg ha-1) in the form of enriched ^{15}N urea. N doses did not affect stalk yield, probably because 91.14% of all N accumulated in culms came from the soil and other sources. The rainfall (1,194.2 mm) was sufficient to meet the water requirements of the crop. The recovery of N-urea was not influenced by the N doses and represented on average of 20.5% of the total amount applied. The soil and other sources were the main providers of N to sugarcane plants regardless of the mineral-N dose applied.

Key words: Cerrado, nitrogen use efficiency, dystrophic Red Latosol, Saccharum spp.

Introduction

Sugarcane (*Saccharum* spp.) is a culture of extreme socio-economic importance. It is considered as one of the main agricultural commodities grown in tropical and subtropical regions. It is mainly used for the production of sugar and biofuel. Brazil is the largest producer of this crop and its derivatives. According to the last survey conducted by the national supply company (April 2017), the estimated production of sugarcane in the 2016/2017 harvest is 657.18 million tons in an area of 9.05 million hectares, providing a yield of 72.62 t ha^{-1}. This value is below the productive potential of sugarcane crop. Such a low productivity is related to several factors, among them mineral nutrition and a poor distribution of rainfalls (Conab, 2017). These data justify studies that use tools such as irrigation and nitrogen fertilization because an adequate management of such tools promotes the productive potential of sugarcane.

Nitrogen (N) has been extensively studied on sugarcane crops because of the benefits and the different responses of this culture to N associated mainly to the management, cycle, variety and soil type. According to Franco et al. (2015), nitrogen fertilization is not focused during the plant-cane cycle because the response to this nutrient is more visible during the ratoon cane cycle. However, the yields of the subsequent ratoon cane and the longevity of the plantation may be significantly impaired when biological nitrogen fixation (BNF) and nutrient reserves in the soil are not sufficient to meet the needs of the crop during its first cycle.

With the increase in cultivation of mechanically harvested sugarcane, there is an increased volume of organic matter arising from straw (dry leaves, tops and stalk pieces). According to Leal et al. (2013), the deposition of this material over the soil is approximately 10-20 t ha^{-1}. This affects the dynamics of the N in the soil in different ways. One of the main benefits of this material is to provide N from an organic origin through mineralization. In view of the mineralization potential of the straw, there are more studies conducted on sugarcane grown in soils rich in organic matter because of the variations in the response of sugarcane to the fertilizers applied, mainly N, which is widely supplied by the uptake of organic matter from the soil. Despite the N from organic matter, the use of mineral-N is widespread in Brazil. The most used fertilizer is urea. However, from all nitrogen applied to sugarcane as fertilizer, only 20-40% are actually recovered by the crop, which negatively affects production costs and the environment (Mariano et al., 2012; Vieira Megda et al., 2015).

To achieve high productivities with low costs and few impacts to the environment, a perfect interaction is required among abiotic factors, management and the genetic potential of a variety. According to Rhein et al. (2016), among several abiotic factors, water deficiency must be highlighted because it is common in sugarcane plantations. Its adverse effects on plant development must be taken into account mainly because of the decrease in cell expansion. To meet the water requirements of sugarcane, irrigation is a fundamental practice, promoting many direct and indirect benefits for plants. However, when it comes to poorly managed irrigation and nitrogen fertilization, great potential losses occur, especially in nitrate leaching (Roberts, 2008).

Knowledge on the recovery of N or the use efficiency of nitrogen (from urea) by irrigated sugarcane may help to understand the dynamics of this nutrient in the soil-plant system. In this study we consider the hypothesis that nitrogen doses are associated with soil water volume which affect the productivity and the amount of N absorbed by sugarcane plants. Therefore, this study aimed to evaluate the stalk yield and the recovery of urea-N by shoots (tops and stalks) of sugarcane (plant-cane) subjected to different levels of water replacements and nitrogen fertilization doses using the ^{15}N isotope dilution technique.

