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DOSE OF TRINEXAPAC-ETHYL AND THE AGE OF SEEDLING INFLUENCE THE STIMULATORY EFFECT IN *EUCALYPTUS*

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Trinexapac-ethyl (TE) has recently been shown to have a stimulatory effect on the initial growth of *Eucalyptus* seedlings. However, the optimal dose and seedling age at which TE should be applied has not been assessed. The objective of our study was to evaluate the effects of sub-doses of TE on the initial growth of eight *Eucalyptus* clones. The experiment was conducted in an open area. The treatments were arranged in an 8 × 3 factorial scheme, meaning eight *Eucalyptus* clones and three doses of TE (0, 15 and 30 g a.i. ha^{-1}) sprayed at 70 days after planting. Plant height, stem diameter, shoot and root dry mass, and leaf area were evaluated. *Eucalyptus* clones differed in their growth regardless TE application. TE did not negatively affect the growth of any clones but doses used in our study were not sufficient to give a significant positive effect on growth when applied at 70 days after planting. We suggest that older *Eucalyptus* seedlings need higher doses of TE to reveal potential stimulatory effects on growth of the plants.

Keywords: Plant growth regulator, gibberellins, chemical ripener, *Eucalyptus* clones.

INTRODUCTION

The forestry sector is of significant importance to Brazil's economy. Forestry accounted for 1.2% of the national GDP and contributed over R$69 billion in 2015. Of the 7.8 million ha of forest planted in Brazil for economic purposes, more than 70% were *Eucalyptus* (Ibá 2016). The large increase in *Eucalyptus* plantations has been attributed to improved silvicultural practices and breeding programme. The market launch of several outstanding clones has made Brazil's *Eucalyptus* plantations the most productive in the world (Ibá 2016). Clones available on the market are of the *Eucalyptus urograndis* hybrid (*E. urophylla* × *E. grandis*), developed for fast growth, disease resistance and drought tolerance (Retief & Stanger 2009). However, such growth was only possible because of the cultural practices improvement as well as the development of breeding programme. Thus, several clones were launched in the market, enabling the country to obtain the highest *Eucalyptus* productivity in the world (Stape et al. 2004, Pereira et al. 2012, Ibá 2016).

Biotic (e.g. competition and herbivory) and abiotic (e.g. water and nutrient deficiencies) stressors can retard the growth of newly planted *Eucalyptus* seedlings in the field (Nambiar & Sands 1993, Garau et al. 2008). As growth disruptions during this critical period of development may result in productivity losses (Sankaran et al. 2004, Garau et al. 2009), a number of studies have focused on boosting seedling growth.

Application of trinexapac-ethyl (TE) to *E. urograndis* clones gave total dry mass gains of up to 60% in Clone 1407 (Bacha et al. 2017). The positive effect resulting from small-dose applications of a chemical that would otherwise be toxic in high quantities is known as hormesis (Calabrese & Baldwin 2002). This phenomenon has been observed previously for several plant species (coffee, pine, corn and soybean), including *Eucalyptus* (Velini et al. 2008, Carvalho et al. 2013, Pereira et al. 2013, Bacha et al. 2018).

TE is a plant growth regulator that acts in the final stages of gibberellic acid biosynthesis (Rademacher 2000). It is often used in sugarcane to accelerate the maturation process and schedule the harvest (Nascimento et al. 2009) and in other monocotyledonous crops, such as rice and wheat, to avoid lodging (Moddus 2020). At the molecular level, TE inhibits the conversion of GA_{20} to GA_1 (a major bioactive gibberellin) by deactivating the enzyme GA_{20} 3β-hydroxylase. TE competes with 2-oxogluteate

for the metabolic substrate Fe^{+2} / ascorbate–dependent dioxygenase (Adams et al. 1992, Rademacher 2016, Hedden 2016).

