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Scientific Article

ECOPHYSIOLOGICAL RESPONSES OF *Eucalyptus* INOCULATED WITH DIFFERENT *Ceratocystis fimbriata* ISOLATES

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ABSTRACT

Ever-increasing industrial demand means that forest stands and the number of areas with *Eucalyptus* plantations are growing rapidly in Brazil and worldwide. This has increased the use of genetic materials, increasing the incidences of pests and diseases. Wilt is caused by the *Ceratocystis fimbriata* fungus, which is one of the most common diseases in *Eucalyptus* plantations. This study aimed to evaluate the ecophysiological responses of clonal seedlings of *Eucalyptus* spp. inoculated with different *C. fimbriata* isolates. The treatments were LPF 1512, 1806, 1607, and 1657 from the states of São Paulo, Minas Gerais, Bahia, and Mato Grosso do Sul, respectively, and a control comprising distilled water. Fungal isolates were inoculated 60 days after seedling planting. After inoculation, the growth, gas, leaf temperature, and chlorophyll a fluorescence were analyzed. Regardless of the isolate, inoculation with *C. fimbriata* increased water flow resistance in the xylem vessels of *Eucalyptus* plants, causing water stress. This results in reduced gas exchange and compromised photosynthetic performance, as evidenced by decreased absorption flux per reaction center and low photosynthetic index values. Furthermore, fungal pathogenicity analysis indicated that the LPF 1657 isolate was most virulent to *Eucalyptus* seedlings, directly affecting the height, photosynthetic CO_2 assimilation, stomatal conductance, and transpiration of the inoculated plants.

Keywords: Virulence; Gas exchange; Pathogenicity; Thermography

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RESPOSTAS ECOFISIOLÓGICAS DE EUCALIPTO INOCULADOS COM DIFERENTES ISOLADOS DE *Ceratocystis fimbriata*

RESUMO – A crescente demanda industrial faz com que os povoamentos florestais e o número de áreas com plantações de eucalipto cresçam rapidamente no Brasil e no mundo. Isto tem aumentado a utilização de diferentes materiais genéticos nas áreas de cultivo, aumentando a incidência de pragas e doenças. Entre elas está a murcha causada pelo fungo *Ceratocystis fimbriata*, uma das doenças mais comuns nas plantações de eucalipto. Diante disto, este estudo teve como objetivo avaliar as respostas ecofisiológicas de mudas clonais de *Eucalyptus* spp. inoculadas com diferentes isolados de *C. fimbriata*. Os tratamentos foram LPF 1512, 1806, 1607 e 1657 dos estados de São Paulo, Minas Gerais, Bahia e Mato Grosso do Sul, respectivamente, e um controle composto por água destilada. Os isolados fúngicos foram inoculados 60 dias após o plantio das mudas. Após a inoculação, o crescimento, o gás, a temperatura da folha e a fluorescência da clorofila a foram analisados. Independentemente do isolado, a inoculação com *C. fimbriata* aumentou a resistência ao fluxo de água nos vasos do xilema das plantas de eucalipto, causando estresse hídrico. Isso resulta em trocas gasosas reduzidas e desempenho fotossintético comprometido, conforme evidenciado pela diminuição do fluxo de absorção por centro de reação e baixos valores de índice fotossintético. Além disso, a análise de patogenicidade fúngica indicou que o isolado LPF 1657 foi mais virulento para mudas de eucalipto, afetando diretamente a altura, assimilação fotossintética de CO_2, condutância estomática e transpiração das plantas inoculadas.

Palavras-Chave: Virulência; Trocas gasosas; Patogenicidade; Termografia.

1. INTRODUCTION

Ever-increasing industrial demand means that forest stands and the number of areas with *Eucalyptus* plantations are growing rapidly in Brazil and worldwide (Alfenas et al., 2009; Silva et al., 2012). This has increased the use of different genetic materials in crop areas, increasing the incidence of pests and diseases, including wilt caused by *Ceratocystis fimbriata* Ellis & Halsted, which is one of the most common diseases affecting *Eucalyptus* plantations (Silva et al., 2018; Roux et al., 2020; Silva et al., 2020; Brito et al., 2021; Harrington et al., 2024). This pathogen is found in several parts of the world. In Brazil, it has been recorded in several states including Bahia, Pará, Maranhão, and Espírito Santo (Alfenas et al., 2009; Gomes et al., 2019; Brito et al., 2021), potentially causing significant yield losses or even plant death (Alfenas et al., 2009; Guimarães et al., 2010; Mafia et al., 2013; Silva et al., 2020). A study conducted to observe the impact of *C. fimbriata* fungus on *Eucalyptus* plantations in four different areas of Brazil showed that losses in wood volume can vary between 11.5 and 29.3%, depending on the location and climatic conditions, suggesting that these factors can affect the degree of severity of the fungus (Fernandes et al., 2014).

Despite the damage caused by this fungus, the infection process in *Eucalyptus* is yet to be fully elucidated. To date, this pathogen is known to mainly infect the parenchyma, phloem, and xylem vessels, causing longitudinal and radial browning in the stem, and consequently, leaf wilting (Ferreira et al., 2006; Silva et al., 2018; Silva et al., 2020). The fungus enters the plant through root or trunk wounds caused by tools or abiotic factors (Ferreira et al., 2006; Oliveira et al., 2015).

When this fungus colonizes a plant, it obstructs the vascular system and prevents normal water and nutrient flow through the xylem vessels. Plants respond to an attack by forming tyloses, gels, or fungal material, which block xylem vessels and increase water flow resistance from the roots to leaves, thereby inducing water stress (Tumura et al., 2012; Park et al., 2013; Bispo et al., 2016a; Silva et al., 2018).

When exposed to water stress, plants close their stomata to reduce water loss to the atmosphere, decrease transpiration, and consequently restrict CO_2 entry into the leaf (Bispo et al., 2016a; 2016b; Silva et al., 2018), in addition to increasing the leaf temperature (Kashiwagi et al., 2008; Biju et al., 2018). Water stress associated with high temperatures may also degrade chlorophyll and damage the

photosynthetic apparatus of plants, leading to reduced photosynthetic CO_2 assimilation (Bhargava et al., 2014; Cavalcante et al., 2018). This hinders plant growth and can cause wilting, cankers, and root rot, leading to plant death (Baker et al., 2003; Firmino et al., 2013; Mafia et al., 2013).

