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Quantifying turgor loss point and leaf water potential across contrasting *Eucalyptus* clones and sites within the TECHS research platform

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ABSTRACT

Understanding the mechanisms governing drought tolerance in highly productive clonal *Eucalyptus* plantations in Brazil will become increasingly important with climate change driven increases in temperature and drought events. We quantified how leaf water potential, hydraulic safety margin (mid-day – pre-dawn leaf water potential, Ψδ) and the physiological parameters obtained from standard pressure–volume curves differed between four contrasting *Eucalyptus* genotypes across a temperature and water availability gradient in Brazil. We hypothesized that genotypes developed in dry areas were more drought tolerant and would exhibit lower mid-day leaf water potentials and turgor loss points than clones developed in wetter regions. Recognizing that standard pressure volume curves are time consuming and may not be a suitable screening tool for forest managers, we also tested if a key physiological parameter from the pressure–volume curve (turgor loss point, πtlp) could be accurately estimated from an osmometer as has been found in other species. We found no support for our first hypothesis; physiological parameters determined (including turgor loss point) from the pressure volume curves were not associated with the supposed drought tolerance of any of the clones. Similarly, mid-day leaf water potentials were not directly correlated with drought tolerance. The lack of support for our hypothesis may be because our measurements were taken during periods of minimum water stress. However, we did find that, overall, turgor loss point tended to be lower at the dry compared to wetter sites we studied suggesting that it may be a useful tool for assessing drought tolerance of *Eucalyptus* plantations in the future. We also found that estimates of osmotic potential at full turgor were similar between the pressure volume curve and osmometer techniques and that turgor loss point can be accurately estimated with an osmometer in highly productive *Eucalyptus* genotypes (R^2 = 0.79).

1. Introduction

Eucalyptus plantations occupy almost 6 million hectares in Brazil (IBA, 2019) and are grown across a broad range of environmental conditions where plantation productivity ranges from 25 to 60 $m^3\ ha^{-1}\ y^{-1}$ (Gonçalves et al., 2013). Advances in tree breeding and silvicultural practices have led to large gains in productivity over the past 25 years however soil water availability is a primary limiting factor to *Eucalyptus* growth in many areas (Ryan et al., 2010). Climate change is predicted to cause increased temperatures and rainfall reductions across much of Brazil and will likely exacerbate water limitation for many *Eucalyptus* plantations (Chou et al., 2014); possibly reducing the suitable area for plantation establishment. However, predicting how climate change will affect *Eucalyptus* plantation productivity and sustainability is difficult because relatively little research has been done on the physiological mechanisms that govern tree survival in *Eucalyptus* plantations and we do not fully understand how clonal *Eucalyptus* integrate the physiological characteristics of the parental species.

Trees have evolved numerous strategies to cope with and survive a lack of water supply or high evaporative demand. For example, some species "avoid" drought by shedding leaves and limiting transpirational water loss while others are able to "tolerate" drought via physiological adjustments or anatomical adaptations that allow maintenance of survival, growth and function during periods where water availability is limited. Because the ultimate goal of plantation forestry is wood production, understanding "drought tolerance" mechanisms is key to

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successful and sustainable plantation management especially in the face of climate change (Kozlowski & Pallardy, 2002).

Water loss occurs during photosynthesis because as plants open their stomata for CO_2 uptake, water is pulled through the soil–plant-atmosphere continuum where it evaporates into the atmosphere. The ability of plants to maintain the integrity of the water column from soil to leaf through the water conducting tissues of the plant during drought is a key determinant of drought tolerance and survival. Research describing the physiological parameters governing the vulnerability of water transport to drought has increased substantially (McDowell et al., 2019) as drought mortality events have become more prevalent across the globe (Choat et al., 2012) and these parameters offer promise in predicting vegetation response to drought. For example, Adams et al. (2013) showed that 26 tree species worldwide that were at or near mortality had 60% or higher loss of hydraulic conductivity and Choat et al. (2012) showed that a large percentage of forest species globally exhibit a narrow hydraulic safety margin to drought through analysis of the P_{50} index which quantifies a 50% loss of xylem conductivity. Bartlett et al. (2012b) also found that leaf water potential at wilting (turgor loss point, πtlp) was highly correlated with water availability within and across biomes and in a separate study (Bartlett et al., 2014) showed that πtlp is highly correlated with drought tolerance for many species.

In spite of the promise P_{50} and πtlp show for predicting the global response of forest vegetation to climate change induced drought, studies that examine the physiological variables that confer drought tolerance in highly productive *Eucalyptus* plantations are relatively few (Blackman et al., 2019). Because *Eucalytus* plantations in Brazil will likely experience warmer, drier climates in the future (Hoegh-Guldberg et al., 2018), we need ecophysiological predictors that can be modeled and are indicative of future performance and drought tolerance. However, quantifying P_{50} in *Eucalyptus* species can be difficult because this genus has long vessels making it difficult to construct reliable vulnerability curves (e.g. Torres-Ruiz et al., 2014). More broadly, robust relationships between physiology and drought tolerance are difficult to determine for *Eucalyptus* clones because the genetic makeup of clonal material changes as new hybrids are developed and planted. New genetic crosses may incorporate new traits with every new clone such that establishing long-term correlations between these traits and drought survival is problematic. Although xylem architecture is correlated with hydraulic safety and drought resistance (Gleason et al., 2016) recent work suggests that vascular traits within the genus *Eucalyptus* are largely genotypic in origin (Pfautsch et al., 2016). New hybrids that include genes from different species further complicates our ability to develop robust relationships between drought survival and anatomical/physiological characteristics.

