

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

4
5 Lucas Aparecido Manzani Lisboa^{1;2}, Reginaldo Sciarra Leonezi¹, Andresa
6 Toledo Chagas¹, João Paulo Basaglia Freschi¹, Paulo Alexandre Monteiro
7 de Figueiredo, Edson Lazarini³

8
9 ¹São Paulo State University (Unesp), College of Technology and Agricultural
10 Sciences, Dracena, São Paulo, Brazil.
11 *e-mail:lucas.lisboa@unesp.br

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

14 ³São Paulo State University (Unesp), School of Natural Sciences and
15 Engineering, Ilha Solteira

16
17 **Authors' contributions**

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

23
24 **Abstract** - The hormones are closely related to the emergence of gemstones
25 contained seedlings of sugarcane, at the time of planting of the stems. The
26 objective of this work was to evaluate the effects of the ethephon associated to
27 the position of gemstones in the cane of sugar cane in the initial development of
28 the culture. In March 2014, at the Rio Vermelho Plant, located in Junqueirópolis,
29 State of São Paulo, a cane plant with a sugar cane plant was selected for
30 seedlings with an approximate age of 11 months. Two areas with dimensions of
31 20x20 meters were demarcated. In one of the areas ethephon was applied. At
32 15 days after application, the seedlings containing 1 and 2 buds were collected
33 to compose two independent experiments. From the area where the product
34 was not applied, seedlings were removed for the control and application

35 treatments of ethephon in the planting groove in pots. The gems were sent to
36 the Faculty of Agrarian and Technological Sciences of the Paulista State
37 University "Júlio de Mesquita Filho" - from Dracena, State of São Paulo. The
38 seedlings came from the apex, middle and base of the canes of sugarcane. In
39 this way, the experimental design was in a 3x3 factorial scheme, that is, the
40 position of the seedlings in the canes of sugarcane and the modes of
41 application of ethephon. The use of ethephon and positions of the seedlings in
42 sugarcane stalks did not influence the Chlorophyll Index and Stomatal
43 Conductance. The use of ethephon in the plant 15 days before planting,
44 together with seedlings from the apex followed by the medium of the canes of
45 sugarcane, presented better results for the ultrastructural characteristics of
46 sugarcane foliage.

47

48 **Keywords:** Hormone. Ethephon. Morphology.

49

50 INTRODUCTION

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

56 The chlorophyll index is determined by the emission of a wavelength of
57 $\lambda=650\text{nm}$, this value is close to the wavelengths that stimulate chlorophyll
58 activity, while the emission of the wavelength $\lambda=940\text{nm}$ acts as an internal
59 reference in the leaf limy to compensate for differences in leaf thickness or
60 water content [4;5]. The photosynthetic efficiency can be estimated by the
61 concentration of chlorophyll pigments present in the leaves, becoming a tool for
62 the actual recommendation of the fertilization need [6]. As a result, the macro
63 and micromorphological modifications of each cultivar, as well as the effects
64 caused by them, should be increasingly studied in order to improve
65 understanding and the use of phyto-regulators makes it a strategy to control
66 foliar activities [7;8].

67 The use of phyto-regulars in agriculture has become an increasingly
68 common process in the beginning of the 21st century. 2-chloroethylphosphonic

69 acid, or ethephon, is a substance classified as a growth regulator with systemic
70 performance in plants [9]. In the plant organism, ethephon rapidly undergoes
71 degradation, being reduced in phosphoric acid, chloride ions and ethylene ions,
72 which act on the growth process [10].

73 In this case, structural aspects help in understanding the mechanisms
74 that cause the injuries. However, it is important to point out that changes visible
75 to the naked eye are derived from changes in the structures of the dermal,
76 fundamental or vascular tissues of plants, making it necessary to have a
77 thorough knowledge of these changes caused by changes in the environment
78 [11]. The symptomatology is widely used to evaluate the damage caused by
79 biotic or abiotic factors [12].

