FEDERAL UNIVERSITY OF ESPÍRITO SANTO
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PHYSIOLOGICAL RESPONSES OF YOUNG *Eucalyptus grandis* × *Eucalyptus urophylla* PLANTS TO SOIL MOISTURE AND VAPOR PRESSURE DEFICIT VARIABLES, AND KAOLIN PARTICLE FILM APPLICATION AS A WATER STRESS MITIGATOR

JERÔNIMO MONTEIRO – ES
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Advisor: Prof. Ph.D José Eduardo Macedo Pezzopane
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Elbya Leão Gibson

Tese apresentada ao Programa de Pós-Graduação em Ciências Florestais do Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo, como parte das exigências para obtenção do Título de Doutora em Ciências Florestais na Área de Concentração Ciências Florestais.

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DEDICATION

To my grandparents Tarcila (in memoriam), Lourival (in memoriam), and Iolanda, and to my parents, Manoel Gibson and Rozana Leão, for being my inspiration and role models. To you all, my eternal love and gratitude.

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"*Education is the most powerful weapon you can use to change the world.*"

(Nelson Mandela)

GENERAL ABSTRACT

GIBSON, Elbya Leão. **Physiological responses of young** *Eucalyptus grandis* × *Eucalyptus urophylla* **plants to soil moisture and vapor pressure deficit variables, and kaolin particle film application as a water stress mitigator.** 2025. Thesis **(**Doctoral Degree in Forestry Sciences) – Federal University of Espírito Santo, Jerônimo Monteiro, ES. Advisor: Prof. Ph.D José Eduardo Macedo Pezzopane. Co-advisors: Prof. Ph.D Manuel Fernández Martínez and Ph.D João Vitor Toledo.

Eucalyptus is the most extensively cultivated forest species on the planet, with a total area of 25 million hectares planted. Brazil is the world's foremost producer, with 7.6 million hectares under cultivation. Among the hybrids, *Eucalyptus grandis* × *Eucalyptus urophylla* (I144) is especially prominent due to its rapid growth, robust resistance to fungal diseases, adaptability and high density. However, climate change can have a severe impact on *Eucalyptus* production, making it essential to look for alternatives to mitigate the impacts of water restrictions and increasingly frequent temperature rises. The present thesis is thus structured in three chapters. The aim of Chapter I was to assess the ecophysiological responses of *Eucalyptus grandis* × *Eucalyptus urophylla* plants grown at different levels of water availability, in order to identify the level at which water limitation begins to affect plant growth. The plants were grown for 30 days in 12-litre pots with commercial substrate and watered daily to keep the water in the substrate close to the maximum water retention capacity (MWRC). Subsequent to this initial period, the plants were subjected to five levels of water availability: 100%, 80%, 60%, 40%, and 20% of the MWRC. The analyses encompassed measurements of leaf water potential (Ψ_{leaf}), leaf gas exchange, branch hydraulic conductivity (K_s) and growth. The results obtained demonstrate that the critical threshold for the growth of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants is between 40% and 60% of the MWRC. Below this range, the plants exhibited signs of water stress, which compromised their growth and development. The aim of Chapter II was to examine the responses of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants to different vapor pressure deficits (VPD) under conditions of water restriction. Plants of the *Eucalyptus grandis* × *Eucalyptus urophylla* clone were grown for 75 days in pots (60 litres) containing commercial substrate, with daily watering. The plants were then exposed to two distinct environments: I) high atmospheric demand (HAD) - high maximum VPD (5.8 kPa) and II) low atmospheric demand (LAD) - low maximum VPD (3.2 kPa) and two levels of soil water availability (control and stressed). Ecophysiological analyses included measurements of $\Psi_{leaf,}$ leaf gas exchange, photosystem II quantum efficiency (F_v/F_m) and K_s. The results obtained suggest that high atmospheric demand exacerbates the negative effects of water stress,

impairing plant hydraulic efficiency and gas exchange. Hydraulic efficiency was reduced by 28% in the HAD treatment compared to the LAD treatment under water deficit, and CO_2 assimilation was reduced by 10%. The aim of Chapter III was to assess the effects of foliar application of kaolin in mitigating the negative effects of water restriction on young *Eucalyptus grandis* × *Eucalyptus urophylla* plants. Plants were grown for 75 days in pots (60 litres) containing commercial substrate, with daily watering. The plants were then subjected to three treatments: 70% of MWRC (control), progressive reduction of water availability (Stress) and progressive reduction of water availability with kaolin application (Stress + KL). Ecophysiological analyses included measurements of reflected solar radiation, leaf temperature, leaf gas exchange, Ψ_{leaf}, F_v/F_m and K_s. The results obtained from this study demonstrate that the foliar application of kaolin constitutes an effective strategy for mitigating the adverse effects of water stress in *Eucalyptus grandis* × *Eucalyptus urophylla* clones, particularly during the initial days of water restriction.

Keywords: Water restriction, Atmospheric demand, Leaf photoprotector, Ecophysiology, *Eucalyptus*

RESUMO GERAL

GIBSON, Elbya Leão. **Respostas fisiológicas de plantas de** *Eucalyptus grandis* × *Eucalyptus urophylla* **às variáveis de umidade do solo e déficit de pressão de vapor, e aplicação de filme de partículas de caolina como mitigador de estresse hídrico.** 2025. Tese (Doutorado em Ciências Florestais) - Universidade Federal do Espírito Santo, Jerônimo Monteiro, ES. Orientador: Prof. Dr. José Eduardo Macedo Pezzopane. Co-orientadores: Prof. Dr. Manuel Fernández Martínez e Dr. João Vitor Toledo**.**

O *Eucalyptus* é o gênero florestal mais plantado no mundo, com 25 milhões de hectares cultivadas, sendo o Brasil o maior produtor mundial, com 7,6 milhões de hectares. Dentre os híbridos, o *Eucalyptus grandis* × *Eucalyptus urophylla* (I144) é altamente cultivado por apresentar crescimento rápido, boa tolerância a doenças fúngicas, adaptabilidade a condições edafoclimáticas variáveis e maior densidade da madeira. As mudanças climáticas podem causar impactos severos na produção dos *Eucalyptus*, tornando-se essencial buscar alternativas que amenizem os impactos da restrição hídrica e aumentos da temperatura cada vez mais frequentes. Esta tese foi estruturada em três capítulos. No capítulo I, objetivou-se avaliar as respostas fisiológicas de plantas jovens de *Eucalyptus grandis* × *Eucalyptus urophylla* cultivadas em diferentes níveis de disponibilidade de água, com vistas a identificar a partir de qual nível de restrição hídrica começa a comprometer o crescimento inicial das plantas. As plantas foram cultivadas em vasos com capacidade de 12 L com substrato comercial e irrigadas diariamente, durante 30 dias, para manter a umidade no substrato próxima à capacidade máxima de retenção de água (CMRA). Após esse período, as plantas foram submetidas a cinco níveis de disponibilidade de água, sendo eles: 100, 80, 60, 40 e 20 % da CMRA. As análises incluíram medições de potencial hídrico foliar (Ψ_{leaf}), trocas gasosas foliares, condutividade hidráulica de ramos (k_s) e atributos biométricos de crescimento. Os resultados indicam que o limiar crítico para o crescimento das plantas jovens de *Eucalyptus grandis* × *Eucalyptus urophylla* encontra-se entre 60% e 40% da CMRA. Abaixo desse intervalo, as plantas estavam sob estresse hídrico, comprometendo seu crescimento e desenvolvimento. No capítulo II, objetivou-se investigar como plantas jovens de *Eucalyptus grandis* × *Eucalyptus urophylla* respondem a diferentes déficits de pressão de vapor (DPV) sob condições de restrição hídrica. As plantas do clone *Eucalyptus grandis* × *Eucalyptus urophylla* foram cultivadas em vasos (60 L) contendo substrato comercial, com irrigação diária durante 75 dias. Em seguida, as plantas foram submetidas a dois ambientes: I) alta demanda atmosférica (HAD) – alto DPV máximo (5,8 kPa) e II) baixa demanda atmosférica (LAD) – baixo DPV máximo (3,2 kPa), e dois níveis de disponibilidade de água no solo (controle e estresse). As análises fisiológicas incluíram medições de Ψ_{leaf}, trocas gasosas foliares, eficiência quântica do fotossistema II (F_v/F_m) e K_s. Os resultados indicam que a alta demanda atmosférica intensifica os efeitos negativos do estresse hídrico, prejudicando a eficiência hidráulica e as trocas gasosas das plantas. Sob déficit

hídrico, a eficiência hidráulica em HAD foi reduzida em 28%, em comparação a LAD, enquanto a assimilação de CO_2 foi reduzida em 10%. No capítulo III, objetivou-se avaliar os efeitos da aplicação foliar do Kaolin na mitigação dos impactos negativos da restrição hídrica em plantas jovens de *Eucalyptus grandis* × *Eucalyptus urophylla.* As plantas foram cultivadas em vasos (60 L) contendo substrato comercial, com irrigação diária durante 75 dias. Em seguida, as plantas foram submetidas a três tratamentos: 70 % da CMRA (controle), redução progressiva da disponibilidade hídrica (Stress) e redução progressiva da disponibilidade hídrica com aplicação de Kaolin (Stress + KL). As análises incluíram medições de radiação solar refletida, temperatura da folha, trocas gasosas foliares, Ψ_{leaf}, F_v/F_m e K_s. Os resultados indicam que a aplicação foliar de Kaolin foi uma estratégia eficiente para mitigar os efeitos negativos do estresse hídrico em plantas do clone *Eucalyptus grandis* × *Eucalyptus urophylla* principalmente nos primeiros dias de restrição hídrica.

Palavras-chave: Restrição hídrica, Espécies arbóreas, Demanda atmosférica, Fotoprotetor foliar, Relações hídricas, Assimilação de CO_2

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CHAPTER I – SENSITIVITY OF *Eucalyptus grandis* × *Eucalyptus urophylla* TO DIFFERENT WATER RESTRICTIONS

SUMMARY

INTRODUÇÃO GERAL

O *Eucalyptus* é o gênero florestal mais plantado no mundo, com 25 milhões de hectares cultivadas (FAO, 2021; MARTINS et al., 2022), sendo o Brasil o maior produtor mundial, com 7,6 milhões de hectares (ELLI et al. 2020; IBÁ 2023). Esse número representa um crescimento de 41% nos últimos dez anos, evidenciando a crescente importância do *Eucalyptus* na economia florestal brasileira. A distribuição dos plantios no território nacional está concentrada principalmente nos estados de Minas Gerais, Mato Grosso do Sul, São Paulo, Paraná e Santa Catarina, que juntos somam 69% da área plantada (IBÁ, 2023).

As espécies ou híbridos desse gênero mais utilizadas em plantios florestais brasileiro incluem *Eucalyptus grandis*, *E. globulus*, *E. urophylla*, *E. saligna*, *E. camaldulensis*, *Corymbia citriodora* e os híbridos *E. urophylla* × *E. grandis*, *E. urophylla* × *E. camaldulensis*, *E. grandis* × *E. camaldulensis* e *E. urophylla* × *E. globulus* (FREITAS et al., 2020; HAKAMADA et al., 2020). Essas espécies e híbridos destacam-se devido à qualidade da madeira, crescimento rápido, ciclos de rotação curtos (<7 anos), alta produtividade e adaptação a diferentes solos e condições climáticas (ELLI; SENTELHAS; BENDER, 2020; MARTINS et al., 2022). Essas características os tornam ideais para a produção de biomassa destinada a indústria de celulose e papel, carvão vegetal e produtos de madeira sólida (FLORÊNCIO; MARTINS; FAGUNDES, 2022; IBÁ, 2022).

Dentre os híbridos, o *Eucalyptus grandis* × *Eucalyptus urophylla*, resultado do cruzamento entre as espécies *Eucalyptus grandis* W. Hill ex Maiden e *Eucalyptus urophylla* S.T. Blake, é amplamente cultivado devido ao fato de apresentar crescimento rápido, boa tolerância a doenças fúngicas, adaptabilidade, maior densidade e boa capacidade de rebrota (KULLAN et al., 2012). Além disso, trata-se de um híbrido tolerante à seca no campo (MÜLLER et al., 2020). No entanto, nos últimos anos, eventos climáticos extremos, como secas e ondas de calor, tornaram-se mais frequentes no sudeste da América do Sul (IPCC, 2023), impactando o crescimento das plantas, especialmente nos estágios iniciais (GUARNASCHELLI; GARAU; LEMCOFF, 2012).

A produtividade do *Eucalyptus* está diretamente relacionada ao atendimento hídrico pelas chuvas (STAPE et al., 2010), sendo que condições de déficit hídrico reduzem seu crescimento e a produção de biomassa (BOURNE et al., 2017). Durante os estágios iniciais dos plantios florestais, por exemplo, as plantas estão sujeitas a maior exposição a cenários climáticos adversos, sendo que essas condições podem acarretar no aumento da taxa de mortalidade e diminuição do crescimento das plantas (ELLI; SENTELHAS; BENDER, 2020; SCOLFORO et al., 2019). De acordo com as últimas projeções do IPCC, os efeitos das

mudanças climáticas poderão prejudicar a produtividade e a qualidade de muitas culturas com forte relevância socioeconômica em todo o mundo, especialmente durante o verão (FRAGA; PINTO; SANTOS, 2019), devido a fatores de estresse intensos e prolongados (ELLI; SENTELHAS; BENDER, 2020). De acordo com a Indústria Brasileira de Árvores – IBÁ (2021), as mudanças climáticas podem ser um dos fatores que impactaram na queda de produtividade média por ano no plantio de *Eucalyptus* de 38,6 m³/ha, em 2019, para 36,8 m³/ha, em 2020.

Embora o aumento global da temperatura seja um fator comum para a mortalidade de árvores, outros fatores importantes para a sobrevivência das plantas em campo também sofrem alterações decorrentes de mudanças climáticas, como o padrão de precipitação, a duração e intensidade de secas e o déficit de pressão de vapor do ar (DPV), podendo afetar negativamente o crescimento e desenvolvimento de plantas do gênero *Eucalyptus* em algumas regiões (IBÁ, 2022; IPCC, 2019; STAPE et al., 2010). Casos recentes de aumento da mortalidade de árvores causada por secas e altas temperaturas levantam a suspeita de que a mortalidade florestal amplificada já pode estar ocorrendo em alguns locais, em resposta às mudanças climáticas globais (BENNETT et al., 2015; BLACKMAN et al., 2019; DUKE et al., 2017).

O efeito de secas futuras certamente será agravado pelo aumento da temperatura do ar, que causa elevação do DPV, favorecendo a evaporação para a atmosfera. Isso resulta em aumento da perda de água por transpiração e consequente fechamento estomático, no caso de espécies isohídricas, ou diminuição da margem de segurança, por falha hidráulica, no caso de espécies anisoídricas (ALLEN et al., 2010; CHOAT et al., 2018). O aumento das temperaturas representa uma tripla ameaça à sobrevivência das plantas, pois promove efeitos diretos do estresse térmico, amplificação da seca atmosférica e intensificação da seca no solo (HANSEN; SATO; RUEDY, 2012; YAO et al., 2013).

O estresse hídrico, por exemplo, ocorre quando a necessidade de água da planta não pode ser totalmente atendida, ou seja, quando o nível de água transpirada excede a quantidade de água absorvida pelas raízes, devido a redução da precipitação e diminuição do nível do lençol freático (ROMEET et al., 2019). Com isso, as plantas realizam ajustes morfoanatômicos, fisiológicos e bioquímicos, que visamatenuar a perda de água e preservar seu estado hídrico (MÜLLER et al., 2020; PITA-BARBOSA et al., 2023; SILVA et al., 2016).

Nesse contexto, é importante destacar que temperaturas mais altas também aumentam o DPV, acelerando a perda de água dos solos e das plantas durante os períodos mais quentes, mesmo quando os estômatos das folhas estão fechados (HAMMOND et al., 2022). O DPV é a força motriz para a transpiração das plantas (GROSSIORD et al., 2020). Portanto, sob DPV

mais alto, o esgotamento mais rápido do solo e da água da planta acelera a desidratação da planta sob a seca (MCDOWELL et al., 2022).

Nesse sentido, desperta-se a relevância de pesquisar novas estratégias silviculturais, com o intuito de minimizar os efeitos das mudanças climáticas, melhorar as respostas fisiológicas durante o crescimento inicial e aumentar a sobrevivência das plantas de *Eucalyptus*, sobretudo nas épocas mais secas do ano. Soma-se a isso a necessidade de reduzir custos com irrigação e diminuir a perda evaporativa das folhas no pós-transplantio, visto que a intensificação dos fatores de estresse no verão, como aumento da temperatura, seca mais intensas e DPV, já está desafiando produtores e pesquisadores a desenvolver estratégias adaptativas para lidar com as mudanças climáticas (BERNARDO et al., 2018).

Uma das estratégias de curto prazo utilizada para mitigar os efeitos das pressões ambientais e reduzir a taxa de transpiração das mudas, tem sido a aplicação de antitranspirantes. Os antitranspirantes foram classificados em três tipos, com base em seu papel de ação: (I) materiais metabólicos, que são compostos químicos que podem atuar sobre as células-guarda ao redor do poro dos estômatos, regulando a sua abertura e reduzindo a perda de vapor d'água das folhas das plantas (ANJUM et al., 2011); (II) materiais formadores de filme, como emulsões de cera, látex ou plásticos secos na folhagem, que formam filmes finos e transparentes, atenuando a perda de vapor d'água das folhas (FARALLI et al., 2016); e (III) materiais refletores, que reduzem a absorção da energia radiante e, consequentemente, a temperatura das folhas e a taxa de transpiração (GLENN et al., 2003).

Vários estudos têm relatado os benefícios do uso do Kaolin em culturas como tomate (ABDALLAH, 2019), maçã (AL-ABSI; ARCHBOLD, 2016; FAGHIH et al., 2021; GLENN, D. M., 2016), mamão (CAMPOSTRINI et al., 2010), uva (BERNARDO et al., 2018; CONDE et al., 2018; DINIS et al., 2018), e café (ABREU et al., 2022; RODA et al., 2022). Os efeitos positivos da aplicação desse produto inclui mecanismos de fotoproteção para os fotossistemas e cadeia de transporte de elétrons (DINIS et al., 2018), ação antitranspirante (BOARI et al., 2015), diminuição da temperatura da folha e aumento da eficiência do uso da água (BOARI et al., 2015; SANTOS et al., 2021), com consequente aumento da produtividade (GHARAGHANI; MOHAMMADI JAVARZARI; VAHDATI, 2018), podendo inclusive controlar a ocorrência de pragas (AMALIN et al., 2015) e patógenos (TUBAJIKA et al., 2007).

Devido ao desempenho da aplicação do Kaolin na agricultura, diante de condições de estresse abiótico, alguns estudos vêm sendo desenvolvidos para espécies florestais, como as do gênero *Eucalyptus*. Santos et al. (2021), observaram que aplicação de Kaolin a 3% é a concentração ideal para promover o crescimento e manter a eficiência fotossintética em plantas jovens de eucalipto, enquanto concentrações mais altas resultaram na diminuição das variáveis

de trocas gasosas. Oliveira et al. (2021a) constataram que aplicação foliar do Kaolin é uma tecnologia promissora para melhorar a produtividade e a qualidade de miniestacas de *Eucalyptus* em viveiros clonais. Além disso, Xavier et al. (2018) indicam que o fotoprotetor foliar pode ser uma ferramenta eficaz para aumentar o crescimento de mudas de eucalipto sob condições ambientais desafiadoras.

Diante do exposto, mesmo com avanços significativos do melhoramento e das práticas silviculturais intensivas, o crescimento e a produtividade do gênero *Eucalyptus* ainda são fortemente influenciados por fatores climáticos, sendo fundamental compreender as respostas ecofisiológicas de plantas de *Eucalyptus* submetidas a condições de estresse e a viabilidade da aplicação foliar do Kaolin como uma estratégia para mitigar os efeitos do estresse nas plantas. Para isso, a tese foi dividida em três capítulos, onde: o capítulo I teve como objetivo identificar o nível de restrição hídrica que começa a impactar a taxa de crescimento de plantas jovens de *Eucalyptus grandis* × *Eucalyptus urophylla*, considerando variáveis fisiológicas como taxa fotossintética, condutância estomática, potencial hídrico e produção de biomassa; o capítulo II objetivou investigar como plantas de *Eucalyptus grandis* × *Eucalyptus urophylla* respondem a diferentes DPV sob condições de restrição hídrica em ambiente controlado; e o capítulo III objetivou avaliar os efeitos da aplicação foliar do Kaolin na mitigação dos impactos negativos da restrição hídrica em plantas jovens de *Eucalyptus grandis* × *Eucalyptus urophylla.*

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CHAPTER I – SENSITIVITY OF *Eucalyptus grandis* × *Eucalyptus urophylla* TO DIFFERENT WATER RESTRICTIONS

ABSTRACT

Water deficit is one of the main challenges for forest productivity, especially in the context of climate change, affecting economically important species such as the *Eucalyptus* genus. In this context, this study aimed to identify the level at which water limitation begins to affect the growth of young *Eucalyptus grandis* × *Eucalyptus urophylla* (clone I144) plants. The experiment was conducted at the Federal University of Espírito Santo, Brazil (latitude 20° 47′ 25" S, longitude 41° 23′ 48" W, and elevation 120 m), between May and July 2021. Plants were grown in pots with commercial substrate and irrigated daily for 30 days to maintain maximum water retention capacity (MWRC). Afterwards, the plants were subjected to 100%, 80%, 60%, 40%, and 20% of MWRC. Leaf water potential (Ψ_{leaf}), leaf gas exchange, crop water stress index (CWSI), branch hydraulic conductivity, and growth-related attributes were measured. The treatment with 20% MWRC resulted in a significant reduction in Ψ_{leaf}, with average values of approximately -2.2 MPa. In addition, both transpiration (E) and stomatal conductance (g_s) showed values close to 0 in the 20% and 40% MWRC treatments. The reduction in soil water availability resulted in a 71% loss in specific hydraulic conductivity (Ks) for the treatment with the lowest water availability (20% MWRC). The CWSI was very discriminating between treatments, with values around 0.9 for the 20% and 40% MWRC treatments, confirming that the plants were under stress, resulting in a reduction in total dry mass of around 77% and 62%, respectively. The results indicate that the growth of *Eucalyptus grandis* × *Eucalyptus urophylla* begins to be affected at 40% MWRC. The plants experience severe stress below this water restriction level which affects their growth and development. The results obtained could support developing more efficient irrigation practices, promoting sustainable use of water resources and optimizing plants growth.

Keywords: Water deficit; Ecophysiology; Growth; woody plants

1. INTRODUCTION

The area dedicated to planted forests has substantially increased on a global scale. *Eucalyptus* has the highest plantings among the various tree genera cultivated for this purpose, with a total of 25 million hectares under cultivation (FAO 2021; Martins et al. 2022). Brazil is noteworthy for its status as the world's leading producer, with 7.6 million hectares dedicated to

forestry, accounting for 76% of the total area under forest cultivation in the country (Elli et al. 2020; Ibá 2023). However, expanding forest plantations has been observed in regions characterized by substantial limitation on plant growth and development, including limited water availability and high local temperatures (Silva et al. 2016). Among the cultivated species and hybrids, *Eucalyptus grandis* × *Eucalyptus urophylla* is notable for its combination of characteristics, including rapid growth, robust tolerance to fungal diseases, and adaptability to diverse climatic conditions (Kullan et al. 2012; Martins et al. 2022).

