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RESEARCH ARTICLE

Sugarcane yield loss due to water and nitrogen deficiencies evaluated by carbon isotopic discrimination method

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ABSTRACT

Water and nitrogen (N) availability are determining factors for crop development and production. Assessments of sugarcane yield loss based on these factors may become more accurate by using the stable carbon (C) isotope technique. The aim of this work was to evaluate the effect of N and water on sugarcane yield, isolating the yield losses (YL) caused by N or by water limitations, and correlating them with the fractionation of C isotopes ($\Delta^{13}C$). The research was carried out in field conditions in the municipality of Jaú, Brazil. A statistical design in randomized block was used, considering the 2 × 2 × 2 factorial arrangement, composed of two cycles [first (2008/09) and second (2009/10)], two N rates (without and with N) and two water supplies (rainfed and irrigated). These variables were evaluated: biometric features (plant height, stalk diameter and tillering), yield elements [stalk yield (STY), sucrose yield (SUY), and stalk dry matter (SDM)], technological quality parameters [fiber content, sucrose juice content (SJC), and total sucrose recovery (TSR)], stalk N concentration (SNC) and accumulation (SNA), and $\Delta^{13}C$. The effect of irrigation associated with N fertilization on STY, SUY, and SDM, resulted in average increases of 53.7, 9.0, and 18.6 Mg ha^{-1}, respectively. The average YL for STY in two crop seasons were 40.5%, 35.2%, and 48.2% due to limitations of N or water or N + water, respectively. The SNC was not affected by the water supply, but it was increased by N fertilization. The highest value of $\Delta^{13}C$ was obtained for the treatment without N fertilization in rainfed conditions, and the least $\Delta^{13}C$ was found for the one with N Fertilization and irrigation. The $\Delta^{13}C$ measurement was effective in identifying N and water deficiencies, presenting potential to be used as an indicator of N and water limitations for sugarcane yield.

Keywords: Loss index of stalk biomass; *Saccharum* ssp.; Water deficit; Water-N interaction; Water supply

INTRODUCTION

Sugarcane (*Saccharum* spp.) is an important crop in tropical and subtropical regions, used for sugar and ethanol production. In addition, it generates byproducts derived from its processing, such as straw and bagasse, which can be used for electricity cogeneration and to obtain second generation ethanol (2G ethanol), increasing its economic feasibility (Cardozo et al., 2013; Dias et al., 2013). Brazil is a major world producer of sugarcane, and its total production has increased in recent decades (Bordonal et al., 2018). However, Brazilian sugarcane yield is still considered low, with an average of 76.1 Mg ha^{-1} in the 2019/20 crop season (Conab, 2019), mainly due to water deficit and nutrient deficiency, especially Nitrogen (N), or the combined N and water deficit (Silva et al. 2020).

Water deficit is the single greatest abiotic stress affecting sugarcane development and productivity for all major sugarcane producing countries (Ferreira et al., 2017). The water deficit is one of the main agriculture problems, even in regions with high annual rainfall but with uneven distribution throughout the crop cycle, which has been intensified in crops under the influence of adverse climatic phenomena, such as El Niño (Inman-Bamber and Smith, 2005; Gava et al., 2011; Santos and Sentelhas, 2012).

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Climate scenario models have shown that reduced water availability will affect crop yield in several agricultural regions worldwide, especially when associated with rising temperatures (Kang et al., 2009).

Nitrogen is the most limiting nutrient for sugarcane yield, as it interferes with sugarcane tillering, growth, and development (Boschiero et al., 2020). Usually, N is frequently applied in responsive sites because the contribution of residual N fertilizer to subsequent crops is very low (<6% of the applied N; Smith and Chalk, 2018). Thus, the N rates applied to sugarcane ratoons are similar to those at planting, that is., 120 to 200 kg N ha^{-1} (Cantarella and Rossetto, 2014). In this context, the demand of N fertilization for sugarcane production has been highly debatable given the lack of a consensus about the precise N rate to reach the highest stalk and sugar yields (Castro et al., 2019). This is explained due to the complex dynamic of N in the soil-plant-atmosphere system, which is highly influenced by environmental conditions, mainly water availability, temperature and type of soil, among other factors.

Under rainfed conditions, the responsiveness of sugarcane to N fertilization is variable and climate-dependent (Vitti et al., 2007; Franco et al., 2010). However, irrigated sugarcane has greater stability and yield in response to N addition, with several studies confirming this positive effect of water on N uptake (Singh and Mohan, 1994; NG Kee Kwong et al., 1999; Thorburn et al., 2003; Wiedenfeld and Enciso, 2008; Kolln et. al. 2016; Mendoça et al. 2020).

According to Caemmerer et al. (2014), photosynthetic carbon isotope discrimination is a non-destructive tool for investigating C_4 metabolism. New tecnologies can used to mensure carbon isotope discrimination and CO_2 assimilation over a range of environmental conditions. Laser absorption spectroscopy provides new opportunities for making rapid, and concurrent measurements. Biomass production by plants with C_4 photosynthetic metabolism is severely affected by water and N limitations, especially when they occur simultaneously (Ranjith et al., 1995; Saliendra et al., 1996; Fravolini et al., 2002; Clay et al., 2005). According to Ranjith et al. (1995) and Mienzer and Zhu (1998), this can be explained by the high positive correlation of water and N availability with the activity of the enzymes Rubisco and PEP carboxylase, which directly influences the rate of carbon (C) fixation (liquid photosynthesis). On the other hand, these authors found a negative correlation between the availability of water and N with C carbon isotopic discrimination ($\Delta^{13}C$). In this context, the stratification of sugarcane yield losses by water deficit or by N deficiency, as well as by the simultaneous action of these two growth factors, could be possible through the correlation between $\Delta^{13}C$ and dry biomass production, considering their evaluation on environments with or without N and/or water limitations (Fravolini et al., 2002; Clay et al., 2005). We hypothesized that $\Delta^{13}C$ can identify, separate and quantify sugarcane yield losses due to water deficit and N deficiency.

The aim of this work was to evaluate by carbon isotopic discrimination the effect of N and water on sugarcane yield, isolating the yield losses caused by N deficiency or by water deficit, and correlating them with the Δ13C.

MATERIALS AND METHODS

Site description

The research was carried out in field conditions at the APTA West Center unit (22°17'S, 48°34'W; altitude 580 m), located in Jaú, São Paulo State, Brazil, during the second (2008/09) and third (2009/10) ratoons of the sugarcane genotype SP80-3280. The landscape is smooth-wavy, and the experimental area had been under continuous sugarcane production for at least 10 years prior to experiment establishment. The soil at the experimental field, according to the USDA Soil Taxonomy (Soil Survey Staff, 2010) is a Typic Hapludox (that is, *Latossolo Vermelho*, in the Brazilian Soil Classification System; Santos et al., 2013). The main physical and chemical soil properties are presented in Table 1.

The predominant climate in the region, according to the Köppen climate classification (Köppen, 1931), is humid tropical (Aw), with dry winter, warmer and rainy summer, mean annual temperature of 22.7 °C, and average annual rainfall of 1,344 mm.

Experimental design and treatments

A statistical design in randomized block (with four replicates) was used, considering the 2 × 2 × 2 factorial arrangement, composed of two cycles [first (2008/09) and second (2009/10)], two N rates (without and with N) and two water supplies (rainfed and irrigated). The first cycle was from September 01, 2008 to September 19, 2009 (367 days), and the second cycle was from September 20, 2009 to October 18, 2010 (394 days). The treatments with N received 150 and 140 kg ha^{-1} N in the first and second cycles, respectively. The N rate in the second cycle was smaller than in the first one because nutrient requirements (and yield) decrease as the ratoons age (Fig. 1).

