Ciência Florestal

UFSM

Ci. Fl., Santa Maria, v. 29, n. 2, p. 740-753, abr./jun. 2019
DOI: https://doi.org/10.5902/1980509830685
ISSN 1980-5098

Artigos

Submissão: 04/01/2018 Aprovação: 24/04/2018 Publicação: 30/06/2019

Photosynthetic responses of *Eucalyptus seedlings* submitted to chemical and mechanical stimulus

Respostas fotossintéticas em mudas de eucalipto submetidas a estímulo químico e mecânico

Paulo Ricardo Lima[I], Ubirajara Contro Malavasi[II], Andre Gustavo Battistus[III], João Alexandre Lopes Dranski[IV], Marlene de Matos Malavasi[V]

Abstract

This work aimed to investigate the photosynthetic responses of *Eucalyptus urophylla* x *Eucalyptus grandis* hybrids 1528 submitted to the application of jasmonic acid (JA) and stem bending. The experiment was conducted in a shade house using a completely randomized design with five replicates of 20 seedlings, with the following treatments: T1: control, T2: 8.0 µmol of JA and T3: 40 stem bending. The net assimilation rate of CO_2 (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), leaf transpiration rate (*E*), water use efficiency (*WUE*), intrinsic efficiency of use of Water (*iWUE*) and carboxylation efficiency (*Fc*) were measured with an IRGA. The dose of JA applied to the seedlings was insufficient to alter the photosynthetic responses in relation to the control treatment. Mechanical stimuli induced by stem bending in eucalyptus hybrid 1528 seedlings promoted an increase in the maximum photosynthetic capacity and maximum assimilation of CO_2, but the seedlings presented a reduction in the efficiency of the water use, that is, lower photosynthetic efficiency when compared to the seedlings submitted to other treatments. Seedlings of eucalyptus hybrid 1528 submitted to mechanical stimuli and jasmonic acid show greater respiration under low flow levels of photosynthetically active photons.

Keywords: Jasmonic acid; Stem bending; Quality of seedlings; Clone 1528

Resumo

Este trabalho objetivou investigar as respostas fotossintéticas em mudas de híbrido 1528 de *Eucalyptus urophylla* x *Eucalyptus grandis* submetidas à aplicação de ácido jasmônico (JA) e flexões caulinares. O experimento foi conduzido em casa de sombra utilizando delineamento inteiramente ao acaso com cinco repetições de 20 mudas, com os seguintes tratamentos: T1: controle, T2: 8,0 µmol de JA e T3: 40 flexões caulinares. A taxa de assimilação líquida de CO_2 (*A*), condutância estomática (*gs*), concentração interna de CO_2 (*Ci*), taxa de transpiração da folha (*E*), eficiência do uso de água (*WUE*), eficiência intrínseca do uso de água (*iWUE*) e eficiência de carboxilação (*Fc*) foram mensuradas com o auxílio do IRGA. A dose de JA aplicada nas mudas foi insuficiente para alterar as respostas fotossintéticas em relação ao tratamento-controle. Estímulos mecânicos induzidos através de flexões caulinares em mudas do híbrido 1528 de eucalipto promoveram incremento da capacidade fotossintética máxima e máxima assimilação de CO_2. Porém, as mudas apresentaram redução na eficiência do uso da água, ou seja, menor eficiência fotossintética quando comparado com as mudas submetidas aos demais tratamentos. Mudas do híbrido 1528 de eucalipto submetidas aos estímulos mecânico e ácido jasmônico apresentaram maior respiração sob níveis baixos de fluxo de fótons fotossinteticamente ativos.

Palavras-chave: Ácido jasmônico; Flexões caulinares; 1ualidade de mudas; Clone 1528

[I] Engenheiro Agrônomo, Dr., Professor do Curso de Tecnologia em Gestão Ambiental, Universidade Estadual do Mato Grosso do Sul, Rodovia BR 163, Km 20,2, CEP 79980-000, Mundo Novo (MS), Brasil. paulorikardoo@hotmail.com (ORCID: 0000-0003-3313-0613)

[II] Engenheiro Florestal, Dr., Professor do Programa de Pós-Graduação em Agronomia, Centro de Ciências Agrárias, Universidade Estadual do Oeste do Paraná, Rua Pernambuco, 1777, CEP 85960-000, Marechal Cândido Rondon (PR), Brasil. biramalavasi@yahoo.com.br (ORCID: 0000-0003-4300-4338)

[III] Engenheiro Agrônomo, DSc., Coordenador de pesquisa e desenvolvimento em nutrição vegetal, Tecnomyl S.A., Ruta Internacional Km 6,5; Av. San Blas esq. c/ Pablo Neruda, Ciudad del Este, Alto Parana, Paraguay. andre_battistus@hotmail.com (ORCID: 0000-0003-4944-8167)

