

1 **EFFECTS OF ETHEPHON ASSOCIATED WITH THE POSITION OF GEMS**
2 **ON THE PLUM OF SUGAR CANE IN THE INITIAL DEVELOPMENT OF**
3 **CULTURE - PART II**

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6
7 **Abstract** - The hormones are closely related to the emergence of gemstones
8 contained seedlings of sugarcane, at the time of planting of the stems. The
9 objective of this work was to evaluate the effects of the ethephon associated to
10 the position of gemstones in the cane of sugar cane in the initial development of
11 the culture. In March 2014, at the Rio Vermelho Plant, located in Junqueirópolis,
12 State of São Paulo, a cane plant with a sugar cane plant was selected for
13 seedlings with an approximate age of 11 months. Two areas with dimensions of
14 20x20 meters were demarcated. In one of the areas ethephon was applied. At
15 15 days after application, the seedlings containing 1 and 2 buds were collected
16 to compose two independent experiments. From the area where the product
17 was not applied, seedlings were removed for the control and application
18 treatments of ethephon in the planting groove in pots. The gems were sent to
19 the Faculty of Agrarian and Technological Sciences of the Paulista State
20 University "Júlio de Mesquita Filho" - from Dracena, State of São Paulo. The
21 seedlings came from the apex, middle and base of the canes of sugarcane. In
22 this way, the experimental design was in a 3x3 factorial scheme, that is, the
23 position of the seedlings in the canes of sugarcane and the modes of
24 application of ethephon. The use of ethephon and positions of the seedlings in
25 sugarcane stalks did not influence the Chlorophyll Index and Stomatal
26 Conductance. The use of ethephon in the plant 15 days before planting,
27 together with seedlings from the apex followed by the medium of the canes of
28 sugarcane, presented better results for the ultrastructural characteristics of
29 sugarcane foliage.

30
31 **Keywords:** Hormone. Ethephon. Morphology.

32
33 **INTRODUCTION**

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

39 The chlorophyll index is determined by the emission of a wavelength of
40 $\lambda=650\text{nm}$, this value is close to the wavelengths that stimulate chlorophyll
41 activity, while the emission of the wavelength $\lambda=940\text{nm}$ acts as an internal
42 reference in the leaf limy to compensate for differences in leaf thickness or
43 water content [4;5]. The photosynthetic efficiency can be estimated by the
44 concentration of chlorophyll pigments present in the leaves, becoming a tool for
45 the actual recommendation of the fertilization need [6]. As a result, the macro
46 and micromorphological modifications of each cultivar, as well as the effects
47 caused by them, should be increasingly studied in order to improve
48 understanding and the use of phyto-regulators makes it a strategy to control
49 foliar activities [7;8].

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

56 In this case, structural aspects help in understanding the mechanisms
57 that cause the injuries. However, it is important to point out that changes visible
58 to the naked eye are derived from changes in the structures of the dermal,
59 fundamental or vascular tissues of plants, making it necessary to have a
60 thorough knowledge of these changes caused by changes in the environment
61 [11]. The symptomatology is widely used to evaluate the damage caused by
62 biotic or abiotic factors [12].

63 Examples demonstrate the importance of the morphophysiological and
64 functional knowledge of the plants; stomatal changes were observed in the
65 leaves of roses with the use of ethylene [13], the use of low concentrations of
66 ethephon provided a momentary decrease in the tillering phase of the crop.

67 length and width of the leaves, but promoted differentiation of the vascular
68 bundles in the leaves, which provided greater efficiency in the transport of sap.

69 Ethylene resulted in a differentiation in the mesophyll cells with doses of
70 100 mg L^{-1} which provided an increase in the outer surface of the cells and
71 allowing a better distribution of the chloroplasts. There was an increase in the
72 number of chloroplasts. According to Li & Solomon 2003, these changes have
73 brought about a significant increase in the total photosynthetic area in the
74 mesophyll cell of sugarcane leaves. Using ethylene provided an acceleration in
75 the differentiation and an increase in the number of vessels floem and xylem
76 species, which provided greater efficiency in the transportation of sap in
77 sugarcane [14].