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

86 Ethylene resulted in a differentiation in the mesophyll cells with doses of
87 100 mg L^{-1} which provided an increase in the outer surface of the cells and
88 allowing a better distribution of the chloroplasts. There was an increase in the
89 number of chloroplasts. According to Li & Solomon 2003, these changes have
90 brought about a significant increase in the total photosynthetic area in the
91 mesophyll cell of sugarcane leaves. Using ethylene provided an acceleration in
92 the differentiation and an increase in the number of vessels floem and xylem
93 species, which provided greater efficiency in the transportation of sap in
94 sugarcane [14].

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

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

103

104 **MATERIAL AND METHODS**

105

106 *Obtaining sugarcane seedlings*

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

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

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

130

131 *Installing the experiment*

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

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

143 The results of the soil chemical analysis were: pH CaCl_2 = 5.0; MO= 14 g
144 dm^{-3} ; P= 8.0 mg dm^{-3} (resin); K= 2.3 mmol dm^{-3} (resin); Ca= 7.0 mmol dm^{-3}
145 (resin); Mg= 5.0 mmol dm^{-3} (resin); H+Al= 20 mmol dm^{-3} ; Al= zero $\text{mmol}_c \text{ dm}^{-3}$;
146 Base Sum= 14.3 mmol dm^{-3} ; CTC= 34.3 mmol dm^{-3} ; Base Saturation (V%)= 42;
147 Saturation Al (m%)= zero; S (SO_4^{2-})= 3.0 mg dm^{-3} ; Cu= 2.8 mg dm^{-3} (DTPA);
148 Fe= 19 mg dm^{-3} (DTPA); Zn= 1.3 mg dm^{-3} (DTPA); Mn= 16.5 mg dm^{-3} (DTPA);
149 B= 0.14 mg dm^{-3} (Hot water); Clay= 75 g kg^{-1} ; Silt= 33 g kg^{-1} and Total sand=
150 893 g kg^{-1} [16;17].

151 All soil corrections were performed, according to [18;19]. On this
152 occasion, in pots of 45 dm^3 containing sifted soil, where sugarcane seedlings
153 were planted in two situations, containing 1 (one) and 2 (two) buds, composing
154 this maniera, 2 (two) independent experiments. During the experiments, all
155 necessary cultural treatments were carried out, such as: phytosanitary control,
156 elimination of invasive plants and cover fertilization. The pots were kept irrigated
157 whenever necessary in order to meet the field capacity.

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

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

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

174

175 *The evaluations*

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

182 All fragments of plant tissues received the pertinent procedures for
183 dehydration, diaphanization, inclusion and embedding and with the help of a
184 microtome, where cross sections of 8 µm were performed on each tissue
185 fragment. The slides were observed in an optical microscope with a camera
186 coupled to perform the measurements of the histological variables through the
187 analysis program, calibrated with a microscopic ruler at the same magnification
188 [20], where limbal thickness; thickness of the epidermis of the abaxial face;
189 thickness of the epidermis of the adaxial face; mesophyll thickness; flolem
190 diameter; diameter of the metaxilematic vessels; abaxial cuticle thickness;
191 adaxial cuticle thickness; diameter of the cells of the sheath and distance
192 between the vascular bundles in the leaf blade, according to [2].

193 In the collected fragments, the impression was also made on the
194 epidermal faces using cyanoacrylate ester [21], where the following
195 characteristics were observed: number of stomata per mm² on the abaxial;
196 number of stomata per mm² in the adaxial face; number of abaxial epidermal
197 cells per mm²; number of adaxial epidermal cells per mm²; stomatal functionality
198 of the abaxial face; stomatal functionality or relation of the adaxial face; and the
199 stomatal index of the adaxial face according to [22;23;2]. For all the
200 characteristics, 5 (five) measurements per slide were performed. The plots were
201 represented by the average value obtained from the measurements of each
202 characteristic.