Results and Discussion

Dry matter of crop residues and nitrogen recovery

Table 2 shows the variables related to sugarcane crop residues (R) during the plant-cane cycle. By analyzing the individual effects of the factors, we noted that water replacement levels did not influence any of the variables. Nitrogen doses affected the amount of dry matter of crop residues (DMR), percentage of crop residues, nitrogen from the fertilizer (%NffR) and amount of nitrogen from crop residues in the soil and other sources (ANsosR). The interaction between these factors did not influence any variable.

Concerning DMR, the 120 kg ha^{-1} ND was relevant because it was statistically equal to the 180 kg ha^{-1} ND and different from the 60 kg ha^{-1} ND. This evidenced that to achieve high dry matter yield of plant residues under these conditions, the best dose which aims a low N fertilizer expenditure and a high yield is the average dose (120 kg ha^{-1}). However, from a commercial point of view, the use of high doses of N to increase the DMR does not have great benefits since the plant part of interest is the stalk (energy drain) and not the leaf (energy source).

The 120 and 180 kg ha^{-1} N doses provided a high volume of N from the fertilizer accumulated in R. Regarding the percentage of fertilizer nitrogen, the 180 kg ha^{-1} ND stood out as presenting the greatest accumulation of N-urea. This indicates that, by increasing the amount of nitrogen applied to the soil, crop residues concentrated more N on the dry matter. Upon analyzing the percentage of N that crop residues offered to the total N accumulated in the shoots of sugarcane (NR/NSh), there was a great demand for N: approximately 50% of N accumulated in the tissues (Table 2). This information is of great value for the replacement of the N stock in the soil in relation to the organic matter from crops without burning. Therefore, a 50% of the N accumulated in sugarcane shoots can be recycled by mineralization of crop residues.

The recovery of nitrogen from urea by crop residues (RNUR) was not influenced by any factor, corroborating the study conducted by Franco et al. (2015). The authors, studying the recovery of nitrogen urea applied to sugarcane in three different cities in the state of São Paulo, reported that in two cities, the recovery of N-urea was not influenced by the increase of nitrogen levels during plant-cane. The values found by the authors and their collaborators for the recovery of N-urea by crop residues ranged from 7.3 to 14.3%. Such values are very close to those found in this study, with average of 9.06%.

Stalk yield, dry matter of crop residues and nitrogen recovery

Table 3 shows the variables related to sugarcane stalks (S). The results obtained were similar to those obtained for crop residues. For water replacement levels and the interaction among factors, there were no differences since N doses only influenced the variables such as ANffS and %NffS.

After analyzing stalks (S), it was observed that the highest accumulation of N-fertilizer was occurred when the amount of applied N increased. This is due to the fact that the highest value was achieved for both ANffS and %NffS by applying the ND 180 kg ha^{-1}, followed by 120 and 60 kg ha^{-1} (Table 3).

As previously mentioned, of all the N accumulated by sugarcane shoots, approximately 50% were allocated to crop residues and 50% were allocated to stalks (Table 3). Vieira-Megda et al. (2015), studied the N extracted by sugarcane. They found an average value for nutrient accumulation in roots of 11.7% in relation to the total N extracted by the plant. Thus, considering values close to those, it can be assumed that approximately 45% of all N extracted by sugarcane exists in stalks as drain as the same percentage was concentrated in crop residues.

Under such conditions, i.e., an organic matter content in the soil around 5% and a good water availability, the plant-cane managed to accumulate average of 327.83 kg ha^{-1} of N in its shoots. Of this value, 163.91 kg ha^{-1} were allocated by stalks and that same amount could potentially return to the soil through crop residues that remained on the soil during green sugarcane crop cycles. However, it is relevant to note that the straw of sugarcane has a high C/N ratio, approximately 100:1. Thus, the mineralization process by chemical organotrophic microbiota (heterotrophic) can be slow. However, the N contained in crop residues is of great importance for the formation of soil organic matter, which is the main component for the maintenance of a long-term potential productive land.

