Variation of photosynthesis and carbohydrate levels induced by ethephon and water deficit on the ripening stage of sugarcane

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Abstract
An important index for the ethanol industry is the sucrose yield in sugarcane, which is affected by plant ability to accumulate sucrose during ripening. Despite the known efficiency of treatments such as water restriction and the application of regulators to stimulate the sucrose storage in culms, little is known about the physiological responses of the plant that lead to ripening. In this context, the aim of this study was to evaluate the physiological responses of sugarcane to different ripening treatments. Two varieties, IACSP95-5000, with high yield, and IACSP94-2094, with moderate yield were subjected to water deficit or application of chemical regulator (ethephon 480 g ha⁻¹) and both treatments associated. Growth, accumulation of carbohydrates in leaves and culms were measured. It can be concluded that the effects of ethephon on sugarcane are genotype-dependent. Ethephon stimulates sucrose accumulation in the culm and the photosynthate supply by the source in the responsive variety (IACSP95-5000). Such effects are not associated with growth restriction. In relation to the drought combined with the application of ethephon, the responsive variety shows increased sucrose content in culm at the same level as when ethephon is applied alone, hence treatments have no additive effects on sugarcane ripening.

Key words: Saccharum spp, source-sink relationships, sucrose, plant growth regulators.

1. INTRODUCTION

An important index for the sugar and alcohol industry is sucrose yield in sugarcane, which varies according to variety (Watt et al., 2014). Sugarcane breeding programs promoted, in recent decades, significant increase in sucrose production by means of the greatest amount of culms per hectare, with little or no change in the concentration of sugar in the culm (Jackson, 2005). The main stage of plant development involved in sucrose accumulation is the ripening, physiological process involving the formation of sugars in the leaves and their transport and storage in the culm (Watt et al., 2014), when the plants almost cease vegetative growth. In turn, the production and transport of assimilates in plants are regulated by the photosynthetic activity and the sink strength (Wardlaw & Moncur, 1976).

Factors such as climate and water availability decisively influence the development of the plant and consequently the production of sucrose by sugarcane. The ideal climate is the one with two distinct seasons: a hot and humid to promote germination, tillering and vegetative development, followed by a cold and dry season to promote natural ripening (Caputo et al., 2008; Moore & Maretzki, 1996). Due to the need of water restriction for ripening, irrigation suspension in irrigation-dependent crops is carried out in the pre-harvest to increase the concentration of sucrose in the culm (Donaldson & Van Staden, 1995; Inman-Bamber & Smith, 2005), a technique that increases up to 18% sucrose yield (Robertson & Donaldson, 1998). The water deficit impairs photosynthesis and
vegetative growth of the plant, with growth affected by water restriction even before significant changes in photosynthesis (Inman-Bamber et al., 2002). Thus, the moderate water deficit benefits the ripening process since the competition for photoassimilates between the plant sinks is reduced by growth restriction.

An alternative to control and optimize ripeness and consequently the production and harvesting of sugarcane is the exogenous application of ripeners. Among these substances, stands out 2-chloroethylphosphonic acid (ethephon), which when in contact with leaf tissue releases ethylene, stimulating the production of this hormone by plants and thus increasing its endogenous concentration. Ethylene is involved in response to different stresses (Yang & Hoffman, 1984), working in the maturation of tissues, seed germination, senescence and causing leaf abscission and variation in the degree of stomatal opening (Abeles et al., 1992; Pallas & Kays, 1982). In sugarcane, ethylene is also associated with reduced growth of immature internodes (Li & Solomon, 2003; Stewart & Freebairn, 1969) and with accumulation of sucrose (Chong et al., 2010). Ethephon also stimulates the sucrose accumulation in culms by interfering with the activity of enzymes involved in sugar synthesis (Wang et al., 2013), thus increasing the demand for photoassimilates by sink.

Donaldson & Van Staden (1995) observed that the imposition of drought combined with the application of ripeners caused no increase in the concentration of sucrose in the sugarcane culms when compared to treatments carried out separately. This response is justified because both treatments induce similar physiological responses, since the decline in growth induced by etylene and water restriction would allow the transport of carbon assimilated in photosynthesis to culms (Kaitaniemi & Honkanen, 1996). Alternatively, plants could not accumulate more sucrose when treated with ripeners and subjected to drought because the biological limit of sucrose accumulation would have already been achieved.

The maintenance of carbon assimilation has a key role in the ripening process, providing the substrate for the synthesis and storage of sucrose in culms. In general, studies on sugarcane ripening focus on evaluation of sucrose accumulation in culms due to the application of ripening promoters (Caputo et al., 2008; Leite et al., 2011; Li & Solomon, 2003; Robertson & Donaldson, 1998), and little research has increased the understanding of the affected physiological processes (Chong et al., 2010; Gronwald, 1991; Jain et al., 2013). Given the above, this study aimed to evaluate the physiological responses to different treatments inducing ripening in sugarcane and thus better understand the physiology of this process. For this, the following hypotheses were tested: (i) ethephon interferes with the source-sink relationship during sugarcane ripening, decreasing the growth of the plant without changing the photosynthesis and thereby increasing the sucrose storage in the culm; and (ii) by affecting different physiological processes, water deficit and ethephon have additive effects in inducing ripening.