The experimental unit consisted of five 30-m rows of sugarcane (270 m^2). The paired-double row planting arrangement was used, with a 1.80-m spacing between double rows and 0.4 m between paired sugarcane rows,

Table 1: Chemical and physical properties of the soil from the experimental area

Soil layer (m)	pH[a]	TOC (g dm^{-3})	N[b] (mg kg^{-1})	P (mg dm^{-3})	K	Ca	Mg	CEC	V (%)	Sand	Silt	Clay
					----- (mmol$_c$ dm^{-3}) ------					--------- (g kg^{-1}) --------		
0–0.25	5.2	9	6.0	17	1.7	15	7	70	56	660	70	270
0.25–0.50	4.8	7	6.6	20	1.2	9	4	32	44	560	100	320

[a]pH in 0.01 mol L^{-1} $CaCl_2$. [b]Inorganic nitrogen (NH_4+ + NO_3-). TOC, total organic carbon. CEC, cation exchange capacity. V, base saturation. Chemical and particle-size analysis according to Raij et al. (2001)

with a dripline (DRIPNET PC 22135 FL model; Adana, Turkey) installed between them for the irrigated treatments. The dripline had a 1.0 L h^{-1} flow rate and was equipped with drip nozzles every 0.5 m, which were buried at a depth of 0.25 m beneath the soil surface (Fig. 2).

In the irrigated treatments, water was supplied to replace 100% of the crop evapotranspiration (CET), according to the Penman–Monteith method (Howell and Evett, 2004). The frequency of irrigation was determined considering the available water capacity (AWC) of the soil of 70 mm, the water supplied by rainfall (R), and the atmospheric demand due to sugarcane CET. The soil moisture was periodically checked by means of four tensiometer sets installed in the experimental area at depths of 0.2, 0.4, and 0.6 m (Fig. 3).

In the irrigated treatments, N as urea was split-applied in small amounts (twice weekly) throughout the crop cycle, by underground drip irrigation. A percentage of the total N rate was added monthly to the sugarcane crop according to the phenological growth stage (Fig. 1). The N fertilization stopped around four months before harvesting, which corresponded to the sugarcane maturation stage. All treatments were fertilized with potassium (K), using 150 kg ha^{-1} K_2O as KCl, a rate that was split-applied by means of fertigation, throughout the crop cycle (Fig. 4). This K fertilization stopped three months before harvesting. In turn, in the rainfed treatments, the N and K fertilizations were performed 30 days after cut (DAC) of each previous ratoon, by placing the fertilizers in a furrow between the paired sugarcane rows 0.4 m. For application double disc fertilizer spreader were used burying 0.05 m of depth afterwards. KCl and Urea were used in this treatment.

Fig 1. Details of the different N treatments separated in 200 L gallons.

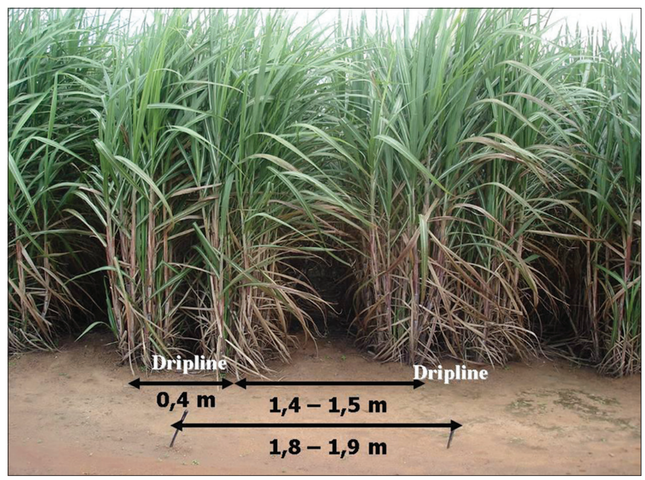

Fig 2. Detail of row spacing of sugarcane used and location of the drippers.

Biomass sampling and analysis

The evaluation of $\Delta^{13}C$ was performed in samples of leaf+1 (first leaf with visible dewlap; according to Meinzer and Zhu, 1998), prior to sugarcane harvesting (355 DAC in the first cycle, and 384 DAC in the second one). Leaf sampling was carried out in the morning, close to 9 am, and consisted of a sample composed of 15 blades of leaf+1 per plot. Then the central rib of the leaves was removed and discarded. For $\Delta^{13}C$ determination, only 20 cm of the middle region of the leaves was used. This fresh vegetable tissue was washed with potable water, dried in a forced-air-circulation oven at 65 °C until constant mass, ground to pass through a 0.5-mm sieve in a Wiley mill (model MA340, Marconi Laboratory Equipment Co., Piracicaba, Brazil), and stored in plastic bottles with a pressure cap.

The value of relative isotopic enrichment of carbon ($\delta^{13}C$)- from the samples of dried vegetable tissue-was obtained by an isotope ratio mass spectrometer (IRMS, model ANCA-GSL Hydra 20-20, SERCON Co., Crewe, GBR). The $^{13}C/^{12}C$ ratio in relation to the international standard Pee Dee Belemnite (PDB; Craig, 1957) was calculated using Equation 1 (Eq. 1); where: $\delta^{13}C$ = relative isotopic enrichment of the sample in relation to standard PDB (dimensionless); R = isotopic ratio $^{13}C/^{12}C$ of the sample and the standard (dimensionless) (Barrie and Prosser, 1996).

$$\delta^{13}C_p\left(\begin{matrix}\text{Sample,}\\ \text{PDB}\end{matrix}\right)=\left(\left(\frac{\text{R sample}}{\text{R Standard}}\right)-1\right)*1000 \quad (1)$$

To obtain the $\Delta^{13}C$ (‰), Equation 2 (Eq. 2) was used, as described by Farquhar (1983), Henderson et al. (1992) and Cernusak et al. (2013); where, $\delta^{13}C_a$ = reference atmospheric CO_2 isotopic composition ($\delta^{13}C_a$ of -8.0 ± 0.01 ‰) in relation to the PDB international standard; $\delta^{13}C_p$ = isotopic composition of the sugarcane leaf+1.

$$\Delta^{13}C = \frac{\delta^{13}C_a + \delta^{13}C_p}{1+\delta^{13}C_p} \quad (2)$$

Samplings of 2-m row of sugarcane per plot at the end of each cycle (harvesting time) were used to measure average plant height and stalk diameter, to count the number of tillers by linear meter, and to determine the aboveground plant biomass. The stalks were disassembled (leaves removed) and weighed. After that, the fresh material was ground in a forage chopper and subsamples were selected to determine moisture and dry biomass, after drying them in a forced-air-circulation oven at 65 °C until constant mass. On the same occasion, plants of five 8-m rows of sugarcane (located in the center of each experimental plot) were manually dehusked and weighed using a load-cell scale to determine fresh biomass, which was converted to dried biomass, according to the moisture previously determined. Fig. 5 Then, considering 5,556 linear meters of sugarcane per hectare, the average yield of stalk dry matter (SDM, Mg ha^{-1}) was estimated. At the same harvesting time, ten stalks were collected per plot to determine sugarcane technological quality attributes [fiber content, sucrose juice content (SJC), and total sucrose recovery (TSR)] using the procedures described by Fernandes (2003). Finally, sucrose yield (SUY, Mg ha^{-1}) was obtained through the product of SDM and the corresponding value of TSR of each plot.

Fig 3. The weather station used for monitoring weather conditions and for irrigation management.

Stalk dried samples were ground in a Wiley mill to pass through a 0.5-mm sieve, and stalk N concentration (SNA, g kg^{-1}) was determined by the Kjeldahl method (Malavolta et al., 1997). After that, the stalk N accumulation (SNA, kg ha^{-1}) was estimated (multiplying SDM by SNA).

