



# Transpiration and growth responses by *Eucalyptus* species to progressive soil drying

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**Abstract** The regulation of plant transpiration is a key factor affecting transpiration efficiency, growth and adaptation of *Eucalyptus* species to limited water availability in tropical and subtropical environments. However, few studies have related this trait to the performance of *Eucalyptus* seedlings and none have investigated the influence of vapor pressure deficit (VPD) on transpiration rates and growth. In this study, the transpiration and growth responses of seedlings of *Eucalyptus urophylla* (S.T. Blake) and *Eucalyptus cloeziana* (F. Muell.) to progressive soil water deficits were evaluated under semi-controlled conditions using the fraction of transpirable soil water (FTSW) method. In addition, the influence of VPD on seedling transpiration, development and growth was also investigated. The FTSW threshold ranged from 0.40 to 0.99 for the transpiration rate and from 0.32 to

0.97 for the development and growth variables. Little or no changes in the FTSW threshold were detected in response to changes in atmospheric VPD. Both *Eucalyptus* species presented a conservation strategy under drought stress. In addition, water-conserving mechanisms during the seedling phase were related to rapid stomatal closure, reduced leaf area, and number of leaves.

**Keywords** Soil water deficit · Fraction of transpirable soil water · Transpiration · Seedling growth · Vapor pressure deficit

## Abbreviations

FTSW	Fraction of transpirable soil water
FTSW <sub>t</sub>	Threshold fraction of transpirable soil water
TR	Transpiration rate
NTR	Normalized transpiration
NNL	Normalized number of leaves
NLA	Normalized leaf area
NH	Normalized height
ND	Normalized diameter
WW	Well-watered
WD	Water deficit
VPD	Vapor pressure deficit
VPD <sub>h</sub>	High vapor pressure deficit
VPD <sub>l</sub>	Low vapor pressure deficit
T <sub>avg</sub>	Mean temperature
T <sub>max</sub>	Maximum temperature
T <sub>min</sub>	Minimum temperature
RH	Relative humidity
es <sub>avg</sub>	Average saturation water vapor pressure using T <sub>min</sub> and T <sub>max</sub>
es <sub>min</sub>	Saturation water vapor pressure using T <sub>min</sub>
es <sub>max</sub>	Saturation water vapor pressure using T <sub>max</sub>

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$ea_{avg}$	Average actual water vapor pressure using $T_{min}$ and $T_{max}$
$ea_{min}$	Actual water vapor pressure using $T_{min}$
$ea_{max}$	Actual water vapor pressure using $T_{max}$
E1	Experiment 1
E2	Experiment 2

## Introduction

Soil water availability is a major driver of forest productivity. Recent projections of climate change have indicated, among other possible consequences, variable trends in precipitation and the intensification of drought worldwide (Santos et al. 2017; Llopart et al. 2020). An issue is how plants—cultivated or in natural environments—will respond to changing conditions. Besides the implication to natural ecosystem functioning, the intensification of drought will have a significant negative impact on agriculture and forestry with increased mortality and decreased growth (Scolforo et al. 2019; Elli et al. 2020; Hu et al. 2020).

Brazil has a global role in agricultural production, including cellulose fiber from *Eucalyptus* as an important forest product. Brazil has the largest area in the world cultivated with *Eucalyptus* with a mean increment of  $36 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$  and rotation of seven years or shorter (IBA 2019; Elli et al. 2020). The *Eucalyptus* plantation sector plays an important role in the country's economy and land use. About 7.5 million hectares are intensively cultivated under high technology (Elli et al. 2020) from which a variety of products such as pulp, paper, charcoal, boards and solid wood are obtained (IBA 2019).

Drought constitutes a major limiting factor in the growth of eucalypts in many regions of Brazil (Abreu et al. 2015; Scolforo et al. 2019), especially where water deficits are associated with high temperatures during particular periods of the year such as in Minas Gerais State, located in Brazil's southeast which has the largest area of eucalypt plantations. Recent climatic studies have shown the possibilities for considerable increases in air temperatures of up to  $5^\circ\text{C}$ , as well as various trends in amounts of precipitation, with projections of reduced rainfall in the northern region and a slight increase in central and south regions of the state (Santos et al. 2017; Martins et al. 2020). Therefore, there will be changes in soil water availability (supply) and in the amount of water consumed by forest plantations (demand).

The imbalance between water supply and demand leads to water-deficit situations (Souza et al. 2014; Abreu et al. 2015), which affects all cellular processes, leaf expansion, stomatal conductance, photosynthetic activities, dry matter remobilization, senescence and foliar abscission, and transpiration rates (TR) (Esmailzade-Moridani et al. 2015; King and Purcell 2017; Santos et al. 2017). In forest

species, all of these effects are even more drastic when they occur during the seedling phase (Abreu et al. 2015) because they impair seedling establishment in the field, reducing drastically forest potential development and yield (Sinclair et al. 2005; Martins et al. 2008; Elli et al. 2020).

In this context, different indexes have been developed to evaluate plant responses to drought stress: total available soil water (TAW), available water capacity (AWC), available water fraction (AWF), plant-available soil water (PAW), and fraction of transpirable soil water (FTSW). Among these, FTSW has been widely used to detect plant responses to drought stress (Sinclair and Ludlow 1986; Sinclair et al. 2005, 2017; Medina et al. 2017).

FTSW has two distinct stages (Sinclair and Ludlow 1986; Sinclair et al. 2005; Souza et al. 2014). Stage I occurs when soil water is maximum and stomatal conductance and water vapor loss are the highest. Stage II begins when soil water decreases and the rate of water uptake from the soil cannot match the potential TR. At stage II, the plants initiate stomatal closure for conserving water (Medina et al. 2017) as the stomata are gateways for gas exchange between the leaf and the atmosphere (Devi and Reddy 2018).

The threshold between stages I and II (FTSW<sub>t</sub>) indicates the point at which the transpiration rate is reduced (Martins et al. 2008; King and Purcell 2017; Adiredjo et al. 2018), i.e., when stomatal closure in response to water deficit is initiated (Sinclair and Ludlow 1986; Souza et al. 2014) and the degree of plant tolerance to drought (Souza et al. 2018b). FTSW<sub>t</sub> is commonly used in crop yield simulation models (Devi et al. 2009; Abreu et al. 2015; Fuentealba et al. 2016; Medina et al. 2019) and is extremely useful for understanding genotypic behavior in the face of water deficits (Medina et al. 2019).

It is reasonable to assume that FTSW<sub>t</sub> is a result of balancing plant water content (Ray et al. 2002; Bimpong et al. 2011; Pang et al. 2017; Devi and Reddy 2018). Therefore, factors influencing plant water demands could influence FTSW<sub>t</sub> (Ray et al. 2002; Schoppach and Sadok 2012; Abreu et al. 2015; Hainaut et al. 2016; Ouattara et al. 2018).

Several studies have demonstrated that FTSW<sub>t</sub> and consequently, plant transpiration are driven by changes in VPD (Bimpong et al. 2011; Schoppach and Sadok 2012; Abreu et al. 2015; Hainaut et al. 2016; Devi and Reddy 2018; Ouattara et al. 2018), which is a function of air temperature and relative humidity (RH) (Abreu et al. 2015; Medina et al. 2019). Theoretically, high vapor pressure deficits, which usually occurs from midday to ~4 pm, influence the water balance and result in an increased rate of transpiration (Devi and Reddy 2018). This situation drives a greater hydraulic conductivity, allowing water to be more readily replenished to leaves (Ray et al. 2002; Lago et al. 2011; Gholipour et al. 2012; Sinclair et al. 2017).

A critical requirement to sustain leaves is to have restricted TR under high VPD by partial stomatal closure so that the rate of transpiration decreases to match water flux into the leaf (Sinclair et al. 2017). However, this response is not universal and differences have been reported among species, cultivars, hybrids and varieties (Sinclair et al. 2017; Medina et al. 2019) such as maize hybrids (Ray et al. 2002), cassava cultivars (Lago et al. 2011) and *Eucalyptus saligna* Smith. (Martins et al. 2008) for which limitations on transpiration rates under high VPD have not been observed and, consequently, no relationship between FTSWt and VPD.

Although FTSWt is an important indicator of plant responses to water-deficit, few studies have looked in forest species. The limited researches were on Scots pine (*Pinus sylvestris* L.) (Irvini et al. 1998), beech (*Fagus sylvatica* L.) (Granier et al. 2000), Norway spruce (*Picea abies* (L.) H. Karst.) (Lagergren and Lindroth 2002), canoe-cedar (*Thuja plicata* Donn ex D. Don), red maple (*Acer rubrum* L.), English holly (*Ilex aquifolium* L.), black locust (*Robinia pseudocacia* L.) (Sinclair et al. 2005), Barbados-nut (*Jatropha curcas* L.) (Ouattara et al. 2018), Lemon-Scented Gum (*Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson) (Abreu et al. 2015), and two *Eucalyptus* species (Martins et al. 2008). All these studies confirmed a two-phase response to soil water availability with little differences in the FTSWt values that represent the transition from stages I to II. However, none considered the influence of VPD on this response.

Therefore, in this study, the following questions arise: (1) Are there *Eucalyptus* seedling defense strategies under increasing soil water deficits?; (2) What is the water use efficiency by *Eucalyptus* seedlings under increasing soil water deficits?; and, (3) What is the dry weight allocation by *Eucalyptus* seedlings under increasing soil water deficits? To answer these questions, this study assessed transpiration rates as well as the development response of *Eucalyptus urophylla* (S.T. Blake) and *Eucalyptus cloeziana* (F. Muell.) seedlings to progressive soil water deficits, taking into account differences in VPD. These two species are widely planted in Brazil and studies show differences in their response to drought. Although highly adaptable to a wide range of environmental conditions (Gonçalves et al. 2017; Elli et al. 2020), *E. cloeziana* is sensitive to soil water reduction (Ngugi et al. 2004), while *E. urophylla*, has been reported to be more drought tolerant (Elli et al. 2020).