Physiological characteristics, such as gas exchange and photosynthetic efficiency of chlorophyll a, are good indicators of the photosynthetic apparatus functioning in plants under adverse conditions, such as *C. fimbriata* infection (Cacique et al., 2017; Silva et al., 2018). However, little is known about how this pathogen affects the physiology of forest plants such as *Eucalyptus*.

Currently, the main control method against *C. fimbriata*-induced wilt is the use of high-resistance genotypes coupled with other methods, such as the removal of diseased plants and tool disinfection (Brito et al., 2021). Furthermore, to increase the efficiency of the development of genotypes with greater resistance to this pathogen, the degree of pathogenicity of different isolates of this fungus must be identified.

Because *C. fimbriata* infection can impede water and nutrient flow in the plant and the intensity of this infection may be associated with the degree of isolate virulence, this study aimed to investigate the ecophysiological responses of clonal *Eucalyptus* seedlings inoculated with different *C. fimbriata* isolates.

2. MATERIAL AND METHODS

2.1 Study area and experiment implementation

The experiment was conducted in a greenhouse at the Maranhão State University (UEMA), located in São Luís, Maranhão (02°35'30.6" S and 44°1243.4" W), Brazil. The climate of the region is characterized as hot and humid (Aw), according to the Köppen and Geiger classification (Trinta, 2007).

We used 90-day-old clonal *Eucalyptus* seedlings (*Eucalyptus grandis* x *Eucalyptus urophylla*) produced in polyethylene tubes with a volume of 53 cm^3, acquired from the Enraize Indústria e Comercio de Produtos Agroflorestais nursery in the municipality of Açailândia, Maranhão.

Seedlings were transplanted to 15 L polypropylene pots and filled with autoclaved soil, where soil analysis revealed 1 g dm^{-3} of organic matter (OM), $pHCaCl_2$ = 4.0; 37.0 mg dm^{-3} of phosphorus 0.9 mmol dm-3 of potassium (K), 2.0 mmol dm^{-3} of calcium, 5.0 mmol dm^{-3} of magnesium (Mg), 29.0 mmolc dm^{-3} of hydrogen (H) plus aluminum (Al), 2.0 mmolc dm^{-3} of sodium, 0 of aluminum, 29.0 mmolc dm^{-3} of hydrogen, and 0.7 g dm^{-3} of carbon. Coarse sand = 410 g kg^{-1}, fine sand = 450 g kg^{-1}, silt = 40 g kg^{-1}, and clay = 100 g kg^{-1}.

Fertilization was performed before transplantation with 100 g of NPK per pot (18-18-18), and daily drip irrigation was performed.

2.2 Environmental variables

Environmental variables, such as temperature, relative humidity, photosynthetically active radiation (PAR), and vapor pressure deficit (VPD) were obtained using a microclimate weather station (WatchDog, series 1000, model 1400, SPECTRUM Technologies Inc., IL, USA) (Figure 1).

2.3 Treatments

The treatments consisted inoculation with four distinct *Ceratocystis fimbriata* isolates (Table 1) and a control (seedlings without fungus inoculation). Each treatment comprised 6 replicates, totaling 30 experimental units.

The *C. fimbriata* isolates used in this study were obtained from four states with a confirmed occurrence of the disease - Bahia, Minas Gerais, Mato Grosso do Sul, and São Paulo, and were donated by the Laboratory of Forest Pathology/Bioagro of the Federal University of Viçosa.

2.4 Inoculation with C. fimbriata

C. fimbriata isolates were cultivated in a petri dish containing PDA (potato-dextrose-agar) culture medium and stored in a biochemical oxygen demand (BOD) incubator at 25 ± 1 °C under a 12-h photoperiod for 15 days (Mafia et al., 2011).

At the end of the fungal growth period,

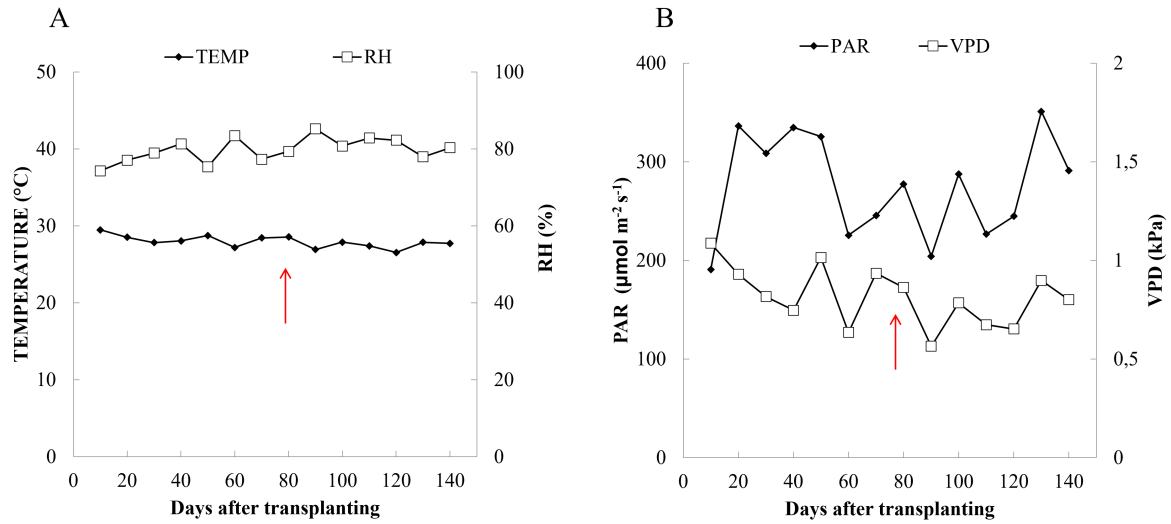

Figure 1. Daily average temperature and relative humidity - RH (A), photosynthetically active radiation - PAR and vapor pressure deficit - VPD (B), during plant cultivation (November 18, 2020 to April 4, 2021). The arrow indicates the moment of inoculation

