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Article

# EFFECT OF TRINEXAPAC-ETHYL, AT TWO APPLICATION TIMINGS, ON THE INITIAL DEVELOPMENT OF EUCALYPTUS UNDER WATER DEFICIT

*Efeito de Trinexapac-ethyl, em Duas Épocas de Aplicação, no Desenvolvimento Inicial do Eucalipto sob Deficiência Hídrica*

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ABSTRACT - Eucalyptus plants are sensitive to stress during their initial development, and water deficit is the most important one. Thus, the hormetic effect caused by the application of chemical products may be a factor that allows plants to tolerate such stresses. The objective of this study was to evaluate the effects of trinexapac-ethyl on the initial growth of *Eucalyptus urophylla* (Clone I-144), under water deficiency, at two application timings (before planting - BP; and after planting - AP). Two experiments were conducted simultaneously in a greenhouse for 74 days after planting (DAP) eucalyptus in 15 L pots. Treatments consisted of three trinexapac-ethyl doses (0.0, 30, and 60 g a.i. $ha^{-1}$) and two water conditions (with and without water deficit). A complete randomized block design was used, in a 3 x 2 factorial arrangement, with five replications. At the BP application timing, trinexapac-ethyl was sprayed at 0 DAP, and at the AP timing, at 24 DAP. In both experiments, plant height, diameter, leaf area, dry matter, total relative chlorophyll content and gas-exchange were evaluated. There was a positive effect for the net $CO_2$ assimilation rate at 27 and 40 DAP, for AP and BP, respectively. Eucalyptus plants, without water deficit, presented higher growth, regardless of the application timing. In conclusion, the application of trinexapac-ethyl before planting caused a positive effect on the height and diameter of eucalyptus; and the application timing influenced, in different ways, the evaluated characteristics, not having harmful effects on any of them.

**Keywords:** *Eucalyptus urophylla*, hormesis, subdose, abiotic stress.

*RESUMO - Plantas de eucalipto são sensíveis a estresses durante o desenvolvimento inicial, sendo a deficiência hídrica o mais importante entre eles. Assim, o efeito hormético ocasionado pela aplicação de produtos químicos pode ser um fator que proporcione às plantas tolerar esses estresses. Objetivou-se neste estudo avaliar o efeito de trinexapac-ethyl no crescimento inicial de* ***Eucalyptus urophylla*** *(Clone I-144) sob deficiência hídrica, em duas épocas de aplicação (antes do plantio – AP; e depois do plantio – DP). Dois experimentos foram conduzidos simultaneamente em casa de vegetação, durante 74 dias após o plantio (DAP) do eucalipto, em vasos de 15 L. Os tratamentos consistiram de três doses de trinexapac-ethyl (0,0, 30 e 60 g i.a. $ha^{-1}$) e duas condições hídricas (com e sem deficiência hídrica). Foi utilizado delineamento casualizado em blocos, em esquema fatorial 3x2, com cinco repetições. Na época de aplicação AP, a pulverização de trinexapac-ethyl ocorreu aos 0 DAP, e na época DP, aos 24 DAP. Foram avaliados: altura das plantas, diâmetro, área foliar, massa seca, teor relativo de clorofila total e trocas gasosas. Houve efeito positivo para a taxa de assimilação líquida de $CO_2$ aos 27 e 40 DAP, para as épocas DP e AP, respectivamente. As plantas de eucalipto na*

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*ausência de deficiência hídrica apresentaram maior crescimento, independentemente da época de aplicação. Conclui-se que a aplicação de trinexapac-ethyl antes do plantio proporcionou efeito positivo para a altura e o diâmetro do eucalipto. As épocas de aplicação influenciaram de formas distintas as características avaliadas, não havendo efeito prejudicial em nenhuma delas.*

**Palavras-chave:** *Eucalyptus urophylla*, hormese, subdose, estresse abiótico.

## INTRODUCTION

Eucalyptus is the most important crop in the Brazilian forestry sector, which generated 69.1 billion BRL in 2015, representing 1.2% of the country's GDP. Of the 7.8 million hectares of planted area, 5.6 millions are occupied by eucalyptus (IBÁ, 2016); most of the extensive commercial forests were formed with clonal seedlings. However, eucalyptus is sensitive to biotic and abiotic stresses during its initial growth and development; this period comprises the first few months after planting (Nambiar and Sands, 1993; Garau et al., 2008).

Several authors point out that the low availability of water in the soil has been the most important abiotic factor in the decrease of eucalyptus productivity (Chaves et al., 2009; Villar et al., 2011; Correia et al., 2014). This occurs because water deficit causes several physiological changes in plants, which, in an attempt to guarantee their survival, reduce growth, thus compromising productivity (Bedon et al., 2011).

In response to water deficit, several physiological, biochemical and molecular changes are observed (Lawlor, 2009; Pinheiro and Chaves, 2011). As soon as the roots detect low availability of water in the soil, abscisic acid is produced, which is transported to the leaves and mediates processes that promote stomatal closure and end up reducing gas exchange processes. As a result, there is a reduction of the $CO_2$ concentration inside the leaf mesophyll, limiting the carbon fixation process and, consequently, reducing leaf growth and productivity (Bedon et al., 2011; Pinheiro and Chaves, 2011; Correia et al. al., 2014). Therefore, studies that seek to elucidate ways to provide gains to eucalyptus, under stress conditions, should be stimulated.

