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Trinexapac-ethyl causes stimulatory effect on eucalyptus initial growth under nutritional deficiency

Allan Lopes Bacha[1]*, Pedro de Figueiredo Rocha Barbosa Martins[2], Pedro Luis da Costa Aguiar Alves[3] and Rinaldo Cesar de Paula[4]

[1,2,3] Sao Paulo State University – UNESP/FCAV, Department of Biology Applied to Agriculture. Via de Acesso Prof. Paulo Donato Castellane, s/n 14884 900 Jaboticabal – SP, Brazil. [1]allan_lb@hotmail.com; [2]martins.pfrb@gmail.com; [3]plalves@fcav.unesp.br

[4] Sao Paulo State University – UNESP/FCAV, Department of Plant Production. Via de Acesso Prof. Paulo Donato Castellane, s/n Bairro Rural. 14884 900 Jaboticabal – SP, Brazil. rcpaula@fcav.unesp.br

*Corresponding author: Allan Lopes Bacha; allan_lb@hotmail.com; +55 16 3209-7206

Abstract – Eucalyptus plants are sensitive to abiotic stresses in their initial growth, and nutritional deficiency is one of the most recurrent among them. Trinexapac-ethyl, which is a plant growth regulator, can positively affect eucalyptus, a response known as hormesis, possibly providing plants greater tolerance to stress. The objective of this study was to evaluate the effect of trinexapac-ethyl at two application times (before planting – BP; after planting - AP) in *Eucalyptus urophylla* under nutritional deficiency conditions (NPK). Two experiments (one for each application time) were conducted simultaneously during 81 days after planting of eucalyptus in 15 L pots. The treatments consisted of three trinexapac-ethyl doses (0, 30 and 60 g a.i. ha^{-1}) and four variations of nutrient supply: complete solution (NPK), solution without N (-N), -P, and -K. The variables of gas exchange, growth and dry matter were evaluated. For both application times, trinexapac-ethyl had a positive effect on the root/shoot ratio of plants grown in -N, and also positively affected some eucalyptus photosynthetic characteristics. In the application AP, the compound provided gains in height and dry matter, regardless of the nutrient supply. Under phosphorus deficiency, trinexapac-ethyl provided gains on total dry matter (BP) and leaf area (AP).

Keywords: *Eucalyptus urophylla*, nitrogen, phosphorus, plant growth regulator, hormesis.

Introduction

The genus *Eucalyptus* is the most important for the Brazilian forestry sector, being the main source of wood for cellulose production, energy, among others (Ibá 2016). Due to genetic enhancement programs and the evolution of silvicultural techniques (Stape et al. 2004; Pereira et al. 2012), in 2015 the eucalyptus crop reached yields of 36 m^3 ha^{-1} $year^{-1}$, in a total planted area of 5.6 million hectares. Thus, Brazil has the world's highest productivity of eucalyptus forests (Ibá 2016).

The initial growth stage of eucalyptus, which includes the first year after seedlings planting, is one of the most critical periods in the crop cycle. During this period, the plants are most susceptible to interference caused by biotic and abiotic stresses, and the nutritional deficiency is one of the most recurrent among them (Nambiar and Sands 1993; Garau et al. 2008). This becomes more relevant due to the crop expansion around Brazilian's diverse agroecosystems, in which low fertility is a natural characteristic of many soils.

The nutritional deficiency affects all plant metabolism, leading to low initial survival, less tolerance to biotic and abiotic stresses (Teixeira et al. 2006) and significant reductions in forest productivity. Thus, studies looking for alternatives to improve the seedlings capacity to overcome this critical period should be supported.

Some studies suggest that the application of trinexapac-ethyl in eucalyptus seedlings may have a positive effect on plant growth, which can also be related with increases in photosynthetic parameters of eucalyptus (Pires et al. 2013; Correia and Villela 2015; Bacha et al. 2017). This phenomenon is known as hormesis, and consists of a stimulatory effect resulting from the application of low doses of a substance that would be toxic in high doses (Calabrese and Baldwin 2002; Belz and Duke 2014).

Trinexapac-ethyl is a plant growth regulator (acylcyclohexanedione), which is used as a ripener in sugar cane. Since it reduces internode elongation, this product is also used to lower the risk of lodging in wheat and other cereal species (Caldas et al. 2009; Nascimento et al. 2009; Rademacher 2015). The main activity of this compound results from reducing the levels of active gibberellins (mainly GA_1) in response to competition with structurally related 2-oxogluterate. The latter is a co-substrate of dioxygenases catalyzing late steps in GA metabolism (Adams et al. 1992; Rademacher 2000; Hedden and Sponsel 2015). As verified in some studies (Pires et al. 2013; Correia and Villela 2015; Bacha et al. 2017), there is no negative effect of low doses of this compound on eucalyptus plants.