Eucalyptus grandis × *Eucalyptus urophylla* demonstrates a certain degree of tolerance to drought; however, the intensity and duration of water restriction affect its ability to adapt. As a fast-growing hybrid, it exhibits high energy demands, resulting in a significant need for water to ensure good survival rates and seedling development (Simões; Silva 2012; Bortolin et al. 2012). It is widely recognized that water availability is the most significant environmental factor influencing plant growth and development, and water restriction conditions limit forest productivity (Gavrilescu 2021; Breda et al. 2006).

Water is an essential component of numerous physiological processes, including photosynthesis, nutrient transport, and maintaining cell turgidity. These processes collectively facilitate leaf expansion and plant growth (Taiz et al. 2017). Plants use different physiological responses to ensure their survival in drought periods. One of the initial responses to water deficit is stomatal closure, which limits transpiration and reduces CO_2 assimilation, thus compromising plant growth (Martin-St.Paul; Delzon; Cochard 2017). Leaf abscission under severe or prolonged drought further reduces transpiration at the whole-plant level. While these strategies are common among *Eucalyptus* species, they also lead to slower growth and substantial reductions in productivity (Ahluwalia et al. 20221; Flo et al. 2021). Many studies focus on plant responses to water stress, especially morphological, physiological, and biochemical aspects, as well as its influence on crop productivity. However, it is essential to understand at what level of soil moisture these responses begin to manifest themselves. This knowledge can serve as a basis for decision-making in water deficit conditions and in climate change scenarios.

Climate change is increasingly recognized as a global challenge not only affecting water availability for the human population, but also for the agricultural and forestry sectors. For example, water scarcity is particularly critical during the seedling establishment stage in the field, where the high evaporative demand in deforested areas with high solar radiation aggravates water stress. Seedlings in these conditions exclusively depend on rainfall and the water retention properties of the upper layers of the soil (Carignato et al. 2020). Although the impact of drought on plant physiological processes is well documented, it is still essential to

understand the effects of soil moisture variability on seedlings, particularly for widely cultivated species such as those of the *Eucalyptus* genus.

Therefore, the aim of this study was to evaluate the ecophysiological responses of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants grown under different water availability levels in order to identify the level at which water restriction begins to compromise the growth of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants. This investigation considered variables such as photosynthetic rate, stomatal conductance, water potential and biomass production at different water availability levels.

2. MATERIALS AND METHODS

2.1. Plant material and experimental conditions

The experiment was conducted in the experimental area of the Department of Forestry and Wood Sciences of the Federal University of Espírito Santo (DCFM-UFES), located in Jerônimo Monteiro, Espírito Santo, Brazil (latitude 20° 47′ 25" S, longitude 41° 23′ 48" W, and elevation 120 m), under outdoor conditions, from May 24 to July 13, 2021.

Eucalyptus grandis × *Eucalyptus urophylla* seedlings were grown in 50 cm^3 tubes filled with substrate consisting of biostabilized pine bark, vermiculite, crushed charcoal, and phenolic foam. Clone I144 was used because it is the most commonly used clone in plantations in the study region. Plants with a height of 24.73 ± 1.2 cm and a diameter of 0.8 ± 0.2 cm (mean ± SE) at 60 days of age were transplanted into 12 L pots (height 25 cm, upper circumference 85 cm, and lower circumference 70 cm) containing commercial pine bark substrate fertilized with 4 g dm^{-3} of Basacote® Mini 9M controlled-release fertilizer, with NPK 16-8-12 formulation. The pots were sealed with a 10 mm thick sheet of white Styrofoam to prevent water evaporation from the soil and to monitor water transpiration by the plants to ensure that water was lost only through transpiration by the plants.

After transplanting, the plants underwent an acclimatization period of 25 days between May 24 and June 17, 2021, receiving enough daily irrigation to keep the substrate at humidity conditions close to the maximum water retention capacity (MWRC). Irrigation management began after this period until the end of the experiment. Plants with a height of 46.40 ± 1.43 cm, a diameter of 6.33 ± 0.80 cm and a leaf area of 1,785.92 ± 415.78 cm^2 (mean ± SE) were subjected to five soil water availability levels: 100%, 80%, 60%, 40% and 20% MWRC, with four replications (20 plants in total, with 4 plants randomly distributed for each treatment). The different soil water availability levels were achieved by stopping irrigation until the desired

level was reached and maintaining the plants in these soil water conditions until the end of the experiment (Figure 1.1).

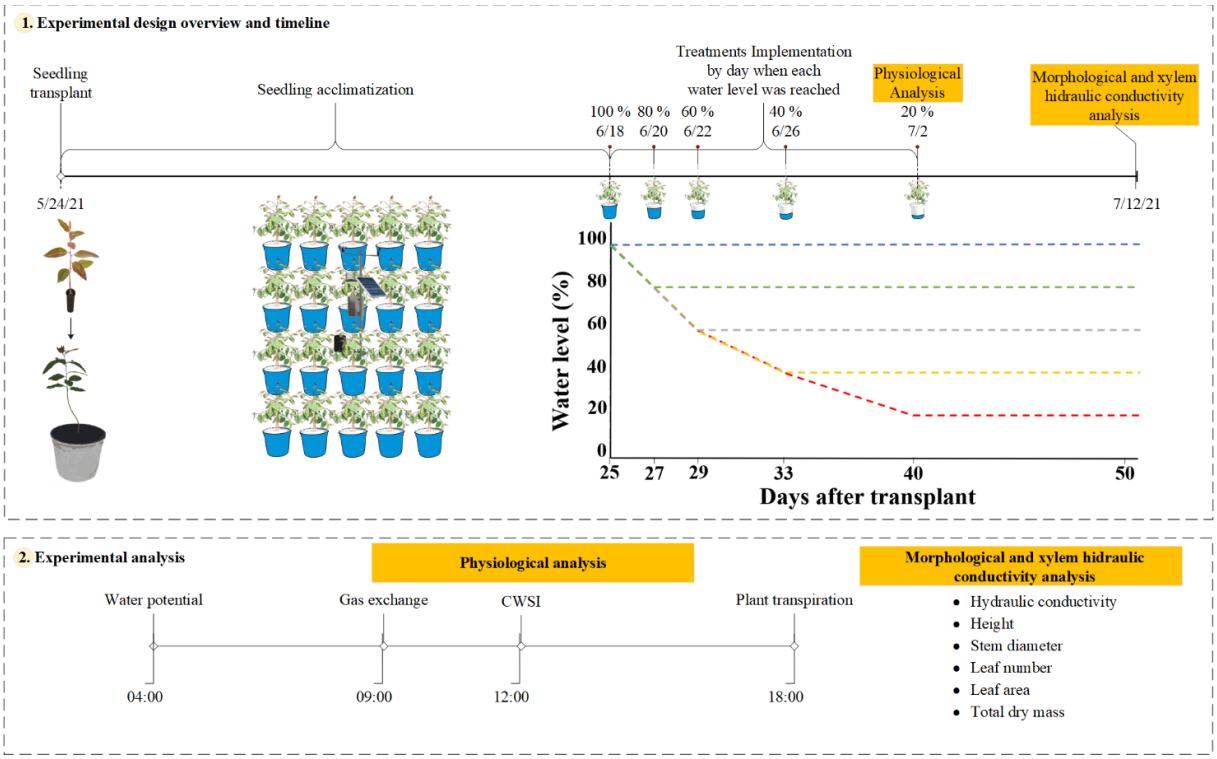

Figure 1 - 1. Experimental design overview and timeline. **2.** Experimental analysis at the end of the study period (physiological analysis on July 02, local time; morphological and hydraulic conductivity on July 12). CWSI = Crop Water Stress Index.

2.2. Monitoring soil water levels

The soil water availability was previously characterized by calculating the maximum water retention capacity of the substrate (MWRC) as follows: a sample of the substrate was taken before adding it to the pots (control sample), which was saturated with water and weighed after free draining to characterize the saturated substrate; then the control sample was placed into a laboratory oven with forced air circulation at a temperature of 105°C for 24 hours to characterize the dry weight, once it was verified that the constant weight of the sample was reached before the final measurement. Equation (I) was used to determine the MWRC value:

$$\mathrm{MWRC} = \mathrm{Wps} - \mathrm{Wp} - \left(\frac{\mathrm{Wsp} * \mathrm{Wds}}{\mathrm{Ws}}\right) \quad \text{(I)}$$

In which: Wps is the weight of the pots with saturated substrate (g); Wp is the weight of the empty pots (g); Wsp is the weight of the substrate inserted in the pots (g) (Wsp = Wps – Wp); Wds is the weight of the dry control sample (g); Ws is the weight of the saturated control sample (g).

All the plants were irrigated in the late afternoon to replace water lost through transpiration based on the daily weight of the pots. Total plant transpiration was calculated from

the difference in mass measured at the beginning and the end of the day. Plants were watered daily for the 100% MWRC treatment, while watering for the 80%, 60%, 40% and 20% MWRC treatments was done to maintain the desired water levels.

2.3. Plant water status and gas exchange

Leaf water potential (Ψ_{leaf}) and gas exchange analyses were performed on the 15th day after irrigation was suspended, the day the lowest soil water content (20% MWRC) was reached, on fully expanded and healthy leaves in the middle third of the plants. The Ψ_{leaf} was measured at 4 a.m. (local time, Figure 1.2) using a Scholander 1505D-EXP pressure chamber (PMS Instrument Co., Albany, OR, USA).

Gas exchange measurements were performed at 9 a.m. using a portable infrared gas analyzer (IRGA, model Li COR 6400, LI-COR Inc., Lincoln, NE, USA) with a 6 cm^2 measuring chamber, a light intensity of 1500 μmol m^{-2} s^{-1}, and a CO_2 concentration of 400 μL L^{-1}. Net CO_2 assimilation rate (*A*, μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}) were measured.

2.4. Crop Water Stress Index and leaf temperature

Thermal images were taken from sun-facing, fully expanded, healthy leaves in the middle third of the plans at noon (12 p.m.) on the 15th day after irrigation was suspended to determine the Crop Water Stress Index (CWSI), being the day with the lowest soil water content (20% MWRC) (Figure 1.2). A FLIR T430sc thermal imaging camera (FLIR Systems, Wilsonville, OR, USA) with a resolution of 320 × 240 pixels was used. The emissivity was set to 0.96 with a distance of 0.5 m between the camera and the plant canopy. FLIR Tools software was used to process the images, taking 10 samples in each replicate to obtain the average temperature in each treatment. In addition to the spot reading at noon, leaf temperature was measured between 8 a.m. and 4 p.m. the following day along with the other physiological measurements (Figure 2).

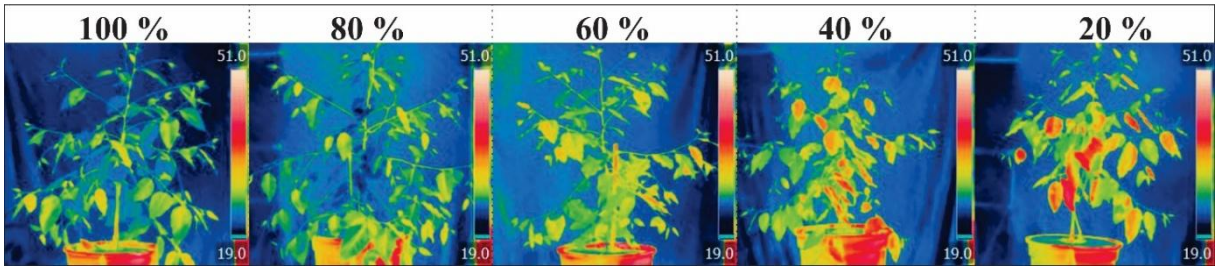

Figure 2 - Thermal images of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels obtained at noon, represented by one plant for each treatment.

The CWSI was calculated according to the methodology proposed by Jones (1999) using the following equation:

$$\text{CWSI} = \frac{(Tleaf - Twet)}{(Tdry - Twet)} \quad \text{(II)}$$

In which: *Tleaf* = the average temperature of the leaf in ambient conditions; *Twet* = the temperature of a leaf saturated with water (saturation was performed with a water spray 5 min before the leaf temperature was measured); and *Tdry* = the temperature of a leaf in non-transpiring conditions, coated with petroleum jelly to simulate stomatal closure, emphasizing that CWSI values close to 1 indicate that the plants are stressed and close to 0 that the plants are not stressed.

2.5. Hydraulic conductivity

Branch segments were collected in the air at pre-dawn on the 25th day after irrigation was suspended in order to quantify the effect of different soil water availability levels on xylem hydraulic conductivity. They were cut 15 cm from the proximal end underwater, obtaining branch segments with a mean diameter of 3.6 ± 0.4 mm (mean ± SE) to assess the native hydraulic conductivity according to the methodology proposed by Sperry et al. (1988).

The segments were connected to a high-pressure reservoir filled with a degassed 5 mM potassium chloride solution, in which the water flow at a hydrostatic pressure of 6 kPa for 10 minutes passing through the branch section was quantified using an analytical scale (0.0001 g) to determine the native hydraulic conductance (k_h).

The native conductance (k_h) was calculated every 30 s, along with an average of the previous ten readings of 30 s, as the ratio of the mass flow of water passing through the branch section to the pressure gradient. The branch specific hydraulic conductivity (K_s) was then calculated as K_s = ((k_h/ branch cross-sectional area) x branch length) (Tyree and Ewers 1991).

2.6. Measuring biometric growth attributes

Measuring biometric growth attributes were performed on the 25th day after irrigation was suspended as well as the increase during the application period of the treatments. For this purpose, the size reached by the plants at the end of the study period was analyzed. The following measurements were taken: height (H, cm), measured with a millimeter ruler; stem diameter (SD, mm), measured with a digital caliper (precision 0.01 mm); leaf number (LN), by counting the number of visible and fully expanded leaves; and total leaf area (LA, cm^2), measured with a LI-3100 leaf area integrator (Li-Cor Inc., Lincoln, NE, USA).

Next, the plant material was divided into shoots (leaves and stem) and roots, packed in kraft paper bags and dried in a laboratory oven with forced air circulation at 65°C for 72 hours, after which it was weighed on a precision scale (0.0001 g precision) to determine the shoot dry mass (SDM, g) and root dry mass (RDM, g). The total dry mass (TDM, g) was then quantified by adding the SDM and RDM values.

2.7. Statistical analysis

The data were subjected to the Shapiro-Wilk test for normality and the Bartlett test for homoscedasticity. Generalized linear mathematical models were fitted for the dependent variables as a function of soil water levels. The data were then subjected to one-way analysis of variance (ANOVA), with water treatment a fixed factor. When significant differences were found using the F-test at 5%, the means were compared using the Tukey's test at 5% probability. The analyses were performed using the R Core Team (2022) program, version 4.2.2. Sigmoidal fits were performed with SigmaPlot 12.0 (2012) software.

3. RESULTS

The evolution pattern of daily plant transpiration generally varied depending on the soil water availability. The treatments with higher water availability, especially 100% and 80% MWRC, increased their daily plant transpiration during the application period of the treatments due to the increase in the leaf area, although there were some lower values on days with low atmospheric demand. The same increase was not observed in the water restriction treatments due to the low water availability in the substrate (Figure 3), which also resulted in lower growth and biomass production (Figure 8).

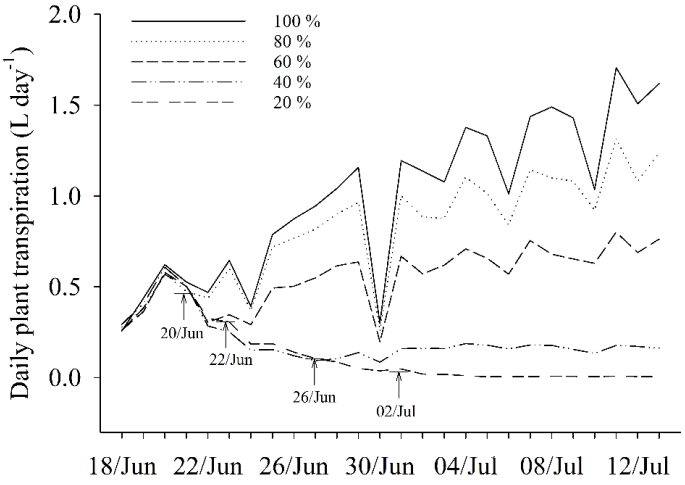

Figure 3 - Daily plant transpiration during the application period of treatments (between June 18, 2021 and July 13, 2021) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels. The dates indicated in the graphs refer to the

days when the desired water levels were reached, as follows: 80% MWRC on June 20, 60% on June 22, 40% on June 26, and 20% on July 2.

The relationship between leaf water potential (Ψ_{leaf}) and soil water availability followed a non-linear model (Figure 4), with higher pre-dawn Ψ_{leaf} in those treatments with high soil water levels (≥ 60% MWRC), followed by a sharp decline and reaching average values of -2.2 MPa for plants subjected to the 20% MWRC treatment.

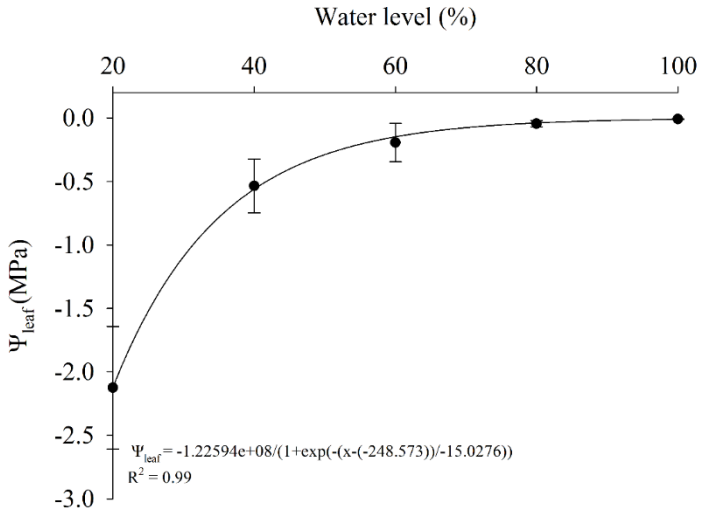

Figure 4 - Leaf water potential (Ψ_{leaf}) measured at 4 a.m. (local time) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants grown as a function of different soil water availability levels on the day the lowest soil water content (20% MWRC) was reached. Vertical bars represent the standard error of the mean (n = 4). The 20% treatment differed significantly ($p < 0.05$) from the other four, while the 40% to 100% treatments did not differ from each other.

The leaf gas exchange parameters followed a model with a sigmoidal trend in relation to soil water availability (Figure 5). A maximum *A* value of 25.6 μmol m^{-2} s^{-1} CO_2 was observed for the treatment with water close to field capacity, with a reduction in *A* of about 80% for the treatment with substrate moisture at 20% MWRC. There was a more drastic reduction regarding *E* and *gs* as a result of the water restriction, as both *E* and *gs* were close to zero for the 20% and 40% MWRC treatments.

The effect of the different soil water availability levels on the young *Eucalyptus grandis* × *Eucalyptus urophylla* plants could be observed through the CWSI (Figure 5), with values close to zero being obtained for the treatments with the highest water availability levels (≥ 60 % MWRC), indicating that the plants were not under stress. However, CWSI values of 0.92 and 0.90 were observed for the treatments below this water level (i.e. 20 and 40% MWRC), respectively, indicating a high level of water stress.

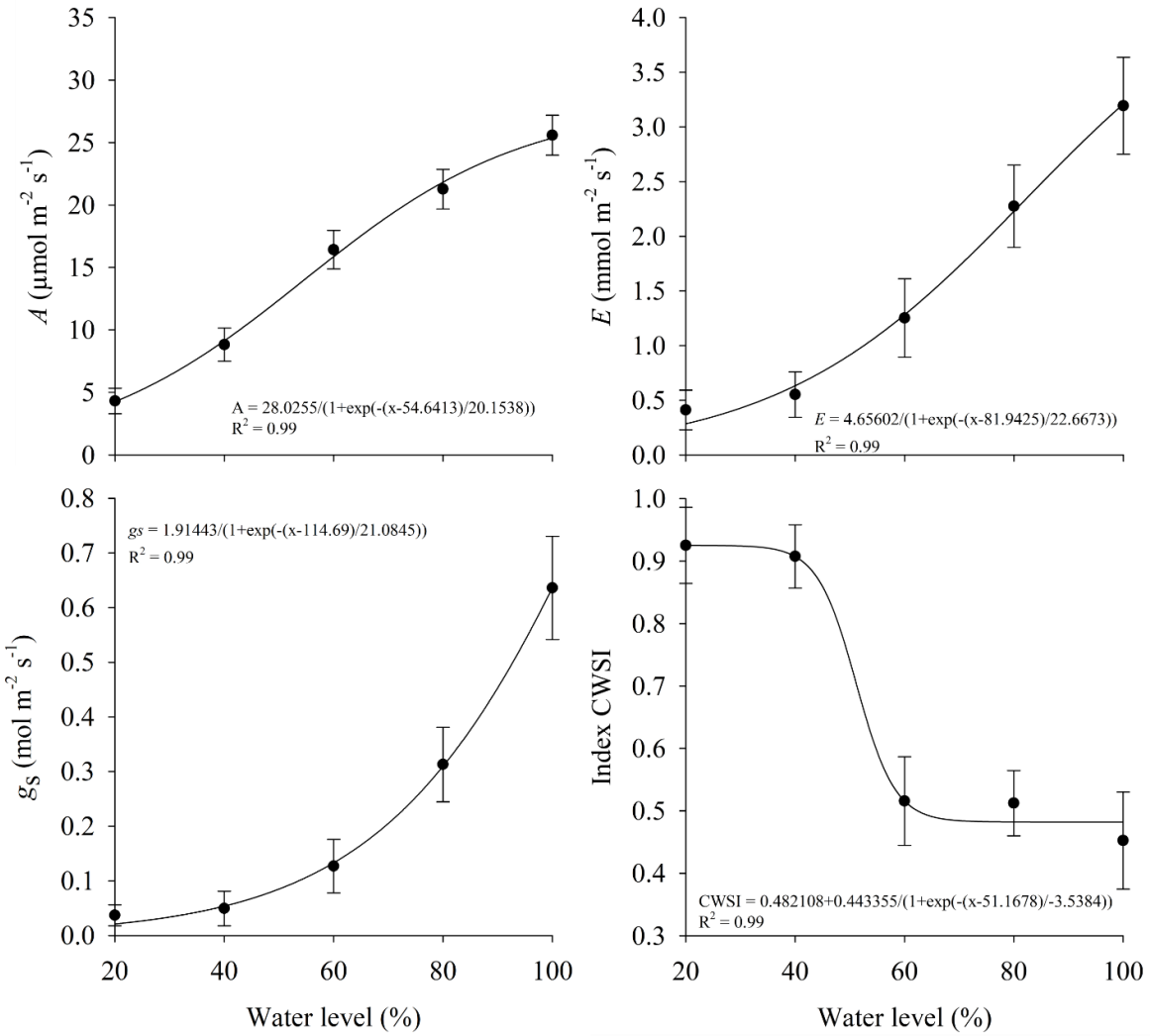

Figure 5 - Net CO_2 assimilation rate (*A*, µmol m^{-2} s^{-1} of CO_2), transpiration rate (*E*, mmol m^{-2} s^{-1} of H_2O), stomatal conductance (*gs*, mol m^{-2} s^{-1} of H_2O) and crop water stress index (CWSI) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels on the day the lowest soil water content (20% MWRC) was reached. Vertical bars represent the standard error of the mean (n = 4). Means followed by different letters are significantly different between treatments ($p \leq 0.05$): for *A* (100% a; 80% b; 60% c; 40% d; 20% e), for *E* (100% a; 80% b; 60% c; 40% d; 20% d), for *gs* (100% a; 80% b; 60% c; 40% c; 20% c), and for CWSI (100% a; 80% a; 60% a; 40% b; 20% b).

