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Growth of *Eucalyptus urocam* Under Different Irrigation Managements

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Abstract

The objective of the present study was to evaluate the effects of different water irrigation managements on the growth of *Eucalyptus urocam* plants. The experiment was conducted in a greenhouse at the Goiás State University, Ipameri campus, Brazil, using 100-day-old *Eucalyptus urocam* seedlings, which were transplanted into pots with 5 kg of substrate. A completely randomized experimental design with five replications was used, in a 2 × 4 factorial arrangement consisting of two daily irrigation depths (50%, and 100% of the substrate retention capacity) and four irrigation times (7:30 a.m., 11:30 a.m., 4:30 p.m., and 7:00 p.m.). The treatments were applied when the plants were 120 days old, and last 18 days, then, the variables were analyzed. *E. urocam* plants subjected to water deficit had high stomatal sensitivity, reduced transpiration rate, and maintained hydration, which are characteristics of isohydric plants. *E. urocam* plants irrigated at 4:30 p.m. or 7:00 p.m. presented low stomatal sensitivity and high biomass accumulation potential. Therefore, irrigations between 4:30 p.m. and 7:00 p.m. are recommended for *Eucalyptus urocam* plants at the initial growth stage.

Keywords: forestry, stomatal sensitivity, water supply

1. Introduction

Areas with forest plantations are increasing worldwide. Restoration of degraded landscapes, conservation of native tree species, wood and cellulose supply, and carbon sequestration are the main reasons for growing forest species (Pozo & Säumel, 2018). In the last decades, plantations have increased from 1675 Mha in 1990 to 2779 Mha in 2015, equivalent to 7% of global forest cover (Payn et al., 2015).

Despite the diversity of tree species, *Eucalyptus* spp. dominate planted forests worldwide and is used in intensive management systems due to its easy establishment, high short-term productivity, and good adaptation to different climatic conditions (Binkley et al., 2017). The world area with eucalyptus crops exceeds 20 million hectares; India accounts for 22%, Brazil 20%, and China 14% of the global production (GIT Forestry, 2008). Most eucalyptus plantations are grown to supply wood for cellulose and timber markets, moreover, interest on other products such as solid wood, laminate wood, charcoal, and biomass, has been growing (Jesus et al., 2017).

Despite the success of planted forests, the sector can grow even more with the use of areas that are unsuitable for many other crops that are less tolerant to abiotic stresses (Lopes et al., 2015). Decreases in available agricultural areas led eucalyptus crops to marginal regions that have low-fertility soils and low water availability (Acuña, Rubilar, Cancino, Albaugh, & Maier, 2018).

Water deficit is the greatest obstacle to increase agricultural productivity. It is one of the most critical abiotic stresses and the one that most limit the growth of eucalyptus plants (Matos et al., 2016). Climate change has intensified drought periods in several regions of the world, increasing problems commonly faced by producers (Schlaepfer et al., 2017). Water deficit significantly hinders biomass accumulation by eucalyptus plants. Changes in water and thermal regimes reduce the plant's metabolic capacity and change their gas and water exchange activity, with significant damage to wood formation and anatomy (Sette Junior, Tomazello Filho, Lousada, & Laclau, 2010).

Water deficit symptoms in eucalyptus plants at the initial stage of development are more severe and visible, affecting plant growth and increasing plant mortality in the field (Cabral et al., 2010). According to Valdés et al.

(2013), multiple responses are activated when trees are under water deficit to synchronize their development with molecular activities and guarantee their survival. Plants subjected to water deficit activate morphological, physiological, or anatomical mechanisms to adapt to drought (Tatagiba, Pezzopane, Dos Reis, & Penchel, 2009). Identifying these mechanisms may explain the resistance to drought of eucalyptus plants.

Evaluating irrigation water depths in different times of the day may assist in avoiding losses caused by water deficit. The irrigation of cultivated plants represents one of the most important advances to obtain high biomass yields, as the water supply in sufficient volumes increases the growth of the plants. Over the years many irrigation systems have been developed and the studies have advanced markedly with determination of the appropriate method of irrigation and volume of water to be applied under various environmental conditions, however, the recommendation of the appropriate irrigation schedule still lacks further scientific investigation.