Thus, with the hypothesis that trinexapac-ethyl confers a stimulatory effect on eucalyptus, this study aimed to evaluate the effect of trinexapac-ethyl applied at two timings (before and after planting) in *E. urophylla* seedlings under nutritional deficiency conditions.

Material and Methods

Growth conditions and plant materials

Two experiments were conducted simultaneously in a greenhouse during 81 days after planting (DAP) of eucalyptus seedlings, in 15 L pots previously filled with sand. During the experimental period, the mean values of relative air humidity were 61.4%, temperature 22.5 °C (maximum 32.3 °C and minimum 14.0 °C) and insolation of 247 hours monthly.

Seedlings of *Eucalyptus urophylla* (clone I-144) provided by Agriflora®, with 90 days of age were used, which had on average 37 cm of height, 3.35 mm of stem diameter and 12 leaves.

Treatments, experimental design and trinexapac-ethyl application

Two trinexapac-ethyl (Moddus® produced by Syngenta® Crop Protection) application times were evaluated: before planting (BP - Experiment 1) and after planting (AP - Experiment 2). In the BP application time, the seedlings were sprayed with trinexapac-ethyl at the doses of 30 and 60 g a.i. ha^{-1} one day before eucalyptus planting. These rates represent, respectively, 10% and 20% of the commercial dose recommended for sugarcane and had previously been shown to cause positive effects on eucalyptus (Bacha et al. 2017). A costal sprayer with constant pressure (CO_2) equipped with a double rod TT 110.02 and adjusted to spray a tank volume of 200 L ha^{-1} has been used. At the time of application, the air temperature was 27.3 °C with a relative humidity of 59.5%. Twenty-four hours after trinexapac-ethyl application, all seedlings (from both experiments) were planted in the pots.

For the AP timing, the application occurred at 33 DAP, and the same doses and application methodology were used as described above. At that time, the air temperature was 25.2 °C and the relative humidity was 61.5%.

In both experiments, a randomized block design with five replications was used, and the treatments were arranged in a 3x4 factorial scheme, which means three doses of trinexapac-ethyl (0%, 10% and 20% of the commercial dose) and four variations of the solution described by Hoagland and Arnon (1950): complete solution; absence of N (-N); absence of P (-P) and absence of K (-K). At the end of the experimental period, the complete solution provided per pot was: 9.44 g of $Ca(NO_3)_2\ 4H_2O$, 4.04 g of KNO_3, 3.94 g of $MgSO_4\ 7H_2O$ and 1.08 g of KH_2PO_4; the -N solution provided 0.98 g of $MgSO_4\ 7H_2O$, 1.00 g of $Ca(H_2PO_4)_2\ H_2O$, 13.9 g of K_2SO_4 and 2.75 g of $CaSO_2\ 2H_2O$; the -P solution provided 14.1 g of $Ca(NO_3)_2\ 4H_2O$, 3.94 g of $MgSO_4\ 7H_2O$ and 13.9 g of K_2SO_4; and the -

K provided 14.1 g of $Ca(NO_3)_2$ $4H_2O$, 3.94 g of $MgSO_4$ $7H_2O$ and 0.1 g of $Ca(H_2PO_4)_2$ H_2O; all variants were additionally supplied per pot with 4 mg of B, Mn and Fe-EDTA, 0.08 mg of Mo, 0.16 mg of Cu and 0.4 mg of Zn.

To guarantee seedlings survival, all of them were irrigated with the complete solution at concentrations of 25% (at 7, 9 and 11 DAP) and 50% until 21 DAP. At 23 DAP the application of the solutions proposed for each treatment began. The pots were daily irrigated with water to field capacity, and 200 mL of each nutrient supply were applied in alternate days. The plants were maintained under these nutrient conditions until 81 DAP.

Assessed variables and statistical analysis

In the AP application time, at 36 DAP (three days after trinexapac-ethyl application), and at 53 DAP in the BP application time, the net CO_2 assimilation rate, intercellular CO_2 concentration and stomatal conductance were evaluated with an infrared gas analyzer (IRGA mod. LI 6400, LiCor®). The working reference conditions adopted for gas exchange evaluations were: 19 mmol H_2O mol^{-1}, 398 µmol CO_2 mol^{-1}, chamber temperature set at 25 °C, flow rate at 400 µmol s^{-1}, atmospheric pressure in 1000 KPa and the photosynthetically active radiation (PAR) at 1100 µmol m^{-2} s^{-1}.

At the end of the experimental period (81 DAP), stem diameter (using digital caliper) and plant height (with ruler graduated in mm) were determined in both experiments. Total chlorophyll content (Falker®, mod. CFL 1030), net CO_2 assimilation rate, intercellular CO_2 concentration and stomatal conductance were also evaluated in the third fully expanded leaf of each plant, using the same methodology as described above. After the evaluations, the plants were cut at the base level and leaves were detached for leaf area determination (LiCor®, mod. LI 3100 A). The roots were washed and, like the

stems and leaves, were dried in a forced air circulation oven (70 ºC) for 96 hours, to determine dry matter with an electronic precision scale.