Figura 1. Temperatura média diária e umidade relativa - UR (A), radiação fotossinteticamente ativa - PAR e déficit de pressão de vapor - VPD (B), durante o cultivo da planta (18 de novembro de 2020 a 4 de abril de 2021). A seta indica o momento da inoculação

Table 1. Species and origin of the *C. fimbriata* isolates

Tabela 1. Espécies e origem dos isolados de *C. fimbriata*

Isolate	Species	Location
LPF 1512	*C. fimbriata*	Suzano/SP
LPF 1806	*C. fimbriata*	Minas Gerais/MG
LPF 1607	*C. fimbriata*	Queimados/BA
LPF 1657	*C. fimbriata*	Três Lagoas/MS

an inoculation suspension was prepared by adding 10 mL sterile distilled water to the plates for fungal growth. Next, the fungal colony surface was scraped to obtain a spore suspension and filtered through a double layer of gauze, and the concentration was determined in a Neubauer chamber at 2.5 × 106 spores mL^{-1} (Laia et al., 2000; Mezzomo et al., 2019).

The conidial suspension was inoculated 60 days after transplanting. Using a scalpel, a 1 cm long and 0.2 cm deep longitudinal incision was made in the plant epidermis at a height of 3 cm from the plant base. An automatic micropipette was used to apply 500 µL of the conidial suspension. The incision site was wrapped in cotton moistened with distilled water and coated with a PVC film to prevent evaporation of the suspension, ensure tissue colonization by the pathogen, and avoid contamination by other microorganisms (Gomes et al., 2019).

2.5 Biometric assessments and gas exchange

Plant height was measured from the base to the point of insertion of the last pair of leaves using a centimeter-graduated ruler. The stem

diameter was determined at the base height using a digital caliper. Biometric evaluations were conducted 0 and 60 days after inoculation (DAI).

Gas exchange was assessed from 7 a.m. to 9 a.m. using an infrared gas analyzer (IRGA, LI-6400XT, Li-Cor, Lincoln, NE, USA). Photosynthetic CO_2 assimilation (A, µmols CO2 $m^{-2}s^{-1}$), stomatal conductance (gs, mol H2O $m^{-2}s^{-1}$) and transpiration (E, mmol H_2O $m^{-2}s^{-1}$) were determined at temperatures ranging from 27 to 30ºC, internal chamber air flow of 500 µmol, CO_2 concentration of 400 ppm and 1500 µmol photons $m^{-2}s^{-1}$ of artificial light. Gas exchanges was performed between 20 and 60 DAI. All measurements were obtained on healthy, fully expanded leaves exposed to solar radiation, selected from the middle third of the canopy.

2.6 Infrared thermography

The average, minimum, and maximum leaf and branch temperatures were evaluated at 13 DAI and then every 7 days until 60 DAI at 10 a.m. A FLIR 8 Wi-Fi mid-wave infrared camera (FLIR Systems, OR, USA) with 0.95 emissivity was used to capture thermal images. A focal plane array detector was used to produce 320 × 240-pixel (76,800 pixels) images with ± 2% accuracy. To that end, the equipment was positioned approximately 0.50 m above the plant. The images were stored in the memory of the equipment and imported using FLIR Tools Software, version 6.4.18039.1003 (copyright 2021 Teledyne FLIR LLC), where they were processed and the parameters were adjusted for atmospheric temperature and relative humidity according to the environmental variables and the distance between the device and the plant.

2.7 Leaf greenness and chlorophyll a fluorescence

Between 1 and 60 DAI, leaf greenness was evaluated weekly using a SPAD-502 portable chlorophyll meter (Konica Minolta, Tokyo, Japan) between 7 am and 9 am. Five fully expanded leaves from the middle third of each plant were recorded to obtain the overall mean.

Chlorophyll fluorescence was also assessed weekly between 0 and 60 DAI on the same leaves used for gas exchange evaluations, using a non-modulated Pocket-PEA portable fluorimeter (Hansatech, Norfolk, UK). The leaves were allowed to adapt to the dark for 30 min with the aid of special leaf clips placed in the intermediate part of the leaf. The maximum quantum yield of photosystem II (Fv/Fm), absorption flux per reaction center (ABS/RC), and photosynthetic index (PI) were evaluated.

2.8 Pathogenic action of *C. fimbriata* isolates in *Eucalyptus* seedlings

The pathogenic action of *C. fimbriata* isolates used in the present study was evaluated at 60 DAI, when plants were cut longitudinally to determine lesion length (LL). The relative pathogenicity (S) of the fungus was determined by the ratio between lesion length (LL) and plant height (PH) using the formula S (%) = LL × 100/PH (Gomes et al., 2019).

2.9 Experimental design and data analysis

The experiment was conducted using a completely randomized design, comprising 5 treatments and 6 replicates, totaling 30 experimental units. Data were subjected to analysis of variance, and the means were compared by Tukey's test at 5% probability using R software version 4.1.1. Linear and quadratic regression analyses were performed for DAI to identify the chronological behavior of infection.

3. RESULTS

Ecophysiological changes were observed in *Eucalyptus* plants in the presence of *C. fimbriata*. Even in plants where lesion symptoms were not visible, it was possible to identify changes in the variables studied, with significant differences in plant height and gas exchange (A, gs, and E) between the isolates and assessment periods. In contrast, stem diameter differed ($p<0.05$) only between assessment days (0 and 60 DAI) (Table 2).

3.1. Biometric assessments

Plant height showed significant differences between the DAI and the isolates studied, with plants inoculated with LPF 1806 being shorter at 60 DAI (103.7 cm), whereas those

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Table 2. Plant height (Height, in cm) and stem diameter (SD, in mm) at 0 and 60 days after inoculation (DAI) of *Eucalyptus* plants inoculated with *C. fimbriata* isolates (n = 6).

