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COMPARING FUNCTIONAL TRAITS OF PLANTED *Eucalyptus* sp. GENOTYPES MANAGED AS HIGH TREE AND COPPICE

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Dissertation presented to the Faculty of Agronomic Sciences of Unesp, Campus of Botucatu, to obtain the degree of Master in Science by the Postgraduate Program in Forest Science.

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TÍTULO DA DISSERTAÇÃO: COMPARING FUNCTIONAL TRAITS OF PLANTED Eucalyptus sp. GENOTYPES MANAGED AS HIGH TREE AND COPPICE

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My December 24th will never be the same without you.
Thank you for everything.
Adailde,
in memorium.

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"It's our choices that reveal who we really are, much more than our qualities."

ROWLING J. K. **Harry Potter and the Chamber of Secrets**. London: Bloomsbury, p. 368, 1998.

ABSTRACT

In this dissertation, we aimed to evaluate the potential of rapidly measured leaf water relations traits for the phenotyping of drought tolerance across the 21 commercial *Eucalyptus* genotypes, and subsequently, to compare the drought tolerance of *Eucalyptus* genotypes managed as high forest and coppice stand. The reason for this hypothesis is that, due to a root system developed from the initial development phase, *Eucalyptus* genotypes managed as coppice experience less water stress in the first years of the rotation. Therefore, *Eucalyptus* genotypes managed as coppice are less resistant to drought, due to plasticity in drought tolerance traits. The field experiment was installed in February 2018 in Itatinga, São Paulo state, Brazil. We found that π_o and Ψ_{tlp} exhibit a significant seasonal plasticity. Only the measurements conducted at the end of the dry season were associated to some extent with the expected drought tolerance of the *Eucalyptus* genetic materials. Although we confirmed that variation in Ψ_{tlp} is mostly driven by variation in π_o across *Eucalyptus* material, the osmometer method was not able to accurately rank drought tolerance among the genotypes of our common-garden experiment. We found that the drought tolerance traits of coppice and high forest are similar. Contrary to expectations, plant drought tolerance predictor traits such as Ψ_{tlp} and Ψ_{50} are not influenced by management in commercial *Eucalyptus* genotypes. Conversely, the stand biomass, and leaf area index are more determinant for differences between silvicultural systems than drought tolerance traits. However, this warrants further investigation of management effects in conditions of higher water stress, in order to draw a more extensive picture of *Eucalyptus* vulnerability to drought.

Keywords: turgor loss point; vulnerability to cavitation; *Eucalyptus* genotypes; drought tolerance.

RESUMO

Nesta dissertação, objetivou-se avaliar o potencial de rapidamente medir as características hídricas foliares para a fenotipagem da tolerância à seca de genótipos de *Eucalyptus*, e posteriormente, comparar a resistência à seca de genótipos de *Eucalyptus* manejados como alto fuste e talhadia. A justificativa para essa hipótese é que, devido a um sistema radicular desenvolvido desde a fase inicial de desenvolvimento, genótipos de eucalipto manejados como talhadia sofrem menos estresse hídrico nos primeiros anos da rotação. Portanto, genótipos de eucalipto manejados como talhadia são menos resistentes à seca, devido à sua plasticidade nas características de tolerância à seca. O experimento de campo foi instalado em fevereiro de 2018 em Itatinga, estado de São Paulo, Brasil. O experimento faz parte do Programa Cooperativo de Produtividade e Fluxos de Carbono e Água em Eucalipto (EUCFLUX). Observou-se que π_o e Ψ_{tlp} exibem uma plasticidade sazonal significativa. Apenas as medições realizadas no final da estação seca foram associadas de alguma forma com a tolerância à seca esperada dos materiais genéticos de eucalipto. Embora tenha-se confirmado que a variação em Ψ_{tlp} é principalmente impulsionada pela variação em π_o em material de eucalipto, o método do osmômetro não foi capaz de classificar com precisão a tolerância à seca entre os genótipos estudados. Foi observado que as características de tolerância à seca da talhadia e alto fuste são semelhantes. Ao contrário do esperado, características preditoras de tolerância à seca como Ψ_{tlp} e Ψ_{50} não são influenciados pelo manejo em genótipos comerciais de eucalipto. Por outro lado, a biomassa do estande e o índice de área foliar são mais determinantes para as diferenças entre os sistemas silviculturais do que as características de tolerância à seca. No entanto, isso merece uma investigação mais aprofundada dos efeitos do manejo em condições de maior estresse hídrico, a fim de traçar um quadro mais amplo da vulnerabilidade do eucalipto à seca.

Palavras-chave: ponto de perda de turgor; vulnerabilidade à cavitação; genótipos de *Eucalyptus*; tolerância à seca.

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SUMMARY

GENERAL INTRODUCTION

Eucalyptus is a fast-growing genus of the Myrtaceae family that is endemic from Australia and surrounding islands but was largely introduced in all the tropics (SAADAOUI et al., 2017). Indeed, *Eucalyptus* dominates the hardwood plantations in tropical and subtropical regions (FAO, 2010). In Brazil, *Eucalyptus* is the most used species in forest plantations, approaching about 7.8 million hectares distributed throughout the territory (IBGE, 2019), with an average above-ground productivity of 36 m^3 ha^{-1} $year^{-1}$ (IBÁ, 2019).

This can be explained by its fast growth under Brazil's climate and soil conditions (FERRAZ et al., 2019; HAKAMADA et al., 2017; LONDERO et al., 2012) and by the high-quality of the resulting wood products (PROTÁSIO et al., 2021). *Eucalyptus* can be used for the production of paper and cellulose (GOMES et al., 2015), as well as in the supply of the panel industry (GONULTAS; CANDAN, 2018) and sawmill (MONTEIRO et al., 2017). Moreover, *Eucalyptus* commercial plantations can decrease the deforestation of natural forests and landscape degradation by substituting wood products from native species for the forest industry. Therefore, *Eucalyptus* plantations can have an important role in protecting native forests (CARLE; DUVAL; ASHFORDC, 2020), although this positive effect is strongly dependent on local and national legal contexts (PIRARD; DAL SECCO; WARMAN, 2016).

After harvest, two options can be favored by forest managers to initiate the next rotation of the plantation: planting seedlings (i.e., high forest stand) or managing the resprout shoots growing from the stumps (i.e., coppice stands) (PIVA et al., 2020). The main advantages of coppice are that it has a lower total cost (PIVA et al., 2020), it is more ecologically sustainable (GONÇALVES et al., 2014) and the new shoots already have a fully developed radicular system (CHRISTINA et al., 2017). Indeed, after a rotation cycle of 5-7 years, the roots of *Eucalyptus* can reach approximately 20 m in depth (CHRISTINA et al., 2011), facilitating the absorption of water and nutrients (WILDY; PATE; SEFCIK, 2004). When managed in short rotations (5-7 years), a prolonged dry period can drastically reduce production (ALMEIDA et al., 2004), especially in the eastern region of Brazil (STAPE; BINKLEY; RYAN, 2004).

Unlike the coppice system, the stands of *Eucalyptus* in the high forest system are more onerous, because of the seedling and planting costs (PIVA et al., 2020). However, the high forest system has become more common in recent decades due to increased productivity (GONÇALVES et al., 2013) and decreased rotation length (GONÇALVES et al., 2014). Indeed, the high productivity and short rotation of *Eucalyptus* plantations in Brazil (typically 6/7 years) allow managers to rapidly change the *Eucalyptus* genotype grown at a given site and to benefit from the last advances of breeding programs.

Recent reports provide evidence that increased drought is already affecting forest productivity worldwide (ANDEREGG et al., 2012; BRESHEARS et al., 2005; ZHAO; RUNNING, 2010) and projections for the XXI century have pointed to an increase in temperature and in the intensity and frequency of drought compared to the last century (IPCC, 2013). Large waves of dieback are already observed in *Eucalyptus* plantations (GONÇALVES et al., 2017). For these reasons, characterizing the ability of tree species to cope with drought is a major scientific challenge, with important implications for forest conservation and management (ANDEREGG; KANE; ANDEREGG, 2013; BARTLETT; SCOFFONI; SACK, 2012; CHOAT et al., 2018; CONTI JUNIOR et al., 2020; LAMERS; DER MEER; TESTERINK, 2020; LARTER et al., 2017).

Hydraulic failure was evidenced as a major cause of drought-induced mortality in trees (ADAMS et al., 2017). Trees transport water from the soil to their leaves to sustain photosynthesis through vessels or tracheids (i.e., the xylem vascular system). This water column is under tension in the xylem allowing water to be pumped up to the leaves at very low energetic costs. When drought occurs, the first tree response is to close stomata, strongly reducing transpiration and dehydration. However, if drought continue to increase, the tension in the xylem water column may become too high, which results in events of cavitation, a sudden phase change from liquid water to gas that creates a bubble and, therefore, the embolism of the vessel (CHOAT et al., 2018). If xylem cavitation spread throughout the xylem, it leads to hydraulic failure and tree death. Previous works showed that tree hydraulic safety margin, i.e., the difference between the water stress actually experienced by trees and the water stress that causes xylem dysfunction, is conserved in many species of the world (CHOAT et al., 2012). This indicates that the risk of hydraulic failure is pervasive in forests over the globe.

Functional traits are morphological, physiological or phenological features that can be used to predict tree performances in optimal or harsh conditions (VIOLLE et al., 2007). In the last years, two key traits were shown to efficiently predict drought resistance in trees. First, the leaf turgor loss point (Ψ_{tlp}, MPa) is the water potential at turgor loss (CHEUNG; TYREE; DAINTY, 1975). The water potential is a measure of water stress that quantifies the potential energy of water per unit volume relative to pure water in reference conditions and becomes increasingly negative during drought. The loss of cell turgor causes stomata to close. Therefore, Ψ_{tlp} correlates with the leaf water potential at stomatal closure (BRODRIBB; HOLBROOK, 2003).

The Ψ_{tlp} has been widely used to directly quantify tree drought resistance (BARTLETT; SCOFFONI; SACK, 2012; CONTI JUNIOR et al., 2020; KOIDE et al., 1989), or to evaluate the extent to which early stomatal closure prevents the risk of hydraulic failure for a species (GUILLEMOT et al., 2022; MARTIN-STPAUL; DELZON; COCHARD, 2017). Plants with high Ψ_{tlp} (less negative) are more susceptible to early stomata closing, preventing water loss by evaporative demand (OLIVEIRA et al., 2021) and maintaining hydration under decreasing soil water potential (SUN et al., 2020). On the other hand, a low Ψ_{tlp} (more negative) can translate into greater drought tolerance due to their capacity to maintain function while the plant dries out (BARTLETT; SCOFFONI; SACK, 2012). The fact that stomata closing occurs before xylem cavitation suggests that avoidance of xylem cavitation is an important mechanism for plant survival under drought (CHOAT et al., 2018).

Another fundamental trait involved in drought resistance is the water potential that causes xylem dysfunction, which is commonly measured as the water potential causing 50% loss of hydraulic conductance (Ψ_{50}) (CHOAT et al., 2012; COCHARD et al., 2008; GUILLEMOT et al., 2022; LARTER et al., 2017; MAHERALI; POCKMAN; JACKSON, 2004; MARKESTEIJN et al., 2011). The vulnerability of the xylem to cavitation strongly varies among species and is a major determinant of drought resistance in trees (ANDEREGG et al., 2016).

Both TLP and Ψ_{50} are classically obtained from pressure-volume curves and vulnerability curves to cavitation, respectively, which are time-consuming methodologies (COCHARD et al., 2013; LENZ; WRIGHT; WESTOBY, 2006). In recent years, new innovative methodologies were proposed allowing faster measurement of these key traits. On one hand, the pneumatron device is used to estimate xylem vulnerability to cavitation by applying a partial vacuum to extract air from a sampled

branch, while monitoring the pressure change over time (PEREIRA et al., 2016, 2020). As the amount of gas extracted is strongly correlated with the percentage loss of xylem conductivity, the measured air discharged is combined with xylem measurement potential to build the vulnerability curves to cavitation and extract Ψ_{50}. On another hand, leaf Ψ_{tlp} can be obtained indirectly via its strong relationship with the osmotic potential at full hydration (π_o). This π_o is rapidly obtained using vapuor-pressure osmometry of freeze-thawed leaf samples, resulting in a fast measurement of TLP (Bartlett et al., 2012). These new methodologies open interesting perspective regarding the screening drought resistance among tree genotypes and the inclusion of drought resistance into the objectives of breeding programs. However, they remain to be properly evaluated for the characterization of *Eucalyptus* genotypes.

For this dissertation, we developed two chapters to evaluate drought tolerance in *Eucalyptus* genotypes. The objectives of our study are organized as follows:

First chapter: evaluate the potential of rapidly measured leaf water relations traits for the phenotyping of drought tolerance across the 21 commercial *Eucalyptus* genotypes found in the EUCFLUX common-garden; and,

Second chapter: comparing the drought tolerance of *Eucalyptus* genotypes managed as high and coppice forests.

With this dissertation, we hope to contribute to the much-needed science basis of the management of *Eucalyptus* under climate change. On the other hand, we hope that this study opens more research issues for future works.

CAPÍTULO 1

THE POTENTIAL OF LEAF WATER RELATION TRAITS FOR DROUGHT TOLERANCE PHENOTYPING IN COMMERCIAL *Eucalyptus* GENOTYPES

1.1 INTRODUCTION

Increased drought associated with climate change has been affecting forest productivity worldwide (ANDEREGG; KANE; ANDEREGG, 2013; BRESHEARS et al., 2005; ZHAO; RUNNING, 2010). Moreover, projections for the 21th century forecast an increase in the intensity and frequency of heat-waves and drought compared to the last century (IPCC, 2021). Therefore, predicting the impacts of climate change on forest growth and productivity is one of the biggest challenges in ecological research.

Tree species exhibit high variation in drought tolerance (GUILLEMOT et al., 2022; MARTIN-STPAUL; DELZON; COCHARD, 2017a). However, little is known on whether and how much drought tolerance varies within species from a same genus, or between varieties/clones of the same species. This question is particularly acute for genetic materials that has been repeatedly selected through breeding programs, e.g., to improve their characteristics such as wood productivity and quality or berry production. Indeed, how the search for productivity could affect drought tolerance remains largely unknown. A recent study found no clear relationship between drought tolerance and growth in maritime pine (SONG et al., 2022), suggesting that drought tolerance needs to be measured and targeted into tree breeding program strategies in order to adapt tree plantations to future climate conditions.

Eucalyptus is a fast-growing genus that is the most planted worldwide (KEENAN et al., 2015). Strikingly, most of the studies assessing the response of *Eucalyptus* to drought were led on natural forests (e.g., MITCHELL et al., 2013; PFAUTSCH et al., 2016), urging for more efforts towards cultivated materials, especially as drought-related large scale mortality events were already observed in tropical *Eucalyptus* plantations (GONÇALVES et al., 2017). However, the variety of drought tolerance strategies within genetic materials of *Eucalyptus* involve many organs and response types, at different time scales (MITCHELL et al., 2013; OLIVEIRA et al., 2022). One crucial challenge is therefore to identify functional traits that 1) efficiently quantify

drought tolerance at the variety/clone level and 2) are scalable, that is, fast and easy enough to measure to be used in large scale breeding programs.

Recent studies suggest that water relation traits are good candidates to predict tree drought tolerance (BLACKMAN, 2018). Indeed, leaf water relation traits correlate with both leaf hydraulic and economic traits, both in large scale datasets (GUILLEMOT et al., 2022; ZHU et al., 2018) and among contrasted *Eucalyptus* varieties (LUCANI et al., 2018; OLIVEIRA et al., 2022). In particular, the leaf water potential at turgor loss (or turgor loss point, Ψ_{tlp}; in MPa) was widely used to assess tree drought tolerance (KUNERT et al., 2021; ZHU et al., 2018). The Ψ_{tlp} is the water potential (Ψ_{leaf}) at which the cell wilts due to gradual loss of water, but it is also well known to correlate with Ψ_{leaf} at stomatal closure (BARTLETT et al., 2016; BRODRIBB; HOLBROOK, 2003; CHOAT et al., 2018). Also known as “permanent wilting point”, the Ψ_{tlp} is a measure of the stringency of stomatal control under reduced soil water potential and may be able to contrast drought tolerance among tree varieties (BARTLETT; SCOFFONI; SACK, 2012). However, Ψ_{tlp} can exhibit plastic adjustments as soil dry, possibly leading to seasonal intra-genotype variation (BARTLETT et al., 2014). This warrants further investigation to explore whether Ψ_{tlp} does change between dry and wet season the *Eucalyptus* genus, and if so, when measurement should be made to best capture drought tolerance. In addition, trees continue losing water from their leaves after stomatal closure (DUURSMA et al., 2019). Beyond the differences in the stomatal control strategies, the intensity of the residual leaf water loss (g_{min}) has been suggested as one important and overlooked determinant of tree drought tolerance (CHOAT et al., 2018). The importance of g_{min} variation in determining drought tolerance in commercial *Eucalyptus* genotypes remains to be quantified.