Understanding the physiological responses to the biophysical environment, particularly water stress, is key for the development of silvicultural prescriptions and to anticipate the response of these two important species and also of other tree species of similar behavior to the projected climate changes.

## Materials and methods

### Observed meteorological data

The daily maximum ( $T_{\max}$ ) and minimum ( $T_{\min}$ ) air temperatures were measured using conventional thermometers with mercury and alcohol, respectively, and a hygrometer recorded the relative humidity (RH). The instruments were installed in a meteorological station at the greenhouse. Daily VPD was obtained as per Abreu et al. (2015):

$$\text{VPD} = e_{s_{\text{avg}}} - e_{a_{\text{avg}}} \quad (1)$$

$$e_{s_{\min}} = 0.6108 \cdot 10^{\left[ \frac{7.5 \cdot T_{\min}}{(237.4 - T_{\min})} \right]} \quad (2)$$

$$e_{s_{\max}} = 0.6108 \cdot 10^{\left[ \frac{7.5 \cdot T_{\max}}{(237.4 - T_{\max})} \right]} \quad (3)$$

$$e_{a_{\min}} = \frac{\text{RH}_{\min} \cdot e_{s_{\min}}}{100} \quad (4)$$

$$e_{a_{\max}} = \frac{\text{RH}_{\max} \cdot e_{a_{\max}}}{100} \quad (5)$$

where  $T_{\min}$  is the minimum temperature ( $^{\circ}\text{C}$ ),  $T_{\max}$  is the maximum temperature ( $^{\circ}\text{C}$ ), VPD is the vapor pressure deficit (kPa),  $e_{s_{\min}}$  is the saturation water vapor pressure using  $T_{\min}$  (kPa),  $e_{s_{\max}}$  is the saturation water vapor pressure using  $T_{\max}$  (kPa),  $e_{s_{\text{avg}}}$  is the average between the  $e_{s_{\min}}$  and  $e_{s_{\max}}$  (kPa),  $e_{a_{\min}}$  is the actual water vapor pressure using  $T_{\min}$  (kPa),  $e_{a_{\max}}$  is the actual water vapor pressure using  $T_{\max}$  (kPa),  $e_{a_{\text{avg}}}$  is the average between  $e_{a_{\min}}$  and  $e_{a_{\max}}$  (kPa),  $\text{RH}_{\min}$  is the minimum and  $\text{RH}_{\max}$  is the maximum relative humidity (%).

### Experimental design

Two greenhouse experiments (E1 and E2) were carried out in Itajubá, Minas Gerais, southeastern Brazil (SEB) (22.50° S, 45.45° W and altitude of 850 m) following similar protocols as in Sinclair and Ludlow (1986) and Abreu et al. (2015). Itajubá has a typical monsoon climate, with a well-defined dry and cold season in the austral winter (June to August), and rainy and hot in the austral summer (December to February) (Reis et al. 2021). E1 was carried out in the spring and summer (from October to March) of 2012–2013 and E2 in the fall and winter (from April to November) of 2013. Both experiments were installed in a completely random design in a 2:2 factorial scheme with two *Eucalyptus* species, *E. urophylla* and *E. cloeziana*, two soil–water treatments (well-watered, WW, and progressive water deficit,

WD), and nine replicates per treatment. The experimental units were pots containing two seedlings.

To establish the experimental units, highly pure seeds ( $\approx 92\%$ ) were obtained from the Society for Forestry Investigations, Minas Gerais. Approximately twenty seeds of both species were sown in each of  $36 \times 25 \text{ cm} \times 22 \text{ cm} \times 20 \text{ cm}$  polypropylene pots filled with 8 kg of a Rhodic Hapludox Oxisol. The soil chemical characteristics included  $2.45 \text{ dag kg}^{-1}$  of organic matter (Walkley–Black),  $0.5 \text{ mg dm}^{-3}$  of potassium and  $4.0 \text{ mg dm}^{-3}$  of phosphorous, obtained by Mehlich extractor 1. Approximately 90 days before sowing, fertility and acidity were corrected according to Barros and Novais (1999) by applying 10.18 g of simple superphosphate (18%), 0.31 g of potassium chloride (60%), 0.20 g of ammonium sulfate (20%) and 12.32 g of calcium carbonate in each pot. After emergence, the seedlings in each pot were periodically removed until only two remained.

### Water regimes and transpiration responses to progressive soil water deficit

Plants were grown under WW conditions until the main stem had formed 25 leaves, which is considered the most proper development plateau for *Eucalyptus* seedlings (Martins et al. 2008). WW conditions were guaranteed by daily watering to 100% of pot weight capacity, never allowing the water content to fall below 50% of the pot weight capacity, following the procedures of Sinclair and Ludlow (1986), Lago et al. (2011), Abreu et al. (2015) and King and Purcell (2017). When all seedlings had produced 25 leaves on the main stem, WD was developed, which occurred on 01/27/2013 and 03/05/2013 in E1, and on 08/14/2013 and 10/28/2013 in E2. The first date of each experiment refers to *E. urophylla* and the second to *E. cloeziana*.

In order to initiate soil–water treatments, all pots were saturated at 04:30 pm, allowed to drain between 24 and 30 h until they reached a constant weight. The pots were then covered with opaque white low-density plastic wrap to avoid water losses by evaporation, so that only water lost by TR was quantified (Sinclair and Ludlow 1986; Bindi et al. 2005; Guha et al. 2018).

All pots were weighed to determine their initial weight, considered as day 1 of E1 and E2 (Sinclair and Ludlow 1986; Guha et al. 2018). For each species, nine pots were assigned to the WW treatment and nine pots to the WD treatment. The pots were weighed daily at 04:30 pm from day 1 to the last day of the experiment which occurred when WD pots reached 10% TR or lower when compared to WW pots (Sinclair and Ludlow 1986; Esmaeilzade-Moridani et al. 2015).

The difference in pot weight for each weighing interval was assumed to be due to water loss through TR ( $\text{g pot}^{-1}$ ) (King and Purcell 2017). The WW pots were watered by

adding as much water as the difference between the weight measured on each day and the initial weight (day 1). The WD pots were not watered until the last day of the experiment because, according to Devi et al. (2009), as soil drought occurs slowly, water addition in the WD pots was not necessary. The final weight (weight measured on the last day of the experiment) was obtained for each pot when the TR of the WD pots was less than 10% when compared to WW pots.

To minimize the influence of day-to-day variations in environmental conditions, daily TR values were normalized as described by King and Purcell (2017), Fuentealba et al. (2016) and Cathey et al. (2013), resulting in normalized transpiration (NTR) values ranging approximately from 0 to 1 (Eq. 6). For the normalization procedure, a correction factor (cf) was calculated to adjust for differences in TR among plants within a species under WW treatment (Eq. 7). The cf was calculated while all plants were well-watered by dividing the average water loss for individual plants by the average water loss of the WW treatment ( $\text{WW}_{\text{avg}}$ ) of the same species before starting WD. For each weighing interval from day 1 to the last day of the experiment ( $\text{TR} \leq 10\%$ ), water loss for individual pots was divided by average loss for WW pots of the same species. This ratio ( $\text{g pot}^{-1}/\text{average g pot}^{-1}$ ) was divided by the cf to give NTR for each pot for a given weighing interval (King and Purcell 2017):

$$\text{NTR} = \frac{\text{g} \cdot \text{pot}^{-1} / \text{WW}_{\text{avg}} \text{g} \cdot \text{pot}^{-1}}{\text{cf} \cdot \text{plant}^{-1}} \quad (6)$$

$$\text{cf} = \frac{\text{g} \cdot \text{pot}^{-1}}{\text{WW}_{\text{avg}} \text{g} \cdot \text{pot}^{-1}} \quad (7)$$

Total transpirable soil water (TTSW,  $\text{g pot}^{-1}$ ) for an individual pot in the WD treatment was defined as the difference between pot-capacity weight and pot weight at the end of the experiment. The FTSW of each pot was calculated according to Sinclair and Ludlow (1986):

$$\text{FTSW} = \frac{\text{AWS}}{\text{TTSW}} \quad (8)$$

where: AWS is the difference between individual pot weight on each day and pot weight at the end of the experiment ( $\text{g pot}^{-1}$ ).

Values of NTR and FTSW obtained during the drying experiment for each pot on each day were combined to determine the FTSWt value and NTR response. The logistic function (Eq. 8) was fitted, relating NTR to FTSW, for each species in each experiment (E1 and E2). To assess the influence of VPD on NTR values, this procedure was carried out considering three cases: (a) NTR values on all days of the experiment, (b) NTR values on days with low vapor pressure deficits (VPD1), and (c) NTR values on days with



high vapor pressure deficits (VPDh). The threshold between VPDh and VPDl was considered as 1.5 kPa according to Ray et al. (2002), Lago et al. (2011), and Abreu et al. (2015). The FTSWt at which the normalized transpiration began to decline in response to soil drying was obtained for the three cases:

$$\text{NTR} = \frac{a}{1 + \exp\{-(\text{FTSW} - c)/b\}} \quad (9)$$

where ‘a’, ‘b’, and ‘c’ are the coefficients of the logistic function estimated by the Gauss–Newton algorithm. Coefficient ‘a’ represents the maximum expected FTSW value (considered = 1), ‘b’ is related to the rate of curve decrease and ‘c’ is the intercept.

