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ARTICLE

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

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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 with greater tolerance to stress. The objective of this study was to evaluate the effect of trinexapac-ethyl at two application times, before planting (BP) or after planting (AP), in *Eucalyptus urophylla* under conditions of nutritional deficiency (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 doses of trinexapac-ethyl (0, 30, and 60 g a.i.·ha^{-1}) and four variations of nutrient supply: complete solution (NPK) and solutions without nitrogen (–N), without phosphorus (–P), and without potassium (–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 AP application, the compound provided gains in height and dry matter, regardless of the nutrient supply. Under phosphorus deficiency, trinexapac-ethyl provided gains in total dry matter (BP) and leaf area (AP).

Key words: Eucalyptus urophylla, nitrogen, phosphorus, plant growth regulator, hormesis.

Résumé : Durant la période initiale de croissance, les plants d'eucalyptus sont sensibles aux stress abiotiques dus le plus souvent à des déficiences nutritionnelles. Le trinexapac-éthyle, un régulateur de croissance des plantes, peut avoir un effet positif chez l'eucalyptus en procurant probablement une plus grande tolérance aux stress chez les plantes, une réaction connue sous le nom d'hormèse. L'objectif de cette étude consistait à évaluer l'effet du trinexapac-éthyle appliqué à deux moments différents, soit avant (BP) ou après (AP) la plantation chez *Eucalyptus urophylla* soumis à des déficiences nutritionnelles (NPK). Deux expériences, une pour chaque moment d'application, ont été menées simultanément durant 81 jours suivant la plantation d'eucalyptus dans des pots de 15 L. Les traitements comprenaient trois doses de trinexapac-éthyle (0, 30 ou 60 g de substance active à l'hectare) et quatre solutions d'apport d'éléments nutritifs : solution complète (NPK) et solutions sans azote (–N), sans phosphore (–P) ou sans potassium (–K). Les variables ayant trait aux échanges gazeux, à la croissance et à la matière sèche ont été évaluées. Peu importe le moment où il a été appliqué, le trinexapac-éthyle a eu un effet positif sur le rapport racines–tige des plants cultivés avec le traitement –N ainsi que sur certaines caractéristiques photosynthétiques de l'eucalyptus. Appliqué après la plantation, le composé a entraîné des gains en hauteur et en matière sèche peu importe l'apport de nutriment. En présence d'une déficience en P, le trinexapac-éthyle a entraîné des gains de matière sèche totale (BP) et de surface foliaire (AP). [Traduit par la Rédaction]

Mots-clés : Eucalyptus urophylla, azote, phosphore, régulateur de croissance des plantes, hormèse.

Introduction

Eucalyptus is the most important genus in the Brazilian forestry sector, being the main source of wood for cellulose production and energy, among other uses (Indústria Brasileira de Árvores (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's eucalyptus forests have the world's highest productivity (Ibá 2016).

The initial growth stage of eucalyptus, which includes the first year after seedlings were planted, 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 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 to eucalyptus seedlings may have a positive effect on plant growth, which can also be related to 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

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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. Because 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 is 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, the aim of this study was to evaluate the effect of trinexapac-ethyl applied at two timings (before planting and after planting) to *Eucalyptus urophylla* seedlings under conditions of nutritional deficiency.

Material and methods

Growth conditions and plant materials

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

Seedlings of 90-day-old *Eucalyptus urophylla* (clone I-144) provided by Agriflora® were used. On average, the seedlings had 12 leaves, were 37 cm in height, and had a stem diameter of 3.35 mm.

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). During BP application, the seedlings were sprayed with trinexapac-ethyl at doses of 30 and 60 g a.i.·ha^{-1} one day before planting. These rates represent, respectively, 10% and 20% of the commercial dose recommended for sugarcane and had previously been shown to have positive effects on eucalyptus (Bacha et al. 2017). A CO_2-pressurized backpack sprayer (Herbicat®, Catanduva-SP, Brazil) equipped with a double-rod Turbo TeeJet® 110.02 and adjusted to spray a tank volume of 200 L·ha^{-1} was used. At the time of application, the air temperature was 27.3 °C and relative humidity was 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 days after planting (DAP), and the same doses and application methodology as described above were used. 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 3 × 4 factorial scheme: 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 nitrogen (–N), absence of phosphorus (–P), and absence of potassium (–K). At the end of the experimental period, the complete solution provided per pot was 9.44 g of $Ca(NO_3)_2{\cdot}4H_2O$, 4.04 g of KNO_3, 3.94 g of $MgSO_4{\cdot}7H_2O$, and 1.08 g of KH_2PO_4; the –N solution provided 0.98 g of $MgSO_4{\cdot}7H_2O$, 1.00 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 13.9 g of K_2SO_4, and 2.75 g of $CaSO_2{\cdot}2H_2O$; the –P solution provided 14.1 g of $Ca(NO_3)_2{\cdot}4H_2O$, 3.94 g of $MgSO_4{\cdot}7H_2O$, and 13.9 g of K_2SO_4; and the –K solution provided 14.1 g of $Ca(NO_3)_2{\cdot}4H_2O$, 3.94 g of $MgSO_4{\cdot}7H_2O$, and 0.1 g of $Ca(H_2PO_4)_2{\cdot}H_2O$; all variants were additionally supplied 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 per pot.