203

204 *Statistical analyzes*

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

207

208 RESULTS AND DISCUSSIONS

209

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

212

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.

213

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.

214

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

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

229

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.

230

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.

231

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

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

245 [25] report the importance of foliar limb cells due to the presence of β -
246 ketothiolase that act in the production of polymer in mesophilic plastids that
247 maximizes the yield of these organelles.

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

254 It is noteworthy that the epidermis function as external coating of the
255 vegetable, which protects its internal tissues. Because it is a simple layer of
256 cells and juxtaposed, this characteristic helps in the process of regeneration of
257 this tissue when subjected to some mechanical or chemical damage [27;8].
258 According to [12], when studying plants of the family Orchidaceae, they affirm
259 that the anatomical characteristics of the epidermis in the plant may be involved
260 with different adaptations to the different environments during evolutionary

261 process. [28], when studying water stress in sugarcane, concluded that after
 262 stress an increase in the thickness of leaf epidermis was observed, showing
 263 resistance of the plant to avoid the loss of water by transpiration.

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

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

276 In the experiment with 1 and 2 buds, this effect of interaction between the
 277 factors was not found as in the experiment with 2 buds for the characteristic
 278 thickness of the mesophyll. But a significant effect was found for the position
 279 factors of the yolk and application of ethephon. Again the results found
 280 corroborate with the other data already discussed, in which gemstones from the
 281 apex and middle with the use of ethephon provided a greater thickness of the
 282 leaf blade of the sugar cane.

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

287

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 F1x F2				
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 F1x F2				
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 F1x F2*	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 F1x F2	-			

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.

288

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 F1x F2*	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 F1x F2	-			
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.

289

290 In Table 5 it is possible to observe a significant effect on the
 291 characteristic diameter of the phloem vessels in the experiment with 1 yolk at 90
 292 days after planting. This result shows that gemstones from the base showed
 293 better results, no significant effect was found on the use of ethephon. However
 294 in the experiment with 2 buds, an interaction effect between the factors was
 295 found. It showed that, with the planting of gemstones at the apex, regardless of
 296 the use of the ethephon, they did not present a significant effect, but gemstones
 297 of the middle of the stem with application of the ethephon at the 15 days before
 298 the planting followed by the control that presented better diameters, in this way
 299 the data of this experiments demonstrate the non-use of ethephon. For base
 300 gems it is indifferent whether or not to use the ethephon.

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

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

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

316 [31], when studying the effects of phytohormones in sugarcane, observed
317 that there was an increase in the number of metaxilems in the vascular bundles
318 of roots in young plants, which may have contributed to the greater survival of
319 tillers, and could have provided in a higher number of crops. All the way to the
320 characteristic thickness of the abaxial cuticle in the experiment with 1 yolk at 90
321 days after planting, it is possible to observe a significant effect of interaction
322 between the factors. When we consider stem apex buds the ethephon
323 application effect did not differ with the control, in the same way it occurred with
324 the stalk medium buds.

325 For gemstones from the base of the stem the application of ethephon at
326 15 days before planting presented better results together with the control. In this
327 way it is recommended not to use ethephon due to the economic values of the
328 applied product. When considering non-use of the product (control) the position
329 of the buds do not differ statistically. For the application of ethephon in the plant
330 to the 15 days before the planting of the gems is recommended the use of the
331 gems of the middle and base of the cane of sugar cane. In the application
332 situation of the ethephon in the planting groove, the positions of the yolk on the
333 stem do not present significant difference.

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

340 For the characteristic thickness of the adaxial cuticle in experiments with
341 1 and 2 buds at 90 days after planting, no significant effect was found between
342 the factors studied (Table 6). The data corroborate that the chemical
343 composition of the cuticle may vary, but with predominance of cutin and wax.
344 Cutin is an insoluble biopolyester that has a high degree of cross-linking
345 between the long chain hydroxyl fatty acids composing them, while the wax is
346 embedded in the polymer or deposited on the outside of the cuticle.