Tabela 2. Altura da planta (altura, em cm) e diâmetro do caule (DP, em mm) aos 0 e 60 dias após a inoculação (DAI) de plantas de eucalipto inoculadas com isolados de *C. fimbriata* (n = 6).

Treatments	Variables			
	Height (0 DAI)	Height (60 DAI)	SD (0 DAI)	SD (60 DAI)
1512	68.58 ± 0.63Ba	119.1 ± 1.99Aab	8.63 ± 1.40Ba	14.07 ± 2.78Aa
1806	74.50 ± 0.78Ba	103.7 ± 2.58Abc	9.43 ± 2.05Ba	15.85 ± 1.68Aa
1607	75.67 ± 1.12Ba	130.9 ± 2.12Aa	8.85 ± 1.33Ba	16.98 ± 3.55Aa
1657	77.33 ± 1.23Ba	104.8 ± 2.66Abc	9.73 ± 2.70Ba	16.38 ± 1.68Aa
Control	68.42 ± 1.17Ba	90.8 ± 0.95Ac	10.05 ±1.43Ba	16.43 ± 2.95Aa

Note: Different uppercase and lowercase letters indicate significant differences between DAI and treatments, respectively, according to Tukey's test at 5% probability.

Nota: Letras maiúsculas e minúsculas diferentes indicam diferenças significativas entre DAI e tratamentos, respectivamente, de acordo com o teste Tukey a 5% de probabilidade.

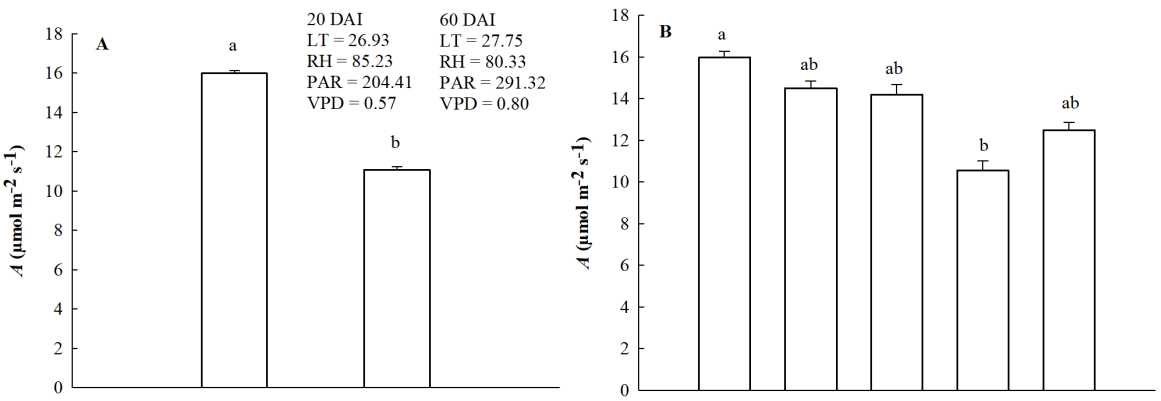
inoculated with LPF 1657 (104.8 cm) did not differ significantly. Regarding stem diameter, there was a significant difference only between DAI.

3.2. Gas exchange

Regarding gas exchange, A, gs, and E decreased by 31, 42, and 32%, respectively, from 20 to 60 DAI in plants inoculated with *C. fimbriata* (Figures 2A, C, and E). When considering the treatments with different isolates, plants inoculated with the LPF 1657 isolate showed 29% less photosynthetic CO2 assimilation than those inoculated with LPF 1512, which exhibited the highest A (Figure 2B). For gs and E, LPF 1657-inoculated plants exhibited lower stomatal conductance and transpiration (42 and 32%, respectively) than plants inoculated with LPF 1806, which displayed higher stomatal conductance and transpiration (Figures 2D and F).

3.3. Infrared thermography

Leaf temperature was affected by the *C. fimbriata* isolates. There was a significant increase ($p<0.05$) in the minimum, average, and maximum temperatures by 20 %, 27 %, and 31 %, respectively, throughout the post-



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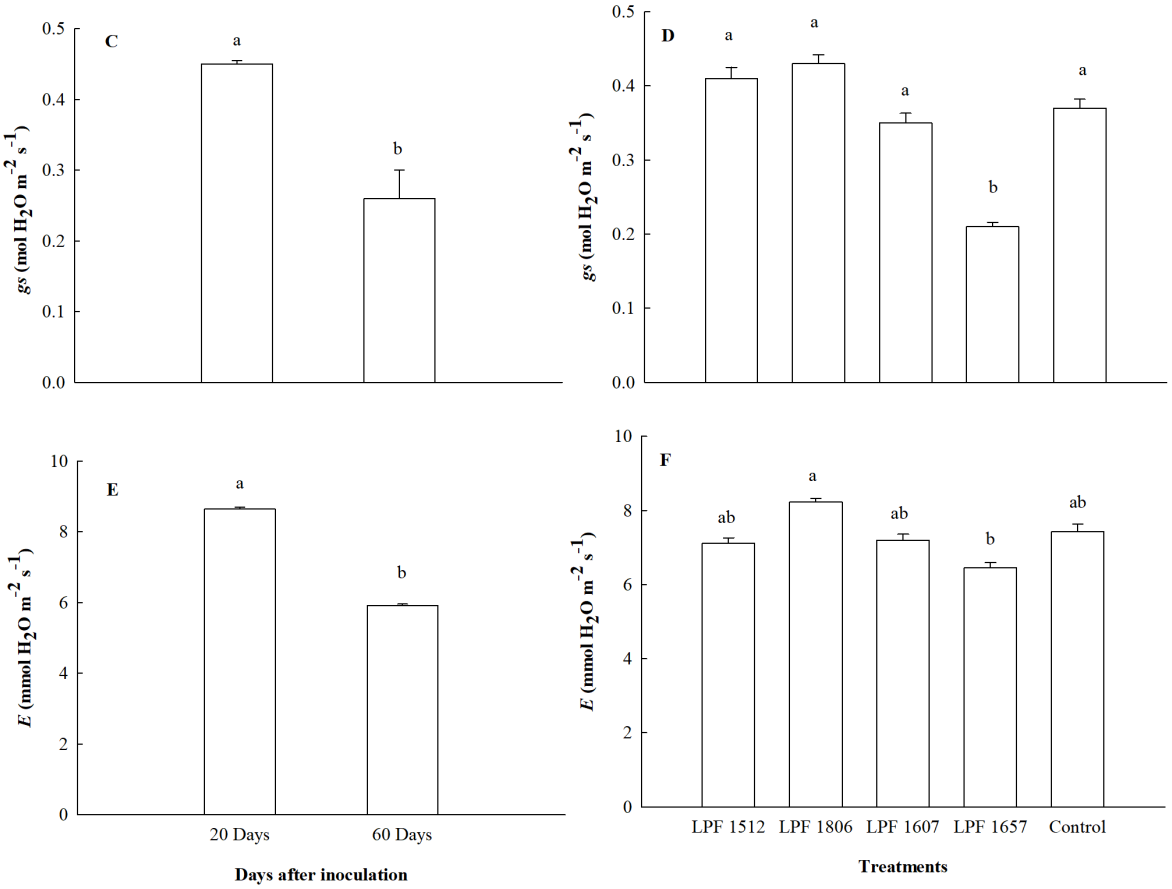

