



Effects of Ethephon Associated with the Position of Gems on the Plum of Sugar Cane in the Initial Development of Culture - Part II

Lucas Aparecido Manzani Lisboa^{1,2*}, Reginaldo Sciarra Leonezi¹,
Andresa Toledo Chagas¹, João Paulo Basaglia Freschi¹,
Paulo Alexandre Monteiro de Figueiredo¹ and Edson Lazarini³

¹College of Technology and Agricultural Sciences, São Paulo State University (UNESP), Dracena, São Paulo, Brazil.

²Integrated College Stella Maris (FISMA) and Educational Foundation of Andradina (FEA), Andradina, São Paulo, Brazil.

³School of Natural Sciences and Engineering, São Paulo State University (UNESP), Ilha Solteira, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. Author LAML designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RSL, ATC and JPBF managed the analyses of the study. Authors PAMF and EL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The hormones are closely related to the emergence of gemstones contained seedlings of sugarcane, at the time of planting of the stems. The objective of this work was to evaluate the effects of the ethephon associated to the position of gemstones in the cane of sugar cane in the initial development of the culture. In March 2014, at the Rio Vermelho Plant, located in Junqueirópolis, State of São Paulo, a cane plant with a sugar cane plant was selected for seedlings with an approximate age of 11 months. Two areas with dimensions of 20x20 meters were demarcated. In one of the areas ethephon was applied. At 15 days after application, the

*Corresponding author: E-mail: lucas.lisboa@unesp.br;

seedlings containing 1 and 2 buds were collected to compose two independent experiments. From the area where the product was not applied, seedlings were removed for the control and application treatments of ethephon in the planting groove in pots. The gems were sent to the Faculty of Agrarian and Technological Sciences of the Paulista State University "Júlio de Mesquita Filho" - from Dracena, State of São Paulo. The seedlings came from the apex, middle and base of the canes of sugarcane. In this way, the experimental design was in a 3x3 factorial scheme, that is, the position of the seedlings in the canes of sugarcane and the modes of application of ethephon. The use of ethephon and positions of the seedlings in sugarcane stalks did not influence the Chlorophyll Index and Stomatal Conductance. The use of ethephon in the plant 15 days before planting, together with seedlings from the apex followed by the medium of the canes of sugarcane, presented better results for the ultrastructural characteristics of sugarcane foliage.

Keywords: Hormone; ethephon; morphology.

1. INTRODUCTION

The sugarcane is classified as a C4 plant, a characteristic that makes the vegetable more efficient in the use and capture of atmospheric CO₂, reactions performed by the sheath cells of the vascular bundles [1,2]. The photosynthetic response to CO₂ is directly linked to PEPcase activity and presents different levels of carbon in different segments along leaf length [3].

The chlorophyll index is determined by the emission of a wavelength of $\lambda=650\text{nm}$, this value is close to the wavelengths that stimulate chlorophyll activity, while the emission of the wavelength $\lambda=940\text{nm}$ acts as an internal reference in the leaf limy to compensate for differences in leaf thickness or water content [4,5]. The photosynthetic efficiency can be estimated by the concentration of chlorophyll pigments present in the leaves, becoming a tool for the actual recommendation of the fertilization need [6]. As a result, the macro and micromorphological modifications of each cultivar, as well as the effects caused by them, should be increasingly studied in order to improve understanding and the use of phyto-regulators makes it a strategy to control foliar activities [7,8].

The use of phyto-regulators in agriculture has become an increasingly common process in the beginning of the 21st century. 2-chloroethylphosphonic acid, or ethephon, is a substance classified as a growth regulator with systemic performance in plants [9]. In the plant organism, ethephon rapidly undergoes degradation, being reduced in phosphoric acid, chloride ions and ethylene ions, which act on the growth process [10].

In this case, structural aspects help in understanding the mechanisms that cause the

injuries. However, it is important to point out that changes visible to the naked eye are derived from changes in the structures of the dermal, fundamental or vascular tissues of plants, making it necessary to have a thorough knowledge of these changes caused by changes in the environment [11]. The symptomatology is widely used to evaluate the damage caused by biotic or abiotic factors [12].

Examples demonstrate the importance of the morphophysiological and functional knowledge of the plants; stomatal changes were observed in the leaves of roses with the use of ethylene [13], the use of low concentrations of ethephon provided a momentary decrease in the tillering phase of the crop. length and width of the leaves, but promoted differentiation of the vascular bundles in the leaves, which provided greater efficiency in the transport of sap.

Ethylene resulted in a differentiation in the mesophyll cells with doses of 100 mg L⁻¹ which provided an increase in the outer surface of the cells and allowing a better distribution of the chloroplasts. There was an increase in the number of chloroplasts. According to Li & Solomon 2003, these changes have brought about a significant increase in the total photosynthetic area in the mesophyll cell of sugarcane leaves. Using ethylene provided an acceleration in the differentiation and an increase in the number of vessels folem and xylem species, which provided greater efficiency in the transportation of sap in sugarcane [14].

To know the foliar morphology, the functions of the vegetal tissues and their possible modifications to the damages caused by the absence or presence of nutrients and hormones can be decisive in the decision making regarding the appropriate management to be employed in

the sugar cane crop, as well as predicting the losses estimated by not knowing these effects [15,7].

The objective of this work was to evaluate the effects of the ethephon associated with the position of gemstones in the cane sugar cane in the initial development of the culture.

2. MATERIALS AND METHODS

2.1 Obtaining Sugarcane Seedlings

In March 2014 an area was chosen that contained a sugar cane plantation at the plant stage approximately 11 months old; destined to molt that presented a homogeneity of plants. The cultivar of sugarcane chosen for the installation of the experiment was RB966928. The area selected belonged to the Agroindustrial Production Unit of the Rio Vermelho Plant, located in Junqueirópolis, State of São Paulo, with geographic coordinates 21°29'35.34"S and 51°16'13.60"W and altitude 416 m. The climate of the region is characterized as Cwa according Köppen, mesothermic, with rainy summers. The average temperature of the region is 24°C, presenting maximum of 31°C and minimum of 19°C.