Loss index of stalk dry matter (LI_{SDM}) due to stresses caused by N deficiency (–N) or water deficit (–W) or both (–N and –W) was calculated according to Equation 3 (Eq. 3), adapted from Clay et al. (2005); where, $SDM_{optimum}$ = SDM in the treatment without stress (+N and +W); SDM_{stress} = SDM in the treatment with some stress (-N or -W or both).

$$LI_{SDM}(\%) = \left(SDM_{optimum} - \frac{SDM_{stress}}{SDM_{optimum}}\right) x\, 100 \quad (3)$$

Statistical analysis

The experimental data for each cycle (2008/09 and 2009/10), individually, were analyzed by the GENES® statistical packages (Cruz, 2013). To analyze model assumptions, Lilliefors' test for normality and Bartlett's test for homogeneity of variance were used. Skewness and kurtosis coefficients were also evaluated. According

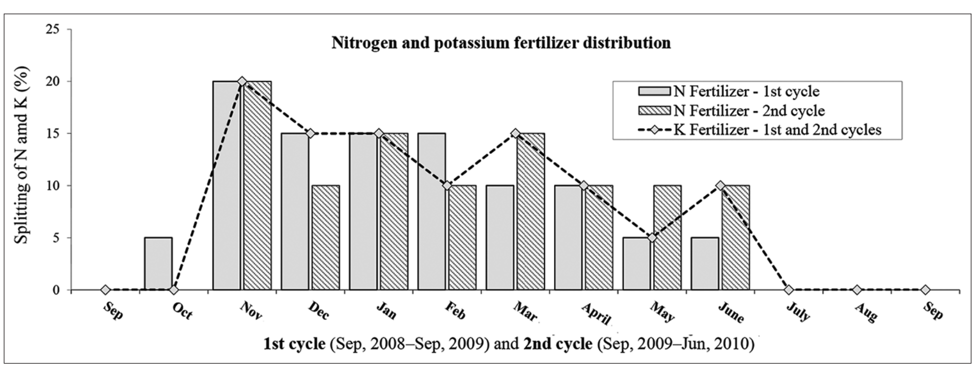

Fig 4. Percentage distribution of nitrogen (N) and potassium (K) fertilizers in the fertigated treatments during two sugarcane cycles (1st = Sep, 2008-Sep, 2009; 2nd = Sep, 2009-Jun, 2010) in Jaú (Brazil).

Fig 5. Samples processing: a) Stalks separate to measure height and diameter; b and c) sample processing to determine dry mass; d and e) Drying and weighing samples; f) measurement of productivity;

to these tests, no data transformation was needed. Since all assumptions required for a valid analysis of variance (ANOVA) were met, the F-test was performed. When the ANOVA resulted in a significant P value ($P \leq 0.05$), Tukey test ($P \geq 0.05$) was used for multiple comparisons of the treatment means, individually for each main factor (cycles or N rates or water supplies) considering their unfolding in case of significant interaction between two factors.

Subsequently, a correlation was determined between $\Delta^{13}C$ with SDM under water deficit (+N and –W) or N deficiency (–N and +W) or both stresses (–N and –W). With these correlation equations, a study of functions was performed, determining the rate of change (RC) (Δ ‰ SDM^{-1}) by means of Equation 4 (Eq. 4), according to Ferreira (1999); where X1 = initial delta; X2 = final delta; Y1 = initial SDM; Y2 = final SDM.

$$RC = \frac{X1 - X2}{Y1 - Y2} \quad (4)$$

RESULTS

Weather conditions and irrigation during experimental sugarcane cycles

The 10-day water balance and the water deficit (DEF) estimated for both cycles are shown in Fig. 6. Total rainfall during the first cycle was 1,741 mm (Fig. 6a and 6b). The rainfed treatments had an accumulated CET of 1,013 mm and water deficit of 142 mm (Fig 6a). In this period, 292 mm of water was applied in the irrigated treatments (total amount of 2,033 mm for rain + irrigation), which increased the CET to 1,182 mm, despite still having a small water deficit of 12 mm (Fig. 6b). In turn, the mean maximum and minimum temperatures observed were 29.3 °C and 15.2 °C, respectively.

For the second cycle, the accumulated rainfall reached 1,436 mm (Fig. 6c and 6d). The rainfed treatments had CET of 1,032 mm and water deficit of 318 mm (Fig. 6c). The water applied in the irrigated treatments was 393 mm, distributed throughout the entire period; therefore, sugarcane crop received a total of 1,829 mm of water, resulting in a CET of 1,320 mm, and water deficit of 29 mm (Fig. 6d). The maximum and minimum temperatures observed during this cycle were 29.1 °C and 15.5 °C, respectively.

Biometric and yield characteristics

Sugarcane plant height was influenced only by N rate, with average increase of 10.4% (0.18 m) due to N fertilization (Table 2). In turn, stalk diameter also increased (11.7%) by the input of N, and it was 20.6% higher in the first crop cycle (average of 25.8 mm) than the second one (average of 21.4 mm). The results of tillering show that it was favored by water supply, since irrigation increased the number of tillers per linear meter by an average of 29.8%.

Stalk and sucrose yields and SDM were affected by all study factors (cycle, N rate, and water supply), which interacted among themselves in several cases (Table 2).

Stalk yield decreased by an average of 45.6% (43.3 Mg ha^{-1}) from the first to the second cycle (Table 2), but it increased 26.4 and 14.2 Mg ha^{-1} due to irrigation in the first and second cycles, respectively (Fig. 7). Nitrogen fertilization increased stalk yield in both conditions of water availability, but the magnitude of the increases was higher when associated with irrigation [74.2% (45.2 Mg ha^{-1})] than under rainfed environment [41.2% (21.6 Mg ha^{-1})].

Sucrose yield and SDM had similar behavior considering the influence of N rate, water supply, and crop cycle, as well as the interactions of these three factors (Table 2 and Fig. 7). Irrigation and N fertilization improved both

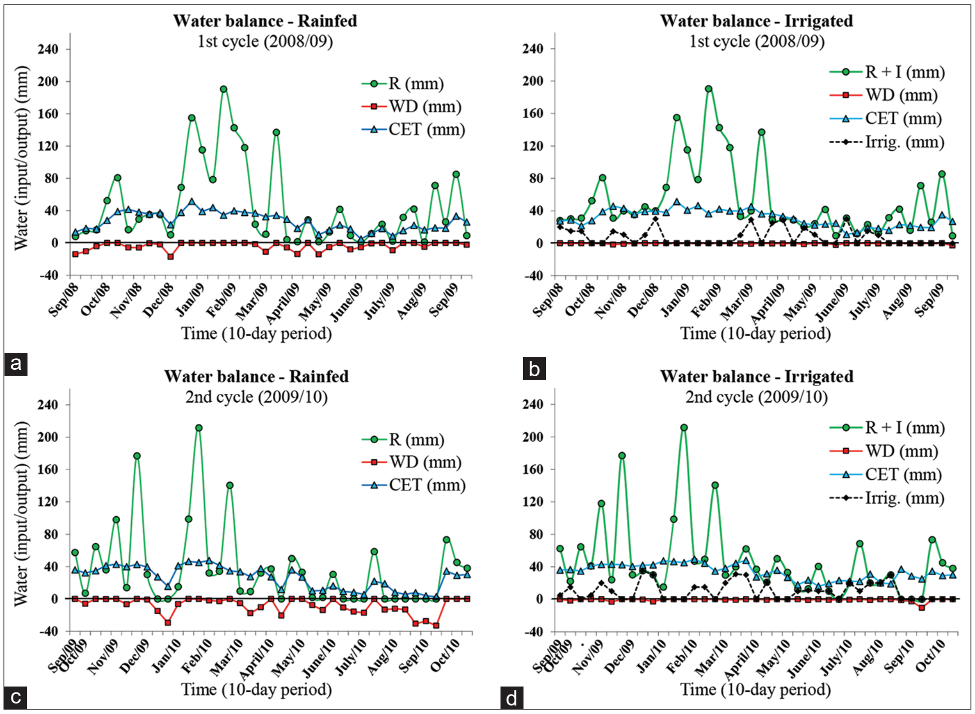

Fig 6. (a-d) Water balance (on the basis of 10-day period) of the sugarcane genotype SP80-3280, grown in Jaú (Brazil) in two cycles [first (2008/09) and second (2009/10)], in response to two water supplies (rainfed and irrigated).