78 To know the foliar morphology, the functions of the vegetal tissues and
79 their possible modifications to the damages caused by the absence or presence
80 of nutrients and hormones can be decisive in the decision making regarding the
81 appropriate management to be employed in the sugar cane crop, as well as
82 predicting the losses estimated by not knowing these effects [15;7].

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

86

87 **MATERIAL AND METHODS**

88

89 *Obtaining sugarcane seedlings*

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

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

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

113

114 *Installing the experiment*

115 Fifteen days after the application of the ethephon in the field, the
116 experiments were started in an unprotected external environment at the FCAT -
117 Faculty of Agrarian and Technological Sciences of the "Júlio de Mesquita Filho"
118 State University, located in the city of Dracena, State of São Paulo, with
119 geographic coordinates 21°46'04"S and 51°55'41"W and altitude 396 m.

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

126 The results of the soil chemical analysis were: pH CaCl₂= 5.0; MO= 14 g
127 dm⁻³; P= 8.0 mg dm⁻³ (resin); K= 2.3 mmol dm⁻³ (resin); Ca= 7.0 mmol dm⁻³
128 (resin); Mg= 5.0 mmol dm⁻³ (resin); H+Al= 20 mmol dm⁻³; Al= zero mmol_c dm⁻³;
129 Base Sum= 14.3 mmol dm⁻³; CTC= 34.3 mmol dm⁻³; Base Saturation (V%)= 42;
130 Saturation Al (m%)= zero; S (SO₄⁻²)= 3.0 mg dm⁻³; Cu= 2.8 mg dm⁻³ (DTPA);
131 Fe= 19 mg dm⁻³ (DTPA); Zn= 1.3 mg dm⁻³ (DTPA); Mn= 16.5 mg dm⁻³ (DTPA);
132 B= 0.14 mg dm⁻³ (Hot water); Clay= 75 g kg⁻¹; Silt= 33 g kg⁻¹ and Total sand=
133 893 g kg⁻¹ [16;17].

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

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

148 To determine the positions of the gems on the stalks were counted all of
149 the high nodes and dividing by three. In this way, the three parts of the stem
150 were obtained, being an apical region; median and baseline. For the stems that
151 presented odd numbers of nodes, we considered the basal third with the largest
152 number.

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

157

158 *The evaluations*

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

165 All fragments of plant tissues received the pertinent procedures for
166 dehydration, diaphanization, inclusion and embedding and with the help of a
167 microtome, where cross sections of 8 µm were performed on each tissue

168 fragment. The slides were observed in an optical microscope with a camera
 169 coupled to perform the measurements of the histological variables through the
 170 analysis program, calibrated with a microscopic ruler at the same magnification
 171 [20], where limbal thickness; thickness of the epidermis of the abaxial face;
 172 thickness of the epidermis of the adaxial face; mesophyll thickness; flolem
 173 diameter; diameter of the metaxilematic vessels; abaxial cuticle thickness;
 174 adaxial cuticle thickness; diameter of the cells of the sheath and distance
 175 between the vascular bundles in the leaf blade, according to [2].

176 In the collected fragments, the impression was also made on the
 177 epidermal faces using cyanoacrylate ester [21], where the following
 178 characteristics were observed: number of stomata per mm² on the abaxial;
 179 number of stomata per mm² in the adaxial face; number of abaxial epidermal
 180 cells per mm²; number of adaxial epidermal cells per mm²; stomatal functionality
 181 of the abaxial face; stomatal functionality or relation of the adaxial face; and the
 182 stomatal index of the adaxial face according to [22;23;2]. For all the
 183 characteristics, 5 (five) measurements per slide were performed. The plots were
 184 represented by the average value obtained from the measurements of each
 185 characteristic.

186

187 *Statistical analyzes*

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

190

191 **RESULTS AND DISCUSSIONS**

192

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

195

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
Medium	219.10
Base	184.20
MFA(F2)	191.63 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.

196

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.