Figure 2. Net photosynthetic CO_2 assimilation - A (A and B), stomatal conductance - gs, (C and D), and leaf transpiration - E (E and F), as a function of assessment periods and treatments evaluated, respectively. Vertical bars are the standard error of the mean (n = 12 for assessment periods and n = 30 for treatments). Different uppercase and lowercase letters indicate significant differences between assessment periods and treatments, respectively, according to Tukey's test at 5% probability. LT: Leaf Temperature, RH: Relative Humidity, PAR: Photosynthetically Active Radiation, VPD: Vapor Pressure Deficit

Figura 2. Assimilação fotossintética líquida de CO_2 - A (A e B), condutância estomática - gs, (C e D) e transpiração foliar - E (E e F), em função dos períodos de avaliação e tratamentos avaliados, respectivamente. Barras verticais são o erro padrão da média (n = 12 para períodos de avaliação e n = 30 para tratamentos). Letras maiúsculas e minúsculas diferentes indicam diferenças significativas entre períodos de avaliação e tratamentos, respectivamente, de acordo com o teste de Tukey a 5% de probabilidade. LT: Temperatura da Folha, RH: Umidade Relativa, PAR: Radiação Fotossinteticamente Ativa, VPD: Déficit de Pressão de Vapor

inoculation period between 14 and 60 DAI (Figure 3A). Similar behavior was observed for branch temperature, with an approximate increase of 11% from 14 to 60 DAI (Figure 3B), demonstrating a positive linear correlation between leaf and branch temperatures (Figure 3C).

Although there were no significant differences between isolates for leaf and branch temperature, the latter varied, with the highest average temperature (34.1 ºC) obtained in inoculated with LPF 1657- inoculated plants (Figure 4D) and the lowest in the controls (30.7 ºC) (Figure 4E).

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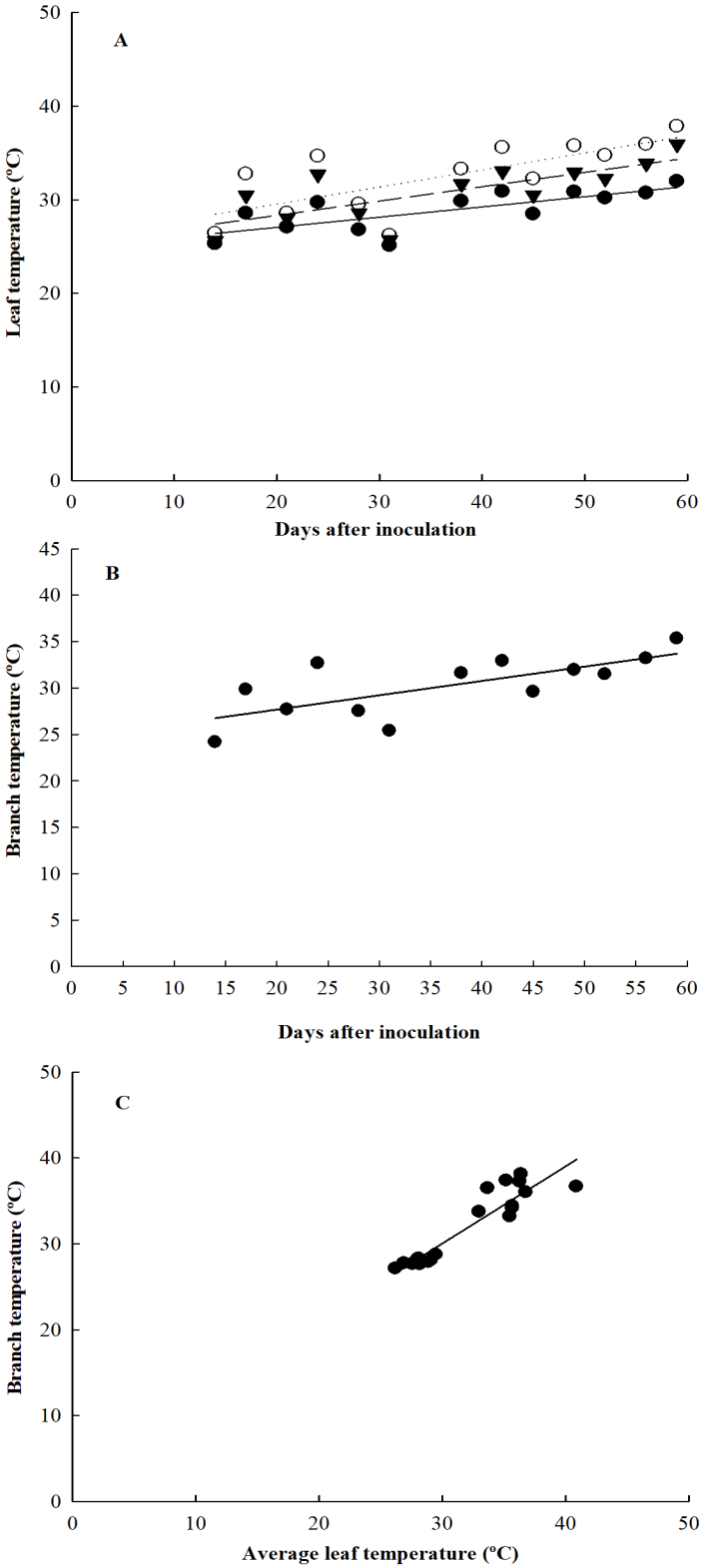