The fit was evaluated with the following statistics: model efficiency (ME, Eq. 10) and root mean square error (RMSE, Eq. 11):

$$\text{ME} = 1 - \frac{\sum_{i=1}^n (\widehat{\text{NTR}} - \text{NTR})}{\sum_{i=1}^n (\text{NTR} - \overline{\text{NTR}})} \quad (10)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (\widehat{\text{NTR}} - \text{NTR})^2}{n}} \quad (11)$$

### *Eucalyptus* responses to progressive soil water deficit

To verify plant responses to progressive water deficits under the WW and WD treatments, physiological and growth variables were evaluated. The physiological variables were: initial, final, and total transpiration (TRi, TRf and TRt, g plant<sup>-1</sup>), total and partitioned dry weight (root, stem, and leaf, g), dry weight allocation, daily water consumption (WC, g plant<sup>-1</sup> day<sup>-1</sup>), and water requirement (WR, g H<sub>2</sub>O g<sup>-1</sup> dry weight). The WR index was used as it indicates water use efficiency in plants under drought conditions (Sinclair 2012; Fuentealba et al. 2016; Souza et al. 2018b).

Because the dry weight was measured by destructive sampling, three extra pots were included in both experiments (E1 and E2). The dry weight was obtained on the first day of the experiment from these extra pots, and at the end of the experiment from all pots under the WW and WD treatments. Roots, stems and leaf samples were dried to constant weight at 65 °C. The dry weight allocation was obtained by the ratio between root (i), stem (ii), and leaf (iii) dry weights, and the total dry weight. The WR represents the amount of water (in L or g) used to produce a unit of dry mass (kg or g), obtained by the ratio between TRt and the dry weight increment (final dry mass minus initial dry mass obtained on day 1). The WR was calculated for WW and WD.

The growth variables measured were: number of leaves (NL), height (H, cm), root collar diameter (D, mm) and leaf area (LA, cm<sup>2</sup>). Height was measured with a ruler and diameter with a digital caliper. Leaf area was obtained by the product of leaf width (cm), length (cm) and the form factor (KA). The KA of *E. urophylla* was 0.68 and of *E. cloeziana* was 0.67 (Abreu 2014).

All physiological (TRi, TRf, TRt, WC and WR), variables except for dry weight allocation and growth variables (NL, H, D, and LA) were analyzed using a two-way analyses of variance (ANOVA) to compare the mean differences between the two species and two soil–water treatments (WW and WD) at 5% probability. Dry weight allocation was analyzed using a three-way ANOVA to evaluate the effect of two species, two soil–water treatments and three dry weight allocation (root, stem and leaf) at 5% probability. When a significant difference was detected in the ANOVA, the treatment means were compared with Tukey’s test at 5% probability. In the Tukey’s test, the following hypotheses were tested: H<sub>0</sub> = mean values are equal ( $P \geq 0.05$ ) versus H<sub>1</sub> = mean values are unequal ( $P < 0.05$ ). Before performing the ANOVA, the assumption of normality of the data was checked by the Shapiro–Wilk test at 5% probability.

In addition, the growth variables (NL, H, D and LA) were normalized by the same procedure as was performed for NTR and related, one at a time, to FTSW with a logistic function (Eq. 8) for both species and experiments (E1 and E2). The previously described a, b, and c cases were considered individually. The fit was also assessed with the statistics RMSE and ME.

## Results

### Growth and physiological variables

*E. urophylla* and *E. cloeziana* were maintained WW through 100 and 137 days in E1, and 107 and 182 days in E2, respectively. The WD treatment was then applied, and each species responded slightly differently in relation to progressive soil water deficit, resulting in different durations between experiments. The WD for *E. urophylla* lasted 15 and 21 days, while for *E. cloeziana* it was 12 and 17 days, in E1 and E2 respectively.

Tables 1 and 2 show, respectively, the responses of the growth and dry weight variables and physiological traits for the WW and WD treatments on day 1 and at the end of the experiments, after the progressive soil water deficits. At the time the soil–water treatments were applied in both experiments, the plants had similar growth, dry weights (Table 1) and TRi (Table 2). This status is desirable as it reduces the differences or errors attributed to WW and WD treatments (Abreu et al. 2015). The effects of soil–water

**Table 1** Growth and dry weight variables measured on the first and on the last day of experiments for seedlings subjected to soil–water treatments (well-watered—WW and progressive soil water deficit—WD)

Species	E1 (Spring/Summer)			E2-(Fall/Winter)		
	Initial values	Soil–Water treatments		Initial values	Soil–Water treatments	
		WW	WD		WW	WD
<i>Initial leaf area (cm<sup>2</sup>)</i>						
<i>E. urophylla</i>	506.4 <sup>aA</sup>	426.7 <sup>aA</sup>	458.6 <sup>a</sup>	154.4 <sup>aA</sup>	171.8 <sup>ba</sup>	164.8 <sup>a</sup>
<i>E. cloeziana</i>	465.5 <sup>aA</sup>	351.9 <sup>aA</sup>	397.3 <sup>a</sup>	182.5 <sup>aA</sup>	249.1 <sup>aA</sup>	225.3 <sup>b</sup>
Mean	485.9 <sup>A</sup>	389.3 <sup>A</sup>		167.2 <sup>A</sup>	210.4 <sup>A</sup>	
<i>Final leaf area (cm<sup>2</sup>)</i>						
<i>E. urophylla</i>	530.6 <sup>aA</sup>	296.9 <sup>aB</sup>	390.3 <sup>a</sup>	195.4 <sup>aA</sup>	74.6 <sup>aB</sup>	122.9 <sup>a</sup>
<i>E. cloeziana</i>	475.0 <sup>aA</sup>	251.6 <sup>aB</sup>	341.4 <sup>a</sup>	219.0 <sup>aA</sup>	121.6 <sup>aB</sup>	156.4 <sup>a</sup>
Mean	503.1 <sup>A</sup>	274.3 <sup>B</sup>		206.1 <sup>A</sup>	98.1 <sup>B</sup>	
<i>Initial height (cm)</i>						
<i>E. urophylla</i>	39.6 <sup>aA</sup>	34.2 <sup>aA</sup>	36.4 <sup>a</sup>	21.3 <sup>aA</sup>	24.3 <sup>aA</sup>	23.1 <sup>a</sup>
<i>E. cloeziana</i>	36.7 <sup>aA</sup>	32.8 <sup>aA</sup>	34.4 <sup>a</sup>	22.9 <sup>aA</sup>	19.9 <sup>aA</sup>	21.0 <sup>a</sup>
Mean	38.3 <sup>A</sup>	33.5 <sup>A</sup>		22.1 <sup>A</sup>	22.1 <sup>A</sup>	
<i>Final height (cm)</i>						
<i>E. urophylla</i>	40.6 <sup>aA</sup>	32.0 <sup>aB</sup>	35.4 <sup>a</sup>	25.0 <sup>aA</sup>	22.3 <sup>aA</sup>	23.4 <sup>a</sup>
<i>E. cloeziana</i>	37.2 <sup>aA</sup>	30.7 <sup>aA</sup>	33.3 <sup>a</sup>	23.4 <sup>aA</sup>	17.7 <sup>ba</sup>	19.8 <sup>b</sup>
Mean	38.9 <sup>A</sup>	31.3 <sup>B</sup>		24.4 <sup>A</sup>	20.0 <sup>B</sup>	
<i>Initial diameter (mm)</i>						
<i>E. urophylla</i>	5.1 <sup>aA</sup>	5.2 <sup>aA</sup>	5.2 <sup>a</sup>	2.8 <sup>aA</sup>	2.9 <sup>aA</sup>	2.9 <sup>b</sup>
<i>E. cloeziana</i>	4.5 <sup>aA</sup>	4.1 <sup>ba</sup>	4.3 <sup>b</sup>	3.6 <sup>aA</sup>	3.4 <sup>aA</sup>	3.5 <sup>a</sup>
Mean	4.8 <sup>A</sup>	4.7 <sup>A</sup>		3.2 <sup>A</sup>	3.2 <sup>A</sup>	
<i>Final diameter (mm)</i>						
<i>E. urophylla</i>	5.8 <sup>aA</sup>	4.3 <sup>aB</sup>	4.9 <sup>a</sup>	3.3 <sup>aA</sup>	2.3 <sup>aB</sup>	2.7 <sup>a</sup>
<i>E. cloeziana</i>	4.7 <sup>aA</sup>	3.8 <sup>aB</sup>	4.1 <sup>b</sup>	3.8 <sup>aA</sup>	2.5 <sup>aB</sup>	2.9 <sup>a</sup>
Mean	5.2 <sup>A</sup>	4.0 <sup>B</sup>		3.5 <sup>A</sup>	2.4 <sup>B</sup>	
<i>Initial number of leaves</i>						
<i>E. urophylla</i>	20.3 <sup>aA</sup>	23.1 <sup>aA</sup>	22.0 <sup>a</sup>	15.4 <sup>aA</sup>	15.3 <sup>aA</sup>	15.4 <sup>a</sup>
<i>E. cloeziana</i>	18.7 <sup>aA</sup>	16.1 <sup>ba</sup>	17.1 <sup>b</sup>	11.4 <sup>ba</sup>	11.1 <sup>ba</sup>	11.2 <sup>b</sup>
Mean	19.5 <sup>A</sup>	19.6 <sup>A</sup>		13.6 <sup>A</sup>	13.2 <sup>A</sup>	
<i>Final number of leaves</i>						
<i>E. urophylla</i>	20.2 <sup>aA</sup>	18.9 <sup>aA</sup>	19.4 <sup>a</sup>	10.8 <sup>ba</sup>	7.9 <sup>ba</sup>	13.1 <sup>a</sup>
<i>E. cloeziana</i>	18.3 <sup>aA</sup>	13.3 <sup>ba</sup>	15.3 <sup>b</sup>	15.6 <sup>aA</sup>	11.4 <sup>aB</sup>	8.9 <sup>b</sup>
Mean	19.3 <sup>A</sup>	16.1 <sup>B</sup>		13.4 <sup>A</sup>	9.7 <sup>B</sup>	
<i>Root dry weight (g per pot)</i>						
<i>E. urophylla</i>	0.9 <sup>ba</sup>	1.6 <sup>aA</sup>	1.3 <sup>a</sup>	1.5 <sup>aA</sup>	1.2 <sup>aAB</sup>	0.8 <sup>a</sup>
<i>E. cloeziana</i>	1.9 <sup>aA</sup>	0.8 <sup>bb</sup>	0.9 <sup>b</sup>	0.9 <sup>aA</sup>	1.1 <sup>aA</sup>	0.9 <sup>a</sup>
Mean	1.4 <sup>A</sup>	1.2 <sup>AB</sup>	0.9 <sup>B</sup>	1.2 <sup>A</sup>	1.1 <sup>A</sup>	0.9 <sup>A</sup>
<i>Stem dry weight (g per pot)</i>						
<i>E. urophylla</i>	0.9 <sup>aB</sup>	1.9 <sup>aA</sup>	1.4 <sup>a</sup>	0.4 <sup>aA</sup>	0.8 <sup>ba</sup>	0.7 <sup>b</sup>
<i>E. cloeziana</i>	1.6 <sup>aA</sup>	1.4 <sup>aA</sup>	1.3 <sup>a</sup>	0.6 <sup>aB</sup>	1.3 <sup>aA</sup>	1.0 <sup>a</sup>
Mean	1.3 <sup>A</sup>	1.6 <sup>A</sup>	1.3 <sup>A</sup>	0.5 <sup>B</sup>	1.2 <sup>A</sup>	0.7 <sup>AB</sup>
<i>Leaf dry weight (g per pot)</i>						
<i>E. urophylla</i>	2.1 <sup>aA</sup>	2.8 <sup>aA</sup>	2.7 <sup>a</sup>	1.2 <sup>aA</sup>	1.9 <sup>aA</sup>	1.5 <sup>a</sup>
<i>E. cloeziana</i>	3.7 <sup>aA</sup>	3.3 <sup>aA</sup>	2.9 <sup>a</sup>	1.8 <sup>aAB</sup>	2.8 <sup>aA</sup>	1.5 <sup>a</sup>
Mean	2.9 <sup>A</sup>	3.1 <sup>A</sup>	2.7 <sup>A</sup>	1.5 <sup>AB</sup>	2.4 <sup>A</sup>	1.0 <sup>B</sup>
<i>Total dry weight (g per pot)</i>						
<i>E. urophylla</i>	4.0 <sup>ba</sup>	6.2 <sup>aA</sup>	5.4 <sup>a</sup>	2.5 <sup>aA</sup>	3.6 <sup>aA</sup>	2.9 <sup>a</sup>
<i>E. cloeziana</i>	7.2 <sup>aA</sup>	5.5 <sup>aB</sup>	5.1 <sup>a</sup>	3.9 <sup>aAB</sup>	5.3 <sup>aA</sup>	3.4 <sup>a</sup>