Quantifying leaf water relations and related physiological traits may be useful tools for identifying or ranking drought tolerance for *Eucalyptus* clones. For example, multiple studies have demonstrated that turgor loss point is highly correlated with both water availability (Bartlett et al., 2012b) and drought tolerance (Marechaux et al., 2015) across plant types and Bourne et al. (2017) showed that πtlp was positively correlated with climate of origin for several *Eucalyptus* species in a common garden experiment. Additionally, Johnson et al. (2018) showed that species exhibiting a larger difference in pre-dawn and mid-day water potentials (Ψδ) are more plastic with respect to several leaf hydraulic parameters (including turgor loss point). Turgor loss point (πtlp), osmotic potential at saturation (π-osm), volumetric modulus of elasticity (ε) and relative water content at turgor loss point RWC_{tlp} are typically quantified by constructing pressure–volume curves but this technique is time consuming and may not be practical for rapid assessments of drought tolerance in newly developed clonal material. However, Bartlett et al. (2012a) showed the πtlp is mainly driven by differences in π-osm due to changing solute concentration in the symplast (osmotic adjustment) allowing πtlp to be estimated using an osmometer resulting in a 50-fold decrease in the time required to quantify πtlp (Griffin-Nolan et al., 2019).

In this study, we quantify differences in πtlp, RWC_{tlp}, predawn and mid-day water potentials and Ψδ for contrasting highly productive *Eucalyptus* genotypes across a range of water availability (within and across sites) within the TECHS research platform (Binkley et al., 2020). We examined four clones that were developed in areas with contrasting temperature and water availability regimes that were planted in each of three sites across a temperature and precipitation gradient within TECHS and compared these same clones within control and rainfall reduction treatments at each site. We hypothesized that clones developed from species derived from arid areas would have the lowest πtlp and RWC_{tlp}, favorable characteristics to withstand water deficit and the widest hydraulic safety margin compared to species originating in more humid environments. We also determined if turgor loss point could be accurately estimated using an osmometer for the clones and sites within our study.

2. Material and methods

2.1. Experimental area and treatments

We selected three tropical sites within the TECHS research platform (Binkley et al. 2017) that spanned a large precipitation gradient (700 – 1600 mm y^{-1}) located in Bocaiúva/MG (30), Mogi-Guaçu/SP (20), Telêmaco Borba/PR (22) (Fig. 1., Alvares et al., 2013). We measured four *Eucalyptus* clones with contrasting productivities and drought sensitivities that were planted at each of our three sites: A1 - *E. urophylla*; B2 - *E. urophylla* × *E. grandis*; C3 - *E. grandis* × *E. camaldulensis*; and P7 - *E. urophylla* × *E. brassiana* (Araujo et al., 2019).

All four of the clones we selected are commonly planted in commercial plantation in Brazil. The different species provides a range of distinct genotypic behavior. A1 and C3 are considered plastic clones (suitable for a wide range of environmental conditions), being A1 recommended for humid areas and C3 for dry areas; B2 and P7 are considered tropical clones (suitable for warmer areas) but B2 was developed for wet areas and P7 for dry and hot areas (Table 1).

At each site, the clones were planted in two water availability treatments: (1) 100% of the local precipitation and (2) 30% rainfall

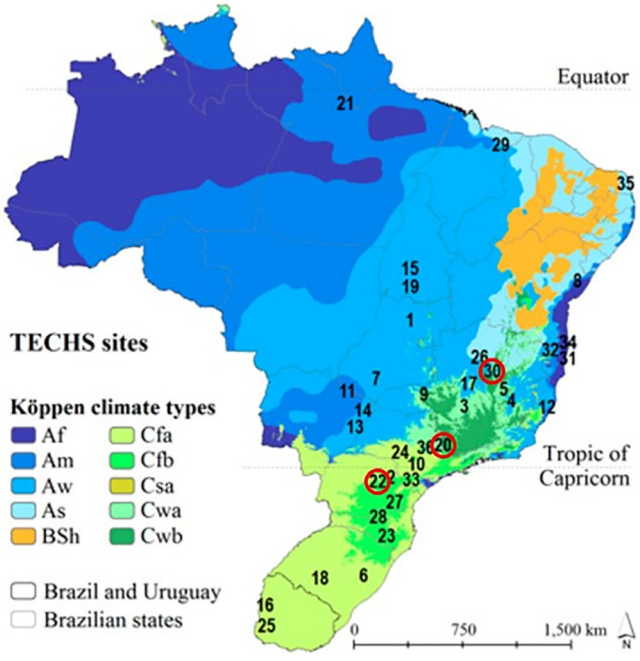

Fig. 1. Location of the 36 sites of the TECHS research project. Circulated areas indicate the sites selected for the study.

Table 1
Mean annual increment ($m^3\ ha^{-1}\ y^{-1}$) and mortality rate (%) at 5 years old for each clone in the three sites of the study.

Clone	Specie	Climate of the clone at origin region	Mean Annual lncrement ($m^3\ ha^{-1}\ y^{-1}$)			Mortality (%)		
			22	20	30	22	20	30
A1	*E. urophylla*	Cwa	69.7	59.1	34.3	2.6	2.5	0.0
B2	*E. urophylla* × *E. grandis*	Aw	81.7	60.3	23.8	1.3	0.0	23.2
C3	*E. grandis* × *E. camaldulensis*	As	71.0	38.4	19.9	0.0	0.0	1.3
P7	*E. urophylla* × *E. brassiana*	As	34.3	28.5	19.5	3.8	7.6	0.0

reduction achieved using plastic tarpaulins to cover an area corresponding to 30% of the spacing between the planting rows. Clones C3 and P7 at site 22 were not planted in a 30% rain exclusion treatment due to operational issues. Temperature, humidity, photosynthetically active radiation, and precipitation were collected hourly at nearby (< 1km) meteorological stations. Climate data were compiled and water deficit was calculated using Thornthwaite & Mather (1957) method and vapor pressure deficit (VPD) and potential evapotranspiration (Eto) were calculated using the Penman-Monteith equation (Allen et al., 1998). We conducted our measurements in 2017 during a rainy (January-February) and dry (August-September) period when trees were 5 to 5.5 years old.