It has been believed since the 14^{th} century that one should not water plants under full sun (Power, 1928), but evidences with scientific basis are needed for such claim. However, few studies show the effects of irrigation in different times of the day on plant growth. In this context, the objective of the present study was to evaluate the effects of different water irrigation managements on the growth of *Eucalyptus urocam* plants.

2. Material and Methods

The experiment was conducted in a greenhouse covered with a transparent plastic at the Goiás State University, Ipameri campus, Brazil (17°43′19″ S, 48º09′35″ W, and altitude of 764 m). The climate of the region is tropical, with dry winter and wet summer (Aw), according to the Köppen classification, with average temperature of 20 °C (Alvares Stape, Sentelhas, Gonçalves, & Sparovek, 2013).

Eucalyptus urocam seedlings of 100 days old from a seedling production nursery were transplanted to pots with 5 kg of a substrate consisted of Oxisol, sand, and manure (3:1:0.5). Based on the chemical analysis of the substrate: pH of 6.4, 19 g dm^{-3} of organic matter, 2.4 mg dm^{-3} of P, 109 $cmol_c$ dm^{-3} of K, 1.5 $cmol_c$ dm^{-3} of H+Al, 3.2 $cmol_c$ dm^{-3} of Ca, 1.6 $cmol_c$ dm^{-3} of Mg, 27.7 mg dm^{-3} of Zn, base saturation of 77.20%, and cation exchange capacity of 6.58—no liming or fertilization were performed.

The experiment was conducted in a completely randomized design with five replications, using a 2×4 factorial arrangement consisted of two daily irrigation depths (50%, and 100% of the substrate retention capacity) and four irrigation times (7:30 a.m., 11:30 a.m., 4:30 p.m., and 7:00 p.m.). The water retention capacity of the substrate was determined by the difference in weight of the pots as described by Sá et al. (2017). The irrigation was done manually with application of pre-determined volume of water using graduated container. The treatments were applied when the plants were 120 days old, and the experiment last 18 days, then, the following variables were analyzed: plant height, stem diameter, relative water content, number of leaves, transpiration rate, total plant weight, root weight ratio, stem weight ratio, shoot weight ratio, specific leaf area, and leaf chlorophyll content.

2.1 Variables

Plant height and stem diameter were measured using a graded ruler and digital pachimeter, respectively. The number of leaves was measured by counting all the leaves on each plant.

For destructive testing, the roots, stems and leaves were separated and dried in an oven at 72 ºC until constant dry weight and then weighed. The dry weight data were used to calculate the root mass ratio (RMR), stem mass ratio (SMR), leaf mass ratio (LMR) and total biomass. To obtain the specific leaf area (SLA), six leaf discs of 12 mm in diameter were removed from the leaves in a drying oven and SLA was then determined by dividing the area of the six discs by the dry mass of the discs.

Total daily plant transpiration was determined by the difference in weight of the pots. First, each pot was placed inside a plastic bag attached to the stem of the plant with a rubber band, leaving only the aerial parts (stem and leaves) exposed. Next, the pot (and bag) was weighed at 12 pm (weight 1) and then again 24 hours later (weight 2). Total transpiration was estimated based on the difference between weight 1 and 2 (Dos Anjos et al., 2017). In order to calculate relative water content, ten 14mm-wide leaf discs were collected, weighed and immersed in distilled water for 8 h in Petri dishes. Next, the discs were weighed and dried at 70 ºC for 72 hours.

To determine the chlorophyll concentration, leaf disks were removed from the known area and placed in jars containing dimethyl sulfoxide (DMSO). Later extraction was carried out in a water bath at 65 ºC for one hour. Aliquots were removed for spectrophotometric reading at 480, 646 and 665 nm. The chlorophyll a (Cl *a*) and chlorophyll b (Cl *b*) contents were determined following the equation proposed by Wellburn (1994).