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

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

356

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.

357

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.

358

359 For the characteristic sheath cell diameter of the experiments with 1 and
 360 2 buds at 90 days after planting, no significant effect was found between the
 361 factors studied. The biochemical reactions of carbon fixation by C4 plants occur
 362 in the cells of the sheath [35]; due to the higher carbon concentration present
 363 within their cytoplasm, and have well-developed sheath cells that provides
 364 greater carbon fixation through the photosynthesis photochemical processes
 365 together with the action of the rubisco molecule [1;36] which may explain the
 366 data obtained [10].

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

375 In Tables 9 and 10, the mean values of the number of stomata per mm^2
 376 in the abaxial face are presented; number of stomata per mm^2 in the adaxial
 377 face; number of abaxial epidermal cells per mm^2 and number of adaxial
 378 epidermal cells per mm^2 from the experiments with 1 and 2 buds at 90 days
 379 after installation of the experiment.

380

Table 9. Mean values of the number of stomata per mm^2 in the abaxial face; 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 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.

381

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.

382

383 For the characteristic number of stomata per mm², in the experiment with
 384 1 yolk at 90 days after planting, a significant effect was found only on the
 385 application factor of the ethephon, shows that the application in the plant at 15
 386 days before planting and followed showed better results. Due to the statistical
 387 equality between the factors there was no effect of ethephon application.

388 In the experiment with 2 buds, a significant effect of interaction between
 389 the factors was found. When the position of the yolk at the top of the stem is
 390 considered, the best results were found when application of ethephon occurs in
 391 the planting groove; as for the position of the yolk in the middle of the stem, no
 392 effect was found regarding the application of the ethephon, for the yolk of the
 393 stem base it was better to use the ethephon when applied to the plant 15 days
 394 before planting.

395 No significant effect of the position of the gem was found on the stem;
 396 but when application of ethephon occurs in the plant 15 days before planting,
 397 gemstones originating from the apex and base present a higher number of
 398 stomata per mm² in the abaxial epidermis; however, already for the use of
 399 ethephon in the planting groove, it was better in the sugarcane culms. For the
 400 characteristic number of stomata per mm² in the adaxial epidermis, in the 1-yolk

401 experiment, a significant effect was found for interaction between the factors
402 (Table 9).

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

409 Without the use of ethephon when base gems were used followed by the
410 yolk of the stalk medium, they presented better values for stomata per mm² in
411 the adaxial epidermis at 90 days after planting. When the ethephon was applied
412 to plant 15 before planting, no effect was found on the position of the yolk on
413 the sugarcane stem; however, when the ethephon was used in the planting
414 groove, the gemstones originating from the apex and the middle of the stem
415 had better means for the said characteristic.

416 In the experiment with 2 buds, the significant effect on the interaction
417 between the factors was also found (Table 10). As for the position of the yolk on
418 the stalk, the yolks of the apex and stalk medium did not suffer from the
419 application of ethephon; However, for the stem base buds the control presented
420 better means for the characteristic number of stomata per mm² in the adaxial
421 epidermis at 90 days after planting.

422 Without the use of ethephon showed that base gems followed by the
423 middle of the sugar cane stalk; presented better means for the same
424 characteristic number of abaxial epidermal cells. When the ethephon was
425 applied to the plant at 15 days before planting, the position of the yolk did not
426 differ statistically. Although, overall, it provided homogeneity in the characteristic
427 number of stomata per mm² in the adaxial epidermis. This same behavior was
428 not found when applied ethephon in the planting groove, demonstrating that
429 gemstones of the apex followed by the medium of sugarcane stalks showed
430 better means for the characteristic in question.