The data were submitted to analysis of variance by the F test and the means were compared by the Tukey test, at the 5% probability level. The software used for statistical analysis was AgroEstat (version 1.1.0.626).

Results

1st Experiment: Application Before Planting – BP

For the eucalyptus stem diameter, both doses of trinexapac-ethyl did not differ from control. For eucalyptus height, leaf area, chlorophyll and dry matter of stem and root, there was no effect of the product (Table 1). Seedlings cultivated in deficiency of nitrogen (-N) obtained the lowest values, with reductions of 35.7% in height, 46.8% in diameter and 85.4% in leaf area, in comparison to the complete solution (Table 1).

For the root/shoot ratio (Table 2), there was a positive effect of the application of 20% of trinexapac-ethyl in the -N treatment. Moreover, this treatment provided higher values for this characteristic in relation to the other nutrient supply at all doses tested. On the other hand, the application of 20% of the product had no positive effect for leaf dry matter (LDM) when the plants were grown in complete solution (Table 2).

For the total dry matter (TDM) in the -P treatment, the dose of 10% of trinexapac-ethyl was beneficial to the plants, matching those that received complete nutrient supply (Table 2). However, for the treatment -K the application of 10% of the product had opposite effect, presenting smaller values in comparison to the control (Table 2).

For the net assimilation rate, the -P solution resulted in a value similar to the complete solution, whereas the -N treatment provided the lowest value, a result that is

155 probably related to the higher amount of intercellular carbon found in this condition (Table
156 3).

157 Under complete solution, the plants sprayed with 10% of trinexapac-ethyl had a
158 higher net assimilation rate at 81 DAP, compared to control (Table 4). This treatment was
159 also significantly different from the treatments with -P and -K, a fact that was not observed
160 in the 0% or 20% doses of trinexapac-ethyl (Table 4). The plants grown in -P solution
161 obtained higher stomatal conductance with the application of 20% of trinexapac-ethyl
162 (Table 4).

163

164 ***2nd Experiment: Application After Planting – AP***

165 The application of 20% of trinexapac-ethyl provided a positive effect on plant
166 height compared to untreated plants, with an increase of 7.4% (Table 5). For the nutrient
167 supply, the plants of -N treatment were 39.4% smaller than that which received a complete
168 solution. The -P treatment equaled the -K and complete solution, a fact that did not occur
169 in the first experiment (Table 5). This may be related to the fact that the plants had the root
170 system already established at the time of treatment, and this may have benefited plants
171 grown under solution -P.

172 Regarding dry matter, both doses of trinexapac-ethyl favored ShootDM and TDM.
173 For StemDM and LDM, only the application of 20% of trinexapac-ethyl provided a
174 positive effect. No difference was observed between treatments for RDM (Table 5).

175 For LDM, the plants that received -P solution were able to compensate for the loss
176 in the other dry matter characteristics, matching the plants that received complete solution
177 and -K (Table 5). This gain was provided by the application of ethyl-trinexapac, as it can
178 be noted in Table 6, in which both doses benefited leaf growth in eucalyptus plants. The
179 application of the compound in plants treated with -P solution provided increases in leaf

area of 19.4% and 28.5% for the doses of 10% and 20%, respectively, compared to control, matching them with treatments -K and complete solution (Table 6).

For root/shoot ratio, the plants from -N treatment that received 10% of the product had higher values than the others (Table 6). Regardless of the application of trinexapac-ethyl, the plants cultivated with -N solution had a higher root/shoot ratio than those grown in the other solutions (Table 6).

For the total chlorophyll content, we found a positive effect of both doses on -K treatment, making these plants equal to those of the -P treatment (Table 6). Regardless of the dose of trinexapac-ethyl, the plants of the -N treatment had the lowest levels of chlorophyll (Table 6).

At 36 DAP, there was a positive effect of trinexapac-ethyl on the net assimilation rate, with an increase of up to 13.3% compared to control (Table 7).

The plants cultivated with -N solution presented less stomatal conductance, independent of the application of the product. However, the lower dose caused lower values for these variables, comparing to the control and the higher dose (Table 8).

Discussion

Regarding the results for plants grown with -P solution, several studies (Hernandez et al. 2007; Huang et al. 2008; Warren 2011) have reported adverse effects on multiple classes of plant metabolites under phosphorus deficiency (e.g., tricarboxylic acid, phenylpropanoids, cycle organic acids, carbohydrates and amino acids). It is likely that these are the main causes for the differences in dry matter found between -P treatments and complete solution in both application times (BP and AP). However, it should be emphasized that for TDM (in BP application time - Table 2) and leaf area (in AP application time - Table 6), the application of trinexapac-ethyl caused a positive effect on

plants with phosphorus deficiency, with gains of 13.5% in TDM, and up to 28.5% in leaf area.