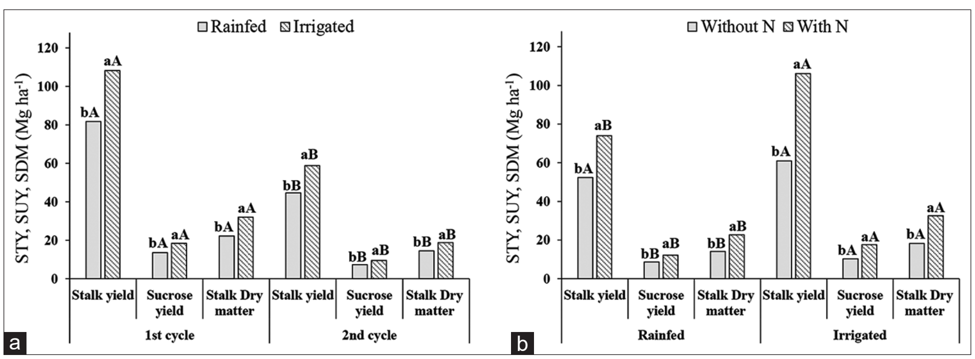

Fig 7. Unfolding of the analysis of variance (ANOVA) for stalk yield (STY), sucrose yield (SUY) and stalk dry matter (SDM) of the sugarcane genotype SP80-3280, grown in Jaú (Brazil) in two cycles [first (2008/09) and second (2009/10)], in response to two nitrogen (N) rates (without and with N) and two water supplies (rainfed and irrigated). In panel a, columns comparing two water supplies (individually for each cycle) folloSWwed by the same lowercase letter, or comparing two cycles (individually for each water supply) followed by the same capital letter, are not statistically significant (Tukey test, $P \geq 0.05$). In panel b, columns comparing two N rates (individually for each water supply) followed by the same lowercase letter, or comparing two water supplies (individually for each N rate) followed by the same capital letter, do not significantly differ (Tukey test, $P \geq 0.05$).

yield elements (Fig 7a and 7b), which were greater in the first cycle (Fig. 7a). Sucrose yield increased by averages of 35.3% and 30.1% in response to water supply in the first and second cycles, respectively, and SDM increased by 44% (9.8 Mg ha^{-1}) and 29.7% (4.3 Mg ha^{-1}) following the same comparisons (Fig. 7a). Nitrogen fertilization was more effective in increasing sucrose yield and SDM when combined with irrigation, as it was previously verified for stalk yield (Fig. 7b). In this context, the addition of N increased sucrose yield up to 40.2% (3.5 Mg ha^{-1}) and 70.9% (7.3 Mg ha^{-1}), and SDM up to 61% (8.6 Mg ha^{-1}) and 79.1% (14.4 Mg ha^{-1}) for rainfed and irrigated conditions, respectively.

Technological quality attributes

There were no significant interactions among the main factors (cycle, N rate, and water supply) for sugarcane fiber content, SJC and TSR (Table 3). Fiber content was 6% higher in the second cycle than in the first, and it increased by 7.6% due to irrigation. On the other hand, SJC and TSR decreased by 4.2% and 3.7% from the first to the second cycles, respectively, and decreased

Table 2: Significance (P-value) of the analysis of variance (ANOVA) of main effects [cycle (C), N rate (N), water supply (W)] and their interactions; averages of plant height, stalk diameter, tillering, stalk yield, sucrose yield, stalk dry matter (SDM) of the sugarcane genotype SP80-3280, grown in Jaú (Brazil) in two cycles [first (2008/09) and second (2009/10)], in response to two nitrogen (N) rates (without and with N) and two water supplies (rainfed and irrigated)

Source of variation	df[b]	Biometric features			Yield elements		
		Plant height	Stalk diameter	Tillering	Stalk yield	Sucrose yield	SDM
Block	3	ns	ns	ns	ns	ns	ns
Cycle (C)	1	ns	***	ns	***	***	***
N rate (N)	1	*	**	ns	***	***	***
Water supply (W)	1	ns	ns	***	***	***	***
C x N	1	ns	ns	ns	ns	ns	ns
C x W	1	ns	ns	ns	ns	*	*
N x W	1	ns	ns	ns	**	**	*
C x N x W	1	ns	ns	ns	ns	ns	ns
CV (%) [a]		14.2	9.2	18.3	12.4	12.3	13.8
Factor	**Treatment**	**Biometric features**			**Yield elements**		
		Plant height (cm)	**Stalk diameter (mm)**	**Tillering (tillers m^{-1})**	**Stalk yield (Mg ha^{-1})**	**Sucrose yield (Mg ha^{-1})**	**SDM (Mg ha^{-1})**
Cycle	First	174	25.8 a	16.3	95.0 a	16.0	27.1
	Second	190	21.4 b	16.1	51.7 b	8.4	16.7
N rate	Without	173 b	22.3 b	15.3	56.6	9.5	16.1
	With	191 a	24.9 a	17.1	90.0	14.9	27.7
Water supply	Rainfed	187	24.1	14.1 b	63.2	10.5	18.4
	Irrigated	177	23.1	18.3 a	83.5	14.0	25.4

[a]CV = coefficient of variation. [b]*df* = degrees of freedom (note: *df* from error = 21). ns,*,** and *** = no significant, and significant at the $P \leq 0.05$, 0.01 and 0.001, respectively, by F-test. Within each factor (cycle, N rate, or water supply), means followed by different lowercase letters in the column differ from each other by F-test

by 3% (SJC) and 2.5% (TSR) due to the N fertilization. Thus, these technological quality attributes showed to be quite stable in response to environmental changes.

Nitrogen uptake by sugarcane

As expected, the well-known dynamic of N in the soil–plant system was expressed by the influence of N rate and water supply throughout both sugarcane cycles, which significantly affected N uptake by plants, affecting several interactions between the evaluated factors (Table 3). Thus, the analysis of the data can be better understood by closely examining these interactions (Fig. 8).

Nitrogen fertilization greatly increased N uptake by sugarcane crop in both cycles (Fig. 8a and 8b). Thus, SNC increased by 94.3% and 71.8%, while SNA improved by 221% and 241% in the first and second cycles, respectively. On average, SNC and SNA were 36.5% and 59.7% lower in the second cycle compared to the first, respectively.

Irrigation improved SNC only in the second cycle, by an average of 56.7% (Fig. 8c). However, SNA increased by 59% (24.2 kg ha-1) and 110% (15.2 kg ha-1) due to irrigation, in the first and second cycles, respectively (Fig. 8d).

The combined effects of N fertilization and irrigation on sugarcane N uptake are shown in Fig. 8e and 8f. Under rainfed conditions, there were SNC and SNA increases of 76.8% and 194%, respectively, due to N fertilization. In turn, when associated with irrigation these responses to N addition increased up to 92% (for SNC) and 248% (for SNA). Besides this, considering an overall analysis, irrigation increased SNC and SNA by averages of 26% and 71.9%, respectively, but considering these outcomes within N rates, there was a synergistic effect between N and water, since SNC and SNA increased 19% and 51% due to irrigation without N, while they increased by 29.2% (SNC) and 78.7% (SNA) when including N in the irrigated treatments.

Carbon isotopic discrimination and loss index of stalk dry matter

The $\Delta^{13}C$ was influenced only by water supply, which did not present interaction with cycle or N rate factors (Table 3). Thus, the increase of water availability reduced $\Delta^{13}C$ by an average of 5.7%. There was an inverse correlation between $\Delta^{13}C$ and SDM, as a function of N fertilization (r = 0.51, $P < 0.05$; Fig. 9a) or water supply (r = 0.47; Fig. 9b). In addition to this, the different equation slopes (-47.7 and -23.8, Fig. 9a and 9b, respectively) show that $\Delta^{13}C$ depended on the dataset used for the correlation calculation. Therefore, in the irrigated treatment, for each increase of a 0.1‰ in $\Delta^{13}C$ due to N deficiency, there was a SDM decrease of 4.8 Mg ha^{-1} (Fig. 9a). On the other hand, for treatments with N fertilization, the reduction of SDM was 2.4 Mg ha^{-1} as a function of 0.1‰ increase in $\Delta^{13}C$ (Fig. 9b).