Stalk yield was not affected by the different nitrogen fertilization doses and by water replacement levels. This corroborates authors who reported a lack of response of plant-cane to the application of N-fertilizer, such as Araújo et al. (2001) and Franco et al. (2015). The high content of organic matter incorporated into the soil during the subsequent cycles, the great vigor of roots associated with high absorptions of native soil N and the BNF are the most common factors that explain the absence of responses of plant-cane to N-fertilizer. This justifies the fact that why there is no significant yield increase of cane-plant stems due to nitrogen fertilization. Because even with an average recovery of 11.44% of N from urea, the soil with good organic matter content and other sources, were responsible for providing 91.14 % of the entire N accumulated in the stems. As for water replacement levels, the results showed that climatic conditions, particularly rainfalls (Fig 1), were sufficient to express the genetic yield potential of the variety under study, since the distribution of rainfalls was favorable for crop development.

Dry matter of shoots and nitrogen recovery

Regarding the effects of factors on plant-cane shoots, there were significant differences regarding nitrogen doses. This

Table 1. Initial chemical characterization of substrate (soil + bovine manure) used in this experiment.

pH	M. O.	P	Ca	Mg	K	S	Al	H + Al
$CaCl_2$	g dm^{-3}	mg dm^{-3}	-------- $mmol_c$ dm^{-3} --------			mg dm^{-3}	$mmol_c$ dm^{-3}	
6.9	51	305	139	33	14.5	70	<1	12
BS	CTC	Cu	Fe	Mn	Zn	B	m	V
$mmol_c$ dm^{-3}		---------------------- mg dm^{-3} ----------------------					--------- % ----------	
186.9	198.8	4.8	38	8.6	9.8	0.33	0	94

Ca – calcium; Mg – magnesium; K – potassium; Al – aluminum; H – hydrogen; P – phosphorus; S – sulfur; Cu – copper; Fe – iron; Mn – manganese; Zn – zinc; B – boron; pH – hydrogen potential; M. O. – organic matter; CEC – cation exchange capacity; BS – base sum; m – aluminium saturation; V – base saturation.

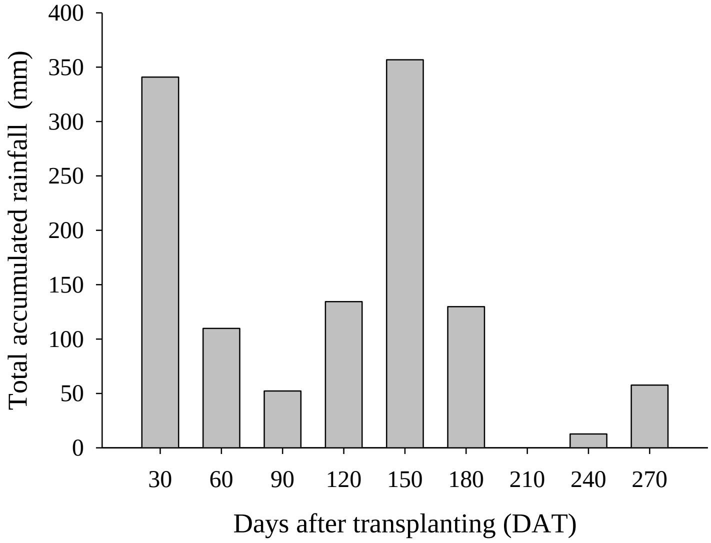

Fig 1. Accumulated rainfall during the plant-cane cycle, in function of days after transplanting (October 2013 to August 2014). Source: INMET (UNIRV Meteorological Station - University of Rio Verde).

Table 2. Summary of the analysis of variance for dry matter (DMR), accumulated nitrogen (ANR), percentage of nitrogen derived from the fertilizer (%NffR), amount of nitrogen derived from fertilizer (ANffR), recovery of nitrogen from urea (RNUR), amount of nitrogen from the soil and other sources (ANsosR) and relation NR/NSh of crop residues sugarcane (cane-plant) subjected to different levels of water replacements (WR) and nitrogen doses (ND).