Pressure-Volume (PV) curves are traditionally built to estimate Ψ_{tlp} (BARTLETT; SCOFFONI; SACK, 2012) along with other key traits linked to drought tolerance strategies of tree species, which are relative water content at turgor loss (RWC_{tlp}, in %), Apoplastic water fraction (a_f, in %), modulus of elasticity (ε, in MPa) and osmotic potential at full turgor (π_o, unit MPa). However, PV curves are a time-consuming methodology, hardly compatible with the requirements of large-scale commercial phenotyping (CALLISTER; ARNDT; ADAMS, 2006; COCHARD et al., 2013; LENZ; WRIGHT; WESTOBY, 2006). In recent years, a new innovative method has been proposed, allowing a faster measurement of Ψ_{tlp} by using osmometry (BARTLETT et al., 2012). Under drought, the cell loses water until it reaches the wilting point (Ψ_{tlp}),

which increase the concentration of cell solute and decrease Ψ_{leaf}. Hence, Ψ_{tlp} can be obtained indirectly via a strong relationship with the osmotic potential at full hydration (π_o) (BALL; OOSTERHUIS, 2005; BARTLETT et al., 2012a; CONTI JUNIOR et al., 2020). The π_o is rapidly obtained using vapor-pressure osmometry (hereafter called πo_{osm}) of freeze-thawed leaf samples, resulting in the fast measurement of Ψ_{tlp} (BALL; OOSTERHUIS, 2005). Bartlett, Scoffoni, Ardy, et al. (2012) showed that πo_{osm} is a good predictor of Ψtlp_{pv} across of range of species widely differing in drought tolerance. The two main identified causes of error influencing the osmometer method are the dilution of the symplastic fluid by the apoplastic fluid, and the influence of the cell wall solutes, both potentially affecting the measurement of symplastic osmotic potential. It has been suggesting that traits related to cell wall investment could help reducing this error and improving the πo_{osm}-Ψtlp_{pv} relationship among species (BARTLETT et al., 2012; PETRUZZELLIS et al., 2019). These traits include apoplastic fraction (a_f), and modulus of elasticity (ε) - obtained using PV curves, or the “soft traits” leaf mass per area (LMA) and leaf dry matter content (LDMC). However, we do not know whether these relationships hold for species from the same genus, or for varieties of the same species.

Here, we build upon a field common garden of commercial *Eucalyptus* genotypes in southern Brazil to explore the potential of leaf water relation traits for drought tolerance phenotyping in commercial *Eucalyptus* genotypes. We hypothesize that:

(1) *Eucalyptus* genotypes exhibit significantly lower Ψ_{tlp} in the dry season than in the wet season.

(2) The *Eucalyptus* genotypes largely differ in Ψ_{tlp}, and Ψ_{tlp} is a good proxy of drought tolerance in *Eucalyptus* genotypes. Ψ_{tlp} measured at the end of the dry season is a better predictor of drought tolerance because leaves acclimated to dry conditions in a different way among genotypes.

(3) πo_{osm} is a good predictor of Ψtlp_{pv} for *Eucalyptus* genotypes, but a specific equation developed for commercial *Eucalyptus* genotype performs better than the generic Bartlett et al. (2012) equation. Ψtlp_{pv} also correlates with traits associated with cell wall investment, which could be used to improve the πo_{osm} – Ψtlp_{pv} relationship.

1.2 MATERIAL AND METHODS

1.2.1 Site description

The field experiment was set up in February 2018 in Itatinga, state of São Paulo, Brazil (22°58'04'' S and 48°43'41'' W, 857 m asl). The experiment is part of the Cooperative Program on Productivity and Carbon and Water Fluxes in *Eucalyptus* (EUCFLUX – https://www.ipef.br/eucflux2/). The local climate is humid mesothermal (Cwa) according to the Köppen's classification, with an average annual temperature of 19 °C and an average annual precipitation of 1300 mm (ALVARES et al., 2013).

1.2.2 Study site, material, and sampling

The experiment used in the study consisted in a common-garden of 21 *Eucalyptus* genotypes managed under high forest stands, and a subset of 10 *Eucalyptus* genotypes managed under coppice stands (Table 1). All 21 genotypes are commercial, genetically-improved materials used by the Brazilian *Eucalyptus* planting industry. Plot were 36 m × 32 m and were planted with one genotype with tree spacing of 3 x 2 m (1666 trees ha^{-1}), totaling 192 individuals. Sampling was only made in the 100 central trees to avoid edge effects. The details of the evaluations carried out, date of measurement and age of the *Eucalyptus* under coppice and high forest are shown in table 2.

Table 1 - Description of the 21 *Eucalyptus* genotypes. States of origin in Brazil: SP - São Paulo, ES - Espírito Santo, MA – Maranhão, MG - Minas Gerais, RS - Rio Grande do Sul, BA – Bahia

Genotype	Species	Origin	Silvicultural System	Provenance (Brazil)	Drought Index
3	*E. grandis* x *E. urophylla*	Clone	Both	SP	2
4	*E. grandis* x *E. urophylla*	Clone	Both	Aracruz-BA	1
5	*E. grandis* x *E. urophylla*	Clone	Both	State of São Paulo	2
6	*E. grandis* x E. urophylla	Clone	Both	Espírito Santo	3
7	*E. grandis*	Clone	High Forest	SP	1
8	*E. grandis* x *E. urophylla*	Clone	Both	Southwest MG	3
9	*E. grandis* x *E. urophylla*	Clone	High Forest	Mogi Guaçu-SP	1
10	*E. grandis*	Clone	Both	SP	2
11	*E. grandis* x *E. urophylla*	Clone	High Forest	SP	2

12	*E. grandis* x *E. urophylla*	Clone	Both	Itamarandiba-MG	3
13	*E. grandis* x *E. urophylla*	Clone	Both	Itamarandiba-MG	3
14	*E. saligna*	Clone	Both	Guaíba-RS	1
15	*E. grandis*	Clone	High Forest	SP	2
16	*E. camaldulensis* x *E. grandis*	Clone	Both	Sátiro Dias-BA	3
17	*E. grandis* x *E. urophylla*	Clone	High Forest	Mogi Guaçu-SP	1
18	*E. grandis* x *E. urophylla*	Clone	High Forest	Casa Branca-SP	1
20	*E. grandis* x *E. urophylla*	Clone	High Forest	SP	2
22	*E. dunnii*	Clone	High Forest	Otacílio Costa-SC	1
23	*E. pilularis*	Seeds	High Forest	Anhembi-SP	2
24	*E. pellita*	Seeds	High Forest	Açailândia-MA	2
25	*E. tereticornis*	Seeds	High Forest	Açailândia-MA	2

Both = arranged under high and coppice forest.

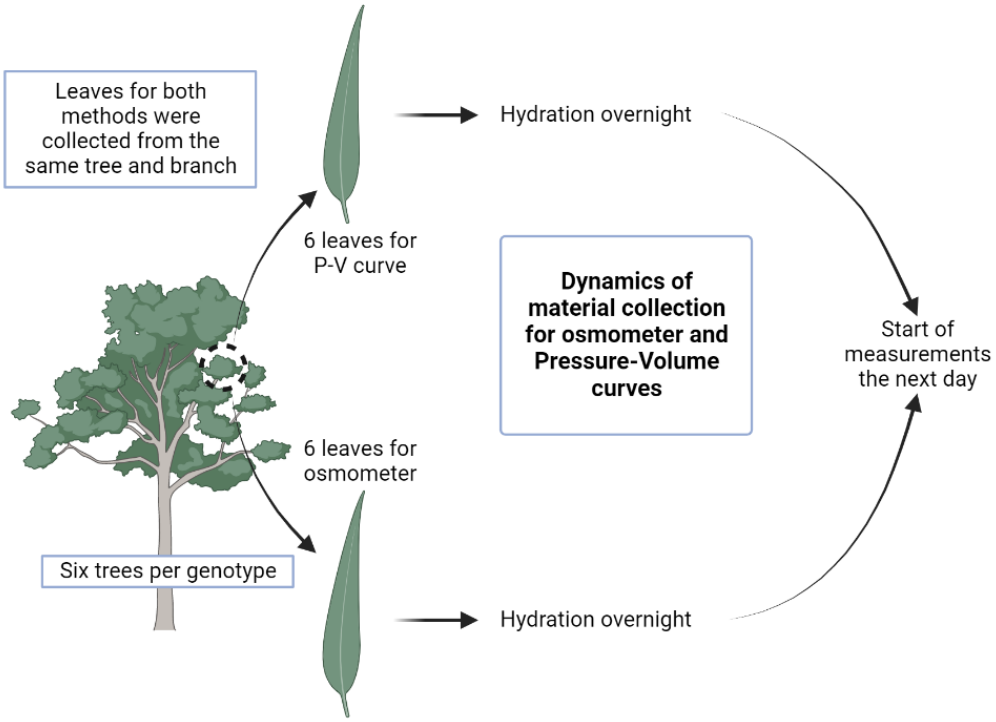
Six trees (replicates) per genotype in both high forest and coppice stands were sampled. For the osmometer and PV curve measurements, we collected leaves from the same tree and branch to avoid inter-individual variation. We collected branches from the upper part of the crown and selected only healthy, fully expanded leaves. The initial steps of osmometer and PV curve data collection can be seen in figure 1.

Table 2 - Details of the assessments carried out, date of measurement and age of *Eucalyptus* under coppice and high forest stands

Traits	Date of measurements	Age of planting (Coppice)	Age of planting (High Forest)
PV curves	May 2021	36 months	41 months
	November 2021	42 months	47 months
g_{min}	May 2021	36 months	41 months
Osmometer	November 2021	42 months	47 months

Where: PV curves is Pressure-Volume curves.

Figure 1 - Data collection for pressure-volume (PV) curve and osmometer methods. Were selected six trees per genotype. A branch from the middle third of the crown was collected and 12 leaves were removed (6 for both PV curve and osmometer methods). Then, the leaves were properly stored under humidity and taken to the laboratory, where they were placed for rehydration overnight



1.2.3 Pressure-volume curves

Pressure-volume (PV) curves were built to derive leaf water relation traits (CHEUNG; TYREE; DAINTY, 1975). A branch was collected from each sampled tree, and two leaves were taken from it, which were immediately stored in a ziplock plastic bag with moistened paper to prevent transpiration. The leaves were taken to the laboratory where leaf size was measured, and petiole was cut under water. The leaves were hydrated by placing their cut ends in de-ionized water. They were covered with plastic bags to limit water loss and stored overnight (Figure 2) (NGUGI et al., 2003). The following morning, PV curves were built, based on weight and water potential (Ψ_w) measurements, following the bench drying method (SACK; PASQUET-KOK; CONTRIBUTORS, 2011). The leaves were weighed using a precision analytical balance, and the Ψ_w measurements were performed using a pressure chamber (PMS Instruments, Albany, OR, USA) (SCHOLANDER et al., 1965). Leaves were repeatedly measured up to 12 times or until the Ψ_w values were < -4 MPa. Leaves were dried in

dark conditions, to induce stomatal closure, allowing to subsequently derive g_{min}. After completion of the PV curve measurements, the leaves were oven-dried at 65 °C for 72 h to obtain dry weight.

In addition to Ψtlp_{pv}, we extracted from the PV curves the Relative Water Potential at turgor loss (RWC_{tlp}, unit percentage), Modulus of Elasticity (ε, unit MPa), Osmotic Potential at full turgor (π_o, unit MPa), Apoplastic fraction (a_f, unit percentage) following (BARTLETT; SCOFFONI; SACK, 2012a). PV curves were fit using the "pvldcurve" R package (RAESCH, 2020).

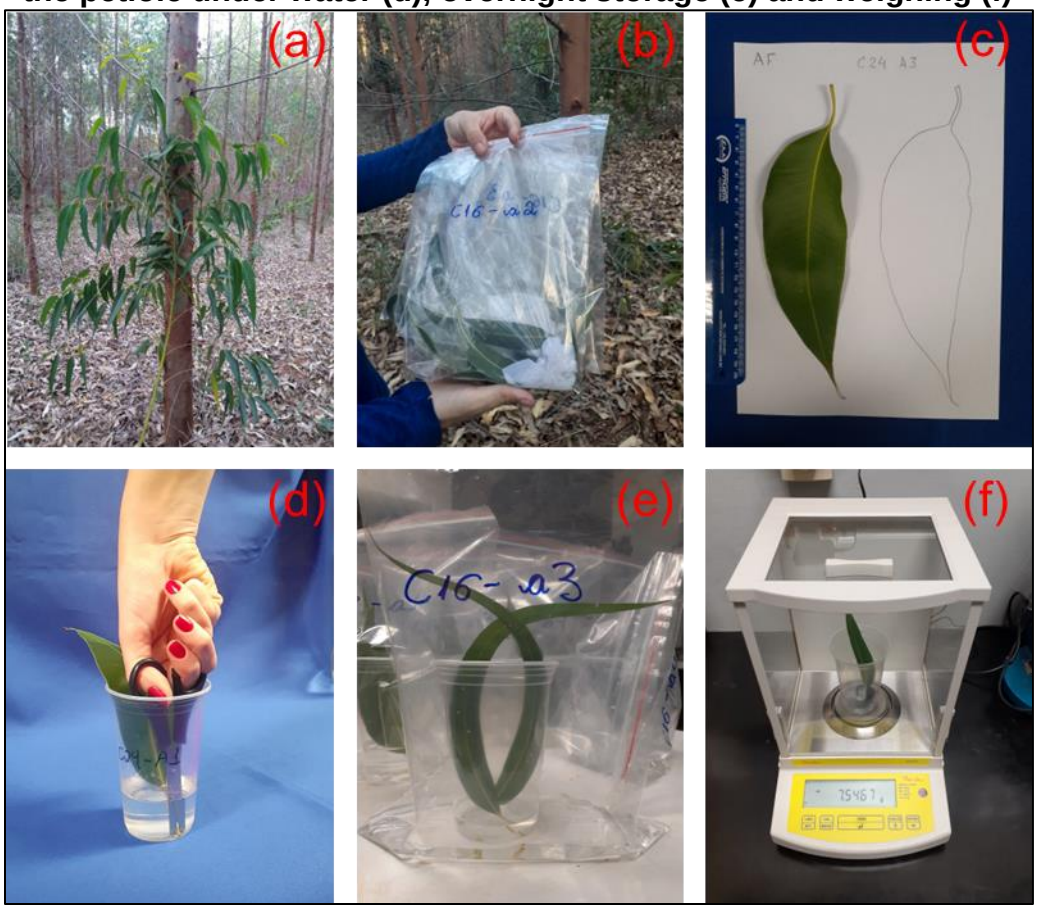
Leaf minimum transpiration was calculated as the slope of water loss *versus* time, normalized by the total leaf surface area. For the slope estimation, only the linear part of the regression was used (i.e., after complete stomatal closure). The value of g_{min} was calculated as the ration between transpiration and the mole fraction gradient in water vapor from the leaf to air, assuming the leaf internal air to be fully saturated (i.e., vapor pressure deficit, MACHADO et al., 2021). Leaf fresh mass, leaf size, and leaf dry mass were used to calculate leaf dry mass per area (LMA; g m^{-2}) and leaf dry matter content (LDMC; g g^{-1}).

1.2.4 Osmometer measurements

We measured the leaf turgor loss point using the linear analysis relationship established with the osmotic potential at full hydration (π_o) with the vapor pressure osmometer (Ψ_{tlposm}; VAPRO 5600, Wescor, Logan, UT, USA) (Bartlett, Scoffoni, Ardy, et al., 2012).

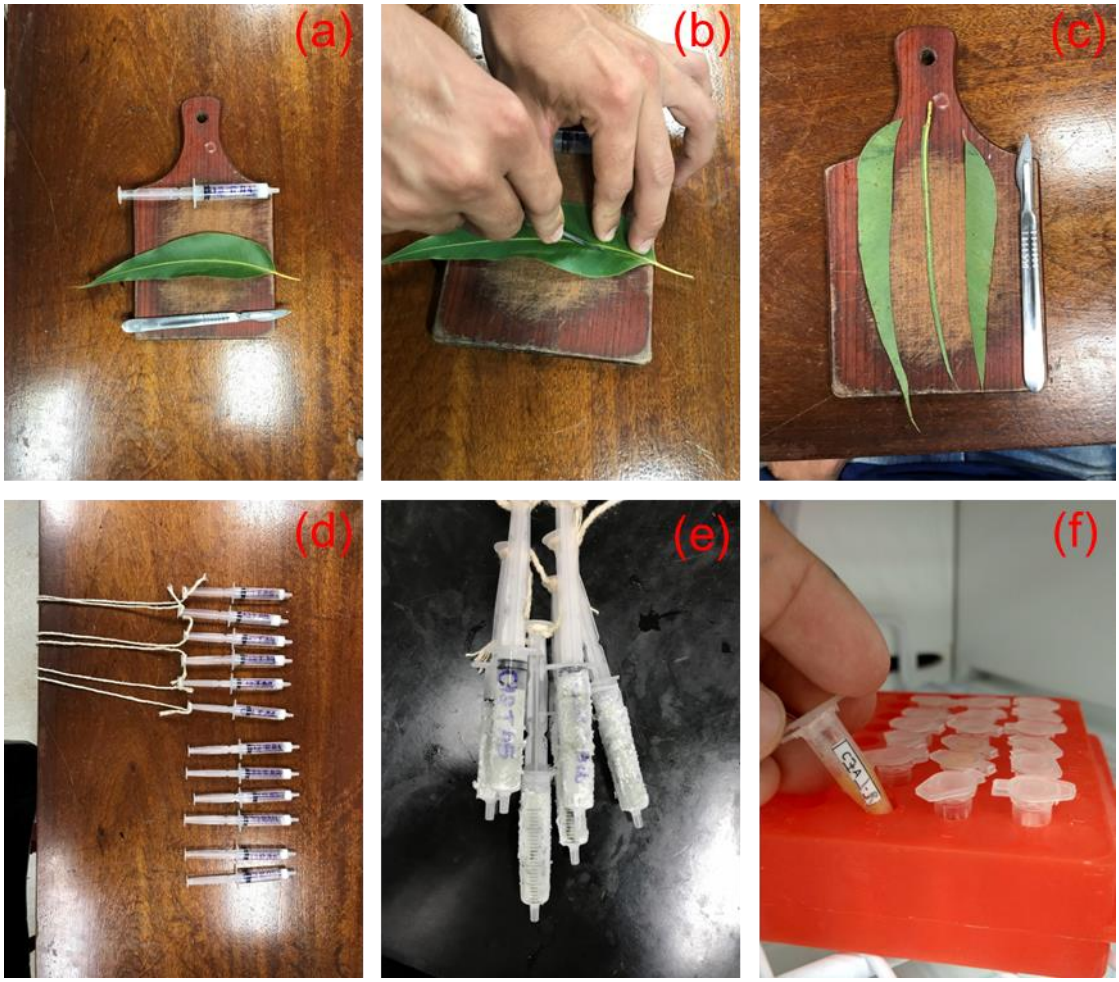
From each sampled tree, we collected three leaves (Fig.2a), which were immediately stored in a plastic ziplock bag with moistened paper (Fig.2b). In the laboratory, we cut the petiole of each leaf in fresh deionized water and let it rehydrate overnight (Fig.2d, 2e).