Trinexapac-ethyl is an acylcyclohexanedione primarily inhibiting the enzyme GA_{20} 3β-hydroxylase, thereby blocking the conversion of GA_{20} (inactive) into GA_1 (highly bioactive) (Adams et al. 1992; Hedden 2016). As a result, longitudinal shoot growth would be reduced. However, Rademacher (2016) points out that in some cases there may be a paradoxical effect of trinexapac-ethyl and related acylcyclohexanediones, namely to intensify shoot growth. The compound may also inhibit the hydroxylation at the 2β-position (Griggs et al. 1991). This it would prevent GA_1, already present in the plant at the time of treatment, to be transformed into inactive GA_8 (Hisamatsu et al. 1998). The extended permanence of GA_1 could account for growth enhancement as observed in the eucalyptus seedlings.

The highest values of root/shoot ratio found in plants cultivated in -N solution, compared to the others nutrient supply, can be explained by the proportionally smaller shoot growth in relation to root growth, as a result of the greater allocation of photoassimilates to this organ. The greater investment in root growth under nutritional deficiency conditions is related to the need for increased soil nutrient uptake, and this increase in root/shoot ratio was also observed by Ferreira et al. (2015) in *E. urophylla* seedlings (clone I-144) under nitrogen restriction. It is induced because nitrogen modulates the production of cytokinins, and a deficiency of this nutrient decreases the production of this type of hormone (Sakakibara 2006). As a result, cytokinin-deficient plants develop smaller shoots, whereas root growth is intensified, resulting in a higher root/shoot ratio (Werner et al. 2001). However, it was also observed that the application of trinexapac-ethyl provided gains for this characteristic in both application times. This response may be related to a sum of cytokinin-related responses and the changes in GA levels caused by the

compound, since only plants under nitrogen deficiency responded positively in this regard (Tables 2 and 6).

In this context, it is important to emphasize that the initial development of eucalyptus is in the period in which the plant is more susceptible to environmental stress (Nambiar and Sands 1993; Garau et al. 2008). Thus, the gains achieved by spraying the product may be a viable alternative to producers so that the plant can cope with adverse environmental conditions during the initial growth phase.

Several authors define hormesis as a stimulatory effect resulting from the application of low doses of a substance that would be toxic in high quantities (Calabrese and Baldwin 2002; Belz and Duke 2014). This effect has already been observed in several plant species, including *E. urograndis* and *E. grandis* (Velini et al. 2008, Pires et al. 2013; Correia and Villela 2015; Bacha et al. 2017).

In the BP application time, there was a positive effect of the application of 20% of trinexapac-ethyl in the root/shoot ratio (-N treatment); and for TDM (in the -P treatment), the application of 10% of trinexapac-ethyl caused an increase of 13.5% (Table 2). For gas exchange at 81 DAP, some positive effects were also observed in the plants under complete and -P solutions (Table 4). Nevertheless, these gains were not reflected in increases in growth and dry matter characteristics of eucalyptus (Table 1). Belz and Duke (2014) elucidate that several factors can influence the occurrence of hormesis resulting from the application of chemicals, such as: species, clone or cultivar used (Dusky et al. 1986; McDonald et al. 2001; Bacha et al. 2017), stage of plant development (Carvalho et al. 2013), environmental conditions (Belz and Cedergreen 2010), and the final evaluation point (Cedergreen et al. 2009; Belz et al. 2011), that is, how long after exposure to the product is the assessment. Thus, this could be the reason for the difference observed between the results obtained in the present study and those reported by Pires et al. (2013),

255 which found gains of 19% in leaf area of *E. urograndis* sprayed with trinexapac-ethyl
256 before planting of seedlings (methodology similar to the BP application time). Also, it
257 should be noted that the authors conducted the experiment during 42 DAP, while in the
258 present work the plants were cultivated over 81 DAP.

259 In the AP application time, there was a positive effect of trinexapac-ethyl for most
260 of the eucalyptus dry matter variables (Table 5), with increases up to 10.5% in ShootDM,
261 and 8.81% in TDM. The height and leaf area also increased significantly as compared with
262 the control, namely by 7.41% and 28.5% (for treatment -P), respectively (Tables 5 and 6).

263 The beneficial effect of the chemical in most of the biometric characteristics of the
264 eucalyptus exposed to the AP application time, is probably related to the fact that the AP
265 plants had the root system completely established at treatment (at 33 DAP). This was not
266 the case with the BP application time. The AP variant could have favored the degradation
267 of trinexapac-ethyl and the consequent disappearance of its metabolites, which might result
268 in an increased net assimilation rate three days after application (Table 7), and this may
269 also be causative for increase in some parameters previously reported (Tables 5 and 6).
270 Altogether, the results support the hypothesis that trinexapac-ethyl at low doses does not
271 cause any deleterious effect on the photosynthetic apparatus of eucalyptus, as suggested by
272 Pires et al. (2013). In addition, the results also support the assertion that the occurrence of a
273 hormetic effect depends on the plant's development stage at the time of application (Belz
274 and Duke 2014).