The work was set up in a completely randomized design in a 24 factorial with five replicates. The data was submitted to analysis of variance and the Tukey test to compare the means. Multivariate analysis was carried out by multiple regression using the *forward stepwise* model (Sokal & Rolf, 1995) and Principal component analysis was performed using a permutational multivariate analysis of variance (Permanova-Anderson, 2001). The R (R Core Team, 2018) and SigmaPlot 10.0 (Systat Software, 2006) software was used to carry out these analyses.

3. Results and Discussion

The results of the analysis of variance and mean test (Table 1) showed that *Eucalyptus urocam* plants irrigated at 4:30 p.m. or 7:00 p.m. presented higher height, stem diameter, and relative water content; however, without significant increases in total plant weight. The results found for number of leaves, transpiration rate, and total plant weight indicate an interaction between water depths and irrigation times (Table 2).

Table 1. Summary of variance analysis and mean test of Eucalyptus urocam irrigated with water volume of 50% and 100% of the retention capacity of the substrate at different hours (7:30 a.m, 11:30 a.m, 4:30 p.m and 7:00 p.m)

	DF	PH (cm)	SD (mm)	LN	RWC (%)	E (g H_2O dia^{-1})	Biom (g)
Variation source				Mean squares			
Hour (H)	3	414.74^{ns}	0.96**	260.20**	156.59*	6865.30**	2.92^{ns}
Irrigation (I)	1	2.63**	1.77**	4644.00*	68.77^{ns}	93286.60**	120.97**
H × I	3	11.74^{ns}	0.11^{ns}	184.40**	54.36^{ns}	2809.20**	6.45*
Erro	32	15.04	0.11	94.90	198.61	519.31	2.11
CV (%)		7.98	7.10	13.76	9.30	15.53	14.99
Hour				Means			
7:30 a.m		48.20a	4.78a	32.00ab	77.74a	133.90b	8.50a
11:30 a.m		48.80a	5.13a	38.50a	70.98b	133.00b	8.80a
4:30 p.m		48.12a	4.41b	26.20b	71.72b	185.99a	8.90a
7:00 p.m		49.20a	4.98a	34.00ab	78.57a	133.80b	9.80a
Irrigation				Means			
50%		45.36b	4.61b	21.90b	76.06a	98.4b	7.30b
100%		51.80a	5.03a	43.45a	73.44a	194.8a	10.70a

Note. PH = plant height; SD = stem diameter; LN = leaf number; RWC = relative water content; E = total transpiration; Biom = total plant biomass. *significant at 5% and at **1% probability; ns = not significant according to the F test. Means followed by the same lower case letter in the column do not differ at 5% probability according to the Tukey test.

The plant height, stem diameter, number of leaves, transpiration rate, and total plant weight of plants irrigated with 100% of the retention capacity of the substrate were higher than those of plants under water deficit 13%, 9%, 50%, 50%, and 32%, respectively. The water deficit resulted in lower availability of water for cell expansion and significant restriction to vegetative growth and corroborate those found by Matos, Freitas, Souza and Lopes, (2018) who identified that the initiation and development of the leaf primordium are dependent on the water status of the plant. The emergence and development of leaf primordia are dependent on the plant's water status.

The reduction of number of leaves recorded in the present study is an important strategy to tolerate water deficit by minimizing transpiration surface, and one of the most efficient forms of delaying dehydration in plants. According to Souza et al. (2015), the reduction in number of leaves, the small variation in relative water content (maintenance of leaf hydration), and the reduction in transpiration rate in *Eucalyptus urophylla* plants in environments with low water availability denote their tolerance to water deficit.

The reduced transpiration rate, and maintenance of tissue hydration found in plants subjected to water deficit are typical of isohydric plants, which minimize water loss through their high stomatal sensitivity and maintain leaf water potential along the day. However, this small stomatal opening reduces the CO_2 influx to carboxylation sites, limiting photosynthesis process and biomass accumulation.