431 For the characteristic number of abaxial epidermal cells per mm² in the 1-
432 yolk experiment at 90 days after planting, a significant effect of interaction
433 between the factors was found (Table 9). It was verified that, when using the
434 gem of the apex with application of ethephon in the groove followed by the

435 control, presented better results for the featured feature. This same behavior of
436 the data was not found with the yolk of the stem medium, where it is possible to
437 observe that the control treatment followed by the application of ethephon in the
438 plant presented better results. And base gem was not found the significant
439 effect between the ethephon application factor.

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

447 The same significant effect of interaction between the factors was
448 observed in the experiment with 2 buds, for the characteristic number of abaxial
449 epidermal cells per mm^2 at 90 days after planting. When the yolk of the stem
450 was used together with the control and followed by the application of the
451 ethephon in the groove, better means are observed. As for the origin of the gem
452 in the middle of the stem was not influenced by the use of the ethephon.
453 However, for stem base buds together with application to the plant followed by
454 application to the planting groove presented better means for the characteristic
455 in question.

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

462 For the characteristic number of adaxial epidermal cells per mm^2 in the 1-
463 yolk experiment, significant effect was found on the yolk position on the stem
464 and on the application mode of ethephon as shown in Table 9. It is possible to
465 observe that gems the apex and the middle of the stem presented higher
466 averages; and also the control and with application of the ethephon in the
467 planting groove respectively presented better results, therefore, the data did not
468 show response with the use of ethephon, it is recommended to repeat new

469 work. However, in the experiment with 2 buds, no significant effect was found
 470 among the factors studied.

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

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

481

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.

482

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.

483

484

485

For the characteristic stomatal functionality of the abaxial face of the 1-yolk experiment at 90 days after planting, no significant effect was found

486 between the factors (Table 11); but it is possible to observe an interaction effect
487 between the factors in the experiment with 2 buds (Table 12).

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

494 When the control was considered, that is, the non-use of ethephon with
495 gemstones originating from the medium, then gem of the stem base, presented
496 better stomatal functionality of the abaxial face; when the ethephon application
497 occurs, in the plant at 15 days before the planting with the yolk of the medium of
498 the stem, better responses of said characteristic were demonstrated; for
499 application of ethephon in the planting groove with base yolks and then medium
500 shoot yolks presented better means for functionality.

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

507 It can be understood that the greater the stomatal functionality, the better
508 the photosynthetic yields the vegetables, due to the greater opening of the
509 stomata, which proportionally gives rise to a greater gas exchange [2] of carbon
510 in the green matter of the vegetable. These values are similar to those found by
511 [37], when studying cassava cultivars with tolerance to water stress, in the
512 same way [38;39] also studying cassava species, observed values similar to the
513 other studies. This shows that even different species stomatal development are
514 similar, and may exhibit the same index of stomatal functionality. For the
515 characteristic stomatal index of the abaxial face, we found significant interaction
516 effects between the factors in the two experiments, with 1 and 2 buds at 90
517 days after planting.

518 In the experiment with 1 yolk, when the yolk was used together with and
519 without the use of ethephon in the plant 15 days before planting, greater

520 stomatal indices were found on the abaxial side of the cane leaf; but for gems
521 from the middle and bottom of the cane sugar cane, no significant effect was
522 found with the ethephon use factor. When the effect of the use of ethephon as a
523 main factor is considered, the control and application of ethephon at 15 days
524 before planting together with the positions of the yolks on the stem showed no
525 significant effects. However, it is possible to observe a significant effect of the
526 position of the yolk when the ethephon was used in the planting groove. In this
527 way, the middle gemstones followed by the base of the sugarcane stem
528 presented better stomatal indices on the abaxial surface (Table 11).

529 As for the interaction effect of the factors studied, in the experiment with
530 2 gems the same characteristic in question, that is, stomatal index in the abaxial
531 face; the gemstones of the apex together with the application of ethephon in the
532 planting groove presented higher averages, which did not occur with the middle
533 and base sugarcane shoots. When the ethephon use factor is considered, in the
534 control the medium and base gems presented better contents. However, for
535 application of the ethephon in the plant at 15 days before planting and
536 application in the groove the bud and base buds present higher averages for
537 the stomatal index in the abaxial face.