Variation source	DF	Mean square						
		DMR	ANR	%NfffR	ANffR	ANsosR	RNUR	NR/NSh
WR	2	10.90^{ns}	203.33^{ns}	0.02^{ns}	5.54^{ns}	167.31^{ns}	0.70^{ns}	60.10^{ns}
Block	2	15.09^{ns}	2318.17^{ns}	0.01^{ns}	6.55^{ns}	2079.53^{ns}	7.82^{ns}	31.23^{ns}
Residue (a)	4	16.01	605.85	2.59	3.94	563.82	1.23	27.08
ND	2	70.98^{**}	1377.52^{ns}	131.81^{**}	363.98^{**}	1355.93^{ns}	8.80^{ns}	7.90^{ns}
WR x ND	4	3.66^{ns}	799.48^{ns}	0.61^{ns}	6.63^{ns}	706.04^{ns}	5.89^{ns}	19.02^{ns}
Residue (b)	12	7.84	803.02	8.84	25.85	894.33	14.25	19.11
CV a (%)	-	17.68	14.67	23.77	17.69	15.17	12.27	10.14
CV b (%)	-	12.37	16.89	43.90	45.29	19.10	41.67	8.52
ND		t ha^{-1}	kg ha^{-1}	%	------- kg ha^{-1} -------		------- % -------	
60		19.87 b	157.87 a	03.05 b	04.75 b	153.12 a	7.92 a	51.55 a
120		25.48 a	181.65 a	06.56 b	11.46 a	170.19 a	9.55 a	50.27 a
180		22.57 ab	163.83 a	10.70 a	17.46 a	146.37 a	9.70 a	52.09 a
General means		22.64	167.79	6.75	11.22	156.56	9.06	51.30
LSD		3.52	35.66	3.74	6.4	37.63	4.75	5.50

**Significant among themselves the 0.01 probability by F test; *Significant 0.05 probability by F test; nsnot significant to 0.05 probability by F test; means followed by the same letter in the columns do not differ statistically at 0.05 probability by Tukey's test; DF, degrees of freedom); LSD, least significant difference.

Table 3. Summary of the analysis of variance for stalk yield (SY), dry matter (DMS), accumulated nitrogen (ANS), percentage of nitrogen derived from the fertilizer (%NffC), amount of nitrogen derived from fertilizer (ANffS), recovery of nitrogen from urea (RNUS), amount of nitrogen from the soil and other sources (ANsosS) and relation NS/NSh of stalks sugarcane (cane-plant) subjected to different levels of water replacements (WR) and nitrogen doses (ND).

Variation source	DF	Mean square							
		SY	DMS	ANS	%NffS	ANffS	ANsosS	RNUS	NS/NSh
WR	2	2142.68^{ns}	50.16^{ns}	1931.82^{ns}	3.77^{ns}	0.07^{ns}	1908.79^{ns}	0.58^{ns}	60.10^{ns}
Block	2	570.83^{ns}	13.66^{ns}	259.86^{ns}	1.74^{ns}	7.52^{ns}	197.31^{ns}	2.30^{ns}	31.23^{ns}
Residue (a)	4	664.05	82.14	1429.24	4.35	12.64	1272.68	10.84	27.08
ND	2	1205.70^{ns}	161.78^{ns}	2889.56^{ns}	303.73**	632.33**	3800.80^{ns}	12.53^{ns}	7.90^{ns}
WR x ND	4	1296.03^{ns}	44.87^{ns}	213.82^{ns}	2.06^{ns}	2.53	229.32^{ns}	2.11^{ns}	19.02^{ns}
Residue (b)	12	777.41	71.28	1218.32	5.17	8.01	1285.49	3.66	19.11
CV a (%)	-	16.59	20.43	23.62	22.75	25.07	24.46	28.78	10.69
CV b (%)	-	17.95	19.03	21.81	24.80	19.96	24.58	16.73	8.98
ND		------ t ha^{-1} ------		kg ha^{-1}	%	------ kg ha^{-1} ------		------ % ------	
0		145.67 a	-	-	-	-	-	-	-
60		150.94 a	42.60 a	149.73 a	04.26 c	06.32 c	143.45 a	10.53 a	48.44 a
120		171.43 a	49.21 a	180.73 a	07.66 b	13.22 b	167.50 a	11.02 a	49.73 a
180		153.30 a	41.31 a	149.61 a	15.59 a	23.00 a	126.61 a	12.78 a	47.90 a
General means		155.33	44.37	160.04	9.17	14.18	145.86	11.44	48.70
LSD		33.16	10.62	43.92	2.86	3.56	45.11	2.41	5.5