Previous studies have reported that the application of trinexapac-ethyl on eucalyptus seedlings favored some of the evaluated characteristics (Pires et al., 2013; Correia and Villela, 2015). This phenomenon is known as the hormetic effect or hormesis, which is characterized as a positive response to the application of sub-doses of a chemical product (Belz and Duke, 2014; Bacha et al., 2017, 2018). This effect was already observed on *Eucalyptus urograndis*, with gains of up to 19% in leaf area and 8% in total dry matter (Pires et al., 2013). However, it is important to observe that this effect depends on several factors, such as the used clone or cultivar, the plant development stage, the environmental conditions, among others (Belz and Duke, 2014).

In spite of the positive effect that the application of trinexapac-ethyl gives to eucalyptus under normal conditions, studies that report how eucalyptus seedlings respond to this product under conditions of water deficit do not exist in literature. Therefore, the application of trinexapac-ethyl may be a viable alternative to provide better responses from eucalyptus seedlings to periods of water stress.

Trinexapac-ethyl is a plant growth regulator, or ripener, often used in crops such as sugarcane and rice, whose application aims at reducing the lodging of these plants, since the product causes reduction in internode elongation (Nascimento et al., 2009).

With the hypothesis that trinexapac-ethyl can provide positive responses to eucalyptus under conditions of abiotic stress, this work aimed at evaluating the effect of this chemical on the initial growth of *E. urophylla* (clone I-144) under water deficit at two application timings.

## MATERIAL AND METHODS

Two experiments were simultaneously conducted in a greenhouse, under semi-controlled conditions, from August to October 2016, in the city of Jaboticabal - São Paulo state, Brazil (altitude of 590 m and geographic coordinates 21°15'17" S and 48°19'20" W). During the experimental period, the average air temperature was 22.5 °C (maximum 32.3 °C and minimum 14 °C), with a relative average humidity of 61.4% and a 247-hour monthly sunshine.

Both experiments were conducted during 74 days after planting (DAP) eucalyptus in 15 L pots, previously filled with a mixture of soil collected from the surface layer of a Dark Red Latosol (Table 1) and coarse sand in the ratio of 2:1 (v/v).

***Table 1*** **-** Chemical analysis of the substrate used in the experimental plots. Jaboticabal - São Paulo state, 2016

| pH ($CaCl_2$) | M.O. (g $dm^{-3}$) | P resin (mg $dm^{-3}$) | K | Ca | Mg | H+Al | SB | T | V % |
|---|---|---|---|---|---|---|---|---|---|
| | | | (mmol$_c$ $dm^{-3}$) | | | | | | |
| 6.0 | 18 | 17 | 3.6 | 23 | 9 | 18 | 35.2 | 53.5 | 66 |

Commercial seedlings of a 90-day-old *Eucalyptus urophylla* clone (clone I-144), which had, on an average, 37 cm in height, 3.35 mm of stem diameter and 12 leaves, were used.

Two trinexapac-ethyl application timings were evaluated: before planting (BP - experiment 1) and after planting (AP - experiment 2). For the before planting timing (BP), eucalyptus seedlings, still conditioned in 50 mL tubes, were sprayed with trinexapac-ethyl (Moddus®) at the 30 and 60 g a.i. $ha^{-1}$ doses (10% and 20% of the recommended commercial dose for sugarcane, respectively). To apply the product, a backpack sprayer was used, at constant pressure ($CO_2$), equipped with a bar with two TT 110.02 nozzles, regulated for a spray volume of 200 L $ha^{-1}$. During the application, the air temperature was 27.3 °C, with a relative humidity of 59.5%. Twenty-four hours after the application of the product, all seedlings were planted in pots.

For the after planting (AP) application timing, spraying occurred at 24 DAP, and the aforementioned doses and application methodology were used. At that time, the air temperature was 29.4 °C, and the relative humidity was 55.2%.

In both experiments, a randomized block design was used, and the treatments were arranged in a 3 x 2 factorial arrangement, with three doses of trinexapac-ethyl (0%, 10% and 20% of the commercial dose) and two water regimes (with and without deficit), with five replications.

In the first eight days after planting, all plants were maintained in field capacity to ensure the survival of the seedlings. Subsequent humidity monitoring of the substrate was performed with the Falker HidroFarm sensor (HFM 2030 model), considering values between 20-22% for plants that were maintained in the absence of water deficit and 3-5% (equivalent to a 20% mean of the field capacity) for plants kept under water deficit conditions. For this, all treatments with water deficit stopped receiving irrigation between 8 DAP and 15 DAP. From this point onwards, the experimental plots were irrigated daily with the following values: 100 mL from 15 DAP to 23 DAP; 200 mL from 24 DAP to 49 DAP; 300 mL from 50 DAP to 69 DAP; and 400 mL from 70 to 74 DAP.

At 27 DAP (three days after the application of trinexapac-ethyl), at the AP application timing, and at 40 DAP, at the BP application timing, the $CO_2$ net assimilation rate, transpiration rate, internal $CO_2$ concentration and stomatal conductance were evaluated in the third fully expanded leaf, with an infrared gas analyzer (IRGA model, LI 6400, LiCor®). For this, using a $CO_2$ reference of 398 µmol $CO_2$ $mol^{-1}$ and $H_2O$ reference of 19 mmol $H_2O$ $mol^{-1}$, the chamber temperature was set at 25 °C; the atmospheric pressure was set at 1,000 KPa; the flow rate was set at 400 µmol $s^{-1}$; and the photosynthetic active photon flux (quantum) was set at 1,100 µmol $m^{-2}$ $s^{-1}$.