Figure 3. Temperatures: minimum-Tmin, maximum-Tmax, and average-Tavg leaf (A) and branch temperatures (B) and ratio between average branch and leaf temperatures (C), at 10 a.m. in *Eucalyptus* plants inoculated with *C. fimbriata* isolates

Figura 3. Temperaturas: Tmin mínimo, Tmax máximo e Tavg médio das folhas (A) e dos ramos (B) e razão entre as temperaturas médias dos ramos e das folhas (C), às 10h em plantas de eucalipto inoculadas com isolados de *C. fimbriata*

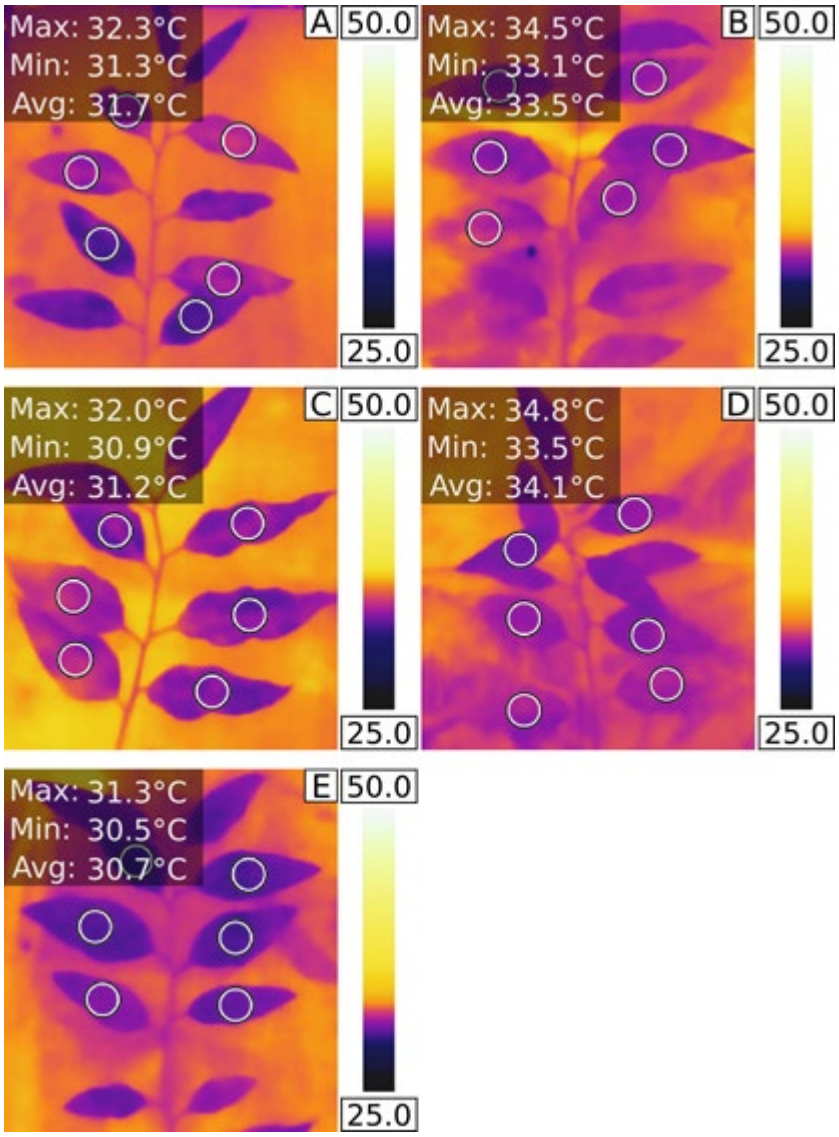

Figure 4. Thermographic images with "iron" palette, 25–50 ºC. Plant branch inoculated with LPF 1512(A), LPF 1806 (B), LPF 1607 (C), and LPF 1657 (D), and control plant branch (E)

Figura 4. Imagens termográficas com paleta "ferro", 25–50 ºC. Ramo da planta inoculado com LPF 1512(A), LPF 1806 (B), LPF 1607 (C) e LPF 1657 (D), e ramo da planta controle (E)

3.4. Chlorophyll a fluorescence and leaf greenness index

C. fimbriata activity in *Eucalyptus* plants caused a significant decline ($p<0.01$) in the SPAD index, ABS/RC, and PI values, and ($p<0.05$) in the Fv/Fm ratio. There was a noticeable increase in the SPAD index until 12 DAI, followed by a gradual decrease until the lowest values were reached at 60 DAI (Figure 5A). In contrast, Fv/Fm displayed a slight reduction across the DAI, remaining above 0.75 (Figure 5B). The ABS/RC and PI also decreased by 57 and 60%, respectively, from 0 to 60 DAI (Figures 5C and D).

3.5. Pathogenic action of *C. fimbriata* isolates on *Eucalyptus* seedlings

A statistically significant difference was observed between the pathogenic actions of the *C. fimbriata* isolates used in our study. LPF 1657 differed from the other isolates, behaving as the most virulent, and external wilt symptoms caused by *C. fimbriata* were identified, including crown wilt, wilting of individual branches, epicormic sprouting, collar darkening, and subsequent plant death.