Table 3: Significance (P-value) of the analysis of variance (ANOVA) of main effects [cycle (C), N rate (N), water supply (W)] and their interactions; averages of fiber content, sucrose juice content (SJC) total sucrose recovery (TSR), stalk N concentration (SNC), stalk N accumulation (SNA), and carbon isotopic discrimination ($\Delta^{13}C$) of sugarcane genotype SP80-3280, grown in Jaú (Brazil) in two cycles [first (2008/09) and second (2009/10)], in response to two nitrogen (N) rates (without and with N) and two water supplies (rainfed and irrigated)

Source of variation	df[b]	Technological quality attributes			Nitrogen and ^{13}C		
		Fiber content	SJC	TSR	SNC	SNA	$\Delta^{13}C$
Block	3	ns	ns	ns	ns	ns	ns
Cycle (C)	1	**	**	**	***	***	ns
N rate (N)	1	ns	*	*	***	***	ns
Water supply (W)	1	***	ns	ns	***	***	***
C x N	1	ns	ns	ns	**	***	ns
C x W	1	ns	ns	ns	*	ns	ns
N x W	1	ns	ns	ns	ns	***	ns
C x N x W	1	ns	ns	ns	ns	ns	ns
CV (%) [a]		4.7	3.8	3.6	16.1	23.0	3.8
Factor	**Treatment**	**Technological quality attributes**			**Nitrogen and ^{13}C**		
		Fiber content (%)	**SJC (%)**	**TSR (kg Mg^{-1})**	**SNC (g kg^{-1})**	**SNA (kg ha^{-1})**	**$\Delta^{13}C$ (‰)**
Cycle	First	13.3 b	16.8 a	163 a	18.1	53.1	4.54
	Second	14.1 a	16.1 b	157 b	11.5	21.4	4.54
N rate	Without	13.8	16.7 a	162 a	10.4	17.5	4.64
	With	13.6	16.2 b	158 b	19.2	57.0	4.53
Water supply	Rainfed	13.2 b	16.6	162	13.1	27.4	4.72 a
	Irrigated	14.2 a	16.2	158	16.5	47.1	4.45 b

[a]CV = coefficient of variation. [b]*df* = degrees of freedom (note: *df* from error = 21). ns,*,** and *** = no significant, and significant at the $P \leq 0.05$, 0.01 and 0.001, respectively, by F-test. Within each factor (cycle, N rate, or water supply), means followed by different lowercase letters in the column differ from each other by F-test

The rate of change in the irrigated treatments (with both, -N and +N) was -0.02 Δ ‰ SDM^{-1} (Fig. 9a). In turn, in the N fertilized treatments (with -W and +W), the RC was -0.04 Δ ‰ SDM^{-1} (Fig. 9b).

In general, the impacts of N deficiency (–N), water deficit (–W) and both (–N and –W) on LI_{SDM} were very similar in the two sugarcane cycles (Fig. 10). The LI_{SDM} attributed to –N or –W were on average 40.5% and 35.3%, respectively (Fig. 10). However, the LI_{SDM} considering both limitations was on average 48.2%; that is, it was just 7.7 and 12.9 percentage points higher than those caused by –N and –W, respectively.

DISCUSSION

Sugarcane biometric and yield characteristics and Sugarcane technological quality as affected by the interaction N-water availability

Water stress has been one of the greatest environmental challenges faced by humanity throughout its history. The recurrent crop loss yield in some regions has transformed some previously well-established agricultural areas on the food production map. In this context, some actions have been taken to mitigate this adverse phenomenon. For example, there are several reports on the effective response of sugarcane yield to N fertilization combined with water supply (Singh and Mohan, 1994, Ng Kee Kwong et al., 1999; Wiedenfeld and Enciso, 2008; Gava et al., 2011; kolln, et al. 2016; Mendoça et al., 2020).

We found sugarcane plant height and stalk diameter increased in response to N fertilization, regardless of water supply. In turn, Wiedenfeld and Enciso (2008) and Uribe et al., (2013) also reported a significant response of sugarcane to N application, but depending on water supplementation through irrigation. However, their experiment was carried out under a condition of severe water deficit. On the other hand, in the current research, there was a significant enlargement of stalk yield by N addition when combined with water supply, which can be attributed to the effectiveness of splitting N fertilizer using fertigation, throughout the sugarcane cycle. Dalri et al. (2008) also verified higher stalk yield increases (up to 67%) as a response to N addition through fertigation, in São Paulo State, Brazil. Regarding rainfed conditions, it is common to observe no significant effect of N fertilization on crop yield (Bosquiero et al., 2020). The current SJC results contradict those obtained by Wiedenfeld (1995), since he verified a SJC decrease of 3.5% with the increase of N rate from zero to 168 kg ha^{-1} N, considering the average of two consecutive ratoons. Singh and Mohan (1994) obtained SJC reduction when N rate ranged from zero to 300 kg ha^{-1} N. They hypothesized that this SJC reduction can be attributed to the increase of invertase activity due to N fertilization,