For both experiments, at 74 DAP, the collar diameter was determined using a digital caliper, and the height of plants, with an mm graduated ruler. The relative content of total chlorophyll (Falker, CFL 1030 model) and gas exchanges were also evaluated in the third fully expanded leaf of each plant, using the aforementioned methodology. After these evaluations, plants were cut at their base and their leaves were separated in order to determine their leaf area (LiCor, LI 3100 A model). Then, leaves and stems were taken to a forced air circulation oven (70 °C) for 96 hours, to determine the dry matter on an electronic precision scale.

The collected data were submitted to analysis of variance by F test, initially by application timing and, later, comparing timings for each water regime, in order to verify the possible beneficial effect of trinexapac-ethyl under water deficiency. Averages were compared by Tukey's test, at a 5% probability level.

## RESULTS AND DISCUSSION

### Experiment 1 – Before Planting Application (BP)

Eucalyptus plants maintained without water deficit, regardless of the applied trinexapac-ethyl doses, presented greater height, diameter, leaf area and total dry matter (TDM) in relation to plants with water deficit, with increases of, respectively, 49.1%, 47.6%, 215.4% and 316.3% (Table 2A). On the other hand, the relative content of total chlorophyll was about 33.6% higher in plants cultivated under water deficit.

***Table 2*** - Effect of trinexapac-ethyl application on *Eucalyptus urophylla* (Clone I-144), without and with water deficit, on eucalyptus height (cm), stem diameter (mm), leaf area ($cm^2$), dry matter (Total DM - g), relative content of total chlorophyll (Chlorophyll - UR), net assimilation rate of $CO_2$ ($A$ – µmol $CO_2$ $m^{-2}$ $s^{-2}$), stomatal conductance ($g_s$ - mol $H_2O$ $m^{-2}$ $s^{-2}$), internal $CO_2$ concentration ($C_i$ - µmol $CO_2$ $mol^{-1}$) and transpiration rate ($E$ - mmol $H_2O$ $m^{-2}$ $s^{-2}$). Before Planting Application (BP)

| | (A) – Growth and Chlorophyll – 74 DAP | | | | |
|---|---|---|---|---|---|
| | Height | Diameter | Leaf Area | TDM | Chlorophyll |
| Without deficit | 81.0 A | 7.25 A | 1728.1 A | 23.11 A | 29.24 B |
| With deficit | 54.3 B | 4.91 B | 547.8 B | 5.55 B | 44.06 A |
| 0% Trinexapac-ethyl | 66.1 B | 5.84 B | 1222.0 A | 13.64 A | 36.72 A |
| 10% Trinexapac-ethyl | 66.1 B | 6.24 A | 1029.3 A | 14.74 A | 35.78 A |
| 20% Trinexapac-ethyl | 70.7 A | 6.15 AB | 1162.6 A | 14.61 A | 37.45 A |
| F (Water Regime - WR) | 486.8** | 347.2** | 75.88** | 1151** | 804.2** |
| F (Trinexapac-ethyl - ET) | 6.39* | 3.68* | $0.71^{ns}$ | $1.77^{ns}$ | $3.42^{ns}$ |
| F (WR x ET) | $2.77^{ns}$ | $0.34^{ns}$ | $0.57^{ns}$ | $0.38^{ns}$ | $1.99^{ns}$ |
| CV (%) | 4.90 | 5.65 | 32.60 | 9.88 | 3.90 |
| | (B) – First IRGA evaluation (40 DAP) | | | | |
| | $A$ | $g_s$ | $C_i$ | $E$ | - |
| Without deficit | 15.83 A | 0.765 A | 320.6 A | 9.57 A | - |
| With deficit | 10.26 B | 0.141 B | 211.9 B | 3.44 B | - |
| 0% Trinexapac-ethyl | 13.85 A | 0.485 A | 275.2 A | 6.60 A | - |
| 10% Trinexapac-ethyl | 12.83 AB | 0.431 B | 269.9 A | 6.80 A | - |
| 20% Trinexapac-ethyl | 12.45 B | 0.444 AB | 253.6 A | 6.11 A | - |
| F (Water Regime - WR) | 200.2** | 1633.7** | 237.9** | 273.4** | - |
| F (Trinexapac-ethyl - ET) | 4.54* | 4.40* | $3.38^{ns}$ | $1.21^{ns}$ | - |
| F (WR x ET) | 37.64** | $2.89^{ns}$ | 3.89* | 5.20* | - |
| CV (%) | 8.25 | 9.32 | 7.24 | 15.61 | - |
| | (C) – Second IRGA evaluation (74 DAP) | | | | |
| | $A$ | $g_s$ | $C_i$ | $E$ | - |
| Without deficit | 12.31 B | 0.710 A | 338.1 A | 8.68 A | - |
| With deficit | 14.69 A | 0.229 B | 242.3 B | 5.19 B | - |
| 0% Trinexapac-ethyl | 13.52 A | 0.476 A | 288.1 A | 6.87 AB | - |
| 10% Trinexapac-ethyl | 14.25 A | 0.468 A | 294.3 A | 7.60 A | - |
| 20% Trinexapac-ethyl | 12.73 A | 0.464 A | 288.3 A | 6.32 B | - |
| F (Water Regime - WR) | 19.29** | 283.1** | 114.2** | 154.2** | - |
| F (Trinexapac-ethyl - ET) | $2.65^{ns}$ | $0.06^{ns}$ | $0.21^{ns}$ | 6.97** | - |
| F (WR x ET) | $1.20^{ns}$ | $3.22^{ns}$ | $2.19^{ns}$ | $0.45^{ns}$ | - |
| CV (%) | 10.98 | 16.67 | 8.45 | 11.09 | - |

Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability. * and ** = significant value at 5% and 1% probability by F test, respectively. $^{ns}$ = non-significant value at 5% probability by the F test; CV = coefficient of variation. DAP = days after planting eucalyptus.