When the internal symptoms and pathogenic action of isolates were identified,

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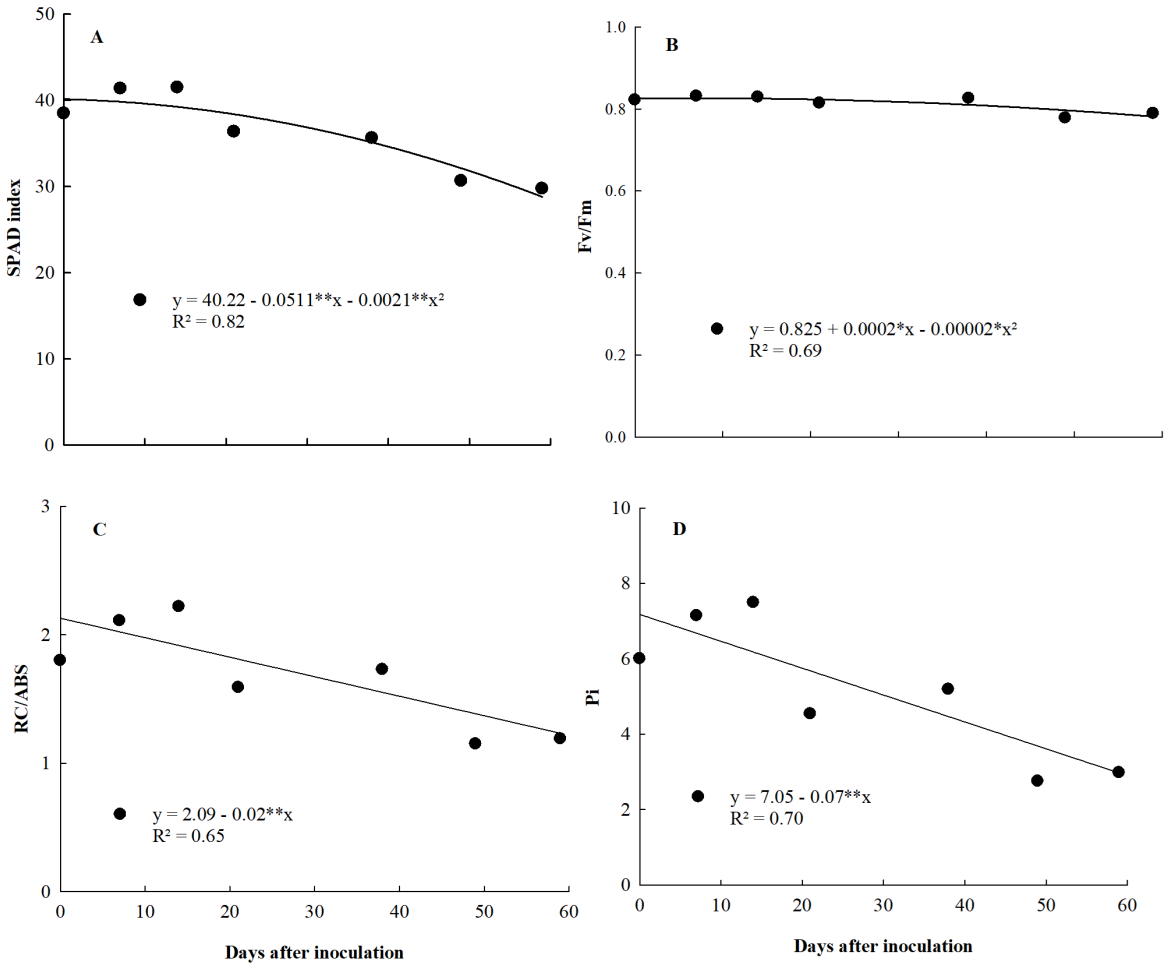

Figure 5. SPAD index (A), maximum quantum efficiency of photosystem II – Fv/Fm (B), active reaction center density - ABS/RC (C), and photosynthetic index (PI) of *Eucalyptus* plants inoculated with *C. fimbriata* isolates

Figura 5. Índice SPAD (A), eficiência quântica máxima do fotossistema II – Fv/Fm (B), densidade do centro de reação ativo - ABS/RC (C) e índice fotossintético (IP) de plantas de eucalipto inoculadas com isolados de *C. fimbriata*

the length of internal lesions caused by the fungus in xylem tissues was evaluated. There was a significant difference ($p<0.05$) between isolates, particularly in plants inoculated with LPF 1657, which exhibited the highest percentage of injured tissue (approximately 10.9%) (Figure 6). The other isolates did not differ significantly, causing a lower infection percentage, particularly the LPF 1607 isolate, with a severity of 2.44%.

4. DISCUSSION

This study found that *C. fimbriata* isolates affected the growth, gas exchange, and photosynthetic efficiency of *Eucalyptus* seedlings, with reductions in photosynthetic CO_2 assimilation, stomatal conductance, and transpiration due to fungal colonization. Compared with other isolates, plants infected with LPF 1657 showed lower photosynthetic CO_2 assimilation, stomatal conductance, and transpiration. These reductions may have resulted from the colonization of xylem and adjacent tissues and radial cells, as partial or total colonization of xylem vessels can cause tissue deterioration, inducing responses such as the formation and accumulation of lignin, gels, and phenolic compounds, blocking xylem vessels, and increasing water flow resistance

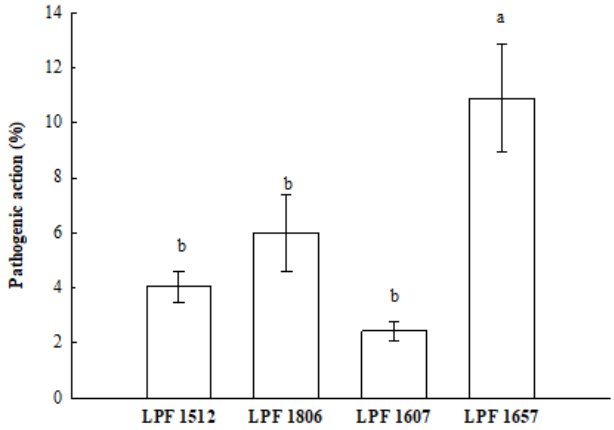

Figure 6. Pathogenic action of *C. fimbriata* in *Eucalyptus* plants. Vertical bars are the standard error of the mean (n = 6). Different lowercase letters indicate significant differences according to Tukey's test at 5% probability between treatments

Figura 6. Ação patogênica de *C. fimbriata* em plantas de eucalipto. Barras verticais são o erro padrão da média (n = 6). Letras minúsculas diferentes indicam diferenças significativas de acordo com o teste de Tukey a 5% de probabilidade entre os tratamentos

from roots to leaves, leading to water stress (Tumura et al., 2012; Park et al., 2013; Bispo et al., 2016a; 2016b; Silva et al., 2018).