For *Eucalyptus*, applying 15 g a.i. ha^{-1} of TE before field-planting seedlings increased stem diameter by up to 17% (Pires et al. 2013). Similarly, a pre-planting TE application of 60 g a.i. ha^{-1} gave gains of 30% in stem dry mass (Bacha et al. 2017). When applied at 200 g a.i. ha^{-1} 46 days after planting, a 29% increase in crown diameter over untreated seedlings was recorded (Correia & Villela 2015). In phosphorus deficient conditions, 30 g a.i. ha^{-1} applied to *E. urograndis* (Clone I-144) at 33 days after planting yielded a 19% increase in leaf area (Bacha et al. 2018). While these studies have shown that TE has a stimulatory effect on the initial growth of *Eucalyptus* seedlings, more studies on optimal dosage and timing of application are needed. Studies assessing the growth response to TE of the many *Eucalyptus* clones that are available on the market, are needed as baseline information for breeding programs. The present study evaluates the effect of TE on the initial growth of eight *E. urograndis* clones.

MATERIALS AND METHODS

The potted trial was conducted in an open area, in the municipality of Jaboticabal-SP (21° 14′ S and 48° 17′ W) in Brazil, from January to May 2014. Mean air temperature was 23.7 °C (range = 18.4–30.8 °C), relative humidity (RH) was 71.6% and insolation of 249 hours per month was recorded (Table 1).

Seedlings of eight commercial *E. urograndis* clones were planted in 10 L plastic pots filled with Dark Red Latosol topsoil and sand (2:1 v/v). The clones were 3203 (designated here as Clone 1), 3487 (Clone 2), I-144 (Clone 3), 3334 (Clone 4), 1407 (Clone 5), 2361 (Clone 6), I-224 (Clone 7) and GG100 (Clone 8). Seedlings had a mean height and stem diameter of 33 cm and 2.8 mm respectively, and about 10 leaves. A one-time application of NPK 4-14-18 fertilizer was given at a rate of 300 kg ha^{-1}, and the experiment was conducted without water restriction. At 70 days after planting, seedlings were sprayed with TE at doses of 0, 15 and 30 g a.i. ha^{-1}. A CO_2-pressurised backpack sprayer equipped with a double rod spray and adjusted for a tank volume of 200 L ha^{-1} was used. The application took place in a spray room with an ambient temperature of 29 °C and RH of 70%. A randomised complete block design was used, and the treatments consisted of a factorial scheme 8 × 3, meaning eight clones and three doses of TE, with four replicates.

At 7, 14, 21, 28 and 35 days after application, plant height was measured (from stem base to apical bud) with a ruler (in mm) and stem diameter was measured with a digital callipers (at 1 cm from stem base). At the end of the experiment, 42 days after application, the plants were cut at the stem base and the leaves detached for determination of total leaf area using a leaf area meter.

To obtain shoot dry matter and root dry matter stems, leaves and roots were separated, then dried in a forced-air convection oven at 70 °C for 96 hours until constant mass of the samples was achieved. The samples were then individually weighed. Absolute Growth Rates (AGR) for the plants were calculated using

Table 1 Meteorological data from the region of the city of Jaboticabal-SP in Brazil, during the experiment months in 2014

Month	P (hPa)	T_{max} (°C)	T_{min} (°C)	T_{ave} (°C)	RH (%)	Precipitation (mm)	NRD	Insolation (h)
Jan	943.8	32.6	19.9	25.5	69.1	99.8	13	294.1
Feb	943.0	32.5	19.9	25.5	67.0	83.0	12	233.9
Mar	943.6	30.9	19.5	24.1	76.8	106.8	10	238.4
Apr	944.4	30.1	17.9	23.0	75.2	63.3	8	241.2
May	945.4	28.0	14.6	20.2	70.0	6.7	4	237.4
Year 2014 (averange)	944.7	30.8	17.3	23.1	66.5	814.6	95	2861.3

RH = relative air humidity, NRD = number of rainy days, T_{max} = maximum temperature, T_{min} = minimum temperature, T_{ave} = average temperature, P = atmospheric pressure

the formula proposed by Benincasa (2003) (equation 1).

$$AGR = W_t - W_o / T \quad (1)$$

where W_t and W_o = the final and initial values, respectively, of the assessed variable and T = total observation period.