[IV] Biólogo, Dr., Professor do Curso de Agronomia, Faculdade Educacional de Medianeira, Rua Rio Branco, 1820, CEP 85884-000, Medianeira (PR), Brasil. joaodranski@yahoo.com.br (ORCID: 0000-0002-2460-7865)

[V] Engenheira Agrônoma, Dra., Professora do Programa de Pós-Graduação em Agronomia, Centro de Ciências Agrárias, Universidade Estadual do Oeste do Paraná, Rua Pernambuco, 1777, CEP 85960-000, Marechal Cândido Rondon (PR), Brasil. marlenemalavasi@yahoo.com.br (ORCID: 0000-0002-6726-6490)

Introduction

Among woody species, *Eucalyptus* stands out for fast growth, adaptability and high productivity (MORA; GARCIA, 2000; GONÇALVES; SOUSA; VELINI, 2015). Brazilian total area with *Eucalyptus* have about 50% of hybrids (ASSOCIAÇÃO BRASILEIRA DE CELULOSE E PAPEL, 2008). Interspecific hybridization has become a fast and efficient manner of obtaining genetic gains in the improvement of *Eucalyptus* species, with direct and significant effects on the forest industry. The complementarity search for technological characteristics of wood, tolerance to biotic and abiotic stresses, as well as the manifestation of heterosis were verified in several hybrid crosses, as for example in the interspecific hybrid *Eucalyptus urophylla* x *Eucalyptus grandis*, called "urograndis" (ASSIS; MAFIA, 2007).

Eucalyptus Cl 1528, known as "super clone" according to Portal Florestal (2016) shows a genetic basis of *Eucalyptus urophylla* x *Eucalyptus grandis* with an annual mean increment of 50 m^{-3} ha^{-1} year^{-1}, basic wood density of 531 kg m^{-3}, total lignin content of 29.24%, holocellulose content of 68.14%, gravimetric yield of 50.41% and mechanical strength of 80.82%. The clone aims at good growth, which is an *Eucalyptus grandis* characteristic, as well as an increase in wood density and improvements in yield and physical properties of cellulose which is an *Eucalyptus urophylla* characteristic (CARVALHO; NAHUZ, 2011).

The natural environment in which plants develop is composed of a complex set of biotic and abiotic stresses. Consequently, productivity below ideal levels of plant responses to those stresses is dynamic and complex (CRAMER, 2010; SKIRYCZ; INZE, 2010). In addition, difficulties in establishing standards and recommendations for obtaining quality seedlings lead to post-planting mortality. Thus, the production of resistant seedlings capable of surviving and tolerating post-planting adversities, among them, water stress and wind gust stand out, is one of the possible alternatives to minimize losses.

Several authors indicated that some practices such as mechanical stimuli and plant growth regulators may be related to improvements of seedling quality from woody species (JACOBS; LANDIS, 2009; ORO *et al.*, 2011; VOLKWEIS *et al.*, 2014; CADORIN *et al.*, 2015; DRANSKI; MALAVASI; MALAVASI, 2015). Thus, such practices could be included in nursery routine, favoring development after planting.

Plants show integrated or induced defenses, which encompass morphological, physiological and biochemical changes. The most common morphological alterations in response to mechanical and hormonal disturbances are leaf area reduction, stomatal closure and abscisic acid-mediated root system (LOPEZ; CHAUHAN; JOHANSEN, 2008; TAIZ; ZEIGER, 2013). Plant responses to stress include low leaf water potential (LIBERATO *et al.*, 2006), reduction of quantum efficiency of photosystem II (SILVA *et al.*, 2007) and relative leaf water content (SILVA *et al.*, 2007; LOBATO *et al.*, 2008), decreases in stomatal conductance and photosynthetic rate (POLIZEL *et al.*, 2011) and adjustment in synthesis of sugars and proline (MCCORMICK; CRAMER; WATT, 2008; CAMPOS *et al.*, 2011).

Mechanical stimuli have been applied to describe mechanically induced responses in plants using stem bending in seedlings (JAFFE, 1973). Current reactions to a significant number of woody species have been reported. Among them are cellular stretching reductions, with consequent reduction in the height of the seedlings and the increase in stem diameter, indicators of seedling quality (KERN *et al.*, 2005; VOLKWEIS *et al.*, 2014; CADORIN *et al.*, 2015; DRANSKI; MALAVASI; MALAVASI, 2015).

However, mechanical stimuli usually used in forest seedlings hardening are difficult to operate in large scale nurseries justifying the search for other methods such as plant growth regulators. These compounds act as resistance inducers, and some are classified as plant hormones. Plants produce a wide variety of plant hormones including auxins, gibberellins, abscisic acid, cytokinins and ethylene (GONÇALVES; SOUSA; VELINI, 2015).