Figure 2 - Data collection procedures for osmometer measurements. Collection of branches (a), storage of leaves (b), drawing and identification (c), cutting of the petiole under water (d), overnight storage (e) and weighing (f)



We then weighted the leaves (Fig.2f) and immediately cut the midrib and place the three leaves inside a common syringe (Fig.3a-d). The leaves were left for approximately 5 minutes in nitrogen (N_2) (Fig.3e). After that, we waited 1 minute for thawing and pressed the syringe to extract the solution from the leaves, that were immediately placed in Eppendorf and in the freezer until osmometer measurement (Fig.3f).

Figure 3 - Procedure for leaf sap extraction using the freeze-thaw method. Choosing the leaf (we use two leaves per syringe; a), cutting and excluding the midrib of the leaf (b, c), inserting the leaves into a syringe, and adding a string to help (d), freezing the leaf sap (e), thawing and storage of the samples until the measurement of the osmotic potential in the VAPRO Osmometer (f)



The solute concentration value (c_0; units mmol kg^{-1}) was recorded from the osmometer when the difference between consecutive 2 min measurements fell below 5 mmol kg^{-1} (MARÉCHAUX et al., 2015). c_0 was converted to π_o values using van't Hoff equation:

$$\pi_o = c_0 * \frac{-2.5}{1000} \quad (1)$$

where 1000 mmol kg^{-1} = 2.5 MPa, and c_0 is the osmotic potential in mmol kg^{-1} obtained by the osmometer.

We estimated the Ψtlp_{osm} value from π_{osm} using the following equation established by Bartlett, Scoffoni, Ardy, et al., 2012:

$$\psi \mathrm{tlp}_{osm} = 0.832 * \pi_{osm} - 0.631 \quad (2)$$

1.2.5 Drought tolerance index

A drought tolerance index was used to rank the 21 studied genotypes. The index included three categories: drought sensitive (DS), moderately drought tolerant (MDT) and drought tolerant (DT). Genotypes were attributed to one category based on the aridity of the region of origin of the different species (FLORES et al., 2018), the aridity of the region of breeding in Brazil (ALVARES et al., 2013), and the recommendation of use in the Brazilian planting industry (*personal communication*).

1.2.6 Statistical analyses

First, we checked whether there was a difference between coppice and high forest stands for all traits. Having confirmed that there was no significant difference, we pooled the data and performed analysis without considering the effect of management.

All traits obtained from the PV curve and leaf traits were tested for normality and homoscedasticity tests (ANDERSON; DARLING, 1954; BARTLETT; KENDALL, 1946; SHAPIRO; WILK, 1965). Two outliers were eliminated, for which data value were obviously outside of the plausible range.

We tested the effect of the seasons (dry and wet) on the 21 *Eucalyptus* genotypes using paired t-test for all leaf traits obtained from PV curves. We did not include g_{min} in this analysis because we did not measure it in the dry period. We tested whether there were differences in traits among the drought tolerance groups by using ANOVA.

To compare PV curves-based and osmometry-based estimates, we used paired *t*-test and Spearman's rank correlations. We finally aimed to test whether Ψtlp_{pv} can be predicted from πo_{osm}. First, we tested the hypothesis that Ψtlp_{pv} and π_{opv} are strongly correlated traits, i.e., leaf turgor loss point is largely controlled by leaf osmotic potential at full turgor among *Eucalyptus* materials. Secondly, we evaluated the correlation between πo_{pv} and $\pi oosm$ on one hand, and between Ψtlp_{pv} and Ψtlp_{osm} on the other hand, Ψtlp_{osm} being estimated from $\pi oosm$ using the generic Bartlett et al., 2012b relationship. Lastly, we evaluated the correlation between $\Psi tlppv$ and $\pi oosm$ to

explore whether a specific equation for *Eucalyptus* could be more powerful in prediction TLP from osmometry than the generic equation from Bartlett et al., 2012.

Additionally, we tested whether LMA, LDMC, a_f and ε alone were correlated with Ψtlp_{pv} and could be used to improve the model predicting Ψtlp_{pv} from πo_{osm} (equation 4).

$$\psi tlp_{pv} \sim \pi o_{osm} * (\mathrm{LMA} + \mathrm{LDMC} + \mathrm{af} + \varepsilon) \quad (4)$$

Model selection was performed using a maximum likelihood selection, where models were compared using the Second-order Akaike information criterion (AICc). The model with the lowest AICc value, and differences >2 in AICc values was considered the final model (BURNHAM, 1998).

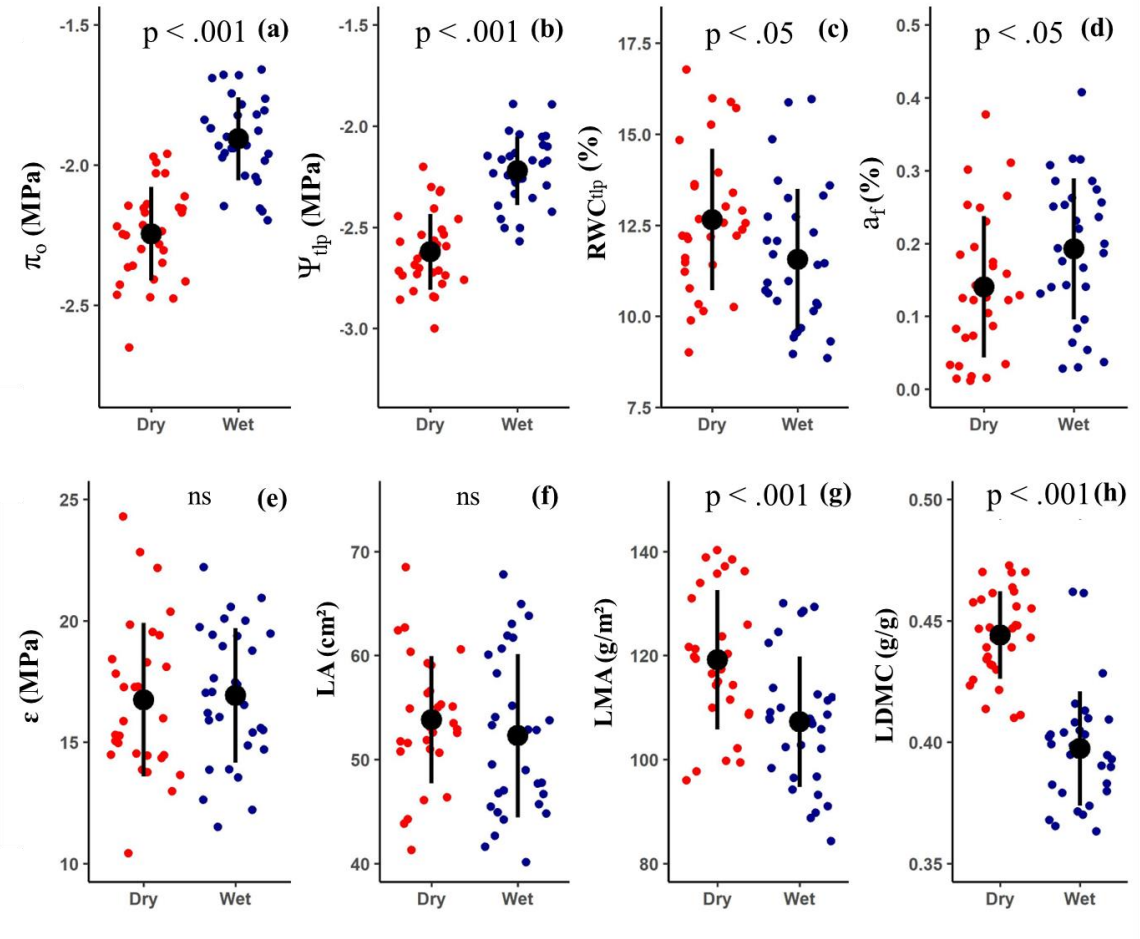
1.3 RESULTS

1.3.1 Leaf trait seasonal plasticity

Several studied traits exhibited significantly different average values between dry and wet season: π_o, Ψ_{tlp}, RWC_{tlp}, and a_f (Fig. 4). The average difference of Ψ_{tlp}, π_o and RWC_{tlp} across seasons was 0.44 MPa (confidence intervals = 0.32 to 0.56), 0.38 MPa (confidence intervals = 0.28 to 0.49) and -0.95 (confidence intervals = -2.25 to 0.96), respectively. By contrast, a seasonal plasticity was not observed for ε (Fig. 4.e). Regarding leaf traits, LMA (fig. 4.g) and LDMC (fig. 4.h) were significantly different between season, while LA was not (fig. 4.f).

In line with Hypothesis 1, Ψ_{tlp} was significantly reduced by 18% in the dry season, along with the osmotic potential at full hydration (15%), the relative water content at turgor loss point (9%), and the apoplastic fraction (26%). Leaf area of *Eucalyptus* genotypes did not vary between seasons. Conversely, the leaf mass per area increased in the wet season (12%), and leaf dry matter content was higher in the dry season in the same proportion (12%).

Figure 4 – Season plasticity of osmotic potential at full hydration (a, π_o), leaf turgor loss point (b, Ψ_{tlp}), relative water potential at turgor loss (c, RWC_{tlp}), modulus of elasticity (d, ε) and apoplastic fraction (e, a_f), leaf area (f, LA), leaf mass per area (g, LMA), and leaf dry matter content (h, LDMC) in *Eucalyptus* genotypes. Black points are average and error bars are standard errors Colors indicate seasons: dry (red) and wet (blue) season. The p indicates p-value, and ns, non-significant difference by paired *t*-test to confidence intervals at 95%



1.3.2 Correlations among leaf traits

We observed a strong negative correlation between Ψtlp_{pv} and LMA (r= -0.51, p < .001) and Ψtlp_{pv} and LDMC (r= -0.76, p < .0001). Contradictorily, the turgor loss point obtained using the osmometer (Ψtlp_{osm}) did not correlate with the LMA and LDMC (Fig.5e and 5f). π_o behave in the same way as Ψtlp, exhibiting significant correlation with LMA and LDMC when measured with PV, but no relationship when measured by osmometry. There was a strong positive correlation between LMA and LDMC (Fig. 6; r= 0.73, p < .0001).

Figure 5. Pearson correlation (r) between water potential at turgor loss point (Ψ_{tlp}) using Pressure-Volume curve and Osmometer with leaf functional traits, i.e., leaf mass per area (LMA) and leaf dry matter content (LDMC) in 21 commercial *Eucalyptus* genotypes (± SE, n = 252). Figures a, b, c, and d include dry and wet season data. Figures e, f, g, and h include data for the dry season only. Blue dashed line is adjusted linear model and confidence intervals is in gray. Black points are average and error bars are standard errors

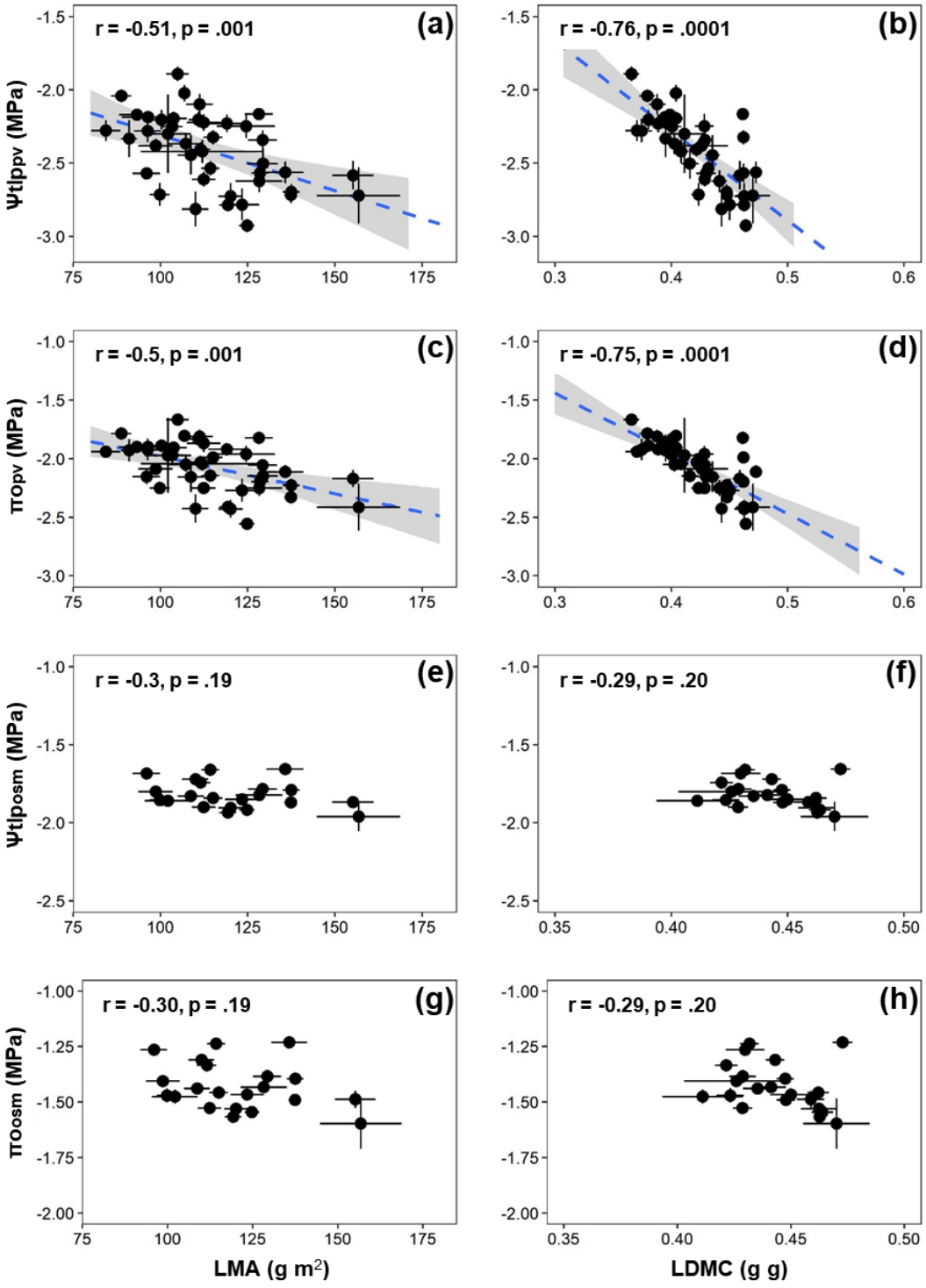
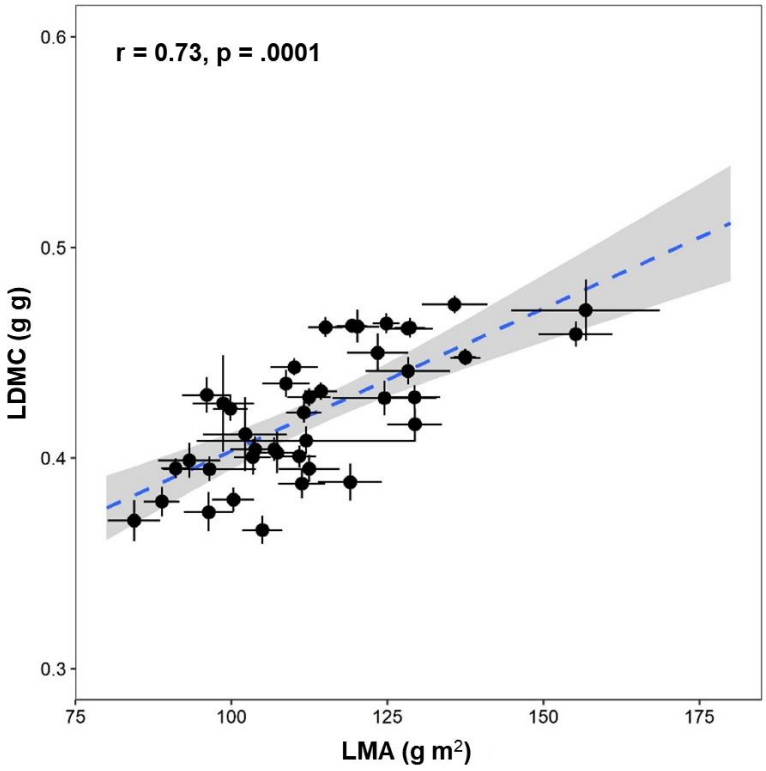


Figure 6. Pearson correlation (r) between leaf functional traits, i.e., leaf mass per area (LMA) and leaf dry matter content (LDMC) in 21 commercial *Eucalyptus* genotypes (± SE, n = 252). Results include dry and wet season data. Blue dashed line is adjusted linear model and confidence intervals is in gray. Black points are average and error bars are standard errors



1.3.3 Leaf traits and drought index

Our second hypothesis was supported: the Ψtlp_{pv} was on average lower in drought-tolerant and moderately drought-tolerant than in drought-sensitive genotypes. However, this was only the case for dry season Ψtlp_{pv} data (Figure 6).