**Table 1** (continued)

Species	E1 (Spring/Summer)			E2-(Fall/Winter)		
	Initial values	Soil–Water treat-ments		Initial values	Soil–Water treat-ments	
		WW	WD		WW	WD
Mean	5.6 <sup>A</sup>	5.9 <sup>A</sup>	4.8 <sup>A</sup>	3.2 <sup>A</sup> B	4.4 <sup>A</sup>	2.3 <sup>B</sup>

Means followed by the same lowercase letter in the column (species) and capital letter in the line (soil–water treatment) for each variable do not differ by the Tukey’s test. Numbers and letters in gray indicate no interaction between soil–water treatments and species. Initial (final) values refer to the first (last) day of the experiment. The normality assumption is not violated by Shapiro–Wilk test at 5% probability. The initial values were measured on 01/27/2013 and 03/05/2013 in E1, and on 08/14/2013 and 10/28/2013 in E2, respectively for *E. urophylla* and *E. cloeziana*. The final values were measured on 02/11/2013 and 03/16/2013 in E1, and on 09/04/2013 and 11/13/2013 in E2, respectively for *E. urophylla* and *E. cloeziana*. The letters (A or B, a or b) were set up in descending order

**Table 2** Initial, final and total transpiration and daily water consumption of *Eucalyptus urophylla* and *Eucalyptus cloeziana* in two soil–water treatments (well- watered–WW and progressive soil water deficit–WD) and two greenhouse experiments

Species	E1-Spring/Summer			E2-Fall/Winter		
	Soil–water treatments		Mean	Soil–water treatments		Mean
	WW	WD		WW	WD	
<i>Initial transpiration (TRi, g plant<sup>-1</sup>)</i>						
<i>E. urophylla</i>	52.1 <sup>aA</sup>	50.8 <sup>aA</sup>	51.3 <sup>a</sup>	38.5 <sup>ba</sup>	47.8 <sup>aA</sup>	44.5 <sup>b</sup>
<i>E. cloeziana</i>	46.3 <sup>aA</sup>	46.4 <sup>aA</sup>	46.3 <sup>a</sup>	70.0 <sup>aA</sup>	61.1 <sup>aA</sup>	64.7 <sup>a</sup>
Mean	49.2 <sup>A</sup>	48.6 <sup>A</sup>		56.0 <sup>A</sup>	54.1 <sup>A</sup>	
<i>Final transpiration (TRf, g plant<sup>-1</sup>)</i>						
<i>E. urophylla</i>	150.4 <sup>aA</sup>	8.3 <sup>aB</sup>	65.7 <sup>a</sup>	147.1 <sup>aA</sup>	11.7 <sup>aB</sup>	65.8 <sup>a</sup>
<i>E. cloeziana</i>	151.7 <sup>A</sup>	11.1 <sup>aB</sup>	67.3 <sup>a</sup>	106.0 <sup>ba</sup>	11.1 <sup>aB</sup>	45.0 <sup>a</sup>
Mean	151.0 <sup>A</sup>	9.7 <sup>B</sup>		128.4 <sup>A</sup>	11.4 <sup>B</sup>	
<i>Total transpiration water (TRt, g plant<sup>-1</sup>)</i>						
<i>E. urophylla</i>	1121.1 <sup>ba</sup>	527.2 <sup>bb</sup>	764.8 <sup>b</sup>	2215.1 <sup>aA</sup>	519.7 <sup>aB</sup>	1265.2 <sup>a</sup>
<i>E. cloeziana</i>	2646.7 <sup>aA</sup>	1059.4 <sup>aB</sup>	1694.3 <sup>a</sup>	907.0 <sup>ba</sup>	631.9 <sup>aB</sup>	658.0 <sup>b</sup>
Mean	1883.9 <sup>A</sup>	793.3 <sup>B</sup>		1620.5 <sup>A</sup>	575.8 <sup>B</sup>	
<i>Daily water consumption (WC, g plant<sup>-1</sup>day<sup>-1</sup>)</i>						
<i>E. urophylla</i>	74.7 <sup>aB</sup>	35.2 <sup>aB</sup>	51.0 <sup>b</sup>	105.5 <sup>aA</sup>	30.1 <sup>aB</sup>	60.3 <sup>a</sup>
<i>E. cloeziana</i>	110.3 <sup>aA</sup>	48.2 <sup>aB</sup>	73.0 <sup>a</sup>	53.4 <sup>ba</sup>	30.6 <sup>aB</sup>	38.7 <sup>b</sup>
Mean	92.5 <sup>A</sup>	41.7 <sup>B</sup>		81.8 <sup>A</sup>	30.3 <sup>B</sup>	
<i>Water requirement (WR, g H<sub>2</sub>O g<sup>-1</sup> dry weight)</i>						
<i>E. urophylla</i>	406.5 <sup>ba</sup>	679.4 <sup>aA</sup>	565.7 <sup>a</sup>	985.7 <sup>aA</sup>	1090.5 <sup>aA</sup>	1052.4 <sup>a</sup>
<i>E. cloeziana</i>	1567.0 <sup>aA</sup>	678.7 <sup>aB</sup>	1034.0 <sup>b</sup>	711.2 <sup>aA</sup>	1000.1 <sup>aA</sup>	866.7 <sup>a</sup>
Mean	1039.5 <sup>A</sup>	679.0 <sup>A</sup>		821.0 <sup>A</sup>	1045.3 <sup>A</sup>	

Means followed by the same lowercase letter in the column (species) and capital letter in line (soil–water treatment), for each variable, do not differ among themselves by Tukey test (5% probability). Numbers and letters in gray indicate no interaction between the two factors (soil–water treatment and species). Initial and final values refer to the first and to the last day of the experiment. No violation of the assumption of normality was detected by the Shapiro–Wilk test at 5% probability. The initial values were measured on 01/27/2013 and 03/05/2013 in E1, and on 08/14/2013 and 10/28/2013 in E2, respectively for *E. urophylla* and *E. cloeziana*. The final values were measured on 02/11/2013 and 03/16/2013 in E1, and on 09/04/2013 and 11/13/2013, E2, respectively for *E. urophylla* and *E. cloeziana*. The letters (A or B, a or b) were set up in descending order

treatments are shown by the final values for leaf area and number, diameters, and heights (Table 1) and TRf, TRt and WC (Table 2). For all these variables, there were significant differences between the WW and WD treatments in

both experiments, with lower values in the WD treatment. Leaf area had the greatest reduction under the WD treatment (~50% for both species and experiments), followed by, in descending order: root (~40%) and leaf (~33%) dry

weight, diameters (~28%), stem dry weight (~25%), number of leaves (~22%) and height (~19%). For the TRf, TRt and WC variables, the reduction was greater than for the growth variables, and ranged from ~55% (TRt and WC) to ~92% (TRf), with slight differences between species and experiments as shown in Table 2.