The area was approximately 20 m wide by 40m long, which was divided into two distinct areas with the same films of 20x20m, one adjacent to another, in order to ensure homogeneity of application of the syrup and to ensure a lower border effect.

In one of the demarcated areas, under field conditions, the ethephon was applied using a CO₂ pressurized costal sprayer with a 6 m long, T-shaped bar with 6 flat AXI 11002 nozzles spaced at 0.5 m, allowing simultaneous application in two lines, the nozzles were approximately 0.5 m from the target with an application pressure of 40 psi pol², at the dosage of 482.4 g ha⁻¹ of the active ingredient of the product, with a volume of 150 L ha⁻¹ and hydrochloric acid was used to adjust the pH to 2.8±2. Simultaneously, a similar, contiguous area received only water as a control. At the time of application, wind velocity was approximately 2.9 km h⁻¹, relative humidity at 77.6% and 25 °C.

2.2 Installing the Experiment

Fifteen days after the application of the ethephon in the field, the experiments were started in an

unprotected external environment at the FCAT - Faculty of Agrarian and Technological Sciences of the "Júlio de Mesquita Filho" State University, located in the city of Dracena, State of São Paulo, with geographic coordinates 21°46'04"S and 51°55'41"W and altitude 396 m.

The soil used in the experiments was classified as Dystrophic Yellow Red Argisol [16] with good drainage. At the time of installation of the experiment in April 2014, soil sampling was performed at depths of 20-40 cm for the physical and chemical analysis. A deeper soil was chosen in order to avoid an incidence of invasive plant seeds and homogeneity in their chemical and physical attributes.

The results of the soil chemical analysis were: pH CaCl₂= 5.0; MO= 14 g dm⁻³; P= 8.0 mg dm⁻³ (resin); K= 2.3 mmol dm⁻³ (resin); Ca= 7.0 mmol dm⁻³ (resin); Mg= 5.0 mmol dm⁻³ (resin); H+Al= 20 mmol dm⁻³; Al= zero mmol_c dm⁻³; Base Sum= 14.3 mmol dm⁻³; CTC= 34.3 mmol dm⁻³; Base Saturation (V%)= 42; Saturation Al (m%)= zero; S (SO₄⁻²)= 3.0 mg dm⁻³; Cu= 2.8 mg dm⁻³ (DTPA); Fe= 19 mg dm⁻³ (DTPA); Zn= 1.3 mg dm⁻³ (DTPA); Mn= 16.5 mg dm⁻³ (DTPA); B= 0.14 mg dm⁻³ (Hot water); Clay= 75 g kg⁻¹; Silt= 33 g kg⁻¹ and Total sand= 893 g kg⁻¹ [16,17].

All soil corrections were performed, according to [18,19]. On this occasion, in pots of 45 dm³ containing sifted soil, where sugarcane seedlings were planted in two situations, containing 1 (one) and 2 (two) buds, composing this maniera, 2 (two) independent experiments. During the experiments, all necessary cultural treatments were carried out, such as: phytosanitary control, elimination of invasive plants and cover fertilization. The pots were kept irrigated whenever necessary in order to meet the field capacity.

The experimental design was a completely randomized design in a 3x3 factorial design with 5 (five) replicates, totaling 45 plots or vessels. The factors pertinent to the treatments, as well as the respective levels were: position of the buds in the stem - apical region; median region and basal region and the form of application of ethephon - control (without ethephon); application of ethephon in the plant with fifteen days before planting and application of ethephon in the groove / pots at the time of planting.

To determine the positions of the gems on the stalks were counted all of the high nodes and

dividing by three. In this way, the three parts of the stem were obtained, being an apical region; median and baseline. For the stems that presented odd numbers of nodes, we considered the basal third with the largest number.

For the treatment in the groove of the pot, the dosage of ethephon occurred according to the technical recommendation of the product, which provides for the dosage of 360 g ha⁻¹ of active ingredient of the product in the planting groove, with application rate of 150 L ha⁻¹.

2.3 The Evaluations

At 90 days after the installation of the experiment the Chlorophyll Index was determined through the use of the OSI model chlorophyll meter CCM-200 through direct reading. Stomatal conductance was determined using the AP-4 model porometer also by direct reading. At the time, one (1) leaf fragment+1 was removed per plant of the main stem, each fragment was 5 (five) cm long drawn from the central part of the limbus.

All fragments of plant tissues received the pertinent procedures for dehydration, diaphanization, inclusion and embedding and with the help of a microtome, where cross sections of 8 µm were performed on each tissue fragment. The slides were observed in an optical microscope with a camera coupled to perform the measurements of the histological variables through the analysis program, calibrated with a microscopic ruler at the same magnification [20], where limbal thickness; thickness of the epidermis of the abaxial face; thickness of the epidermis of the adaxial face; mesophyll thickness; flolem diameter; diameter of the metaxilematic vessels; abaxial cuticle thickness; adaxial cuticle thickness; diameter of the cells of the sheath and distance between the vascular bundles in the leaf blade, according to [2].

In the collected fragments, the impression was also made on the epidermal faces using cyanoacrylate ester [21], where the following characteristics were observed: number of stomata per mm² on the abaxial; number of stomata per mm² in the adaxial face; number of abaxial epidermal cells per mm²; number of adaxial epidermal cells per mm²; stomatal functionality of the abaxial face; stomatal functionality or relation of the adaxial face; and the stomatal index of the adaxial face according

to [22,23,2]. For all the characteristics, 5 (five) measurements per slide were performed. The plots were represented by the average value obtained from the measurements of each characteristic.

2.4 Statistical Analysis

The results were submitted to analysis of variance by the F test ($p \leq 0.05$) and their means by the Tukey test at 5% of significance, according to [24].

3. RESULTS AND DISCUSSION

Tables 1 and 2 show the chlorophyll index and stomatal conductance of experiments with 1 and 2 buds at 90 days after installation of the experiment.

For the characteristic chlorophyll index, a significant effect was found only on the position of the yolk on the sugarcane stem in the experiment with 1 yolk at 90 days after planting. It was observed that gemstones originating from the base and medium showed higher averages. These results were not expected due to the more intense activity in the younger tissues present in the sugarcane culms. This effect was not observed in the experiment with 2 buds, showing that the factors studied were not significant.