197

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

205 It is possible to observe in Tables 1 and 2 the mean values of stomatal
 206 conductance in the experiments with 1 and 2 buds at 90 days after planting the
 207 sugarcane buds, no significant effect was found for this characteristic. Tables 3
 208 and 4 show the mean limbal thickness values; thickness of the epidermis of the
 209 abaxial face; thickness of the epidermis of the adaxial face; and mesophyll
 210 thickness of the experiments with 1 and 2 buds at 90 days after installation of
 211 the experiment.
 212

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 ($0.01 \leq p < 0.05$). ** 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 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.

214

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

222 For the same characteristic thickness of the leaf limb, in the experiment
 223 with 2 buds, a significant effect was found between the position of the yolk on

224 the sugarcane stem and the use of ethephon (Table 4). It showed that
225 gemstones originating from the middle of the stem and applying the ethephon in
226 the plant to the 15 days before the planting or the furrow of planting, presented
227 better results.

228 [25] report the importance of foliar limb cells due to the presence of β -
229 ketothiolase that act in the production of polymer in mesophilic plastids that
230 maximizes the yield of these organelles.

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

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

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

254 Basal gemstones present a greater accumulation of sucrose, which can
255 make a source of glucose, later converting energy into tissues in full cell
256 division. This process of sugar conversion requires a greater energy demand,

257 that the plant stops investing in the growth of the aerial part and the speed of
 258 emergency as reported by [29;5].

259 In the experiment with 1 and 2 buds, this effect of interaction between the
 260 factors was not found as in the experiment with 2 buds for the characteristic
 261 thickness of the mesophyll. But a significant effect was found for the position
 262 factors of the yolk and application of ethephon. Again the results found
 263 corroborate with the other data already discussed, in which gemstones from the
 264 apex and middle with the use of ethephon provided a greater thickness of the
 265 leaf blade of the sugar cane.

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

270

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.

271

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 ($0.01 \leq p < 0.05$). **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.

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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

276 better results, no significant effect was found on the use of ethephon. However
277 in the experiment with 2 buds, an interaction effect between the factors was
278 found. It showed that, with the planting of gemstones at the apex, regardless of
279 the use of the ethephon, they did not present a significant effect, but gemstones
280 of the middle of the stem with application of the ethephon at the 15 days before
281 the planting followed by the control that presented better diameters, in this way
282 the data of this experiments demonstrate the non-use of ethephon. For base
283 gems it is indifferent whether or not to use the ethephon.

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

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

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

299 [31], when studying the effects of phytohormones in sugarcane, observed
300 that there was an increase in the number of metaxilems in the vascular bundles
301 of roots in young plants, which may have contributed to the greater survival of
302 tillers, and could have provided in a higher number of crops. All the way to the
303 characteristic thickness of the abaxial cuticle in the experiment with 1 yolk at 90
304 days after planting, it is possible to observe a significant effect of interaction
305 between the factors. When we consider stem apex buds the ethephon
306 application effect did not differ with the control, in the same way it occurred with
307 the stalk medium buds.

308 For gemstones from the base of the stem the application of ethephon at
309 15 days before planting presented better results together with the control. In this

310 way it is recommended not to use ethephon due to the economic values of the
 311 applied product. When considering non-use of the product (control) the position
 312 of the buds do not differ statistically. For the application of ethephon in the plant
 313 to the 15 days before the planting of the gems is recommended the use of the
 314 gems of the middle and base of the cane of sugar cane. In the application
 315 situation of the ethephon in the planting groove, the positions of the yolk on the
 316 stem do not present significant difference.

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

323 For the characteristic thickness of the adaxial cuticle in experiments with
 324 1 and 2 buds at 90 days after planting, no significant effect was found between
 325 the factors studied (Table 6). The data corroborate that the chemical
 326 composition of the cuticle may vary, but with predominance of cutin and wax.
 327 Cutin is an insoluble biopolyester that has a high degree of cross-linking
 328 between the long chain hydroxyl fatty acids composing them, while the wax is
 329 embedded in the polymer or deposited on the outside of the cuticle.

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

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

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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.

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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.