In this context, plant markers such as brassinosteroids, polyamines, salicic acid and jasmonic acid have been mentioned as a new class of plant growth regulators related to several morphological and physiological effects on plant development. Among those effects are defense, cell division, reproduction, growth and development (FRANCISCO *et al.*, 2008; BORCIONI; NEGRELLE, 2012; TAIZ *et al.*, 2017).

Jasmonic acid (JA) and its jasmonate derivatives are endogenous regulators of plant growth originating from linolenic acid, which occur in several plant species (FRANK; ROSS, 1991; KERBAUY, 2012). The exogenous application of jasmonates in plants produces a wide variety of effects, including inhibition of root stretching, chlorophyll degradation and stomatal closure under stress conditions (SANCHEZ, 2008).

In addition, JA is involved in regulating defense responses and signaling of plants faced abiotic stresses (LORENZO; SOLANO, 2005; YASUDA *et al.*, 2008). The signaling pathways of these plant hormones are not isolated, but interconnected by a complex network involving several defense responses and development processes (MARTÍN, 2009). According to Deuner *et al.* (2015) induction or application of jasmonic acid provides resistance in plants under stress, resulting in better development and crop production.

Although stress induction by mechanical and chemical stimuli has the potential to represent an immediate advantage, since they may stimulate plant defense system. Kerbauy (2012) has indicated that stress situations can also directly affect assimilation of CO_2 and consequently net photosynthetic rate. A direct influence that stress can cause in plant photosynthesis is through the impact on the stomatal opening (MARENCO; LOPES, 2009). As the stomata close in response to stress, the resistance to CO_2 diffusion within the leaves increases.

Stress can also affect photosynthesis through a number of other mechanisms: by affecting the metabolic intermediates level, by inhibiting the photosynthetic electron transport system, and by changing the respiratory rate. Additionally, mechanical perturbations may decrease leaf temperature, as they reduce the resistance of the boundary layer promoted by the vapor pressure, affecting the diffusion of gases and water vapor (CHAVARRIA; SANTOS, 2012). On the other hand, JA application can compromise photosynthesis, reducing the expression of genes located in the nucleus and in the chloroplasts, besides being potential to degrade chlorophyll in leaves (SANCHEZ, 2008).

Understanding the behavior of defense and protection of plants is essential to obtain organisms resistant to several stresses (SOARES; MACHADO, 2007). In this perspective, studies are need to understand the physiological responses of plants as a function of stress induction promoted by the application of mechanical and hormonal disturbances, in order to better understand the regulatory role of these defense inducers in the adaptation mechanisms of plants, as well as the possibility of estimating the management reflexes on plant metabolism.

Therefore, this study aimed to investigate the photosynthetic responses in hybrid seedlings of *Eucalyptus urophylla* vs *Eucalyptus grandis* subjected to the application of jasmonic acid and stem bendings.

Material and methods

The experiment was conducted from May to October 2016, in a shade house located at 24°33'S and 54°2' W with and average altitude 414 m. The climate of the region, according to Köppen is Cfa subtropical, with average temperature of the coldest month lower than 18°C and the warmest month above 22°C, with infrequent frost and tendency of concentration of rainfall in the summer but with no defined dry season. The annual precipitation is around 1,600 to 1,800 mm (CAVIGLIONE *et al.*, 2000).

We used seedlings of *Eucalyptus* clone 1528 (*Eucalyptus urophylla* vs *Eucalyptus grandis*) propagated in 50 cm^3 conical plugs filled with commercial substrate (Plantmax®) made of pinus bark with the addition of 300 g m^{-3} of controlled-release fertilizer (Basacote® Plus 6M).

Shade house consisted of a galvanized iron structure with arc-shaped roof of 7 x 30 m and

3.5 m of high. The ceiling is covered with a low-density polyethylene (LDPE) and anti-UV 150 µm thick plastic film. The sides were closed with a 30% white shading screen.

The experimental design was a completely randomized with five replicates of twenty seedlings each submitted to the following treatments for 4 consecutive weeks: T1: control with deionized water + nonionic surfactant applied weekly; T2: 8.0 µmol L^{-1} jasmonic acid (JA) + deionized water + nonionic surfactant applied weekly; T3: 40 stem bendings (thigmomorphogenesis) + deionized water and nonionic surfactant (AGRAL®) applied weekly.

The application of the chemical stimulus occurred at weekly intervals by means of leaf pulverizations of jasmonic acid in the concentration according to each treatment, with a total solution volume equivalent to 7 mL per seedling until runoff. The solutions consisted of jasmonic acid, deionized water and nonionic surfactant, applied with a manual sprayer, working at a pressure of 0.3 MPa.