The data were subjected to analysis of variance by the F-test and the means were compared by the Tukey's test at the 5% level of probability. Statistical analysis was performed using AgroEstat software (version 1.1.0.626) (Barbosa & Maldonado 2011).

RESULTS

From 7 to 35 days after TE application, no significant effect of the interactions between clones and doses on plant height was observed (Table 2). At 7 days after application, Clone 1 plants were still taller than plants of Clones 3, 5 and 6, which in turn were taller than Clone 2, 7, 4 and 8 plants. The last two clones presented lower height (Table 2), thus maintaining the same behaviour observed at 0 days after application. These clone heights were consistent up to 35 days after application, at which point Clone 1 was 132% taller than Clones 2 and 8.

Averaged across TE dosages, height absolute growth rate of Clone 1 was greatest at 0.55 cm day^{-1}, followed by 0.52 cm day^{-1} for Clone 6 while the remaining clones was 0.46 cm day^{-1} on average (Table 3). Height absolute growth rate of Clone 8 at 0.36 cm day^{-1} was lowest of all the clones.

Stem diameter of Clone 8 at the time of application was smaller than that of the other clones (Figure 1) but from 7 days after application onward Clone 8 stem diameter was not significantly smaller than that of the other clones (Table 4). It is noted that at 21 days after application, stem diameters (averaged across all clones) for plants receiving 15 and 30 g a.i. ha^{-1}, were significantly different, while not differing from the untreated control. This response was not observed during the remainder of the experimental period (Table 4).

Table 2 Height of eight *Eucalyptus urograndis* clones at 7, 14, 21, 28 & 35 days after application (DAA) of trinexapac-ethyl

Clones	Height (cm)				
	7 DAA	14 DAA	21 DAA	28 DAA	35 DAA
(1) - 3203	72.33a	77.58a	81.12a	84.58a	86.62a
(2) - 3487	57.33d	60.75d	62.92de	65.17cd	67.04de
(3) - I-144	65.58b	68.91b	71.17bc	73.42b	76.17b
(4) - 3334	59.33cd	63.33cd	65.92cd	68.33bc	69.67cde
(5) - 1407	62.83bc	67.50bc	70.25bc	72.25b	74.80bc
(6) - 2361	63.75bc	68.33bc	72.00b	73.50b	75.67bc
(7) - I-224	60.08cd	63.50cd	66.25cd	67.80bcd	70.00cd
(8) - GG100	55.41d	58.17d	60.25e	62.20d	63.87e
Trinexapac-ethyl					
D1 (15 g a.i. ha^{-1})	62.90a	66.71a	69.16a	71.39a	73.89a
D2 (30 g a.i. ha^{-1})	60.90a	65.15a	67.90a	70.23a	72.26a
D3 (0 g a.i. ha^{-1})	62.71a	66.15a	69.14a	71.09a	72.78a
F clones (C)	21.43**	24.10**	27.74**	27.75**	26.70**
F doses (D)	2.09^{ns}	1.11^{ns}	0.91^{ns}	0.57^{ns}	1.00^{ns}
F C × D	1.34^{ns}	1.30^{ns}	0.90^{ns}	0.85^{ns}	1.17^{ns}
CV (%)	6.43	6.42	6.18	6.34	6.45

Means followed by the same letter in the same column do not differ from each other by Tukey's test at 5% probability; ^{ns} = not significant, ** = significant at 1% probability by the F-test; CV = coefficient of variation

Table 3 Absolute growth rate (AGR) of eight *Eucalyptus urograndis* clones from 0 to 35 days after application of trinexapac-ethyl