The means of number of leaves and transpiration rate found (Table 2) indicated that plants irrigated at 4:30 p.m. or 7:00 p.m. had intermediate number of leaves and higher transpiration rate. Irrigation at these times probably reduced the stomatal sensitivity of the *E. urocam* plants. When the plants were subjected to water deficit, the greater stomatal opening at these times increased CO_2 influx, generating a greater biomass accumulation.

Table 2. Average values of Eucalyptus urocam plants irrigated with water volume referring to 50% and 100% of the capacity substrate at different hours (7:30 a.m, 11:30 a.m, 4:30 p.m and 7:00 p.m)

Hour	Means					
	LN		Biom (g)		*E* (g H_2O dia^{-1})	
	50%	100%	50%	100%	50%	100%
7:30 a.m.	15.00 bC	49.00 aA	6.63 bA	10.39 aA	80.00 bBC	187.96 aB
11:30 a.m.	28.40 bA	48.60 aA	6.15 bA	11.63 aA	70.00 bC	196.00 aB
4:30 p.m.	18.80 bBC	33.60 aB	8.27 aA	9.59 aA	134.00 bA	273.98 aA
7:00 p.m.	25.40 bAB	42.60 aA	7.97 bA	11.55 aA	109.60 bAB	158.00 aB
CV (%)	13.76		15.00		15.53	

Note. LN = leaf number; Biom = total biomass; *E* = total transpiration. Means followed by the same lower case letter in the line of each variable at 50% and 100% and by capital letter in the column do not differ to a 5% probability, according to the Tukey test.

The means of the variables evaluated presented low difference when irrigated with 100% of the water volume; however, plants under water deficit that were irrigated at 4:30 p.m. or 7:00 p.m. (under milder weather conditions) presented higher transpiration rates and total plant weights. According to Tatagiba, Pezzopane, Reis (2015), when eucalyptus plants reach critical levels of leaf water potential, the stomata are partially or almost completely closed.

Table 3. Summary of analysis of variance and mean test of irrigated Eucalyptus urocam plants with water volume of 50% and 100% of the retention capacity of the substrate at different hours (7:30 a.m, 11:30 a.m, 4:30 p.m and 7:00 p.m)

	DF	RMR (%)	SMR (%)	LMR (%)	SLA (m^3 kg^{-1})	Chl (*a*+*b*) (mg g^{-1} MF)
Variation source	Mean squares					
Hour (H)	3	82.01^{ns}	23.67**	157.59**	55.30**	0.36**
Irrigation (I)	1	438.31**	65.53**	1194.98**	248.50**	2.47**
H × I	3	37.07^{ns}	16.08**	34.31**	106.91**	0.63**
Erro	32	40.10	4.57	7.30	18.99	0.10
CV (%)		20.98	7.44	5.00	12.50	19.5
Hour	Means					
7:30 a.m		30.13a	26.60a	39.67c	28.82a	1.39a
11:30 a.m		31.62a	26.17a	42.19bc	24.18a	1.11a
4:30 p.m		26.38a	24.55ab	49.06a	27.60a	1.06a
7:00 p.m		33.04a	23.26b	43.80b	24.29a	1.43a
Irrigation	Means					
50%		33.60b	26.42a	38.21b	23.73b	1.00b
100%		26.98b	23.86b	49.15a	28.72a	1.50a

Note. RMR = for root mass ratio; SMR = stem mass ratio; LMR = leaf mass ratio, SLA = specific leaf area; Chl *a*+*b* = foliar concentration of total chlorophylls. *significant at 5% and at **1% probability; ns = not significant according to the F test. Means followed by the same lower case letter in the column do not differ at 5% probability according to the Tukey test.

The irrigation times did not affect root weight ratio (Table 3). The results found for root weight ratio, leaf weight ratio, specific leaf area, and foliar chlorophyll content indicate an interaction between water depths and irrigation times (Table 4). The leaf weight ratio, specific leaf area, and chlorophyll content of plants irrigated with 100% of the retention capacity of the substrate were respectively 23%, 18%, and 34% higher than those of plants under water deficit. The root weight ratio and stem weight ratio of plants under water deficit were respectively 20% and 10% higher than those of plants irrigated with 100% of the water volume.