2.2. Leaf water potential (Ψw)

Leaf water potential was measured at predawn (Ψpd) between 3 and 5 a.m. and mid-day (Ψmd) between 11 and 2p.m. using a pressure chamber (PMS Instrument Co – USA; Scholander et al., 1965). Water potentials were measured during cloud free periods that spanned at least 2 h before each measurement. Four medium trees were pre-selected per treatment and trained climbers collected three well-formed, fully expanded leaves from branches located in the middle third of the crown on the north face of each selected tree. Leaf samples were cut at the base of the petiole and were immediately (< 1 min) taken to the pressure chamber for measurement. The difference between the Ψpd and Ψmd potentials was also calculated as a measure of hydraulic safety (Ψδ) as suggested by Johnson et al. (2018).

2.3. Leaf turgor loss point (πtlp) using the Pressure-Volume curve Technique

From the same trees sampled for water potentials, a leafy branch was collected for estimating πtlp from a pressure–volume curve (PV). After sampling, each branch was re-cut under water, placed in a water filled bucket and allowed to rehydrate in the dark 4 to 8 h (Arndt et al., 2015). In the laboratory, a fully expanded and healthy leaf was selected and weighed on an analytical balance to determine the turgid leaf mass/weight, immediately followed by a leaf water potential measurement using a pressure chamber (PMS Instrument Co – USA; Scholander et al., 1965). Subsequently, the leaves were exposed to air at gradually increased time intervals (8 to 60 min) to allow natural water loss from the tissues. This procedure was repeated for all samples, and the whole sequence repeated several times until water potential reached −3.0 to −4.0 MPa. At the end of the measurement, samples were oven dried at 70 °C for 48 h to determine the dry mass/weight and calculate the relative water content (RWC).

PV curves were constructed from measures of the above changes in RWC and leaf water potential during leaf dehydration (Tyree and Hammel, 1972). A non-linear curve (quadratic) was used to model the turgor pressure values (Schulte & Hinkley, 1985) and the turgor loss point (πtlp) was taken as the point where the turgor pressure equals the osmotic pressure - intersection of the linear (osmotic pressure) and non-linear (turgor pressure) lines. Calculations were done using the Excel spreadsheet provided at Lanflux.org (PV_Curve_Fitting_5.6.xls, Landflux.org). Each curve created was carefully analyzed and there was no evidence of intercellular water uptake during the hydration period from the shape of the resulting pressure volume curves.

2.4. Quantifying osmotic potential at saturation (πo-osm)

Adjacent to the leaf selected for determining the P-V curve parameters, another completely expanded leaf was sampled and a 10 mm-diameter leaf disc was removed between the main vein and leaf margin. Each disc was immersed in liquid nitrogen for 1 min and then punched 10 times with a sharp tip forceps to facilitate evaporation through the cuticle (Bartlett et al., 2012b). Subsequently, the disc was immediately placed into the osmometer chamber (VAPRO® Vapor Pressure Osmometer - Model 5600) for measurement. A reading was recorded approximately every 2 min until reaching a constant value (variation < 0.01 mmol/kg), totaling a maximum of 10 measurements. A mean value per sample was calculated and the values were then transformed into MPa (1000 mmol/kg = 2.5 MPa). To avoid cross contamination between sites/samples the sample chamber was cleaned after each measurement. The osmometer was calibrated using three standard solutions (100, 290, 1000 mmol) at the beginning of each measurement day.

2.5. Statistical analysis

The combination of variables (clones, sites, water treatments and season) was analyzed together and/or separately using R software version 3.30 (R Core Team, 2017). All data were tested for homoscedasticity (Bartlett, 1937; Bartlett and Kendall, 1946; Levene, 1961; Brown & Forsythe, 1974) and normality (Anderson & Darling, 1954) tests. Also, the verification of outliers and/or influential values was done. Where assumptions were violated, the relevant transformations were carried out (Box & Cox, 1964) and discrepant data were removed.

Data were submitted to analysis of variance using the F test ($p < 0.05$) (GLM, with Gaussian distribution). When statistically significant fixed effects were detected, Tukey's multiple comparison tests ($\alpha = 0.05$) were undertaken with the "lsmeans" function of the lsmeans R package (Lenth, 2017). We tested the significance of the relationship between πtlp, RWC_{tlp} and osmotic potential at full turgor using linear regression analysis.

3. Results

3.1. Climatic characterization of sites

Weather was significantly different between sites but was typical of long-term patterns (Table 2). Two months prior measurement precipitation was 188.2 and 141.6 mm at site 22, 281.6 and 75.6 mm at site 20, and 199.6 and 0.0 mm at site 30, for the wet and dry seasons, respectively. During the experimental period, no water deficit was observed at site 22 but sites 20 and 30 had a deficit of −17.1 mm and −47.2 mm in the dry season, respectively. The vapor pressure deficit (VPD) on measurement days reached maximum values close to midday and the highest VPD was observed at site 20 in the dry season while the lowest values were recorded in the wet season at site 30. (Table 2).

Table 2
Climatic variables at the three sites of the study for each season evaluated.