Figure 6 shows that there was an abrupt drop in gas exchange rates for this experiment, mainly *E* and *gs*, as well as in CWSI, when the pre-dawn leaf water potential dropped to around -0.5 MPa, mainly affecting the 20% and 40% soil moisture treatments (Figure 4).

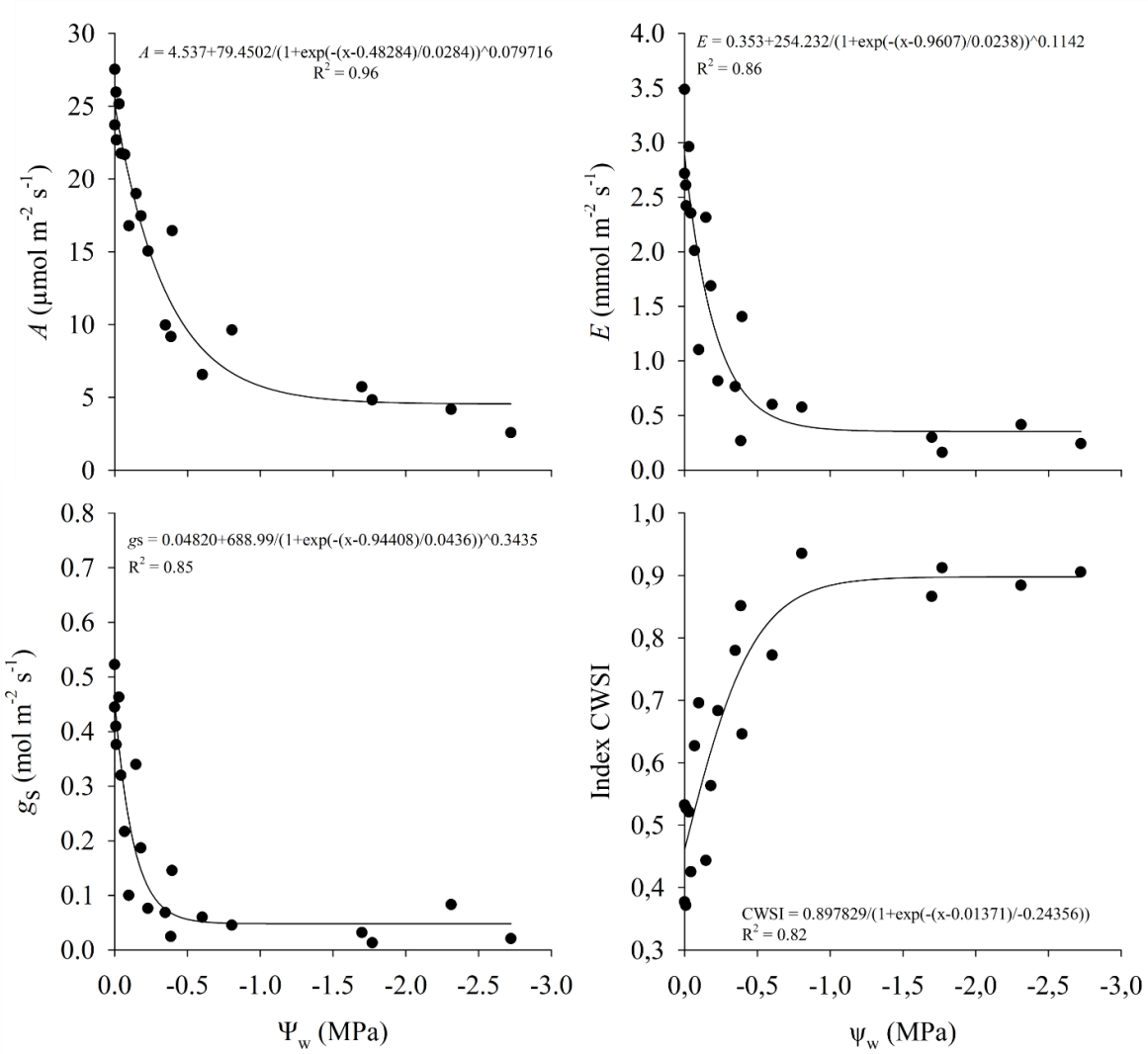

Figure 6 - Net CO_2 assimilation rate (*A*), transpiration rate (*E*) and stomatal conductance (*gs*) and crop water stress index (CWSI) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of pre-dawn leaf water potential.

The different soil water availability levels resulted in changes in the heat dissipation and leaf cooling processes of the young *Eucalyptus grandis* × *Eucalyptus urophylla* plants, in turn resulting in an increase in leaf temperature throughout the day, with higher temperature values being observed in the hottest hours of the day for the 20% and 40% MWRC treatments, the treatments with higher water stress (Figure 7). The most pronounced differences among treatments occurred in the middle hours of the day, between 10 am and 2 pm.

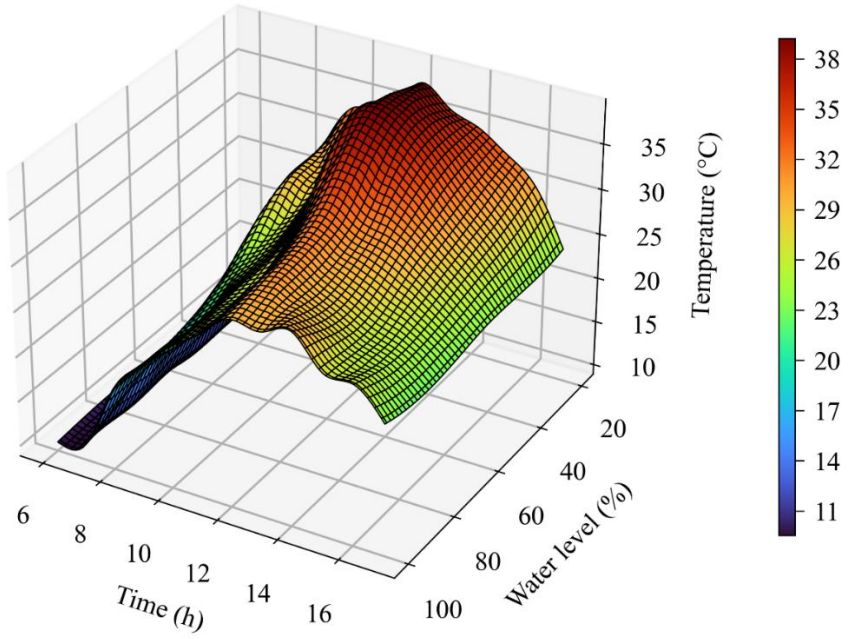

Figure 7 – Leaf temperature throughout the day for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels on the day the lowest soil water content (20% MWRC) was reached. Average of 4 plants per treatment and measurement hour.

Regarding the branch hydraulic conductivity, the 100% MWRC treatment showed the highest mean value at the end of the experiment (3.8 kg m^{-1} s^{-1} MPa^{-1}), with a decrease as the soil water availability level decreased (Figure 8). The treatment with the lowest water level in the soil (20% MWRC) showed a hydraulic conductivity percentage loss of 75 % compared to the treatment with moisture close to field capacity (100% MWRC).

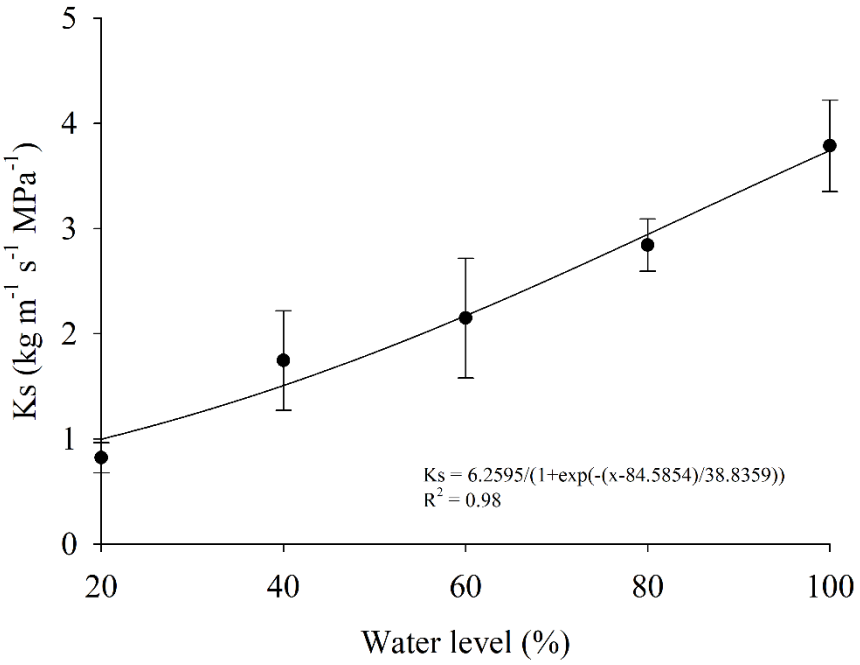

Figure 8 - Specific hydraulic conductivity for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels on the 25th day after

irrigation was suspended. Vertical bars represent the standard error of the mean (n = 4). Means followed by different letters are significantly different between treatments ($p < 0.05$): 100% a; 80% b; 60% c; 40% cd; and 20% d.

It can be seen that the relationship between the vegetative growth of the young *Eucalyptus grandis* × *Eucalyptus urophylla* plants and the soil water availability level at the end of the experiment followed a model with a sigmoidal trend, with greater values at the two highest water availability levels, followed by a decrease as the soil water availability level decreased (Figure 9). Water restriction significantly limited growth and biomass production. Figure 10 shows a plant of each treatment at the end of the study period, and allows us to visually understand the effect of each soil water availability level on growth.

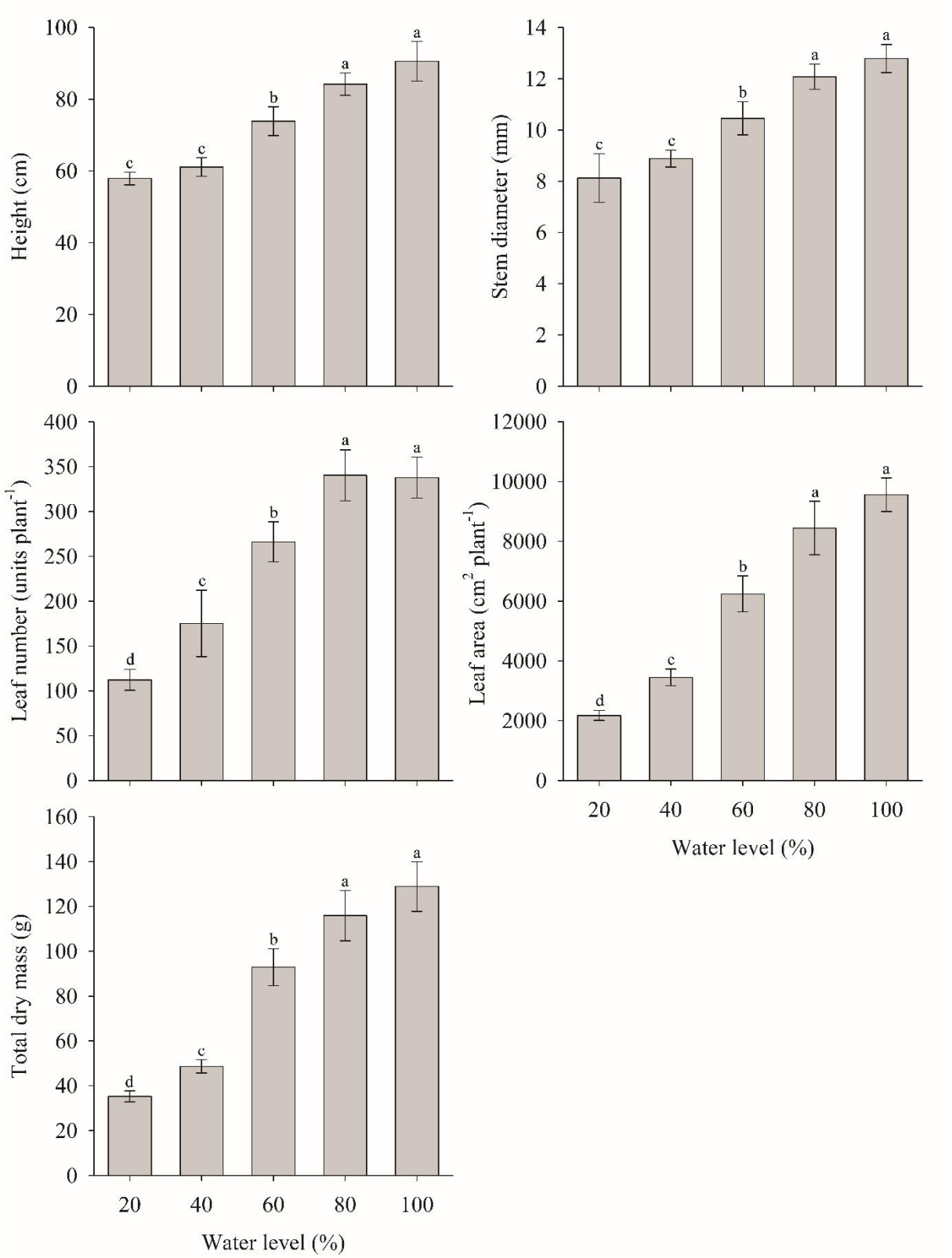

Figure 9 - Height (H), stem diameter (SD), leaf number (LN), leaf area (LA) and total dry mass (TDM) of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels on the 25th day after irrigation was suspended. Vertical bars represent the standard error of the mean (n = 4). Means followed by different letters are significantly different between treatments ($p \leq 0.05$).

Table 1 - Increase (Inc) in stem diameter (SD), height (H), number of leaves (NL), leaf area (LA) and total dry mass (TDM) of *Eucalyptus* × *urograndis* seedlings as a function of different soil water availability levels during the application of treatments. Means followed by different letters in the same column are significantly different between treatments ($p \leq 0.05$).

MWRC	Inc SD (mm)	Inc H (cm)	Inc LN (units plant^{-1})	Inc LA (cm^2 plant^{-1})	Inc TDM (g)
100%	6.38 ± 0.36 a	46.50 ± 3.44 a	269.25 ± 28.34 a	7777.44 ± 562.10 a	112.63 ± 11.07 a
80%	5.60 ± 0.17 b	42.75 ± 0.97 a	246.75 ± 22.91 a	6660.79 ± 894.75 a	99.6 ± 11.21 a
60%	4.20 ± 0.18 c	27.75 ± 2.46 b	185.00 ± 22.26 b	4457.24 ± 600.01 b	76.65 ± 8.24 b
40%	2.45 ± 0.15 d	17.55 ± 0.89 c	89.00 ± 13.05 c	1662.33 ± 278.14 c	32.34 ± 2.95 c
20%	1.94 ± 0.32 d	11,85 ± 1.11 d	31.00 ± 11.73 d	390.15 ± 164.57 c	18.97 ± 2.44 c

Figure 10 - Morphological characteristics of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants as a function of different soil water availability levels at the end of the experiment, 50 days after transplanting. Plants subjected to five soil water availability levels (20%, 40%, 60%, 80% and 100% MWRC) in the same order from left to right.

Accumulated plant transpiration during the application period of the water availability treatments (June 18 to July 13) was approximately 25 liters for the 100% MWRC treatment, with a drastic reduction as the soil water levels decreased (Figure 11). An even more drastic reduction was observed for the transpiration per leaf area ratio on the last day of the experiment (TLD/LA) as soil water availability decreased, showing that plant transpiration is not only conditioned by soil water availability, but also by the leaf area. Figure 12 also shows that the relationship between TDM and AT followed a linear model, which represents the high efficiency of water use for biomass production (4.2 g/L on average) by the I144 clone.

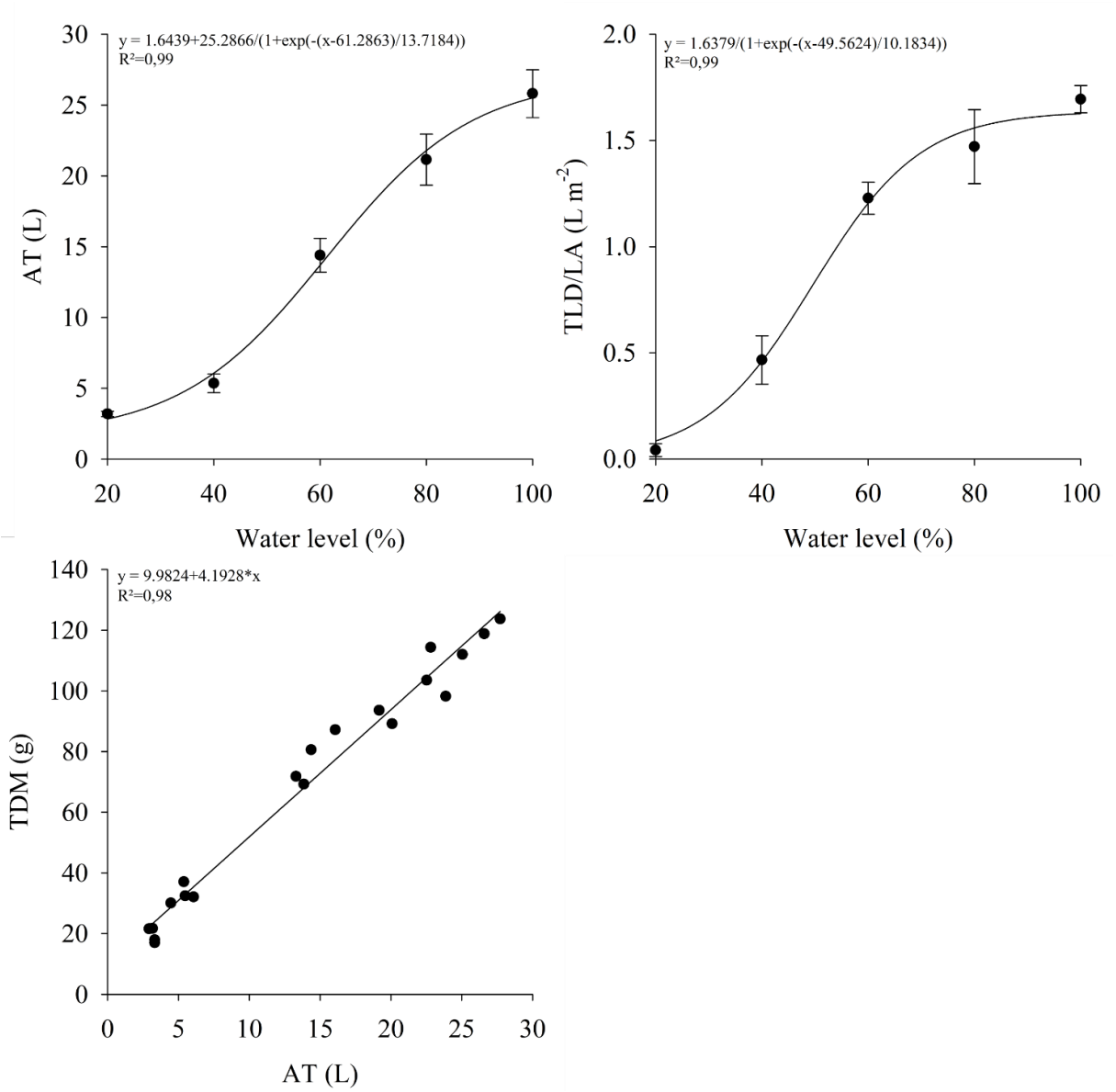

Figure 11 - Accumulated transpiration (AT), transpiration on the last day of the experiment (TLD) per leaf area (LA), and the ratio of total dry mass (TDM) to accumulated transpiration for *Eucalyptus grandis* × *Eucalyptus urophylla* seedlings as a function of different soil water availability levels. Vertical bars represent the standard error of the mean (n = 4). Means followed by different letters are significantly different between treatments ($p \leq 0.05$): for AT (100% a; 80% b; 60% c; 40% d; 20% d) and for TDL/LA (100% a; 80% a; 60% b; 40% c; 20% d).

4. DISCUSSION

The results obtained in this study show that the growth of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants (clone I144) was significantly affected by the level of water restriction imposed. Young *Eucalyptus grandis* × *Eucalyptus urophylla* plants started to show impaired growth and very strong reductions in *E*, *gs* and CWSI when the Ψ_{leaf} at dawn was ≤ -0.5 MPa, corresponding to the 20% and 40% MWRC treatments. These water restriction levels have an effect on physiological parameters which are essential for plant growth and development.

Leaf water potential followed a non-linear decreasing pattern with decreasing substrate moisture. Leaf water potential for treatments ≥ 60% MWRC was > -0.5 MPa, indicating that the plants were not under stress (Garcia et al. 2023). However, the average Ψ_{leaf} values for the 20% MWRC treatment were about -2.2 MPa at pre-dawn, indicating susceptibility to hydraulic failure. These results are consistent with studies showing that values below -2.0 MPa are sufficient to cause a 50% loss of hydraulic conductivity (PLC50) for clone I144 (*E. grandis* × *E. urophylla*) (Santos 2023). The interaction between hydraulic conductivity and Ψ_{leaf} sets limits on the hydraulic transport capacity of the plant and has a significant influence on the stomatal regulation of water use during drought and consequently on growth.

Hydraulic conductivity decreased significantly with decreasing substrate moisture, resulting in a 71% loss of hydraulic conductivity in the 20% MWRC treatment compared to the 100% MWRC treatment. This indicates a severe restriction of water movement in the xylem, compromising leaf water supply and exacerbating the effects on photosynthesis and plant growth (Tyree & Zimmermann 2002). Plant hydraulics limit ecosystem productivity by imposing physical constraints on water transport (Manzoni et al. 2013). In addition, accumulated transpiration over the course of the experiment was significantly lower in the low water availability treatments (20% and 40% MWRC). According to Manzoni et al. (2013), plant transpiration depends on plant hydraulics, stomatal conductance, and/or environmental conditions and plays a critical role in plant physiological processes, affecting carbon assimilation by leaves, growth, and productivity.

There was a significant reduction in leaf gas exchange parameters in this study, including net CO_2 assimilation rate, transpiration rate, and stomatal conductance as a result of reduced substrate water availability. Both *E* and *gs* took values close to 0 in the treatments with the lowest water availability levels in the substrate (20% and 40% MWRC). This suggests a defense mechanism to minimize water loss through transpiration, but at the same time it limited the CO_2 assimilation, compromising photosynthesis and plant growth (Nóia Júnior et al. 2020) (McDowell et al. 2011; Chaves et al. 2016). According to Kapoor et al. (2020), stomatal closure is considered to be one of the first plant strategies to avoid excessive dehydration under water stress, which affects plant growth. Although plants in the 40% MWRC treatment maintained a higher mean leaf water potential (approximately -0.5 MPa) than the 20% MWRC treatment, they responded by adjusting stomatal aperture and closure to reduce transpiration and avoid water potentials that could induce hydraulic failure and compromise plant growth; however, if the drought is prolonged, reductions in carbon assimilation and subsequent consumption of reserve carbohydrates may occur until a limit is reached, after which plants may die from carbon starvation (McDowell et al. 2011; Chaves et al. 2016).