275 In this sense, the work of Cedergreen (2008) is worth mentioning, who found that
276 low doses of glyphosate, applied at the two-leaf stage, did not lead to gains in productivity
277 in barley. In contrast, Cedergreen et al. (2009) reported gains of 12-15% in barley
278 productivity after treatment with 2.5-20 g a.i. ha^{-1} of glyphosate at the grain filling stage.

Correia and Villela (2015) also observed a hormetic effect in *E. urograndis* at 45 days after spraying 200 g a.i. ha^{-1} of trinexapac-ethyl, with a 29.2% increase in the crown diameter of the plants. In addition, it should be noted that the application of the product took place 73 days after seedlings planting, which means that the application mode was similar to the AP of the present study, but the seedlings were older. Thus, the difference between the results obtained in these studies could also be due to the fact that the hormetic response is related to plant age. This means that older plants need higher doses than younger plants (Belz and Duke 2014). This view was supported by Velini et al. (2008), who obtained the maximum hormetic response of *Commelina benghalensis* having two expanded leaves and using a dose five times smaller than that used in plants with four expanded leaves.

The processes underlying hormetic effects in response to the application of trinexapac-ethyl have not yet been clarified, but are likely to be related to several signaling steps and physiological responses in the plant resulting from modulated GA metabolism. Thus, the results found in the present study, in which the plant growth regulator provided gains of 28.5% in leaf area (AP) and 13.5% in TDM (BP) under phosphorus deficiency, can provide important information for future studies aiming to understand the hormetic process in more detail. This might enable productivity increases in the near future, especially in the cultivation of crops under non ideal conditions. However, despite these initial positive results, further research is needed, especially studies evaluating the effects of this compound until harvest. By this, it will be possible to verify gains in productivity under applied conditions.

Conclusion

303 At both application times, trinexapac-ethyl had a positive effect on the root/shoot
304 ratio of plants grown in nitrogen-deficient solution.

305 Under phosphorus deficiency, trinexapac-ethyl had a positive effect on total dry
306 matter and leaf area when the application times were "before planting" and "after planting",
307 respectively.

308 In the application after planting the compound provided gains in height and dry
309 matter of the eucalyptus plants, regardless of the nutrient supply used.
310

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TABLES

Table 1. Effect of trinexapac-ethyl on stem height, stem diameter, leaf area, root / shoot ratio (Root/Shoot), total chlorophyll content (Chlorophyll), stem dry matter (StemDM), leaf dry matter (DML), shoot dry matter (ShootDM), root dry matter (RDM) and total dry matter (TDM) of *Eucalyptus urophylla* (Clone I-144) seedlings grown in different nutrient supply at 81 days after planting. Time of Application Before Planting (BP).

Treatments	Height (cm)	Diameter (mm)	Leaf Area (cm^2)	Root/Shoot	Chlorophyll (UR)
Complete	86.9 A	10.1 A	3941.2 A	0.564	28.8 B
-N	55.8 C	5.37 C	573.1 C	1.243	21.5 C
-P	80.6 B	8.62 B	3467.1 B	0.568	34.2 A
-K	90.0 A	9.46 A	3820.1 A	0.494	29.5 B
0% Trinexapac-ethyl	77.3	8.51 AB	3027.8	0.716	28.1
10% Trinexapac-ethyl	78.9	8.03 B	2957.1	0.669	28.0
20% Trinexapac-ethyl	78.7	8.63 A	2866.1	0.768	29.4
F Nutrients	229.9**	135.5**	396.8**	306.3**	76.2**
F Trinexapac-ethyl (TE)	0.90^{ns}	4.10*	1.36^{ns}	8.16**	2.33^{ns}
F (Nutrients x TE)	1.78^{ns}	0.78^{ns}	1.50^{ns}	12.1**	1.40^{ns}
C.V. (%)	5.04	8.33	10.5	10.8	8.14
	StemDM	LDM	ShootDM	RDM	TDM
			(g)		
Complete	16.5 A	21.8	38.6	21.7 A	60.3
-N	2.77 C	4.82	7.59	9.85 C	17.4
-P	12.2 B	19.7	31.9	18.9 B	50.6
-K	16.6 A	21.9	38.6	19.6 AB	57.7
0% Trinexapac-ethyl	11.8	17.4	29.3	17.4	46.7
10% Trinexapac-ethyl	11.7	17.2	29.1	17.2	46.4
20% Trinexapac-ethyl	12.5	16.6	29.1	17.8	46.4
F Nutrients	310.1**	389.9**	421.6**	57.1**	501.3**
F Trinexapac-ethyl (TE)	1.95^{ns}	1.50^{ns}	0.02^{ns}	0.27^{ns}	0.06^{ns}
F (Nutrients x TE)	1.37^{ns}	2.96*	2.32*	1.87^{ns}	7.52**
C.V. (%)	11.9	9.45	9.52	15.3	7.36

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% of probability. * and ** = significant values at 5% and 1% of probability by F test, respectively. ^{ns} = non-significant value at 5% of probability by F test. C.V. = Coefficient of variation. F = F test value.