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342 For the characteristic sheath cell diameter of the experiments with 1 and
 343 2 buds at 90 days after planting, no significant effect was found between the
 344 factors studied. The biochemical reactions of carbon fixation by C4 plants occur
 345 in the cells of the sheath [35]; due to the higher carbon concentration present
 346 within their cytoplasm, and have well-developed sheath cells that provides

347 greater carbon fixation through the photosynthesis photochemical processes
 348 together with the action of the rubisco molecule [1;36] which may explain the
 349 data obtained [10].

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

358 In Tables 9 and 10, the mean values of the number of stomata per mm²
 359 in the abaxial face are presented; number of stomata per mm² in the adaxial
 360 face; number of abaxial epidermal cells per mm² and number of adaxial
 361 epidermal cells per mm² from the experiments with 1 and 2 buds at 90 days
 362 after installation of the experiment.

363

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
Medium	91.43
Base	88.07
MFA(F2)	95.36 A
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.

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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

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366 For the characteristic number of stomata per mm², in the experiment with
367 1 yolk at 90 days after planting, a significant effect was found only on the
368 application factor of the ethephon, shows that the application in the plant at 15
369 days before planting and followed showed better results. Due to the statistical
370 equality between the factors there was no effect of ethephon application.

371 In the experiment with 2 buds, a significant effect of interaction between
372 the factors was found. When the position of the yolk at the top of the stem is
373 considered, the best results were found when application of ethephon occurs in
374 the planting groove; as for the position of the yolk in the middle of the stem, no
375 effect was found regarding the application of the ethephon, for the yolk of the
376 stem base it was better to use the ethephon when applied to the plant 15 days
377 before planting.

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

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

392 Without the use of ethephon when base gems were used followed by the
393 yolk of the stalk medium, they presented better values for stomata per mm² in
394 the adaxial epidermis at 90 days after planting. When the ethephon was applied
395 to plant 15 before planting, no effect was found on the position of the yolk on
396 the sugarcane stem; however, when the ethephon was used in the planting

397 groove, the gemstones originating from the apex and the middle of the stem
398 had better means for the said characteristic.

399 In the experiment with 2 buds, the significant effect on the interaction
400 between the factors was also found (Table 10). As for the position of the yolk on
401 the stalk, the yolks of the apex and stalk medium did not suffer from the
402 application of ethephon; However, for the stem base buds the control presented
403 better means for the characteristic number of stomata per mm^2 in the adaxial
404 epidermis at 90 days after planting.

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

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

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

430 The same significant effect of interaction between the factors was
431 observed in the experiment with 2 buds, for the characteristic number of abaxial
432 epidermal cells per mm^2 at 90 days after planting. When the yolk of the stem
433 was used together with the control and followed by the application of the
434 ethephon in the groove, better means are observed. As for the origin of the gem
435 in the middle of the stem was not influenced by the use of the ethephon.
436 However, for stem base buds together with application to the plant followed by
437 application to the planting groove presented better means for the characteristic
438 in question.

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

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

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

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

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.

466

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

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

477 When the control was considered, that is, the non-use of ethephon with
 478 gemstones originating from the medium, then gem of the stem base, presented
 479 better stomatal functionality of the abaxial face; when the ethephon application
 480 occurs, in the plant at 15 days before the planting with the yolk of the medium of
 481 the stem, better responses of said characteristic were demonstrated; for

482 application of ethephon in the planting groove with base yolks and then medium
483 shoot yolks presented better means for functionality.

484 No significant effect was found for the characteristic stomatal functionality
485 of the adaxial face of experiments with 1 and 2 buds at 90 days after planting.
486 This result was not expected; due to the position of the epidermis on the sheet,
487 it consequently received first the syrup of the ethephon applied, in this way
488 greater effects of the active ingredient of the applied product in the epidermis of
489 the adaxial face were expected.