The plant water stress index (CWSI) registered values close to zero in the treatments with high water availability (≥ 60% MWRC), indicating an absence of stress. Conversely, the 20% and 40% MWRC treatments exhibited values of 0.92 and 0.90, respectively, signifying elevated water stress. A concurrent and significant increase in leaf temperature was also observed, particularly during the middle hours of the day due to the diminished capacity of the plants to regulate heat through evapotranspiration. The loss of canopy evaporative cooling is widely regarded as a sensitive indicator of water stress, and it can be represented by an increase in leaf temperature (Grant et al. 2006; Garruña-Hernández et al. 2014). This phenomenon is particularly pronounced during the middle hours of the day, as the plants exhibit a diminished capacity to regulate heat through evapotranspiration. Transpiration serves as the primary mechanism for cooling the leaves in plants receiving adequate hydration. Conversely, stomata are closed under conditions of water deficit, and transpiration occurs exclusively through the cuticle, leading to an increase in leaf temperature (Schuster et al. 2016; Chaves et al. 2016; Carignato et al. 2020).

According to Silva et al. (2016), limited water conditions result in a significant reduction in the morphological characteristics of *Eucalyptus* species. An average reduction of 35% and 31% was observed in the present study for both height and stem diameter at levels of 20% and 40% of MWRC, respectively. The decline in growth is attributed to water stress, which has been shown to reduce the stomata opening, thereby limiting CO_2 absorption and consequently reducing photosynthetic activity (Osakabe et al. 2014). Reduced growth has been posited as an adaptive strategy for plant survival under stressful conditions. This strategy enables plants to redirect assimilated resources and energy to withstand water stress or to maintain root growth by improving water acquisition (Chaves and Oliveira 2004). Furthermore, a reduction in the water availability resulted in a 47% and 63% decrease in the number of leaves and a 64% and 77% decrease in leaf area for the 40% and 20% MWRC treatments, respectively. According to Nadal-Sala et al. (2021), this reduction is considered a morphological adjustment which allows plants to conserve water during drought periods, thereby preventing dehydration through transpiration.

It has been observed that the leaf expansion process is reduced under conditions where water availability becomes a limiting factor. This reduces carbon and energy use and allows a greater proportion of plant assimilates to be used for root and stem growth (Maseda and Fernández 2016; Taiz et al. 2017; Hubbard et al. 2020). In addition, water limitation resulted in greater reductions in total dry mass accumulation than in height and diameter. There was a 62% and 73% reduction in total dry mass for the 40% and 20% MWRC treatments, respectively.

This may be due to a reduction in leaf area and consequently a reduction in carbon assimilation by the plant to produce biomass (Silva et al. 2015).

Although reducing substrate water availability from 100% to 60% of MWRC did not cause significant reductions in leaf water potential and CWSI variables, there were reductions in certain plant characteristics such as height (18%), stem diameter (18%), leaf area (35%), total dry mass (28%), and number of leaves (19%). These results suggest that this soil moisture level was sufficient to induce mild water stress in *Eucalyptus* plants. Conversely, substrate humidity levels of 40% MWRC or lower resulted in severe water stress, leading to substantial reductions in plant growth. These findings indicate that ≤ 40% MWRC represent a highly restrictive condition for plant development. Furthermore, levels of 40% and 20% MWRC resulted in reductions exceeding 50% in all the characteristics evaluated when compared to the condition considered optimal for water availability in the substrate (100% MWRC). These findings evidence the high sensitivity of the young *Eucalyptus grandis* × *Eucalyptus urophylla* plant to these low water availability levels in the substrate.

Fast growing genotypes of the genus *Eucalyptus* have been shown to exhibit less conservative hydraulic strategies. Stomata in these genotypes show reduced sensitivity to plant water status and remain open even under water deficit conditions (Mitchell et al. 2013). However, it is important to identify the level at which water limitation begins to affect plant growth rates. For example, our results indicate that soil water levels at ≤ 40% MWRC begin to affect the growth of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants, indicating that plants are experiencing severe stress that affects their growth and development. Identifying this limit is essential for water management of plants in nurseries and reforestation, allowing more efficient use of water resources and optimizing plant growth.

5. CONCLUSIONS

The results of this study showed that the growth of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants is highly sensitive to the water availability. This sensitivity was evidenced by a significant reduction in physiological and morphological parameters under water restriction conditions. The results indicate that water availability at 40% MWRC or less was highly restrictive to plant growth, causing reductions of more than 50% in all characteristics evaluated, including height, stem diameter, leaf area, total biomass, and number of leaves.

Plants exposed to the 20% and 40% MWRC treatments exhibited physiological responses, such as stomatal closure, and morphological responses, such as a reduction in leaf area and number of leaves, to minimize water loss and increase survival under prolonged drought conditions.

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CHAPTER II – THE ECOPHYSIOLOGICAL RESPONSE OF *Eucalyptus grandis* × *Eucalyptus urophylla* PLANTS DEPEND ON THE VAPOR PRESSURE DEFICIT AND SOIL WATER AVAILABILITY INTERACTION

ABSTRACT

Changes in temperature, vapor pressure deficit (VPD), and precipitation patterns leads to increased drought intensity, affecting plant survival, growth, and productivity. In this context, this study aimed to investigate how young *Eucalyptus grandis* × *Eucalyptus urophylla* plants respond to different atmospheric demands and water availability regimes. The experiment was conducted at the Federal University of Espírito Santo, Brazil (latitude 20° 47′ 25" S, longitude 41° 23′ 48" W, and elevation 120 m), between April and July, 2023. Plants of clone I144 were grown for 75 days in 30 L pots containing commercial substrate, with daily irrigation to maintain the water levels at maximum water retention capacity (MWRC). The plants were then exposed to two environments: I) High Atmospheric Demand (HAD) - high temperature, with a maximum daily temperature of 40°C, and high VPD, with a maximum daily value of about 5.8 kPa; and II) Low Atmospheric Demand (LAD) - high temperature (40°C) and low VPD (3.2 kPa), and two soil water availability levels (Control and Stress, without and with soil water restriction, respectively). An automatic weather station was used to monitor microclimatic conditions. Ecophysiological analyses included leaf water potential (Ψ_{leaf}), leaf gas exchange, daily plant transpiration, crop water stress index (CWSI), quantum efficiency of photosystem II (F_v/F_m), and branch hydraulic conductivity measurements. The results showed that the plants grown without water restriction had high transpiration and CO_2 assimilation, but maintained a stable and lower performance in the LAD environment than in HAD. There was a drastic decrease in all physiological parameters under water restriction in the HAD environment, including Ψ_{leaf} and leaf gas exchange, resulting in partial recovery after rehydration, with serious effects on leaf area and hydraulic conductivity. However, the decline in physiological parameters was less pronounced for water-restricted plants in the LAD environment, and recovery after rehydration was more effective, but still affected leaf area. The CWSI and F_v/F_m variables indicated that the plants under water restriction in the HAD environment showed a high stress level compared to the LAD environment. Our results indicate that high atmospheric demand exacerbates the negative effects of water stress and impairs the hydraulic efficiency and gas exchange of plants, i.e. the hydraulic efficiency was reduced by 28% in the HAD treatment compared to the LAD treatment under water deficit, and CO_2 assimilation was reduced by 10%. An understanding of these responses is critical for developing management strategies that will mitigate the effects of climate change on *Eucalyptus* plantations.

Keywords: Atmospheric demand; drought; photosynthesis; greenhouse; climate change.

1. INTRODUCTION

Eucalyptus plantations in Brazil occupied approximately 7.6 million hectares in 2022, representing 76% of planted forests. The preference for commercial plantations of this genus is associated with high growth rates, adaptability to different soil and climatic conditions, and multiple uses of the wood (IBÁ 2023). There are more than 25 million hectares of commercial *Eucalyptus* plantations worldwide, mostly clones or hybrid clones (FAO, 2021; CABI, 2024). However, climate change is one of the greatest challenges of our time. The world is searching for solutions and alternatives to mitigate its effects. According to the *Industria Brasileira de Árvores* (IBÁ 2021), climate change could be one of the factors influencing the decline in the average annual productivity of *Eucalyptus* plantations from 38.6 m^3/ha in 2019 to 36.8 m^3/ha in 2020.

The productivity of planted forests is directly related to the climatic conditions of the environment where they are located (Bourne et al. 2017). According to the latest IPCC projections, the effects of climate change could reduce the productivity and quality of many crops of high socio-economic importance around the world, especially during the summer season due to intense and prolonged stress factors (Elli et al. 2020; IPCC 2023). Although temperature increase is the most noticeable effect of climate change, other important factors for plant survival in the field have been highlighted, such as precipitation patterns, drought duration and intensity, and air vapor pressure deficit (VPD), which can negatively affect the hydraulic efficiency of *Eucalyptus* plants (Stape et al. 2010; Fraga et al. 2019; McDowell et al. 2022; IPCC 2023).

Temperature and water availability are directly related to evapotranspiration processes and metabolic responses, affecting plant survival, growth, and development (Oliveira et al. 2012; Taiz et al. 2017). Plants respond physiologically under drought conditions to prevent excessive water loss, and this response includes a decrease in stomatal conductance to prevent leaf water potentials from falling below a critical threshold (Van Gorsel et al. 2013). Water uptake by roots in the absence of effective stomatal regulation under drought may not compensate for water loss, resulting in high tension in the plant's vascular system, which can lead to xylem embolism, and if the embolism extends throughout the xylem, water transport becomes limited and plants may eventually die from hydraulic failure (Choat et al. 2018).

As mentioned above, another critical factor affected by climate change is the vapor pressure deficit (VPD), which is the difference between the amount of moisture present in the

air and the moisture capacity at saturation. VPD is one of the main drivers of stomatal conductance, and the response can be characterized by a negative logarithmic function, i.e. stomatal conductance decreases as VPD increases (Zeppel et al. 2012; Resco De Dios et al. 2018; Chowdhury et al. 2022). Severe droughts with higher VPD may especially promote a faster decline in water content than in carbon supply rate, thus exceeding the water content limit on mortality before the carbon supply limit.

The imbalance between the plant water supply and demand leads to water deficit situations which affect all cellular processes, leaf expansion, stomatal conductance, photosynthetic activities, leaf senescence and abscission, and transpiration rates (Esmaeilzade-Moridani et al. 2015; King and Purcell 2017). Given this scenario, this study was conducted to investigate how *Eucalyptus grandis* × *Eucalyptus urophylla* plants, one of the hybrids widely grown in commercial plantations around the world (CABI, 2024), respond to different vapor pressure deficits under water limitation conditions in a controlled environment. Thus, we hypothesize that plants in a High Atmospheric Demand (HAD) environment will experience greater physiological stress, resulting in faster reductions in soil water content, transpiration, and stomatal conductance compared to those in a Low Atmospheric Demand (LAD) environment.

2. MATERIALS AND METHODS

2.1. *Plant material and experimental conditions*

The experiment was conducted in climate-controlled vegetation houses (Poly Venlo model, Van der Hoeven®), with temperature and relative humidity control, located at the Department of Forestry and Wood Science of the Federal University of Espírito Santo (DCFM-UFES), in Jerônimo Monteiro, Espírito Santo, Brazil (latitude 20° 47′ 25" S, longitude 41° 23′ 48" W, and elevation 120 m). The experimental period was from April 13 to July 17, 2023 (Fig. 1).

Rooted cuttings of *Eucalyptus grandis* × *Eucalyptus urophylla* clone I144 were grown in 50 cm^3 tubes filled with a substrate of biostabilized pine bark, vermiculite, crushed charcoal and phenolic foam. Plants with a height of 26.33 ± 1.40 cm and an average stem diameter of 1.0 ± 0.3 mm (mean ± SE) at 90 days of age were transplanted into 60-litre pots (height 45 cm, upper circumference 150 cm and lower circumference 125 cm) containing commercial substrate and fertilized with 4 g dm^{-3} of Basacote® Mini 9M controlled-release fertilizer with NPK 16-8-12 formulation. After transplanting, the plants underwent a 75-day acclimatization period (from

April 13 to June 26, 2023) in an outdoor environment with sufficient daily irrigation to maintain the substrate at moisture conditions close to the maximum water retention capacity (MWRC).

The plants had a height of 1.7 ± 0.2 m, a stem diameter of 17.0 ± 1.2 mm and a leaf area of 1.2 ± 0.2 m^2 at 76 days after transplanting (June 27, 2023). They also looked healthy and the instantaneous gas exchange rates (measured at 9 a.m., at a temperature of 36.6ºC, a light intensity of 1500 μmol m^{-2} s^{-1} and a CO_2 concentration of 400 μL L^{-1}) were: photosynthesis 23.1 ± 1.7 μmol m^{-2} s^{-1} CO_2, transpiration 11.4 ± 03 mmol m^{-2} s^{-1} H_2O, stomatal conductance 0.5 ± 0.05 mol m^{-2} s^{-1} H_2O and an internal CO_2 concentration of 275.6 ± 12 ppm (mean ± SE). At this point, they were saturated to obtain the weight at field capacity of each pot. The pots were then allowed to reach 70% MWRC (June 30, 2023) before being taken into the greenhouse and randomly distributed in two environments: environment I - High temperature and high vapor pressure deficit (High Atmospheric Demand; HAD); and environment II - High temperature and low vapor pressure deficit (Low Atmospheric Demand; LAD). Two soil water availability levels were used in each environment: Control – 70% Maximum Water Retention Capacity (MWRC); and Stress - progressive reduction of water availability, with irrigation stopped. The experimental design was completely randomized with five replications and the experimental unit consisted of one plant, giving a total of 20 plants.

Daily gas exchange measurements were taken during the progressive reduction of water availability period using a portable infrared gas analyzer (IRGA, LI-COR, model LI-6400) until net CO_2 assimilation rate approached zero (water restriction period - July 01 to 05, 2023). When net CO_2 assimilation rate approached to 0, irrigation was restored to field capacity (rehydration period - July 06 to 18, 2023) to assess recovery from stress.

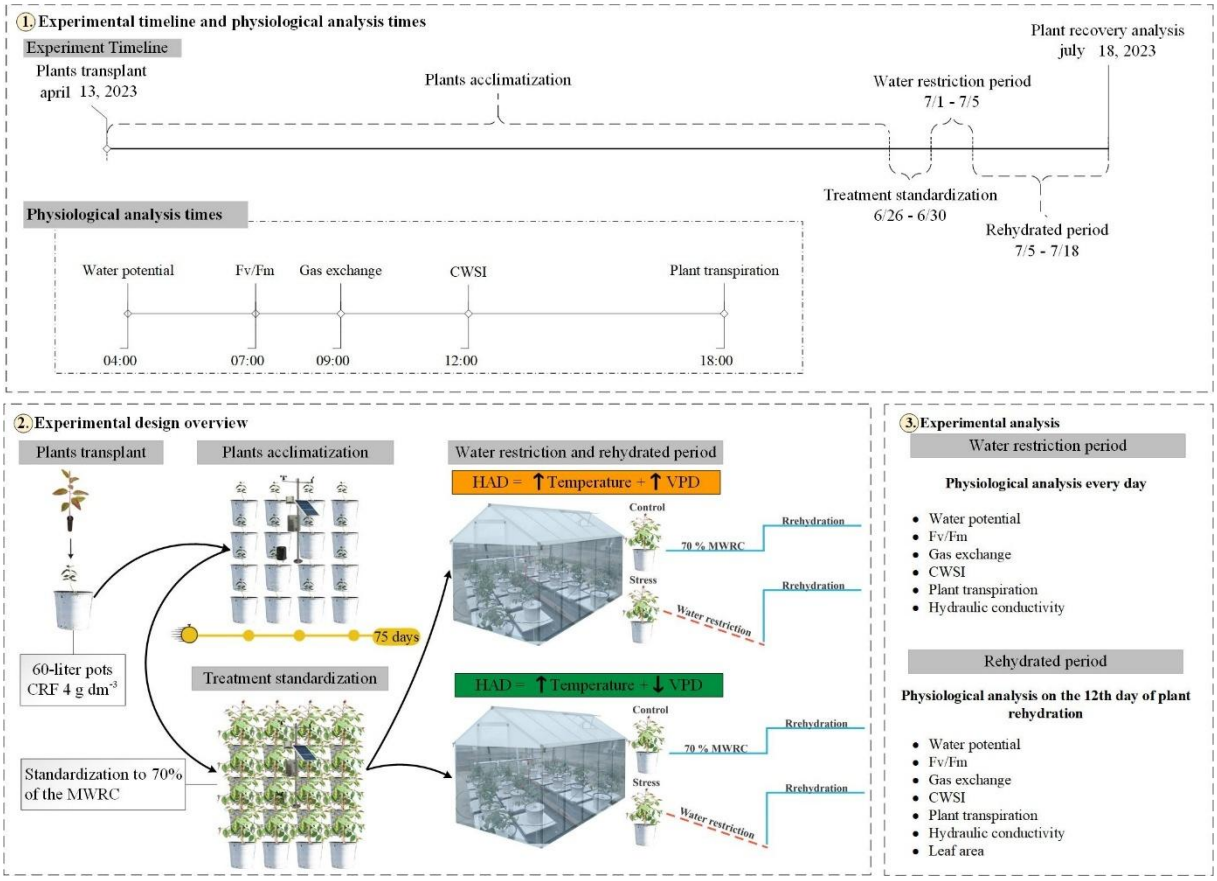

Figure 1-1. Experimental timeline and physiological analysis time. **2**. Experimental design overview. 3. Experimental analysis during the water restriction period (July 01 to 05, 2023) and at the end of the plant recuperation period (July 17, 2023).

2.2. Microclimatic characterization

The greenhouse temperature was controlled using an evaporative cooling system (pad cooling) and heaters controlled by the MT-543RiPlus controller (Full Gauge®). Relative air humidity was controlled by a fogging system activated by a humidity controller (Psychrometer model AHC-80 plus, Full Gauge®).

The variations in temperature and relative humidity values of the systems were controlled and maintained through Sistrad® software. The temperature control system was programmed every 30 minutes during the day and every hour at night to maintain the desired environmental conditions in each greenhouse, while the desired humidity was programmed at a fixed value according to the treatment in each greenhouse.

An automatic weather station with temperature and relative humidity sensors (model CS500, Campbell Scientific, Inc., Logan, UT, USA) was installed to characterize the microclimatic conditions of the environments during the water restriction period and the recovery period. Data were stored in a data logger (model CR1000, Campbell Scientific, Inc., Logan, UT, USA), powered by a 12-volt battery connected to a solar panel and programmed to take readings every 10 seconds and store averages every 5 minutes. The vapor pressure deficit

(VPD) was calculated from the difference between the water vapor saturation pressure (es) and partial water vapor pressure (ea) values, following the methodology of Pereira et al. (2002).

The microclimatic characterization of the two environments was performed during the water restriction period (July 01 to 05, 2023) and the recovery period (July 06 to 18, 2023). The high atmospheric demand (HAD) environment had a maximum daily temperature of 40.3 ± 0.6°C and a maximum daily VPD of 5.8 ± 0.4 kPa (mean ± SE) and the low atmospheric demand (LAD) environment had a maximum daily temperature of 39.9 ± 1.6°C and a maximum daily VPD of 3.2 ± 0.3 kPa (mean ± SE) (Figure 2).

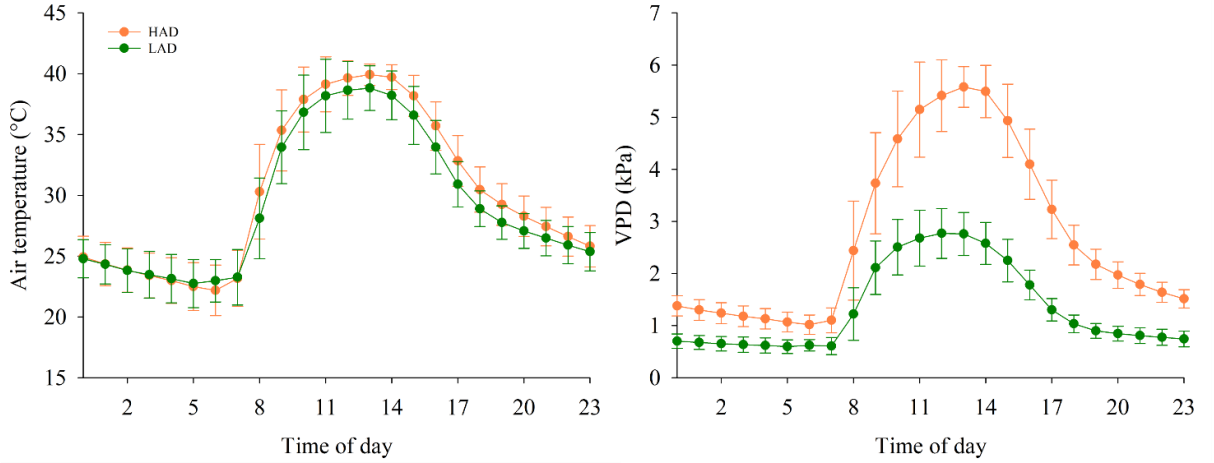

Figure 2 - Daily variation of air temperature and vapor pressure deficit (VPD) during the water restriction (July 01 to 05, 2023) and recovery (July 6 to 18, 2023) periods, simulating two environmental conditions, high atmospheric demand (HAD) and low atmospheric demand (LAD). Values represent mean ± standard deviation.

2.3. Monitoring soil water levels

Soil water levels were characterized using a lysimetric electronic weighing system during the water restriction and recovery periods. This system consisted of a set of precision scales (model Prix 2098, Toledo do Brasil, São Bernardo do Campo, São Paulo, BR) that directly measured plant transpiration based on the difference in the weight of the pots at the beginning and end of the day. The scales were connected to microcontrollers (model ESP8266, Espressif Systems) that were responsible for reading and storing the water consumption of the plants every five minutes.

The plants were watered in the late afternoon (06 p.m., local time) based on daily weighing of pots to replace water lost through transpiration. Plants in the control treatment were watered daily, while there was no watering during the water restriction period in the water-stressed treatment. All plants were watered daily during the rehydration period. Daily plant transpiration was represented by the difference in mass measured at the beginning (06 am) and end (06 pm) of the day. The pots were sealed with a 10 mm thick sheet of white Styrofoam to

monitor water transpiration by the plants and ensure that water was lost only through transpiration, preventing water evaporation from the soil.

2.4. Plant water status and gas exchange

Leaf water potential (Ψ_{leaf}) and gas exchange analyses were performed daily during the water restriction period (July 02 to 05, 2023) and on the 12th day of recovery (July 17, 2023). Ψ_{leaf} was measured at 04 am (local time) using a Scholander 1505D-EXP pressure chamber (PMS Instrument Co., Albany, OR, USA) on fully expanded and healthy leaves in the middle third of the plant height.

Gas exchange measurements were performed at 09 am on fully expanded and healthy leaves from the middle third of the plant height using a portable infrared gas analyzer (IRGA, model Li COR 6400, LI-COR Inc., Lincoln, NE, USA) with a 6 cm^2 measuring chamber, a light intensity of 1500 μmol m^{-2} s^{-1}, and a CO_2 concentration of 400 μL L^{-1}. Net CO_2 assimilation rate (*A*, μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1} H_2O), and internal CO_2 concentration (Ci, ppm) were determined.

2.5. Crop Water Stress Index

Thermal images of fully expanded and healthy sun-facing leaves in the middle third of the plants were taken at 12 noon each day during the water restriction period and on the 12th day of recovery using a FLIR T430sc thermographic camera (FLIR Systems, Wilsonville, OR, USA) with a resolution of 320 × 240 pixels to determine the Crop Water Stress Index (CWSI). The distance between the camera and the plant canopy was set to 0.5 m, and the emissivity used was 0.96. FLIR Tools software was used to process the images, generating 10 samples in each replicate to obtain the average temperature in each treatment.