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

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

549 When considering the use of ethephon, the control together with the
550 application of ethephon in the plant at 15 days before planting, a difference in
551 the stomatal index was not observed due to the positions of the gems in the
552 cane sugarcane. However, when the ethephon is applied to the planting groove,

553 the gemstones originating from the apex and the middle of the stem presented
554 higher stomatal indices on the adaxial side of the cane leaves.

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

563

564

565

566

567 **CONCLUSIONS**

568

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

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

575

576 **REFERENCES**

- 577 1. Taiz, L. & Zeiger, E. 2013. Plant physiology. 5. ed. Porto Alegre: Artemed,
578 918 p.
- 579 2. Castro, E.M. ; Pereira, F.J. & Paiva, R. 2009. Vegetative histology: structure
580 and function of vegetative organs. Lavras: UFLA, 234p.
- 581 3. Mattiello, L. ; Riano-Pachon, D.M. ; Martins, M.C.M. ; Cruz, L.P. ; Bassi, D. ;
582 Marchiori, P.E.R. ; Ribeiro, R.V. ; Labate, M.T.V. ; Labate, C.A. & Menossi,
583 M. 2015. Physiological and transcriptional analyzes of developmental
584 stages along sugarcane leaf. BMC Plant Biology 15: 1-21.

- 585 4. Markwell, J .; Osterman, J.C. & Mitchell, J.L. 1995. Calibration of the Minolta
586 SPAD-502 leaf chlorophyll meter. *Photosynthesis Research* 46 (3): 467-
587 472.
- 588 5. Wekesa, R .; Onguso, J.M .; Nyende, B.A. & Wamocho, L.S. 2015.
589 Sugarcane in Vitro Culture Technology: Applications for Kenya's Sugar
590 Industry. *Journal of Biology, Agriculture and Healthcare* 5 (17): 127-134.
- 591 6. Capuani, S .; Rigon, J.P.G .; Brito Neto, J.F .; Beltrão, N.J.E.M. & Almeida, D.
592 2011. Chlorophyll content during the development of the castor bean under
593 nitrogen and silica fertilization. *Encyclopedia Biosphere* 7 (13): 656-662.
- 594 7. Lisboa, L.A.M .; Ramos, S.B .; Viana, R.S .; Heinrichs, R .; Segati, D.F .;
595 Figueiredo, P.A.M. 2013. Foliar morphological changes of sugarcane as a
596 function of herbicide application strategies. *STAB: Sugar, Alcohol and*
597 *Byproducts* 31 (3): 33-36.
- 598 8. Roberto, G.G .; Cunha, C .; Sales, C.R.G .; Silveira, N.M .; Ribeiro, R.V .;
599 Machado, E.C. & Lagôa, A.M.M. 2015. Variation of photosynthesis and
600 carbohydrate contents induced by etefom and water deficit in the maturation
601 stage of sugarcane. *Bragantia* 74 (4): 379-386.
- 602 9. Faria, A.T .; Silva, A.F .; Ferreira, E.A .; Rocha, P.R.R .; Silva, D.V .; Silva,
603 A.A. & Tironi, S.P. 2014. Changes in the physiological characteristics of
604 sugarcane caused by trinexapac-ethyl. *Brazilian Journal of Agricultural*
605 *Sciences* 9 (2): 200-204.
- 606 10. Chang, C. & Williams, M. 2016. Ethylene. *The Plant Cell*, p.1-14.
- 607 11. Castro, P.R.C. 2002. Effects of luminosity and temperature on
608 photosynthesis and production and accumulation of sucrose and starch in
609 sugarcane. *STAB: sugar, alcohol and by-products* 20 (5): 32-33.
- 610 12. Moreira, A.S.F.P. & Isaias, R.M.S. 2008. Comparative anatomy of the
611 absorption roots of terrestrial and epiphytic orchids. *Brazilian archives of*
612 *biology and technology* 51 (1): 83-93.
- 613 13. Wang, K.L. W.; Li, H. & Ecker, J.R. 2002. Ethylene biosynthesis and
614 signaling networks. *Plant Cell* 14: 131-151.
- 615 14. Lí, Y.J .; Yang, L.T .; Li, Y.R. & Ye, Y.P. 2002. Influence of ethephon
616 sprayed at different stages on growth, agronomic traits and drought
617 resistance of sugarcane. *Sugarcane* 9 (1): 12-18.