To guarantee survival of the seedlings, all pots 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 irrigated daily to field capacity with water, and 200 mL of each nutrient supply was applied on alternate days. The plants were maintained under these nutrient conditions until 81 DAP.

Assessed variables and statistical analysis

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

At the end of the experimental period (81 DAP), stem diameter (using digital caliper) and plant height (with ruler graduated in millimetres) were determined in both experiments. Total chlorophyll content (Falker®, model 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 and leaves were detached for leaf area determination (LiCor®, model LI 3100 A). The roots were washed and, as with the stems and leaves, dried in a forced-air circulation oven (70 °C) for 96 h to determine dry matter with an electronic precision scale.

The data were submitted to analysis of variance (ANOVA) 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; Barbosa and Maldonado 2011).

Results

Experiment 1: application before planting (BP)

For eucalyptus stem diameter, there was no effect from either dose of trinexapac-ethyl compared with the control. For eucalyptus height, leaf area, chlorophyll, and stem and root dry matter, there was no significant effect from trinexapac-ethyl (Table 1). Seedlings cultivated in conditions of nitrogen deficiency (–N) obtained the lowest values, with reductions of 35.7% in height, 46.8% in diameter, and 85.4% in leaf area in comparison with the complete solution (Table 1).

For 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 supplies at all doses tested. On the other hand, the application of 20% of the product had no positive effect on leaf dry matter (LDM) when the plants were grown in complete solution (Table 2).

For 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 –K treatment, the application of 10% of trinexapac-ethyl had the opposite effect, presenting smaller values in comparison with the control (Table 2).

For net assimilation rate, the –P treatment resulted in a value similar to that of the complete solution, whereas the –N treatment provided the lowest value, a result that is probably related to the higher amount of intercellular carbon found in this condition (Table 3).

Under the complete solution, the plants sprayed with 10% of trinexapac-ethyl had a higher net assimilation rate at 81 DAP compared with the control (Table 4). This treatment also differed significantly from the treatments with –P and –K, a fact that was not

Table 1. Effect of trinexapac-ethyl on stem height, stem diameter, leaf area, root–shoot ratio, 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 supplies at 81 days after planting. Time of application: before planting (BP).

	Height (cm)	Diameter (mm)	Leaf area (cm²)	Root–shoot ratio	Chlorophyll (UR)	StemDM (g)	LDM (g)	ShootDM (g)	RDM (g)	TDM (g)
Complete	86.9A	10.1A	3941.2A	0.564	28.8B	16.5A	21.8	38.6	21.7A	60.3
–N	55.8C	5.37C	573.1C	1.243	21.5C	2.77C	4.82	7.59	9.85C	17.4
–P	80.6B	8.62B	3467.1B	0.568	34.2A	12.2B	19.7	31.9	18.9B	50.6
–K	90.0A	9.46A	3820.1A	0.494	29.5B	16.6A	21.9	38.6	19.6AB	57.7
Trinexapac-ethyl (TE)										
0%	77.3	8.51AB	3027.8	0.716	28.1	11.8	17.4	29.3	17.4	46.7
10%	78.9	8.03B	2957.1	0.669	28.0	11.7	17.2	29.1	17.2	46.4
20%	78.7	8.63A	2866.1	0.768	29.4	12.5	16.6	29.1	17.8	46.4
F(nutrients)	229.9**	135.5**	396.8**	306.3**	76.2**	310.1**	389.9**	421.6**	57.1**	501.3**
F(TE)	0.90ns	4.10*	1.36ns	8.16**	2.33ns	1.95ns	1.50ns	0.02ns	0.27ns	0.06ns
F(nutrients × TE)	1.78ns	0.78ns	1.50ns	12.1**	1.40ns	1.37ns	2.96*	2.32*	1.87ns	7.52**
CV (%)	5.04	8.33	10.5	10.8	8.14	11.9	9.45	9.52	15.3	7.36

Note: Means followed by the same letter in the column do not differ from each other at the 5% probability level as determined by the Tukey test; * and **, significant values at probabilities of 5% and 1%, respectively (*F* test); ns, nonsignificant value at a probability of 5% (*F* test); CV, coefficient of variation; *F*, *F* test value.

