Hydrogel in the seedling growth of *Eucalyptus dunnii* Maiden under different irrigation management

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**ABSTRACT**

This study evaluated the effect of using hydrogel in the planting of *Eucalyptus dunnii* seedlings in pots, under different water-management regimens. Seedlings of approximately 25 cm height were planted in pots filled with 4.5 L of soil. The experiment was of Completely Randomized Design, in a 2x3 factorial, with absence and presence of hydrogel related to different water-management regimens (irrigation only in planting, every 3 to 6 days). From planting until the 47th day the seedlings were evaluated for the symptoms of water stress, chlorophyll content and gas exchange. Finally, the development of the plants was evaluated using the root dry weights and shoots, as well as by determining the water retention curve. As a result, the use of the polymer yielded an increase in water retention and a delay in the symptoms of drought stress, consequently increasing chlorophyll levels. One can observe the direct effect gas exchange of this kind, where recovery of the variables analyzed 24 h after irrigation was visible. Reviews of dry biomass demonstrated that treatment with the use of hydrogel showed the best development. However, the use of hydro retainer polymer increased the quality of *Eucalyptus dunnii* seedlings, besides being an alternative planting method in dry periods of the year.

**Keywords:** hydro retainer polymer, symptoms of water stress, water management.
aumentando assim os níveis de clorofila. Houve efeito direto nas trocas gasosas, sendo visível a recuperação das variáveis analisadas, 24 h após a irrigação. Avaliações da biomassa demonstraram que os tratamentos com o uso do hidrogel, apresentaram melhor desenvolvimento. O uso do polímero aumentou a qualidade das mudas, sendo uma alternativa de plantio em épocas secas do ano.

Palavras-chave: manejo hídrico, polímero hidroretentor, sintomatologia do estresse hídrico.

1. INTRODUCTION

A combination of several factors is necessary for the plantation of productive forests, such as site-adapted genetic materials, favorable edaphoclimatic conditions, proper use of management techniques and high-standard seedlings (Davide and Faria, 2008). Among the most widely cultivated forestry species in Brazil, the genus *Eucalyptus stands out*, currently occupying 6.97 million hectares of planted forest area, representing 71.9% of the total (IBA, 2020).

Soil and humidity conditions are dominant factors in the forest establishment, because they affect both planting and regeneration of plants (Sarvas *et al*., 2007). However, one of the main causes of mortality rates of forestry seedlings species, just after transplant, is their inability to stay properly hydrated (Thomas, 2008).

The use of hydro retainer polymers as soil humidity conditioners may therefore be beneficial. According to Mendonça *et al.* (2013), a “hydro retainer polymers” is a polymer that helps mainly in water retention for newly planted seedlings, increasing water availability for crops and local productivity, and minimizing production costs, contributing to the viability of planting throughout the year.

Based on this, the objective of this experiment was to evaluate the effects of hydrogel on *Eucalyptus dunnii* seedlings with different water-management regimens.

2. MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Santa Catarina State University Forest Nursery, located at coordinates 27°47’33.62” S e 50°18’4.60” W, with an approximate altitude of 900 meters above sea level.

According to the Köppen classification, the climate of the municipality of Lages (Santa Catarina) is humid mesothermal with a mild summer (Cfb-temperate). The annual average temperature is 15°C and the annual average precipitation stands between 1300 and 1500 mm.

The *Eucalyptus dunnii* seedlings used were grown from seed, and after being properly developed for planting, with a height close to 25 cm and at least 2 mm of stump diameter, were transferred to vases containing soil classified as Aluminium Humic Cambisol. Analysis (0-40 cm) showed the soil had the following chemical assignments: pH: 4.7; SMP: 4.9; Ca: 2.2 (cmolc/dm³); Mg: 1.2 (cmolc/dm³); Al: 4.9 (cmolc/dm³); H+Al: 9.6 (cmolc/dm³); effective CEC (Cation Exchange Capacity): 13.1 (cmolc/dm³); Aluminium saturation: 58.2%; base saturation: 26.8%; Clay: 47.6%; P (Mehlich): 3.7 (mg/dm³); K: 48.0 (mg/dm³); Cu (Mehlich): 1.8 (mg/dm³) and Zn (Mehlich): 3.4.