AGR	Clone							
	1	2	3	4	5	6	7	8
Height (cm day^{-1})	0.55	0.43	0.47	0.47	0.49	0.52	0.47	0.36
Diameter (mm day^{-1})	0.061	0.082	0.071	0.067	0.066	0.077	0.091	0.084

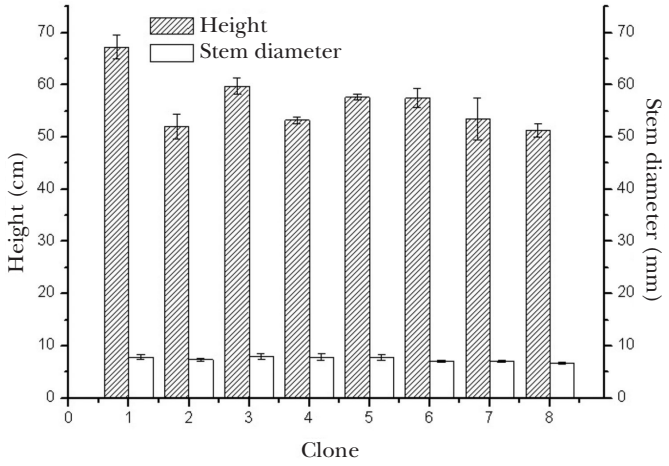

Figure 1 Height and stem diameter of eight *Eucalyptus urograndis* clones at 70 days after planting with the application of trinexapac-ethyl

Table 4 Stem diameter of eight *Eucalyptus urograndis* clones at 7, 14, 21, 28 & 35 days after application (DAA) of trinexapac-ethyl

Clones	Stem diameter (mm)				
	7 DAA	14 DAA	21 DAA	28 DAA	35 DAA
(1) - 3203	7.94a	8.42a	8.90a	9.33a	9.98a
(2) - 3487	8.01a	8.72a	9.10a	9.54a	10.17a
(3) - I-144	8.19a	8.83a	9.12a	9.73a	10.47a
(4) - 3334	8.00a	8.64a	8.99a	9.53a	10.16a
(5) - 1407	8.18a	8.76a	9.27a	9.73a	10.07a
(6) - 2361	7.64a	8.44a	8.80a	9.23a	9.77a
(7) - I-224	8.11a	8.90a	9.34a	9.71a	10.24a
(8) - GG100	7.58a	8.30a	8.77a	9.10a	9.61a
Trinexapac-ethyl					
D1 (15 g a.i. ha^{-1})	8.03a	8.75a	9.24a	9.70a	10.25a
D2 (30 g a.i. ha^{-1})	7.77a	8.53a	8.81b	9.25a	9.90a
D3 (0 g a.i. ha^{-1})	7.80a	8.60a	9.10ab	9.50a	10.02a
F clones (C)	2.95^{ns}	1.53^{ns}	1.56^{ns}	1.37^{ns}	1.44^{ns}
F doses (D)	1.50^{ns}	1.14^{ns}	4.09*	3.08^{ns}	4.90^{ns}
F C × D	0.93^{ns}	0.79^{ns}	1.04^{ns}	0.62^{ns}	1.48^{ns}
CV (%)	8.38	7.02	6.63	7.03	6.81

Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability; ^{ns} = not significant; * = significant at 5% probability by the F-test; CV = coefficient of variation

Stem diameter absolute growth rate was highest in Clones 7, 8 and 2 with values of 0.091, 0.084 and 0.082 mm day^{-1}, respectively followed by Clones 6 and 4 at 0.077 and 0.067 mm day^{-1}, respectively (Table 3).