Because it is a trait strongly linked to Ψtlp_{pv}, the πo_{pv} also showed a significant difference between the drought tolerant groups, where the drought sensitive genotypes were less negative than the other two more drought tolerant groups. No other traits exhibited differences among drought tolerance groups, regardless of the season (Table 3). The LMA and LDMC, which showed strong correlation with Ψtlp_{pv} (Figure 5), was also not significantly different among the drought tolerance groups (Table 3). Conversely, the turgor loss point obtained using the osmometer was not able to rank the drought tolerance groups among *Eucalyptus* genotypes. Indeed, Ψtlp_{osm} and πo_{osm} were not significantly different among drought tolerance groups.

Table 3. Differences in leaf water relation traits between drought tolerance groups of commercial *Eucalyptus* genotypes measured in the dry season (Drought sensitive – DS; moderate drought tolerant – MDT; and drought tolerant – DT). Water potential at turgor loss point (Ψtlp_{pv}), osmotic potential (πo_{pv}), relative water potential at turgor loss (RWC_{tlp}), modulus of elasticity (ε), and apoplastic fraction (a_f) are traits obtained from Pressure-Volume curves; leaf area (LA), leaf mass per area (LMA), and leaf dry matter content (LDMC) minimum conductance (gmin) at stomata closure, turgor loss point (Ψtlp_{osm}) and osmotic potential (πo_{osm}). Data are mean (± standard error). Different letters in line means significant difference by LSD test ($p < 0.05$)

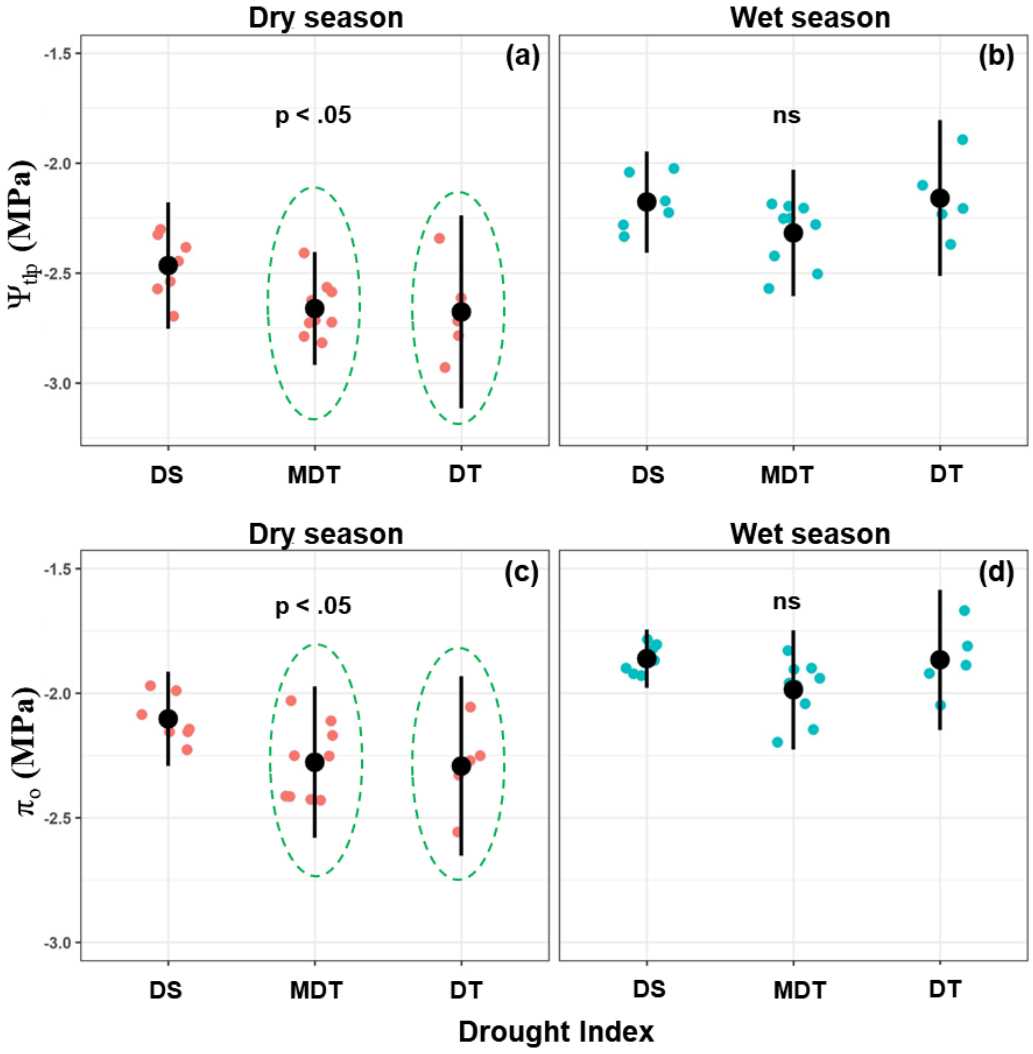
	DS (n = 7)	MDT (n = 9)	DT (n = 5)
Ψtlp_{pv} (MPa)	-2.47 (± 0.05) a	-2.66 (± 0.04) b	-2.68 (± 0.10) b
πo_{pv} (MPa)	-2.10 (± 0.36) a	-2.28 (± 0.51) b	-2.29 (± 0.81) b
RWCtlp (%)	12.86 (± 0.49) a	13.06 (± 0.91) a	12.27 (± 0.65) a
ε (MPa)	16.80 (± 1.35) a	17.21 (± 1.73) a	17.38 (± 1.95) a
a_f (%)	0.15 (± 0.02) a	0.14 (± 0.04) a	0.14 (± 0.04) a
LA (cm^2)	56.78 (± 2.28) a	52.74 (± 4.57) a	55.07 (± 2.80) a
LMA ($g\ cm^2$)	110.36 (± 5.32) a	126.32 (± 6.59) a	125.48 (± 4.07) a
LDMC ($mg\ g^1$)	0.43 (± 0.01) a	0.45 (± 0.01) a	0.44 (± 0.01) a
Ψtlp_{osm} (MPa)	-1.78 (± 0.03) a	-1.83 (± 0.03) a	-1.86 (± 0.02) a
πo_{osm} (MPa)	-1.38 (± 0.04) a	-1.44 (± 0.04) a	-1.48 (± 0.03) a
gmin ($mmol\ m^{-2}\ s^{-1}$)	4.75 (± 0.42) a	5.76 (± 0.53) a	7.08 (± 1.73) a

The traits obtained of the Pressure-Volume curve and leaf functional traits did not show significant difference between drought tolerance groups of *Eucalyptus* genotypes in the wet season (Table 4; Figure 7b and 7d).

Table 4. Differences in leaf water relation traits between drought tolerance groups of commercial *Eucalyptus* genotypes measured in the wet season (Drought sensitive – DS; moderate drought tolerant – MDT; and drought tolerant – DT). Water potential at turgor loss point (Ψtlp_{pv}), osmotic potential (πo_{pv}), relative water potential at turgor loss (RWC_{tlp}), modulus of elasticity (ε), and apoplastic fraction (a_f), leaf area (LA), leaf mass per area (LMA), and leaf dry matter content (LDMC) were evaluated. Data are mean (± SE, n = 252). Different letters in line means significant difference by LSD test ($p < 0.05$)

	DS (n=7)	MDT (n =9)	DT (n =5)
Ψ_{tlp} (MPa)	-2.18 (± 0.04) a	-2.32 (± 0.05) a	-2.16 (± 0.08) a
π_o (MPa)	-1.86 (± 0.02) a	-1.99 (± 0.04) a	-1.87 (± 0.06) a
RWCtlp (%)	11.74 (± 0.77) a	12.36 (± 0.59) a	10.43 (± 0.25) a
ε (MPa)	16.53 (± 1.12) a	16.89 (± 0.65) a	17.53 (± 0.56) a
a_f (%)	0.19 (± 0.04) a	0.15 (± 0.03) a	0.22 (± 0.02) a
LA (cm^2)	55.74 (± 2.67) a	54.02 (± 3.82) a	52.69 (± 2.76) a
LMA (g cm^2)	102.42 (± 5.39) a	110.37 (± 5.08) a	108.56 (± 3.16) a
LDMC (g g)	0.40 (± 0.01) a	0.41 (± 0.01) a	0.38 (± 0.01) a

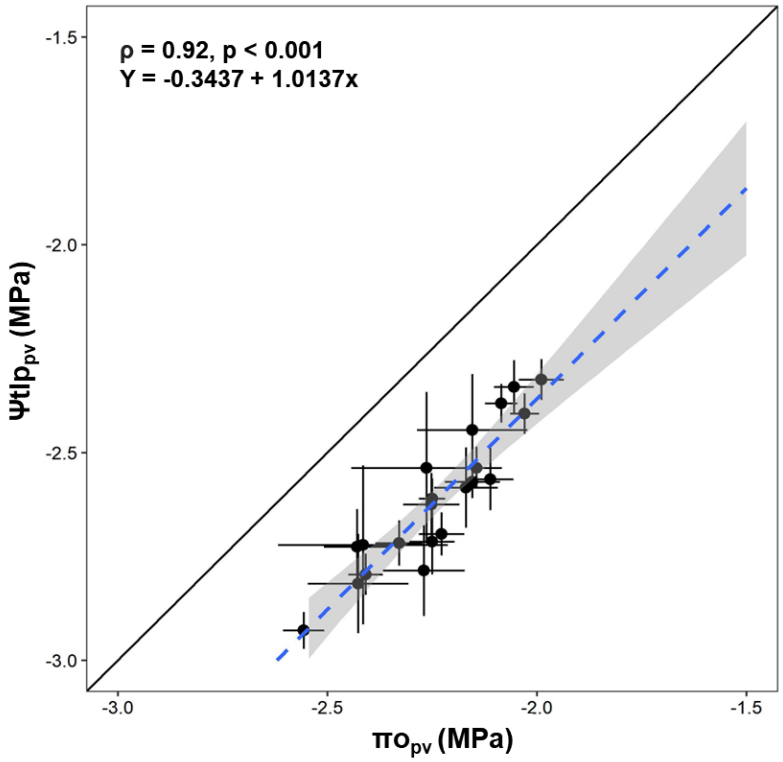
Figure 7 - Turgor loss point (Ψ_{tlp}) and osmotic potential at full hydration (π_o) are predictors of drought tolerance of commercial *Eucalyptus* genotypes in the dry season using Pressure-Volume curves. DS (Drought sensitive), DT (Drought tolerant) and MDT (moderate drought tolerant) are drought index levels; Red and blue points are dry (a, c) and wet (b, d) season, respectively; green circles are groups that showed significant difference by LSD test (p < 0.05)



1.3.4 Osmometer method validation for *Eucalyptus* genotypes

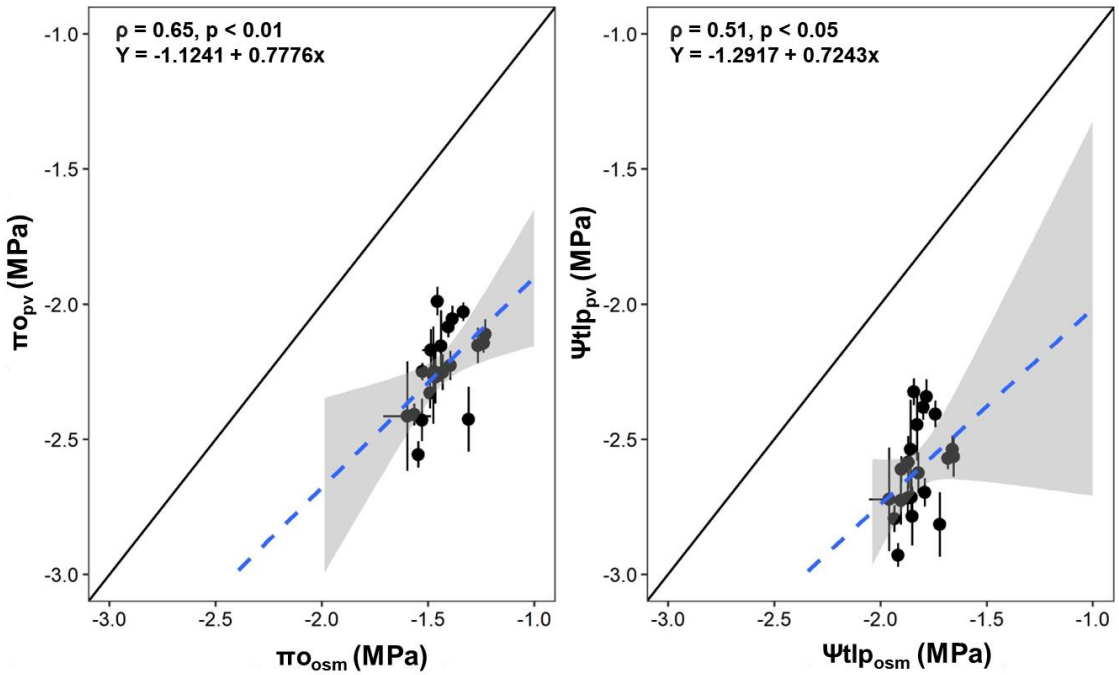
As expected, Ψtlp_{pv} and πo_{pv} of *Eucalyptus* genotypes were strongly correlated using pressure-volume curves (Figure 8, $\rho = 0.92$, $p < 0.001$).

Figure 8 - Measurements of water potential at turgor loss (Ψtlp_{pv}) and osmotic potential at full hydration (πo_{pv}) from the pressure-volume (π_{pv}) curve for *Eucalyptus* genotypes. Results from the π_{pv} shown strong correlation ($\rho = 0.92$; p-value < 0.001) between both variables by the Spearman's rank correlation. Black solid line is 1:1 line (Y = -0.3437 + 1.0137 x); blue dashed line is adjusted linear model, and confidence intervals in gray



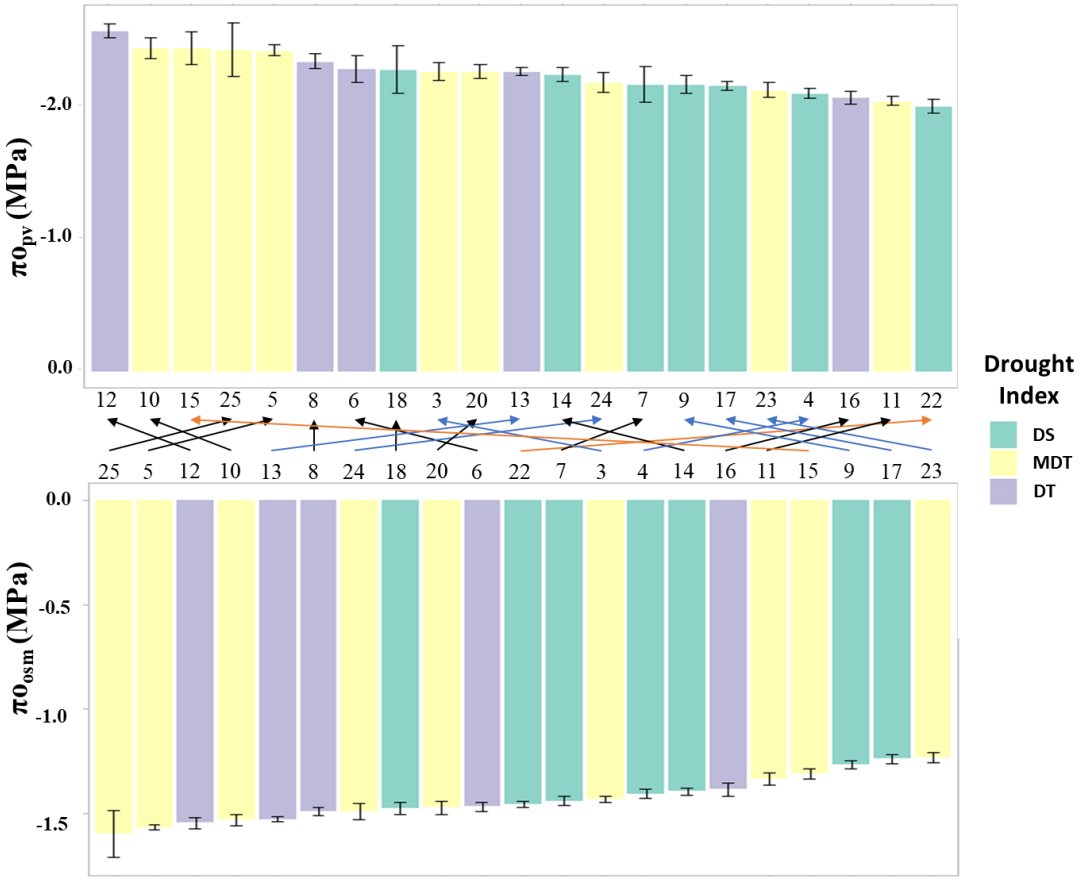
We compared π_o measured from PV curves and derived from osmometer, as well as Ψ_{tlp} measured from PV curves and derived from osmometer to evaluate the potential of osmometer for fast water relation phenotyping in *Eucalyptus* genotype. (Figure 9). πo_{osm} and Ψtlp_{osm} (derived from πo_{osm} and the generic equation from Bartlett et al. 2012b) exhibited significant but relatively weak correlation with πo_{pv} and Ψtlp_{pv}, respectively (for πo_{osm} - Ψtlp_{osm}, Fig. 9a, $\rho = 0.65$, $p < 0.01$, $n = 21$; for πo_{pv} -Ψtlp_{pv}, Fig. 9b, $\rho = 0.51$, $p < 0.05$, $n = 21$).