Local	Seasons	Tmed	Tmin	Tmax	PPT	Exc	Def	Qleaf	DPV
		°C			mm			MJ $m^{-2}h^{-1}$	KPa
20	Wet	24.6	19.4	32.2	281.6	13.4	0.0	3.6	2.89
	Dry	21.0	12.7	31.9	75.6	78.1	−17.1	2.9	4.68
22	Wet	22.2	18.4	27.9	188.2	37.7	0.0	3.8	2.64
	Dry	19.6	13.0	27.3	141.6	17.3	0.0	3.1	3.31
30	Wet	23.2	18.9	30.2	199.6	0.0	−12.1	3.3	1.84
	Dry	19.7	12.5	27.2	0.0	0.0	−47.2	3.1	2.59

Tmed = Average temperature; Tmin = Lowest temperature of the coldest month; Tmax = Higher temperature of the hottest month; PPT = Accumulated rainfall; Exc = Cumulative water surplus in the evaluation months; Def = Accumulated water deficit in the evaluation months; Qleaf = maximum active photosynthetically radiation during the evaluation days; DPV = maximum vapor pressure deficit during the evaluation days.

3.2. Physiological parameters

3.2.1. Determination of leaf turgor loss (πtlp) and correlation with osmotic potential at the saturation point (πo-osm)

Contrary to our first hypothesis, πtlp determined from the P-V curve did not correlate strongly with the apparent drought tolerance of the clones we measured at each site. (Fig. 2). Water availability treatments (control and rainfall reduction) were not significant for πtlp so we combined these two treatments for analysis. Turgor loss point differed significantly by season for at least some of the clones at all sites and was generally more negative in the dry season with the exception of clone P7 at the wet site (22) which exhibited a less negative πtlp in the dry season (Fig. 3). Seasonal differences in πtlp were apparent in three of the four clones (B2, C3, P7) at site 20. All four clones exhibited a lower πtlp in the dry season at our driest site (30) where clone C3 exhibited the widest range of πtlp between wet and dry seasons.

There was a significant difference between at least some of the genotypes at each site but this difference could not be attributed to specific differences in drought tolerance. Clone P7 at site 20 had a lower πtlp than other clones at the site in the wet season but a higher πtlp in the dry season (Fig. 3). Clone A1 had a lower πtlp in the wet season compared to clones B2 and C3 at site 20 but was similar to clone P7. There were no significant differences in πtlp between clones in the dry season at site 20. Clone C3 exhibited a lower πtlp than clone A1 in the dry season at site 30 and clones A1 and P7 had a lower πtlp in the wet season compared to clones B2 and C3 (Fig. 3).

During the dry season, all four clones we measured had a significantly higher turgor loss point at the wet site compared to the two drier sites where turgor loss point was similar for all clones (Fig. 3). During the wet season, clone A1 had a higher turgor loss point at the wet site compared to the two drier sites while clone B2 had a similar turgor loss point across all three sites. Clone C3 had a lower turgor loss point at site 20 compared to sites 22 and 30. In contrast to the other clones, clone P7 had a lower turgor loss point at the wettest site versus the two drier sites. Combining clone treatment and season, πtlp was statistically different between the wet site and the other two sites and decreased from wet to dry sites (22 = −2.04 MPa; 20 = −2.50 MPa; 30 = −2.52 MPa).

Similar to πtlp, we did not find large differences in RWC_{tlp}. The maximum difference between clones was 0.05 (lowest value found for clone B2 at site 20 on the dry season, RWC_{tlp} = 0.87, and higher value at clone C3 at site 22 on the wet season RWC_{tlp} = 0.92). We also found estimates of leaf capacitance (slope of leaf water content versus mid-day leaf water potential) from the PV curve did not differ by clone.

We found a significant linear relationship between πtlp and VPD or Eto for each location, and there was a significant site by evapotranspiration interaction. Site 22 showed higher πtlp across the range of VPD or Eto present at the site which was expected considering the low evaporative demand at this site. On the other hand, πtlp decreased with VPD and Eto at site 20 and 30 confirming at least a small amount of water stress at these sites (Fig. 4).

We also analyzed and compared the πo-osm obtained using the VAPRO osmometer with πtlp and osmotic potential (πo-PV) values derived from the PV curves for each site (Fig. 5) using linear regression to see if the two assessments of leaf turgor were similar. The values obtained with the osmometer were strongly correlated with both estimates from PV curves. Including all sites and clones, we observed an r^2 = 0.79 and r^2 = 0.69, between πo-osm × πtlp and πo-osm × πo-PV, respectively. In both cases, site 30 presented the best fit (r^2 = 0.83 and r^2 = 0.84).

3.2.2. Leaf water potential

Pre-dawn (Ψpd) and mid-day (Ψmd) leaf water potential were not affected by the rainfall reduction treatments so we combined trees