To simulate field conditions, we used polyethylene pots with 5-liter capacity and 4.5 liters of previously unsealed and dry soil. After filling with soil, the pots were watered until they reached field capacity (approximately 1.5 liters). Then a hole of approximately 300 cm³ was manually opened in the center of each pot for planting. In the treatments using hydrogel, 250 mL were added in the hydrated form before seedling planting. Hydration was performed with running well water 30 minutes before the planting; the hydrogel dosage used was 3 g L⁻¹. After the hole opening, with or without hydrogel, began the planting of *Eucalyptus dunnii* seedlings.
After planting, besides the 1500 mL to moisten the soil, 250 mL of water were added to each pot for the treatments without hydrogel, to compensate for the volume added by the hydrogel to the other treatments. For the treatments with irrigation, 250 mL of water were added in each pot, following the previously set frequency, according to each treatment. All irrigation treatments received 250 mL of water during the course of the experiment. This value was obtained by the average field capacity of the experiment by Felippe et al. (2016). The irrigation of the pots was carried out manually, with the amount of water measured in a beaker, and after carefully pouring over the soil (pot), an attempt was made to spread it as much as possible over the entire surface.

The experimental design was the Completely Randomized, in bifactorial scheme, where the “A” factor levels referred to presence or absence of hydrogel, and the “B” factor levels are referred to water management (only at planting, intervals of three and six days between each irrigation). Ten replicates composed of one plant from each replication were used.

2.1. Water retention curve

To determine the water retention curve, we used five soil samples with the presence of hydrogel incorporated to the substrate and five samples with the absence of the hydrogel, totaling ten samples, collected in cylindrical rings of approximately 50 cm³, in order to preserve the maximum original soil structure. The available soil water content was calculated by subtracting the soil volumetric humidity value corresponding to the field capacity (FC), obtained at the water tension of 10 kPa, by the humidity value at the permanent withering point (PWP), obtained at the tension of 1500 kPa, according to Moniz (1972). From these limits, it was possible to determine the water storage capacity available in the soil (Bergamaschi et al., 1992).

In the laboratory, the samples with preserved structure were initially saturated by capillarity for 24 hours with a water layer of approximately 3 cm and then weighed. The samples were subsequently submitted to a sand column to obtain the humidity at the equilibrium tensions of 1, 6 and 10 kPa (Reinert and Reichert, 2006) and at the tensions of 33, 100, 500, 1000 and 1500 kPa in Richards pressure chambers (Klute, 1986). The volumetric humidity of the samples was obtained by the relation between the amount of retained water at a determined tension and the volume of the cylinder. At the end the samples were taken to the drying oven for about two days, where the soil dry weight was obtained, also estimating the density of the soil (Blake and Hartge, 1986). During the elaboration of the soil water retention curve with the addition of hydrogel, the variation of the sample volume was not considered as the humidity varied according to the expansion of the hydrogel.

With the humidity values at the tensions of 1, 6, 10, 33, 100, 500, 1000 and 1500 kPa, the soil water retention curve was adjusted, using the Van Genuchten (1980) model through the SWRC (Soil Water Retention Curve) software.

2.2. Physiological analysis

Until the 47th day after planting, daily evaluations of water stress symptoms, chlorophyll contents and greenhouse temperature verification were performed. For visual symptomatology, analysis criterion of Navroski et al. (2014) was adopted, noting the number of days the plant remained in each condition: SEM- days without symptoms (turgid plant, visually vigorous, with no evidence of water deficit); SLM- days with mild withering symptoms; SMM- days with moderated symptoms (plant in permanent withered state, with curved and darkened apex); SSM- days with severe wilting symptoms (dry leafs/in abscission). For survival, the code PPV was adopted – the number of days the plant remained alive.

Chlorophyll content was obtained using the portable chlorophyll meter SPAD-502 (Minolta Camera Co. Ltd.), measured in two physiologically mature leaves on opposite sides, in the middle portion of the plant top, the leaves were marked for further measures. From the
four readings, the average was calculated using the portable chlorophyll meter itself. The measurements were performed in ten repetitions, in all treatments, always in the pre-irrigation period.