2. MATERIALS AND METHODS

Plant material

This study used ten-month-old plants of two commercial varieties of sugarcane (Saccharum spp.) with different yields and resilience against environmental constraints. The variety IACSP95-5000 has high agricultural yield and is indicated for favorable environments (Landell et al., 2007), the variety IACSP94-2094 has lower yield and is indicated for restrictive environments (Ribeiro et al., 2013). Plants were obtained from mini-stalks of the varieties, which were germinated in trays containing commercial substrate (Carolina Soil®, Vera Cruz, RS, Brazil).

Experimental design

The experiment was conducted in a greenhouse; the treatments were applied in April to minimize the effect of low air temperature to induce ripening in plants. The height of the greenhouse varies from 2.5 to 4 m, the sides are open for continuous renewal of air inside. Seedlings were planted in 16 masonry tanks (eight for each variety) of 2 m$^2$ (4.0 x 0.5 m) and capacity of 1.54 m$^3$. Plants were conducted only with the primary culm, removed all tillers from planting until the end of the experiment. The tanks contained soil as substrate analyzed for nutritional composition and fertilized following the recommendations of van Raij et al. (1996). Each tank contained 15 plants of the same variety, which have been subjected to one of the following treatments: control (C); water deficit (WD); application of ethephon (EN); and drought + application of ethephon (WD + EN). Each treatment was induced in four tanks, two for each variety.

Water deficit was promoted by the gradual reduction of irrigation, with substrate moisture monitored to reach 50% of the maximum water capacity, which occurred after 23 days. At this time, irrigation was resumed for the recovery of plants. The moisture of the substrate was monitored by gravimetric method and the total potential of water in the substrate ($\Psi$) determined with a soil moisture sensor (WaterMark® 200SS, Irrometer, Riverside, CA, EUA).

Ethephon (Ethrel®, Bayer Crop Science, Leverkusen, Germany) was applied on the same day of the beginning of water restriction, in the late afternoon. We used an automated
pressurized backpack sprayer 16L Jett (Sanmaq, São Leopoldo, RS, Brazil) with three nozzles sprayer TP8002VK, and spraying carried out for 80 s with a maximum nominal pressure of 20 bar and total flow of 420 mL min\(^{-1}\). The dose applied was equivalent to 480 g ha\(^{-1}\), concentration recommended by the manufacturer and applied to commercial crops. To prevent contamination between treatments with and without the regulator, plastic sheeting separated the plants during application and in subsequent days. Treatments without ethephon were sprayed with water and surfactant at 1 mL L\(^{-1}\) (Haiten\(^a\), Arysta Lifescience, Salto de Pirapora, SP, Brazil), used for the preparation of ethephon solution.

**Biometric evaluations**

After 30 days of treatment, the leaf area was evaluated with a planimeter (LI-3100C, Li-Cor, Lincoln, NE, USA), the leaves were counted and weighed for fresh weight determination. Simultaneously, the culms were harvested and weighed. Subsamples of leaves and culms were oven dried (60 °C) to constant mass to determine dry matter. These subsamples were used to calculate the tissue moisture content \([(FW - DW) \times FW^{-1}]\), used to estimate the total dry weight of plants from the fresh weight determined.

The accumulation of dry matter in culms and leaves was evaluated with measurements at the beginning and end of the experimental period. This difference was divided by the period (30 days) to obtain daily variation of dry weight of leaves, culm and total (leaf + culm).

**Gas exchange**

Gas exchange was evaluated on leaf+3 (third leaf with apparent ligule) at 1, 2, 5, 15, 23 and 30 days of treatment. The CO\(_2\) assimilation \((P_n)\) was evaluated with an infrared gas analyzer (LI-6400, Li-Cor, Lincoln, NE, USA). The measurements were performed under constant concentration of CO\(_2\) in the air (400 µmol mol\(^{-1}\)), photo synthetically active radiation (PAR) of 2000 µmol m\(^{-2}\) s\(^{-1}\), and natural variation of temperature and relative humidity (Figure 1), between 13 and 15 hours. The PAR was measured with a quantometer LI-190 (Li-Cor, Lincoln, NE, USA) and the temperature and humidity were continuously recorded throughout the experimental period with a data acquisition system LI-1400 (Li-Color, Lincoln, NE, USA). Subsequently, \(P_n\) data were integrated over the experimental period considering the average photoperiod of 12 hours to estimate the amount of CO\(_2\) absorbed by the plants during 30 days \((P_{ni})\) as described by Ribeiro et al. (2013).