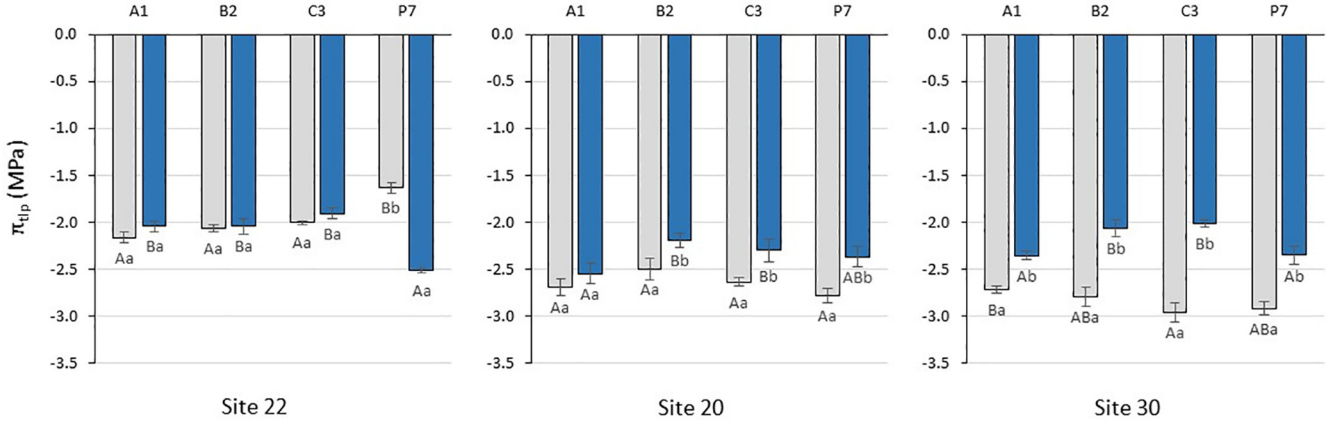

Fig. 2. Leaf turgor loss point (πtlp) in four *Eucalyptus* clones in the dry season () and wet season () at three evaluation sites (20, 22 and 30). Lower case letters compare the seasons in each clone, and uppercase letters compare the clones within each season by Tukey test ($p < 0.05$).

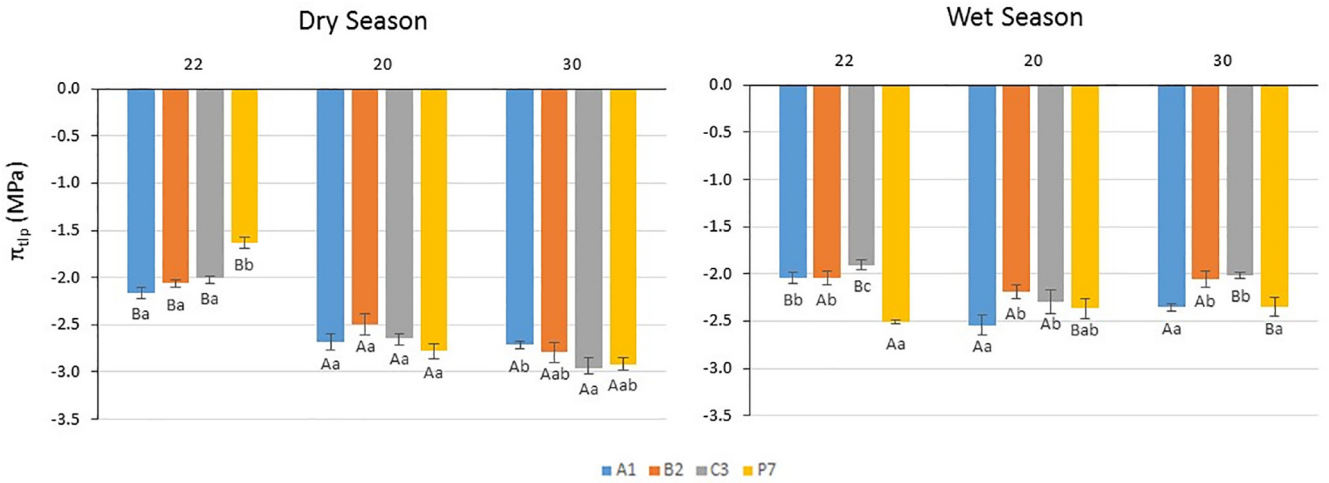

Fig. 3. Leaf turgor loss point (πtlp) comparison in four *Eucalyptus* clones in the dry (left) and wet (right) season. Lower case letters compare the seasons in each clone, and uppercase letters compare the clones within each season by Tukey test ($p < 0.05$).

within each of these treatments for analysis. Pre-dawn leaf water potentials for all clones did not fall below −1.0 Mpa at any of the sites for either season indicating a minimum amount of water stress during our measurement period. Pre-dawn leaf water potential did not differ between clones at the site 22 in neither the wet nor dry season (Fig. 6). Pre-dawn water potential was lower in the dry season for all clones at site 20 but patterns differed at site 30, where two of the clones (A1 and P7) exhibited lower water potentials in the wet season while water potential did not differ with season for clones B2 and C3 (Fig. 6). Pre-dawn leaf water potential did not differ between clones in dry season within each site with the exception of clone C3 in site 20, which had a higher (less negative) pre-dawn water potential compared to all other clones at the site (Fig. 6).

Similar to pre-dawn results, mid-day leaf water potentials did not differ by season or between clones at the wettest site (Fig. 6). Interestingly, mid-day leaf water potential was lower in the wet compared to the dry season for all clones at sites 20 and 30. As we found for our pre-dawn measurements, clone C3 had the least negative mid-day leaf water potential in the dry season at site 20 (Fig. 6). For site 30, mid-day leaf water potential for clone A1 did not differ from the other 3 clones while water potential for clone P7 was lower than clone B2 and C3.

The greatest difference between the water potentials at midday and pre-dawn (Ψδ) was found in the rainy season (−2.18 MPa and −1.66 MPa at the site 20 and 30, respectively) and, especially for clone P7, which differed from clone B2 at the sites 20 and 30 (Table 3).

4. Discussion

Turgor loss point was not correlated with the expected drought tolerance of the clones we measured across three sites, refuting our first hypothesis. This is likely because none of the sites/clones experienced significant water stress as evidenced by the high pre-dawn leaf water potentials we observed during our measurement period. However, we did find that turgor loss point and osmotic potential at full turgor can be accurately estimated with the Vapro osmometer suggesting that this instrument could be useful for future screening of *Eucalyptus* clones and their potential drought tolerance to a changing climate.