**Significant among themselves the 0.01 probability by F test; *Significant 0.05 probability by F test; nsnot significant to 0.05 probability by F test; means followed by the same letter in the columns do not differ statistically at 0.05 probability by Tukey's test; DF, degrees of freedom); LSD, least significant difference.

Table 4. Summary of the analysis of variance for dry matter (DMSh), accumulated nitrogen (ANSh), percentage of nitrogen derived from the fertilizer (%NffSh), amount of nitrogen derived from fertilizer (ANffSh), recovery of nitrogen from urea (RNUSh), amount of nitrogen from the soil and other sources (ANsosSh) of shoots sugarcane (cane-plant) subjected to different levels of water replacements (WR) and nitrogen doses (ND)

Variation source	DF	Mean square				
		DMSh	ANSh	ANsosSh	ANffSh	RNUSh
WR	2	54.07^{ns}	1868.54^{ns}	1996.25^{ns}	4.48^{ns}	0.33^{ns}
Block	2	21.58^{ns}	3859.33^{ns}	3244.84^{ns}	28.10^{ns}	18.58^{ns}
Residue (a)	4	145.04	3164.67	2955.93	9.15	8.33
ND	2	402.89^{ns}	8135.34^{ns}	9649.79^{ns}	1947.84**	36.53^{ns}
WR x ND	4	51.78^{ns}	1343.64^{ns}	1343.47^{ns}	6.99^{ns}	7.63^{ns}
Residue (b)	12	107.41	3261.38	3649.12	48.05	24.58
CV a (%)	-	17.97	17.16	17.98	11.91	14.08
CV b (%)	-	15.47	17.42	19.98	27.28	24.19
ND		t ha^{-1}	------------------ kg ha^{-1} ----------------			%
60		62.46 a	307.64 a	296.58 a	11.07 c	18.45
120		74.70 a	362.40 a	337.68 a	24.69 b	20.57
180		63.88 a	313.45 a	292.98 a	40.46 a	22.48
General means		67.01	327.83	302.41	25.41	20.50
DMS		13.04	43.92	76.00	8.72	6.24

**Significant among themselves the 0.01 probability by F test; *Significant 0.05 probability by F test; nsnot significant to 0.05 probability by F test; means followed by the same letter in the columns do not differ statistically at 0.05 probability by Tukey's test; DF, degrees of freedom); LSD, least significant difference.

influenced the amount of N from fertilizer and followed the results found for crop residues and stalks (Table 4).

Although the plant accumulates more N from fertilizer as the dose of nutrient increased, there were no effects on the total accumulated N and on the production of dry matter, possibly because the stock of soil N and N from other sources was sufficient to meet the needs of the crop. According to Urquiaga et al. (2012), the contribution of BNF to sugarcane is on average 40 kg ha^{-1} year^{-1}. Assuming this value and the values found for N from fertilizer, the contribution of the native N in the soil was approximately 251.34 kg ha^{-1}, 237.72 kg ha^{-1} and 221.95 kg ha^{-1} for the doses 60, 120 and 180 kg ha^{-1}, respectively. Considering such accumulation of all N in shoots, 13.23% were from BNF, 78.37% were from the soil and 8.4% were from the fertilizer applied. This estimate is supported by Sousa and Lobato (2004), who reported that a soil with 5% of organic matter in the Cerrado region can potentially mineralize approximately 150-250 kg ha^{-1} of N. According to Franco et al. (2011), the soil N is efficiently used by sugarcane because of certain characteristics of this species such as the long time it remains in the field and the extensive root system. There are several studies using fertilizers marked with ^{15}N evidencing that most of the N absorbed by sugarcane comes from the soil, since the contribution of nitrogen fertilizers is low compared to the total N accumulated in plants.