A considerable range in TRt and WC was measured between soil–water treatments (Table 2). In WW, the total water transpired throughout the experiments ranged from 1121 to 2215 g plant<sup>-1</sup> (*E. urophylla*) and 907 to 2646 g plant<sup>-1</sup> (*E. cloeziana*). In the WD treatment, TRt water ranged from 519 to 1059 g plant<sup>-1</sup>. Thus, in WW treatment, the WC ranged from 105 to 53 g plant<sup>-1</sup> d<sup>-1</sup>, while in WD the WC values were around 30 g plant<sup>-1</sup> d<sup>-1</sup>.

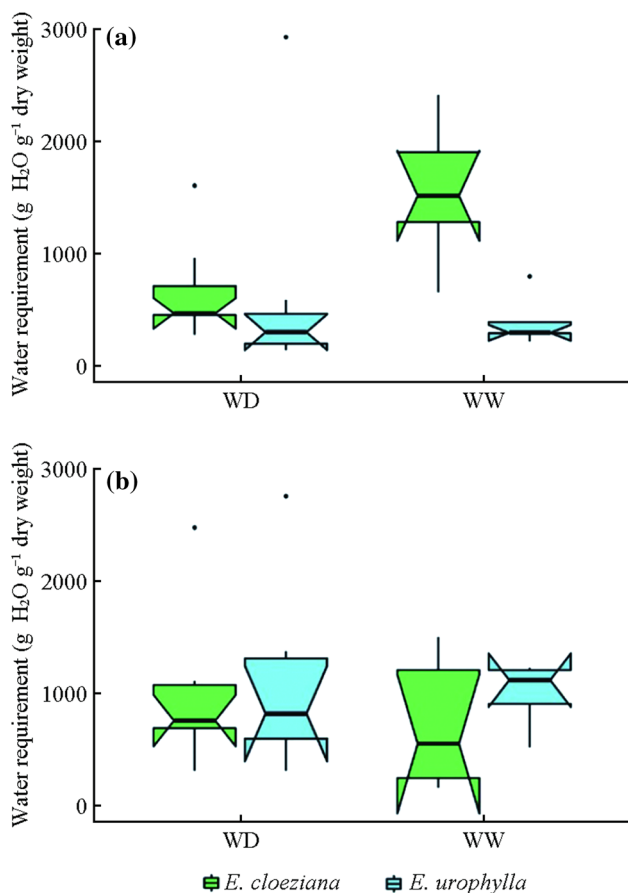
Despite the large variability in water requirements between both species in WW, especially for *E. cloeziana* (Fig. 1), which ranged from 143.98 to 2941.88 g H<sub>2</sub>O g<sup>-1</sup> dry weight, the soil–water treatments did not have a large impact on WR for either species and, except for *E. cloeziana*

in E1, differences in water requirements were not significant (Table 2).

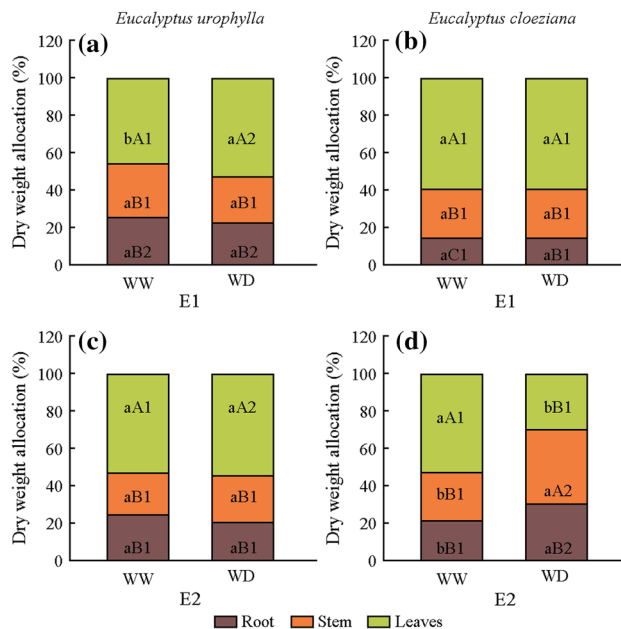
With regards to dry weight allocation (Fig. 2), there was a significant interaction between species, soil–water treatments and dry weight allocation ( $P < 0.05$ ). In general, *E. urophylla* and *E. cloeziana* showed similar dry weight allocation in both experiments and soil–water treatments, prioritizing the dry weight to leaves (except for *E. cloeziana* in E2 and in the WD treatment), although the percentages differed slightly and higher for *E. cloeziana*. In E2 and WD treatment, *E. cloeziana* had greater dry weight allocation to stems compared to roots or leaves. Regarding both species, *E. cloeziana* had slightly higher dry weight allocation to roots and stems, while *E. urophylla* had higher allocation to leaves. Therefore, there was no difference in prioritizing dry weight allocation under water deficit conditions, i.e., it is not a conservation strategy under progressive soil water deficit.

**Transpiration and growth responses to fraction of transpirable soil water (FTSW)**

During the application of the WW and WD treatments, there were differences in meteorological conditions in both greenhouse experiments observed (Fig. 3). Although the



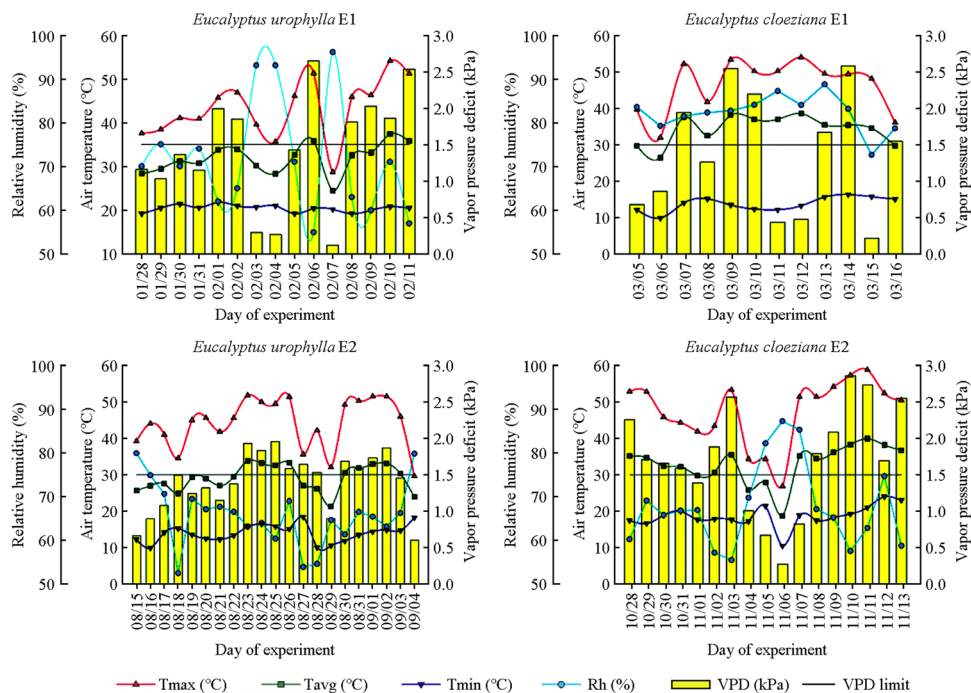
**Fig. 1** Box-plot considering water requirement of *Eucalyptus urophylla* and *Eucalyptus cloeziana* in two soil–water treatments (well-watered–WW and progressive soil water deficit –WD), for two experiment periods: E1 (panel a) and E2 (panel b)



**Fig. 2** Dry weight allocation among root, stem and leaf in soil–water treatments (well-watered –WW and progressive soil water deficit–WD) for *Eucalyptus urophylla* and *Eucalyptus cloeziana* in two experiments E1 and E2. Lowercase letters indicate statistically significant differences in soil–water treatments, capital letters indicate statistically significant differences in dry weight allocation, and different numbers indicate statistically significant differences between species; the same letters or numbers do not differ among themselves by Tukey test (5% probability)



**Fig. 3** Daily maximum (T<sub>max</sub>), minimum (T<sub>min</sub>), mean (T<sub>avg</sub>) air temperatures, relative humidity (RH) and vapor pressure deficit (VPD) during two greenhouse experiments (E1 and E2) of *Eucalyptus urophylla* and *Eucalyptus cloeziana* seedlings; VPD line is the threshold between VPD1 (< 1.5 kPa) and VPDh (≥ 1.5 kPa)



meteorological variation was small in terms of average values between E1 and E2, the absolute daily temperatures ranged from 9.9 °C (minimum) to 59 °C (maximum), absolute RH from 52 to 96%, VPD from 0.12 kPa to 19.54 kPa during both experiments.

The *E. urophylla* and *E. cloeziana* species were exposed, respectively, to VPD1 (VPDh) conditions on 45% (55%) and 53% (47%) of the days in E1, while in E2, the VPD1 (VPDh) conditions occurred on 52% (48%) and 29% (71%) of the days. Therefore, the number of days the species tolerated WD conditions was different between species and experiments. In E1, *E. urophylla* tolerated water deficits for 15 days and *E. cloeziana* for 12 days, while in E2, *E. urophylla* tolerated water deficits for 21 days and *E. cloeziana* for 17 days.