- 618 15. Pincelli, R.P. & Silva, M.A. 2012. Leaf morphological changes in sugarcane
619 cultivars in response to water deficiency. *Bioscience Journal* 28 (4): 546-
620 556.
- 621 16. Brazilian Agricultural Research Corporation - Embrapa. 2006. National Soil
622 Agricultural Research Center. Brazilian system of soil classification. Rio de
623 Janeiro. 412 p.
- 624 17. Raij, B .; Andrade, J.C .; Cantarella, H. & Quaggio, J.A. 2001. Chemical
625 analysis for fertility evaluation of tropical soils. Campinas: Agronomic
626 Institute. 285 p.
- 627 18. Raij, B .; Cantarella, H .; Quaggio, J.A. & Furlani, A.M.C. 1996.
628 Recommendations of fertilization and liming for the State of São Paulo. 2.
629 ed. Campinas: IAC. 285 p. (Technical Bulletin, 100).
- 630 19. Sousa, D.M.G .; Lobato, E. & Rein, T.A. 2004. Use of agricultural gypsum in
631 cerrado soils. Planaltina: Embrapa Cerrados. 20 p. (Technical Circular, 32).
- 632 20. Pereira, F.J .; Castro, E.M .; Souza, T.C. & Magalhães, P. C. 2008.
633 Evolution of root anatomy of 'Saracura' maize in successive selection
634 cycles. *Brazilian Agricultural Research* 43 (12): 1649-1656.
- 635 21. Ceolin, G.B .; Rücker, A. & Kray, J.G. 2007. Leaf epidermal analysis on
636 seedling differentiation of *Geonoma schottiana* and *Euterpe edulis*
637 (Arecaceae). *Brazilian Journal of Biosciences*, 5 (1): 18-20.
- 638 22. Carlquist, S. 1975. Ecological strategies of xylem evolution. Berkeley:
639 University of California, 259 p.
- 640 23. Segatto, F.B .; Bisognin, D.A .; Benedetti, M .; Costa, L.C .; Rampelotto,
641 M.V. & Nicoloso, F.T. 2004. Technique for the study of the anatomy of the
642 potato leaf epidermis. *Rural Science* 34 (5): 1597-1601.
- 643 24. Gomes, F.P. 2000. Course of experimental statistics. 4. ed. Piracicaba:
644 ESALQ, 477p.
- 645 25. Mcqualter, R.B .; Petrasovits, L.A .; Gebbie, L. K .; Schweitzer, D .;
646 Blackman, D.M. Chrysanthopoulos, P .; Hodson, M.P .; Plan, M.R .; Riches,
647 J.D .; Snell, K.D .; Brumbley, S. M. & Nielsen, L.K. 2015. The use of an
648 acetoacetyl-CoA synthase in place of the β -ketothiolase enhances poly-3-
649 hydroxybutyrate production in sugarcane mesophyll cells. *Plant*
650 *Biotechnology Journal* 13: 700-707.