For the *Eucalyptus* clones we studied, πtlp was highest at the wettest site in our study (22) during the dry season but there was no difference in πtlp between the more mesic and driest site (20 and 30). Although there were some differences in πtlp between sites during the wet season for three of the four clones we measured, πtlp was not clearly correlated with water availbility (Fig. 3). While Bartlett et al. (2014) found that measurements of πtlp during a single season could capture differences in drought tolerance between species, our results suggests that sampling πtlp during the dry season may be more informative. Additionaly, there are significant site by Eto and VPD interaction effect as shown in the regression analysis (Fig. 4), indicating that measuring πtlp in a wet site

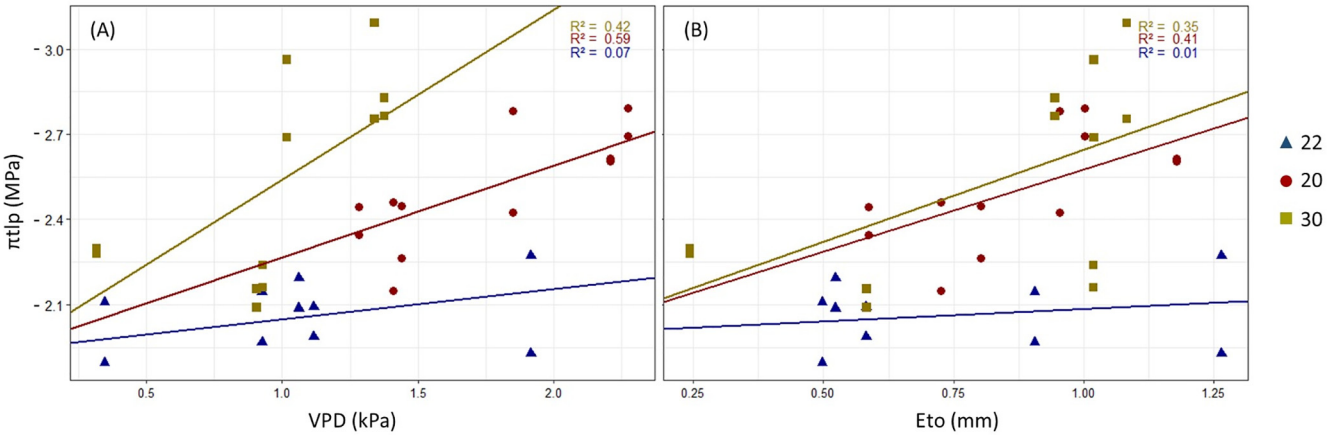

Fig. 4. Linear regression analysis for site effect comparing leaf turgor loss point (πtlp) obtained through the volume pressure curve with vapor pressure deficit (VPD, A) and potential evapotranspiration (Eto, B) calculated using the Penman-Monteith equation and local climate data in four *Eucalyptus* clones at three sites (20, 22 and 30).