Table 2. Means of interactions of factors nutrient supply × dose of trinexapac-ethyl for root–shoot ratio, leaf dry matter (LDM), shoot dry matter (ShootDM), and total dry matter (TDM) of *Eucalyptus urophylla* (clone I-144) submitted to the application of trinexapac-ethyl and grown in different nutrient supplies. Time of application: before planting (BP).

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

Note: Means followed by the same uppercase letter in the column and same lowercase letter in the row do not differ from each other at the 5% probability level as determined by the Tukey test; **, significant value at a probability of 1% (*F* test); ns, nonsignificant value at a probability of 5% (*F* test).

observed in the 0% or 20% doses of trinexapac-ethyl (Table 4). The plants grown in –P solution obtained higher stomatal conductance with the application of 20% of trinexapac-ethyl (Table 4).

Experiment 2: application after planting (AP)

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

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

For LDM, the plants that received the –P solution were able to compensate for the loss in other dry matter characteristics, matching the plants that received the complete solution and the –K treatment (Table 5). This gain was provided by the application of ethyl-trinexapac; as can be noted in Table 6, leaf growth in eucalyptus plants benefited from both doses. The application of the compound in plants treated with the –P solution increased leaf area by 19.4% and 28.5% for 10% and 20% doses, respectively, compared with the control, matching them with –K and complete solution treatments (Table 6).

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

For total chlorophyll content, we found a positive effect of both doses on plants of the –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 with the control (Table 7).

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

Discussion

Regarding the results for plants grown with the –P solution, several studies (Hernández 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 of the differences in dry matter found between –P treatments and the complete solution in both application times (BP and AP). However, it should be emphasized that for TDM (in BP application time in Table 2)

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 supplies at 53 and 81 days after planting. Time of application: before planting (BP).

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

Note: Means followed by the same letter in the column do not differ from each other at the 5% probability level as determined by the Tukey test; * and **, significant values at probabilities of 5% and 1%, respectively (*F* test); ns, nonsignificant value at a probability of 5% (*F* test); CV, coefficient of variation; *F*, *F* test value.

Table 4. Means of the interactions of the factors nutrient supply × dose 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 supplies. Time of application: before planting (BP).

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

Note: Means followed by the same uppercase letter in the column and same lowercase letter in the row do not differ from each other at the 5% probability level as determined by the Tukey test; **, significant value at a probability of 1% (*F* test); ns, nonsignificant value at a probability of 5% (*F* test); DAP, days after planting of eucalyptus.

and leaf area (in AP application time in 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 the –N solution, compared with the other nutrient supplies, 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 conditions of nutritional deficiency 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, as 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 when the plant is more susceptible to environmental stress (Nambiar and Sands 1993;

Table 5. Effect of trinexapac-ethyl on height, stem diameter, leaf area, root–shoot ratio, 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 supplies 81 days after planting. Time of application: after planting (AP).

	Height (cm)	Diameter (mm)	Leaf area (cm²)	Root–shoot ratio	Chlorophyll (UR)	StemDM (g)	LDM (g)	ShootDM (g)	RDM (g)	TDM (g)
Complete	89.4A	9.28AB	3096.1	0.607	29.4	16.2A	21.3A	37.8A	22.9A	60.8A
–N	54.1B	4.97C	406.9	1.338	20.2	2.77C	4.68B	7.45C	9.59C	16.9C
–P	83.1A	8.49B	2801.9	0.587	33.3	12.8B	21.2A	34.1B	19.9B	54.5B
–K	88.8A	9.50A	2939.6	0.564	31.6	16.4A	21.2A	37.7A	21.3AB	59.1A
Trinexapac-ethyl (TE)										
0%	75.5B	8.10	2210.2	0.758	29.1	11.5B	16.1B	27.6B	17.7	45.4B
10%	79.3AB	8.10	2281.8	0.842	28.4	12.2AB	17.2AB	29.6A	19.2	49.4A
20%	81.1A	7.98	2441.3	0.723	29.1	12.5A	18.1A	30.5A	18.3	48.6A
F(nutrients)	90.5**	102.0**	423.1**	385.9**	222.7**	513.1**	409.5**	659.5**	86.7**	558.6**
F(TE)	4.32*	0.16ns	4.86*	13.6**	1.36ns	4.15*	7.39**	8.99*	1.89ns	7.86**
F(nutrients × TE)	1.06ns	1.37ns	3.13*	9.58**	4.87**	1.55ns	1.83ns	1.69ns	0.24ns	1.32ns
CV (%)	8.64	10.0	10.3	9.57	5.30	9.09	9.27	7.54	13.5	7.11