Gas exchange evaluations were performed only in treatments with presence and absence of hydrogel, with a frequency of three days of irrigation, using five repetitions, and it was not possible to evaluate the other treatments, due to mortality that gradually occurred during the experiment. The evaluations were performed using an Infrared Gas Analyzer portable photosynthesis meter (Li-6400xt), determining the assimilation values of CO₂ (A), stomatal conductance (gs), transpiration (E), relation between intercellular and atmospheric concentration CO₂ (Ci/Ca) and water-use efficiency (WUE). The water-use efficiency (WUE) was obtained by dividing A by E. Inside the greenhouse, the plants received, during the measurements, an irradiance of approximately 900 mmol of photons m⁻² s⁻¹. Gas exchange evaluation was performed immediately before and 24 hours after irrigation, and 43 days after planting and the start of intermittent irrigation. Assessments on both days began around 8:00 am.

2.3. Dry biomass
At the end of the experiment (48 days), by a destructive method, the aerial and root biomass of the plants was manually collected. Collection was performed only in treatments that received intermittent irrigation, in the frequencies of three and six days, regardless of the absence or presence of the hydrogel. These were separated and stored in paper bags, kept in a forced air circulation oven (65 ± 3°C) until they reached constant mass, after weighing to determine the dry matter content by using a precision balance (0.01 g).

2.4. Statistical Analysis
After confirming the normality and homogeneity of the data of the analyzed variables, a parametric variance analysis was performed at a 5% error probability level. When the value of “F” was significant, the qualitative treatments had their averages compared by the Scott-Knott test or t-test, at 5% probability of error. The SISVAR software was used to perform the data analysis and for graphics the Origin Pro 9 and the Graphed 8 softwares were used.

3. RESULTS AND DISCUSSION

3.1. Available water retention curve
By determining the soil’s water retention curve, it was found that the use of hydrogel incorporated in the soil caused a significant in the amount of water retention when compared to the same soil with the absence of the polymer, although this increase was more evident at tensions below 10 kPa (Figure 1). In any case, the addition of hydro-retaining polymers positively influenced the soil water-storage capacity.

The field capacity (FC) was higher (0.427 m³ m⁻³) in the presence of hydrogel than in the absence (0.349 m³ m⁻³). Regarding the permanent wilting point (PWP), hydrogel presence promoted inferior results when soil received the polymer (0.189 m³ m⁻³) compared to when it did not (0.206 m³ m⁻³). As a result, the available soil water storage capacity (WSC) was higher in the presence of the polymer (0.238 m³ m⁻³) compared to the absence (0.143 m³ m⁻³), resulting in a relative increase of 66%. The available water storage capacity increased due to the hydro-retaining polymer having a greater activity in ion exchange capacity with the soil solution, as it has a larger diffuse double layer than the sandy particles that are predominant in the soil used in the experiment. Therefore, it is expected that this effect will be more pronounced in sandy-textured soils, as in the present study, than in clayey-textured soils.
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This result can be explained by the high water absorption capacity of the hydrogel. According to Prevedello and Loyola (2007), the hydrogel has the capacity to absorb 150 to 400 times its dry mass and can be used to increase water storage capacity, minimizing problems associated with irregular or deficient water availability of water in the soil, when it may negatively affect plant development.

### 3.2. Photosynthetic analysis

Analyzing the hydrogel efficiency before irrigation and 24 hours afterwards, with the absence or presence of this polymer in the cultivation soil, the direct effect on gas exchange of this species can be observed (Table 1). Overall, there was no significant difference in the results of photosynthetic analyses collected prior to irrigation using the hydrogel. However, there was a difference (p<0.05) when analyses occurred 24 hours after irrigation. It was observed that the treatments without hydrogel presented the best values for all analyzed variables.

This fact can be explained by the drought-tolerance mechanism that the plant develops at the cellular level where the production or accumulation of osmotically active solutes occurs, a process known as “osmotic adjustment,” in order to maintain turgor and water potential balance in the cell (Nunes, 2007). For Serraj and Sinclair (2002), this mechanism allows the survival of the water-deficient plant, but does not maintain plant growth and productivity.