As for the trinexapac-ethyl doses, regardless of the water status, the highest dose provided a height that was 6.9% greater than the other treatments (Table 2A). The largest diameter was observed in plants that received the 10% dose of trinexapac-ethyl, differing significantly from the control treatment. There was no effect of trinexapac-ethyl on leaf area, TDM and chlorophyll content (Table 2A).

In the first gas exchange evaluation, performed at 40 DAP (Table 2B), plants that had not been under water stress obtained higher values for all evaluated parameters. In the evaluation performed at the end of the experimental period (74 DAP), except for the net $CO_2$ assimilation rate (NAR), all other variables maintained the same pattern observed in the previous evaluation (40 DAP), in which plants without water stress obtained higher values (Table 2C). In addition, plants with water restriction showed higher NAR values at 74 DAP, probably due to the fact that, by growing less (due to water restriction), they accumulated more chlorophyll in their leaves, resulting in a higher carbon fixation (Table 2C).

In the NAR evaluation at 40 DAP (Table 3A), it was possible to observe a differentiated effect of the ripener, so that, when under water restriction, trinexapac-ethyl provided reductions in this variable, whereas, in the absence of water stress, a higher dose favored the occurrence of higher values (Table 3A). In spite of the low NAR values of plants treated with the ripener under water restriction, there was no damage to their growth, as it is possible to observe in Table 2A, where there was no significant difference in relation to the control treatment for the TDM.

***Table 3*** **-** Unfolding of the interaction "irrigation regime x trinexapac-ethyl doses" for the $CO_2$ net assimilation rate ($A$ – µmol $CO_2$ $m^{-2}$ $s^{-2}$), internal $CO_2$ concentration ($C_i$ - µmol $CO_2$ $mol^{-1}$) and transpiration rate ($E$ - mmol $H_2O$ $m^{-2}$ $s^{-2}$) in *Eucalyptus urophylla* (clone I-144), submitted to the application of trinexapac-ethyl and cultivated with and without water deficit, 40 days after planting (DAP). Before Planting Application (BP)

| | (A) – *A* (40 DAP) | | | |
|---|---|---|---|---|
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 14.46 Ab | 15.80 Aab | 17.23 Aa | 8.23** |
| With deficit | 13.24 Aa | 9.87 Bb | 7.67 Bc | 33.9** |
| F | 3.20$^{ns}$ | 75.70** | 196.6** | - |
| | (B) – $C_i$ (40 DAP) | | | |
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 329.9 Aa | 312.0 Aa | 319.8 Aa | 1.08$^{ns}$ |
| With deficit | 220.5 Ba | 227.7 Ba | 187.5 Bb | 6.19** |
| F | 80.32** | 47.70** | 117.6** | - |
| | (C) – *E* (40 DAP) | | | |
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 8.90 Aa | 10.56 Aa | 9.26 Aa | 2.73$^{ns}$ |
| With deficit | 4.30 Ba | 3.04 Ba | 2.96 Ba | 3.68$^{ns}$ |
| F | 51.14** | 136.6** | 96.07** | - |

Averages followed by the same letter, uppercase in the column and lowercase in the line, do not differ from each other by Tukey's test at 5% probability. ** = significant value at 1% probability by F test. $^{ns}$ = non-significant value at 5% probability by F test.

For the internal $CO_2$ concentration and transpiration (Table 3B, C), the result pattern was the same in relation to treatments with and without water stress, in which plants cultivated without water restriction obtained higher values. As for the application of trinexapac-ethyl, a higher dose of the ripener provided lower values of internal carbon under a water deficit situation (Table 3B).

**Experiment 2 – After Planting Application (AP)**

There was no statistical difference among trinexapac-ethyl doses in terms of height, leaf area and TDM (Table 4A). As for the water regimes, the pattern was the same as the one observed in the BP season, when only chlorophyll values were higher in the water restriction treatments (Table 4A). The presence of water in the system provided gains of 57.2% in height, 59.5% in diameter, 157.45% in leaf area and 286.95% in TDM, in relation to plants that were kept under water deficit.

***Table 4 -*** Effect of the application of trinexapac-ethyl on *Eucalyptus urophylla* (clone I-144), without and with water deficit, on eucalyptus height (cm), stem diameter (mm), leaf area ($cm^2$), total dry matter (TDM – g), relative content of total chlorophyll (Chlorophyll - UR), net assimilation rate of $CO_2$ ($A$ – µmol $CO_2$ $m^{-2}$ $s^{-2}$), stomatal conductance ($g_s$ - mol $H_2O$ $m^{-2}$ $s^{-2}$), internal $CO_2$ concentration ($C_i$ - µmol $CO_2$ $mol^{-1}$) and transpiration rate ($E$ - mmol $H_2O$ $m^{-2}$ $s^{-2}$). After Planting Application (AP)