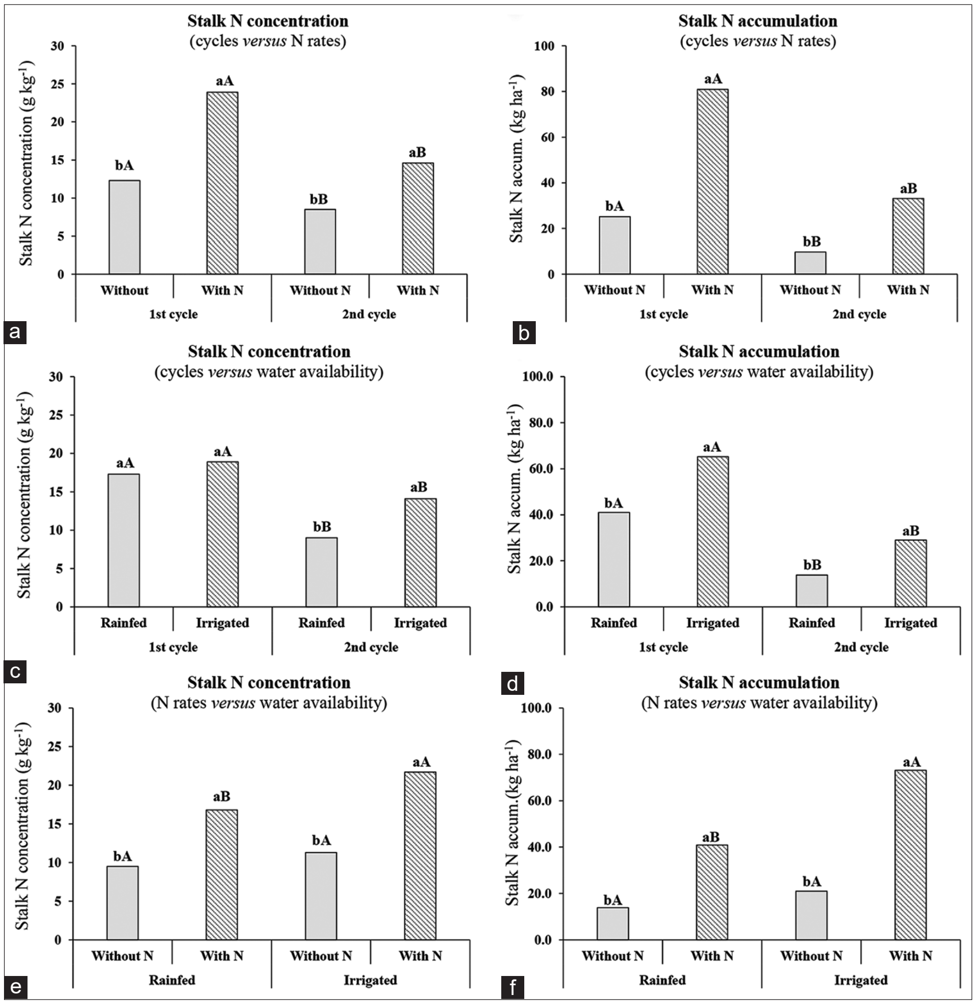

Fig 8. Stalk N concentration (SNC) and stalk N accumulation (SNA) of the sugarcane genotype SP80-3280, grown in Jaú (Brazil) in two cycles [first (2008/09) and second (2009/10)], in response to two nitrogen (N) rates (without and with N) and two water supplies (rainfed and irrigated). In panels a and b, columns comparing two N rates (individually for each cycle) followed by the same lowercase letter; or comparing two cycles (individually for each N rate) followed by the same capital letter, are not statistically significant (Tukey test, $P \geq 0.05$). In turn, in the panels c and d, columns comparing two water supplies (individually for each cycle) followed by the same lowercase letter; or comparing two cycles (individually for each water supply) followed by the same capital letter, are not statistically significant. Finally, in panels e and f, columns comparing two N rates (individually for each water supply) followed by the same lowercase letter, or comparing two water supplies (individually for each N rate) followed by the same capital letter, do not significantly differ .

since this enzyme is responsible for reducing sugars, which are converted to glucose and fructose. Moura et al. (2005) reported that the technological quality of sugarcane decreases with water supply. On the other hand, Weidenfeld (1995) verified the SJC increase in irrigated treatments.

Nitrogen uptake by sugarcane as affected by the interaction N-water availability

The higher SNA obtained in irrigated treatments is attributed to the increase of ion mass flow transport in soil solution, since $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ predominantly use this process to move to the root surface. Considering that the mass flow is favored by water availability, the amount of nutrient uptake by roots depends on the concentration of anions and cations in soil solution and its volumetric water content (Epstein and Bloom, 2004). In addition, the application of small rates of N throughout the sugarcane cycle favored the N uptake efficiency from N-fertilizer (that is, better synchronization between plant demand and fertilizer supply), which benefitted even more from subsurface fertigation; that is, N and water were added closer to the root system Furthermore, Gava et. al., (2018) wasn't found effect of N fixing into the plant system fertigation for sugarcane ratoon cultivar SP80-3280..

Wiedenfeld and Enciso (2008) studying N rates associated with irrigation, verified an interaction between these factors. Nitrogen fertilization increased SNA and stalk yield per unit of applied N. According to Wiedenfeld (1995), SNA is directly related to SDM, which is increased by water supply.

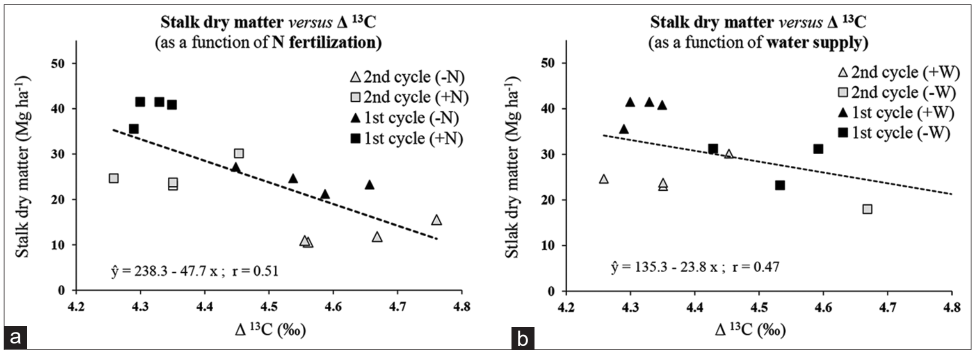

Fig 9. Correlations between stalk dry matter with carbon isotopic discrimination (Δ ^{13}C) as a function of N fertilization (panel a) or water supply (panel b), for the sugarcane genotype SP80-3280, grown in Jaú (Brazil) in two cycles [first (2008/09) and second (2009/10)], in response to two nitrogen (N) rates [without (-N) and with (+N)] and two water (W) supplies [rainfed (-W) and irrigated (+N)].

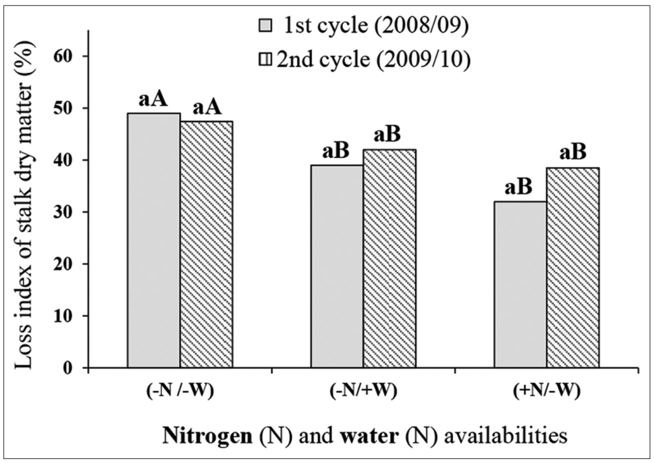

Fig 10. Loss index of stalk dry matter of the sugarcane genotype SP80-3280, grown in Jaú (Brazil) during two cycles [first (2008/09) and second (2009/10)], in response to two nitrogen (N) rates [without (–N) and with (+N)] and two water (W) supplies [rainfed (–W) and irrigated (+W)]. Columns comparing two cycles (individually for each N/W treatment) followed by the same lowercase letter, or comparing three N/W treatment (individually for each cycle) followed by the same capital letter, do not significantly differ (Tukey test, $P \geq 0.05$).

Carbon isotopic discrimination as a tool to determine sugarcane loss by N deficiency and/or water deficit

Mechanisms involved in N uptake (such as nitrate transporters and enzyme activities) and its relationship with photosynthesis have been extensively studied for several plant species, including sugarcane crop. However, data on the relationship between N availability and water supply influencing the effectiveness of N uptake by plants is still lacking. Thus, our study contributed important findings by identifying by Δ^{13}C the impact of N availability and water supply on SDM, considering their individual or combined effects.

Based on the obtained results, we can verify that N deficiency was more limiting for SDM production than the water deficit, which was at a moderate level. This statement can be confirmed considering the estimated loss of SDM using the methodology proposed by Clay et al. (2005), which uses the treatment without limitation (+N/+W) as a reference to quantify the productivity losses of other treatments. Thus, the most stressful treatment (-W/-N) was the most severe on stalk productivity, with losses close to 50%. In this context, the second most limiting treatment was that with N deficiency (+W/-N), with a SDM loss of 40.5%, followed by the treatment with water deficit (-W/+N) with 35.2% of SDM loss (Fig. 10). These findings are in line with the results obtained through Δ^{13}C analysis on sugarcane leaves at the end of the cycle, which were able to estimate the impacts of water deficit and N deficiency on the stalk productivity (Fig. 9).

In fact, Δ^{13}C is an efficient tool to quantify the impacts caused by water deficit and N deficiency on plant productivity. Recently, Endres et al. (2010) found a close negative relationship between Δ^{13}C and the photosynthetic rate of different sugarcane genotypes subjected to water stress. In turn, Minzer and Zhu (1998) found a negative correlation between Δ^{13}C and N application rates. Finally, in the current experiment, it was possible to isolate these two effects (water deficit and N deficiency) in sugarcane productivity using Δ^{13}C technique, which is an important finding. Although the Δ^{13}C values found in this experiment cannot be used as a reference for other studies (because they are specific for each environmental condition), our results showed that Δ^{13}C can be used to estimate productivity losses caused by water deficit and N deficiency.