It is possible to observe in Tables 1 and 2 the mean values of stomatal conductance in the experiments with 1 and 2 buds at 90 days after planting the sugarcane buds, no significant effect was found for this characteristic. Tables 3 and 4 show the mean limbal thickness values; thickness of the epidermis of the abaxial face; thickness of the epidermis of the adaxial face; and mesophyll thickness of the experiments with 1 and 2 buds at 90 days after installation of the experiment.

For the characteristic leaf blade thickness of the 1-yolk experiment at 90 days after planting, a significant effect on the interaction between the factors was found. For the buds at the culmination, there was no significant difference in relation to the control when ethephon was applied. For the gems in the middle of the stem, there was a significant difference in relation to the control. For the stem base buds, the application of ethephon in the planting groove had significantly lower mean values (Table 3).

Table 1. Mean values of chlorophyll index and stomatal conductance of the experiment with 1 yolk at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Chlorophyll index				
Apex	16.50	15.80	15.42	15.90 b
Medium	22.96	16.98	17.84	19.26 ab
Base	22.64	20.82	19.46	20.97 a
MFA(F2)	20.70 A	17.86 A	17.57 A	
CV (%)	22.00			
DMS F1**e F2	3.67			
DMS F1xF2	-			
Stomatal conductance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Apex	171.60	166.90	198.70	179.06 a
Medium	219.10	210.30	185.20	204.86 a
Base	184.20	211.60	214.70	203.50 a
MFA(F2)	191.63 A	196.26 A	199.53 A	
CV (%)	37.31			
DMS F1e F2	65.14			
DMS F1xF2	-			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

Table 2. Mean values of the chlorophyll index and stomatal conductance of the experiment with 2 buds at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Chlorophyll index				
Apex	14.16	13.32	12.83	13.43 a
Medium	10.73	14.78	12.59	12.70 a
Base	9.81	14.11	12.45	12.12 a
MFA(F2)	11.56 A	14.07 A	12.62 A	
CV (%)	24.76			
DMS F1e F2	2.8156			
DMS F1xF2	-			
Stomatal conductance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Apex	286.10	189.20	346.50	273.93 a
Medium	294.30	243.60	333.10	290.33 a
Base	270.20	333.70	270.75	291.55 a
MFA(F2)	283.53 A	255.50 A	316.78 A	
CV (%)	29.47			
DMS F1e F2	74.9491			
DMS F1xF2	-			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

Table 3. Limb thickness mean values; thickness of the epidermis of the abaxial face; thickness of the epidermis of the adaxial face; mesophyll thickness; of the experiment with 1 yolk at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Limb thickness (µm)				
Apex	268.90bA	234.11cA	222.65bA	241.88 c
Medium	285.28bB	359.32bAB	382.49aA	342.36 b
Base	451.24aA	463.10aA	270.88bB	395.07 a
MFA(F2)	335.14 AB	352.18 A	292.00 B	
CV (%)	14.92			
DMS F1**e F2**	43.42			
DMS F1xF2**	75.21			
Thickness of the epidermis of the abaxial face (µm)				
Apex	16.20	12.91	14.82	14.64 a
Medium	15.44	14.17	13.03	14.21 a
Base	15.77	19.61	14.13	16.50 a
MFA(F2)	15.80 A	15.56 A	13.99 A	
CV (%)	22.80			
DMS F1e F2	3.08			
DMS F1xF2	-			
Thickness of the epidermis of the adaxial face (µm)				
Apex	18.53	16.32	15.93	16.93 a
Medium	16.00	13.52	12.72	14.08 a
Base	12.77	19.63	15.80	16.07 a
MFA(F2)	15.77 A	16.49 A	14.82 A	
CV (%)	27.70			
DMS F1e F2	3.87			
DMS F1xF2	-			
Mesophyll thickness (µm)				
Apex	228.06bA	195.95bA	263.44aA	229.15 b
Medium	281.79bA	354.81aA	324.09aA	320.23 a
Base	422.79aA	421.41aA	265.83aB	370.01 a
MFA(F2)	310.88 A	324.06 A	284.45 A	
CV (%)	18.78			
DMS F1**e F2	51.32			
DMS F1xF2**	88.89			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level (0.01 ≤ p < 0.05). ** Significant at the 1% probability level (p < 0.01). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

For the same characteristic thickness of the leaf limb, in the experiment with 2 buds, a significant effect was found between the position of the yolk on the sugarcane stem and the use of ethephon (Table 4). It showed that gemstones originating from the middle of the stem and applying the ethephon in the plant to the 15 days before the planting or the furrow of planting, presented better results.

Mcqualter et al. [25] report the importance of foliar limb cells due to the presence of β-ketothiolase that act in the production of polymer

in mesophilic plastids that maximizes the yield of these organelles.

For the characteristics of the abaxial and adaxial epidermis of the experiments with 1 and 2 buds, no significant effects were found between the factors (Tables 3 and 4). Studies on the effect of gibberellin and ethephon by [26] reported that ethephon at high doses of 1200 mg L⁻¹ provided epidermal changes in leaves of young plants. According to [14] observed changes in leaf epidermal structures of sugarcane, which was not verified in this study.

Table 4. Limb thickness mean values; thickness of the epidermis of the abaxial face; thickness of the epidermis of the adaxial surface and mesophyll thickness of the experiment with 2 buds at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Limb thickness (μm)				
Apex	310.11	453.66	408.29	390.69 ab
Medium	344.28	419.41	425.43	396.37 a
Base	276.43	307.71	383.75	322.63 b
MFA(F2)	310.27 B	393.59 A	405.82 A	
CV (%)	21.82			
DMS F1*e F2**	71.9535			
DMS F1xF2	-			
Thickness of the epidermis of the abaxial face (μm)				
Apex	17.98	17.07	20.31	18.46 a
Medium	15.75	18.41	15.94	16.70 a
Base	19.15	17.34	20.35	18.95 a
MFA(F2)	17.63 A	17.61 A	18.87 A	
CV (%)	26.70			
DMS F1e F2	4.2934			
DMS F1xF2	-			
Thickness of the epidermis of the adaxial face (μm)				
Apex	20.34	21.08	21.47	20.96 a
Medium	16.60	19.86	17.31	17.92 a
Base	18.92	17.10	21.46	19.16 a
MFA(F2)	18.62 A	19.34 A	20.08 A	
CV (%)	25.83			
DMS F1e F2	4.4575			
DMS F1xF2	-			
Mesophyll thickness (μm)				
Apex	289.83	396.45	357.31	347.86 a
Medium	307.91	371.05	379.18	352.71 a
Base	233.73	264.72	327.97	275.47 b
MFA(F2)	277.15 B	344.07 AB	354.82 A	
CV (%)	24.60			
DMS F1*e F2*	71.3694			
DMS F1xF2	-			

Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author

It is noteworthy that the epidermis function as external coating of the vegetable, which protects its internal tissues. Because it is a simple layer of cells and juxtaposed, this characteristic helps in the process of regeneration of this tissue when subjected to some mechanical or chemical damage [27,8]. According to [12], when studying plants of the family Orchidaceae, they affirm that the anatomical characteristics of the epidermis in the plant may be involved with different adaptations to the different environments during evolutionary process. [28], when studying water stress in sugarcane, concluded that after stress an increase in the thickness of leaf epidermis

was observed, showing resistance of the plant to avoid the loss of water by transpiration.

For the characteristic mesophilic thickness at 90 days after planting in the experiment with 1 and 2 buds, a significant interaction effect was found between the studied factors. It was verified that the gem of the apex and the middle of the stem with application of ethephon in the plant and in the furrow presented better results. For yolk of the stem base, the best means are found when the ethephon was applied in the plant 15 days before planting is statistically equal to the control that was not applied.

Basal gemstones present a greater accumulation of sucrose, which can make a source of glucose, later converting energy into tissues in full cell division. This process of sugar conversion requires a greater energy demand, that the plant stops investing in the growth of the aerial part and the speed of emergency as reported by [29,5].

In the experiment with 1 and 2 buds, this effect of interaction between the factors was not found as in the experiment with 2 buds for the characteristic thickness of the mesophyll. But a significant effect was found for the position

factors of the yolk and application of ethephon. Again the results found corroborate with the other data already discussed, in which gemstones from the apex and middle with the use of ethephon provided a greater thickness of the leaf blade of the sugar cane.

In Tables 5 and 6, the mean values of floematic vessel diameter are presented; diameter of the metaxilematic vessels; adaxial and abaxial cuticle thickness of the experiments with 1 and 2 buds at 90 days after the installation of the experiment.

Table 5. Mean values of flolem diameter; diameter of the metaxilematic vessels; abaxial and adaxial cuticle thickness of the experiment with 1 yolk at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Flolem diameter (µm)				
Apex	7.12	6.42	6.06	6.53 b
Medium	7.68	6.99	7.13	7.27 ab
Base	7.23	9.06	6.93	7.74 a
MFA(F2)	7.34 A	7.49 A	6.71 A	
CV (%)	18.09			
DMS F1*e F2	1.15			
DMS F1xF2	-			
Diameter of the metaxilematic vessel (µm)				
Apex	34.07	33.86	33.56	33.83 a
Medium	29.12	38.31	38.71	35.34 a
Base	32.87	50.53	34.09	39.16 a
MFA(F2)	32.02 A	40.90 A	35.45 A	
CV (%)	28.49			
DMS F1e F2	9.17			
DMS F1xF2	-			
Abaxial cuticle thickness (µm)				
Apex	4.13 aA	3.95 bA	4.64 aA	4.24 b
Medium	4.45 aA	5.53 aA	5.24 aA	5.07 ab
Base	5.51 aAB	6.92 aA	4.84 aB	5.76 a
MFA(F2)	4.70 A	5.47 A	4.98 A	
CV (%)	20.11			
DMS F1**e F2	0.90			
DMS F1xF2*	1.56			
Adaxial cuticle thickness (µm)				
Apex	5.08	5.04	4.94	5.02 a
Medium	4.63	4.96	5.20	4.93 a
Base	5.55	6.17	5.24	5.65 a
MFA(F2)	5.08 A	5.39 A	5.12 A	
CV (%)	16.34			
DMS F1e F2	0.75			
DMS F1xF2	-			

Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. *Significant at the 5% probability level ($0.01 \leq p < 0.05$). **Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author

Table 6. Mean values of flolem diameter; diameter of the metaxilematic vessels; abaxial and adaxial cuticle thickness of the experiment with 2 buds at 90 days after the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Flolem diameter (µm)				
Apex	8.55 aA	7.44 bA	9.14 aA	8.38 a
Medium	8.31 aAB	10.21 aA	6.32 bB	8.28 a
Base	6.88 aA	6.87 bA	7.26 abA	7.00 a
MFA(F2)	7.91 A	8.17 A	7.57 A	
CV (%)	21.63			
DMS F1e F2	1.5216			
DMS F1xF2*	2.6356			
Diameter of the metaxilematic vessel (µm)				
Apex	34.66	41.41	42.74	39.60 a
Medium	34.56	36.71	36.56	35.94 a
Base	33.93	37.31	44.39	38.54 a
MFA(F2)	34.38 A	38.48 A	41.23 A	
CV (%)	26.92			
DMS F1e F2	9.1298			
DMS F1xF2	-			
Abaxial cuticle thickness (µm)				
Apex	5.40	6.39	6.54	6.11 a
Medium	4.79	5.99	5.48	5.42 a
Base	5.17	5.74	7.28	6.06 a
MFA(F2)	5.12 B	6.04 AB	6.43 A	
CV (%)	20.54			
DMS F1e F2*	1.0751			
DMS F1xF2	-			
Adaxial cuticle thickness (µm)				
Apex	5.95	7.05	7.21	6.74 a
Medium	5.41	6.11	6.11	5.88 a
Base	5.52	6.04	6.63	6.07 a
MFA(F2)	5.63 A	6.40 A	6.65 A	
CV (%)	24.18			
DMS F1e F2	1.3434			
DMS F1xF2	-			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. *Significant at the 5% probability level (0.01 ≤ p < 0.05). **Significant at the 1% probability level (p < 0.01). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

In Table 5 it is possible to observe a significant effect on the characteristic diameter of the phloem vessels in the experiment with 1 yolk at 90 days after planting. This result shows that gemstones from the base showed better results, no significant effect was found on the use of ethephon. However in the experiment with 2 buds, an interaction effect between the factors was found. It showed that, with the planting of gemstones at the apex, regardless of the use of the ethephon, they did not present a significant effect, but gemstones of the middle of the stem with application of the ethephon at the 15 days before the planting followed by the control that presented better diameters, in this way the data

of this experiments demonstrate the non-use of ethephon. For base gems it is indifferent whether or not to use the ethephon.