Clones differed in shoot and root growth as seen in shoot dry matter, root dry matter and leaf area (Table 5). Shoot dry matter (averaged across TE doses) of Clones 6 and 7 was greater than that of Clones 3 and 4, while the others presented intermediate behaviour, independently of TE doses (Table 5). The two highest root dry matter values were from Clones 1 and 6 (18.3 and 18.5 g, respectively), significantly higher than that of Clone 4 (15.2 g), which had the lowest root dry matter. Clone 7 recorded the largest leaf area (2338 cm^2), significantly greater than that of the other clones, followed by Clones 1 and 3, while Clone 8 recorded the lowest leaf area (1511 cm^2), significantly lower than that of the Clones 7, 1 and 3 (Table 5). There was no significant difference in shoot dry matter, root dry matter or leaf area for the TE doses tested.

DISCUSSION

Plant growth is a result of the interaction between genetic and environmental factors, and refers to irreversible changes in the physical dimensions of plant organs such as mass, volume, length and area (Wilhelm & McMaster 1995, Fagundes et al. 2007). Thus, due to the different genetic materials present in the clones, the *Eucalyptus* presented distinct growth characteristics. This fact may influence, for example, nutrient extraction capacity from the environment, resulting in clonal differences in competitive capacity, directly influencing plant architecture (Cruz et al. 2010, Graat et al. 2015, Colmanetti et al. 2017).

As an example of the different behaviors among genetic materials in relation to the allocation of photoassimilates, we discuss the growth performance of Clones 6 and 7. The former invested more in stem and root growth than in leaf production, resulting in higher values for shoot dry matter and root dry matter, with intermediate leaf area. Conversely, Clone 7 recorded a leaf area 26% higher than that of Clone 6, but not significantly different shoot dry matter and root dry matter (Table 5).

In addition, different responses of clones to stressors, such as water stress, were also observed in other studies (Costa e Silva et al. 2004, Valadares et al. 2014). In these conditions, the plants differed in their response to stomatal closure, which is related to the signalling of the abscisic acid hormone, being a direct result of the genetic material selected (Correia et al. 2014). In *Eucalyptus*, clonal differences also affect allocation speed of photoassimilates to the root (i.e., plant architecture), which leads to greater drought tolerance, by means of higher exploration capacity and soil penetration (Costa e Silva et al. 2004). The results obtained from studies that compared the response of *Eucalyptus* clones under different growth conditions may guide the selection of new materials to be tested in breeding programme (Vellini et al. 2008, Valadares et al. 2014, Colmanetti et al. 2017).

Since TE did not significantly influence the growth of the eight clones in our study, growth responses recorded are therefore attributed to clonal genetic differences (Tables 2, 4 and 5).

The positive effects of a low dose of a chemical compound that would be toxic at high doses, known as the hormetic effect (hormesis), was originally proposed by Southam and Erlich (1943). However, Belz and Duke (2014) point out that the occurrence of the hormetic phenomenon is influenced by several intrinsic and extrinsic characteristics to the plant's metabolism, such as climatic conditions that the plants are exposed after the application of the product (Belz & Cedergreen 2010), cultivar or clone studied (McDonald et al. 2001, Bacha et al. 2017), the evaluation end point where hormesis is verified, i.e., a period of time after product application (Cedergreen et al. 2009) and the stage of development of the plant (Carvalho et al. 2013). This last factor may have been why the positive effects of TE application on *Eucalyptus* seedlings in our study were not observed, even while reported by previous studies (Pires et al. 2019, Correia & Villela 2015, Bacha et al. 2019).

When treated with 30 g a.i. ha^{-1} TE before planting, Clone 5 seedlings showed a total dry mass gain that was 67% higher than the control, 90 days after planting (Bacha et al. 2017). The application of the same dose to Clone 5 seedlings 70 days after planting in the present study, however, showed no stimulatory effect on growth. Thus, to observe the beneficial effect of TE, we suggest that older plants need higher doses of the same product than do younger plants. The same was reported by Velini et al. (2008) for *Commelina*

benghalensis treated with glyphosate where plants with 4 tillers needed five times more glyphosate than plants with 2 tillers to obtain similar shoot dry weight of about 42% when compared to non-treated plants.