Figure 9 - Correlation between leaf water relation traits derived from PV curves and osmometer across 21 commercial *Eucalyptus* genotypes. In the left: Osmotic potential at full hydration (πo_{osm}) obtained using osmometer (π_{osm}) plotted against osmotic potential at full hydration (πo_{pv}) from the pressure-volume (π_{pv}) curve. In the right: water potential at turgor loss (Ψtlp_{osm}) made using osmometer (π_{osm}) plotted against water potential at turgor loss (Ψtlp_{pv}) from the pressure-volume (π_{pv}) curve. Black solid line is 1:1 line, blue dashed line is adjusted linear model, confidence intervals are in gray



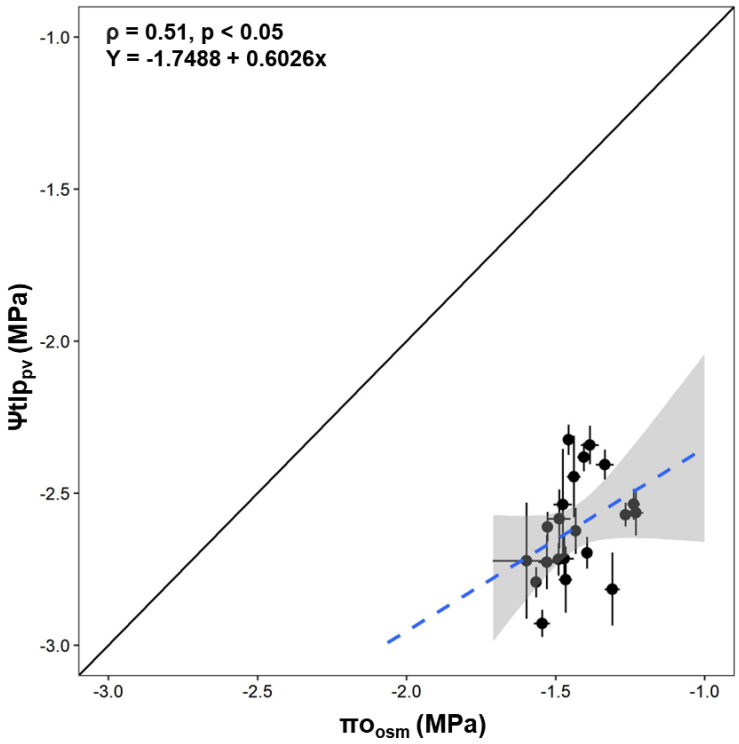
As a consequence of the relatively weak correlation, genotype ranking based on πoosm or Ψtlposm differed substantially. We observed that 42% of the *Eucalyptus* genotypes showed considerable variation in terms of their position, and only *ca.* 10% of the genotypes showed had the same position in the two rankings (Figure 10). We note that in 10 genotypes that presented the most negative values of π_o (below the average of -1.43 and -2.24 MPa for the osmometer and PV curve, respectively), 8 presented comparable ranking between methods. Therefore, our results suggest that methods were more consistent for genotypes with negative π_o.

Figure 10 – Correlation between osmotic potential (π_o) measured using osmometer (πo_{osm}) and Pressure-Volume (πo_{pv}) curves. Colors identify the drought index classification of the genotypes: drought sensitive (DS; green), moderate drought-tolerant (MDT; yellow), and drought tolerant (DT; purple). Each number represents a *Eucalyptus* genotype and arrows link a genotype to itself. Black arrows mean that the genotype ranks were comparable; Blue arrows mean that the genotype ranks were moderately different; Orange arrows mean that the genotype ranks were very different



Finally, we tested whether our dataset could be used to propose a new equation linking Ψtlp_{pv} and πo_{osm}, which would be specific to commercial *Eucalyptus* genotypes. We found a relationship that, although significant ($p < 0.05$), has a weak explicative power ($\rho = 0.51$) (Figure 11).

Figure 11 – Correlation between leaf water potential at turgor loss (Ψtlp_{pv}) derived from pressure-volume curves (Ψtlp_{pv}) and osmotic potential at full turgor (πo_{osm}) measurements derived from osmometer in commercial *Eucalyptus* genotypes



We tested whether LMA, LDMC, a_f and ε could help predict Ψtlp_{pv} from πo_{osm}. The final model resulting from the maximum likelihood selection included only LDMC (AICc = -74.89). Consequently, although LDMC is on its own a good predictor of Ψtlp_{pv} in our dataset (Fig. 5), no additional physiological traits or traits representing leaf structural investment improved the Ψtlp_{pv} – πo_{osm} relationship.

1.4 DISCUSSION

We aimed at exploring the potential of leaf water relation traits for fast phenotyping in the *Eucalyptus* genus. We found that π_o and Ψ_{tlp} exhibit a significant seasonal plasticity. Only the measurements conducted at the end of the dry season were associated to some extent with the expected drought tolerance of the *Eucalyptus* genetic materials. Although we confirmed that variation in Ψ_{tlp} is mostly driven by variation in π_o across *Eucalyptus* material, the osmometer method was not able to accurately rank drought tolerance among the genotypes of our common-garden experiment. Overall, our results suggest that the use of osmometer method, and more

generally, the use of water relation traits for fast screening of drought tolerance in commercial *Eucalyptus* breeding programs deserve further scrutiny.

1.4.1 *Eucalyptus* leaf water traits show high phenotypic plasticity between dry and wet season

Our results showed that on average π_o was significantly lower in the dry than in the wet season across genotype of *Eucalyptus*. As variation in Ψ_{tlp} was mostly driven by variation in π_o, this osmotic adjustment resulted in lower Ψ_{tlp} at the end of the dry season. Lower Ψ_{tlp} is associated with a larger leaf operating range under water stress and can be interpreted as a proxy for higher drought tolerance. Therefore, changes in leaf solute during drought can be seen as an adaptive phenotypic plasticity promoting persistence under contrasting environmental conditions (NIINEMETS, 2020; GHALAMBOR et al., 2007). This is also corroborated by the fact that the average difference between Ψ_{tlp} measured in the wet and dry season (i.e., a measure of Ψ_{tlp} phenotypic plasticity) tended to be higher ($p < 0.1$) in the drought tolerance than in the drought sensitive index group (*result not shown*). This genotype-by-environment interaction is another observation of intraspecific variation in phenotypic plasticity under variable water availability in *Eucalyptus* (ASPINWALL et al., 2015; CONTI JUNIOR et al., 2020; DUTKOWSKI; POTTS, 2012; SILVA et al., 2004). This mounting body of evidence paves the way for the use of intraspecific (e.g., variety, clone, provenance) variation in drought tolerance in breeding programs to sustain *Eucalyptus* production under climate change (ARNOLD; KRUUK; NICOTRA, 2019; NICOTRA et al., 2010).

In line with a previous study, we observed that only traits measured at the end of the dry season was able to inform on drought tolerance (CONTI JUNIOR et al., 2020). Consequently, the phenotypic plasticity of leaf traits we report here should be accounted for when designing measurement protocols aiming to charactered drought tolerance in improved *Eucalyptus* materials. Another limitation of Ψ_{tlp} is that tree species operate within a limited range of stomatal regulation, and, therefore, a limited range of Ψ_{tlp} (GUILLEMOT et al., 2022; MARTIN-STPAUL; DELZON; COCHARD, 2017b). As a consequence, Ψ_{tlp} is commonly less able to capture differences across species originating from very contrasted climate of origin than e.g., the xylem resistance to embolism, another trait commonly related to drought tolerance (e.g.,

GUILLEMOT et al., 2022; LI et al., 2019; MARTIN-STPAUL; DELZON; COCHARD, 2017b). It is also striking that the 21 genotypes included in our common-garden experiment exhibited a relatively limited range of Ψ_{tlp}. This contrasts with the wide range of drought strategies observed in naturally occurring *Eucalyptus* species originating from contrasted climate (LI et al., 2019; PFAUTSCH et al., 2016). Therefore, it is possible that the genetic improvement through breeding conducted on commercial *Eucalyptus* genotypes, and the selection of highly productive species and varieties resulted in a limited range of leaf water traits among the studied genotypes, reducing the explicative power of trait-drought tolerance correlations.

Finally, our results also question the way we *a priori* determine the expected drought tolerance when studying commercial *Eucalyptus* genotypes. Indeed, studies conducted in natural *Eucalyptus* forests commonly use the climate of the species spatial distributions ("climate-of-origin") as a reference of drought tolerance (LI et al., 2019; PFAUTSCH et al., 2016). However, commercial *Eucalyptus* genotypes are often hydrides of two species, and several generations of breeding programs most probably changed substantially their performances compared to the original materials. It is likely that the climate and soil conditions of the site where breeding was conducted also impacted the final drought tolerance and performances of the materials, but this remain to be quantified. Previous studies on cultivated *Eucalyptus* generally use expert knowledge to rank *a priori* the drought tolerance of the materials, and the same was done here. However, this does not allow to integrate results from different studies into a common metrics that could be used by practitioners. This indicates that we need more observations of drought-induced tree mortality in commercial *Eucalyptus* stands in order to build robust predictions. Another way forward would be to conduct a more extensive measurements of drought-related traits, and to incorporate them in a state-of-the-art water relation model (e.g., COCHARD et al., 2021) in order to obtain an integrated indicator of drought tolerance. This will allow further quantifying the relative importance of leaf traits in capturing drought tolerance patterns across genotypes.

1.4.2 The osmometer method was not able to adequately rank drought tolerance among the studied genotypes

We found that the – easily measured - "soft trait" LMA and LDMC were good predictors of Ψ_{tlp} derived from PV curves across commercial *Eucalyptus* genotypes.

This was previously reported from datasets including very contrasting species (GRIFFIN-NOLAN et al., 2019; MÁJEKOVÁ et al., 2021), but is not universally observed (MARÉCHAUX et al., 2020). One common explanation for the Ψ_{tlp} - LDMC relationship is that in dry conditions, water stress simultaneously results in the increase of leaf simplistic solute and smaller, more lignified, cell expansion and, therefore, greater LDMC. The very good relationship we obtained across the studied materials warrants further investigation on how it could be used in breeding program for a first screening of drought tolerance.

By contrast, and contrary to our working hypothesis, we found no evidence that the osmometer method can adequately rank drought tolerance among the studied genotypes. Although very significant, the relationship had a low explicative power, leading to numerous discrepancies compared to the ranking derived from PV curves. In addition, 0 from osmometer was not different on average between the drought tolerance groups, in contrast to Ψ_{tlp} derived from PV curves. Overall, these results suggest that 1) the ability of Ψ_{tlp} to rank drought tolerance across the studied materials is significant by limited (as discussed above) and 2) the additional error associated with the osmometer method compared to PV curves is sufficient to completely alter Ψ_{tlp} predictions. This result contrasts with a body of studies reporting good correlation between both methods (BARTLETT et al., 2012; CONTI JUNIOR et al., 2020; GRIFFIN-NOLAN et al., 2019).

Two main explanations can be suggested for the poor performance of the osmometer method in our case. First, two different samplings have been suggested for use with the osmometer method: the sampling of leaf discs or the extraction of press sap. The relative merit of both sampling is a long-standing debate (GRANGE, 1983; KIKUTA; RICHTER, 1992). Leaf discs could contain, on average, a smaller amount of apoplastic water than the saps. This is in agreement with the offset we found in the $\Psi tlp_{osm} - \Psi tlp_{pv}$ relationship (where $\Psi tlp_{osm} > \Psi tlp_{pv}$), which is larger than previous leaf-disk studies (BARTLETT et al., 2012; CONTI JUNIOR et al., 2020). On another hand, the press sap sampling is an integrated measurement over several leaves, thus reducing the inter-disk variability observed when measuring separately small areas of individual leaves. Whether the press sap sampling is causing the poor performance of the osmometer method in *Eucalyptus* remain to be tested. Second, as stated above, the Ψ_{tlp} range studied in our study is strikingly more limited than the range used in previous validation of the osmometer method. For example, Bartlett et al. 2012 and

Griffin-Nolan et al. 2019 use in their validation a range of Ψ_{tlp} more than double of what we observed in this study. Conti Júnior et al. 2020 recently published a validation of the osmometer method in commercial *Eucalyptus* genotypes, where they found a good agreement with PV curves. However, their validation include data from both wet and dry seasons. In line with our results, they found that a large fraction of the Ψ_{tlp} variation in their dataset were explained by seasonal phenotypic plasticity rather than by differences among genetic materials. Consequently, adding both wet and dry data in the validation considerably extended the range of Ψ_{tlp}, in contrast to our study. Therefore, it is possible that the osmometer method only presents a limited potential in ranking drought tolerance among materials exhibiting small absolute differences in Ψ_{tlp}. We also note that Banks and Hirons (2019) recently reported an important discrepancy between the drought tolerance ranking of both osmometer and PV curves methods across *Acer* genotypes. Overall, our results suggest that the potential of the osmometer methods to discriminate the drought tolerance of commercial *Eucalyptus* genotypes deserve further scrutiny before to be operationalized in breeding programs.

1.5 CONCLUSION

Commercial *Eucalyptus* genotypes showed high phenotypic plasticity between the dry and wet seasons with the Ψ_{tlp} becoming more negative in the dry season. The Ψ_{tlp} did not differ widely between *Eucalyptus* genotypes because it is possible that the genetic improvement through breeding conducted on commercial *Eucalyptus* genotypes, and the selection of highly productive species and varieties resulted in a limited range of leaf water traits among the studied genotypes, reducing the explicative power of trait-drought tolerance correlations. However, Ψ_{tlp} measured at the end of the dry season is a better predictor of drought tolerance of genotypes. Although we confirmed that variation in Ψ_{tlp} is mostly driven by variation in π_o across *Eucalyptus* material, the osmometer method was not able to accurately rank drought tolerance among the genotypes of our common-garden experiment. Overall, our results suggest that the use of osmometer method, and more generally, the use of water relation traits for fast screening of drought tolerance in commercial *Eucalyptus* breeding programs deserve further scrutiny.

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CHAPTER 2

DO HIGH FOREST AND COPPICE *Eucalyptus* STANDS DIFFER IN DROUGHT TOLERANCE? A COMMON-GARDEN COMPARISON IN 10 CONTRASTED GENOTYPES

2.1 INTRODUCTION

Forests are strongly affected by drought worldwide (ALLEN et al., 2010; GONÇALVES et al., 2017; IPCC, 2021; KLEIN; HARTMANN, 2018). As a consequence, the mechanisms determining tree drought tolerance have been under intense scrutiny since the beginning of the 21st century (ALLEN et al., 2010; DE KAUWE et al., 2022; ETZOLD et al., 2019; GUARÍN; TAYLOR, 2005; KHARUK et al., 2021; PEÑUELAS; LLORET; MONTOYA, 2001). These studies generally predict an important impact of increased drought under future climate change on wood productivity and survival (BRODRIBB et al., 2020), making the understanding of tree drought tolerance mechanisms one of the biggest challenges of our time.

Eucalyptus dominates the hardwood plantations in tropical and subtropical regions (FAO, 2010). In Brazil, it is the most used species in forest plantations, approaching about 7.8 million hectares distributed throughout the territory (IBGE, 2019), with an average above-ground productivity of 36 m^3 ha^{-1} $year^{-1}$ (IBÁ, 2019). Beyond wood supply, *Eucalyptus* stands can have an important role in protecting native forests (CARLE; DUVAL; ASHFORDC, 2020), by substituting wood products from native species for the forest industry, although this positive effect is strongly dependent on local and national legal contexts (PIRARD; DAL SECCO; WARMAN, 2016). However, large drought-related dieback waves were reported in Brazilian *Eucalyptus* plantations (GONÇALVES et al., 2017), which questions management prescriptions for sustainable production under climate change.

After harvest, two management options can be used to initiate the next rotation of the plantation: planting seedlings (hereafter called high forest stand) or managing the resprout shoots growing from the stumps (hereafter called coppice stands). The main advantages of coppice are that it has a lower total cost than high forest rotations and usually exhibits high initial growth, because the stems benefit from an existing and fully developed root apparatus (CHRISTINA et al., 2011; CROMBIE, 1997;

FORRESTER; BERTRAM; MURPHY, 2012; PIVA et al., 2020). By contrast, high forest stands are more onerous, because of the seedling and planting costs (PIVA et al., 2020). However, high forest is more commonly used, because the high productivity and short rotation of *Eucalyptus* plantations in Brazil (typically 6-7 years) allow managers to frequently change the *Eucalyptus* genotype grown at a given site and, therefore, to benefit from the last advances of breeding programs (GONÇALVES et al., 2014).

The way coppice management affect tree drought tolerance and water stress compared to high forests remains largely unexplored. Previous studies suggest that the low root-to-shoot ratio of coppice in the first years of the rotation is not only associated with high growth, but also to high water use and reduced water stress, as observed in *Eucalyptus* sp. (DRAKE et al., 2009; WILDY; PATE; SEFCIK, 2004), as well as in others genus (MATOUŠKOVÁ et al., 2022; ŘEHOŘKOVÁ; KUČERA; GEBAUER, 2022; STOJANOVIĆ et al., 2017). However, tree drought tolerance traits have been shown to exhibit phenotypic plasticity, increasing tree viability under intense water stress (CHALLIS et al., 2022; PRITZKOW et al., 2020). Consequently, if trees managed in coppice experience less water stress in the first years of growth, they could express traits less adapted to survival under drought. This could be of importance for *Eucalyptus* plantations in southern Brazil. Indeed, these plantations usually exhibit high water use and continuous soil water depletion from two years after planting until harvest (CHRISTINA et al., 2017; HUBBARD et al., 2020). Therefore, in these systems, the initial phase of soil water replenishment that occurs in the two first years after harvest as a consequence of low evapotranspiration is crucial for the water availability throughout the rotation. It is likely that coppicing reduces the initial soil water replenishment as a consequence of higher initial growth and deep soil root exploration (DRAKE et al., 2009; WILDY; PATE; SEFCIK, 2004) possibly resulting in more severe water stress at the end of the rotation.