The CWSI was calculated according to the methodology proposed by Jones (1999) using the following equation:

$$\mathrm{CWSI} = \frac{(Tleaf - Twet)}{(Tdry - Twet)} \quad \text{(I)}$$

In which: *Tleaf* = the average temperature of the leaf under ambient conditions; *Twet* = the temperature of a leaf saturated with water (saturation was performed by spraying water 5 min before measuring the leaf temperature); and *Tdry* = the temperature of a leaf under non-respiring conditions, coated with petroleum jelly to simulate stomata closure, emphasizing that CWSI values close to 1 indicate that the plants are stressed and close to 0 that the plants are not stressed.

2.6. Chlorophyll fluorescence

The quantum efficiency of photosystem II (F_v/F_m) was measured using a portable FluorPen FP110 (Photon Systems Instruments, Drásov, Czech Republic). Analyses were performed at dawn (07 am) every day during the water restriction period and on the 12th day of recovery on fully expanded and healthy sun-facing leaves from the middle third of the plants. The selected leaves were dark adapted for 30 min and then exposed to a saturating pulse of 3000 μmol m^{-2} s^{-1} actinic light.

2.7. Hydraulic conductivity

Branch segments were collected in the air on July 6, 2023, the day after the last day of the water restriction period, and on the 12th day of the rehydration period at dawn, and then cut underwater 15 cm from the proximal end with a mean branch diameter (± SE) of 4.3 mm (± 0.4 mm) to obtain the native hydraulic conductivity in order to determine the effects of water restriction and atmospheric demand on hydraulic conductivity, according to the methodology proposed by Sperry et al. (1988).

The segments were connected to a high-pressure reservoir filled with a degassed 5 mM potassium chloride solution to determine the native hydraulic conductance (k_h). With the branch attached to the reservoir, the flow of water passing through the branch section was quantified using an analytical scale (0.0001 g) at a hydrostatic pressure of 6 kPa for 10 minutes.

The native conductance (k_h) was calculated every 30 s, together with an average of the 10 previous 30 s readings, and is the ratio between the mass water flow passing through the branch section and the pressure gradient. The specific hydraulic conductivity of the branch (K_s) was then calculated as K_s= (k_h / branch cross-sectional area) x branch length) (Tyree and Ewers 1991).

2.8. Leaf area

Measurements of the total leaf area (LA, m^2) were performed when the pots reached 70% MWRC (June 30, 2023). The leaf area was estimated using the equation:

$$LA = NL^* [f (L \times W)]$$

In which: NL = total number of leaves on the plant, dimensionless; f = shape factor, dimensionless; L and W = length and width, respectively, in cm, referring to an average value of 100 leaves of various sizes in the different extracts of the plant. The “f” factor was determined by simple regression analysis between the leaf area and the product of its respective dimensions.

In addition, the total leaf area (LA, m^2) was measured at the end of the experiment using a leaf area integrator (model LI-3100, Li-Cor Inc, Lincoln, Nebraska, USA).

2.9. Statistical analysis

The data were subjected to the Shapiro-Wilk test for normality and the Bartlett's test for homoscedasticity. The data were then subjected to two-way analysis of variance (ANOVA), with atmospheric demand and water restriction constituting the two fixed factors. When significant differences were found by the F-test at 5%, the means were compared using the Tukey's test at 5% probability level. Analyses were performed using R version 4.2.2 (R Core Team, 2022). Graphs were generated using SigmaPlot 12.0 (2012) software.

3. RESULTS

The results obtained show that there was greater reduction in the soil water content (Fig. 3) during the water restriction period and after two weeks of rehydration for the Control treatment in the HAD environment, reaching values close to 50% MWRC due to the high transpiration of the plants under these conditions. However, the reduction for plants from the stress treatment in the same environment was even more drastic from the first day of the water restriction period, reaching values close to 10% MWRC after the 2^{th} day of restriction, while plants from the stress treatment in the LAD environment showed a moderate reduction, reaching soil water content values close to 10% MWRC only from the 4^{th} day of the water restriction period. All the treatments returned to a moisture content close to the maximum water retention capacity (MWRC) after the rehydration period. However, due to the high transpiration of the control treatment in HAD environment, there were greater reductions in soil water content throughout the day, reaching 70% MWRC by the end of the day. The soil moisture corresponding to complete stomatal closure in the HAD environment was approximately 21%, beginning on the 2^{nd} day of water stress. However, the aforementioned 21% soil moisture was not reached in the LAD environment until noon on the 3^{rd} day of the water restriction period due to the lower evaporative demand.

Daily plant transpiration (Fig. 3) was higher for the control treatment in the HAD environment, with higher transpiration rates during the hottest hours of the day (between 11 am and 2 pm), reaching average hourly values of 0.45 L h^{-1} and average daily values of 3.2 L day^{-1}, while the control treatment in the LAD environment maintained maximum hourly transpiration rates of 0.3 L h^{-1} and daily values of 2.0 L day^{-1}. However, the stress treatment maintained similar transpiration rates in the LAD environment to the control treatment until the

2nd day of water restriction, followed by a gradual decrease in transpiration, while transpiration decreased drastically in the HAD environment from the 2nd day of water restriction, reaching values close to zero.

Control treatment plants in the HAD environment had higher transpiration rates during the hottest hours of the day after two weeks of rehydration, with average hourly values of 0.67 L h^{-1}, while the control treatment in LAD had a transpiration rate of 0.37 L h^{-1} at the same time. Stress treatment plants in the HAD environment did not fully recover the whole plant transpiration due to an observed reduction of 54% compared to the control treatment, while this reduction was 28% in the LAD environment.

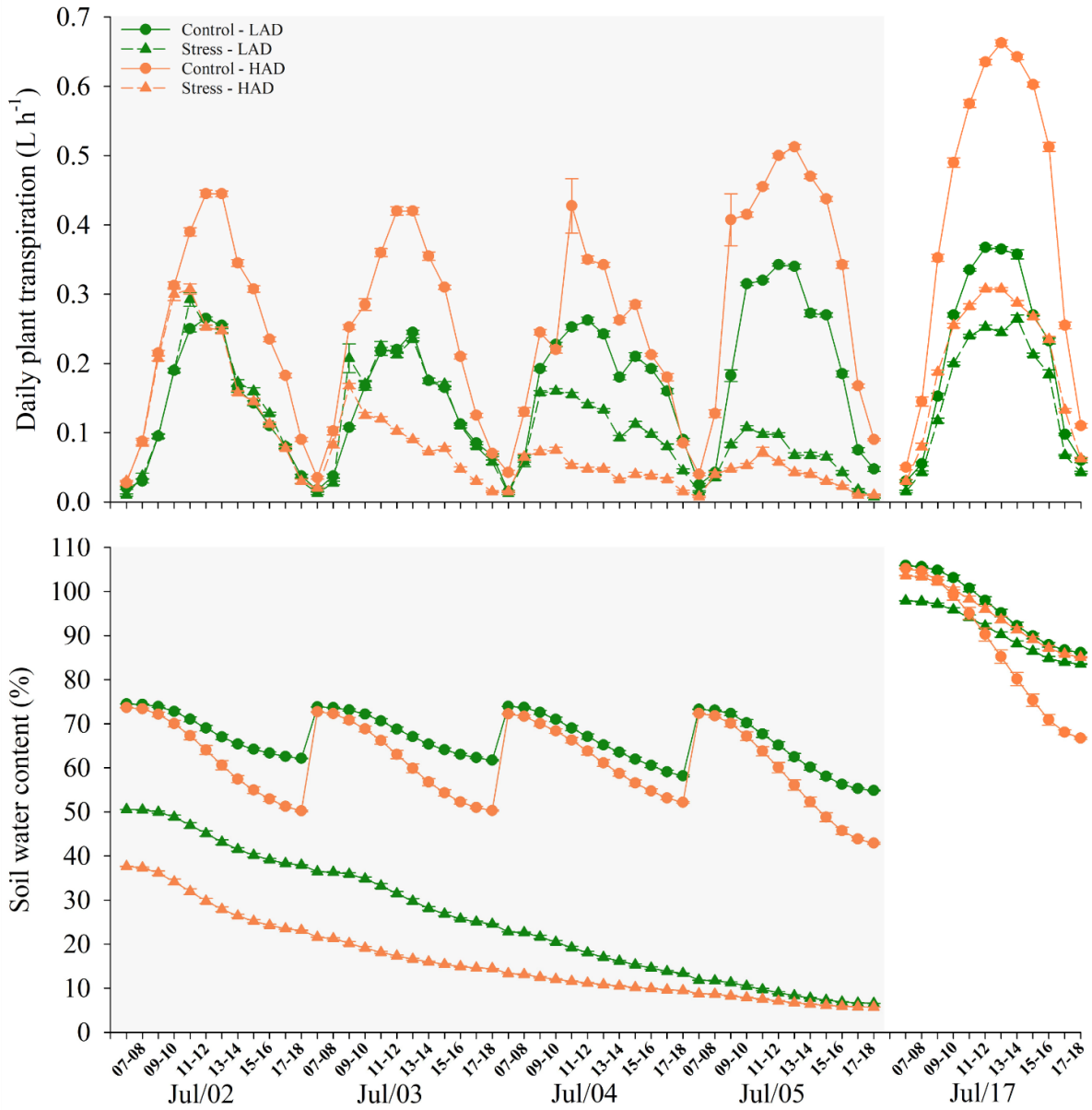

Figure 3 - Hourly measurements on plant transpiration and soil water content for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to two soil water availability regimes (Control-circles and Stress-triangles) in two environments: low atmospheric demand (LAD-green) and high atmospheric demand (HAD-orange), during the water restriction period

between July 1 and 5, 2023 (gray area) and on the 12th day after rehydration, July 17, 2023 (white area). Vertical bars represent the standard error of the mean (n = 4).

Control plants stayed hydrated in both high and low atmospheric demand conditions throughout the evaluation period, with Ψ_{leaf} values at pre-dawn of around -0.3 MPa. Plants in the stress treatment in both the HAD and LAD environments showed a significant decrease ($p \leq 0.05$) in Ψ_{leaf} over the water restriction period. However, the plants under stress treatment in HAD were more affected, showing the biggest drops in leaf water potential at pre-dawn reaching -1.8 MPa, while the plants under soil moisture stress in LAD reached -1.2 MPa on the last day of water restriction. Then on the 12th day of rehydration, all plants returned to a similar leaf water potential to the initial one (-0.2 MPa), indicating that there had been a recovery after the water restriction period (Fig. 4).

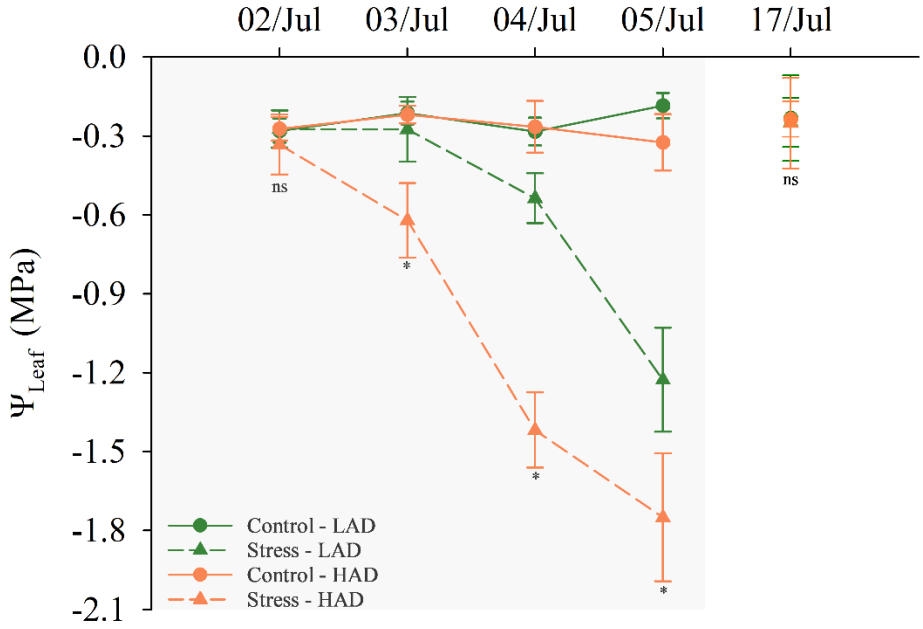

Figure 4 - Leaf water potential (Ψ_{leaf}) at pre-dawn for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to two soil water availability regimes (Control- circles and Stress- triangles) in two environments: low atmospheric demand environment (LAD-green) and high atmospheric demand environment (HAD- orange), during the water restriction period between July 1 and 5, 2023 (gray area) and on the 12th day after rehydration, July 17, 2023 (white area). Vertical bars represent the standard error of the mean (n = 4). for Ψ_{leaf} (100% a; 80% b; 60% c; 40% d; 20% e)

Regarding the gas exchange parameters, there was a significant decrease ($p \leq 0.05$) in the CO_2 assimilation rate over time. Plants from the control treatment in HAD showed a higher CO_2 assimilation rate, with values of 30 µmol m^{-2} s^{-1} on the first day, followed by a slight decrease, reaching values of 18 µmol m^{-2} s^{-1} on the 4th day of the water restriction period; then for plants from the control treatment in LAD, *A* values were lower, reaching values of 9 µmol m^{-2} s^{-1} of CO_2 on the 4th day of the water restriction period (Fig. 5). However, plants from the

stress treatment in the HAD environment showed the greatest reduction in *A*, reaching values of -0.5 µmol m^{-2} s^{-1} CO_2 on the last day of the water restriction period. However, all plant groups converged to high and similar *A* levels of 21 µmol CO_2 m^{-2} s^{-1} after two weeks of rehydration, suggesting a recovery response of the plants to the imposed stress.

With regard to *E* and *gs*, plants from the control treatment in both environments (LAD and HAD) maintained relatively high and constant *E* values close to 11 and 12 mmol H_2O m^{-2} s^{-1}, respectively, and with g_s of 0.6 mol m^{-2} s^{-1} in both environments. Plants in the HAD environment and stress treatment showed a drastic decrease in *gs* and *E* from the second day of the water restriction period, reaching values close to zero, while the LAD stress treatment also decreased but less accentuated, reaching values close to zero, especially for *gs*, and only on the 4th day of the water restriction period (Fig. 5). Figures 4 and 5 show that complete stomatal closure was not achieved (*gs* close to zero) until the leaf water potential at dawn reached values close to -0.6 or -0.7 MPa, at least for the HAD condition. The *gs* and *E* values were equalized between the control groups after two weeks of rehydration, while the stress treatment maintained low values, indicating that the plants had not fully recovered from the imposed stress (Fig. 5).

The control treatment in HAD and LAD environments maintained high Ci values, while the stress treatments showed a significant decrease ($p \leq 0.05$). The stress treatment in HAD showed a reduction in Ci from the first day of water restriction, while in LAD it showed a reduction on the 4th day. After rehydration, the stress treatments, especially in HAD, remained below the control treatments, indicating that they had not fully recovered.

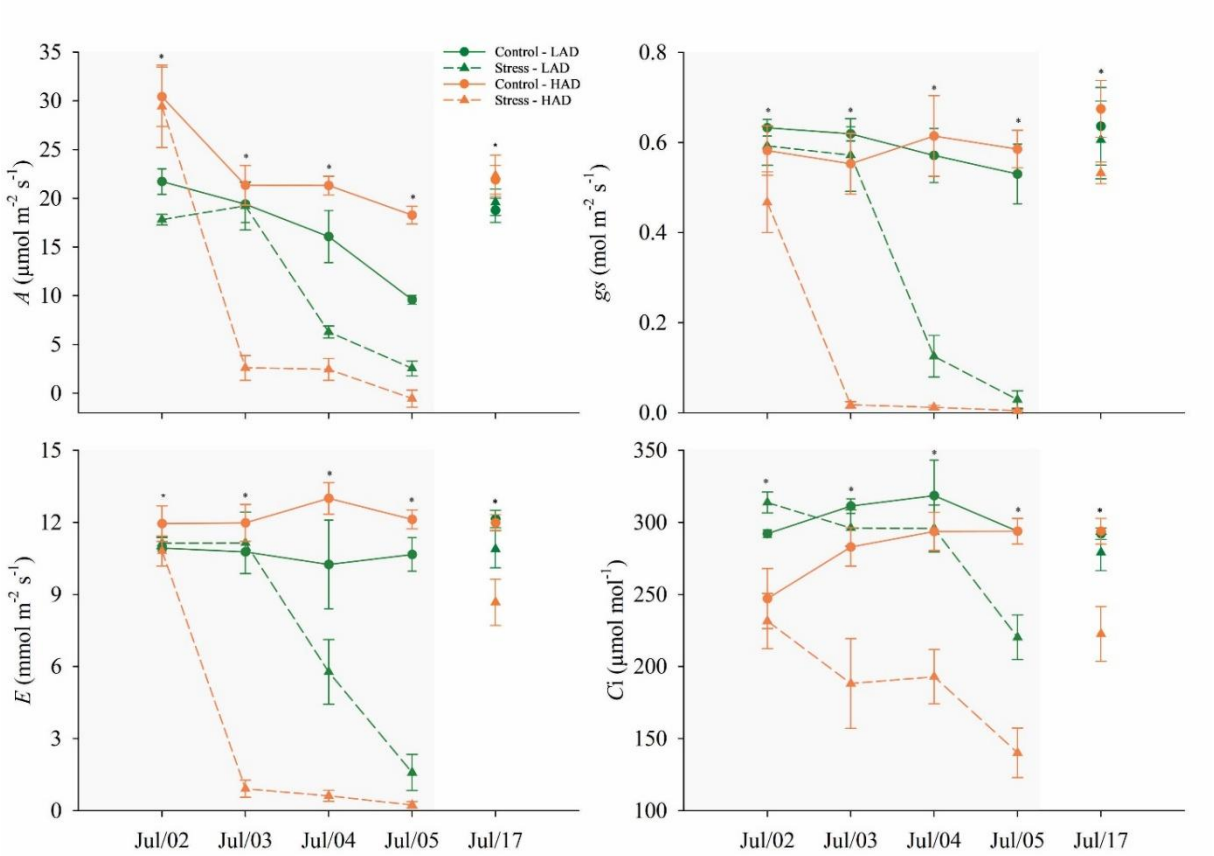

Figure 5 - Net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and intracellular CO_2 concentration (Ci) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants exposed to two soil water availability regimes (Control-circles and Stress- triangles) in two environments: low atmospheric demand (LAD- green) and high atmospheric demand (HAD- orange) environments, during the period of water restriction between July 1 to 5, 2023 (gray area) and on the 12th day after rehydration, July 17, 2023 (white area). Vertical bars represent the standard error of the mean (n = 4).

Control treatment plants in LAD had a specific hydraulic conductivity of 5.7 kg m^{-1} s^{-1} MPa^{-1} After four days of water restriction, which was higher than that observed in the HAD environment (Fig. 6). There was a significant decrease for the stress treatments ($p \leq 0.05$) in the water transport capacity of the plants, with lower K_s values observed in HAD, about 0.9 kg m^{-1} s^{-1} MPa^{-1}. Plants in both environments did not recover for the stress treatments after rehydration.

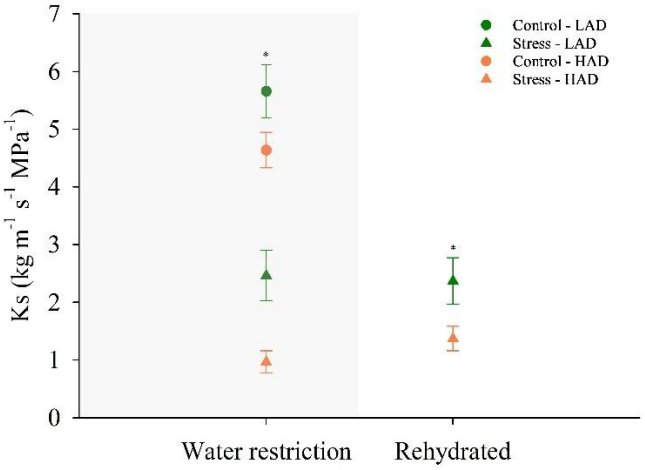

Figure 6 - Hydraulic conductivity for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to two soil water availability regimes (Control- circles and Stress- triangles) in two environments: low atmospheric demand (LAD- green) and high atmospheric demand (HAD- orange). After the period of water restriction between July 1 to 5, 2023 (gray area) and on the 12th day after rehydration, on July 17, 2023 (white area). Vertical bars represent standard error of the mean (n = 4).

The control treatment plants in both environments maintained stable and relatively low CWSI values throughout the application period of the treatments, around 0.40, while there was a progressive increase in the CWSI value for plants of the stress treatment; higher averages were observed for the stress treatment in HAD, reaching average values of 0.93 on the last day of the water restriction period, indicating greater water stress in these conditions, while the stress treatment in LAD reached maximum values of 0.77. All treatments had a reduction in CWSI values after rehydration, indicating recovery from stress (Fig. 7).

There were no significant differences ($p > 0.05$) between treatments during the first two days of the water restriction period regarding the F_v/F_m variable, but there was a significant decrease in F_v/F_m for the stress treatment in HAD from the 3rd day, indicating a reduction in the photosynthetic efficiency of photosystem II (Fig. 7). All plants showed similar F_v/F_m values around 0.8 after rehydration, indicating that the plants had recovered.

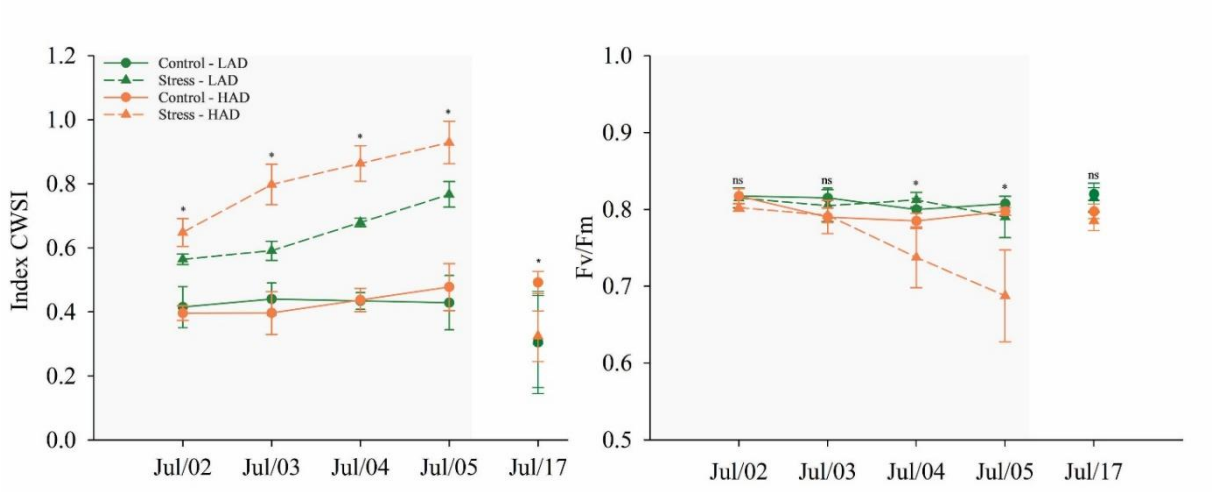

Figure 7 - Crop water stress index (CWSI) and F_v/F_m for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to two soil water availability regimes (Control- circles and Stress- triangles) in two environments: low atmospheric demand (LAD- green) and high atmospheric demand (HAD-orange), during the water restriction period from July 1 to 5, 2023 (gray area) and on the 12th day after rehydration, July 17, 2023 (white area). Vertical bars represent the standard error of the mean (n = 4).