| | (A) – Growth and Chlorophyll – 74 DAP | | | | |
|---|---|---|---|---|---|
| | Height | Diameter | Leaf Area | TDM | Chlorophyll |
| Without deficit | 78.0 A | 6.97 A | 2167.8 A | 23.06 A | 29.37 B |
| With deficit | 49.6 B | 4.37 B | 842.3 B | 5.96 B | 41.72 A |
| 0% Trinexapac-ethyl | 63.3 A | 5.66 A | 1467.8 A | 14.63 A | 35.45 A |
| 10% Trinexapac-ethyl | 63.3 A | 5.63 A | 1464.2 A | 13.78 A | 35.91 A |
| 20% Trinexapac-ethyl | 65.0 A | 5.73 A | 1583.2 A | 15.12 A | 35.28 A |
| F (Water Regime - WR) | 455.7** | 431.6** | 148.5** | 1133.0** | 562.3** |
| F (Trinexapac-ethyl - ET) | $0.73^{ns}$ | $0.20^{ns}$ | $0.52^{ns}$ | $2.38^{ns}$ | $0.52^{ns}$ |
| F (WR x ET) | $0.28^{ns}$ | 4.33* | $0.10^{ns}$ | $0.44^{ns}$ | 13.51** |
| CV (%) | 5.70 | 6.03 | 19.78 | 9.58 | 4.01 |
| | (B) – First IRGA evaluation (27 DAP) | | | | |
| | $A$ | $g_s$ | $C_i$ | $E$ | - |
| Without deficit | 17.68 A | 0.670 A | 298.3 A | 12.56 A | - |
| With deficit | 7.16 B | 0.060 B | 171.8 B | 2.50 B | - |
| 0% Trinexapac-ethyl | 12.80 A | 0.343 B | 232.7 A | 7.39 AB | - |
| 10% Trinexapac-ethyl | 10.73 B | 0.332 B | 236.7 A | 6.98 B | - |
| 20% Trinexapac-ethyl | 13.73 A | 0.419 A | 235.8 A | 8.21 A | - |
| F (Water Regime - WR) | 706.0** | 1000.6** | 249.2** | 1093.5** | - |
| F (Trinexapac-ethyl - ET) | 20.11** | 8.08** | $0.09^{ns}$ | 5.64* | - |
| F (WR x ET) | 6.49** | $2.93^{ns}$ | $0.19^{ns}$ | $0.27^{ns}$ | - |
| CV (%) | 8.72 | 14.4 | 9.32 | 11.06 | - |
| | (C) – Second IRGA evaluation (74 DAP) | | | | |
| | $A$ | $g_s$ | $C_i$ | $E$ | - |
| Without deficit | 10.88 B | 0.678 A | 312.0 A | 10.32 A | - |
| With deficit | 15.04 A | 0.284 B | 248.5 B | 6.46 B | - |
| 0% Trinexapac-ethyl | 12.68 B | 0.453 B | 282.3 A | 8.16 B | - |
| 10% Trinexapac-ethyl | 14.77 A | 0.541 A | 287.9 A | 9.04 A | - |
| 20% Trinexapac-ethyl | 11.43 B | 0.448 B | 284.0 A | 7.97 B | - |
| F (Water Regime - WR) | 53.76** | 265.8** | 205.9** | 194.3** | - |
| F (Trinexapac-ethyl - ET) | 11.84** | 6.28** | $0.42^{ns}$ | 5.68* | - |
| F (WR x ET) | 5.45* | $0.52^{ns}$ | $1.60^{ns}$ | 3.71* | - |
| CV (%) | 11.96 | 13.74 | 4.85 | 9.04 | - |

Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability. * and ** = significant values at 5% and 1% of probability by F test, respectively. $^{ns}$ = non-significant value at 5% probability by F test; CV = coefficient of variation. DAP = days after planting eucalyptus.

Concerning the gas exchanges evaluated three days after the application of the ripener (27 DAP), it was observed that a higher dose of trinexapac-ethyl provided a beneficial effect in relation to a lower dose as for stomatal conductance and transpiration, differing from the control treatment only in stomatal conductance values (Table 4B). When comparing water regimes, plants without water restriction obtained higher values for all variables (Table 4B).

In the gas exchange evaluation at the end of the experimental period (74 DAP), the application of 10% of trinexapac-ethyl favored stomatal conductance, differing significantly from the control treatment (Table 4C). However, as in the BP timing, NAR was the only variable with higher values under water deficit conditions, regardless of the ripener dose (Table 4C), which is probably due to the higher amount of chlorophyll in the leaves (Table 4A). However, it is worth mentioning that, even with higher NAR values, water was still a limiting factor to the growth of plants under this water condition; therefore, they presented lower biometric characteristics at the end of the experimental period, even with greater assimilation of carbon in that period (Table 4A).

As for the significant interactions between water regimes and doses of trinexapac-ethyl, it was observed for the diameter, just like NAR at 27 DAP and transpiration at 74 DAP (respectively, Table 5A, C and E), that plants without water stress showed higher values, regardless of the trinexapac-ethyl dose. As for the chlorophyll content (Table 5B), the application of 10% of the ripener provided a beneficial effect in relation to the control treatment, but did not differ from the higher dose. On the other hand, under water restriction, trinexapac-ethyl provided lower values as for chlorophyll content, compared to the control treatment (Table 5B).