In the last decades, two key traits were shown to efficiently predict drought tolerance in trees. First, turgor loss point (Ψ_{tlp}, units MPa), is the water potential at turgor loss (CHEUNG; TYREE; DAINTY, 1975; MARÉCHAUX et al., 2015). Therefore, the Ψ_{tlp} is associated with wilting, stomata closure, gas exchanges and growth cessation (BARTLETT; SCOFFONI; SACK, 2012; BRODRIBB; HOLBROOK, 2003; CHOAT et al., 2018). The Ψ_{tlp} has been widely used as a proxy of drought tolerance,

as it defines the capacity to maintain function while the plant dehydrates (BARTLETT; SCOFFONI; SACK, 2012).

Another fundamental trait involved in drought tolerance is the water potential that causes xylem dysfunction, which is commonly measured as the water potential causing 50% loss of hydraulic conductance (Ψ_{50}, units MPa) (CHOAT et al., 2012; COCHARD et al., 2008; GUILLEMOT et al., 2022; LARTER et al., 2017; MAHERALI; POCKMAN; JACKSON, 2004; MARKESTEIJN et al., 2011). The vulnerability of the xylem to cavitation strongly varies among species and was shown to be a major determinant of drought resistance in trees (ANDEREGG et al., 2016).

Stomata closing occurs before xylem cavitation, which suggests that avoidance of xylem cavitation is an important mechanism for plant survival under drought (CHOAT et al., 2018). Therefore, the risk of plant hydraulic failure during drought can be measured as the difference between Ψ_{tlp} and Ψ_{50}, which is commonly referred to as Ψ_{tlp}-based hydraulic safety margin (Ψ_{tlp}-based HSM). Another definition of HSM put Ψ_{50} in the context of the maximum water stress actually experienced by the trees, using the difference between Ψ_{50} and the minimum tree water potential (Ψ_{min}-based HSM). These two different HSMs are widely used to quantify different facets of drought risk in trees worldwide (CHOAT et al., 2012; GUILLEMOT et al., 2022; MARTIN-STPAUL; DELZON; COCHARD, 2017; POWERS et al., 2020).

Here, we measured a range of leaf water relation traits (including Ψ_{tlp}), xylem trait (Ψ_{50}), *in situ* leaf water potential, tree size and standing biomass to compare the growth and drought tolerance of 10 *Eucalyptus* genotypes managed as high forest and coppice. Our working hypothesis is that *Eucalyptus* genotypes managed as coppice are less drought tolerant than high forests because they experience less water stress in the first years of the rotation.

2.2 MATERIAL AND METHODS

2.2.1 Site description

The field experiment was set up in 2018 in Itatinga, state of São Paulo, Brazil (22°58’04’’ S and 48°43’41’’ W, 857 m asl). The experiment is part of the Cooperative Program on Productivity and Carbon and Water Flows in *Eucalyptus* (EUCFLUX – https://www.ipef.br/eucflux2/). The previous rotation of the common-garden included

16 genotypes replicated in 10 blocks (LE MAIRE et al., 2019). It was clear-cut in July 2018. Five blocks were replanted with seedlings in December 2018, and four blocks were managed as coppice. Six out of the 16 original genotypes were no more available in the nurseries at the time of planting. Therefore, the common-garden allowed for a comparison between high forest and coppice management for 10 genotypes. The local climate is humid mesothermal (Cwa) according to the Köppen's classification, with an average annual temperature of 19 °C and an average annual precipitation of 1300 mm (ALVARES et al., 2013).

The soil at the site is classified as Typical Dystrophic Red Latosol and Typical Dystrophic Red-Yellow Latosol, both with medium sandy texture, presence of moderate A horizon, kaolinitic and hypoferric and Typical Dystrophic Red Latosol, of clayey texture, presence of moderate A horizon, kaolinic or kaolinitic oxide and mesoferric.

2.2.2 Experimental area and treatments

The experiment used in the study consisted in a common-garden of 10 *Eucalyptus* genotypes managed in high forest and coppice stands (Table 1). Plots were 36 m × 32 m and were planted with one genotype with tree spacing of 3 x 2 m (1666 trees ha^{-1}), totaling 192 individuals. Sampling was only made in the 100 central trees to avoid edge effects. All 10 genotypes are commercial, genetically-improved materials used by the Brazilian *Eucalyptus* planting industry. A description of the genotype can be found in Table 1.

Table 1 - Description of the 10 *Eucalyptus* genotypes arranged under coppice and high forest systems. States: SP - São Paulo, ES - Espírito Santo, MG - Minas Gerais, RS - Rio Grande do Sul, BA – Bahia

Genotype	Species	State of origin	Climate	Minimum temperature (°C)	Mean annual temperature (°C)	Maximum temperature (°C)	Annual precipitation (mm)
3	*E. grandis x E. urophylla*	SP	Cwa	17.1	20.7	23.2	1463
4	*E. grandis x E. urophylla*	SP	Cfa	15.9	19.7	22.5	1336
5	*E. grandis x E. urophylla*	SP	Cwa	17.1	20.7	23.2	1463
6	*E. grandis x E. urophylla*	ES	Aw	20.2	23.4	26.1	1304
8	*E. grandis x E. urophylla*	MG	Aw	19.4	22.6	24.9	1370
10	*E. grandis x E. urophylla*	SP	Cfa	15.4	19.3	22.4	1245
12	*E. urophylla x sp*	MG	Cwb	16.5	19.7	21.7	1180
13	*E. grandis x E. urophylla*	MG	Cwb	16.5	19.7	21.7	1180
14	*E. saligna*	RS	Cfa	13.2	18.4	24.2	1594
16	*E. grandis x E. camaldulensis*	BA	As	22.4	24.7	26.1	1045

Adapted from (LE MAIRE et al., 2019).

2.2.3 Stand inventories

Height (H) and diameter at breast height (DBH, 1.3 m aboveground) were performed at the end of the dry season, in November 2021, the driest period of the year. The stands were at mid-rotation, 3 years after planting. Tree height and circumference at breast height (CBH) of the trees in the central part of all plots in all blocks were measured using an electronic hypsometer and a tape measure, respectively. Plot basal area was calculated based on diameter measurements, assuming circular trunks.

2.2.4 Leaf Area Index (LAI) and standing biomass

A destructive sampling of trees was conducted outside of the inner part of the plots in November 2021 for each genotype in both high forests and coppice. Height trees per genotypes were selected, encompassing the DBH ranges observed in the inventories. Tree leaf area and standing biomass (branches and trunk) were destructively measured, following the protocol described in Le Maire et al. (2019). Allometric relationships between leaf area and DBH, and between tree standing

biomass and CBH were built for each genotype/management and combined with the full DBH inventories to obtain the leaf area index (LAI) and the plot standing biomass.

2.2.5 Leaf Water Potential

We measured leaf water potential at predawn (Ψ_{pd}) between 3 and 5 a.m., and mid-day (Ψ_{md}) between 11 and 1 p.m., using a pressure chamber (PMS Instruments, Albany, OR, USA) (SCHOLANDER et al., 1965). The measurements were performed in October 2021, during the period of maximum annual water deficit. Four trees were selected per clone, and one healthy and fully expanded leaf from a branch located in the middle third of the crown was sampled. Each leaf was quickly (< 1 min) taken to the pressure chamber for water potential measurement. The difference between the Ψ_{pd} and Ψ_{md} values was calculated to obtain the sap flow driving force ($\Delta\Psi$), with the following equation (GUILLEMOT et al., 2021).

$$\Delta\Psi = \Psi\mathrm{pd} - \Psi\mathrm{md} \quad (1)$$

2.2.6 Calculation of the hydraulic safety margin

The HSM is the risk that a plant will experience hydraulic failure in extremely dry conditions (CHOAT et al., 2012). Two main definitions of HSM are found in the literature: (1) the difference between the water potential at the point of loss of turgor closure (Ψ_{tlp}) and the water potential causing 50% loss of hydraulic conductance (Ψ_{50}), hereafter Ψ_{tlp}-based HSM, which quantifies the extent to which early stomatal closure prevents the risk of hydraulic failure for a species (MARTIN-STPAUL; DELZON; COCHARD, 2017); (2) the difference between the minimum water potential (Ψ_{min}) and Ψ_{50}, hereafter Ψ_{min}-based HSM. The Ψ_{min} results from the environmental conditions and the different strategies that allows plants to tolerate water stress (BRODRIBB et al., 2020). As consequence, Ψ_{min}-based HSM put the resistance of the trees in the contexts of the water stress they actually experience in the field (CHOAT et al., 2012). Here, we use Ψ_{md} to represent the minimum water potential (see section 3.2.5).

2.2.7 Pressure-Volume curves (PV curves)

PV curves measurements were conducted in May 2021 and November 2021, corresponding to the maximum and minimum annual water stress. For each genotype

and management, one mature and healthy leaf was sampled from 6 trees (Fig. 1a). After collecting the leaves, we stored them in plastic bags with moistened paper to prevent transpiration and took them to the laboratory (Fig. 1b). In the laboratory, the leaf outlines were quickly drawn (fig. 1c). Then the petioles were re-cut underwater (Fig. 1d) and leaves were left to rehydrate overnight (Fig. 1e, f). In the next day, measurements were carried out in the following steps:

i) leaf weighing before water potential measurement.

ii) leaf water potential measurement.

iii) leaf weighing after water potential measurement.

This process was repeated several times until the leaf water potential reached -3.0 to -4.0 MPa (CONTI JUNIOR et al., 2020). The leaves were weighed using a precision analytical balance, and the water potential were measured using a pressure chamber (PMS Instruments, Albany, OR, USA) (SCHOLANDER et al., 1965). During the measurements, a datalogger was used to obtain data on the temperature and relative humidity of the place. At the end of the measurements, the leaves were placed in an oven dried at 65 °C for 72 h to obtain the dry mass weight.

PV curves constructed from the above measurements were used to extract the parameters Turgor Loss Point (Ψtlp, units MPa), Relative Water Potential at turgor loss (RWC_{tlp}, unit percentage), Modulus of Elasticity (ε, unit MPa), Osmotic Potential (π_o, unit MPa) and Apoplastic fraction (a_f, unit percentage). Leaf minimum transpiration was calculated as the slope of water loss *versus* time, normalized by the total leaf surface area. For the slope estimation, only the linear part of the regression was used (i.e., after complete stomatal closure). The value of g_{min} was calculated as the ration between transpiration and the mole fraction gradient in water vapor from the leaf to air, assuming the leaf internal air to be fully saturated (i.e., vapor pressure deficit, MACHADO et al., 2021). All these analyzes were performed using the "pvldcurve" R package (RAESCH, 2020).

Figure 1 – Pressure-Volume curve workflow: collection of branches (A), storage of leaves in a plastic bag with damp paper to avoid transpiration during transport to the laboratory (B), leaf hand drawing to measure the leaf area later (C), cutting the petiole under water (D), re-storing the samples in a plastic bag under deionized water (E), and finished procedures with leaves rehydrating for 13 h until the next day (F)

We obtained the individual leaf area for each leaf using the LI-3100 AC Area Meter. Finally, we combined the leaf area values with the fresh weight and dry weight of each leaf to obtain, respectively, leaf dry mass per area (LMA; g m^{-2}) and leaf dry matter content (LDMC; mg g^{-1}) using the equations:

$$LMA\ (g\ m^{-2}) = \frac{leaf\ dry\ mass\ (g)}{leaf\ area\ (m^2)} \quad (2)$$

$$LMDC\ (g\ g^{-1}) = \frac{leaf\ dry\ mass\ (g)}{leaf\ fresh\ mass\ (g)} \quad (3)$$

2.2.8 Xylem vulnerability curves

Collections and measurements took place in March 2022, during the wet season, a period of minimum water stress, in order to avoid native embolism in the branches. Before sunset, at a time of relatively high vapor pressure deficit, we sampled one upper branch of approximately 1 meter in 6 trees of each genotype (from the two management systems; hence twelve branches). Branches were bagged in black plastic bags with moistened paper towels to reduce transpiration. In the laboratory, we cut 5 cm of the branch under water, and out them to rehydrate in water buckets overnight (Fig. 2A, B and C).

Figure 2 – Workflow of the Pneumatron measurements

In the next day, we installed the branches in plastic tubes and connected them to the Pneumatron (PEREIRA et al., 2020). The Pneumatron is a device that allows the automatic measurement of gas discharge from the xylem tissue of trees, by combining a vacuum pump driven by a microcontroller and a pressure transducer (PALIGI et al., 2021; PEREIRA et al., 2020; TRABI et al., 2021). We followed the recommendations of Pereira et al. 2020, and automated the Pneumatron to perform air discharge measurements every 15 min. The amount of air discharged into the

reservoir was calculated based on pressure measurement, air temperature and the ideal gas law (PEREIRA et al., 2016).

In addition to using plastic tubing to make the connection, we used glue and adhesive tape to seal any air input/output. This procedure was carried out to ensure that the measured air discharges are exclusively from the branch (Fig. 2D). After installing the branches, automatic measurements were started using the Pneumatron (Fig. 2E). Concomitantly to Pneumatron measurements, we regularly measured the xylem water potential as the branches dried on the bench for approximately 72 h (Fig. 2F). We performed eight to twelve repeated ψ_{xyl} measurements for the same branch during slow dehydration under ambient conditions. We packed the branches for 20 minutes before extracting a leaf to perform the water potential using a pressure chamber (PMS Instruments, Albany, OR, USA) (SCHOLANDER et al., 1965). To prevent the injuries caused by leaf picking from interfering with the gas discharge, we sealed the wound with glue and tape (Fig. 2G).

Vulnerability curves were subsequently fitted between the standardized (%) air discharges and xylem water potential, using a sigmoidal model, and Ψ_{50} was extracted (PEREIRA et al., 2020).

2.2.9 Statistical analysis

Biomass, basal area, height, and water potential were analyzed using the Tukey test ($p < 0.05$). The hydraulic safety margin was analyzed using Anova ($p < 0.05$). The leaf area index between management and genotype was analyzed using the paired t-test ($p < 0.05$).

We tested the differences between coppice and high forest stand using paired *t*-test and Pearson's correlation (r). For traits derived from PV curves, tests were conducted separately for wet and dry season data.

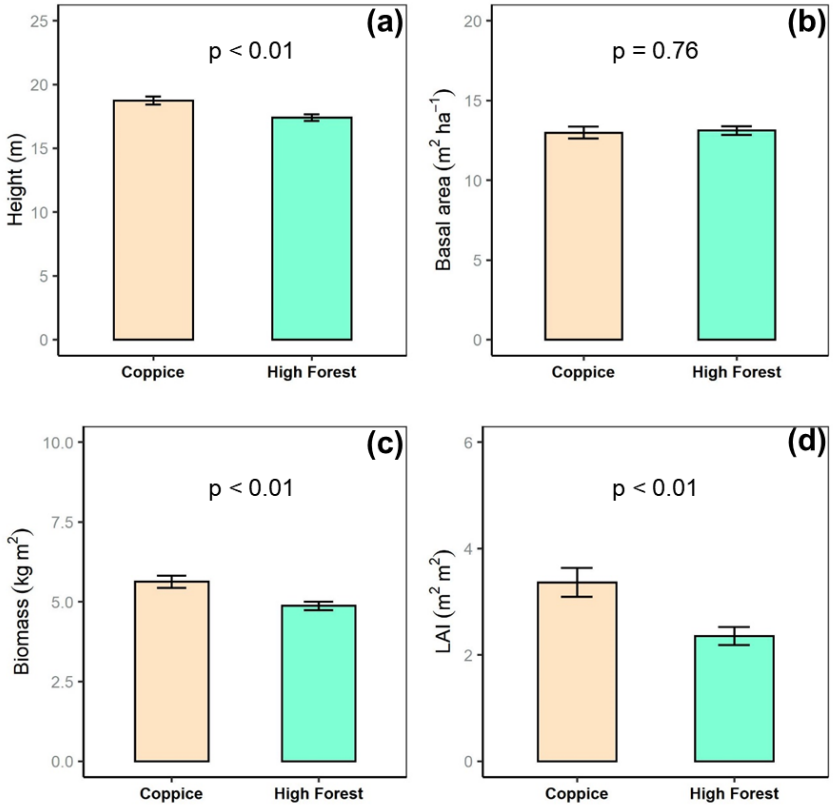
All data were performed using R software version 4.2.1 (R CORE TEAM, 2021), through the “Tidyverse” (WICKHAM et al., 2019), “pvldcurve” (RAESCH, 2020) and “ggsignif” packages (AHLMANN-ELTZE; PATIL, 2021).

2.3 RESULTS

2.3.1 Inventory measurements

Height, biomass production, and leaf area index at stand level were higher for coppice than high forest stand (Fig. 3a, c and d; $p < 0.01$, $n = 1552$; < 0.01, $n = 89$; and < 0.01, $n = 19$; respectively). The average difference for height, biomass and LAI was 7.5, 13.5, and 30%, respectively (Figure 3). Only basal area was not significantly different between the two silvicultural systems evaluated (Fig. 3.b, $p = 0.76$, $n = 89$).