According to Wegner (2017), the first biophysical effect of water stress is a decrease in cellular volume, affecting mainly the elongation of roots. Under these conditions, the *E. urocam* plants allocate assimilates to increase root growth (root weight ratio) and hydraulic conductivity (stem weight ratio) to the detriment of leaf weight ratio and chlorophyll pigments (Chl *a* + Chl *b*). Moreover, *E. urocam* plants under water deficit had reduced specific leaf area, probably due to their increased leaf thickness—increases in cuticle to minimize transpiration and maintain hydrated tissues.

Plants irrigated at 4:30 p.m. or 7:00 p.m. presented higher leaf weight ratio, specific leaf area, and chlorophyll (*a* and *b*) contents, and lower stem weight ratio under water deficit (Table 4). Despite the small difference between the means of the variables in the treatments irrigated with 100%, plants irrigated at 4:30 p.m. or 7:00 p.m. were less acclimated to the water deficit with minor morphophysiological changes. In addition, the higher specific leaf area found indicates a lower leaf thickness and, therefore, lower protection from tissue dehydration.

Table 4. Interaction average test of irrigated Eucalyptus urocam plants with water volume of 50 % and 100% of the retention capacity of the substrate at different hours (7:30 a.m, 11:30 a.m, 4:30 p.m and 7:00 p.m)

Hour	Means							
	Chl *a+b* (mg g^{-1} MF)		SMR (%)		LMR (%)		SLA (m^2 kg^{-1})	
	50%	100%	50%	100%	50%	100%	50%	100%
7:30 a.m	0.97bA	1.81aAB	28.25aA	24.96bA	33.85bB	45.49aB	24.56bA	33.10aA
11:30 a.m	0.82bA	1.41aB	28.29aA	24.07bAB	35.35bB	49.04aAB	18.01bB	30.36aAB
4:30 p.m	1.18aA	0.94aC	23.95aB	25.15aA	46.30bA	51.82aA	28.31aA	26.91aBC
7:00 p.m	1.03bA	1.84aA	25.22aAB	21.30bB	37.36bB	50.24aA	28.31aA	24.51aC
CV (%)	19.50		7.44		5.00		12.50	

Note. Chl *a+b* = foliar concentration of total chlorophylls; SMR = stem mass ratio; LMR = leaf mass ratio; SLA = specific leaf area. Averages followed by the same lowercase letter in the rows and upper case in the columns do not differ from each other by the Tukey test at 5% probability.

The multiple regression model used to evaluate the effects of the variables on the total plant weight of *E. urocam* plants (Table 5) showed that the stem diameter, stem weight ratio, transpiration rate, and total chlorophylls explained 77% of the variance of the total plant weight of the *E. urocam* plants.

Table 5. Multiple regression model to evaluate the effect of variables on the biomass of irrigated *Eucalyptus urocam* plants with water volume referring to 50% and 100% of the retention capacity of the substrate at different hours (7:30 a.m, 11:30 a.m, 4:30 p.m and 7:00 p.m)

Biomas (g)	$R^2 = 0.77$	F (6.33) = 18.667	$p < 0.0001$			
	Beta	Std. Err. of Beta	B	Std. Err. of B	t (19)	p-level
Intercept			6.826	3.963	1.72	0.09
SD	0.275	0.115	1.23	0.56	2.38	0.02**
SMR	-0.234	-0.195	-0.195	0.086	-2.26	0.03**
E	0.337	0.115	0.013	0.004	2.91	0.006*
Chl (*a+b*)	0.384	0.133	2.035	0.705	2.88	0.006*

Note. ** Significant at 5%; * Significant at 1%; Explanation of the R^2 model = 0.77; SD = Stem diameter; SMR = stem mass ratio; *E* = Transpiration; Chl (*a+b*) = Total Chlorophyll.

The stem diameter, stem weight ratio, transpiration rate, and chlorophyll contents were the most determinant variables for biomass production. Plants irrigated at 4:30 p.m. or 7:00 p.m. had a greater biomass accumulation and less sensitivity to water stress than those in the other irrigation times.