### Table 1. Liquid CO$_2$ assimilation ($A$), stomatal conductance ($g_s$), transpiration ($E$), relation between intercellular and atmospheric CO$_2$ concentration ($Ci/Ca$), and water use efficiency ($WUE$) in *Eucalyptus dunnii* before, and 24 hours after irrigation and with or without hydrogel in the soil.

| Period | Hydrogel | $A$ (µmol m$^{-2}$ s$^{-1}$) | $g_s$ (mol m$^{-2}$ s$^{-1}$) | $E$ (mmol m$^{-2}$ s$^{-1}$) | $Ci/Ca$ (mol mol$^{-1}$) | WUE (µmol mmol$^{-1}$) |
|--------|----------|----------------------------|-----------------------------|----------------------------|------------------------|-------------------------|
| Before | With     | 2.44 Aa*                   | 0.016 Aa                    | 0.247 Aa                   | 0.337 Aa               | 9.82 Ba                 |
| Before | Without  | 2.89 Aa                    | 0.021 Aa                    | 0.335 Aa                   | 0.429 Aa               | 8.06 Aa                 |
| After  | With     | 5.38 Ab                    | 0.043 Aa                    | 0.949 Ab                   | 0.422 Aa               | 5.79 Ab                 |
| After  | Without  | 8.18 Bb                    | 0.092 Bb                    | 1.563 Bb                   | 0.557 Bb               | 5.59 Ab                 |
| CV (%) |          | 21.5                       | 19.2                        | 20.4                       | 26.1                   | 16.8                    |

*Averages followed by the same letter, uppercase for hydrogel and lowercase for period do not differ between each other by t-test (0.05).*
Irrespective of the presence or not of irrigation, the plants with the absence of the hydro-
retaining polymer presented the best physiological performance, presenting the better stomatal
control and consequently higher values of $A$, $E$ and $Ci/Ca$.

With the addition of the polymer, $A$, $E$ and $g_s$ recovery was visible 24 hours after irrigation,
demonstrating that $A$ limitation probably occurred due to stomatal limitation, given the rapid
recovery. These results show that all physiological processes of the cell are directly or indirectly
affected by water supply. However, Agaba et al. (2011), state that the hydrogel’s ability to hold
and make water available more slowly may favor the ability of seedlings to return to normal
after water stress has occurred.

As for the $WUE$ values, they maintained their higher values in the presence of hydrogel,
both before irrigation and 24 hours after it. This fact may be justified due to the strategy that
the plant develops to tolerate the water deficit. The control of plant water loss by stomatal
closure keeps the water potential and relative leaf water content high (Souza et al., 2004). As a
consequence, there are restrictions (or reductions) in stomatal conductance and gas exchange,
which reduces the rate of transpiration and assimilation of $CO_2$ as observed.

The first and most sensitive response to water deficit is the decrease in turgidity, through
which stomata closure, photosynthesis reduction and cell elongation decrease, especially in
extension (Larcher, 2006). This stress caused by water deficiency is produced by both soil water
limitation and excessive transpiration loss in relation to root absorption, and these processes are
influenced by environmental factors and plant characteristics such as species or genotype
(Sant’Anna, 2009).

### 3.3. Chlorophyll

The chlorophyll level of *Eucalyptus dunnii* leaves in both treatments (presence and
absence) presented statistically higher averages (t-test) regarding the use of hydrogel in the
three irrigation frequencies, and the highest rates were observed in the treatments with the
presence of this polymer (Figure 2).

![Figure 2. Chlorophyll content in plants as a function of the presence or absence of hydrogel and different irrigation frequencies. A-irrigation only at planting; B-irrigation every 3 days; C-irrigation every 6 days.](image)

In the analysis of chlorophyll between irrigation frequencies, it was not possible to perceive
behavior change between treatments. The chlorophyll content over time showed a quadratic
behavior, with values close to 35 at the beginning of the experiment, reaching 45-48 around 25
days and decreasing again to close to 40 at the end of evaluation (47 days). And at all periods
and over time, the values were higher for hydrogel treatment, as already discussed.