490 It can be understood that the greater the stomatal functionality, the better
491 the photosynthetic yields the vegetables, due to the greater opening of the
492 stomata, which proportionally gives rise to a greater gas exchange [2] of carbon
493 in the green matter of the vegetable. These values are similar to those found by
494 [37], when studying cassava cultivars with tolerance to water stress, in the
495 same way [38;39] also studying cassava species, observed values similar to the
496 other studies. This shows that even different species stomatal development are
497 similar, and may exhibit the same index of stomatal functionality. For the
498 characteristic stomatal index of the abaxial face, we found significant interaction
499 effects between the factors in the two experiments, with 1 and 2 buds at 90
500 days after planting.

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

512 As for the interaction effect of the factors studied, in the experiment with
513 2 gems the same characteristic in question, that is, stomatal index in the abaxial
514 face; the gemstones of the apex together with the application of ethephon in the
515 planting groove presented higher averages, which did not occur with the middle

516 and base sugarcane shoots. When the ethephon use factor is considered, in the
517 control the medium and base gems presented better contents. However, for
518 application of the ethephon in the plant at 15 days before planting and
519 application in the groove the bud and base buds present higher averages for
520 the stomatal index in the abaxial face.

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

525 In the experiment with 2 buds, a significant effect of interaction between
526 the factors was found (Table 12). It is observed that gemstones of the apex and
527 the middle of sugarcane stalk with the use of ethephon did not alter the stomatal
528 index on the adaxial side of the leaf. However, the stem base buds and control
529 later with application of the ethephon in the plant presented better stomatal
530 indexes, in this way the non-use of the ethephon makes a more economic
531 activity.

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

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

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550 **CONCLUSIONS**

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

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

558

559 **REFERENCES**

- 560 1. Taiz, L. & Zeiger, E. 2013. Plant physiology. 5. ed. Porto Alegre: Artemed,
561 918 p.
- 562 2. Castro, E.M .; Pereira, F.J. & Paiva, R. 2009. Vegetative histology: structure
563 and function of vegetative organs. Lavras: UFLA, 234p.
- 564 3. Mattiello, L .; Riano-Pachon, D.M .; Martins, M.C.M .; Cruz, L.P .; Bassi, D .;
565 Marchiori, P.E.R .; Ribeiro, R.V .; Labate, M.T.V .; Labate, C.A. & Menossi,
566 M. 2015. Physiological and transcriptional analyzes of developmental
567 stages along sugarcane leaf. BMC Plant Biology 15: 1-21.
- 568 4. Markwell, J .; Osterman, J.C. & Mitchell, J.L. 1995. Calibration of the Minolta
569 SPAD-502 leaf chlorophyll meter. Photosynthesis Research 46 (3): 467-
570 472.
- 571 5. Wekesa, R .; Onguso, J.M .; Nyende, B.A. & Wamocho, L.S. 2015.
572 Sugarcane in Vitro Culture Technology: Applications for Kenya's Sugar
573 Industry. Journal of Biology, Agriculture and Healthcare 5 (17): 127-134.
- 574 6. Capuani, S .; Rigon, J.P.G .; Brito Neto, J.F .; Beltrão, N.J.E.M. & Almeida, D.
575 2011. Chlorophyll content during the development of the castor bean under
576 nitrogen and silica fertilization. Encyclopedia Biosphere 7 (13): 656-662.
- 577 7. Lisboa, L.A.M .; Ramos, S.B .; Viana, R.S .; Heinrichs, R .; Segati, D.F .;
578 Figueiredo, P.A.M. 2013. Foliar morphological changes of sugarcane as a
579 function of herbicide application strategies. STAB: Sugar, Alcohol and
580 Byproducts 31 (3): 33-36.
- 581 8. Roberto, G.G .; Cunha, C .; Sales, C.R.G .; Silveira, N.M .; Ribeiro, R.V .;
582 Machado, E.C. & Lagôa, A.M.M. 2015. Variation of photosynthesis and