The principal component analysis (PCA) (Figure 1) explained 79.8% of the data variance, and formed two groups: the first for the right of axis 1, with plants irrigated with water depths corresponding to 50%, and the second for the left of axis 1, with plants irrigated with 100% of the retention capacity of the substrate.

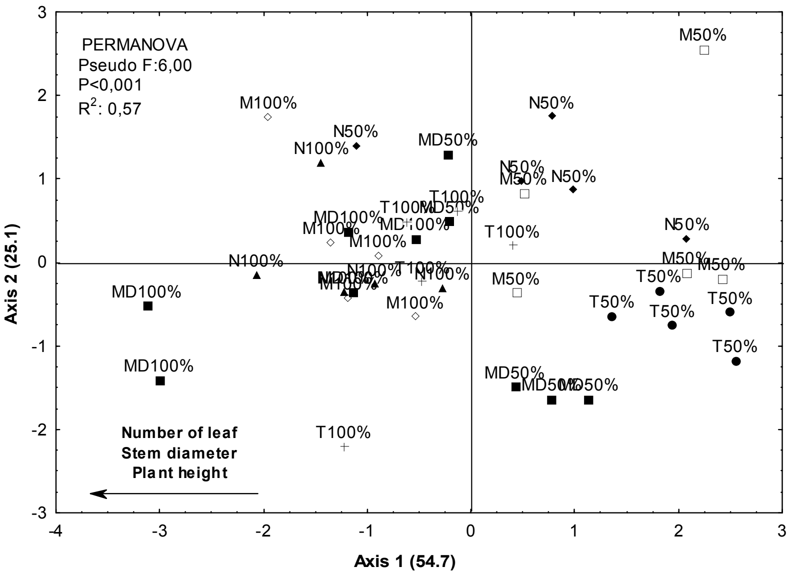

Figure 1. Principal components analysis for all variables studied in Eucalyptus urocam plants irrigated with water volumes referring to 50% and 100% of the retention capacity of the substrate at different times (M = 7:30 a.m., MD =11:30 a.m., T = 16:30 p.m. and N = 19:00 p.m.)

This indicates that the difference in water supply was sufficient to alter the growth of *E. urocam* plants, which, in general, have an isohydric mechanism to maintain tissue hydration, minimizing transpiration by reducing stomatal opening (Matos et al., 2016).

The similarity in total plant weight between treatments irrigated with water depths corresponding to 100% of the retention capacity of the substrate was due to the reduced time of the experiment. However, the marked changes in specific leaf area, transpiration rate, stem weight ratio, and chlorophyll (*a*+*b*) contents indicated that the plants irrigated at 4:30 p.m. or 7:00 p.m. had less stomatal sensitivity and fewer signs of protection from water deficit. Therefore, they have higher growth potential, by allowing greater CO_2 influx and, consequently, significant increases in the photosynthesis, and biomass accumulation.

Irrigations at 7:30 a.m. or 11:30 a.m. caused significant water losses by evaporation because of the high air temperature and low air relative humidity during the day. Irrigations at these times compromise the water inflow to the soil-plant-atmosphere system due to the reduced plant stomatal opening. In these conditions, plants have lower water availability at the end of the afternoon and at night. Contrastingly, irrigations at 4:30 p.m. or 7:00 p.m. resulted in lower losses by direct water evaporation, the plants remain hydrated and with opened stomata until at least the following morning due to low transpiration and evaporation at night times. According to Kerbauy et al. (2013), maintenance of tissue turgidity at night is essential for growth.

4. Conclusions

Eucalyptus urocam plants subjected to water deficit presented high stomatal sensitivity, reduced transpiration rate, and maintained hydration, which are characteristics of isohydric plants. However, *E. urocam* plants irrigated at 4:30 p.m. or 7:00 p.m. presented lower stomatal sensitivity and higher biomass accumulation potential; therefore, irrigations between 4:30 p.m. and 7:00 p.m. are recommended for *Eucalyptus urocam* plants at their initial growth stage.

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