Figure 3 - Height (a), basal area (b), biomass production (c), and leaf area index (d) of 10 *Eucalyptus* genotypes under high forest and coppice stand (± standard error). Colors are high forest (green) and coppice (orange) stand. Above bars are p-value using Tukey test

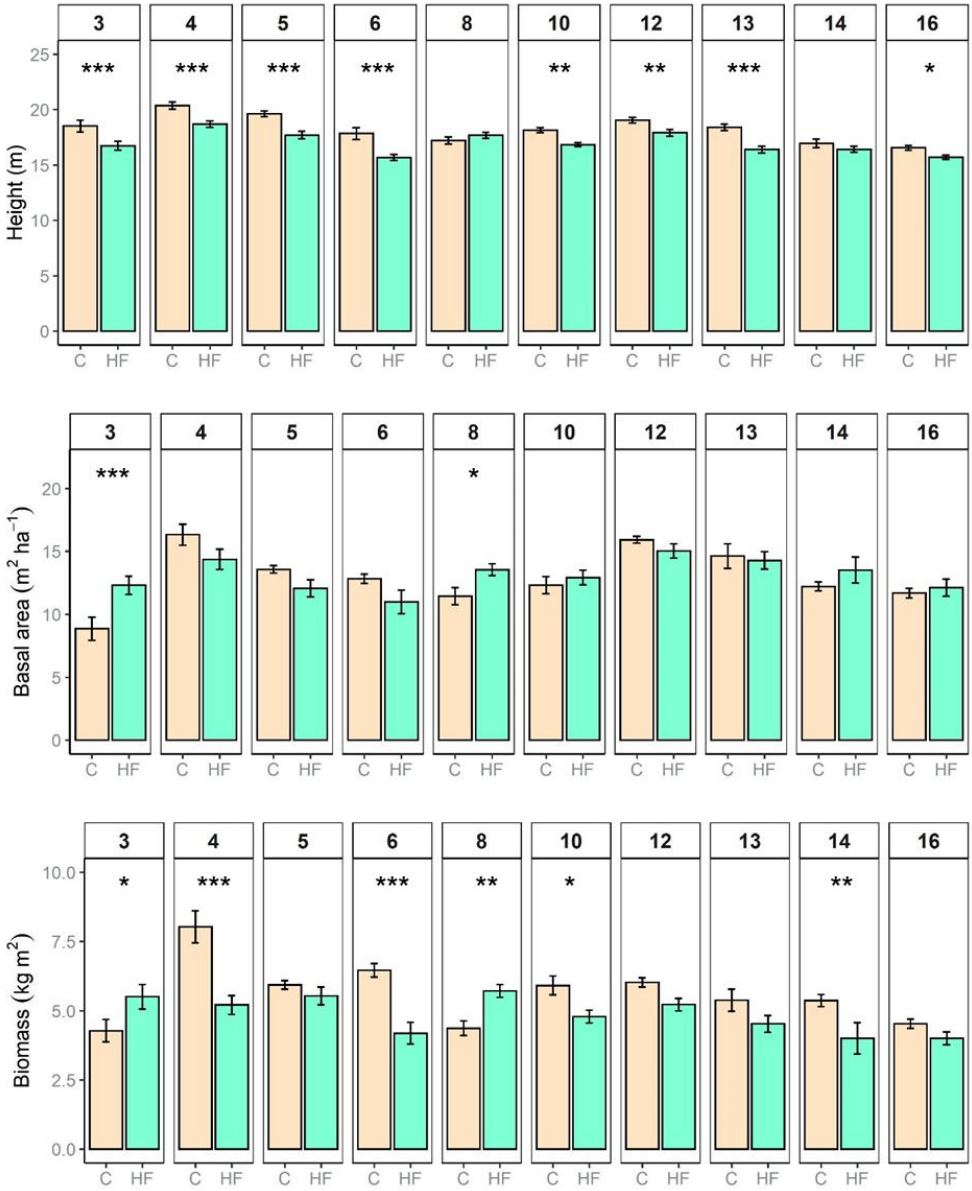


Mean genotype height showed different behaviors depending on the management. Genotypes G3, G4, G5, G6 and G13 ($p < 0.001$), G10 and G12 ($p < 0.01$), and G16 ($p < 0.05$) were different in terms of stand. Only genotypes G8 ($p = 0.26$) and G14 ($p = 0.11$) were no different. Whenever there was a difference, the coppice was higher than the high forest (Fig.4).

The clones that showed the greatest differences between the means were G6, G13, and G3 (12.83, 11.58, and 11.52%, respectively), and the smallest differences were G16 and G12 (5.47 and 5.66%, respectively). Genotypes G5, G4 and G10 had a difference between means of 9.73, 8.83 and 7.37%, respectively. Among the genotypes that did not show significance, the mean difference was 2.82 and 3.53% for G8 and G14, respectively (Fig.4).

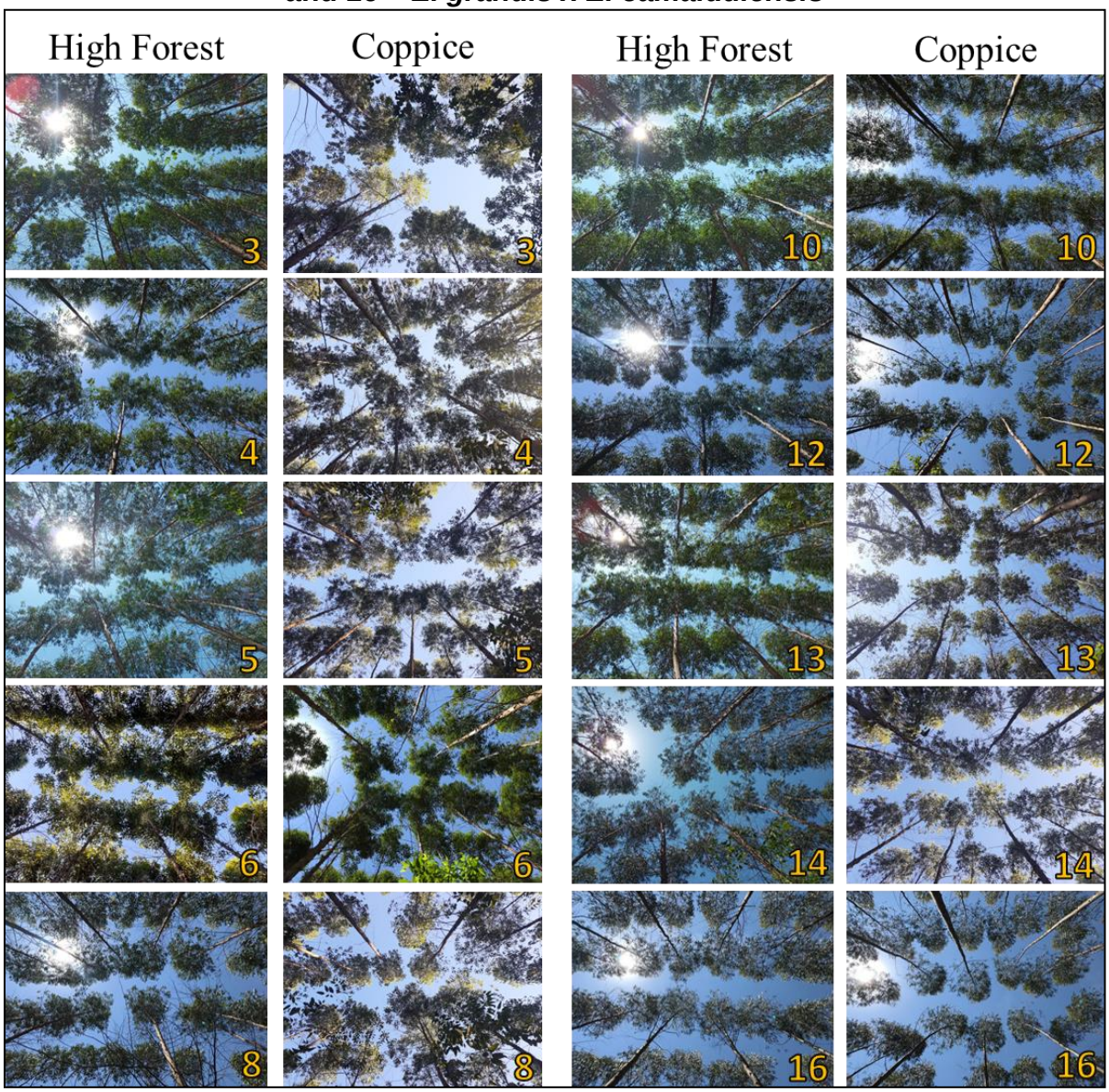
Evaluating the silvicultural system x genotypes interaction, we found a significant difference, where genotypes G3 and G8 presented greater basal area in high forest than coppice (Fig. 4). However, most genotypes showed similar behavior for BA, which justifies the high p-value in the general analysis (= 0.76). For total biomass production, we found that genotypes G3, G4, G6, G8, G10 and G14 showed a significant difference, where only genotype G3 and G8 in high forest was greater than coppice (Fig. 4).

Figure 4 – Height, basal area, and biomass production (± standard error) of 10 *Eucalyptus* genotypes under high forest (HF; green) and coppice (C; orange) stand. Each number represents the respective genotype. Above bars is the significance using Tukey test where: * = p-value < 0.05; ** = p-value < 0.01; *** = p-value < 0.001



The leaf area index measured at the end of the dry season (November 2021) was predominantly higher in coppice than high forest, which may explain the superior results in height in coppice management (Fig. 3a; fig. 5).

Figure 5 – Leaf area index of the 10 *Eucalyptus* genotypes under high forest and coppice stand. Each number represents a genotype: 3 – *E. grandis x E. urophylla*, 4 – *E. grandis x E. urophylla,* 5 – *E. grandis x E. urophylla,* 6 – *E. grandis x E. urophylla,* 8 – *E. grandis x E. urophylla*, 10 – *E. grandis x E. urophylla,* 12 – *E. urophylla* x sp, 13 – *E. grandis x E. urophylla*, 14 – *E. saligna*, and 16 – *E. grandis x E. camaldulensis*

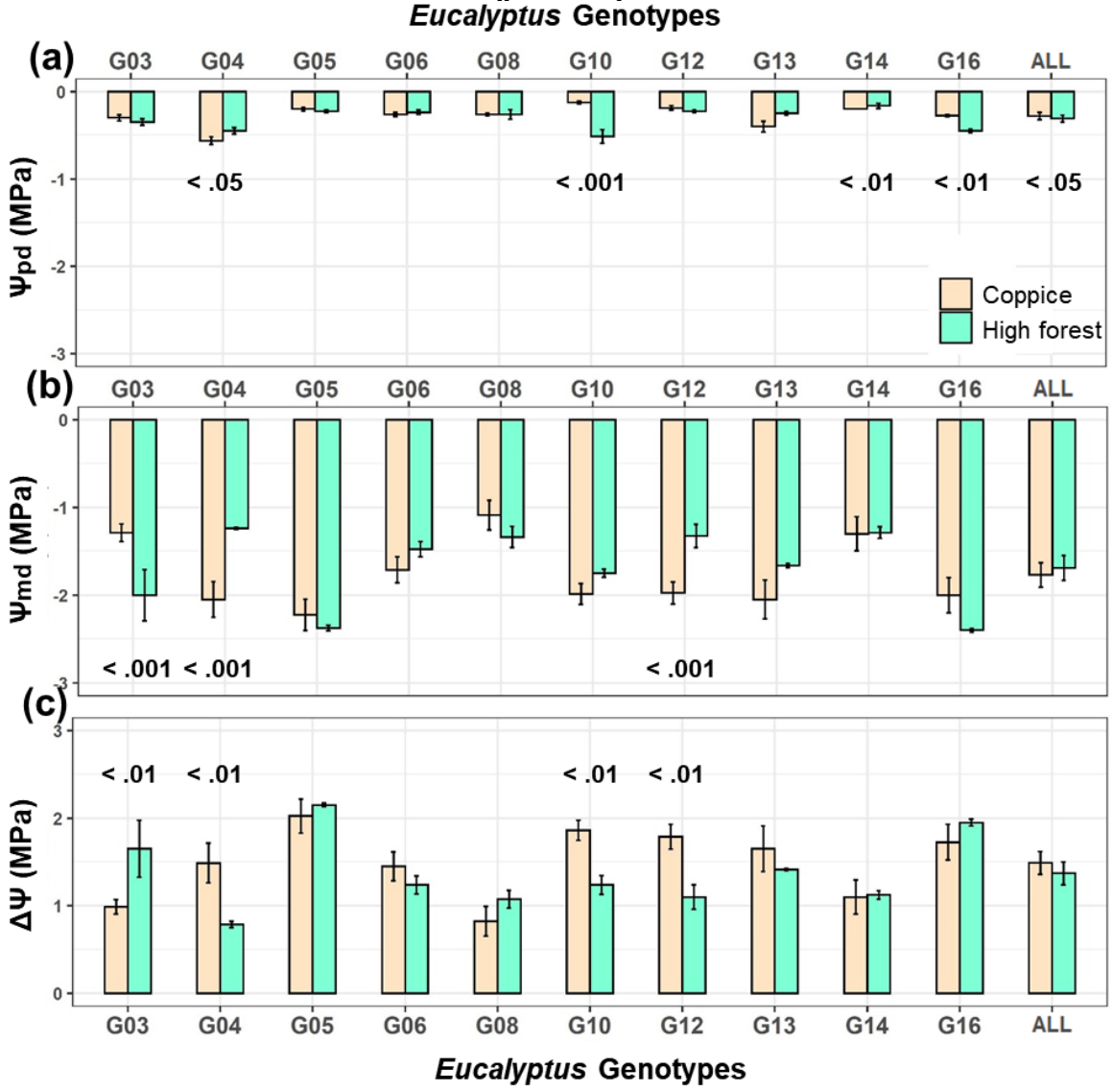


2.3.2 Leaf water potential

Overall, coppice and high forest showed significant difference only for Ψ_{pd} (Fig.6. a; $p < 0.05$, $n = 79$). Ψ_{md} showed similar behavior between managements ($p = 0.21$, $n = 79$), although some genotypes showed differences between managements. Most genotypes did not exhibit difference between high forests and coppice for Ψ_{pd}, Ψ_{md} or $\Delta\Psi$. For the genotypes that did exhibit a difference, we did not observed a consistent trend: in some instances coppice showed lower Ψ_{pd} (GEN 4 and 13) and

Ψ_{md} (GEN 4 and 12) and higher $\Delta\Psi$ (GEN 4, 10 and 12), in some other instances high forests showed lower Ψ_{pd} (GEN 10 and 16) and Ψ_{md} (GEN 3) and higher $\Delta\Psi$ (GEN 3).

Figure 6 – Leaf water potential at pre-dawn (Ψ_{pd}), mid-day (Ψ_{md}), and the different between Ψ_{pd} – Ψ_{md} ($\Delta\Psi$) in *Eucalyptus* genotypes under coppice and high forest system (± SE, n = 79). The p-value above or below the columns indicates significant difference between coppice and high forest by Tukey test (p < 0.05)

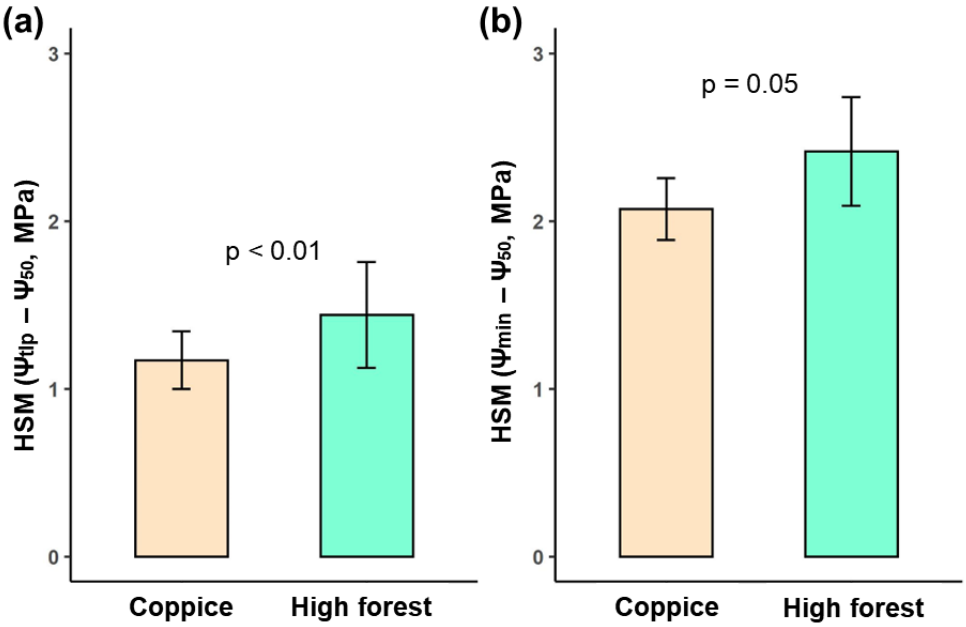


2.3.3 Hydraulic safety margin

Ψ_{tlp}-based HSM, but not Ψ_{min}-based HSM, was significantly different between coppice and high forest. High forest (1.44 MPa) had a higher hydraulic safety margin compared to coppice (1.17 MPa). The mean difference between managements was

0.27 MPa ($p < 0.01$; Fig. 7a). Ψ_{min}-based HSM was marginally significant between managements ($p < 0.1$) and showed the same tend as Ψ_{tlp}-based HSM: high forest (2.41 MPa) was higher than coppice (2.07 MPa). The mean difference between managements for Ψ_{min}-based HSM was 0.34 MPa (Fig. 7b).