**Carbohydrate concentration**

Carbohydrate concentration was determined in samples of dried leaves+2 and culm (internodes 2, 6 and 10) collected after 30 days of treatment. For the determination of total soluble sugars (TSS), the samples were extracted in methanol: chloroform: water solution (Bieleski & Turner, 1966) and quantified by phenol-sulfuric method (Dubois et al, 1956), using glucose as a standard. The sucrose concentration (SAC) was determined by the method described by Van Handel

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**Figure 1.** Minimum, average and minimum air temperature \((T_{\text{air}})\), minimum relative humidity \((\text{RH}_{\text{min}})\) and daily photosynthetic active radiation \((\text{RFA}_{\text{t}})\) in a greenhouse during the experimental period. The treatments started on day 0. Arrows on the axis x indicate the days when photosynthesis was measured.
(1968) and the dosage by the phenol-sulfuric method using sucrose as a standard. The concentration of reducing sugars (RS) was estimated as RS = TSS - SAC. The quantification of starch in leaves was performed using the enzymatic method described by Amaral et al. (2007).

**Statistical analysis**

The experimental design was a randomized block, split plot, with four replications (plants) per treatment for each variety. The variation factors were the varieties, the water conditions and the application of ripener. The results were statistically tested using analysis of variance and when there was significant difference, the mean values were compared by Tukey’s test (p≤0.05).

**3. RESULTS AND DISCUSSION**

During the study period, the average daily temperature varied between 18.3 and 24.7 °C, with a minimum of 12.5 °C on day 12 and a maximum of 32.7 °C on day 30 (Figure 1a). The minimum relative humidity ranged from 34% to 87%, with a declining trend on day 10 after the start of the experiment (Figure 1b). From the 10th day, the total PAR increased, reaching 20.4 mol m⁻² d⁻¹ on day 25 (Figure 1c). Water restriction caused progressive decrease in Ψ, reaching the minimum observed - 159.3 kPa on day 23 of treatment (Figure 2).

Compared to the control, the EN treatment increased growth of IACSP95-5000, being determined by the increase in dry matter of shoots (Figure 3a). The treatment WD+EN caused decrease in mass accumulation, mainly due to a significant decrease in total leaf dry weight (Figures 3a,b).

![Figure 2](image2.png)

**Figure 2.** Total potential of water in the substrate (Ψ) during the experimental period. The reduction of irrigation started on day 0. Arrows on the axis x indicate the days when gas exchange was measured. Each symbol represents the mean of 8 repetitions ± standard deviation.

![Figure 3](image3.png)

**Figure 3.** Variation in the dry weight of shoots (ΔSDW in (a)) and culm (ΔCDW, in (b, c) and leaf (ΔLDW, (b, c)) of IACSP95-5000 (b) and IACSP94-2094 (c) subjected to the following treatments: control; water deficit (WD); ethylene (EN, application of ethephon at 480 g ha⁻¹); WD + EN = combination of treatments WD and EN. Mean values of three replications. In (a), different letters indicate significant effect of the treatments within each variety. In (b) and (c), different capital letters indicate significant effect of treatments on culms and different lower case letters, on the leaves, by Tukey's test (p<0.05).
In IACSP94-2094, the treatment WD caused a reduction in dry matter accumulation due to lower dry matter accumulation in the leaves (Figures 3a,c) and the treatment WD + EN caused a reduction in growth compared to the control (Figures 3a) driven by lower mass accumulation in culms and significant reduction in leaf dry weight (Figures 3a,c).

There was an increase in the total CO$_2$ assimilation in IACSP95-5000 in the treatment EN, but a decrease in the treatment WD (Figure 4a). When the plants were subjected to the treatment WD + EN, there was no decrease in $P_{\text{in}}$ compared to control (Figure 4a). In IACSP94-2094, $P_{\text{in}}$ was reduced in treatments WD and WD + EN when compared to the control and the treatment EN did not affect $P_{\text{in}}$ (Figure 4b). The treatments WD and WD + EN caused reduction in total leaf area at the end of the experiment in both varieties (Figures 4c,d).