The Δ^{13}C in C_4 plants (e.g., sugarcane) quantify the stress in plants mainly determined by the leakiness of CO_2 (Φ) and by the increase of partial pressure of CO_2 in the intracellular space (stomatal chamber) (*pi*) and in the environment (*pa*), that is, by the ratio *pi*/*pa* (Farquhar, 1983; Saliendra et al., 1996). In turn, N deficiency decreases the production and activity of the enzymes Rubisco and PEP carboxylase. Thus, a proportion of the CO_2 synthesized by PEP carboxylase is transported to the sheath cells and not used by Rubisco, returning to the mesophyll and increasing Φ and $\Delta^{13}C$ (Farquhar, 1983; Farquhar et al., 1989; Henderson et al., 1992; Meinzer and Zhu, 1998).

Saliendra et al. (1996), evaluated the influence of soil water availability on sugarcane growth, production and partition of photoassimilates, and enzyme activities, as well as their relationships with $\Delta^{13}C$. They verified the increase of $\Delta^{13}C$ due to reduced irrigation, but Rubisco and PEP carboxylase activities decreased in the same proportion. Therefore, the $\Delta^{13}C$ in leaves of C_4 plants can be used as an indicator of environmental stress, which can be used for monitoring and identifying plants under water deficit and/or N deficiency (Meinzer and Zhu, 1998; Clay et al., 2001a, b; Fravolini et al., 2002; Smeltekop et al., 2002).

The $\Delta^{13}C$ in C_4 plants was also studied by Clay et al. (2001a), who verified that this technique could be used to evaluate the reduction of corn yield caused by water deficit. They showed that a corn yield reduction (due to water deficit) of 1% resulted in an increase of 0.0117‰ of $\Delta^{13}C$. Meinzer and Zhu (1998) found that $\Delta^{13}C$ linearly decreased (r = 0.84, $P < 0.05$) when the plant quantum yield (photon absorption) decreased with the lower N availability for sugarcane crop. They also obtained a high positive correlation (r = 0.94, $P < 0.05$) between $\Delta^{13}C$ and Φ.

There was no synergistic effect between N and water limitations (Fig. 10). This can be explained by Liebig's minimum law, that is, sugarcane yield was limited by the resource (N or water) that was less available in the soil, even if all others were available and in adequate quantities. Therefore, to improve the beneficial effects of N fertilization and irrigation it is necessary to supply both resources simultaneously, in order to achieve potential economic benefits.

CONCLUSIONS

The addition of N associated with subsurface fertigation promoted increases in stalk and sugar productivity greater than 70% compared to the control treatment (rainfed and without N fertilization), indicating that this management is highly efficient in increasing sugarcane yield. The $\Delta^{13}C$ measurement was effective in identifying N deficiency and water deficit. Although the $\Delta^{13}C$ values found in this experiment cannot be used as a reference for other studies, our results showed that $\Delta^{13}C$ can be used to estimate productivity losses caused by water deficit and N deficiency. The total loss of stalk yield, due to the combined effect of water deficit and N deficiency, was almost 50%. However, when these two effects were isolated, it was found that the losses caused by N deficiency may be greater than those caused by a moderate water deficit.

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Abbreviations

SDM, stalk dry matter; SJC, sucrose juice content; TSR, total sucrose recovery; SUY, sucrose yield; SNC, stalk N concentration; SNA, stalk N accumulation; $\Delta^{13}C$, carbon isotopic discrimination; $\delta^{13}C$, isotopic enrichment of carbon; CET, crop evapotranspiration; LI_{SDM}, loss index of stalk dry matter.

Authors' Contributions

Oriel Tiago Kölln: writing original draft preparation, data curation. Glauber José de Castro Gava: Conceptualization, Methodology; Heitor Cantarella: Resources and Writing. Sérgio Ricardo Silva: Writing, review and Editing.Paulo Cesar Ocheuze Trivelin: Supervision.

REFERENCES

Barrie, A. and S. J. Prosser. 1996. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: T. W. Boutton and S. Yamasaki (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York, pp. 1-46.

Bordonal, R. O., J. L. N. Carvalho, R. Lal, E. B. Figueiredo, B. G. Oliveira and N. L. Jr. Scala. 2018. Sustainability of sugarcane production in Brazil. A review. Agron. Sustain. Dev. 38: 13.

Boschiero, B. N., E. Mariano, L. O. Torres-Dorante, T. M. S. Sattolo, R. Otto, P. L. Garcia, C. T. S. Dias, and P. C. O. Trivelin. 2020. Nitrogen fertilizer effects on sugarcane growth, nutritional status, and productivity in tropical acid soils. Nutr. Cycl. Agroecosyst. 117: 367-382.

Caemmerer, S. V., O. Ghannoum, J. J. L. Pengelly, A. B. Cousins, 2014. Carbon isotope discrimination as a tool to explore C4 photosynthesis. J. Exp. Bot. 65: 3459-3470.

Cantarella, H. and R. Rossetto. 2014. Fertilizers for sugarcane. In: L. A. B. Cortez (Ed.), Sugarcane Bioethanol: R&D for Productivity and Sustainability. Edgard Blücher, São Paulo, pp. 405-422.

Cardozo, N. P. and P. C. Sentelhas. 2013. Climatic effects on sugarcane ripening under the influence of cultivars and crop age. Sci. Agric. 70: 449-456.

Castro, S. G. Q., J Rossi Neto, O. T. Kölln, B. M. M. N. Borges and H. C. J. Franco. 2019. Decision-making on the optimum timing for nitrogen fertilization on sugarcane ratoon. Sci. Agric. 76: 237-242.

Cernusak, L. A., N. Ubierna, K. Winter, J. A. M. Holtum, J. D. Marshall and G. D. Farquhar. 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. New Phytol. 200: 950-965.

Clay, D. E., S. A. Clay, Z. Liu and C. Reese. 2001a. Spatial variability of 13C isotopic discrimination in corn. Commun. Soil Sci. Plant Anal. 32: 1813-1827.

Clay, D. E., R. Engel, D. S. Long and Z. Liu. 2001b. Nitrogen and water stress interact to influence carbon-13 discrimination in wheat. Soil Sci. Soc. Am. J. 65: 1823-1828.

Clay, D. E., S. A. Clay, D. J. Lyon and J. M. Blumenthal. 2005. 13C discrimination in corn grain can be used to separate and quantify yield losses due to water and nitrogen stresses. Weed Sci. 53: 23-29.

Conab. 2019. Acompanhamento da Safra Brasileira de cana-de-açúcar, v.6-Safra 2019/2020-N.4, Quarto Levantamento. Brasília: Companhia Nacional de Abastecimento. Available from: https://www.conab.gov.br/info-agro/safras/cana/boletim-da-safra-de-cana-de-acucar [Last accessed on 2021 May 25].

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12: 133-149.

Cruz, C. D. 2013. GENES a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35: 271-276.

Dalri, A. B. and R. L. Cruz. 2008. Produtividade da cana-de-açúcar fertirrigada com N e K via gotejamento subsuperficial. Eng. Agríc. 28: 516-524.

Dias, M. O. S., T. L. Junqueira, O. Cavalett, M. P. Cunha, C. D. F. Jesus, P. E. Mantelatto, C. E. V. Rossell, R. M. Filho and A. Bonomi. 2013. Cogeneration in integrated first and second generation ethanol from sugarcane. Chem. Eng. Res. Des. 91: 1411-1417.

Endres, L., J. V. Silva, V. M. Ferreira and G. V. S. Barbosa. 2010. Photosynthesis and water relations in Brazilian sugarcane. Open Agric. J. 4: 31-37.

Epstein, E. and A. J. Bloom. 2004. Mineral Nutrition of Plants: Principles and Perspectives. Sinauer Associates, Sunderland.