- 583 carbohydrate contents induced by etefom and water deficit in the maturation
584 stage of sugarcane. *Bragantia* 74 (4): 379-386.
- 585 9. Faria, A.T .; Silva, A.F .; Ferreira, E.A .; Rocha, P.R.R .; Silva, D.V .; Silva,
586 A.A. & Tironi, S.P. 2014. Changes in the physiological characteristics of
587 sugarcane caused by trinexapac-ethyl. *Brazilian Journal of Agricultural*
588 *Sciences* 9 (2): 200-204.
- 589 10. Chang, C. & Williams, M. 2016. Ethylene. *The Plant Cell*, p.1-14.
- 590 11. Castro, P.R.C. 2002. Effects of luminosity and temperature on
591 photosynthesis and production and accumulation of sucrose and starch in
592 sugarcane. *STAB: sugar, alcohol and by-products* 20 (5): 32-33.
- 593 12. Moreira, A.S.F.P. & Isaias, R.M.S. 2008. Comparative anatomy of the
594 absorption roots of terrestrial and epiphytic orchids. *Brazilian archives of*
595 *biology and technology* 51 (1): 83-93.
- 596 13. Wang, K.L. W.; Li, H. & Ecker, J.R. 2002. Ethylene biosynthesis and
597 signaling networks. *Plant Cell* 14: 131-151.
- 598 14. Lí, Y.J .; Yang, L.T .; Li, Y.R. & Ye, Y.P. 2002. Influence of ethephon
599 sprayed at different stages on growth, agronomic traits and drought
600 resistance of sugarcane. *Sugarcane* 9 (1): 12-18.
- 601 15. Pincelli, R.P. & Silva, M.A. 2012. Leaf morphological changes in sugarcane
602 cultivars in response to water deficiency. *Bioscience Journal* 28 (4): 546-
603 556.
- 604 16. Brazilian Agricultural Research Corporation - Embrapa. 2006. National Soil
605 Agricultural Research Center. Brazilian system of soil classification. Rio de
606 Janeiro. 412 p.
- 607 17. Raij, B .; Andrade, J.C .; Cantarella, H. & Quaggio, J.A. 2001. Chemical
608 analysis for fertility evaluation of tropical soils. Campinas: Agronomic
609 Institute. 285 p.
- 610 18. Raij, B .; Cantarella, H .; Quaggio, J.A. & Furlani, A.M.C. 1996.
611 Recommendations of fertilization and liming for the State of São Paulo. 2.
612 ed. Campinas: IAC. 285 p. (Technical Bulletin, 100).
- 613 19. Sousa, D.M.G .; Lobato, E. & Rein, T.A. 2004. Use of agricultural gypsum in
614 cerrado soils. Planaltina: Embrapa Cerrados. 20 p. (Technical Circular, 32).

- 615 20. Pereira, F.J .; Castro, E.M .; Souza, T.C. & Magalhães, P. C. 2008.
616 Evolution of root anatomy of 'Saracura' maize in successive selection
617 cycles. Brazilian Agricultural Research 43 (12): 1649-1656.
- 618 21. Ceolin, G.B .; Rücker, A. & Kray, J.G. 2007. Leaf epidermal analysis on
619 seedling differentiation of *Geonoma schottiana* and *Euterpe edulis*
620 (*Arecaceae*). Brazilian Journal of Biosciences, 5 (1): 18-20.
- 621 22. Carlquist, S. 1975. Ecological strategies of xylem evolution. Berkeley:
622 University of California, 259 p.
- 623 23. Segatto, F.B .; Bisognin, D.A .; Benedetti, M .; Costa, L.C .; Rampelotto,
624 M.V. & Nicoloso, F.T. 2004. Technique for the study of the anatomy of the
625 potato leaf epidermis. Rural Science 34 (5): 1597-1601.
- 626 24. Gomes, F.P. 2000. Course of experimental statistics. 4. ed. Piracicaba:
627 ESALQ, 477p.
- 628 25. Mcqualter, R.B .; Petrasovits, L.A .; Gebbie, L. K .; Schweitzer, D .;
629 Blackman, D.M. Chrysanthopoulos, P .; Hodson, M.P .; Plan, M.R .; Riches,
630 J.D .; Snell, K.D .; Brumbley, S. M. & Nielsen, L.K. 2015. The use of an
631 acetoacetyl-CoA synthase in place of the β -ketothiolase enhances poly-3-
632 hydroxybutyrate production in sugarcane mesophyll cells. Plant
633 Biotechnology Journal 13: 700-707.
- 634 26. Martins, M.B.G. & Castro, P.R.C. 1999. Effects of gibberellin and ethephon
635 on the anatomy of sugarcane plants. Pesquisa Agropecuária Brasileira 34
636 (10): 1855-1863.
- 637 27. Ramos, S.B .; Viana, R.S .; Lisboa, L.A.M .; Ventura, G .; Segati, D.F .;
638 Assumpcao, A.C.N.D; Fruchi, V.M .; Magalhaes, A.C. & Figueiredo, P.A.M.
639 2014. Leaf morphoanatomic characteristics of sugarcane cultivars. STAB:
640 Sugar, Alcohol and Byproducts 32: 28-30.
- 641 28. Zhang, F .; Zhang, K .; Du, C .; Li, J .; Xing, Y .; Yang, L. & Li, Y. 2015.
642 Effect of drought stress on anatomical structure and chloroplast
643 ultrastructure in leaves of sugarcane. Sugar Tech 17 (1): 41-48.
- 644 29. Aude, M.I.S. 1993. Stages of development of sugarcane and its relationship
645 with productivity. Rural Science 23 (2): 241-248.
- 646 30. Gloria, B.A. & Guerreiro, S.M.C. 2012. Vegetable anatomy. 3.ed. Viçosa: Ed
647 UFV. 404 p.