Furthermore, when TE was applied at doses of 2 to 200 g a.i. ha^{-1} to *E. urograndis* (clone GG-100) seedlings at 46 days after planting, 100 and 200 a.i. ha^{-1} gave increases in crown diameter of 12% and 33%, respectively, compared with the control (Correia & Villela 2015). The results of that study showed that doses three and almost seven times higher than the one used in the present study (30 g a.i. ha^{-1}) stimulated a 12% and 33% increase in crown diameter, respectively.

An optimal timing and rate of field applications can increase leaf area production (Pires et al. 2013, Correia & Villela 2015, Bacha et al. 2018) and, ultimately, higher wood production from the increase in photosynthetically active area. Thus, the finding that older seedlings need higher doses compared to younger plants, may inform future studies examining the effect of TE on *Eucalyptus* in the context of productivity gains.

TE is an acylcyclohexanedione, which acts by inhibiting the final phases of gibberellin synthesis, due to the structural similarity between the chemical compound and 2-oxogluterate (Rademacher 2000, Rademacher 2016). The inhibition of hydroxylation of GA_{20} at the 3β-position by TE, interrupts the formation of GA_1, which is one of the main biologically active gibberellins in the plant (Adams et al. 1992, Hedden 2016, Rademacher 2016). TE also inhibits the hydroxylation of GA_1 at position 2β (Griggs et al. 1991), which prevents it from transforming into GA_8 (inactive gibberellin) (Hisamatsu et al. 1998). Maintaining GA_1 for a longer period in its bioactive conformation is possibly one of the causes for the beneficial effect provided by TE in *Eucalyptus*. We hypothesise that the accumulation of GA_{20}, due to the inhibition of its conversion to GA_1, may lead to overactive GA_1 formation after degradation of the product by the plant.

The physiological processes that cause the stimulatory effect as a result of the application of TE have not yet been elucidated. Further research studying the effects of this plant growth regulator on *Eucalyptus* is needed, especially work that evaluates the hormonal changes in plants. Understanding this process can lead to increases in productivity.

Table 5 Shoot dry matter (SDM), roots dry matter (RDM) and leaf area of *Eucalyptus* clones at 42 days after application of trinexapac-ethyl

Clones	SDM (g)	RDM (g)	Leaf area (cm^2)
(1) - 3203	19.77abc	18.27a	1978.08b
(2) - 3487	19.29abc	16.27ab	1643.43bcd
(3) - I-144	17.58bc	15.77ab	1919.11bc
(4) - 3334	17.33c	15.24b	1618.65cd
(5) - 1407	19.22abc	16.97ab	1813.14bcd
(6) - 2361	21.17a	18.46a	1850.18bcd
(7) - I-224	20.10ab	17.77ab	2338.45a
(8) - GG100	19.19abc	16.84 ab	1511.34d
Trinexapac-ethyl			
D1 (15 g a.i. ha^{-1})	19.94a	17.69a	1848.01a
D2 (30 g a.i. ha^{-1})	18.84a	16.38a	1862.31a
D3 (0 g a.i. ha^{-1})	18.87a	16.92a	1791.82a
F clones (C)	4.10^{**}	3.07^{*}	11.6^{**}
F doses (D)	2.69^{ns}	2.75^{ns}	0.64^{ns}
F C × D	0.97^{ns}	0.92^{ns}	0.39^{ns}
CV (%)	11.24	13.24	14.33

Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability; ^{ns} = not significant; ** = significant at 1% probability by the F-test; * = significant at 5% probability by the F-tes; CV = coefficient of variation

CONCLUSION

Eucalyptus clones differed in their growth regardless TE application. The chemical did not negatively affect any clone tested. However, the doses used here were not sufficient to cause a positive effect on *Eucalyptus* growth when applied at 70 days after planting. It is suggested that older *Eucalyptus* seedlings need higher doses of TE to show potential stimulatory effects on the growth of plants.

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