The changes in leaf area are shown in Figure 8, where it can be seen that there were no significant differences ($p > 0.05$) in leaf area between treatments prior to the water restriction period. However, after the water restriction and recovery period, plants in the control treatment showed an increase in leaf area, with greater leaf production observed in the HAD environment, constituting a total increase of 65% compared to the initial leaf area. Plants in the soil moisture stress treatment lost leaves. This resulted in a reduction in leaf area in both the HAD and LAD environments.

When analyzing the transpiration ratio on the last day of the experiment per leaf area (TLD/LA) after the rehydration period, it can be seen that the plants in the soil moisture stress treatment in both the HAD and LAD environments, had higher TLD/LA values due to the reduction in leaf area (Fig. 8). However, the HAD environment showed a higher value for both the control and stress treatments compared to the LAD environment. Figure 9 features representative plants from each treatment, and allows us to visually understand the effect of atmospheric demand on the leaf area of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants when they are under water restriction.

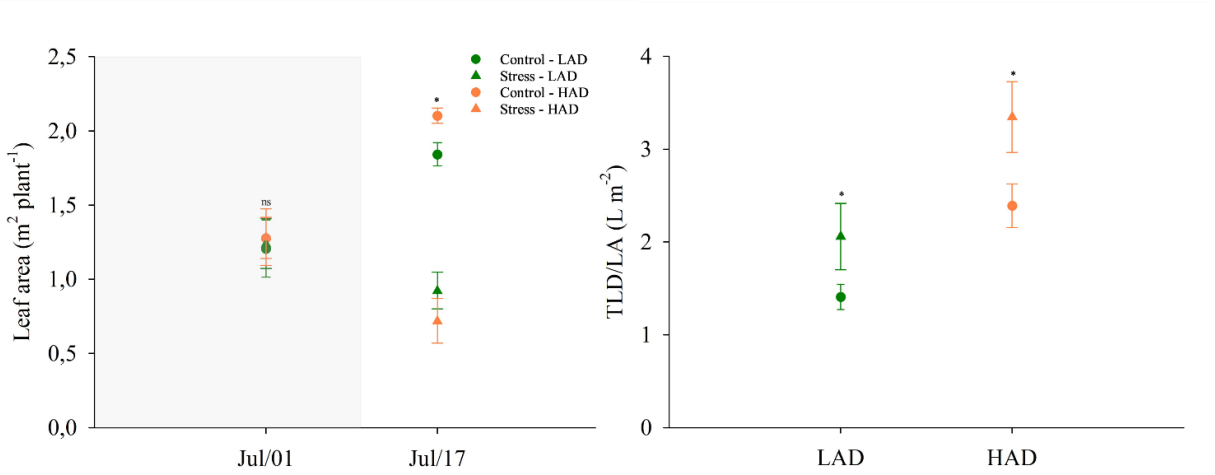

Figure 8 - Leaf area (LA) and transpiration on the last day of the experiment per unit leaf area (TLD/LA) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to two soil water availability regimes (Control - circles and Stress - triangles) in two environments: low atmospheric demand (LAD - green) and high atmospheric demand (HAD - orange), before water restriction, on June 30, 2023 (gray area) and 12th day after rehydration, on July 17, 2023 (white area). Vertical bars represent the standard error of the mean (n = 4).

Figure 9 - Morphological characteristics of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to two soil water availability regimes (Control and Stress) in two environments: low atmospheric demand (LAD) and high atmospheric demand (HAD), at the end of the experiment.

4. DISCUSSION

Our results provide information on how young *Eucalyptus grandis* × *Eucalyptus urophylla* plants physiologically respond to different VPD conditions and low water availability, and indicate the challenges that plantations of this hybrid may face given the impacts of climate change. Leaf water potentials showed that there was no water stress for plants grown in soil moisture conditions close to 70% MWRC under high and low atmospheric demand, indicating that the water availability adopted as a control was sufficient to maintain the physiological activity of plants, especially in high atmospheric demand conditions, being in

agreement with the results found by Santos (2023). However, plants under low atmospheric demand conditions (VPD < 3.2 kPa) showed low water consumption (2 L day^{-1}) compared to plants under high atmospheric demand (VPD < 5.8 kPa), which maintained an average daily consumption of 4 L day^{-1} (3.34 L m^{-2} leaf area day^{-1}). These results are reflected in the growth and productivity of the plants under these conditions.

Plants under water limitation showed increasingly lower leaf water potentials overtime, especially for the high atmospheric demand environment, indicating greater difficulty in maintaining leaf water content. According to Ouyang et al. (2022), plants close their stomata under high VPD to avoid hydraulic failure caused by a decrease in leaf water potential due to high transpiration rates. It is worth noting that we measured predawn Ψ_{leaf}, when transpiration is at its lowest and the water potential of the plant is closer to equilibrium with that of the soil; this water potential would possibly be even lower at midday, as it is influenced by any cuticular or stomatal transpiration (Bartlett et al. 2016).

The stomatal closure observed in plants under water restriction, especially under high atmospheric demand conditions, resulted in a decrease of all physiological parameters over time, reaching values close to zero for *gs*, *E* and *A* on the 4th day of water restriction. These results are in agreement with those obtained by other authors (Bleby et al. 2012; Santos 2023). According to Van Gorsel et al. (2013), plants under drought conditions physiologically respond to avoid excessive water loss; this response includes a decrease in stomatal conductance to prevent leaf water potentials from falling below a critical level, which ensures their survival for longer periods under these conditions (Nóia Júnior et al. 2020). Gu et al. (2021) studied leaf water status, stomatal and hydraulic behavior, and transpiration of the whole plant of *Eucalyptus grandis* × *Eucalyptus urophylla* plants in seasons with different water availability, observing that stomatal conductance (*gs*) decreased with increasing VPD (VPD ranged from 0.5 to 3.0 kPa). Similar results were found by Duursma et al. (2014) for *E. saligna*, in which the highest *gs* values were obtained at VPDs below 1 kPa.

A lack of water in the soil can lead to hydraulic failure, but the reduction in plant water transport is even greater under high atmospheric demand conditions. This occurs because the water uptake rate by the roots is not sufficient to meet the transpiration demand of the plant, resulting in high tension in the plant's vascular system, which can cause embolism in the xylem, and if the embolism extends throughout the xylem, water transport is restricted and the plant may eventually die from hydraulic failure (McDowell et al. 2011; Choat et al. 2018). The stomatal conductance of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants in our study readily adjusted to regulate transpiration daily according to the imposed conditions. Plants under stress in high atmospheric demand conditions responded immediately to VPD, reducing

transpiration values close to zero after two days of water restriction, with leaf water potential at dawn between -0.6 and -0.7 MPa, affecting the plant's water transport capacity, thereby resulting in a reduction in hydraulic conductivity of more than 80%.

In addition, stomatal opening and closing are strongly related to leaf temperature, suggesting that different atmospheric demands and/or water limitation conditions may lead to an increase in the CWSI variable. The CWSI in our study was very efficient in detecting stress in both environments, where it was observed that the stress treatment in the high atmospheric demand environment reached values above 0.7 from the second day of water restriction, while this only happened on the fourth day in the low atmospheric demand environment, and in both cases coinciding with soil relative moisture content of 20%. The threshold for stress is 0.7, but this can vary among species (Bellvert et al. 2014; Matese et al. 2018). Stress can also be detected by the maximum efficiency of the quantum yield of photosystem II(PSII), F_v/F_m, with this value ranging from 0.77 to 0.81 for most species under optimal growth conditions (Zha et al. 2017). Plants exposed to high atmospheric demand conditions after 2 days of water restriction in our study showed values lower than 0.77, considering that this value was found at dawn, indicating that the plants were unable to recover PSII efficiency during the night and probably reached even lower values at midday.

The impact of vapor pressure deficit and water restriction can be visually observed by leaf area. Herein, we observed that in addition to regulating stomatal conductance, leaf area was also reduced to prevent greater water loss through transpiration. Some plants tightly regulate stomatal conductance under stress conditions to prevent water loss which could lead to desiccation, while others drop leaves to reduce transpiration surface area (Choat and Way 2013; Taiz et al. 2017; Hubbard et al. 2020).

The recovery of Ψ_{leaf} to values close to prestress levels after rehydration generally indicates plant resilience, although the high atmospheric demand environment imposed greater stress. Furthermore, the recovery of *A* after rehydration suggests that plants can quickly resume photosynthetic activities after stress relief, unlike *gs* and *E*, which remained low. This finding was observed by Galmés et al. (2007), suggesting that despite an apparent recovery in CO_2 assimilation, water stress in high atmospheric demand environments can have lasting effects on plant health and productivity in the long term, and this will certainly worsen if plants suffer a second cycle of water stress.

These findings have important implications for the management of *Eucalyptus* plantations in climate change scenarios where episodes of drought and high temperatures may become more frequent and severe. According to Reis Junior et al. (2023), future climate projections for the state of Espírito Santo indicate an increase in temperatures. The predicted

additional warming ranges from 1.5°C in the RCP 4.5 scenario (stabilisation of average GHG emissions) to 6°C in the most extreme scenario, RCP 8.5, around the 2080s. Furthermore, a significant reduction in rainfall is expected, with up to a 1000 mm decrease in annual volume, which would result in an increase in the vapor pressure deficit. Given this, our results show that there are significant impacts on plant physiology under water deficit conditions associated with high vapor pressure deficits. This indicates that this clone may face difficulties in adapting to projected climate change.

5. CONCLUSION

The results of this study show that young *Eucalyptus grandis* × *Eucalyptus urophylla* plants exposed to different atmospheric demands and without soil water restriction showed excellent physiological performance, especially under high VPD conditions as represented by high gas exchange rates and high transpiration, which can lead to high productivity. However, the combination of atmospheric demand and water restriction caused severe stress to the plant, limiting physiological and hydraulic performance, especially under high VPD conditions. In addition, the CWSI, F_v/F_m tools have been shown to be effective in detecting plant stress.

Recovery after rehydration was more effective for plants under low VPD conditions. These results highlight how young *Eucalyptus grandis* × *Eucalyptus urophylla* plants respond to different VPD conditions and water availability, and indicate the challenges this hybrid may face under various climate change scenarios.

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CHAPTER III – KAOLIN PARTICLE FILM MITIGATES THE EFFECTS OF WATER RESTRICTION IN *Eucalyptus grandis* × *Eucalyptus urophylla* PLANTS

ABSTRACT

Kaolin-based particle film technology, a reflective antiperspirant material, has been used in agriculture as a strategy to mitigate the adverse effects of abiotic stress. However, its applicability to the *Eucalyptus* genus has not been fully explored. In this context, this study aimed to evaluate the effects of foliar kaolin application in mitigating the negative impacts of water restriction on young *Eucalyptus grandis* × *Eucalyptus urophylla* plants. The experiment was conducted at the Federal University of Espírito Santo, Brazil (latitude 20° 47′ 25' S, longitude 41° 23′ 48' W, and elevation 120 m), between April and July 2023. Plants of clone I144 were grown for 75 days in 60-litre pots containing commercial substrate, with daily irrigation to maintain substrate humidity close to the maximum water retention capacity (MWRC). The plants were then subjected to three treatments: 70% MWRC (control), progressive reduction of water availability (stress) and progressive reduction of water availability with kaolin application (stress + KL). Ecophysiological analyses included reflected solar radiation, leaf temperature, daily plant transpiration, leaf gas exchange, leaf water potential (Ψ_{leaf}), crop water stress index (CWSI), chlorophyll fluorescence and branch hydraulic conductivity measurements. Foliar kaolin application (KL) attenuated the effects of water restriction on young *Eucalyptus grandis* × *Eucalyptus urophylla* plants by increasing reflected radiation by approximately 15%, leading to a reduction in leaf temperature of up to 4.5°C. Kaolin also reduced plant transpiration during the initial days of water restriction, maintaining higher Ψ_{leaf} levels compared to the stress treatment, thereby mitigating photosynthetic damage, such as decreases in F_v/F_m and impairment of photosystem II. The stress + KL treatment partially mitigated the negative effects of water restriction, showing better A, g_S and E values than the stress treatment. Kaolin minimized hydraulic conductivity losses during water restriction, thus facilitating recovery after rehydration. Our results indicate that foliar kaolin application was an effective strategy to mitigate the negative effects of water stress on plants of the young *Eucalyptus grandis* × *Eucalyptus urophylla* plants.

Keywords: Water restriction; Foliar spray; chlorophyll fluorescence; gas exchange.

1. INTRODUCTION

Brazil exceeded 10 million hectares of the total area planted with trees in 2023, which corresponded to 3% expansion compared to 2022 and leverage by new commercial plantations. The *Eucalyptus* genus stands out among the species used in commercial plantations, covering 7.8 million hectares, which corresponds to 76% of the total area. These trees are destined for different segments of the industry, such as paper, pulp, wood panels, laminate flooring and charcoal for the steel industry (IBA 2024). Water deficit remains the main limiting factor for *Eucalyptus* productivity despite advances in silvicultural management and the genetic improvement of plantations (Elli et al. 2019; Gonçalves et al. 2017; Conti Junior et al. 2020). This challenge is intensified by climate change, which increases the frequency and intensity of extreme events such as prolonged droughts and irregular rainfall distribution (Geirinhas et al. 2021). Additionally, the expansion of *Eucalyptus* plantations into new regions, often characterized by unfavorable climatic conditions, low fertility soils and high susceptibility to water shortages, poses further challenges for the sustainable management of these forests (Oliveira et al. 2021b).

New silvicultural strategy development gains more importance every year due to drought events intensifying which cause increasing forest mortality (Blackman et al. 2019). Compounding this, producers and researchers are challenged to adopt management strategies to cope with climate change, which has increased frequency and intensity of summer water stress periods, associated with high solar radiation, temperature and vapor pressure deficit (VPD) (Bernardo et al. 2018). The use of particle films based on calcined kaolin is one of the short-term strategies developed to mitigate the effects of environmental pressures.

Kaolin is a reflective, antiperspirant material that has been used to mitigate the damage caused by thermal and water stress, as well as excess solar radiation, on the leaves and fruits of different crops. It has therefore shown growing potential for use in agriculture (Cantore et al. 2009; Dinis et al. 2018a, b; Frioni et al. 2019; Faghih et al. 2021). Kaolin forms a thin film comprising nanoparticles when applied to leaf surfaces. This film acts as a physical barrier, reflecting excess solar radiation and preventing the build-up of heat. Consequently, the risk of damage to leaves and fruit due to high temperatures and sun radiation exposure is reduced (Glenn 2010, 2016; Brillante et al. 2016).

A number of studies have reported the benefits of using kaolin on crops such as tomatoes (da Silva et al. 2019; AbdAllah 2019), apples (Al-Absi and Archbold 2016; Glenn 2016; Faghih et al. 2021), papaya (Campostrini et al. 2010), grapes (Dinis et al. 2018b; Conde et al. 2018; Bernardo et al. 2018), and coffee (Abreu et al. 2022; Roda et al. 2022). The positive effects of

applying these products include photoprotection mechanisms for photosystems and the electron transport chain (Dinis et al. 2018b), antiperspirant properties (Boari et al. 2015), a decrease in leaf temperature and an increase in water use efficiency (Boari et al. 2015; Santos et al. 2021), with a consequent increase in productivity (Gharaghani et al. 2018). Kaolin application can even help control pests (Amalin et al. 2015) and pathogens (Tubajika et al. 2007).

Some studies have been conducted on forest species such as those of the *Eucalyptus* genus due to kaolin's performance in agriculture under abiotic stress conditions. Seeking to evaluate the effects of different kaolin concentrations (3, 5, 7 and 10%) applied to *Eucalyptus* plants, Santos et al. (2021) observed that a 3% kaolin application is ideal for promoting growth and maintaining photosynthetic efficiency in young *Eucalyptus* plants, while higher concentrations resulted in a decrease in gas exchange variables. Oliveira et al. (2021a) found that foliar application of kaolin is a promising technology for improving the productivity and quality of *Eucalyptus* mini-cuttings in clonal nurseries. Furthermore, Xavier et al. (2018) suggest that foliar photoprotectants may serve as an effective alternative to enhance the growth of *Eucalyptus* plants under challenging environmental conditions.

Based on the above, the aim of this study was to evaluate the effects of applying kaolin on *Eucalyptus grandis* × *Eucalyptus urophylla* plant leaves in mitigating the negative effects of water restriction. To this end, we studied the effects of kaolin application on (i) solar radiation reflectance, transpiration and whole plant temperature; (ii) physiological variables; and (iii) hydraulic functionality.

2. MATERIAL AND METHODS

2.1. Plant material and experimental conditions

The experiment was conducted in the experimental area of the Department of Forestry and Wood Sciences at the Federal University of Espírito Santo (DCFM-UFES), located in Jerônimo Monteiro, Espírito Santo, Brazil (latitude 20° 47′ 25" S, longitude 41° 23′ 48" W, and elevation 120 m), under outdoor conditions. The experimental period lasted from April 13 to July 18, 2023 (Figure 1).

Eucalyptus grandis × *Eucalyptus urophylla* hybrid clone plants were grown in 50 cm^3 tubes filled with a substrate of biostabilized pine bark, vermiculite, crushed charcoal and phenolic foam. Clone I144 was selected because it is the most commonly used clone in plantations within the study region. Plants with a height of 25.23 ± 1.4 cm and a stem diameter of 1.2 ± 0.4 mm (mean ± SE) at 60 days of age were transplanted into 60-litre pots (height 45 cm, upper circumference 150 cm and lower circumference 125 cm) containing commercial pine

bark substrate. The substrate was fertilized with 4 g dm^{-3} of Basacote® Mini 9M controlled-release fertilizer (NPK 16-8-12 formulation). The pots were sealed with a 10 mm thick sheet of white polystyrene to prevent water evaporation from the substrate and to monitor plant transpiration.

After transplantation, the plants were grown outdoors for 75 days, during which they received sufficient daily irrigation to maintain the substrate at a moisture level close to its maximum water retention capacity (MWRC). Thereafter, the plants were saturated to determine the weight of each pot at field capacity and to standardize the substrate moisture. The substrate moisture content was allowed to reach 70% MWRC prior to treatment application (July 1, 2023) to ensure that plants in the control treatment did not experience water stress. The plants had a height of 1.5 ± 0.71 m, a diameter of 16.65 ± 0.34 mm, and a leaf area of 1.06 ± 0.2 m^2 (mean ± SE) at the treatment application time.

The experiment followed a completely randomized design with three treatments: 70% MWRC (control), progressive reduction in water availability (stress), and progressive reduction in water availability with kaolin application (stress + KL). Each treatment had six replicates, resulting in a total of 18 plants. The particle film based on calcined kaolin and composed of 95% kaolin and 5% inert materials (Surround WP, Engelhard Corp., Iselin, NJ) was applied on July 1, 2023, as an aqueous solution at a 3% concentration, without any surfactant (Santos et al. 2021). It was applied using a hand-held knapsack sprayer, ensuring uniform kaolin distribution and homogeneity among the plants.

The experiment was divided into two periods, with the first started on July 2, 2023, and ended on July 10, 2023. This first period was characterized by water restriction, while the second period was rehydration, which started on July 11, 2023, and ended on July 18, 2023. All plants were irrigated daily during the rehydration phase to ensure the substrate was maintained a humidity level close to its maximum water retention capacity.

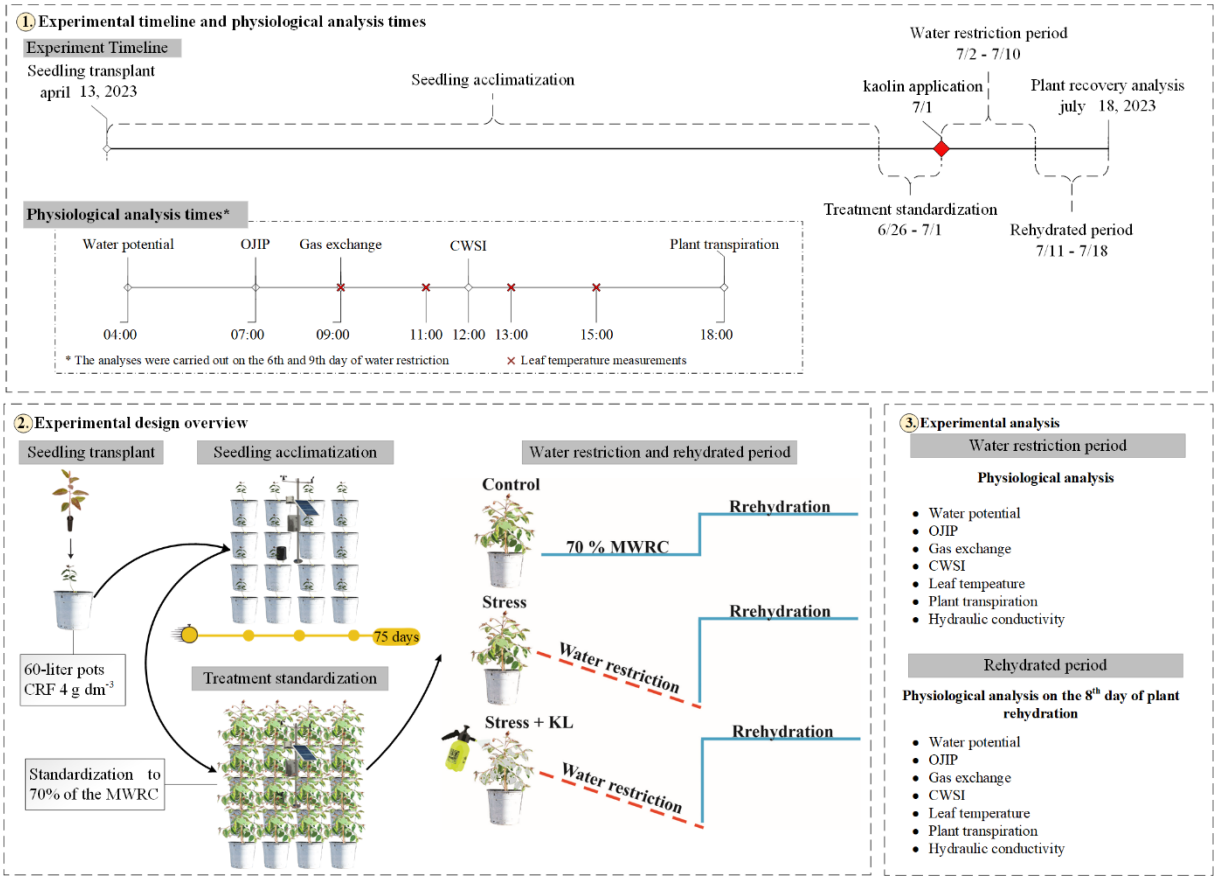

Figure 1 - 1. Experimental timeline and physiological analysis schedule. 2. Overview of the experimental design. 3. Experimental analysis conducted during the water restriction period (July 2–10, 2023) and at the end of the plant rehydration period (July 18, 2023). OJIP: Analysis of the polyphasic OJIP chlorophyll fluorescence transient; CWSI: Crop Water Stress Index; CRF: Controlled-release fertilizer; MWRC: Maximum Water Retention Capacity; KL: Kaolin.

2.2. Microclimate characterization

An automatic weather station was installed with sensors for global solar radiation (Rg) (model SP-Lite, brand Kipp & Zonen, Delft, Netherlands), temperature and relative humidity (model CS500, brand Campbell Scientific, Inc., Logan, UT, USA) to characterize the microclimatic conditions of the environment during the water restriction period. The data was stored in a data logger (model CR1000, Campbell Scientific, Inc., Logan, UT, USA). This data logger was powered by a 12-volt battery that was connected to a solar panel. The data logger was programmed to take readings every 10 seconds and store the average values every 5 minutes. The vapor pressure deficit (VPD) was calculated from the difference between the water vapor saturation pressure (es) and partial water vapor pressure (ea) values, following the methodology of Pereira et al. (2002).