Farquhar, G. D. 1983. On the nature of carbon isotope discrimination in C_4 species. Aust. J. Plant Physiol. 10: 205-226.

Farquhar, G. D., J. R. Ehleringer and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 503-537.

Fernandes, A. C. 2003. Cálculos na Agroindústria da Cana-de-açúcar. 2nd ed. Sociedade dos Técnicos Açucareiros e Alcooleiros do Brasil, Piracicaba.

Ferreira, R. S. 1999. Matemática Aplicada às Ciências Agrárias: Análise de Dados e Modelos. Universidade Federal de Viçosa, Viçosa.

Ferreira, T. H., M. S. Tsunada, D. Bassi, P. Araújo, L. Mattiello, G. V. Guidelli, G. L. Righetto, V. R. Gonçalves, P. Lakshmanan and M. Menossi. 2017. Sugarcane water stress tolerance mechanisms and its implications on developing biotechnology solutions. Front. Plant Sci. 8: 1077.

Franco, H. C. J., P. C. O. Trivelin, C. E. Faroni, A. C. Vitti and R. Otto. 2010. Stalk yield and technological attributes of planted cane as related to nitrogen fertilization. Sci. Agric. 67: 579-590.

Fravolini, A., D. G. Williams and T. L. Thompson. 2002. Carbon isotope discrimination and bundle sheath leakiness in three C_4 subtypes grown under variable nitrogen, water and atmospheric CO_2 supply. J. Exp. Bot. 53: 2261-2269.

Gava, G. J. C., M. A. Silva, M. A. Silva, R. C. Silva, E. M. Jeronimo, J. C. S. Cruz and O. T. Kölln. 2011. Produtividade de três cultivares de cana-de-açúcar sob manejos de sequeiro e irrigado por gotejamento. Rev. Bras. Eng. Agríc. Ambient. 15: 250-255.

Gava, G.J.C., F. V. Scarpare, H. Cantarella, O. T. Kolln, S. T. Ruiz-Corea, A. B. Arlach and P. C. O. Trivelin. 2018. Nitrogen source contribution in sugarcane-inoculated plants with diazotrophic bacterias under urea-N fertigation management. Sugar Tech. 21: 462-470.

Henderson, S. A., S. V. Caemmerer and G. D. Farquhar. 1992. Short-term measurements of carbon isotope discrimination in several C_4 species. Aust. J. Plant Physiol. 19: 263-285.

Howell, T. A. and S. R. Evett. 2004. The Penman-Monteith Method. USDA Agricultural Research Service, Washington, DC.

Inman-Bamber, N. G. and D. M. Smith. 2005. Water relations in sugarcane and response to water deficits. Field Crops Res. 92: 185-202.

Kang, Y., S. Khan and X. Ma. 2009. Climate change impacts on crop yield, crop water productivity and food security a review. Prog. Nat. Sci. 19: 1665-1674.

Kolln, O. T., G. J. C. Gava, H. Cantarella, H. C. J. Franco, R. A. M. Uribe, L. E. R. Panutti and P. C. O. Trivelin. 2016. Fertigated sugarcane yield and carbon isotope discrimination (Δ13C) related to nitrogen nutrition. Sugar Tech. 18: 391-400.

Köppen, W. 1931. Grundriss der Klimakunde. Walter de Gruyter, Berlin.

Malavolta, E., G. C. Vitti and S. A. Oliveira. 1997. Avaliação do Estado Nutricional de Plantas: Princípios e Aplicações. Potafós, Piracicaba.

Meinzer, F. C. and J. Zhu. 1998. Nitrogen stress reduces the efficiency of the C_4 CO_2 concentrating system, and therefore quantum yield, in *Saccharum* (sugarcane) species. J. Exp. Bot. 49: 1227-1234.

Mendoça, M. F., J. Dantas Neto, P. F. Silva and E. C. A. Oliveira. 2020. Yield and technological quality of sugarcane under irrigation depths and nitrogen fertilization. Rev. Bras. Eng. Agríc. Ambient. 24: 482-489.

Moura, M. V. P., C. H. A. Farias, C. A. V. Azevedo, J. Dantas Neto, H. M. Azevedo and R. V. Pordeus. 2005. Doses de adubação nitrogenada e potássica em cobertura na cultura da cana-de-açúcar, primeira soca, com e sem irrigação. Ciênc. Agrotec. 29: 753-760.

Ng Kee Kwong, K. F., J. P. Paul and J. Deville. 1999. Drip-fertigation a means for reducing fertilizer nitrogen to sugarcane. Exp. Agric. 35: 31-37.

Ranjith, S. A., F. C. Meizer, M. H. Perry and M. Thom. 1995. Partitioning of carboxylase activity in nitrogen-stressed sugarcane and its relationship to bundle sheath leakiness to CO_2, photosynthesis and carbon isotope discrimination. Aust. J. Plant Physiol. 22: 903-911.

Saliendra, N. Z., F. C. Meinzer, M. Perry and M. Thom. 1996. Associations between partitioning of carboxylase activity and bundle sheath leakiness to CO_2, carbon isotope discrimination, photosynthesis, and growth in sugarcane. J. Exp. Bot. 47: 907-914.

Santos, D. L. and P. C. Sentelhas. 2012. Climate change scenarios and their impact on the water balance of sugarcane production areas in the State of São Paulo, Brazil. Rev. Ambient. Água 7: 7-17.

Santos, H. G., P. K. T. Jacomine, L. H. C. Anjos, V. A. Oliveira, J. F. Lumbreras, M. R. Coelho, J. A. Almeida, T. J. F. Cunha and J. B. Oliveira. 2013. Sistema Brasileiro de Classificação de Solos. 3rd ed. Embrapa Informação Tecnológica, Brasília.

Silva, N. F. Silva, E. C. Muraoka, T. Teixeira, M. B. Soares, F. A. L. Cunha, F. N. Adu-Gyamfi, J. and Cavalcante, W. S. S. 2020. Nitrogen utilization from ammonium nitrate and urea fertilizer by irrigated sugarcane in Brazilian Cerrado Oxisol. Agriculture. 10: 323.

Singh, P. N. and S. C. Mohan. 1994. Water use and yield response of sugarcane under different irrigation schedules and nitrogen levels in a subtropical region. Agric. Water Manag. 26: 253-264.

Smeltekop, H., D. E. Clay and S. A. Clay. 2002. The impact of intercropping annual “Sava” snail medic on corn production. Agron. J. 94: 917-924.

Smith, C. J. and P. M. Chalk. 2018. The residual value of fertiliser N in crop sequences: An appraisal of 60 years of research using 15N tracer. Field Crops Res. 217: 66-74.

Soil Survey Staff. 2010. Keys to Soil Taxonomy. United States Department of Agriculture-Natural Resources Conservation Service, Washington, DC.

Thorburn, P. J., I. K. Dart, I. M. Biggs, C. P. Baillie, M. A. Smith and B. A. Keating. 2003. The fate of nitrogen applied to sugarcane by trickle irrigation. Irrig. Sci. 22: 201-209.

Uribe, R. A. M., G. J. C. Gava, J. C. C. Saad and O. T. Kölln. 2013. Produtividade de soqueira de cana-de-açúcar integrada à irrigação por gotejamento e à fertilização nitrogenada. Eng. Agríc. 33: 1024-1033.

Vitti, A. C., P. C. O. Trivelin, G. J. C. Gava, C. P. Penatti, I. R. Bologna, C. E. Faroni and H. C. J. Franco. 2007. Produtividade da cana-de-açúcar relacionada ao nitrogênio residual da adubação e do sistema radicular. Pesqui. Agropecu. Bras. 42: 249-256.

Wiedenfeld, R. P. 1995. Effects of irrigation and N fertilizer application on sugarcane yield and quality. Field Crops Res. 43: 101-108.

Wiedenfeld, B. and J. Enciso. 2008. Sugarcane responses to irrigation and nitrogen in semiarid South Texas. Agron. J. 100: 665-671.