- 651 26. Martins, M.B.G. & Castro, P.R.C. 1999. Effects of gibberellin and ethephon
652 on the anatomy of sugarcane plants. *Pesquisa Agropecuária Brasileira* 34
653 (10): 1855-1863.
- 654 27. Ramos, S.B. ; Viana, R.S. ; Lisboa, L.A.M. ; Ventura, G. ; Segati, D.F. ;
655 Assumpcao, A.C.N.D; Fruchi, V.M. ; Magalhaes, A.C. & Figueiredo, P.A.M.
656 2014. Leaf morphoanatomic characteristics of sugarcane cultivars. *STAB:*
657 *Sugar, Alcohol and Byproducts* 32: 28-30.
- 658 28. Zhang, F. ; Zhang, K. ; Du, C. ; Li, J. ; Xing, Y. ; Yang, L. & Li, Y. 2015.
659 Effect of drought stress on anatomical structure and chloroplast
660 ultrastructure in leaves of sugarcane. *Sugar Tech* 17 (1): 41-48.
- 661 29. Aude, M.I.S. 1993. Stages of development of sugarcane and its relationship
662 with productivity. *Rural Science* 23 (2): 241-248.
- 663 30. Gloria, B.A. & Guerreiro, S.M.C. 2012. *Vegetable anatomy*. 3.ed. Viçosa: Ed
664 UFV. 404 p.
- 665 31. Pereira, M.A. 2010. Thiamethoxam in sugarcane, bean, soybean, orange
666 tree and coffee plants development parameters and biochemical aspects.
667 2010. 124 f. Thesis (Doctorate) - School of Agriculture "Luiz de Queiroz",
668 University of São Paulo, Piracicaba.
- 669 32. Ferreira, E.A. ; Demuner, A.J. ; Silva, A.A. ; Santos, J.B. ; Ventrella, M.C.,
670 Marques, A.E. & Procópio, S.O. 2005. Chemical composition of epicuticular
671 wax and characterization of leaf surface in sugarcane genotypes. *Weed* 23
672 (4): 1-6.
- 673 33. Ferreira, E.A. ; Ventrella, M.C. ; Santos, J.B. ; Barbosa, M.H.P. ; Silva, A.A
674 ; Procópio, S.O. & Silva, E.A.M. . 2007. Leaf blade quantitative anatomy of
675 sugarcane cultivars and clones. *Plant* 25 (1): 25-34.
- 676 34. Li, Y.R. & Solomon, S. 2003. Ethephon: a versatile growth regulator for
677 sugar cane industry. *Sugar Technology* 5 (4): 213-223.
- 678 35. Souza, A. ; Moraes, M.G. & Ribeiro, R.C.L.F. 2005. Cerrado grasses: non-
679 structural carbohydrates and ecophysiological aspects. *Acta Botanica*
680 *Brasilica*, 19 (1): 81-90.
- 681 36. Tobin, A.K. 1992. *Plant organelles: compartmentation of metabolism in*
682 *photosynthetic cells*. Cambridge: Seminar beings. 101p.

- 683 37. Ribeiro, M.N.O .; Carvalho, S.P .; Pereira, F.J. & Castro, E.M .. 2012. Foliar
684 anatomy of cassava in function of the potential for tolerance to different
685 environmental conditions. Agronomic Science 43 (2): 354-361.
- 686 38. Oliveira, E.C .; Miglioranza, E. 2014. Density and stomatal distribution in
687 manihot *Manihot esculenta Crantz* cultivar IAC 576-70. Scientia
688 Agropecuaria 5: 135-140.
- 689 39. Oliveira, E.C. & Miglioranza, E. 2013. Dimensions and stomatal density in
690 different varieties of cassava. Cultivating Knowledge 6 (4): 201-213.
- 691 40. Kouwenberg, L.L.R .; Kürschner, W.M. & Visscher, H. 2004. Changes in
692 stomatal frequency and size during elongation of *Tsuga heterophylla*
693 Needles. Annals of Botany 94 (4): 561-569.

UNDER PEER REVIEW