With these results, it is believed that the addition of this polymer possibly decreases
nutrient loss by leaching (Navroski et al., 2015b), providing higher photosynthetic rate due to
higher nitrogen contents, where this nutrient is directly linked to higher photosynthetic rate of
the plant. In this sense, for the soil with higher water availability the mineralization rate of
organic matter increases in relation to the soil with lower humidity, thus making a larger amount of nitrogen available to the plant.

Tohidi-Moghadam et al. (2009), studying the response of six canola genotypes to water stress and hydrogel application, observed that water deficiency also reduced the chlorophyll content and that under the field conditions the hydrogel increased the performance of physiological characters, water deficiency and absence of hydrogel led to a decrease in all parameters evaluated. According to these authors, these results may be related to the reduction of photosynthesis and also chlorophyll content.

3.4. Symptomatology

Analyzing the irrigation frequencies for the classification days without symptoms (WOT), the obtained data showed there was interaction ($p<0.05$) between the factors of the evaluated variables. There was no effect of the presence or absence of hydrogel on irrigation use only at planting. In the presence of hydrogel, the frequencies of 3 and 6 days showed a relatively high gain without the appearance of symptoms, compared to the absence of this polymer, which increased the time for the emergence of stress compared to irrigation only at planting, demonstrating once again that the use of hydrogel responds positively to water scarcity in the soil.

No interaction was observed between hydrogel use and irrigation frequency in the evaluation of plants with mild withering symptoms (MWS), independent of irrigation frequency. With the presence of hydrogel, *Eucalyptus dunnii* seedlings remained more days without the onset of water stress, delaying the appearance of symptoms at all levels, compared to the absence of the polymer, where symptoms appeared much earlier (Table 2).

Regarding irrigation in the presence of the polymer, the frequency of 6 days showed a gain in days without the onset of these symptoms. Not using it, even with irrigation, there was no accelerated occurrence of these symptoms. It is noteworthy that at this stress level the seedlings still have a total physiological condition to recover in the occurrence of rain. The use of irrigation had a positive effect, as expected, and at the frequency of 3 days there was the greatest delay in the onset of symptoms.

In the appearance of the moderate withering symptoms class (MWS), where the plant was close to the permanent wilting point, the analyzed data showed that there was interaction between the factors of the variables. In this class, there was also a delay in stress symptoms when hydrogel was present.

In the class of severe withering symptoms (SWS) where, according to Navroski et al. (2014), at this level of stress a recovery of the plant with new irrigations or rainfall is already difficult, the hydrogel still continued to be efficient, and there was a delay of more than five days in the onset of these symptoms when using irrigation only in the planting, in relation to not using it.

These results corroborate the findings of Navroski et al. (2014) in the study of the influence of the hydro retainer polymer on the survival of *Eucalyptus dunnii* seedlings under different water-management regimens, where its use mitigated water scarcity in the soil, allowing the delay of water deficit symptoms, with its influence being greater when irrigation was performed less frequently.

It’s important to highlight that, in the implantation of *Eucalyptus* forests, it is usually observed that the most critical period for the loss of seedlings due to lack of water is in the first 20 days, when the plant is being established and its root system is still in formation (Fernández et al., 2010). In the field planting, it can be assumed that in cases where irrigation is frequent, or where rainfall is higher, the use of the polymer could be discarded (Navroski et al., 2015a). However, under less frequent rain or more-spaced irrigation, the use of hydrogel may be recommended.
Table 2. Averages results of duration of symptoms of water stress, in days, evaluated from planting until the 47th day after planting *Eucalyptus dunnii* in pots, due to the presence or absence of hydrogel and different irrigation frequencies, where: WOT – no withering symptoms; MLS – mild withering symptoms; MWS – moderate withering symptoms; SWS – severe withering symptoms and PRA – plants remained alive.