To prevent water loss to the atmosphere, plants initially increase the synthesis and concentration of abscisic acid (ABA), which signals stomatal closure. If the stomata remain closed for a prolonged period, there is a decline in plant transpiration and CO_2 entry into the leaves, which are essential substrates for photosynthesis (Bispo et al., 2016a; Bispo et al., 2016b; Silva et al., 2018), leading to reduced plant growth. The same behavior may have occurred in the clone plants used in our study, which showed reductions in A, gs, and E, and consequently, lower growth when inoculated with *C. fimbriata* isolates, according to Silva et al. (2018), who reported a decline in gas exchange in *Eucalyptus* plants infected with *C. fimbriata*. These responses have also been observed in mango cultivars inoculated with the same fungus (Bispo et al., 2016a; 2016b). Reduced gas exchange is a common response to infection by vascular pathogens due to obstruction of the vascular system, as well as lower stomatal activity and water stress (Pascual et al., 2010; Ploetz et al., 2015; Cacique et al., 2017). Stomatal closure causes changes in leaf temperature, which is an important variable in determining plant water status. Under normal conditions, with open stomata and transpiration, leaf temperature and physiological activity remain stable; however, under stress conditions, where stomatal closure and transpiration decrease, leaf temperature increases (Biju et al., 2018; Abreu et al., 2021). This may have occurred in our study, given that *C. fimbriata* colonization and infection occurred gradually and simultaneously throughout the post-inoculation period, which probably obstructed the xylem vessels and induced water stress, triggering stomatal closure, reducing transpiration, and increasing the minimum, maximum, and average leaf and branch temperatures of *Eucalyptus* plants. Similar results were reported by Biju et al. (2018), in which the leaf temperature of lentil genotypes increased under water stress due to stomatal closure and reduced transpiration, resulting in a decline in photosynthetic CO_2 assimilation.

In some cases, reductions in photosynthetic CO_2 assimilation are also associated with chlorophyll degradation, which may lead to disorders in the plant light-harvesting complex and, consequently, photosynthetic apparatus damage (Bhargava et al., 2014; Cavalcante et al., 2018). This was demonstrated by reductions in the leaf greenness index (SPAD), maximum quantum yield of photosystem II (Fv/Fm), ABS/RC, and photosynthetic index (PI), as observed in *Eucalyptus* plants inoculated with *C. fimbriata* isolates.

After inoculation, the SPAD index increased

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for up to 12 days, followed by a decline until 60 DAI. This may partially explain the reduction in Fv/Fm across DAI, as chlorophyll degradation affects the photochemical phase of photosynthesis. However, this reduction was insufficient to cause photosynthetic apparatus damage in *Eucalyptus* plants, because, according to Bolhàr-Nordenkampf et al. (1989), Fv/Fm variation between 0.75 and 0.85 indicates photosynthetic apparatus integrity and no photoinhibition damage in photosystem II. The values found here were within this range, suggesting that a reduction in A was not associated with a decline in Fv/Fm but with stomatal factors. Similar results were reported by Bispo et al. (2016b) and Silva et al. (2018), who studied *C. fimbriata*-infected mango and *Eucalyptus* plants, respectively, where variations in Fv/Fm did not exceed 0.75 to 0.85. However, even in the Fv/Fm range, which demonstrates photosynthetic apparatus integrity, there was a decline in the ABS/RC and PI values in *Eucalyptus* plants inoculated with *C. fimbriata*, indicating that the fungus affects reaction center activity (Zhao et al., 2017), which, along with stomatal closure, may have influenced the decline in photosynthetic CO_2 assimilation.

Based on the pathogenic action obtained by evaluating the length of the internal lesions caused by *C. fimbriata* in xylem tissues, all isolates were pathogenic to the *Eucalyptus* clone used in the study. The LPF 1657 isolate obtained from the state of Mato Grosso do Sul affected plants to a greater extent, suggesting that disease severity may vary according to the virulence of the isolate. In addition, plants containing this isolate showed lower photosynthetic CO_2 assimilation, stomatal conductance, transpiration rates, and consequently higher branch temperatures, indicating that it caused the greatest blockage of sap flow through the xylem, thereby affecting gas exchange. Our findings corroborate those of Guimarães et al. (2010) and Oliveira et al. (2015), who investigated *Eucalyptus* clones infected with *C. fimbriata*, also observing variations in fungal isolate virulence.

5. CONCLUSION

In conclusion water flow resistance in xylem vessels caused by *C. fimbriata* isolates in *Eucalyptus* seedlings resulted in water stress in leaves and a reduction in stomatal opening, with declines in transpiration rates and photosynthetic CO_2 assimilation, regardless of the isolate used. Photosynthetic performance was also compromised by the fungus, with reductions in reaction center density (ABS/RC) and PI, which affect plant growth.

Fungus severity analysis indicated that LPF 1657 had the greatest effect on the physiology and growth of *Eucalyptus* seedlings, which was confirmed by the lower height and A, gs, and E values of the inoculated plants.

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AUTHOR CONTRIBUTIONS

P.C.S. - Performance of the experiment and data analysis; M. F. F. - Review the manuscript; J. R. A. – Data analysis, supervision, writing, review and editing; S. O. M. J. – Writing and review; D. L. A. - Performance of the experiment; C. A. M. - Performance of the experiment; E. K. C. S. Review the manuscript and data analysis; A. A. C. R. - Review the manuscript; T. M. F. - Review the manuscript; F. A. M. M. A. F. – Orientation, methodology, writing, review and editing.

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