The mean minimum and maximum temperatures recorded during the water restriction period were 14.4 and 29.4°C, respectively. The mean hourly global radiation was 367 W m^{-2}

day^{-1}, and the mean maximum vapor pressure deficit was 2.64 kPa (Figure 2).

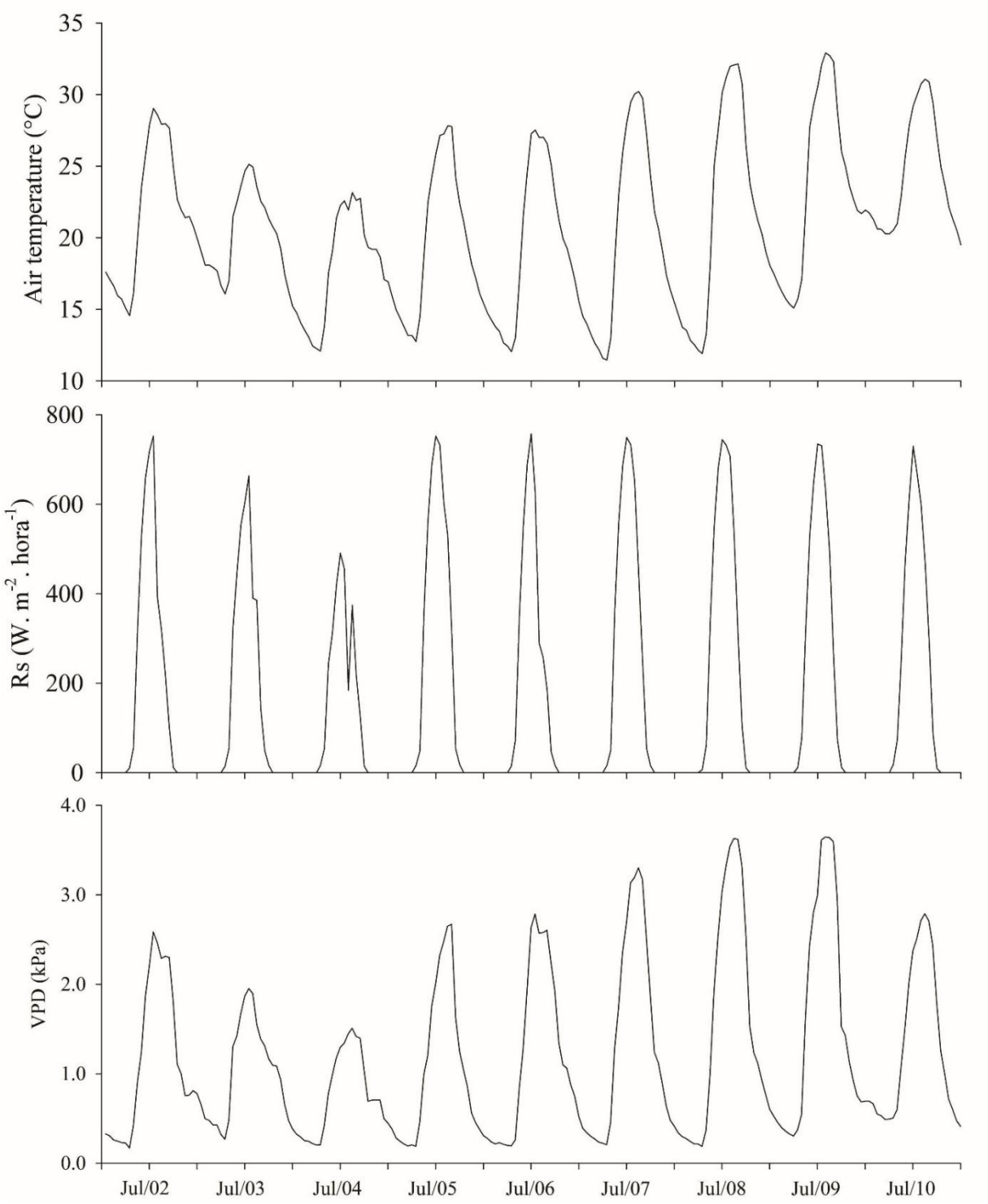

Figure 2 - Daily characterization of air temperature, global radiation (Rs) and maximum vapor pressure deficit (VPD) during the water restriction period from July 2 to 10, 2023. Jerônimo Monteiro, Espírito Santo, Brazil.

2.3. Spectral characterization of incident, transmitted and reflected solar radiation

Spectroradiometers were used in the visible light wavelengths (SpectraPen/LM 510-H/UVIS, from 380 nm to 790 nm) and near infrared wavelengths (SpectraPen/LM 510-H/NIR, from 640 nm to 1050 nm) to characterize the incident, transmitted, and reflected solar radiation

by the leaves on the 4th day of water restriction. Absorbed radiation was calculated as the difference between incident radiation minus transmitted and reflected radiation.

2.4. Leaf temperature

The methodology employed to ascertain the leaf temperature involved acquiring thermal images at two-hour intervals between 9:00 am and 3:00 pm on the 6th and 9th days of water restriction (DWR) and on the 8th day of rehydration. An FLIR T430sc thermographic camera (FLIR Systems, Wilsonville, OR, USA), which possesses a resolution of 320 × 240 pixels, was utilized to obtain the images. The distance between the camera and the plant canopy was established at 1.5 meters, and the emissivity coefficient was set to 0.96. The FLIR Tools software was utilized to process the images, yielding 10 samples in each repetition to ascertain the mean temperature in the respective treatments.

2.5. Hourly whole-plant transpiration

Whole-plant transpiration was measured using an electronic weighing lysimeter system during the water restriction period. This system consisted of a set of precision scales (model Prix 2098, Toledo do Brasil, São Bernardo do Campo, São Paulo, BR) that directly measured plant transpiration based on the difference in the weight of the pots at the beginning and end of the day. The scales were connected to microcontrollers (model ESP8266, Espressif Systems), which were responsible for reading and storing the plants' water consumption every five minutes.

The plants were irrigated daily, with the pots being weighed in the late afternoon (6 pm) to ascertain the amount of water lost through transpiration, which was then replenished. The control treatment plants were irrigated daily. In contrast, irrigation was withheld during the water restriction period for the stress and stress + KL treatments. Daily transpiration of the plants was determined by calculating the difference in mass measured at the beginning (6:00 am) and end (6:00 pm) of the day.

2.6. Plant water status and gas exchange

Analyses were performed on the 6th and 9th day of water restriction (DWR) and on the 8th day of rehydration, on fully expanded and healthy leaves from the middle third of the plants. Leaf gas exchange was analyzed at 09:00, 12:00 and 15:00 (local time) using a portable infrared gas analyzer (IRGA, model Li COR 6400, LI-COR Inc., Lincoln, NE, USA), with a 6 cm^2 measuring chamber, at a light intensity of 1500 μmol m^{-2} s^{-1} and a CO_2 concentration of 400 μL

L^{-1}. Net CO_2 assimilation rate (*A*, μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), and stomatal conductance (*g*s, mol m^{-2} s^{-1} H_2O) were determined. Leaf water potential analyses (Ψ_{leaf}) were performed at 4 am (local time) using a Scholander 1505D-EXP pressure chamber (PMS Instrument Co., Albany, OR, USA).

2.7. Crop Water Stress Index

Thermal images were taken at 12 noon on leaves oriented towards the sun to determine the Crop Water Stress Index (CWSI). The images were taken using a FLIR T430sc thermal imaging camera (FLIR Systems, Wilsonville, OR, USA), which has a resolution of 320 × 240 pixels. The distance between the camera and the plant canopy was set at 0.5 meters, and the emissivity adopted was 0.96. The FLIR Tools software was utilized to process the images, producing 10 samples in each repetition to obtain the average temperature in the respective treatments.

The CWSI was calculated according to the methodology proposed by Jones (1999) using the following equation:

$$\mathrm{CWSI} = \frac{(Tleaf - Twet)}{(Tdry - Twet)} \quad \text{(I)}$$

In which: *Tleaf* = the average temperature of the leaf under ambient conditions; *Twet* = the temperature of a leaf saturated with water (saturation was performed by spraying water 5 min before measuring the leaf temperature); and *Tdry* = the temperature of a leaf under non-respiring conditions, coated with petroleum jelly to simulate stomata closure, emphasizing that CWSI values close to 1 indicate that the plants are stressed and close to 0 that the plants are not stressed.

2.8. Chlorophyll fluorescence

Chlorophyll transient fluorescence was analyzed at dawn (7 am) using a portable FluorPen FP110 device (Photon Systems Instruments) on selected dark-adapted leaves with specific clips for a period of 30 minutes. Thereafter, the chlorophyll *a* fluorescence transients of dark-adapted leaves were measured. The induction of these transients was facilitated by applying a saturating pulse of 3,000 μmol m^{-2} s^{-1} of actinic light. Dark adaptation was implemented to ensure complete oxidation of the photosystem II (PSII) reaction centers, causing them to acquire the so-called “open” condition (Strasser et al., 2000).

Next, an estimation of the JIPTest variables was performed using the basic variables of emitted fluorescence. Fluorescence intensities were recorded on four time scales, including

50 μs (O), 2 ms (J), 60 ms (I) and maximum fluorescence around 1 s (P) (Strasser et al. 2000). Various fluorescence attributes were calculated utilizing these measurements, including: maximum quantum efficiency of photosystem II (F_v/F_m); maximum quantum yield for primary photochemistry (φ_{Po}); probability of electron transport after the reduction of the quinone electron acceptor, QA (ψ_{Eo}); quantum yield for electron transport (φ_{Eo}); quantum efficiency of energy dissipation (φ_{Do}); electron transport flux (further than QA) per reaction center (ET_0/RC); dissipated energy flux per reaction center (DI_0/RC); and performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors (PI_{ABS}) (Strasser et al. 2000, 2010; Stirbet et al. 2018; Tsimilli-Michael 2020).

2.9. Hydraulic conductivity

Branch segments were collected in the air on the 9th day in the water restriction phase and on the 8th day in the rehydration phase,at pre-dawn, then cut 15 cm from the proximal end, with an average diameter (± SE) of 3.4 mm (± 0.4 mm) underwater to obtain the native hydraulic conductivity in order to quantify the impact of water restriction and the effects of applying kaolin-based particle film on hydraulic conductivity, according to the methodology proposed by Sperry et al. (1988).

The branch segments were connected to a high pressure reservoir filled with a degassed 5 mM potassium chloride solution to determine the native hydraulic conductance (k_h). With the branches attached to the reservoir, the water flow through the branch section was quantified using an analytical scale (0.0001 g) at a hydrostatic pressure of 6 kPa for 10 minutes.

The native conductance (k_h) was calculated every 30 s, along with an average of the previous ten 30 s readings, and is the ratio of the mass water flow passing through the branch section to the pressure gradient. The specific hydraulic conductivity of the branch (Ks) was then calculated as K_s= (k_h / branch cross-sectional area) x branch length) (Tyree and Ewers 1991).

2.10. Statistical Analysis

The data were subjected to the Shapiro-Wilk test for normality and the Bartlett's test for homoscedasticity. The data were then subjected to analysis of variance (ANOVA) for a single factor, treatment as a fixed factor, and the means were compared using the Tukey's test at 5% probability when significant differences were found using the F test at 5%. Analyses were performed with the R Core Team (2022) program, version 4.2.2.

3. RESULTS

Kaolin application to the plant leaves in the stress + KL treatment significantly increased the reflectance of solar radiation in the blue and yellow spectral region (400 to 700 nm) and in the near infrared region (750 to 1000 nm) by about 15% and 12%, respectively, compared to the control treatment (Figure 3). The stress treatment showed similar values to the control. In addition, less solar radiation absorption was observed in leaves treated with kaolin, within the visible spectrum of solar radiation (>700).

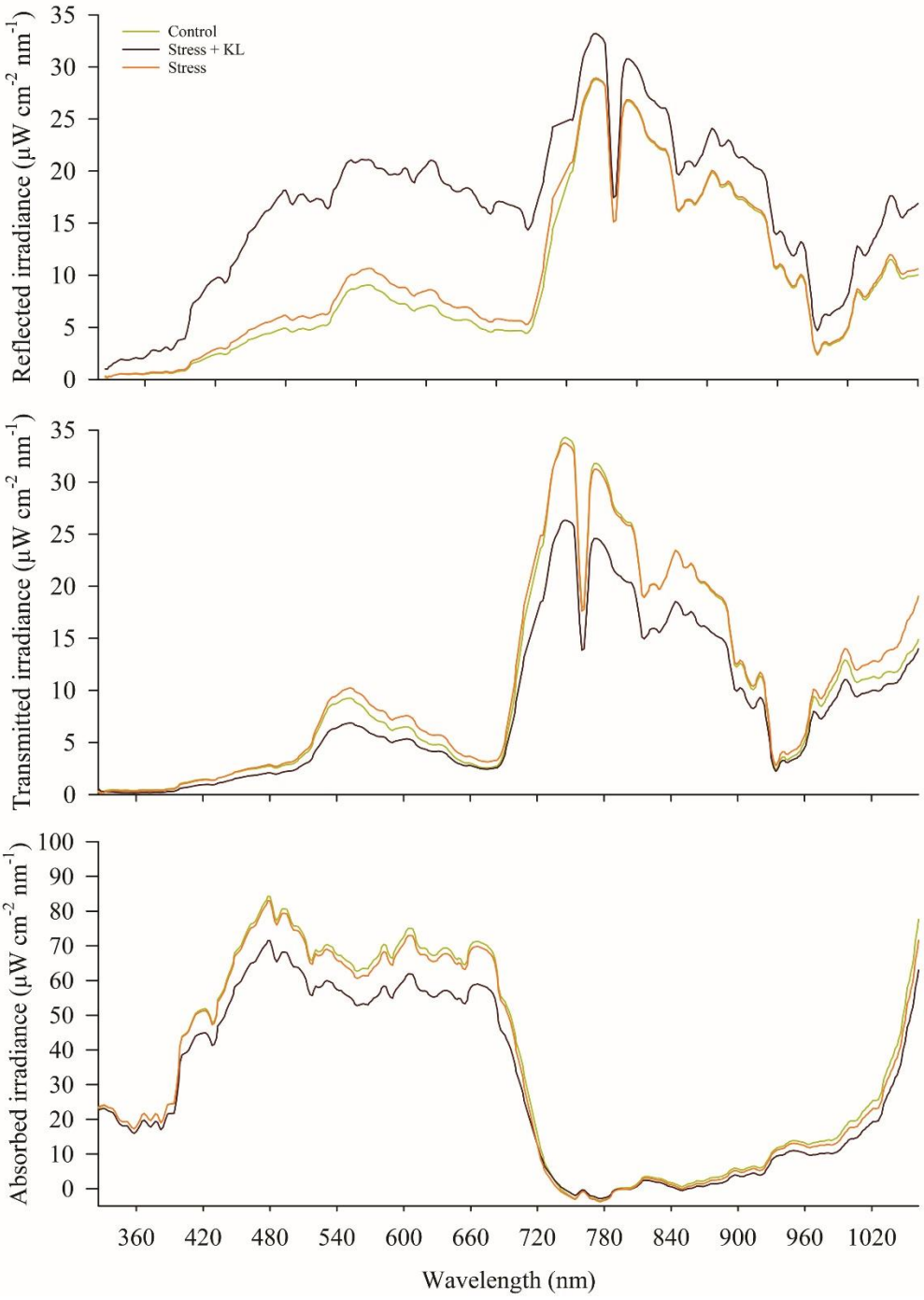

Figure 3 - Characterization of reflected, transmitted and absorbed irradiance by visible and near-infrared wavelengths (nm) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants.

The leaf temperature of the young *Eucalyptus grandis* × *Eucalyptus urophylla* plants was significantly ($p \leq 0.05$) affected by the water restriction (Figure 4). The stress treatment had a higher temperature on the 6th and 9th day of restriction, with values above 40°C during the hottest hours of the day (from 11 am to 1 pm). The kaolin application (stress + KL) reduced leaf temperature compared to the stress treatment by about 4.5 and 3.1°C in the morning and 1.3 and 1.2°C in the afternoon, on the 6th and 9th day of water restriction, respectively. All treatments showed similar leaf temperature values on the 8th day of rehydration.

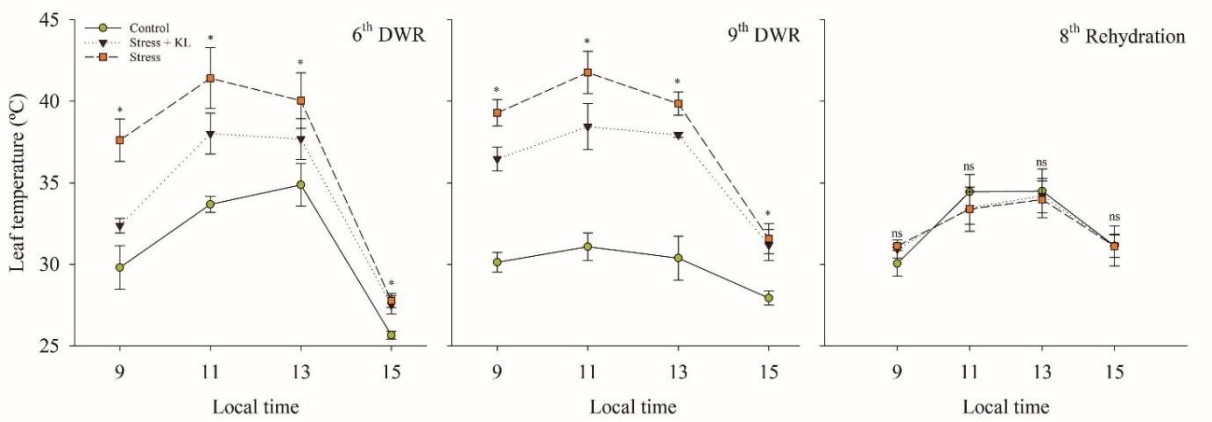

Figure 4 - Leaf temperature throughout the day (between 9 am and 3 pm) for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to three treatments: control (70% maximum water retention capacity - MWRC); stress (progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and foliar kaolin application), on the 6th and 9th days of water restriction (DWR) and on the 8th day of rehydration. Vertical bars represent the standard error of the mean (n = 6).

With respect to the whole-plant transpiration variable, young *Eucalyptus grandis* × *Eucalyptus urophylla* plants in the control treatment showed transpiration of approximately 0.4 L h^{-1} during the hottest periods, with a total average of 3.0 L.day^{-1}. Plants in the stress and stress + KL treatments showed significant reductions in transpiration as the water available in the substrate decreased (Figure 5). However, there was a difference between the stress and stress + KL treatments during the first few days after irrigation was stopped. Plants receiving kaolin (Stress + KL) showed a 59% reduction in transpiration compared to the control treatment during the first three days of water restriction, while the reduction for the stress treatment was approximately 29%. Plants in the stress and stress + KL treatments stabilized transpiration at about 0.5 L day^{-1} from the 6th day of water restriction.

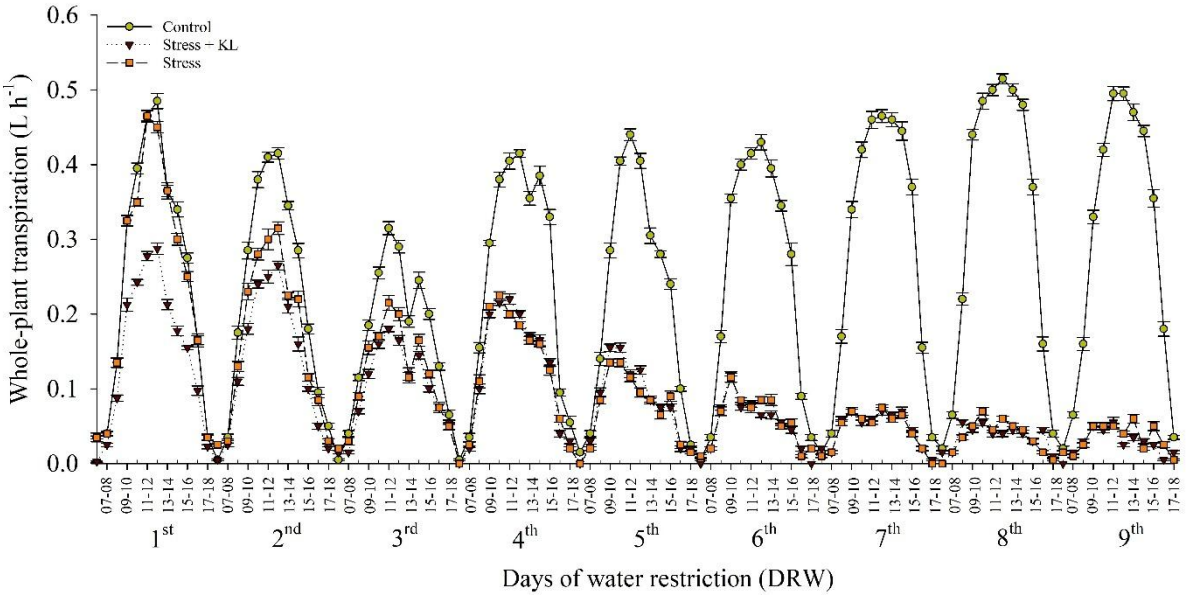

Figure 5 - Hourly whole-plant transpiration for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to three treatments: control (70% maximum water retention capacity - MWRC); stress (progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and foliar kaolin application) during the water restriction period between July 2 and 10, 2023.

There was a significant decrease ($p \leq 0.05$) in gas exchange parameters in isolated leaves of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants during the water restriction period (Figure 6). Irrigated plants showed the highest *A* values at all evaluation times, reaching a maximum of approximately 18.5 μmol m^{-2} s^{-1} CO_2 at 9 am, followed by a progressive decrease throughout the day, probably due to increased environmental stress such as higher temperature and lower relative humidity in the afternoon. Plants in the stress treatment showed a reduction in *A* compared to the control treatment of 81 and 89% in the morning and 92 and 96% in the afternoon on the 6th and 9th day of water restriction, respectively. The reduction in *A* with the foliar kaolin application (stress + KL) was 49 and 80% in the morning and 80 and 89% in the afternoon, respectively, on the 6th and 9th day of water restriction. This suggests that kaolin partially reduced the negative effects of water deficit, possibly by reducing water loss through transpiration and reflecting solar radiation, reducing heat stress. All treatments showed a significant recovery in the carbon assimilation rate after rehydration.

Stomatal conductance (*gs*) was significantly ($p \leq 0.05$) higher in the irrigated plants, reaching maximum values of 0.3 mol m^{-2} s^{-1} H_2O at 9 am, followed by a decrease during the day (Fig. 6). This decrease can be attributed to the increase in vapor pressure deficit during the day, leading to partial closure of the stomata to prevent excessive water loss. Plants in the stress treatment had extremely low *gs* values at all times, indicating strong stomatal closure in response to the water deficit. This behavior is expected as stomatal closure is one of the first

strategies used by plants to reduce transpiration during drought. Plants in the stress + KL treatment showed a significant difference ($p \leq 0.05$) for the *gs* variable on the 6th day of water restriction compared to plants in the stress treatment, although the *gs* values were very low. Stomatal conductance recovered in all treatments after rehydration.