The logistic function described closely the NTR and the growth responses (NNL, NLA, NH and ND) for FTSW for both species in both experiments, with higher ME values ranging from 0.77 to 0.95 for the NTR, and from 0.32 to 0.90 for the growth variables (Table 3), except in E2 for *E. cloeziana* (height and diameter). As FTSW decreased, the NTR also varied around the maximum, and after reaching FTSW<sub>t</sub>, the normalized transpiration started to decrease due to stomatal closure (Schoppach and Sadok 2012; Souza et al. 2014; Abreu et al. 2015; King and Purcell 2017).

In general, *E. urophylla* showed a large variation in FTSW<sub>t</sub> values from 0.41 (E1) to 0.89 (E2), while *E. cloeziana* showed a smaller variation with values from 0.85 (E1) to 0.99 (E2). *E. cloeziana* also had higher FTSW<sub>t</sub> values than *E. urophylla*. At first, this result might indicate greater

sensitivity of *E. cloeziana* to detect soil water deficit and, at the same time, faster stomatal closure to reduce transpiration, even in conditions of available soil water.

Variability was also observed in FTSW<sub>t</sub> between the species in E1 and E2, especially for normalized height (NH) from 0.42 to 0.85, and normalized leaf area (NLA) from 0.32 to 0.83 (Table 3). In addition, for *E. urophylla* in E1, the normalized number of leaves (NNL), NH, and normalized diameter (ND) responded to soil water deficit before any change in transpiration rate (FTSW<sub>t</sub> ≥ 0.75). At the same time, the strategy of *E. cloeziana* to adjust to soil water deficit was to first reduce TR (FTSW<sub>t</sub> from 0.85 to 0.99) and NNL (FTSW<sub>t</sub> from 0.85 to 0.97). However, regarding *E. cloeziana* in E1 and E2 and *E. urophylla* in E2, the FTSW<sub>t</sub> values for NNL, NH (except *E. urophylla* in E2), ND and NLA (except *E. cloeziana* in E2) were similar to the FTSW<sub>t</sub> values for the NTR.

When VPD effect on NTR was considered, slight differences in FTSW<sub>t</sub> values were observed between both species (Table 3 and Figs. 4 and 5) in the two experiment periods. In general, the FTSW<sub>t</sub> value was lower on high vapor pressure (VPDh) days, and higher on low vapor pressure (VPD1) days, although this difference was only ~0.03 in the FTSW<sub>t</sub>. For example, considering *E. urophylla* in E1, the FTSW<sub>t</sub> was 0.50 (VPD1), 0.32 (VPDh) and 0.41 for all days, and for *E. cloeziana* in E1, the FTSW<sub>t</sub> was 0.86 (VPD1), 0.80 (VPDh) and 0.85 for all days. The FTSW<sub>t</sub> values did not differ for *E. cloeziana* in E2, with a value of 0.99 for all three tested cases (a, b, and c; see Materials and methods). Despite this, NTR began to decrease earlier or at the same time at higher FTSW

**Table 3** Parameters of the logistic equation, threshold fraction of transpirable soil water, and model fit by the root mean square error (RMSE) and model efficiency (ME)

Normalized variables	Parameter	<i>Eucalyptus urophylla</i>						<i>Eucalyptus cloeziana</i>						
		Experiment 1			Experiment 2			Experiment 1			Experiment 2			
		Case a	Case b	Case c	Case a	Case b	Case c	Case a	Case b	Case c	Case a	Case b	Case c	
Transpiration	A	1	1	1	1	1	1	1	1	1	1	1	1	1
	B	0.085*	0.110*	0.061*	0.168*	0.162*	0.173*	0.155*	0.162*	0.147*	0.194*	0.182*	0.198*	
	C	0.158*	0.184*	0.140*	0.382*	0.387*	0.378*	0.380*	0.390*	0.370*	0.454*	0.573*	0.446*	
	FTSWt	0.41	0.50	0.32	0.88	0.86	0.88	0.85	0.86	0.80	>0.99	>0.99	>0.99	
	RMSE	0.131	0.149	0.106	0.066	0.083	0.039	0.143	0.162	0.125	0.143	0.162	0.125	
	ME	0.856	0.811	0.914	0.908	0.925	0.948	0.883	0.853	0.917	0.78	0.616	0.945	
	Number of leaves	A	1	1	1	1	1	1	1	1	1	1	1	1
B		0.161*	0.177*	0.152*	0.170*	0.296*	0.146*	0.119*	0.100*	0.122*	0.186*	0.179 <sup>ns</sup>	0.198*	
C		0.317*	0.313*	0.319*	0.324*	0.521*	0.240*	0.533*	0.584*	0.492*	0.436*	0.269 <sup>ns</sup>	0.479*	
FTSWt		0.78	0.82	0.76	0.84	0.99	0.67	0.88	0.88	0.86	0.97	0.85	0.99	
RMSE		0.291	0.271	0.306	0.208	0.131	0.204	0.33	0.316	0.346	0.386	0.323	0.414	
ME		0.621	0.601	0.632	0.663	0.357	0.555	0.644	0.684	0.607	0.441	0.099	0.446	
Leaf area		A	1	1	1	1	1	1	1	1	1	1	1	1
	B	0.065*	0.018*	0.064*	0.182*	0.271*	0.162*	0.172*	0.179*	0.161*	0.139*	0.344 <sup>ns</sup>	0.135*	
	C	0.124*	0.101*	0.126*	0.306*	0.401*	0.258*	0.261*	0.266*	0.255*	0.256*	-0.418 <sup>ns</sup>	0.270*	
	FTSWt	0.32	0.15	0.32	0.83	0.99	0.75	0.78	0.78	0.74	0.66	0.63	0.66	
	RMSE	0.131	0.149	0.142	0.224	0.217	0.21	0.211	0.244	0.18	0.28	0.142	0.325	
	ME	0.845	0.894	0.839	0.479	0.223	0.564	0.706	0.643	0.766	0.739	0.031	0.553	
	Height	A	1	1	1	1	1	1	1	1	1	1	NF	1
B		0.169*	0.201*	0.155*	0.100*	0.133*	0.068*	0.175*	0.169*	0.166*	0.197*	NF	0.195*	
C		0.280*	0.221*	0.306*	0.123*	0.139*	0.112*	0.346*	0.397*	0.307*	0.276*	NF	0.302*	
FTSWt		0.78	0.82	0.76	0.42	0.54	0.32	0.85	0.88	0.79	0.84	NF	0.88	
RMSE		0.234	0.255	0.216	0.228	0.27	0.163	0.185	0.196	0.173	0.39	NF	0.455	
ME		0.663	0.528	0.734	0.479	0.388	0.598	0.788	0.781	0.81	0.322	NF	0.376	
Diameter		A	1	1	1	1	1	1	1	1	1	NF	NF	1
	B	0.165*	0.181*	0.156*	0.194*	0.480 <sup>ns</sup>	0.184*	0.161*	0.139*	0.150*	NF	NF	0.077 <sup>ns</sup>	
	C	0.362*	0.362*	0.373*	0.394*	0.888*	0.292*	0.309*	0.404*	0.237*	NF	NF	0.181*	
	FTSWt	0.85	0.88	0.82	0.95	0.99	0.85	0.80	0.82	0.69	NF	NF	0.410	
	RMSE	0.182	0.152	0.2	0.242	0.214	0.198	0.28	0.303	0.245	NF	NF	0.763	
	ME	0.769	0.713	0.784	0.626	0.068	0.585	0.641	0.658	0.669	NF	NF	0.201	

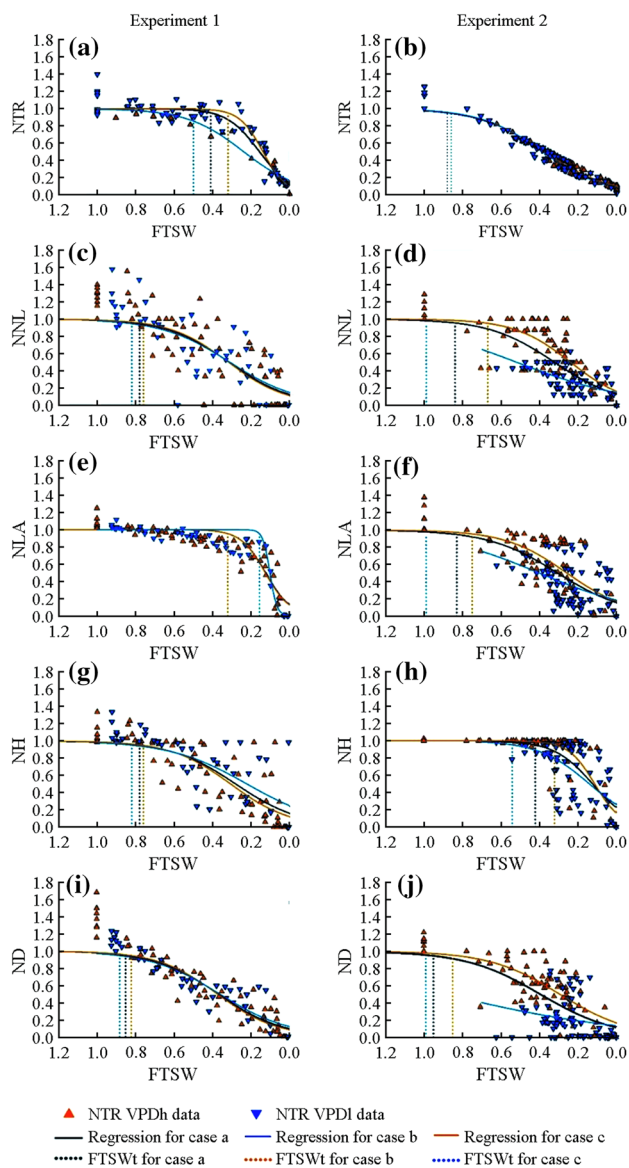
\*or <sup>ns</sup> = significant or non-significant, respectively, according to the test ( $H_0$ : parameter=0) at 5% probability; *NF*=no fit

values on VPDh days for *E. urophylla* and *E. cloeziana* in E2. In other words, the FTSWt from stage I to stage II, which indicates the point when TR is reduced, was anticipated under VPDh conditions.