436 **Table 2.** Means of interaction of factors nutrient supply x doses of trinexapac-ethyl for
437 root/shoot ratio (Root/Shoot), leaf dry matter (LDM), (ShootDM) and total dry matter
438 (TDM) of *Eucalyptus urophylla* (Clone I-144) submitted to the application of trinexapac-
439 ethyl and grown in different nutrient supply. Time of Application Before Planting (BP).

	0% Trinexapac-ethyl	10% Trinexapac-ethyl	20% Trinexapac-ethyl	F
		Root/Shoot		
Complete	0.524 Ba	0.554 BCa	0.615 Ba	1.75^{ns}
-N	1.205 Ab	1.057 Ac	1.468 Aa	35.6**
-P	0.613 Ba	0.618 Ba	0.474 Cb	5.54**
-K	0.520 Ba	0.446 Ca	0.517 BCa	1.45^{ns}
F	89.2**	59.3**	181.9**	---
		LDM (g)		
Complete	23.3 Aa	22.2 Aab	19.9 Bb	5.61**
-N	5.14 Ca	4.88 Ba	4.44 Ca	0.24^{ns}
-P	19.0 Ba	21.0 Aa	19.1 Ba	2.44^{ns}
-K	22.2 Aa	20.8 Aa	22.8 Aa	2.08^{ns}
F	134.7**	130.8**	130.2**	---
		ShootDM (g)		
Complete	39.9 Aa	39.0 Aa	36.9 Aa	1.52^{ns}
-N	7.97 Ca	7.59 Ca	7.22 Ca	0.09^{ns}
-P	30.7 Ba	33.7 Ba	31.4 Ba	1.62^{ns}
-K	38.6 Aab	36.2 ABb	41.0 Aa	3.77**
F	141.5**	136.4**	148.2**	---
		TDM (g)		
Complete	60.7 Aa	60.7 Aa	59.6 Aa	0.18^{ns}
-N	18.2 Ca	16.3 Ca	17.7 Ca	0.43^{ns}
-P	49.5 Bb	56.2 ABa	46.2 Bb	11.1**
-K	58.4 Aa	52.3 Bb	62.3 Aa	10.8**
F	163.6**	176.1**	176.6**	---

440 Means followed by the same uppercase letter in the column, and lower case in the row, do
441 not differ from each other by Tukey test at 5% of probability. ** = significant values at 1%
442 of probability by the F test. ^{ns} = non-significant value at 5% of probability by F test. F = F
443 test value.

444

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Table 3. Effect of trinexapac-ethyl on the net assimilation rate (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of *Eucalyptus urophylla* (Clone I-144) seedlings grown in different nutrient supply at 53 and 81 days after planting. Time of Application Before Planting (BP).

Treatments	A µmol CO_2 m^{-2} s^{-2}	g_s mol H_2O m^{-2} s^{-2}	C_i µmol CO_2 mol^{-1}
	53 days after planting		
Complete	16.7 AB	0.776 A	309.3 B
-N	8.34 C	0.441 B	332.7 A
-P	17.7 A	0.736 A	310.1 B
-K	16.6 B	0.760 A	311.8 B
0% Trinexapac-ethyl	14.7 AB	0.690	316.6
10% Trinexapac-ethyl	14.4 B	0.679	317.9
20% Trinexapac-ethyl	15.3 A	0.665	313.4
F Nutrients	243.3**	75.3**	45.7**
F Trinexapac-ethyl (TE)	3.59*	0.60^{ns}	2.59^{ns}
F (Nutrients x TE)	1.52^{ns}	1.19^{ns}	0.45^{ns}
C.V. (%)	7.30	10.4	2.03
	81 days after planting		
Complete	15.4	0.825	332.4 B
-N	6.55	0.487	362.7 A
-P	13.2	0.615	337.9 B
-K	15.1	0.776	334.3 B
0% Trinexapac-ethyl	12.4	0.679	342.9
10% Trinexapac-ethyl	12.7	0.669	341.2
20% Trinexapac-ethyl	12.5	0.681	341.3
F Nutrients	249.7**	103.9**	33.6**
F Trinexapac-ethyl (TE)	0.40^{ns}	0.21^{ns}	0.20^{ns}
F (Nutrients x TE)	2.43*	4.22**	0.60^{ns}
C.V. (%)	8.06	8.69	2.75

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% of probability. * and ** = significant values at 5% and 1% of probability by F test, respectively. ^{ns} = non-significant value at 5% of probability by F test. C.V. = Coefficient of variation. F = F test value.