Considering the lack of response to N doses regarding the yield of DMR and DMS, even with an increase in the N-accumulated in plant tissue, we infer that with the increase in N-fertilizer in plant-cane, the crop absorbs less N from other sources for a short time period, possibly because the N-fertilizer is widely available and easy to access compared with the N fixed from the atmosphere. However, with the growth and development of the crop, the BNF and the absorption of N native from the soil resume to meet the needs of the plant. This assumption is reaffirmed by the fact that there was an increase in DMR according to N doses (Table 2). This implies a high N-fertilizer absorption at the beginning of the sugarcane cycle, when there is a predominance of leaves and the N-fertilizer is available to be absorbed and assimilated by crop residues. The availability of N-fertilizer decreases over time through losses and immobilization by microorganisms, which then contributes for further absorptions of N native in the soil and BNF throughout the cycle. So, that such sources will be largely responsible for the nutritional maintenance of stalks. The lowest %NffR, compared to %NffS, does not disqualify this hypothesis because the dry matter of crop residues concentrated more than twice the N of stalks.

The recovery of N-urea by the shoots of plant-cane (stalk + leaves and tops) was 20.50% (Table 4). This value is considered low when compared with the literature mentioning an average recovery of 20-40% by sugarcane. However, the recovery is variable depending on the cycle. Thus, many studies generalize the recovery of N-urea for cultivation of sugarcane due to a non-discrimination of crop cycles. The recovery value of 20.50% looks significant when compared to other plant-cane studies such as Franco et al. (2011) and Vieira-Megda et al. (2015), who found a recovery of up to 10 and 13.6%, respectively, for sugarcane crops during the first cycle. Such a high recovery is supposedly due to good local weather conditions, especially related to water availability and irrigation. According to Malavolta (2006), in irrigated crops, the losses of N by volatilization are practically null if the irrigation is performed after the application of urea.

Overall, the low recovery of N-urea, mainly by the different soil inputs and outputs of the nutrient, is due to nitrogen (N) mineralized through straw (organic matter), high rate of immobilization by microorganisms and losses in the system, especially when sources containing urea are applied to the soil surface. According to Sousa and Lobato (2004), a great water percolation in the soil profile associated with a low natural CEC of Red Latosols may cause the nutrient leaching, such as N as nitrate (most prevalent inorganic form of soil N). Based on experimental data, a nitrate leaching index between 1 and 1.5 mm is estimated per mm of water applied to clay soils (Suhet et al., 1986).

This study corroborates with Franco et al. (2015) when authors reported a significant difference of recovery by plant-cane shoots after applying N doses to three regions in the state of São Paulo. They also reported no huge differences of recovery, ranging from 19.5 to 24.7%, which was very close to the results obtained in this study (20.5%). This further supports the rationale that the environment is the main factor influencing the recovery of N-urea or the use efficiency of urea nitrogen. It is noteworthy that the values of recovery of N from urea obtained in this study does not consider the N exist in the root system, therefore representing an underestimation of the total recovered N.

Materials and methods

Location, soil conditions and climate

The experiment was conducted in the southwest region of the state of Goiás, city of Rio Verde, from October 2013 to August 2014. Plastic pots with a 0.6 m diameter × 0.46 m lower diameter × 0.5 m height were used. They were filled with crushed stone no. 2 (0.05 meters at the bottom of the pots) and soil mixed with cattle manure above the crushed stone layer, forming a layer of 0.45 m deep. The pots were placed in the open space at the experimental area of Goiano Federal Institute, Rio Verde campus, GO, located at 17°48'28" S and 50°53'57" W, at an average altitude of 720 meters. The climate is classified (Köppen) as Aw (tropical), with rainfalls from October to May and a drought period from June to September. The average annual temperature ranges from 20 to 35°C, and rainfalls vary from 1,500 to 1,800 mm annually. Data for total accumulated rainfall during the plant-cane cycle are shown in Figure 1. The accumulated rainfall was 1,194.2 mm. The experiment consisted of a dystrophic Red Latosol, Cerrado phase (Santos et al., 2013), mixed with cattle manure at a ratio of 3:1 v/v, respectively. The chemical characteristics of the substrate (soil + manure) in the pots are shown in Table 1.