With regards to the growth variables, both species had different responses when under VPDi and VPDh (Figs. 4 and 5). The NNL responded earlier in VPDh than VPDi days. The FTSWt values for NLA, NH and ND were similar in VPDh and VPDi days.

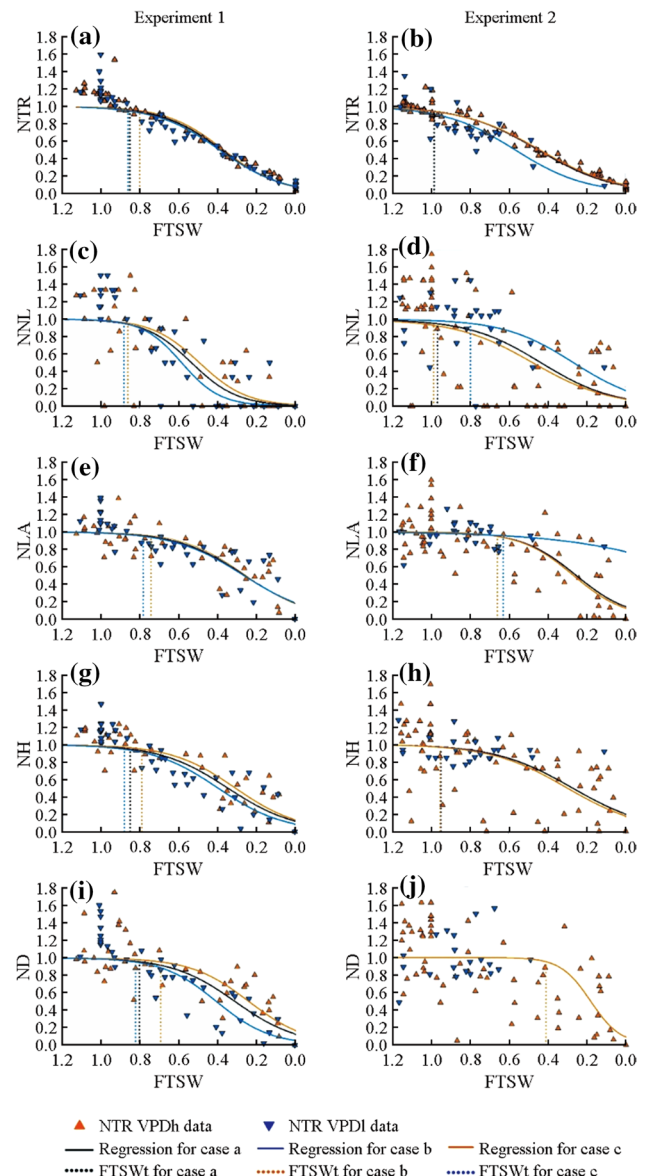
**Discussion**

This study evaluated the transpiration and growth responses of two *Eucalyptus* species to progressive soil water deficit and verified the influence of daily variations of VPD in these responses. The hypotheses were that physiological plant processes, such as transpiration, are influenced by progressive soil water deficits, that there are genotypic differences in the adaptation of species to drought stress, and that the VPD



**Fig. 4** Normalized transpiration and growth variables responses to fraction of transpirable soil water in *Eucalyptus urophylla* in two greenhouse experiment periods, E1-Spring/Summer and E2-Fall/Winter considering three cases: all days of the experiment (case a), days with low vapor pressure deficit ( $VPD < 1.5$  kPa) (case b), and days with high vapor pressure deficit ( $VPD \geq 1.5$  kPa) (case c). FTSW<sub>t</sub>=threshold values of fraction of transpirable soil water from stage I to II indicates the point when transpiration rate and growth variables are reduced; NTR=normalized transpiration; NNL=normalized number of leaves; NLA=normalized leaf area; NH=normalized height; ND=normalized diameter

influences these responses. The transpiration and growth responses (Table 3 and Figs. 4 and 5) showed differences in the FTSW<sub>t</sub> values and slopes from stage I to II of both species between the two greenhouse experiments (E1 and E2). The response of transpiration, number of leaves, leaf area, height and diameter to FTSW and the influence of VPD on



**Fig. 5** Normalized transpiration and growth variable responses to fraction of transpirable soil water in *Eucalyptus cloeziana* in two greenhouse experiment periods (E1-Spring/Summer and E2-Fall/Winter) considering three cases: all days of the experiment (case a), days with low vapor pressure deficit ( $VPD < 1.5$  kPa) (case b), and days with high vapor pressure deficit ( $VPD \geq 1.5$  kPa) (case c). FTSW<sub>t</sub>=threshold values of fraction of transpirable soil water from stage I to II indicates the instant when the transpiration rate, and growth variables are reduced; NTR=normalized transpiration; NNL=normalized number of leaves; NLA=normalized leaf area; NH=normalized height; ND=normalized diameter

these responses have been studied in several crop species, such as maize (Ray et al. 2002), peanut (Devi et al. 2010), potato (Lago et al. 2012; Souza et al. 2014), cassava (Lago et al. 2011; Pinheiro et al. 2014), cotton (Devi and Reddy 2018), rice (Heinemann et al. 2011), sorghum (Gholipour et al. 2012) and millet (Esmailzade-Moridani et al. 2015;

Medina et al. 2017). However, these responses had not been studied in *E. urophylla* and *E. cloeziana* species, and this study is the first for these two important commercial species.

The results of the two greenhouse experiments showed that both species reduced transpiration and growth rates under progressive soil water deficit. These results were expected due to reduced soil water availability resulting in lower growth and development, transpiration and dry weight (Souza et al. 2018b; Hubbard et al. 2020). In addition, reductions in heights and diameters are expected in plants under water deficit conditions as observed in this study (Table 1).

The progressive reduction of soil water causes plants to lose their cellular turgidity. With lower turgidity, there is less cell expansion, inhibiting growth and leaf expansion (Shao et al. 2008). When water deficits are prolonged, senescence and leaf abscission take place (Martins et al. 2008; Abreu et al. 2015), resulting in lower photosynthesis and less biomass production (Elli et al. 2020). Furthermore, it has been shown that plants under soil water deficits tend to first reduce their total transpiration and WC in order to maintain plant water content by conserving soil water content early (Sinclair et al. 2017).

There was a difference in normalized transpiration and growth between the two species. *E. cloeziana* showed higher FTSWt values and started to decrease before the transpiration, number of leaves, height and diameter, and leaf area, compared to *E. urophylla*. However, both species (except *E. urophylla* in E1) had higher FTSWt values ( $\geq 0.84$  to NTR) than other species such as *Vitis vinifera* L. (Bindi et al. 2005) and *Thuja plicata* Donnex D. Don (0.35), *Acer rubrum* L. (0.26), *Robina pseudoacacia* L. (0.37), *Hibiscus* sp. (0.29), *Ilex aquifolium* L. (0.33) (Sinclair et al. 2005) and *Jatropha curcas* L. (0.30 to 0.44) (Ouattara et al. 2018).

Although this study did not determine stomatal index, higher FTSWt values for NTR suggest that the hydraulic signals emitted by leaves cause early stomatal closure (McAdam and Brodribb 2014; Sinclair et al. 2017) to minimize water loss and maintain the plant water status (Souza et al. 2014). In other words, when hydraulic conductance becomes limiting to water transport, the plant is unable to meet transpiration demand, and this results in decreased stomatal conductance. Higher FTSWt values suggest that there is a combination of limited water flux due to soil and plant conductance (early reduction in NTR) to match hydraulic flux (Sinclair 2012; Abreu et al. 2015). Stomatal closure may be the main defense strategy of both *Eucalyptus* species to progressive (Marrou et al. 2015) and prolonged (Abreu et al. 2015) water deficits.

Since the FTSWt values for normalized transpiration were slightly higher than for the normalized number of leaves (0.64 to 0.89), normalized leaf area (0.30 to 0.86), normalized height (0.31 to 0.84) and normalized diameter (0.63 to 0.88) (except for NLA in E1 and NH in E2, both

for *E. urophylla*) for both species, it was possible to confirm that NTR responded to soil water deficit prior (or close) to the growth variables (NNL, NLA, NH and ND), mainly in VPDh. An early transpiration, number of leaves, leaf area, height, and diameter reduction at higher FTSWt values result in benefits and improvements in water requirement (Souza et al. 2018a), as they aim to maintain soil water (Schopach and Sadok 2012; Sinclair et al. 2017) and to match the hydraulic flux (Sinclair 2012). These results suggest that both *Eucalyptus* species have, almost at the same time, stomatal control mechanisms and other strategies such as early leaf senescence and early growth reduction to avoid progressive soil water deficits.