Table 4. Means of the interaction of the factors nutrient supply x doses of trinexapac-ethyl for the net assimilation rate (*A*) and stomatal conductance (g_s) in *Eucalyptus urophylla* (Clone I-144) submitted to the application of trinexapac-ethyl and grown in different nutrient supply. Time of Application Before Planting (BP).

	***A* (μmol CO_2 m^{-2} s^{-2}) – 81 DAP**			
	0% Trinexapac-ethyl	10% Trinexapac-ethyl	20% Trinexapac-ethyl	F
Complete	14.8 ABb	16.4 Aa	15.1 Aab	3.58**
-N	6.12 Ca	7.23 Ca	6.31 Ba	1.70^{ns}
-P	13.2 Ba	12.8 Ba	13.6 Aa	0.79^{ns}
-K	15.6 Aa	14.5 Ba	15.2 Aa	1.63^{ns}
F	91.4**	76.1**	87.1**	---
	***g_s* (mol H_2O m^{-2} s^{-2}) – 81 DAP**			
Complete	0.792 Aa	0.860 Aa	0.824 Aa	1.65^{ns}
-N	0.535 Ba	0.470 Ba	0.455 Ca	2.59^{ns}
-P	0.602 Bb	0.549 Bb	0.694 Ba	7.84**
-K	0.777 Aa	0.799 Aa	0.752 Aa	0.79^{ns}
F	23.7**	51.7**	36.9**	---

Means followed by the same uppercase letter in the column, and lower case in the row, do not differ from each other by Tukey test at 5% of probability. ** = significant values at 1% of probability by the F test. ^{ns} = non-significant value at 5% of probability by F test. DAP = days after planting of eucalyptus. F = F test value.

Table 5. Effect of trinexapac-ethyl on height, stem diameter, leaf area, root/shoot ratio (Root/Shoot), total chlorophyll content (Chlorophyll), stem dry matter (StemDM), leaf dry matter (LDM), shoot dry matter (ShootDM), root dry matter (RDM) and total dry matter (TDM) of *Eucalyptus urophylla* (Clone I-144) seedlings grown in different nutrient supply 81 days after planting. Time of Application After Planting (AP).

Treatments	Height (cm)	Diameter (mm)	Leaf Area (cm^2)	Root/Shoot	Chlorophyll (UR)
Complete	89.4 A	9.28 AB	3096.1	0.607	29.4
-N	54.1 B	4.97 C	406.9	1.338	20.2
-P	83.1 A	8.49 B	2801.9	0.587	33.3
-K	88.8 A	9.50 A	2939.6	0.564	31.6
0% Trinexapac-ethyl	75.5 B	8.10	2210.2	0.758	29.1
10% Trinexapac-ethyl	79.3 AB	8.10	2281.8	0.842	28.4
20% Trinexapac-ethyl	81.1 A	7.98	2441.3	0.723	29.1
F Nutrients	90.5**	102.0**	423.1**	385.9**	222.7**
F Trinexapac-ethyl (TE)	4.32*	0.16^{ns}	4.86*	13.6**	1.36^{ns}
F (Nutrients x TE)	1.06^{ns}	1.37^{ns}	3.13*	9.58**	4.87**
C.V. (%)	8.64	10.0	10.3	9.57	5.30
	StemDM	LDM	ShootDM	RDM	TDM
			(g)		
Complete	16.2 A	21.3 A	37.8 A	22.9 A	60.8 A
-N	2.77 C	4.68 B	7.45 C	9.59 C	16.9 C
-P	12.8 B	21.2 A	34.1 B	19.9 B	54.5 B
-K	16.4 A	21.2 A	37.7 A	21.3 AB	59.1 A
0% Trinexapac-ethyl	11.5 B	16.1 B	27.6 B	17.7	45.4 B
10% Trinexapac-ethyl	12.2 AB	17.2 AB	29.6 A	19.2	49.4 A
20% Trinexapac-ethyl	12.5 A	18.1 A	30.5 A	18.3	48.6 A
F Nutrients	513.1**	409.5**	659.5**	86.7**	558.6**
F Trinexapac-ethyl (TE)	4.15*	7.39**	8.99*	1.89^{ns}	7.86**
F (Nutrients x TE)	1.55^{ns}	1.83^{ns}	1.69^{ns}	0.24^{ns}	1.32^{ns}
C.V. (%)	9.09	9.27	7.54	13.5	7.11

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% of probability. * and ** = significant values at 5% and 1% of probability by F test, respectively. ^{ns} = non-significant value at 5% of probability by F test. C.V. = Coefficient of variation. F = F test value.

Table 6. Means of the interaction of the factors nutrient supply x doses of trinexapac-ethyl for leaf area, root/shoot ratio (Root/Shoot) and total chlorophyll content (Chlorophyll) in *Eucalyptus urophylla* (Clone I-144) submitted to the application of trinexapac-ethyl and grown in different nutrient supply. Time of Application After Planting (AP).