When the non-use of ethephon (control) is considered, the positions of the yolk on the stem do not present significant differences. With the application of the ethephon in the plant at 15 days before planting, the buds of the middle of the stem have better diameters of the floematic vessels, however, when we apply the ethephon in the groove, gemstones of the apex and base present better answers for this characteristic.

The use of ethephon did not influence the diameter of phloem vessels. The action of ethylene within plants is not well defined, especially its metabolic routes as described in the scheme proposed by [13]. The phloem vessels, because they are a tissue that acts directly on the translocation of metabolized sap from the leaves to other regions in the plant [2,30] which did not occur in a more pronounced way.

In the experiments with 1 and 2 buds at 90 days after the installation of the experiment, no significant effect was found between the effects studied for the characteristic diameter of the metaxilematic vessels.

Pereira [31], when studying the effects of phytohormones in sugarcane, observed that there was an increase in the number of metaxilems in the vascular bundles of roots in young plants, which may have contributed to the greater survival of tillers, and could have provided in a higher number of crops. All the way to the characteristic thickness of the abaxial cuticle in the experiment with 1 yolk at 90 days after planting, it is possible to observe a significant effect of interaction between the factors. When we consider stem apex buds the ethephon application effect did not differ with the control, in the same way it occurred with the stalk medium buds.

For gemstones from the base of the stem the application of ethephon at 15 days before planting presented better results together with the control. In this way it is recommended not to use ethephon due to the economic values of the applied product. When considering non-use of the product (control) the position of the buds do not differ statistically. For the application of ethephon in the plant to the 15 days before the planting of the gems is recommended the use of the gems of the middle and base of the cane of sugar cane. In the application situation of the ethephon in the planting groove, the positions of the yolk on the stem do not present significant difference.

In the experiment with 2 buds at 90 days after planting, a significant effect was found only on the application factor of the ethephon in the characteristic thickness of the abaxial cuticle, highlighting the way the ethephon was applied in the planting groove and then applied to the plant at 15 days before planting. planting of the gemstones that presented better abaxial cuticle thickness.

For the characteristic thickness of the adaxial cuticle in experiments with 1 and 2 buds at 90 days after planting, no significant effect was found between the factors studied (Table 6). The data corroborate that the chemical composition of the cuticle may vary, but with predominance of cutin and wax. Cutin is an insoluble biopolyester that has a high degree of cross-linking between the long chain hydroxyl fatty acids composing them, while the wax is embedded in the polymer or deposited on the outside of the cuticle.

Layer or plaque deposition may occur; the wax acts as a protective barrier against water loss through perspiration; the action of pathogens; solar radiation and leaf absorption of chemicals and contaminants, which corroborates [32,33]. Even [34] stated that doses above 300 mg L⁻¹ cause short-term growth of stem blades, but in the long run increased silicon accumulation in the epidermal structures and provides a greater leaf expansion.

The mean values of sheath cell diameter and distance between vascular bundles in the leaf limbus, from the experiments with 1 and 2 buds at 90 days after installation of the experiment, are presented in Tables 7 and 8.

For the characteristic sheath cell diameter of the experiments with 1 and 2 buds at 90 days after planting, no significant effect was found between the factors studied. The biochemical reactions of carbon fixation by C₄ plants occur in the cells of the sheath [35]; due to the higher carbon concentration present within their cytoplasm, and have well-developed sheath cells that provides greater carbon fixation through the photosynthesis photochemical processes together with the action of the rubisco molecule [1,36] which may explain the data obtained [10].

In Tables 7 and 8, it can be observed that for the characteristic distance between vascular bundles in the leaf limbus, in experiments with 1 and 2 buds at 90 days after planting, no significant effect was found between the factors. For [2] the vascular vulnerability index of the plant is inversely proportional to the distance of the vascular bundles of the leaves, when it presents greater distance between the bundles, less vascular vulnerability. The mean values found for the distance between the vascular bundles are similar to those found by [27] when studying leaf morphology of sugarcane cultivars.

Table 7. Mean values of sheath cell diameter and distance between vascular bundles in leaf blade, from the experiment with 1 yolk at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Sheath cell diameter (μm)				
Apex	18.70	16.48	18.34	17.84 a
Medium	17.78	15.88	15.32	16.32 a
Base	16.38	26.08	18.77	20.41 a
MFA(F2)	17.62 A	19.48 A	17.47 A	
CV (%)	27.81			
DMS F1e F2	4.51			
DMS F1xF2	-			
Distance between vascular bundles in leaf (μm)				
Apex	43.04	46.62	48.48	46.05 a
Medium	39.56	48.14	43.61	43.67 a
Base	50.14	43.90	41.17	45.07 a
MFA(F2)	44.25 A	46.22 A	44.42 A	
CV (%)	22.26			
DMS F1e F2	8.92			
DMS F1xF2	-			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

Table 8. Mean values of sheath cell diameter and distance between vascular bundles in leaf limb, from the experiment with 2 buds at 90 days after the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Sheath cell diameter (μm)				
Apex	20.98	23.22	25.16	23.12 a
Medium	17.89	17.95	20.82	18.88 a
Base	21.46	21.67	25.88	23.00 a
MFA(F2)	20.11 A	20.95 A	23.95 A	
CV (%)	25.74			
DMS F1e F2	4.9740			
DMS F1xF2	-			
Distance between vascular bundles in leaf (μm)				
Apex	51.97	52.23	65.83	56.68 a
Medium	46.12	53.86	53.81	51.27 a
Base	57.14	54.47	53.12	54.91 a
MFA(F2)	51.74 A	53.52 A	57.59 A	
CV (%)	17.69			
DMS F1e F2	8.5653			
DMS F1xF2	-			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

In Tables 9 and 10, the mean values of the number of stomata per mm^2 in the abaxial face are presented; number of stomata per mm^2 in the adaxial face; number of abaxial epidermal cells per mm^2 and number of adaxial epidermal cells per mm^2 from the experiments with 1 and 2

buds at 90 days after installation of the experiment.