As water requirement is the tradeoff between water use and biomass production, drought tolerant species such as *E. urophylla* might be expected to use water more conservatively than other species (Martins et al. 2008; Abreu et al. 2015; Hubbard et al. 2020), i.e., with lower WR values. But, this was not the case for these experiments. The hypothesis that water use requirements would be lower for drought tolerant species such as *E. urophylla* was invalid, similar to the findings of Martins et al. (2008) and Hubbard et al. (2020). Water requirement values were similar between well-watered and water deficit treatments and *E. urophylla* and *E. cloeziana* species, demonstrating that both species have similar water use efficiencies under well-watered and water deficit conditions. In addition, water requirements for both species (406 to 1567 g H<sub>2</sub>O g<sup>-1</sup> dry weight) were superior to other eucalypt species such as *Eucalyptus grandis* (240 to 280 g H<sub>2</sub>O g<sup>-1</sup> dry weight), *Eucalyptus saligna* (220 to 280 g H<sub>2</sub>O g<sup>-1</sup> dry weight) (Martins et al. 2008), and *Corymbia citriodora* (100 to 420 g H<sub>2</sub>O g<sup>-1</sup> dry weight) (Abreu et al. 2015).

Our results suggest that *E. urophylla* and *E. cloeziana* have a conservative strategy in which plants react to a soil water deficit by: (1) reducing leaf, stem and diameter growth and/or increasing leaf senescence), (2) reducing transpiration rates, TRt, and WC, and (3) by closing stomata when FTSWt is still relatively high under high soil moisture. This is opposite to a productive strategy in which: (1) plants keep growing despite increasing soil water deficits, as observed for example, for *E. saligna* (Martins et al. 2008) and *Corymbia citriodora* (Abreu et al. 2015), and in which (2) plants reduce water requirements under water deficit conditions.

Genotypic variability in transpiration within forest species is quite wide: responses of FTSW thresholds range from 0.25 to 0.90 (Sinclair et al. 2005; Martins et al. 2008; Abreu et al. 2015), but in *Eucalyptus* species, FTSWt ranged from 0.70 to 0.90 (Martins et al. 2008; Abreu et al. 2015). The FTSWt values obtained in this study for NTR declined (from 0.40 to 0.99), similar to what was observed for *E. grandis* (0.90), *E. saligna* (0.70) (Martins et al. 2008), and *Corymbia citriodora* (from 0.32 to 0.85) (Abreu et al. 2015). This demonstrates that early decreases in transpiration during



the soil drying cycle promote soil water conservation. In *E. urophylla* and *E. cloeziana*, there was first stomatal closure, a first defense strategy under water deficit, followed by disruption of the emission of young leaves, leaf abscission and senescence (FTSWt 0.73 to 0.96), a second defense strategy. Growth (leaf area, height, and diameter) was then reduced (FTSWt 0.31 to 0.86), being the third defense strategy (Shao et al. 2008; Schoppach and Sadok 2012; Kelling et al. 2015).

In this study, the plants were grown in a greenhouse without controlling air temperature and relative humidity. Vapor pressure deficit values were based on daily natural fluctuations, similar that of Casadebaig et al. (2008), Lago et al. (2011, 2012), and Abreu et al. (2015). Despite the weak difference ( $\sim 0.03$ ), the FTSWt value for NTR was slightly higher in VPDl days ( $0.99 \geq \text{FTSWt} \geq 0.50$  for both species), and slightly lower in VPDh days ( $0.99 \geq \text{FTSWt} \geq 0.32$ ) (Table 3 and Figs. 4 and 5), corroborating Ray et al. (2002), Martins et al. (2008) and Lago et al. (2011).

The second hypothesis of this study, in which increased atmospheric vapor pressure deficits may alter the FTSWt to initiate the decline in transpiration and growth was not greatly influenced by VPD. As a rule, it was expected that higher FTSWt values should occur under VDPH conditions, hence with initiated fast regulation of stomata (Ray et al. 2002; Devi and Reddy 2018) and decline in transpiration rate, number of leaves, leaf area, height and diameter variables (Abreu et al. 2015) in wet and dry soils (Devi et al. 2010). In spite of this, an anticipated reduction in transpiration rate in both *Eucalyptus* species was observed when the evaporative demand was higher (VDPH). This anticipated reduction may occur to match water flux into the leaves (Sinclair et al. 2017) and to conserve soil water (Devi and Reddy 2018). This response is a possible approach for enhanced adaptation to water deficits by *Eucalyptus* species. However, it is not possible to confirm that VPD changes FTSWt values in both *Eucalyptus* species.

The responses of FTSW to VPD varied in magnitude and pattern of FTSWt values, which shows genotypic variations. For example, studies carried out by Devi et al. (2010) on peanut genotypes, Lago et al. (2011) for cassava cultivars, Gholipour et al. (2012) for sorghum genotypes, and Abreu et al. (2015) on *Corymbia citriodora*, found decreases in FTSWt with increased VPD. However, all these studies showed differences among the genotypes studied. In contrast, Zaman-Allah et al. (2011), Souza et al. (2014) and Devi and Reddy (2020), showed the opposite, i.e., an increase of FTSWt for TR decline under VDPH and a low FTSWt for TR decline under VPDl. Ray et al. (2002), Casadebaig et al. (2008), and Schoppach and Sadok (2012) showed that FTSWt should not be affected by vapor pressure deficit, a similar response to our results. Even so, higher vapor pressure deficit resulted in greater amounts of water transpired regardless of soil–water treatments. At the same

time, there was a consistent decrease in dry weight (Fig. 2), with increasing VPD by both *Eucalyptus* species in well-watered and water deficit treatments.

Although there is no well-defined pattern described in the literature between the FTSWt response under VPDl and VPDh conditions and among genotypes, lower FTSWt values (from 0.30 to 0.60) are suitable under weak to moderate water deficit (short to medium-term water deficit stress) conditions (Abreu et al. 2015). On the other hand, under a prolonged soil water deficit, high FTSWt values ( $\geq 0.70$ ) (Martins et al. 2008) are expected because a decline in TR at high soil moisture contributes to a conservative water use (Zaman-Allah et al. 2011; Sinclair et al. 2017) and may maintain soil water availability for a longer period, favoring plant survival (Fuentelba et al. 2016; Yan et al. 2017). With species such as *E. urophylla* and *E. cloeziana*, which are managed under rotation cycles of 6 to 8 years (Cook et al. 2016; Scolforo et al. 2019), the period in which they may be subjected to soil water deficits may be lengthy, especially in regions such as southeastern Brazil, which has a well-defined dry season (Santos et al. 2017; Martins et al. 2020).

In such regions, higher FTSWt values are desirable and contribute to water conservation in the soil. This conserved water would be useful to plants in the juvenile phase to maturity phase ( $> 3$  years in *Eucalyptus* species) and therefore, for yield improvement in the dry season. On a daily scale, high FTSWt values may be indicative of rapid stomatal regulation and early closure at critical periods to reduce transpiration, mainly under high vapor pressure deficits (Sinclair 2012; McAdam and Brodrribb 2014; Sinclair et al. 2017). Regulation of stomata to vapor pressure deficits is a process by which plants adjust their transpiration during daytime (Devi and Reddy 2018) and according to soil water content (Casadebaig et al. 2008; Sinclair et al. 2017).

In spite of several studies, the mechanisms of stomatal responses to vapor pressure deficits are poorly understood (McAdam and Brodrribb 2014) and highly variable among crops. Some studies have suggested limited transpiration rates due to hydraulic limitations (Sinclair 2012; Souza et al. 2014; Abreu et al. 2015; Sinclair et al. 2017), and the involvement of water channel proteins (Sinclair et al. 2017). Stomatal regulation of leaf water balance has been proposed to be controlled by active metabolic processes along with a passive hydraulic process (McAdam and Brodrribb 2014). Recent studies observed the expression of abscisic acid (ABA) synthesizing genes in guard cells and regulating stomatal responses to VPD (McAdam and Brodrribb 2016; Devi and Reddy 2018). According to Devi and Reddy (2018), these metabolic processes are prompted by low leaf water potential due to ABA (McAdam and Brodrribb 2016) and might result in limiting the transpiration rate. For this reason, both *Eucalyptus* species in this study had a reduction in stomatal conductance due to early stomatal closure at high



FTSW for both high and low VPD in order to maintain leaf potential to reduce leaf damage. Moreover, both species showed an early reduction in growth (Table 3 and Figs. 4 and 5).

In summary, both *Eucalyptus* species showed a conservative strategy to progressive soil water deficits. Water-conserving mechanisms during the seedling phase showed that three traits contributed to water savings under progressive soil drought: (1) higher FTSW thresholds for the decline in transpiration to avoid rapid soil water depletion, (2) higher FTSW thresholds for the decline in NL to reduce the contact area and to avoid the water and water vapor loss to the atmosphere, and (3) higher growth rates (LA, H, and D) when soil moisture was non-limiting and a restriction of growth under progressive soil water deficits (Tables 1 and 3).

## Conclusions

The results indicate a range in the response by transpiration and growth variables under progressive soil water deficits for *E. urophylla* and *E. cloeziana*. The threshold values of fraction of transpirable soil water ranged from 0.40 to 0.99 for transpiration rate, 0.78 to 0.97 for number of leaves, 0.32 to 0.83 for leaf growth, 0.42 to 0.85 for height growth, and 0.80 to 0.95 for diameter growth, with the highest values for *E. cloeziana*. The limitation in transpiration rate and growth contributes to soil water savings. There was little to no change in the fraction of transpirable soil water threshold detected in response to changes in atmospheric vapor pressure deficits. These results indicate that the general decline in transpiration rate may be assumed without major concern for the vapor pressure deficits. Nevertheless, future studies need to confirm the limited transpiration in the field under a wide range of vapor pressure deficits.

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