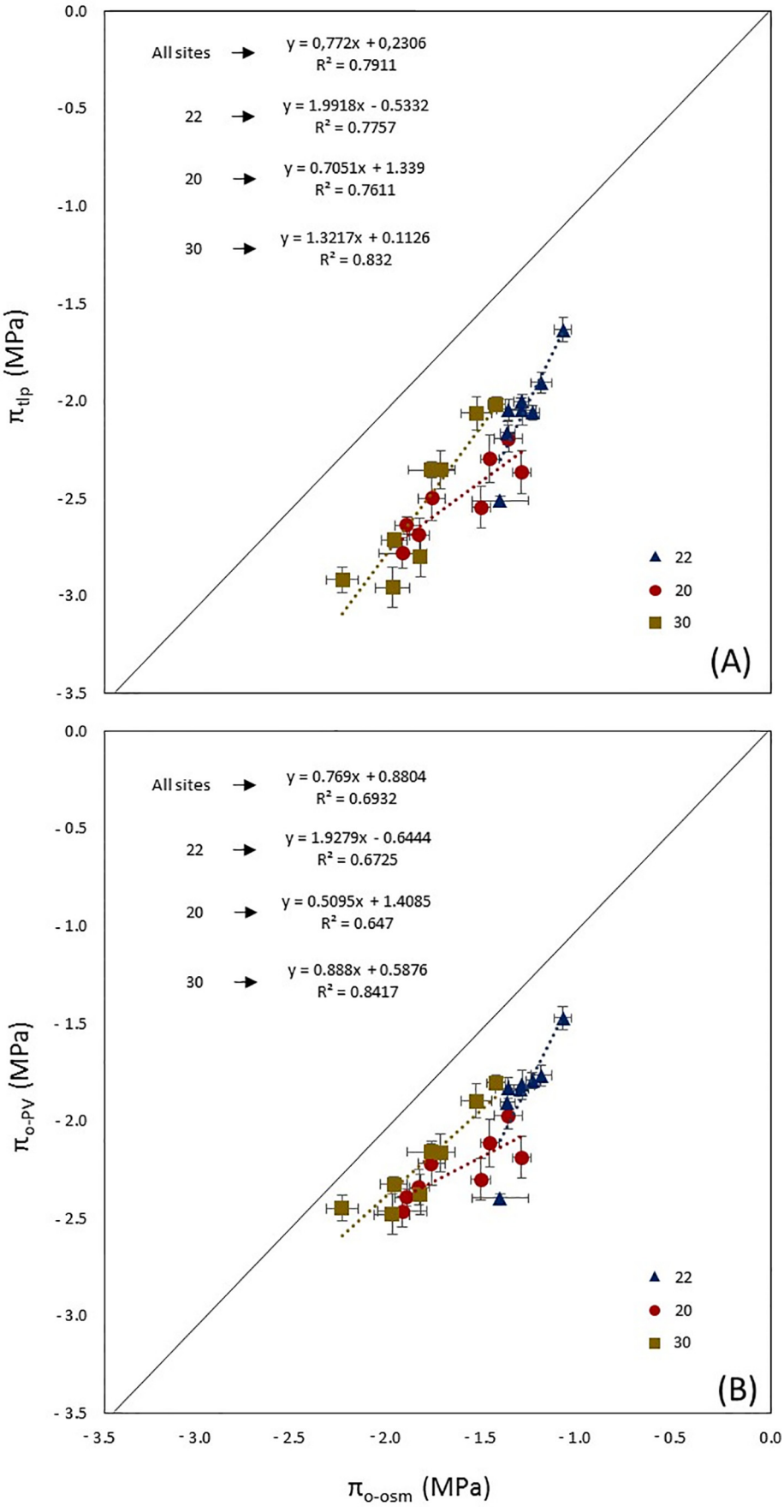

Fig. 5. Leaf turgor loss point (πtlp) obtained through the volume pressure curve versus the osmotic potential at the saturation point (πo-osm) using the osmometer (A) and osmotic potential at full turgor (πo-PV) derived from the pressure volume curve versus the osmotic potential at the saturation point (πo-osm) using the osmometer (B). Data points represent average tree measurements across both seasons (dry and wet) for four *Eucalyptus* clones at three sites (20, 22 and 30).

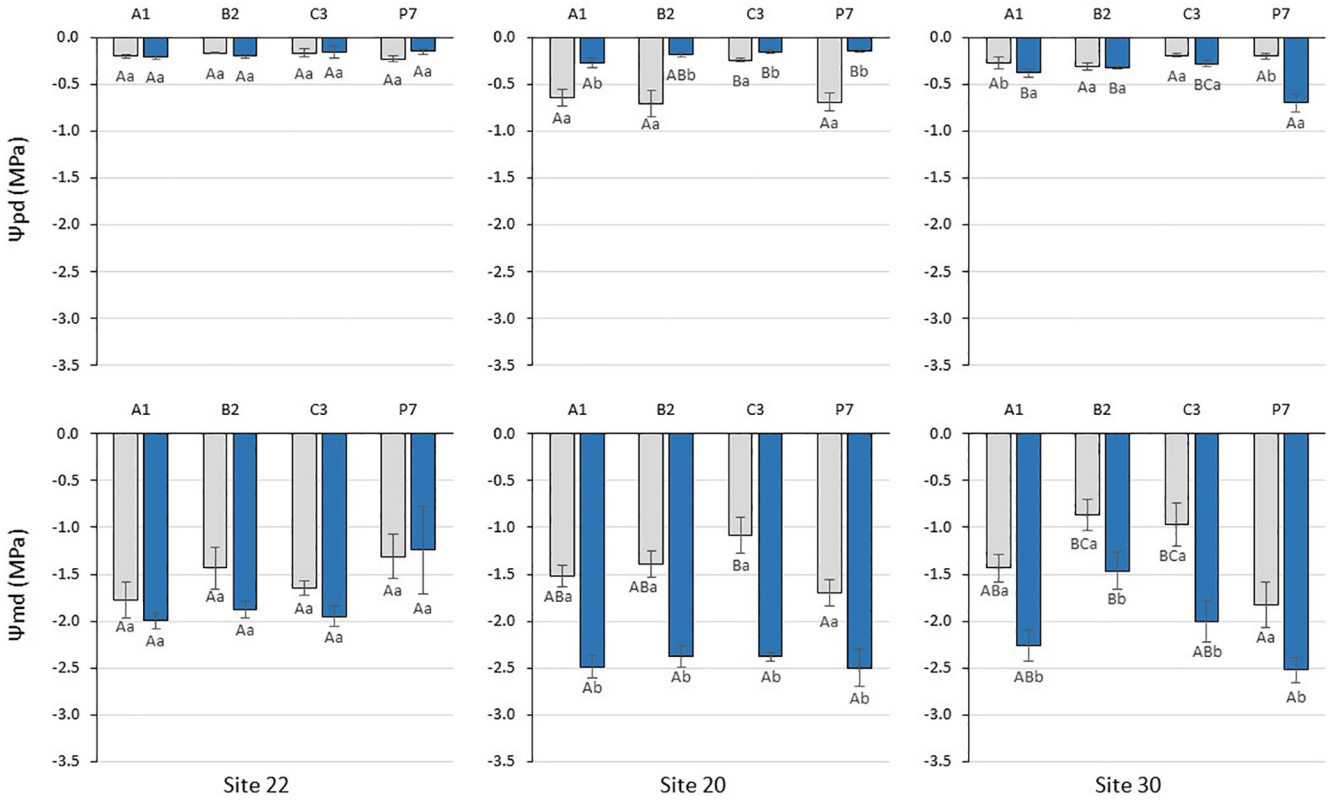

Fig. 6. Leaf water potential: Ψpd (up) and Ψmd (down) in four *Eucalyptus* clones in the dry season () and wet season () at three sites (20, 22 and 30). The bars indicate the standard error. Lower case letters compare the seasons in each clone, and uppercase letters compare the clones within each season by Tukey test ($p < 0.05$).

Table 3
Reduction of water potential (Ψδ, taken as the difference between water potentials at Ψmd and Ψpd) in five *Eucalyptus* clones at two sites (20 and 30).

20			**30**		
Clone	Ψδ		Clone	Ψδ	
P7	−1,76 (0,098)	a	P7	−1,70 (0,177)	a
C3	−1,53 (0,093)	ab	A1	−1,51 (0,158)	a
A1	−1,49 (0,096)	ab	C3	−1,25 (0,147)	ab
B2	−1,44 (0,093)	b	B2	−0,84 (0,147)	b
Season			Season		
Wet	−2,18 (0,066)	a	Wet	−1,66 (0,112)	a
Dry	−0,83 (0,069)	b	Dry	−0,93 (0,106)	b

The letters indicate the comparison between clones and seasons for the Ψδ character at the statistical significance level of 5%. Number in parentheses indicate standard error.

to evaluate drought tolerant genetic material would not be expressive.