Plant material

The planting of the sugarcane was performed in tubes containing vermiculite as substrate. The method used for crop establishment was pre-sprouted seedlings (PSS). The transplanting of seedlings to pots was therefore necessary. The transplantation was performed 15 days after sowing. The variety IACSP95-5000 was used. Its characteristics are a very high agricultural production, rusticity and precocity, indicated for favorable environments (A1 - C2), upright position, optimal shooting from ratoons, good tillering, interrow closing, resistance to major diseases and no tipping and flowering.

Experimental design and characterization of treatments

The experimental design was complete randomized blocks with three replications, analyzed in 3 × 3 split plots. The treatments consisted of a combination of three levels of water replacements (75, 50 and 25% of the field capacity) and three doses of nitrogen enriched with ^{15}N (60, 120 and 180 kg ha^{-1}, equivalent for pots, represented by ND60, ND120 and ND180, respectively) in the form of urea enriched with ^{15}N. The area of the pots was used to calculate the applied doses. The plots represented the water replacement levels (WR) and the subplots represented nitrogen doses (ND). For productive variables, a ND0 was added (no N application), becoming 3 × 4 split plots.

Fertilizations

All fertilizations were performed manually with N (urea), divided into one planting fertilization and three cover fertilizations performed at 45, 60 and 90 days after transplanting (DAT). The urea used in fertilizations had an abundance of 2.00% of the isotope ^{15}N. From this value, the natural abundance of ^{15}N was subtracted. The macronutrients potassium (K_2O) as potassium chloride, and phosphorus (P_2O_5) as triple superphosphate were applied equally at doses of 150 and 100 kg ha^{-1}, respectively. There was no need to perform any application of micronutrients via fertilization, following the recommendations described for the crop (Sousa and Lobato, 2004).

Water replacement

Irrigation was managed using the lysimeter drainage method. Four pots were used for each replication, resulting in twelve reference pots (lysimeters). In these pots, the crop was also implanted. The calculated field capacity (FC) was 25 L. Every two days (irrigation interval), this volume of water was applied to the lysimeters and after collecting the drained volume of the pots, the data were processed using Equation 1.

$$FC = AW - DW \quad (1)$$

Where:
FC = Field capacity;
AW = volume of water applied;
DW = volume of drained water.

The volume of water applied to the pots was always proportional to the volume sufficient to raise the moisture of the respective treatments to 75, 50 and 25% of the field capacity. At the end of the experiment, the net water blade applied to the soil (by irrigation) was recorded during the experimental period. The water depths for the WRs 75, 50 and 25% were 486.75 mm, 162.25 mm and 324.5 mm, respectively. Irrigation was performed using drippers with a turbulent flow at a flow rate of 1.65 L h^{-1} and a service pressure of 1.0 bar.

Variables analyzed

Throughout the experiment, all dry leaves were collected. At harvest, the fresh mass of plant shoots and stalk yield were determined (kg pot^{-1}). Subsequently, samples of stalks and crop residues were collected and dried in a forced-air ventilation oven at 65°C until constant mass to determine the dry matter. To avoid overestimation of stalk yield and dry matter (DM) due to the small area of the pot, the area occupied by the plant canopy and the spacing between each plot was taken into account, obtaining an area of 1,595 m^2. The area, DM and stalk yield (SY) (in kg pot^{-1}) were taken into account for the extrapolation of t ha^{-1} of stalk yield and dry matter. The plant material was then ground using a Wiley mill, identified, hermetically sealed and sent to the Stable Isotope Laboratory of CENA/USP to determine the N concentration in plant material (g kg^{-1}) and the percentage atoms of ^{15}N in excess in the plant (%) using a mass spectrometer (IRMS) coupled to a 20-20 ANCA-SL automatic analyzer (Europe Scientific, Crewe), according to the methodology described by Barrie and Prosser (1996).