In the evaluation three days after the application of trinexapac-ethyl (27 DAP), the highest dose of the product had a positive effect on NAR when eucalyptus plants were under water deficit (Table 5C). At the end of the experimental period (74 DAP), except for the 20% dose of trinexapac-ethyl, all plants under water deficit presented higher NAR values in relation to plants without deficit (Table 5D). There was no difference between the doses tested under the no water deficit condition, whereas, with water restriction, the treatment with 20% of the ripener provided lower

***Table 5*** **-** Unfolding of the interaction "irrigation regime x trinexapac-ethyl doses" for the stem diameter (mm), total chlorophyll content (Chlorophyll - UR), $CO_2$ net assimilation rate ($A$ – μmol $CO_2$ $m^{-2}$ $s^{-2}$), and transpiration rate ($E$ - mmol $H_2O$ $m^{-2}$ $s^{-2}$) in *Eucalyptus urophylla* (clone I-144), submitted to the application of trinexapac-ethyl and cultivated with and without water deficit. After Planting Application (AP)

| | (A) – Diameter (74 DAP) | | | |
|---|---|---|---|---|
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 7.19 Aa | 6.71 Aa | 7.02 Aa | $2.08^{ns}$ |
| With deficit | 4.13 Ba | 4.56 Ba | 4.44 Ba | $2.46^{ns}$ |
| F | 199.1** | 98.9** | 142.1** | - |
| | (B) – Chlorophyll (74 DAP) | | | |
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 27.44 Bb | 31.12 Ba | 29.56 Bab | 8.39** |
| With deficit | 43.46 Aa | 40.70 Ab | 41.00 Ab | 5.64* |
| F | 315.6** | 112.8** | 160.9** | - |
| | (C) – *A* (27 DAP) | | | |
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 18.92 Aa | 16.03 Ab | 18.10 Aa | 9.44** |
| With deficit | 6.69 Bb | 5.43 Bb | 9.36 Ba | 17.16** |
| F | 317.9** | 238.9** | 162.2** | - |
| | (D) – *A* (74 DAP) | | | |
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 10.04 Ba | 11.94 Ba | 10.67 Aa | $1.94^{ns}$ |
| With deficit | 15.32 Aa | 17.60 Aa | 12.19 Ab | 15.36** |
| F | 28.96** | 33.32** | $2.39^{ns}$ | - |
| | (E) – *E* (74 DAP) | | | |
| | 0% trinexapac-ethyl | 10% trinexapac-ethyl | 20% trinexapac-ethyl | F |
| Without deficit | 10.03 Aa | 10.55 Aa | 10.39 Aa | $0.61^{ns}$ |
| With deficit | 6.29 Bb | 7.54 Ba | 5.55 Bb | 8.77** |
| F | 60.59** | 39.30** | 101.8** | - |

Averages followed by the same letter, uppercase in the column and lowercase in the line, do not differ from each other by Tukey's test at 5% probability. * and ** = significant values at 5% and 1% of probability by F test, respectively. $^{ns}$ = non-significant value at 5% probability by F test F. DAP = days after planting eucalyptus.

values in comparison to the other doses (Table 5D). Plants treated with 10% of the product, when under water deficit, transpired more than the other treatments, while under the no water deficit condition, there was no significant difference for this variable (Table 5E).

### Comparison between BP and AP Application Timings

When eucalyptus plants were under water deficit, regardless of the application timing (BP or AP), the trinexapac-ethyl spraying had a positive effect on the height, diameter and dry matter of the stem, providing maximum gains of 7.2%, 10.8%, and 20.9%, respectively (Table 6A). However, the BP timing provided higher height and diameter values compared to AP, probably due to the fact that the product acted precociously. However, the AP timing allowed a leaf area that was 53.8% greater than the BP timing (Table 6A). There was no difference between the two trinexapac-ethyl application timings on the production of dry matter.

***Table 6 -*** Comparison between application timings (Before Planting and After Planting) of trinexapac-ethyl on *Eucalyptus urophylla* (Clone I-144) as for height (cm), stem diameter (mm), leaf area ($cm^2$), total dry matter (TDM - g), dry matter of the stem (Stem DM - g) and leaf dry matter (Leaf DM - g), under two irrigation regimes (with and without water deficit)