Turgor loss point varied by site and season as all of the clones we studied showed some degree of plasticity in their regulation of πtlp. Seasonal adjustments were most apparent at the driest site where all four clones had significantly higher πtlp in the wet versus the dry season. If our results are indicative of other clones within TECHS, plasticity in πtlp may help explain why temperature was a more important factor than water availability in controlling productivity across all TECHS sites (Binkley et al. 2020) because osmotic adjustments in πtlp enable plants to maintain higher rates of photosynthesis and stomatal conductance where water availability is limited (Brodribb and Holbrook, 2003, Bartlett et al., 2014).

Consistent with other studies (Bartlett et al., 2014; Mart et al., 2016; Griffin-Nolan et al., 2019) we found that estimates of πtlp determined from pressure volume curve analysis were highly correlated with estimates of πo-osm obtained with an osmometer. Both techniques captured the variability in πtlp within and across sites (Fig. 5). As other studies have shown (Bartlett et al., 2012a), the variation in πtlp was generally correlated with water availability where the lowest turgor loss points estimated with both methods occurred at the driest site while the highest πtlp values were observed at the wettest site.

Leaf water potential was generally more responsive to water availability and evaporative demand during the months more suitable for plant growth compared to drier months (Gleason et al., 2013). For example, Ψmd was lower in the rainy season and Ψδ was larger in the wet compared to the dry season. During the dry season, trees usually grow slower in response to reduced stomatal conductance as water availability declines (Bourne et al., 2017) which likely resulted in the less negative water potentials we observed. During the rainy season, clones seek to maximize carbon gain (Stape et al., 2010; Gleason et al., 2013), allowing the leaf water potential to become more negative. Queiroz et al. (this issue), showed that the optimum temperature (18 °C – 22 °C) for maximum tree growth is modulated in part by water supply in the soil. During 2017, all four clones grew in diameter 6.2% / 4.1%, 4.7% / 1.1% and 7.0% / 0.0% at site 22, 20 and 30 (wet/dry season) respectively.

The parameter Ψpd is generally considered the best representation of water status at the plant level. Assuming no transpiration at this time Ψpd can be used to represent soil water potential within the root zone (Tyree and Jarvis, 1982). We found no significant difference in Ψpd values between the wet and the dry season at site 22 as this site experienced high precipitation and little soil water deficit between seasons. In contrast, at site 20, Ψpd was more negative during the dry compared to the rainy season and at site 30 the opposite, likely because there was no significant soil water deficit during both periods.

Turgor loss point for individual clones showed a weak to no relationship with site water availability, i.e., clones that are thought to be more tolerant to drought did not show the lowest values of turgor loss

point or leaf water potential. These results differ from Li et al. (2018, 2019) when studying drought and growth in contrasting native species. The patterns seem to be clearer with pure *Eucalyptus* species. White et al. (2000) reported πtlp values of −3.9 MPa for 10 year old *E. platypus* in the dry season while Bourne et al. (2017) observed more negative πtlp for the subhumid species (*E. crebra* = −2.99 MPa) compared to humid (*E. saligna* = −2.50 MPa and *E. grandis* = −2,34 MPa) at 5 years old.

Although the lack of significant water stress during our study likely contributed to the weak correlation between drought tolerance of individual clones and πtlp, the weak correlation may also be related to the fact that all clones used in this study were selected from a well-consolidated *Eucalyptus* genetic breeding program, which is focused on selecting highly productive genotypes. As the potential growth of a clone is driven by the origin of its species, and the origin in turn drives its drought tolerance (Pfautsch et al., 2016, Li et al., 2019), the selection of highly productive genotypes may substantially narrow the range of πtlp expression for the genotypes we studied, since they were selected by their wood productivity and not their drought tolerance.

Clone P7 had the greatest reduction of water potential (Ψδ) and the lowest mortality even in dry environments (Binkley et al, 2020), suggesting a high hydraulic safety margin. The fact that P7 is a cross with *E. brassiana*, which comes from dry regions, may have incorporated this drought tolerance trait into this hybrid clone. On the other hand, clone B2 which is an *E.urophylla* × *E.grandis* had the lowest reduction of water potential (Ψδ) and the highest mortality in dry environments (Binkley et al 2020). C3 is considered a drought tolerant clone, and had less negative Ψpd and Ψmd values compared to the other clones (except for B2 due to high plant mortality at the 30 site), that may be explained by the greater carbon allocation in root production (Campoe et al., 2018), allowing access to deeper soil layers and, consequently, reducing water stress.

Aside from the lack of significant water stress during our study, another important limitation of our study for both leaf water potential and πtlp measurements is that we were only able to sample twice within a single year. For example, single measurements of leaf water potential only capture a small snapshot of plant water status and do not encompass the range of variability within and between clones across years that are caused by differences in soil water availability, temperature and solar radiation. Given the plasticity of πtlp we observed between single measurements in the wet and dry season, more frequent measurements throughout the year may have yielded different results. We suggest that the tight correlation between πtlp and π-osm we found in this study may be able to be used for more detailed assessments of the clonal variation in πtlp across water availability gradients and between clones with the ultimate goal of assessing relative drought tolerance for clonal *Eucalyptus* hybrid clones as climate change impacts increase.

CRediT authorship contribution statement

José Luiz Ferraresso Conti Junior: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing - original draft, Writing - review & editing. **Márcio José de Araujo:** Methodology, Data curation, Formal analysis. **Rinaldo Cesar de Paula:** Formal analysis, Supervision, Writing - review & editing. **Túlio Barroso Queiroz:** Methodology, Data curation. **Rodrigo Eiji Hakamada:** Conceptualization, Supervision, Writing - review & editing. **Robert M. Hubbard:** Conceptualization, Investigation, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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