The transpiration rate (*E*) was significantly ($p \leq 0.05$) higher in the control treatment, with a mean value of 3.5 mmol m^{-2} s^{-1} H_2O (Fig. 6). Extremely low *E* values, around 0.4 mmol m^{-2} s^{-1} H_2O, were obtained at all times due to the progressive reduction in water availability (stress). Stomatal closure limited transpiration as a strategy to minimize water loss under drought conditions. The foliar kaolin application (stress + KL) showed no clear advantages in maintaining transpiration in isolated leaves compared to the stress treatment during the water restriction period. All treatments showed complete recovery in the transpiration rate after rehydration.

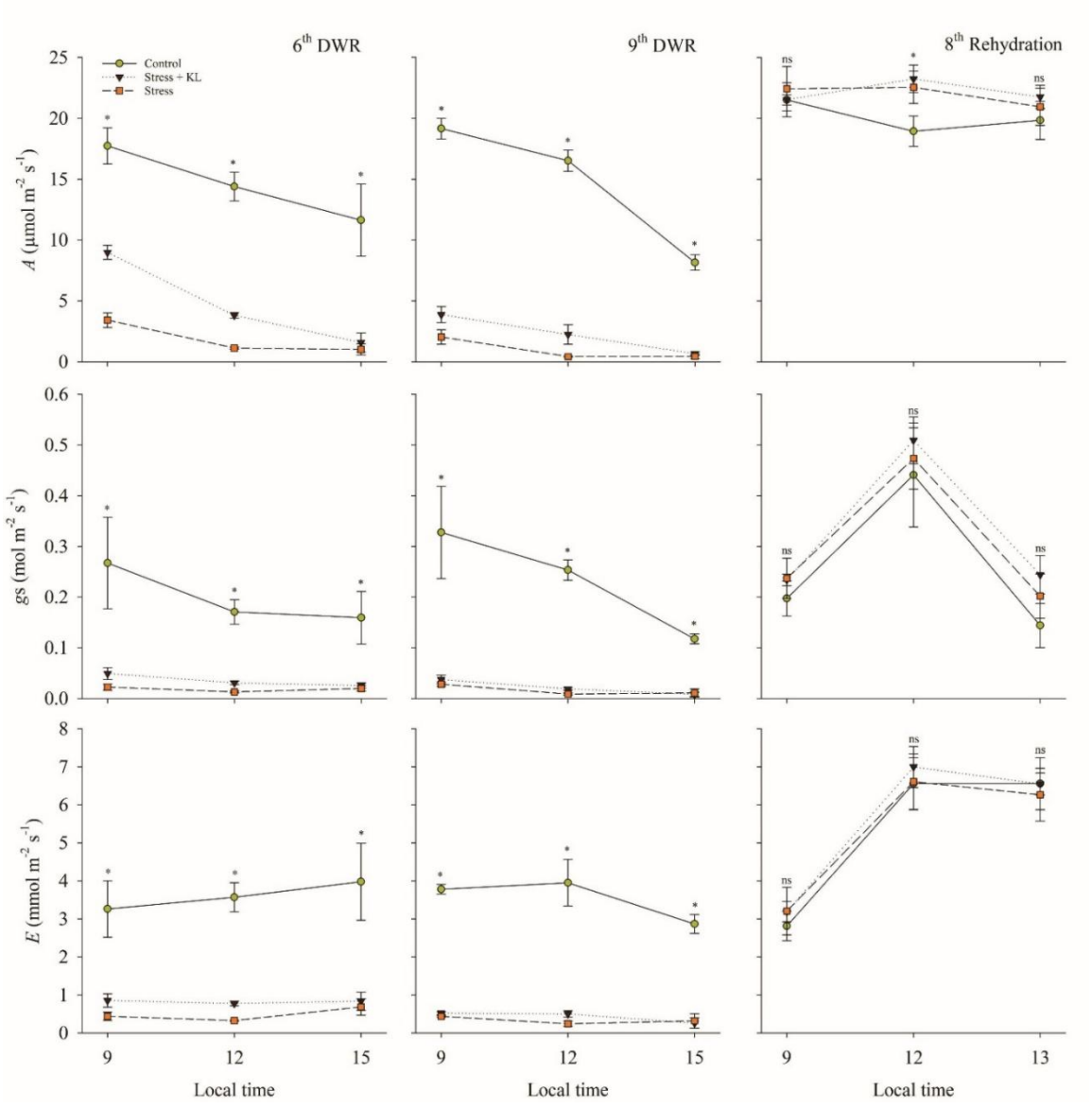

Figure 6 - Net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*) at 09 am, 12 am and 3 pm for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to

three treatments: control (70% maximum water retention capacity - MWRC); stress (progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and foliar kaolin application), on the 6th and 9th days of water restriction (DWR) and on the 8th day of rehydration. Vertical bars represent the standard error of the mean (n = 6).

In turn, there was a significant difference ($p \leq 0.05$) between the treatments for the leaf water potential variable of the young *Eucalyptus grandis* × *Eucalyptus urophylla* plants. The plants in the control treatment had a leaf water potential close to zero, indicating that they were not under water stress. The plants in the stress treatment had a lower leaf water potential, with values of -0.7 and -2.4 MPa on the 6th and 9th day of water restriction, while plants in the treatment receiving kaolin (stress + KL) had intermediate values of -0.3 and -1.7 MPa, respectively (Figure 7). All treatments showed water potential values very close to zero on the 8th day of rehydration.

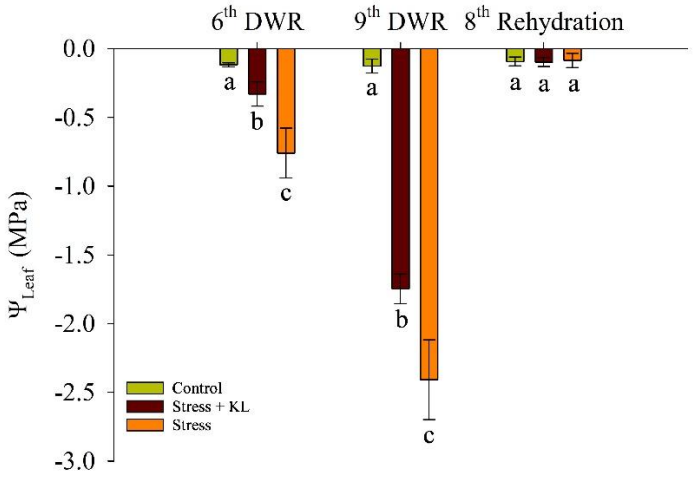

Figure 7 - Leaf water potential (Ψ_{leaf}) at pre-dawn for young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to three treatments: control (70% maximum water retention capacity - MWRC); stress (progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and foliar kaolin application), on the 6th and 9th day of water restriction (DWR) and on the 8th day of rehydration. The vertical bars represent the standard error of the mean (n = 6).

Water restriction resulted in a significant increase ($p \leq 0.05$) in the CWSI index, reaching values close to 1.0 on the 6th and 9th day of water restriction for the stress treatment (Figure 8). However, the foliar kaolin application (stress + KL) resulted in lower CWSI values compared to the stress treatment, showing that kaolin minimized water stress by reducing absorbed radiation and transpiration. Plants in the stress + KL treatment showed CWSI values of 0.4 on the 8th day of rehydration, similar to the control, while plants in the stress treatment showed values close to 0.7, indicating signs of stress.

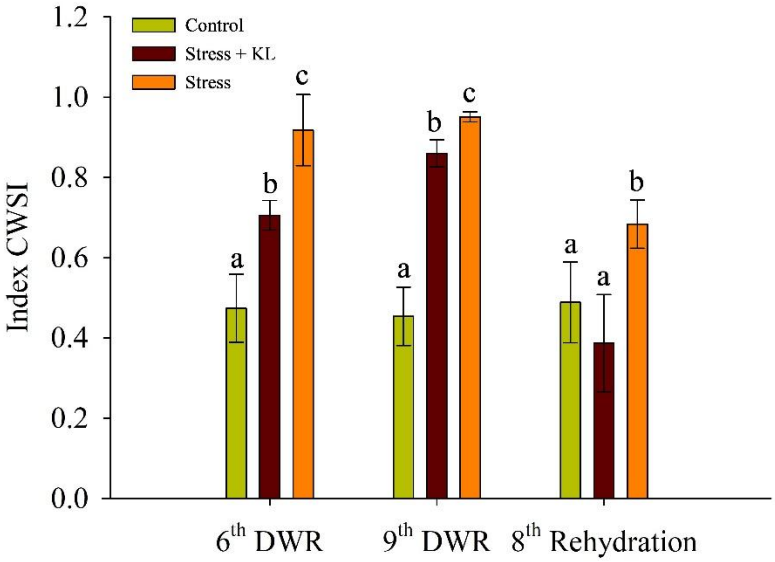

Figure 8 - Crop water stress index (CWSI) of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to three treatments: control (70% of maximum water retention capacity - MWRC); stress (progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and foliar kaolin application), on the 6th and 9th day of water restriction (DWR) and on the 8th day of rehydration. The vertical bars represent the standard error of the mean (n = 6).

There was a significant decrease ($p \leq 0.05$) regarding chlorophyll a fluorescence parameters in the performance index based on light energy absorption (PIABS), in the electron transport flux per reaction center ET_0/RC, in the probability that an electron moves further than quinone electron acceptor, QA (ψ_{Eo}), the maximum quantum yield of primary photochemistry (φPo), the quantum efficiency of electron transport (φEo) and the maximum quantum yield of photosystem II (F_v/F_m), accompanied by an increase in the quantum efficiency of energy dissipation (φDo) and dissipated energy flux per reaction center (DI_0/RC) in plants from the stress treatment to the 9th day of water restriction (Figure 9). These results indicate greater photosynthetic impairment and greater dissipation of energy as heat and fluorescence due to water restriction. The plants that received the foliar kaolin application showed intermediate values.

All treatments showed similar F_v/F_m values (~0.8) with no significant differences ($p \leq 0.05$) between them on the 6th day of water restriction, indicating that the water restriction was not sufficient to cause irreversible damage to the photochemical apparatus (Figure 10). However, plants in the stress treatment showed a significant reduction in F_v/F_m on the 9th day of water restriction, with values below 0.7, indicating photochemical stress. The foliar kaolin application (Stress + KL) minimized this reduction in F_v/F_m, demonstrating the protective effect of the product. Kaolin treatment showed F_v/F_m values above 0.7 during the water restriction

period. All treatments had similar F_v/F_m values (~0.8), with no significant differences after eight days of rehydration.

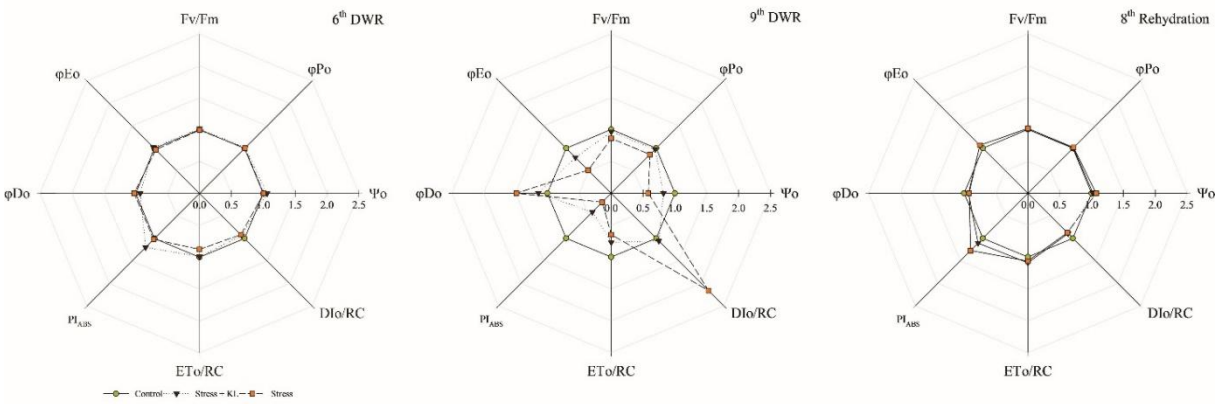

Figure 9 - Radar plots of JIP parameters deduced from chlorophyll a fluorescence OJIP transients of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants, subjected to three treatments: control (70% of maximum water retention capacity - MWRC); stress (progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and leaf kaolin application), on the 6th and 9th day of water restriction (DWR) and on the 8th day of rehydration. Maximum quantum yield of photosystem II (F_v/F_m); maximum quantum yield of primary photochemistry (φ_{Po}); probability of electron transport after the reduction of the quinone electron acceptor, QA (ψ_{Eo}); quantum efficiency of electron transport (φ_{Eo}); quantum efficiency of energy dissipation (φ_{Do}); electron transport flux per reaction center (ET_o/RC); dissipated energy flux per reaction center (DI_o/RC); and performance index based on light energy absorption (PI_{ABS}).

Water restriction caused a significant reduction ($p \leq 0.05$) in the hydraulic conductivity of the branches, reaching values of 1.3 and 2.6 kg m^{-1} s^{-1} MPa^{-1} for the stress and stress + KL treatments, respectively, which corresponds to a conductivity loss of 72 and 44%, respectively, compared to the control (Figure 11). This suggests that kaolin partially mitigated the effects of water stress, thus preventing more severe damage to the xylem. Plants in the stress and stress +KL treatments were unable to recover water transport after rehydration.

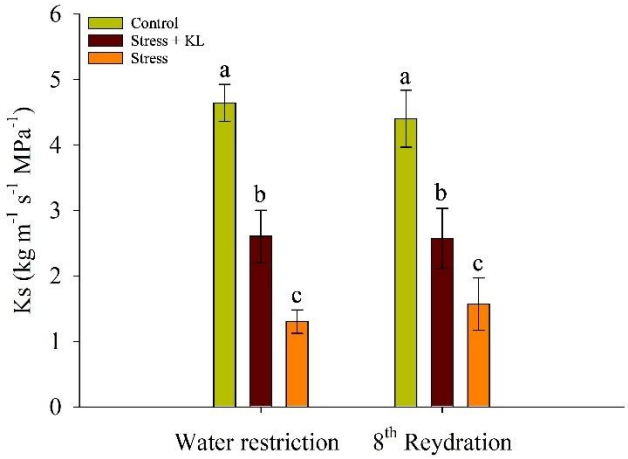

Figure 10 - Hydraulic conductivity of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants subjected to three treatments: control (70% maximum water retention capacity - MWRC); stress

(progressive reduction of water availability in the substrate); stress + KL (progressive reduction of water availability and foliar kaolin application), at the end of water restriction (DWR) and on the 8th day of rehydration. Vertical bars represent the standard error of the mean (n = 6).

4. DISCUSSION

The findings of this study demonstrated the efficacy of employing Kaolin as a mitigation strategy to reduce the adverse effects of water stress on young *Eucalyptus grandis* × *Eucalyptus urophylla* plants. Kaolin film functioned (worked) as a protective layer by increasing the solar radiation reflectance and consequently reducing radiation absorption incidence on the leaves. This phenomenon can mitigate oxidative stress in low water availability conditions in the substrate. The enhanced solar radiation reflectance in the blue and yellow spectral range (400-700 nm) and near infrared (750-1000 nm) observed in the Kaolin treatment (15% and 12%, respectively) resulted in a reduction in canopy temperature compared to the stress treatment.

Water restriction caused a significant increase in leaf temperature of young *Eucalyptus grandis* × *Eucalyptus urophylla* plants, with the highest temperatures (> 40°C) observed in the stress treatment, particularly during the hottest hours of the day. This increase in leaf temperature can be attributed to stomatal closure, which reduces transpirational cooling of the leaves and increases their surface temperature (Glenn 2010; Shellie and King 2013; AbdAllah 2019). However, the foliar kaolin application resulted in a reduction in leaf temperature of approximately 3.5°C in the morning and 1.2°C in the afternoon compared to the stress treatment. This reduction can be attributed to the product's ability to reflect solar radiation with wavelengths in the ultraviolet, near infrared, and photosynthetically active regions (Dinis et al. 2018b; Tosin et al. 2019; Salvatierra-Miranda et al. 2023). Consistent with these findings, studies by Rosati et al. (2006); Shellie and King (2013); and Dinis et al. (2018b) reported that the kaolin application reduced the temperature of grapevine, walnut, and almond trees by up to 6°C.

Furthermore, the foliar kaolin application led to a 30% reduction in the transpiration of the entire plant during the initial three days of water restriction when compared to the stress treatment. This finding suggests that a foliar kaolin application could result in a substantial benefit in terms of water savings. As indicated by Boari et al. (2015), the impact of kaolin in reducing transpiration and enhancing the water status of plants serves to mitigate the deleterious effects of drought, thereby contributing to enhanced water use efficiency. These outcomes are consistent with studies which point to kaolin as an effective antitranspirant agent, reducing transmitted irradiance and therefore water loss through the leaf (Glenn et al., 2015; Aganchich et al., 2020).

When analyzing the gas exchange measurements in isolated leaves, it was observed that the highest values for the net CO_2 assimilation rate (*A*) variable were found in the early morning, with a decrease in the afternoon for all treatments, with the plants treated with kaolin (stress + KL) showing higher values than the plants in the stress treatment. The lower *A* values in the stress treatment may be related to stomatal closure due to lower leaf water potential. According to Centritto et al. (2005), stomatal closure in adult olive leaves limits CO_2 entry into the substomatal cavity, which leads to a decrease in *A* as a result of reduced CO_2 availability.

The JIP test has been used as a stress indicator in studies of the response of plants to environmental conditions (Baker 2008; Tsimilli-Michael 2020). Exposure to water limitation can alter light energy absorption by PSII, resulting in changes in chlorophyll fluorescence (Lichtenthaler et al. 2005, 2007). The F_v/F_m parameter was within the range of 0.75-0.85 on the 6th day of water restriction, indicating that there was no photoinhibition (Bolhàr-Nordenkampf and Öquist 1993). The value of 0.75 is considered the limit for a fully functional PSII (Strasser et al. 2000). Plants in the stress treatment had F_v/F_m values below 0.7 on the 9th day of water restriction, while plants treated with kaolin remained in the range (> 0.75), indicating less PSII impairment. This confirms the role of kaolin in alleviating water stress, as previously demonstrated in other crops such as grapevine and olive (Nogués & Baker 2000; Zhou et al. 2019).

Plants under water limitation receiving a foliar kaolin application (stress + KL) showed a lower effect on the probability of electron transport after reduction of QA (ψ_{Eo}) and a lower quantum efficiency of electron transport (φ_{Eo}) compared to plants in the stress treatment. Reductions in ψ_{Eo} and φEo under water limitation conditions indicate impaired electron transport between photosystems, which can lead to excessive accumulation of excitation energy in chloroplasts (Wang et al. 2017), causing an imbalance between light capture and energy utilization, leading to the formation of reactive oxygen species that cause oxidative stress and degrade chloroplast structures (Razi and Muneer 2021).

The performance index based on light energy absorption (PIABS) evaluates the overall efficiency of PSII in converting light energy into chemical energy. The treatment that received kaolin (stress + KL) showed a higher value on the 9^{th} day under water restriction compared to the stress treatment. The decrease in this parameter, as observed in the stress treatment, is mainly related to a decrease in photochemical efficiency or photosynthetic electron transport (Kalaji et al. 2017). In other words, the decrease in PIABS can be attributed to an increase in the dissipated energy flow (DI0/RC) and a decrease in the electron transport (ET_o/RC) per reaction center, resulting in a lower efficiency in the quantum yield of absorbed photons (Appenroth et al. 2003; Oukarroum et al. 2007).

In addition, DI0/RC is related to the partial deactivation of PSII reaction centers. An increase of this parameter was observed in our results for the stress treatment on the 9th day of water restriction, while the values for the stress + KL treatment were similar to the control treatment. This increase typically occurs when the reaction centers of PSII are unable to transfer energy upstream due to damage to the thylakoid membrane (Rapacz et al. 2015). High DI0/RC values are generally associated with reduced F_v/F_m, as observed for stress treatment, which can lead to photoinhibition of plants (Martins et al. 2015; Hazrati et al. 2016).

The damage caused by water restriction in young *Eucalyptus grandis* × *Eucalyptus urophylla* plants can be seen in the loss of hydraulic conductivity, in which stressed plants showed a 72% reduction in conductivity, while this reduction was lower in plants treated with kaolin, at around 44%. Under water restriction periods or high transpirational demand, negative pressures in the xylem can "seed" the water column with tiny bubbles pulled into conduits, which can nucleate cavitation of the metastable water in the xylem conduits (Kiorapostolou et al. 2019). These cavitation events disable the conduits in which they occur by blocking them first with water vapor and eventually with air. Embolized conduits diminish the hydraulic capacity of the plant, which in turn can limit its photosynthetic capability (Sperry 2000).

5. CONCLUSIONS

The foliar kaolin application was an effective strategy for mitigating the adverse effects of water stress on the *Eucalyptus grandis* × *Eucalyptus urophylla* hybrid clone. The primary effects observed following foliar kaolin application to *Eucalyptus* plants included a reduction in leaf temperature, decreased water loss through transpiration, preservation of hydraulic conductivity, and attenuation of damage to PSII. These effects collectively contribute to maintain physiological functionality during drought periods.

The potential benefits of using kaolin in *Eucalyptus* cultivation during water scarcity require further investigation. Additionally, challenges associated with its application in commercial plantations must be addressed.

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GENERAL CONCLUSIONS

Water deficiency, caused by low water availability in the soil, has been increasingly recurrent in Brazilian forestry. More intense and stressful climatic events, such as extreme temperatures (both low and high) and reduced rainfall, are of current concerns. Consequently, studies that seek to enhance our comprehension of the consequences of abiotic stress factors on plants have witnessed a marked increase in their importance in recent years. This study advances our understanding of the ecophysiological responses of *Eucalyptus grandis* × *Eucalyptus urophylla* under varying water availability, vapor pressure deficits, and stress mitigation strategies using kaolin-based particle films. The findings are highly relevant in the

context of climate change, which is expected to impose increasingly severe drought and heat stress conditions on forest plantations.

This study observed critical water availability thresholds of water availability that significantly affect the physiological performance and growth of young Eucalyptus grandis × Eucalyptus urophylla plants. The results indicated that water limitation below 40% of maximum water retention capacity (MWRC) induces stress, reducing stomatal conductance, leaf gas exchange, and hydraulic conductivity. T These findings underscore the vulnerability of this hybrid to soil water deficits, underscoring the necessity for enhanced water management strategies in forestry plantations.

The study further underscores the pivotal interplay between soil water availability and vapor pressure deficit (VPD) in modulating plant physiological responses. Under conditions of elevated VPD, plants exhibited increased water loss, reduced leaf area, and impaired photosynthetic efficiency. Although plants exhibited partial recovery upon rehydration, the exposure to consecutive cycles of water stress could have long-term negative consequences for productivity. This underscores the necessity of incorporating both soil and atmospheric drought considerations when assessing plant performance, particularly in climate change scenarios that predict increased VPD levels.

The application of kaolin led to a reduction in leaf temperature, an enhancement in water-use efficiency, and a preservation of hydraulic conductivity during periods of drought. Furthermore, the treatment contributed to preserving the functionality of photosystem II, thereby mitigating photoinhibition and oxidative stress. These results suggest that kaolin can be an effective tool to enhance plant resilience under water-limited conditions, although further research is needed to optimize application methods for large-scale forestry operations.

This study underscores the physiological constraints imposed by water stress and atmospheric demand on *Eucalyptus grandis* × *Eucalyptus urophylla*, while concurrently proposing pragmatic solutions to mitigate these effects. In light of the escalating frequency of drought events, the strategic incorporation of adaptive measures such as kaolin application could prove pivotal in sustaining eucalyptus plantation productivity. Future research endeavors should prioritize field-scale implementation and long-term assessments of these strategies' impacts on plant health and wood production.