	0% Trinexapac-ethyl	10% Trinexapac-ethyl	20% Trinexapac-ethyl	F
	Leaf Area (cm^2)			
Complete	2998.7 Aa	3140.6 Aa	3148.9 Aa	0.62^{ns}
-N	390.5 Ca	361.8 Ba	468.4 Ba	0.26^{ns}
-P	2415.4 Bb	2885.3 Aa	3105.1 Aa	10.7**
-K	3036.2 Aa	2739.5 Aa	3043.1 Aa	2.60^{ns}
F	134.6**	144.4**	150.2**	---
	Root/Shoot			
Complete	0.634 Ba	0.596 Ba	0.592 Ba	0.48^{ns}
-N	1.214 Ab	1.579 Aa	1.221 Ab	39.6**
-P	0.620 Ba	0.592 Ba	0.550 Ba	1.12^{ns}
-K	0.563 Ba	0.600 Ba	0.528 Ba	1.18^{ns}
F	84.9**	219.3**	100.8**	---
	Chlorophyll (UR)			
Complete	28.8 Ba	29.2 Ba	30.2 Ba	1.00^{ns}
-N	21.7 Ca	18.4 Cb	20.4 Cab	6.18**
-P	33.5 Aa	33.0 Aa	33.6 Aa	0.21^{ns}
-K	29.3 Bb	33.2 Aa	32.2 ABa	8.61**
F	51.1**	104.4**	76.8**	---

Means followed by the same uppercase letter in the column, and lower case in the row, do not differ from each other by Tukey test at 5% of probability. ** = significant values at 1% of probability by the F test. ^{ns} = non-significant value at 5% of probability by F test. F = F test value.

Table 7. Effect of trinexapac-ethyl on the net assimilation rate (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of *Eucalyptus urophylla* (Clone I-144) seedlings grown in different nutrient supply at 36 and 81 days after planting. Time of Application After Planting (AP).

	36 days after planting		
Treatments	A µmol CO_2 m^{-2} s^{-2}	g_s mol H_2O m^{-2} s^{-2}	C_i µmol CO_2 mol^{-1}
Complete	17.8 A	0.729 A	301.6
-N	11.9 B	0.486 B	305.3
-P	18.6 A	0.708 A	297.5
-K	18.6 A	0.739 A	304.8
0% Trinexapac-ethyl	15.7 C	0.630	301.2
10% Trinexapac-ethyl	16.6 B	0.695	305.2
20% Trinexapac-ethyl	17.8 A	0.671	300.5
F Nutrients	108.3**	26.2**	1.07^{ns}
F Trinexapac-ethyl (TE)	15.1**	2.55^{ns}	0.73^{ns}
F (Nutrients x ET)	1.45^{ns}	1.13^{ns}	1.61^{ns}
C.V. (%)	7.24	13.7	4.43
	81 days after planting		
Complete	14.9 A	0.781	312.9 BC
-N	5.53 B	0.412	334.8 A
-P	14.8 A	0.655	308.6 C
-K	15.1 A	0.751	316.4 B
0% Trinexapac-ethyl	13.3 A	0.671	316.2
10% Trinexapac-ethyl	12.4 AB	0.629	317.2
20% Trinexapac-ethyl	12.1 B	0.649	321.1
F Nutrients	236.7**	104.9**	32.5**
F Trinexapac-ethyl (TE)	5.75**	2.15^{ns}	2.13^{ns}
F (Nutrients x TE)	1.98^{ns}	4.31**	0.99^{ns}
C.V. (%)	9.41	9.71	2.45

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% of probability. * and ** = significant values at 5% and 1% of probability by F test, respectively. ^{ns} = non-significant value at 5% of probability by F test. C.V. = Coefficient of variation. F = F test value.

Table 8. Means of the interaction of the factors nutrient supply x doses of trinexapac-ethyl for stomatal conductance (g_s) in *Eucalyptus urophylla* (Clone I-144) submitted to the application of trinexapac-ethyl and grown in different nutrient supply. Time of Application After Planting (AP).

	g_s (mol H_2O m^{-2} s^{-2}) – 81 DAP			
	0% Trinexapac-ethyl	10% Trinexapac-ethyl	20% Trinexapac-ethyl	F
Complete	0.800 Aa	0.769 Aa	0.773 Aa	0.36^{ns}
-N	0.452 Ca	0.303 Bb	0.482 Ca	11.4**
-P	0.641 Ba	0.688 Aa	0.635 Ba	1.04^{ns}
-K	0.790 Aa	0.757 Aa	0.706 ABa	2.19^{ns}
F	33.2**	60.8**	19.5**	---

Means followed by the same uppercase letter in the column, and lower case in the row, do not differ from each other by Tukey test at 5% of probability. ** = significant values at 1% of probability by the F test. ^{ns} = non-significant value at 5% of probability by F test. DAP = days after planting of eucalyptus. F = F test value.