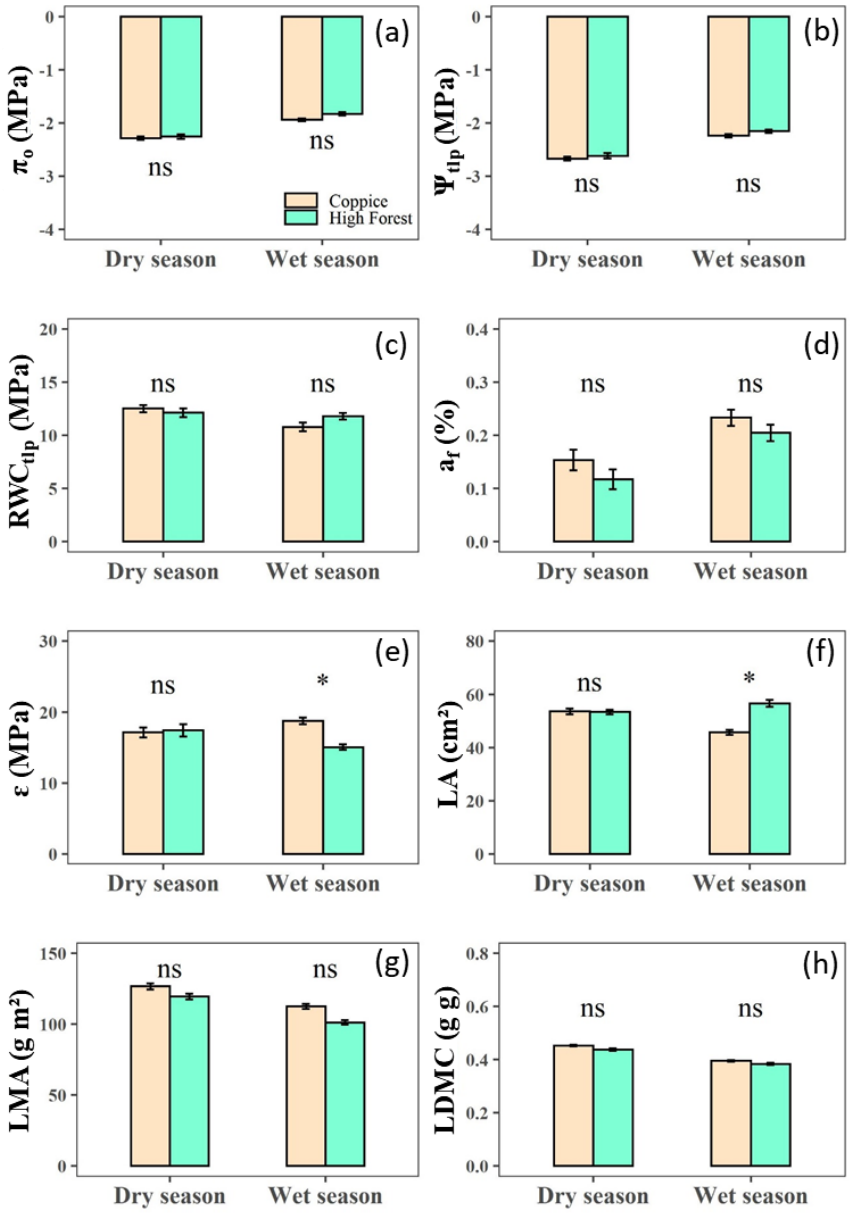
Figure 7 – Influence of management on the hydraulic safety margin based on Ψ_{tlp} (Ψ_{tlp}-based HSM, a) and Ψ_{min} (Ψ_{min}-based HSM, b) in *Eucalyptus* genotypes (± SE, n = 16). colors indicate the silvicultural system: coppice (orange) and high forest (green). Bars are standard error. p-values above the bars were obtained through analysis of variance (ANOVA)



2.3.4 Seasonal effects on drought tolerance traits of *Eucalyptus* stands

All leaf water relation traits, except for ε (Fig. 8e; $p < .01$) and LA (Fig. 8f; $p < .0001$), were similar between high forest and coppice stands, in both dry and wet seasons. For the modulus of elasticity, the coppice stand was higher than the high forest stand. Conversely, the high forest had a larger leaf area than the coppice stand. In these two traits, differences were only significant when measurements occurred in the dry season.

Figure 8 - Effect of dry and wet season on functional traits of *Eucalyptus* genotypes under coppice (orange) and high forest (green) stand (± SE, n = 216). Below or above bars are significance by paired *t*-test ($p < 0.05$), where: * - significantly difference, and ^{ns} – not significant



2.3.5 Management effects on drought tolerance traits

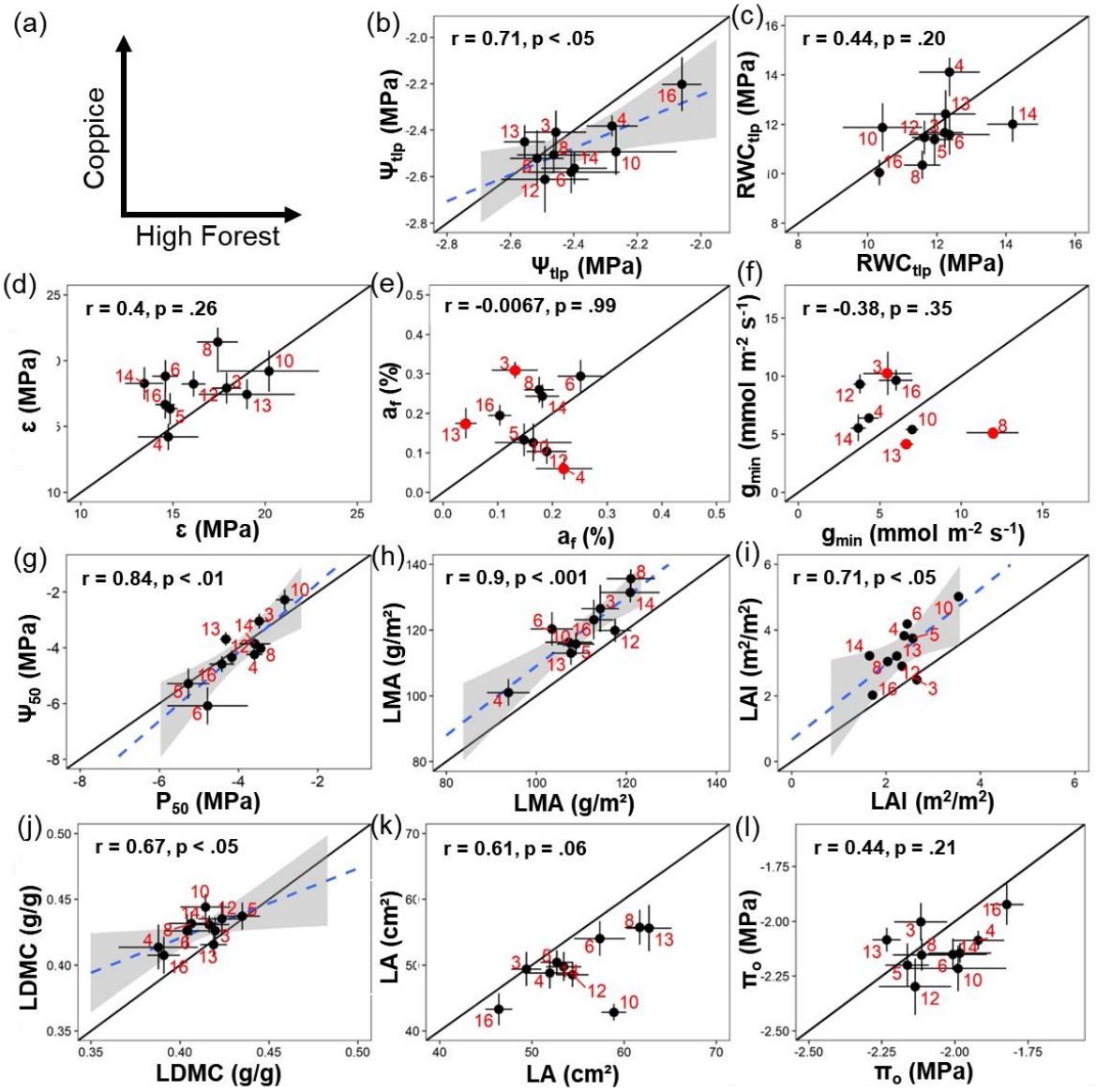
The management (high forest versus coppice) did not affect most of the drought tolerance traits in the studied *Eucalyptus* genotypes. Coppice stands showed a greater leaf area index and stand biomass than high forest stands (Table 2).

Table 2 - Behavior of *Eucalyptus* genotypes under high forest and coppice stands in view of the variables evaluated in this work: does management interfere with the performance and drought tolerance of genotypes? An evaluation using paired *t*-test ($p < 0.05$). The average values per stand are followed by the standard error (± SE). The p-value in bold indicates the significant difference between high forest and coppice stand. The degree of freedom demonstrates the sample number for each variable

Parameters	High Forest Stand	Coppice Stand	p-value	Degree of freedom
ψ_{tlp} (MPa)	-2.41 (± 0.02)	-2.46 (± 0.03)	0.5016	216
Ψ_{50} (MPa)	-3.99 (± 0.15)	-4.15 (± 0.17)	0.7042	139
π_o (MPa)	-2.06 (± 0.02)	-2.11 (± 0.03)	0.3937	216
RWC_{tlp} (%)	12.34 (± 0.19)	11.63 (± 0.27)	0.1916	216
ε (MPa)	16.59 (± 0.35)	17.92 (± 0.44)	0.1301	216
a_f (%)	0.15 (± 0.01)	0.19 (± 0.01)	0.1429	216
LMA (g m^2)	111.85 (± 1.36)	119.77 (± 1.59)	0.0601	216
LDMC (mg g^{-1})	0.42 (± 0.002)	0.42 (± 0.003)	0.5232	216
LAI (m^2 m^2)	2.35 (± 0.17)	3.36 (± 0.27)	**< 0.01**	19
LA (cm^2)	55.74 (± 0.77)	49.52 (± 0.83)	**< 0.01**	216
g_{min} (mmol m^{-2} s^{-1})	5.66 (± 0.04)	6.91 (± 0.05)	0.1297	27
BA (m^2 ha^{-1})	13.12 (± 0.27)	12.98 (± 0.38)	0.7200	89
Biomass (kg m^2)	4.87 (± 0.13)	5.63 (± 0.19)	**< 0.01**	88

Next, we used Pearson's correlation (r) to explore the effect of high forest and coppice management for all PV curve parameters, Ψ_{50} and leaf traits. Overall, Fig. 9 illustrates that the variability observed among genotypes for the different traits is much greater than the variability explained by difference of management (high forest *versus* coppice). For LMA and LAI, we observed a systematic effect of management, with high forests exhibiting lower LAI and LMA than coppice for all genotypes, even though difference was not significative at the genotype level. For some other traits, no clear effect of management was detected. For example, g_{min} showed significant effects of management at the plot level, but in some instances high forests exhibited higher gmin than coppice (GEN 13 and 8), and in some other instance the opposite was observed (GEN 3).

Figure 9 - Pearson's correlation (r) for *Eucalyptus* genotypes under coppice (a, axis y) and high forest (a, axis x) stand. The variables evaluate were leaf turgor loss point (b, Ψ_{tlp}), relative water potential at turgor loss (c, RWC_{tlp}), modulus of elasticity (d, ε), apoplastic fraction (e, a_f), minimum conductance (f, g_{min}), water potential at 50% loss of hydraulic conductance (g, Ψ50), leaf mass per area (h, LMA), leaf area index (i, LAI), leaf matter dry content (j, LDMC), leaf area (k, LA) and osmotic potential (l, π_o). Black solid lines are 1:1 line. Blue dashed lines are linear regression. Black point: there was no significant genotype difference (p > 0.05; Tukey test); Red points: there was a significant difference in genotype (p < 0.01; Tukey test). Confidence intervals are in gray. Red numbers are each *Eucalyptus* genotype



2.4 DISCUSSION

In this study, we aimed at evaluating the drought tolerance of 10 commercial *Eucalyptus* genotypes managed in coppice and high forests. Both the exposure (*in situ* leaf water potential) and the vulnerability (Ψ_{tlp}, Ψ_{50}, HSM) to water stress were measured to quantify the management effects on drought risks.

No intense water stress was observed in our experiment, even during the period of maximum annual water deficit (Ψ_{pd} > 0.6 MPa), regardless of the management. This result is in line with previous studies conducted in the same region that indicate that on very deep tropical soil, *Eucalyptus* seedlings exhibit a very fast root soil exploration and access the deep soil horizon and water table only two years after planting, which strongly limits water stress (BATTIE-LACLAU et al., 2014; CHRISTINA et al., 2017; GUILLEMOT et al., 2021). Therefore, the huge initial differences in the root apparatus of high forest and coppice are only substantial in the first two years after planting, which is also a period of low water stress because of the replenishment of soil water following clear cut of the previous rotation and low initial stand evapotranspiration (CHRISTINA et al., 2017). We report than, when grown in the deep tropical soils of southern Brazil, *Eucalyptus* managed in high forests and coppice experience similar exposure to water stress.

This similarity in drought exposure between high forests and coppice likely explain why we did not report substantial effects of management on the studied drought tolerance traits. Although Ψ_{50} is often reported to be rather conserve traits, leaf water relations commonly exhibit substantial phenotypic plasticity (BARTLETT et al., 2014; CHALLIS et al., 2022; FUCHS et al., 2021; LIMOUSIN et al., 2022). In our study, although it was higher on Ψ_{tlp} than on Ψ_{50}, the effect of management was not significant on these two traits, when evaluated individually, indicating similar drought vulnerability. However, when integrated over all the genotype, we did report a significantly higher Ψtlp-based HSM (Ψ_{tlp}-Ψ_{50}) in high forest than in coppice. This suggests that stomatal control protection of xylem integrity is higher on average in *Eucalyptus* managed as coppice than in *Eucalyptus* managed as high forests. This warrants further investigation of management effects in conditions of higher water stress and shallow soil, in order to draw a more extensive picture of *Eucalyptus* vulnerability to drought.

Our results confirms that coppice exhibit a higher biomass growth than high forests in the first 3 years after planting (DRAKE et al., 2009). However, the same was

not true for basal area, which was similar between coppice and high forests. This suggests that management induce changes in tree allometry that deserve to be further explored. The initial higher growth of coppice is associated with a higher LAI in most genotypes. This high LAI in coppice affects the dynamics of water potential: the difference of LAI between coppice and high forest is positively related to the difference of Ψ_{min} between coppice and high forest ($p < 0.1$, *result not shown*). Therefore, when coppice exhibit greater LAI, they also tend to exhibit lower Ψ_{min}, which suggests that the soil-to-leaf tree conductance is not substantially affected by management.

Predictions point to a reduction in the rainfall volume and an increase in temperature in areas of forest plantations in Brazil (CHOU et al., 2014), and if so, these conditions could significantly affect the productivity of *Eucalyptus* forest plantations (CAMPOE et al., 2020) because they increase the risk of tree drought mortality (ALLEN; BRESHEARS; MCDOWELL, 2015). In conclusion, our study showed that in deep tropical soils, coppice and high forest of commercial *Eucalyptus* genotype exhibit a similar (low) exposure to water stress and a mostly similar vulnerability to water stress. This means that both the initial high forest rotation and subsequent coppice rotation are expected to have similar drought risk. However, management strongly affects stand structure, with higher stand biomass and LAI three years after planting, with could have important consequences for drought tolerance under dryer conditions.

2.5 CONCLUSION

We explored the differences between *Eucalyptus* stands under coppice and high forest through the drought tolerance traits that govern plant strategies in the face of contrasting climatic conditions. We found that the drought tolerance traits of coppice and high forest are similar. Contrary to expectations, plant drought tolerance predictor traits such as Ψ_{tlp} and Ψ_{50} are not influenced by management in commercial *Eucalyptus* genotypes. Conversely, the stand biomass, and leaf area index are more determinant for differences between silvicultural systems than drought tolerance traits. However, this warrants further investigation of management effects in conditions of higher water stress, in order to draw a more extensive picture of *Eucalyptus* vulnerability to drought.

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FINAL CONSIDERATIONS

Based on the results obtained, it was possible to conclude that:

- Commercial *Eucalyptus* genotypes showed high phenotypic plasticity between the dry and wet seasons with the Ψ_{tlp} becoming more negative in the dry season (**supported hypothesis**).

- The Ψ_{tlp} did not differ widely between *Eucalyptus* genotypes because it is possible that the genetic improvement through breeding conducted on commercial *Eucalyptus* genotypes. Also, the selection of highly productive species and varieties resulted in a limited range of leaf water traits among the studied genotypes, reducing the explicative power of trait-drought tolerance correlations. However, Ψ_{tlp} measured at the end of the dry season is a better predictor of drought tolerance of genotypes (**partially supported hypothesis**).

- Although we confirmed that variation in Ψ_{tlp} is mostly driven by variation in π_o across *Eucalyptus* material, the osmometer method was not able to accurately rank drought tolerance among the genotypes of our common-garden experiment (**unsupported hypothesis).**

- Coppice and high forest of commercial *Eucalyptus* genotype exhibit a similar (low) exposure to water stress and a mostly similar vulnerability to water stress. This means that both the initial high forest rotation and subsequent coppice rotation are expected to have similar drought risk (**unsupported hypothesis)**. However, management strongly affects stand structure, with higher stand biomass and LAI three years after planting, with could have important consequences for drought tolerance under dryer conditions.

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