| Variable | Hydrogel | Appearance of stress symptoms (days) | Irrigation frequency | Averages |
|----------|----------|--------------------------------------|----------------------|----------|
|          |          |                                      | Planting 6 days 3 days |          |
|           | **WOT**  | Abundance of stress symptoms (days)  |                      |          |
| Absence   | 23.10 aB  | 24.80 bB  | 31.70 bA   | 26.54    |
| Presence  | 24.10 aC  | 34.12 aB  | 44.90 aA   | 24.37    |
| Average   | 23.60     | 29.21     | 38.30      |          |
| MLS       | Absence   | 27.10     | 32.70      | 44.00    | 34.60    |
| Absence   | 31.00     | 34.33     | 46.00      | 37.20    |
| Average   | 29.05B    | 33.47 B   | 45.00 A    |          |
| MWS       | Absence   | 32.00 bC  | 36.50 bB   | 44.50 aA | 37.66    |
| Absence   | 36.50 aB  | 45.56 aA  | 46.00 aA   | 42.58    |
| Average   | 34.25     | 40.78     | 45.25      |          |
| SWS       | Absence   | 35.40 bC  | 40.80 bB   | 44.70 aA | 40.30    |
| Absence   | 41.70 aB  | 46.00 aA  | 46.00 aA   | 44.52    |
| Average   | 38.55     | 43.26     | 45.35      |          |
| PRA       | Absence   | 35.40 bC  | 40.80 bB   | 44.70 aA | 40.30    |
| Absence   | 41.70 aB  | 45.55 aA  | 46.00 aA   | 44.37    |
| Average   | 38.55     | 43.18     | 45.35      |          |

* Averages followed by the same letter, uppercase letter in the line (Scott-Knott test), and lowercase in the column (t-test) do not differ from each other at 5% probability of error.

In the evaluation of the number of days the plants remained alive (PRA), it was noticed that the use of hydrogel presented a difference when irrigation occurred every 6 days and only at planting. In this case, with the presence of the hydrogel in the soil, an increase of approximately 5 to 6 days in which they remained alive was obtained, thus justifying the expenses with the use of the polymer.

In the use of post-planting irrigations, frequently no significant effect of hydrogel was observed. This may indicate that the use of hydrogel is beneficial in delaying seedling mortality when frequent or infrequent irrigation is used only at planting, allowing a gain of a few days waiting for rain to occur.

3.5. Dry biomass

The results showed that for the root dry mass variable, the hydrogel interaction and irrigation frequency time was not significant (p>0.05), there was only difference for the isolated factors. In the presence of hydrogel, there was a significant difference, where the root dry mass was higher, presenting 2.17 g and in the absence only 1.12 g. As expected, the addition of the hydrogel contributed to the development of *Eucalyptus dunnii* seedlings, presenting a significant increase in root dry mass. Eloy *et al.* (2013) state that this variable is of great importance in the development of plants, since when there is a good rooting, they have a greater potential for growth and field survival.
In the evaluation of the presence of hydrogel, within the frequencies of 3 and 6 days, there was no significant difference. These results indicate that regardless of irrigation time, the use of hydrogel favored the increase of root dry mass of seedlings. In the absence of the hydrogel, there was a significant difference between the frequencies, where the frequency of 3 days had 1.97 g of root dry mass and for the frequency of irrigation of 6 days the root dry mass was lower, with 1.33 grams.

For the variable dry mass of the aerial parts, the hydrogel interaction and irrigation frequency were not significant (p>0.05). In the presence of hydrogel there was significant difference, and the highest values were found for the treatment with hydrogel, in which the dry mass of the aerial parts was higher (4.35 g), compared to the absence (2.34 g).

For the frequency of irrigation, there was a significant difference, and in the frequency of 3 days the dry mass of the aerial parts was higher (3.99 g) compared to the frequency of 6 days (2.7 g). The results indicate that the use of hydrogel incorporated into the soil and associated with different irrigation frequencies positively influenced the development of aerial parts of *Eucalyptus dunnii* seedlings.

### 4. CONCLUSION

- The use of hydrogel promotes a significant increase in water retention in the soil, allowing the delay of water deficit symptoms, although the effect of retention was greater at lower tensions (less than 10 kPa).
- The presence of the hydro retainer polymer in the soil presents higher averages for morphological characteristics, and in general decreased symptoms of water deficit in plants.
- There is a direct effect on gas exchange, with recovery of the variables analyzed 24 hours after irrigation. However, plants grown in the absence of the hydrogel exhibit the best physiological performance, so further studies should be conducted (preferably in the field) to understand physiological processes.
- In general, the hydrogel allows the reduction of the frequency of irrigation, showing a better result when the plants are under water restriction.

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