| | (A) – With Water Deficit | | | | | |
|---|---|---|---|---|---|---|
| | Height | Diameter | Leaf Area | TDM | Stem DM | Leaf DM |
| Before Planting | 54.3 A | 4.91 A | 547.8 B | 5.55 A | 1.99 A | 3.61 A |
| After Planting | 49.6 B | 4.37 B | 842.3 A | 5.96 A | 2.19 A | 3.61 A |
| 0% Trinexapac-ethyl | 50.0 B | 4.36 B | 703.6 A | 5.49 A | 1.86 B | 3.72 A |
| 10% Trinexapac-ethyl | 52.2 AB | 4.83 A | 667.9 A | 5.80 A | 2.25 A | 3.49 A |
| 20% Trinexapac-ethyl | 53.6 A | 4.73 A | 714.7 A | 5.99 A | 2.17 A | 3.62 A |
| F (Application Timing) | 19.07** | 26.67** | 84.88** | $2.56^{ns}$ | $4.26^{ns}$ | $0.00^{ns}$ |
| F (Trinexapac-ethyl - ET) | 3.90* | 7.44** | $0.82^{ns}$ | $1.29^{ns}$ | 6.20** | $0.33^{ns}$ |
| F (Application x ET) | $1.20^{ns}$ | $0.11^{ns}$ | $1.18^{ns}$ | $2.93^{ns}$ | $1.57^{ns}$ | $1.48^{ns}$ |
| CV (%) | 5.58 | 6.09 | 12.59 | 12.27 | 12.57 | 17.06 |
| | (B) – Without Water Deficit | | | | | |
| | Height | Diameter | Leaf Area | TDM | Stem DM | Leaf DM |
| Before Planting | 81.06 A | 7.25 A | 1728.1 B | 23.11 A | 7.88 A | 15.21 A |
| After Planting | 78.06 B | 6.97 A | 2167.8 A | 23.06 A | 7.68 A | 15.37 A |
| 0% Trinexapac-ethyl | 79.40 AB | 7.14 A | 1986.3 A | 22.79 A | 7.52 A | 15.63 A |
| 10% Trinexapac-ethyl | 77.20 B | 7.05 A | 1826.6 A | 22.72 A | 7.87 A | 14.32 A |
| 20% Trinexapac-ethyl | 82.10 A | 7.15 A | 2031.1 A | 23.73 A | 7.94 A | 15.79 A |
| F (Application Timing) | 6.67* | $2.86^{ns}$ | 6.00* | $0.01^{ns}$ | $0.68^{ns}$ | $0.19^{ns}$ |
| F (Trinexapac-ethyl - ET) | 5.95** | $0.14^{ns}$ | $0.48^{ns}$ | $1.45^{ns}$ | $1.14^{ns}$ | $2.57^{ns}$ |
| F (Application x ET) | $1.10^{ns}$ | $1.89^{ns}$ | $0.41^{ns}$ | $2.01^{ns}$ | $0.21^{ns}$ | $0.23^{ns}$ |
| CV (%) | 3.99 | 6.26 | 25.23 | 6.44 | 8.54 | 10.44 |

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

As for the comparison between application timings under no water stress condition, the observed pattern was similar to the previous one, so that BP provided greater height to eucalyptus plants (3.8% increase), while AP allowed a larger leaf area, with a 25.4% increase (Table 6B). As for trinexapac-ethyl doses, it was observed that the highest dose provided greater height in comparison to the lowest dose, but did not differ significantly from the control treatment (Table 6B).

The reduced growth observed in some eucalyptus variables, in treatments with water deficit, such as in terms of height, diameter, leaf area and TDM, regardless of the application of trinexapac-ethyl (Tables 2A and 4A), is due to the fact that water is an essential factor for the cellular metabolism of the plant and this corroborates other previous works (Susiluoto and

Berninger, 2007; Granda et al., 2011; Correia et al., 2014). Taiz and Zeiger (2013) emphasize that the physiological processes are affected by water availability, because the properties of water molecules directly influence the cell components, such as the structures of proteins, nucleic acids, membranes, among others.

Eucalyptus plants under water restriction tend to have lower photosynthetic rates, as observed in this study (Tables 3B and 5B), since water molecules act as donors of electrons in the photochemical phase of this process and, with this, they have a direct influence on the production of ATP, since $H^+$ protons, resulting from the photolysis of water, are released inside the thylakoid, for later use by the ATP-synthase pump, as well as directly affecting the activity of Rubisco and the RuBP regeneration (Lawlor, 2002; Parry et al., 2002).

Again, under water restriction, it was possible to observe the reduction of the transpiration and stomatal conductance due to the closure of the stomata, which implied a lower internal carbon concentration (Tables 2B and 4B). In addition (Hanba et al., 2004; Flexas et al., 2006, 2008; Miyazawa et al., 2008), the reduction of the photosynthetic rate is not only related to the stomatal closure, but also to the reduction of $CO_2$ conductance in the leaf mesophyll, in which aquaporins appear to be involved, especially under water deficit conditions. Thus, it is important to observe that the closure of the stomata, whose purpose is to avoid water loss through transpiration, is the result of a complex network of signaling, with the abscisic acid hormone (ABA) having a primary function in this process (Zhang et al. 2006; Jiang and Hartung, 2008); this was also observed in eucalyptus plants under water stress conditions (Granda et al., 2011; Correia et al., 2014).

However, unlike what was observed in the first gas exchange evaluations, the net assimilation rate at the end of the experimental period (74 DAP) was higher in plants with water deficit compared to those without stress, regardless of the application timing of trinexapac-ethyl (Tables 2C and 4C). This result is related to the amount of chlorophyll found in leaves of eucalyptus plants under water restriction, which was higher under water deficit than in treatments irrigated daily until field capacity (Tables 2A and 4A).

A similar result was also found by Correia et al. (2014), who related the higher concentration of this pigment to the lower leaf growth observed in eucalyptus plants under water deficit, corroborating the results of this study (Tables 2A and 4A). These authors also suggest that the presence of high concentration of carotenoids, also due to the lower leaf expansion, may have protected chlorophylls from degradation, thus preserving the photosynthetic capacity of plants.

On the other hand, Susiluoto and Berninger (2007) point out another justification for the persistence of high concentrations of this pigment under conditions of water stress: the increase of the root/shoot ratio, also reported by other authors (Blake and Suiter-Filho, 1988; Li and Wang, 2003), increases soil nutrient uptake capacity, resulting in increased nitrogen uptake by the root and, consequently, maintaining high levels of chlorophyll and photosynthesis in the leaf.

In this experiment, at the BP timing, gains of 6.96% in height and 6.85% in diameter were observed for plants that received the application of 20% and 10% of trinexapac-ethyl, respectively (Table 2A). This stimulatory response is characterized as hormesis. The term “hormesis” is defined as a stimulatory effect resulting from the action of low doses of an originally toxic chemical (Belz and Duke, 2014), which has already been reported in several species and products, including eucalyptus (Velini et al., 2008; Pereira et al., 2013; Pires et al., 2013; Correia and Villela, 2015).