For the characteristic number of stomata per mm^2 , in the experiment with 1 yolk at 90 days after planting, a significant effect was found only

on the application factor of the ethephon, shows that the application in the plant at 15 days before planting and followed showed better results. Due to the statistical equality between the factors there was no effect of ethephon application.

In the experiment with 2 buds, a significant effect of interaction between the factors was found. When the position of the yolk at the top of the stem is considered, the best results were found when application of ethephon occurs in the planting groove; as for the position of the yolk in the middle of the stem, no effect was found regarding the application of the ethephon, for the yolk of the stem base it was better to use the

ethephon when applied to the plant 15 days before planting.

No significant effect of the position of the gem was found on the stem; but when application of ethephon occurs in the plant 15 days before planting, gemstones originating from the apex and base present a higher number of stomata per mm² in the abaxial epidermis; however, already for the use of ethephon in the planting groove, it was better in the sugarcane culms. For the characteristic number of stomata per mm² in the adaxial epidermis, in the 1-yolk experiment, a significant effect was found for interaction between the factors (Table 9).

Table 9. Mean values of the number of stomata per mm² in the abaxial face; number of stomata per mm² in the adaxial face; number of abaxial epidermal cells per mm² and number of adaxial epidermal cells per mm² from the 1-yolk experiment at 90 days after the experiment was set up

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Number of stomata in the abaxial face (mm²)				
Apex	179.91	180.13	160.60	173.54 a
Medium	166.17	176.23	159.93	167.44 a
Base	155.38	180.22	166.85	167.49 a
MFA(F2)	167.16 AB	178.86 A	162.46 B	
CV (%)	9.01			
DMS F1e F2*	13.62			
DMS F1xF2	-			
Number of stomata in the adaxial face (mm²)				
Apex	90.31bA	99.90aA	103.17aA	97.79 a
Medium	100.97abA	88.17aA	95.47aA	94.87 a
Base	112.75aA	93.85aA	70.13bB	92.24 a
MFA(F2)	101.34 A	93.97 A	89.59 A	
CV (%)	14.19			
DMS F1e F2	12.01			
DMS F1xF2**	20.80			
Number of abaxial epidermal cells (mm²)				
Apex	297.63 aAB	286.83 aB	356.03 aA	313.50 a
Medium	329.43 aA	316.50 aAB	263.77 bB	303.23 a
Base	294.82 aA	286.30 aA	323.94 abA	301.69 a
MFA(F2)	307.30 A	296.55 A	314.58 A	
CV (%)	13.08			
DMS F1e F2	35.6927			
DMS F1xF2**	61.8216			
Number of adaxial epidermal cells (mm²)				
Apex	106.57	90.36	98.68	98.54 a
Medium	91.43	93.21	88.15	90.93 a
Base	88.07	67.44	81.92	79.14 b
MFA(F2)	95.36 A	83.67 B	89.58 AB	
CV (%)	13.26			
DMS F1**e F2*	10.5867			
DMS F1xF2	-			

Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author

Table 10. Mean values of the number of stomata per mm² in the abaxial face; number of stomata per mm² in the adaxial face; number of abaxial epidermal cells per mm² and number of adaxial epidermal cells per mm² from the experiment with 2 buds at 90 days after the experiment was set up

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Number of stomata in the abaxial face (mm²)				
Apex	187.90 aB	190.36 aB	249.12 aA	209.13 a
Medium	167.94 aA	152.68 bA	160.73 cA	160.45 c
Base	173.55 aB	211.70 aA	186.02 bB	190.42 b
MFA(F2)	176.46 B	184.91 B	198.63 A	
CV (%)	7.64			
DMS F1**e F2**	12.7140			
DMS F1xF2**	22.0214			
Number of stomata in the adaxial face (mm²)				
Apex	105.32 bA	103.66 aA	116.26 aA	108.41 a
Medium	110.27 abA	93.59 aA	98.56 abA	100.81 a
Base	125.74 aA	103.39 aB	81.77 bC	103.63 a
MFA(F2)	113.77 A	100.21 B	98.86 B	
CV (%)	11.96			
DMS F1e F2**	11.12594			
DMS F1xF2**	19.2707			
Number of abaxial epidermal cells (mm²)				
Apex	423.47 aA	358.15 aB	383.49 aAB	388.37 a
Medium	359.28 bA	349.92 aA	326.14 bA	345.11 b
Base	299.51 cB	361.25 aA	338.06 abAB	332.94 b
MFA(F2)	360.75 A	356.44 A	349.23 A	
CV (%)	9.89			
DMS F1**e F2	31.3581			
DMS F1xF2**	54.3138			
Number of adaxial epidermal cells (mm²)				
Apex	222.41	235.08	199.15	218.88 a
Medium	240.64	215.50	215.17	223.77 a
Base	209.65	226.82	230.59	222.35 a
MFA(F2)	224.23 A	225.80 A	214.97 A	
CV (%)	17.21			
DMS F1e F2	34.0192			
DMS F1xF2	-			

Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author

When considering the position of the yolk on the stem, it was possible to observe that the gemstones originating from the apex and the middle of the cane sugarcane had no effect on the use of ethephon; but for yolk of the stem base and with application of ethephon in the plant at 15 days before planting together with the control presented better averages of number of stomata per mm² in the adaxial epidermis.

Without the use of ethephon when base gems were used followed by the yolk of the stalk medium, they presented better values for

stomata per mm² in the adaxial epidermis at 90 days after planting. When the ethephon was applied to plant 15 before planting, no effect was found on the position of the yolk on the sugarcane stem; however, when the ethephon was used in the planting groove, the gemstones originating from the apex and the middle of the stem had better means for the said characteristic.