The following variables were also determined: dry matter (DM), accumulated nitrogen (AN), percentage of nitrogen derived from the fertilizer (%Nff), amount of nitrogen derived from fertilizer (ANff), recovery of nitrogen from urea (RNU), amount of nitrogen from the soil and other sources (ANsos), and ratio between total accumulated nitrogen in crop residues and stalks compared to the total N accumulated in plant shoots (NR/NSh, NS/NSh). All variables were determined for crop residues (straw + tops), stalks and plant shoots (stalk + crop residues). The yield was determined only for stalks (SY).

The recovery of N by plant from urea, the amount of N accumulation and the amount of N from other sources were calculated using the following sequence of equations:

a) Accumulated nitrogen by the plant (AN, kg ha^{-1})

$$AN = N \times MS \quad (2)$$

Where:
N = Nitrogen concetration in the plant (g kg^{-1});
DM = Dry matter (t ha^{-1}).

b) Percentage of nitrogen derived from the fertilizer (%Nff)

$$\%Nff = \frac{\%\text{Atoms of } ^{15}\text{N in excess in the plant}}{\%\text{Atoms of } ^{15}\text{N in excess in the fertilizer}} \text{ x } 100 \quad (3)$$

Where:
%Atoms of ^{15}N in excess in the plant = %Atoms of ^{15}N in excess in the plant minus the natural concentration of ^{15}N (0.3663%);
%Atoms of ^{15}N in excess in the fertilizer = %Atoms of ^{15}N in excess in the plant minus the natural concentration ^{15}N (0.3663%).

c) Amount of nitrogen derived from fertilizer (ANff, kg ha^{-1})

$$ApNff = \frac{\%Nff \times AN}{100} \quad (4)$$

Where:
AN = accumulated nitrogen by plant (kg ha^{-1});
%Nff = percentage of nitrogen derived from the fertilizer.

d) Recovery of nitrogen from urea (RNU, %)

$$RNU = \frac{ANff}{ANA} \times 100 \quad (5)$$

Where:
ANff = amount of nitrogen derived from fertilizer (kg ha^{-1});
ANA = amount nitrogen applied in the form of marked fertilizer (kg ha^{-1}).

e) Amount of nitrogen from the soil and other sources (ANsos, kg ha^{-1})

$$ANsos = AN - ANff \quad (7)$$

Where:
ANff = amount of nitrogen derived from fertilizer (kg ha^{-1});

To calculate the total accumulated nitrogen in crop residues compared to the total N accumulated in plant shoots (NR/NSh, %), and the total N accumulated in stalks compared to the total N accumulated in plant shoots (NS/Nsh, %), equations 8 and 9 were used, respectively.

Statistical analyses

Data were subjected to analysis of variance and when the F test was significant, a comparison of means by Tukey test at 0.05 probability was performed for all factors. The statistical software SISVAR-ESAL® and SigmaPlot® 11 (Systat Software Inc.) were used.

Conclusion

Nitrogen doses influenced only the dry matter of crop residues and the amount and the percentage of N in the plant. Water replacement levels did not affect stalk yield and crop residues (leaves and tops), nor the recovery of N from urea.
The recovery of nitrogen from urea was not influenced by the different doses applied and represented on average 20.5% of the amount applied. The soil and other sources were the main providers of N for sugarcane regardless of the N-mineral dose applied.

Acknowledgments

The authors would like to thank the Brazilian National Council for Scientific and Technological development (CNPq) for funding the current study, the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Goiano Federal Institute, Campus Rio Verde, GO, Brazil (IFgoiano).

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