Pires et al. (2013) found even more significant gains when spraying the same product at the dose of 20 g a.i. $ha^{-1}$ on *E. urograndis* plants. On this occasion, these authors found gains of 8% in diameter, 8% in total dry matter, 19% in leaf area and 13% in leaf dry matter, at 42 DAP. Belz and Duke (2014) point out that the hormesis resulting from the application of chemical products depends on several factors, among which: environmental conditions (Belz and Cedergreen, 2010), plant development stage (Carvalho et al., 2013), chosen clone or cultivar (McDonald et al., 2001) and the final evaluation point (Cedergreen et al., 2009), that is, how long after the exposure to the product the evaluation will take place. This justifies the difference of the results found in this work, conducted for 74 DAP with clone I-144, and those found by Pires et al. (2013), who have conducted the experiment for 42 DAP.

Also, at 40 DAP, the net assimilation rate in plants submitted to 20% trinexapac-ethyl, which were not under water deficit, was 19.1% higher than that of the control treatment (Table 3A).

This result supports the hypothesis raised by Pires et al. (2013), who suggested that trinexapac-ethyl had no harmful effect on the photosynthetic properties of *E. urograndis*.

At the AP timing, in which eucalyptus plants had already been planted 24 days before the application, it was possible to observe that spraying 20% trinexapac-ethyl had a beneficial effect on the NAR, three days after the application (27 DAP), in treatments under water stress (Table 5C), suggesting that the degradation of the chemical product by plants has a beneficial effect when their root system has already established. It is worth mentioning that stomatal conductance was also higher in plants sprayed with the same dose of the ripener, regardless of the water condition to which they were submitted (Table 4B).

However, this observed gain in gas exchange evaluations was not sufficient to significantly increase the biometric characteristics of eucalyptus, such as height, diameter, leaf area and total dry matter (Table 4A). Correia and Villela (2015) found a 29.2% increase in crown diameter of *E. urograndis* (clone GG100) 45 days after the application of 200 g a.i. $ha^{-1}$ of trinexapac-ethyl. It is worth highlighting that the authors sprayed the product at 46 DAP the seedlings, that is, the application timing was similar to the AP of this study, but the seedlings were older.

The difference found between the studies is due to the fact that the occurrence of hormesis is possibly related to the age of plants, so that younger plants seem to need smaller doses, whereas older plants need larger doses of the same compound (Belz and Duke, 2014). In this sense, it is worth mentioning the work of Velini et al. (2008), who observed that *Commelina benghalensis* plants with two expanded leaves had a maximum hormetic response (they grew 98% more than the control plant) at a dose five times smaller than plants with four expanded leaves (which obtained a growth that was 42% greater than the control plant). Thus, for the hormetic effect to be significant in the production, it is important that the application of the product occurs at the correct development stage of the plant. Cedergreen (2008) applied low doses of glyphosate to barley at the two-leaf stage and did not observe any gain in productivity. On the other hand, Cedergreen et al. (2009) observed 12% to 15% gains in the productivity of barley when applying 2.5 to 20 g a.e. $ha^{-1}$ of glyphosate at the grain filling stage.

Comparing BP and AP, it was observed that trinexapac-ethyl, at the BP timing, benefited the height of plants under both water conditions and also the diameter under water stress, whereas in the AP timing, there was a gain in leaf area (Table 6A and 6B). The trinexapac-ethyl compound is an acyl-cyclohexanedione that causes reduction in the internode elongation of some species (such as rice, wheat and barley), thus reducing the lodging of these plants (Nascimento et al., 2009). Adams et al. (1992) elucidate that this compound is related to the inactivation of the enzyme $GA_{20}$ 3β-hydroxylase, due to the competition between the ripener and the 2-oxogluterate by cosubstrate $Fe^{+2}$/ascorbate-dependent dioxygenase, reducing, therefore, the level of active gibberellins, mainly $GA_1$. However, in eucalyptus, there is no negative effect resulting from the application of low doses of this product, as observed in this study and also verified by other authors (Pires et al., 2013, Correia and Villela, 2015, Bacha et al., 2017, 2018).

Despite the greater leaf area observed in eucalyptus plants at the AP timing compared to the BP timing, there was no significant difference in terms of leaf dry matter (Table 6A and 6B); this indicates that when exposed to the application of trinexapac-ethyl after planting, eucalyptus plants change the production of their leaf structures, so that when in AP, eucalyptus invests in a larger size or quantity of thin leaves, whereas when in BP, it invests in the production of smaller leaves (or in smaller quantity) and thicker, obtaining, therefore, equivalent matter, but with a distinct leaf area.

The physiological processes that cause the hormetic effect through the application of trinexapac-ethyl have not been elucidated yet, but are likely to involve several signaling steps and physiological responses in the plant, since this molecule modulates the production of gibberellins. Thus, further studies in this sense must be conducted, so that understanding this process can lead to productivity increases in the near future.

With this, it is possible to conclude that eucalyptus plants without water deficit presented higher growth, regardless of the application timing of trinexapac-ethyl. The application of trinexapac-ethyl before planting seedlings provided a positive effect on the height and diameter of eucalyptus. The application timing influenced in different ways the evaluated characteristics, with no harmful effect on any of them.

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