In the experiment with 2 buds, the significant effect on the interaction between the factors was also found (Table 10). As for the position of the

yolk on the stalk, the yolks of the apex and stalk medium did not suffer from the application of ethephon; However, for the stem base buds the control presented better means for the characteristic number of stomata per mm^2 in the adaxial epidermis at 90 days after planting.

Without the use of ethephon showed that base gems followed by the middle of the sugar cane stalk; presented better means for the same characteristic number of abaxial epidermal cells. When the ethephon was applied to the plant at 15 days before planting, the position of the yolk did not differ statistically. Although, overall, it provided homogeneity in the characteristic number of stomata per mm^2 in the adaxial epidermis. This same behavior was not found when applied ethephon in the planting groove, demonstrating that gemstones of the apex followed by the medium of sugarcane stalks showed better means for the characteristic in question.

For the characteristic number of abaxial epidermal cells per mm^2 in the 1-yolk experiment at 90 days after planting, a significant effect of interaction between the factors was found (Table 9). It was verified that, when using the gem of the apex with application of ethephon in the groove followed by the control, presented better results for the featured feature. This same behavior of the data was not found with the yolk of the stem medium, where it is possible to observe that the control treatment followed by the application of ethephon in the plant presented better results. And base gem was not found the significant effect between the ethephon application factor.

For the same featured feature, the control and with application of ethephon in the plant at 15 days before planting was not found the significant effect as to the position of the yolk on the high. When ethephon was applied to the planting groove, the position of the yolk on the stem was significant, showing that gemstones originating from the apex followed by the base of the stem showed a higher number of abaxial epidermal cells per mm^2 , these results corroborate with the information of [29].

The same significant effect of interaction between the factors was observed in the experiment with 2 buds, for the characteristic number of abaxial epidermal cells per mm^2 at 90 days after planting. When the yolk of the stem was used together with the control and followed by the application of the ethephon in the groove,

better means are observed. As for the origin of the gem in the middle of the stem was not influenced by the use of the ethephon. However, for stem base buds together with application to the plant followed by application to the planting groove presented better means for the characteristic in question.

The non-application of the ethephon, that is, the control and with gemstones of the apex presented better means for the characteristic number of epidermal cells abaxial. With the application of ethephon in the plant at 15 days after planting again the position of the buds, no significant effect was found on the highlighted feature. However, for the application of ethephon in the planting groove, again, gemstones of the apex presented higher averages.

For the characteristic number of adaxial epidermal cells per mm^2 in the 1-yolk experiment, significant effect was found on the yolk position on the stem and on the application mode of ethephon as shown in Table 9. It is possible to observe that gems the apex and the middle of the stem presented higher averages; and also the control and with application of the ethephon in the planting groove respectively presented better results, therefore, the data did not show response with the use of ethephon, it is recommended to repeat new work. However, in the experiment with 2 buds, no significant effect was found among the factors studied.

The characteristics number of cells and stomata in the epidermis are directly related to the characteristic stomatal density as proposed by [2]. The greater number of stomata in relation to the number of epidermal cells has a higher density, which may contribute to a greater efficiency in the absorption of carbon by the leaves.

The mean values of the stomatal functionality or relation of the abaxial face; stomatal functionality or relation of the adaxial face; stomatal index of the abaxial face; and stomatal index of the adaxial surface of experiments with 1 and 2 buds at 90 days after installation of the experiment are presented in Tables 11 and 12.

For the characteristic stomatal functionality of the abaxial face of the 1-yolk experiment at 90 days after planting, no significant effect was found between the factors (Table 11); but it is possible to observe an interaction effect between the factors in the experiment with 2 buds (Table 12).

Table 11. Mean values of the stomatal functionality or relation of the abaxial face; stomatal functionality or relation of the adaxial face; stomatal index of the abaxial surface and stomatal index of the adaxial surface of the experiment with 1 yolk at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Stomatal functionality or relation of the abaxial face				
Apex	2.10	2.30	2.13	2.18 a
Medium	2.03	2.07	2.18	2.10 a
Base	2.07	2.28	2.14	2.16 a
MFA(F2)	2.07 A	2.22 A	2.15 A	
CV (%)	8.00			
DMS F1e F2	0.1533			
DMS F1xF2	-			
Stomatal functionality or relation of the adaxial face				
Apex	2.07	2.11	2.05	2.08 a
Medium	2.23	1.91	2.10	2.08 a
Base	2.05	1.98	2.03	2.02 a
MFA(F2)	2.11 A	2.00 A	2.02 A	
CV (%)	9.64			
DMS F1e F2	0.1772			
DMS F1xF2	-			
Stomatal index of the abaxial surface				
Apex	37.78 aA	38.93 aA	31.23 bB	35.98 a
Medium	33.81 aA	36.08 aA	37.77 aA	35.88 a
Base	34.71 aA	38.61 aA	33.95 abA	35.76 a
MFA(F2)	35.43 AB	37.87 A	34.32 B	
CV (%)	10.68			
DMS F1*e F2*	3.41			
DMS F1xF2*	5.91			
Stomatal index of the adaxial surface				
Apex	38.02	33.47	39.63	37.04 ab
Medium	39.57	43.14	37.74	40.15 a
Base	34.78	29.30	37.7	33.94 b
MFA(F2)	37.46 A	35.30 A	38.37 A	
CV (%)	14.18			
DMS F1*e F2	4.68			
DMS F1xF2	-			

*Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author*

In the experiment with 2 gems, when referring to the gemstones of the apex and the middle of the stem, along with the factor of application of ethephon, no significant effect was found for the ethephon factor; however, for stem base yolk with the use of ethephon or not in the planting groove, significant effect was found between them; showing that due to the economic value is unfeasible the use of it.

When the control was considered, that is, the non-use of ethephon with gemstones originating from the medium, then gem of the stem base,

presented better stomatal functionality of the abaxial face; when the ethephon application occurs, in the plant at 15 days before the planting with the yolk of the medium of the stem, better responses of said characteristic were demonstrated; for application of ethephon in the planting groove with base yolks and then medium shoot yolks presented better means for functionality.