Carbohydrate concentration in the culms and leaves was also affected by the treatments (Figure 5). In the culm of IACSP95-5000, the treatments EN and WD + EN caused higher sucrose content, without changing the concentration of reducing sugars in plants. When compared to the control, the treatment WD caused a reduction in the content of both sucrose and reducing sugars in the culms (Figure 5a). The treatments WD and WD + EN caused a decrease in leaf starch concentrations in IACSP95-5000 without affecting the levels of sucrose and reducing sugars (Figure 5b). In the culms of IACSP94-2094, the treatments WD and WD + EN reduced the sucrose concentration compared to the control, with stability in reducing sugars content (Figure 5c). In the leaves of IACSP94-2094, the sucrose content was reduced in the treatment EN, being even more affected in treatments WD and WD + EN (Figure 5d). The starch content in leaf in IACSP94-2094 was reduced

![Figure 4](image-url)
in the treatments WD and WD + EN with the reducing sugar content decreasing only in plants of the treatment WD (Figure 5d). Before the application of the treatments, the concentration of sucrose in the culms of the varieties IACSP95-5000 and IACSP94-2094 was 216.7 ± 4.8 and 228.0 ± 9.3 mg (g FW)\(^{-1}\), respectively. As these values were similar, the differences in sucrose concentrations observed after 30 days of treatment reflect the accumulation of sucrose during the study period.

Under the influence of ethephon, accumulation of dry matter was found in the culms of IACSP95-5000 (Figure 3b) along with an increase in total carbon assimilation (Figure 4a). Higher photosynthesis may be associated with higher demand for carbon by culms. In fact, culm is a high priority sink in the allocation of photoassimilates (Pammenter & Allison, 2002) and the activity of sinks in sugarcane regulates the activity of the source (Inman-Bamber et al., 2011). Thus, ethephon seems to have stimulated culm growth (Figure 3b), which started to accumulate more sucrose (Figure 5a). This increased demand for assimilates would have induced and increased photosynthesis in IACSP95-5000 (Figure 4a). The effect of ethephon on the sink strength was remarkable when considering that even with higher accumulation of sucrose in the culm of IACSP95-5000, the sucrose content in the leaves remained unchanged (Figure 5b).

The response to ethephon was dependent on the variety: IACSP95-5000 was more responsive. In IACSP94-2094, growth and sucrose accumulation in culms were not amended by the treatment EN (Figures 3b and 5c). Actually, the differential response of sugarcane varieties to ripeners is known (Caputo et al., 2008; Donaldson & Van Staden, 1995; Li & Solomon, 2003), as well as the differential susceptibility to environmental stresses (Ribeiro et al., 2013; Sales et al., 2013).

In sugarcane, growth suppression favors photoassimilate partitioning for storage (Chong et al., 2010), increasing...
the concentration of sucrose in the culm due to shading, partial defoliation, drought or cold (Huang et al., 2015; Li & Solomon, 2003; Pammenter & Allison, 2002; Robertson & Donaldson 1998). In this study, the imposition of drought did not affect the dry matter accumulation in the culms (Figures 3b,c) but decreased the concentration of sucrose in the culms of both varieties (Figures 5a,c). With a significant decrease in leaf area of plants subjected to water deficit (Figures 4c,d), sucrose accumulation in this condition could increase if the plants showed an increase in $P_{ni}$. This condition was not met in this study (Figures 4a,b) and thus there was a reduction in the sucrose content in the culm of plants under water deficit (Figures 4c,d). Taking into consideration that the CO$_2$ absorbed by the plant canopy declined significantly due to reduced leaf area, it could be suggested that the photoassimilate supply was reduced by drought in IACSP95-5000.

Low water availability observed between 15 and 23 days after induction of treatment (Figure 2) was sufficient to reduce $P_{ni}$ (Figure 4a) and it is known that even short periods of water deficit can compromise the accumulation of sucrose in the culm (Inman-Bamber, 2004). The drought caused a reduction of photosynthesis in two varieties (Figure 4a, b) and this response was associated with stomatal closure (results not shown). The reduction in leaf starch content in both varieties under water deficit (Figure 5b, d) indicates that the plants used foliar reserves available to meet the demand of the sink and/or bear the costs of maintaining metabolic homeostasis under stressful condition.

Similar concentrations of sucrose were registered in the culms of IACSP95-5000 subjected to the treatments WD+EN and EN, despite the first reducing CO$_2$ assimilation (Figures 4a and 5a). This suggests that ethylene also acted in the ripening process by stimulating the activity of the sink and allowing the culm to continue to store sucrose even without increased CO$_2$ assimilation. Thus, our results indicate that water stress was less restrictive to the yield of sugarcane occurred when under the influence of ethephon. Could sucrose accumulation in culms be increased by applying ethephon before the imposition of drought? Wu et al. (2004) reported that the ethephon applied in the early stages of plant development increases drought resistance during sugarcane development. Nevertheless, its effect on sucrose accumulation in the culm under non-limiting environmental conditions must be evaluated in future research.

4. CONCLUSION

The genotype-dependent effect of ethephon on sugarcane stimulates sucrose accumulation in the culm and the photoassimilate supply by the source in the responsive range (IACSP95-5000). Such effects are not associated with growth restriction. Regarding the application of ethephon combined with the imposition of drought, the hypothetical additive effect on sucrose accumulation in the culm was not found in the ripening of sugarcane.

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