Note: Means followed by the same letter in the column do not differ from each other at the 5% probability level as determined by the Tukey test; * and **, significant values at probabilities of 5% and 1%, respectively (*F* test); ns, nonsignificant value at a probability of 5% (*F* test); CV, coefficient of variation; *F*, *F* test value.

Table 6. Means of the interactions of the factors nutrient supply × dose of trinexapac-ethyl for leaf area, root–shoot ratio, and total chlorophyll content (Chlorophyll) in *Eucalyptus urophylla* (clone I-144) submitted to the application of trinexapac-ethyl and grown in different nutrient supplies. Time of application: after planting (AP).

	Trinexapac-ethyl			
	0%	10%	20%	*F* value
Leaf area (cm²)				
Complete	2998.7Aa	3140.6Aa	3148.9Aa	0.62ns
–N	390.5Ca	361.8Ba	468.4Ba	0.26ns
–P	2415.4Bb	2885.3Aa	3105.1Aa	10.7**
–K	3036.2Aa	2739.5Aa	3043.1Aa	2.60ns
F value	134.6**	144.4**	150.2**	—
Root–shoot patio				
Complete	0.634Ba	0.596Ba	0.592Ba	0.48ns
–N	1.214Ab	1.579Aa	1.221Ab	39.6**
–P	0.620Ba	0.592Ba	0.550Ba	1.12ns
–K	0.563Ba	0.600Ba	0.528Ba	1.18ns
F value	84.9**	219.3**	100.8**	—
Chlorophyll (UR)				
Complete	28.8Ba	29.2Ba	30.2Ba	1.00ns
–N	21.7Ca	18.4Cb	20.4Cab	6.18**
–P	33.5Aa	33.0Aa	33.6Aa	0.21ns
–K	29.3Bb	33.2Aa	32.2ABa	8.61**
F value	51.1**	104.4**	76.8**	—

Note: Means followed by the same uppercase letter in the column and same lowercase in the row do not differ from each other at the 5% probability level as determined by the Tukey test; **, significant value at a probability of 1% (*F* test); ns, nonsignificant value at a probability of 5% (*F* test).

Garau et al. 2008). Thus, the gains achieved by spraying trinexapac-ethyl 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 *Eucalyptus urograndis* and *Eucalyptus 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, e.g., species, clone, or cultivar used (Dusky et al. 1985; 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), i.e., 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), which found gains of 19% in leaf area of *E. urograndis* sprayed with trinexapac-ethyl before planting of seedlings (methodology similar to the BP application time). Also, it should be noted that the authors conducted the experiment during 42 DAP, whereas in the present work, the plants were cultivated over 81 DAP.

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

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

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

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 supplies at 36 and 81 days after planting. Time of application: after planting (AP).

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

Note: Means followed by the same letter in the column do not differ from each other at the 5% probability level as determined by the Tukey test; * and **, significant values at probabilities of 5% and 1%, respectively (*F* test); ns, nonsignificant value at a probability of 5% (*F* test); CV, coefficient of variation; *F*, *F* test value.

Table 8. Means of the interactions of the factors nutrient supply × dose of trinexapac-ethyl (TE) for stomatal conductance (g_s) in *Eucalyptus urophylla* (clone I-144) submitted to the application of trinexapac-ethyl and grown in different nutrient supplies. Time of application: after planting (AP).

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

Note: Means followed by the same uppercase letter in the column and same lowercase letter in the row do not differ from each other at the 5% probability level as determined by the Tukey test; **, significant value at a probability of 1% (*F* test); ns, nonsignificant value at a probability of 5% (*F* test); DAP, days after planting of eucalyptus.

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 trinexapac-ethyl took place 73 days after seedling 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

At both application times, trinexapac-ethyl had a positive effect on the root–shoot ratio of plants grown in the nitrogen-deficient solution. Under phosphorus deficiency, trinexapac-ethyl had a positive effect on total dry matter and leaf area when the application times were BP and AP, respectively. In the AP application, trinexapac-ethyl provided gains in height and dry matter of the eucalyptus plants, regardless of the nutrient supply used.

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