No significant effect was found for the characteristic stomatal functionality of the adaxial face of experiments with 1 and 2 buds at 90 days

after planting. This result was not expected; due to the position of the epidermis on the sheet, it consequently received first the syrup of the ethephon applied, in this way greater effects of the active ingredient of the applied product in the epidermis of the adaxial face were expected.

It can be understood that the greater the stomatal functionality, the better the photosynthetic yields the vegetables, due to the

greater opening of the stomata, which proportionally gives rise to a greater gas exchange [2] of carbon in the green matter of the vegetable. These values are similar to those found by [37], when studying cassava cultivars with tolerance to water stress, in the same way [38,39] also studying cassava species, observed values similar to the other studies. This shows that even different species stomatal development are similar, and may exhibit the same index of

Table 12. Mean values of the stomatal functionality or relation of the abaxial face; stomatal functionality or relation of adaxial face; stomatal index of the abaxial surface and stomatal index of the adaxial surface of the experiment with 2 buds at 90 days after installation of the experiment

	Control	Plant/15DAP	Groove/Vases	MFP(F1)
Stomatal functionality or relation of the abaxial face				
Apex	2.02 bA	1.89 bA	2.06 bA	1.99 b
Medium	2.31 aA	2.30 aA	2.30 abA	2.30 a
Base	2.27 abA	1.87 bB	2.38 aA	2.17 a
MFA(F2)	2.20 A	2.02 B	2.24 A	
CV (%)	8.29			
DMS F1**e F2**	0.1595			
DMS F1xF2*	0.2764			
Stomatal functionality or relation of the adaxial face				
Apex	1.88	1.94	2.05	1.96 a
Medium	2.05	2.04	1.96	2.02 a
Base	1.94	2.07	2.04	2.02 a
MFA(F2)	1.96 A	2.02 A	2.02 A	
CV (%)	9.89			
DMS F1e F2	0.1765			
DMS F1xF2	-			
Stomatal index of the abaxial surface				
Apex	30.75 bC	34.61 aB	39.38 aA	34.91 a
Medium	31.86 bA	30.39 bA	33.43 bA	31.89 b
Base	36.68 aA	37.04 aA	35.67 abA	36.46 a
MFA(F2)	36.10 B	34.01 AB	36.16 A	
CV (%)	7.18			
DMS F1**e F2**	2.2056			
DMS F1xF2**	3.8202			
Stomatal index of the adaxial surface				
Apex	32.56 aA	29.47 aA	36.93 aA	32.99 a
Medium	31.69 aA	30.13 aA	31.96 abA	31.26 a
Base	37.66 aA	31.61 aAB	26.73 bB	33.00 a
MFA(F2)	33.97 A	30.41 A	31.87 A	
CV (%)	15.90			
DMS F1e F2	4.5480			
DMS F1xF2*	7.8774			

Note: Lowercase averages followed by the same letter in the column do not differ statistically from one another. Capital averages followed by the same letter on the line do not differ statistically from one another. * Significant at the 5% probability level ($0.01 \leq p < 0.05$). ** Significant at the 1% probability level ($p < 0.01$). MFA - Average of the application factor of the ethephon. MFP - Mean of the position factor of the yolk on the sugarcane stem. DAP - Days after application. Source: Prepared by the author

stomatal functionality. For the characteristic stomatal index of the abaxial face, we found significant interaction effects between the factors in the two experiments, with 1 and 2 buds at 90 days after planting.

In the experiment with 1 yolk, when the yolk was used together with and without the use of ethephon in the plant 15 days before planting, greater stomatal indices were found on the abaxial side of the cane leaf; but for gems from the middle and bottom of the cane sugar cane, no significant effect was found with the ethephon use factor. When the effect of the use of ethephon as a main factor is considered, the control and application of ethephon at 15 days before planting together with the positions of the yolks on the stem showed no significant effects. However, it is possible to observe a significant effect of the position of the yolk when the ethephon was used in the planting groove. In this way, the middle gemstones followed by the base of the sugarcane stem presented better stomatal indices on the abaxial surface (Table 11).

As for the interaction effect of the factors studied, in the experiment with 2 gems the same characteristic in question, that is, stomatal index in the abaxial face; the gemstones of the apex together with the application of ethephon in the planting groove presented higher averages, which did not occur with the middle and base sugarcane shoots. When the ethephon use factor is considered, in the control the medium and base gems presented better contents. However, for application of the ethephon in the plant at 15 days before planting and application in the groove the bud and base buds present higher averages for the stomatal index in the abaxial face.

In the experiment with 1 yolk, significant effect was only found in the position of the yolk on the stem, for the characteristic stomatal index on the adaxial side at 90 days after planting sugarcane (Table 11). It is observed that gem of the apex followed by gem of the stem showed better stomatal indices.

In the experiment with 2 buds, a significant effect of interaction between the factors was found (Table 12). It is observed that gemstones of the apex and the middle of sugarcane stalk with the use of ethephon did not alter the stomatal index on the adaxial side of the leaf. However, the stem base buds and control later with application of the ethephon in the plant presented better

stomatal indexes, in this way the non-use of the ethephon makes a more economic activity.

When considering the use of ethephon, the control together with the application of ethephon in the plant at 15 days before planting, a difference in the stomatal index was not observed due to the positions of the gems in the cane sugarcane. However, when the ethephon is applied to the planting groove, the gemstones originating from the apex and the middle of the stem presented higher stomatal indices on the adaxial side of the cane leaves.

The mean values found for stomatal index were lower than those found by [15], studying foliar morphological changes in sugarcane cultivars subjected to water stress. The authors point out that, in tolerant cultivars, water deficiency promoted less damage in the number of green leaves and leaf area, and also promoted an increase in the stomatal index [10]. According to [40], the stomatal density of a leaf occurs through the process of leaf growth and even its quantity in plant species, besides some external factors such as differences in light intensity and water availability affect this quantity.

4. CONCLUSIONS

The use of ethephon and positions of the seedlings in sugarcane stalks did not influence the Chlorophyll Index and Stomatal Conductance.

The use of ethephon in the plant 15 days before planting, together with seedlings from the apex followed by the medium of the canes of sugarcane, presented better results for the ultrastructural characteristics of sugarcane foliage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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