- 648 31. Pereira, M.A. 2010. Thiamethoxam in sugarcane, bean, soybean, orange
649 tree and coffee plants development parameters and biochemical aspects.
650 2010. 124 f. Thesis (Doctorate) - School of Agriculture "Luiz de Queiroz",
651 University of São Paulo, Piracicaba.
- 652 32. Ferreira, E.A .; Demuner, A.J .; Silva, A.A .; Santos, J.B .; Ventrella, M.C.,
653 Marques, A.E. & Procópio, S.O. 2005. Chemical composition of epicuticular
654 wax and characterization of leaf surface in sugarcane genotypes. *Weed* 23
655 (4): 1-6.
- 656 33. Ferreira, E.A .; Ventrella, M.C .; Santos, J.B .; Barbosa, M.H.P .; Silva, A.A
657 .; Procópio, S.O. & Silva, E.A.M .. 2007. Leaf blade quantitative anatomy of
658 sugarcane cultivars and clones. *Plant* 25 (1): 25-34.
- 659 34. Li, Y.R. & Solomon, S. 2003. Ethephon: a versatile growth regulator for
660 sugar cane industry. *Sugar Technology* 5 (4): 213-223.
- 661 35. Souza, A .; Moraes, M.G. & Ribeiro, R.C.L.F. 2005. Cerrado grasses: non-
662 structural carbohydrates and ecophysiological aspects. *Acta Botanica*
663 *Brasilica*, 19 (1): 81-90.
- 664 36. Tobin, A.K. 1992. Plant organelles: compartmentation of metabolism in
665 photosynthetic cells. Cambridge: Seminar beings. 101p.
- 666 37. Ribeiro, M.N.O .; Carvalho, S.P .; Pereira, F.J. & Castro, E.M .. 2012. Foliar
667 anatomy of cassava in function of the potential for tolerance to different
668 environmental conditions. *Agronomic Science* 43 (2): 354-361.
- 669 38. Oliveira, E.C .; Miglioranza, E. 2014. Density and stomatal distribution in
670 manihot *Manihot esculenta* Crantz cultivar IAC 576-70. *Scientia*
671 *Agropecuaria* 5: 135-140.
- 672 39. Oliveira, E.C. & Miglioranza, E. 2013. Dimensions and stomatal density in
673 different varieties of cassava. *Cultivating Knowledge* 6 (4): 201-213.
- 674 40. Kouwenberg, L.L.R .; Kürschner, W.M. & Visscher, H. 2004. Changes in
675 stomatal frequency and size during elongation of *Tsuga heterophylla*
676 Needles. *Annals of Botany* 94 (4): 561-569.