The stem bending treatments comprised daily mechanical stimuli using a structure composed of a 25 mm diameter PVC bar arranged horizontally and fixed in a metallic structure with bearings. The seedlings were vertically bended not more than 45º by passing the bar in the upper third of the foliage, always at the same time, at a speed 0.10 m sec^{-1} (VOLKWEIS *et al.*, 2014). During treatment application, irrigation was provided twice a day by aspersion until substrate saturation.

After two weeks of the treatments application, gas exchange response curves with an IRGA model LI-6400XT (Licor Inc. Lincoln, Nebraska, USA) were determined as a function of the photosynthetic active photon flux density (PPFD) by reduction from 2,000 µmol m^{-2} s^{-1} to 0.1 µmol m^{-2} s^{-1} and CO_2 set at 380 µmol m^{-2} s^{-1} using 25 seedlings per treatment between 9 a.m. and 11 a.m. We sampled fully developed leaves from the middle crown.

As a function of PPFD, the net assimilation rate of CO_2 (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*) and leaf transpiration rate (*E*) were determined according to (ZHANG *et al.*, 2001), where *WUE* corresponds to the efficiency of water use, *iWUE* is the intrinsic efficiency of water use and *Fc* is the carboxylation efficiency.

The CO_2 liquid assimilation response curves as a function of PPFD were preferentially adjusted to the equation: *A*= *A*max [1-e-k(Q-Ic)], allowing to estimate the maximum net assimilation (A_{max}) (PRADO; MORAES, 1997). Data of *A* originated from PPFD below 200 µmol m^{-2} s^{-1} were used to calculate the apparent quantum efficiency (*Φ*), adjusting linear equation from 0 to 200 µmol m^{-2} s^{-1} PPFD. Light compensation point (Γ) at the point of intersection of the line with the X-axis was also determined.

Data was tested for normality of the distribution of residues, variance homogeneity and submitted to ANOVA followed by regression analysis at 5% error probability. For *A* as a function of PPFD, we used equation proposed by Prado and Moraes (1997) while other variables had their models adjusted by significant equations, based on the highest coefficient of determination. The apparent quantum efficiency and light compensation point was submitted by Tukey test at 5% error probability.

In parallel, was quantified the increment of the number of leaves, being obtained by counting all the leaves. The increments were calculated by the difference between the values before and after the imposition of the treatments, that is, between a time interval of four weeks.

Results and discussion

Stem bending promoted an increase in the maximum photosynthetic capacity of eucalyptus seedlings (Figure 1a), with the maximum CO_2 assimilation (A_{max}) of 14.80 µmol of CO_2 m^{-2} s^{-1}, whereas the control and the treatment that received jasmonic acid (JA) resulted in A_{max} of 9.65 and 9.98 µmol of CO_2 m^{-2} s^{-1}, respectively (*p*-valor = 0.0138).

This photosynthetic capacity promoted by the mechanical disturbance enabled the control by feedback of the stomatal opening (Figure 1b), increasing in approximately 50% the capacity of gas and water exchanges between plant-environment in relation to control and JA seedlings. Therefore, CO_2 input in the sub stomatal chamber in seedlings subjected to stem bending was increased (Figure 1c). However, greater stomatal conductance promotes greater water loss to the atmosphere than CO_2 diffusion. Therefore, transpiration showed high values for seedlings submitted to stem bending (Figure 1d), which caused greater leaf cooling (Figure 1e).

The above transpiration event relates to the difference in leaf area between treatments, since mechanically disturbed seedlings had lower leaf area ($p<0.05$) in relation to the control and JA seedlings. The reduction in leaf area of seedlings perturbed by stem bending in relation to the control and JA was 14.68% and 21.55%, respectively. Plants with smaller leaf area and adequate water status of the substrate (condition during this study) tend to increase stomatal conductance consequently favoring greater transpiration. Thus, this sequence of events favors CO_2 diffusion to the intercellular spaces of the leaf mesophyll, in contrast to the cost of water loss in the form of vapor.

Koo *et al.* (2009) observed in *Arabidopsis* that induction of damage in leaves with a hemostat led to a marked decrease in the expression of JA response genes during a period in which JA levels remain elevated. This observation suggests that the damaged leaves become desensitized to the JA-Ile (Jasmonoyl-L-isoleucine) signal. However, several authors have reported that JA responses typically depend on large-scale changes in gene expression, noting that in non-stressed cells with low JA levels, JA-responsive gene expressions are repressed by jasmonate zim (JAZ) proteins (CHINI *et al.*, 2007; THINES *et al.*, 2007; YAN *et al.*, 2007). Therefore, when taking into account that plant cells use additional mechanisms to attenuate stress responses, possibly the applied dose of JA in this experiment was not enough for the plant to present responsive expressions of genes in relation to the control treatment. For Chung and Howe (2009), JA repressors that are stabilized against hormone-induced degradation may be involved in this form of negative feedback control.

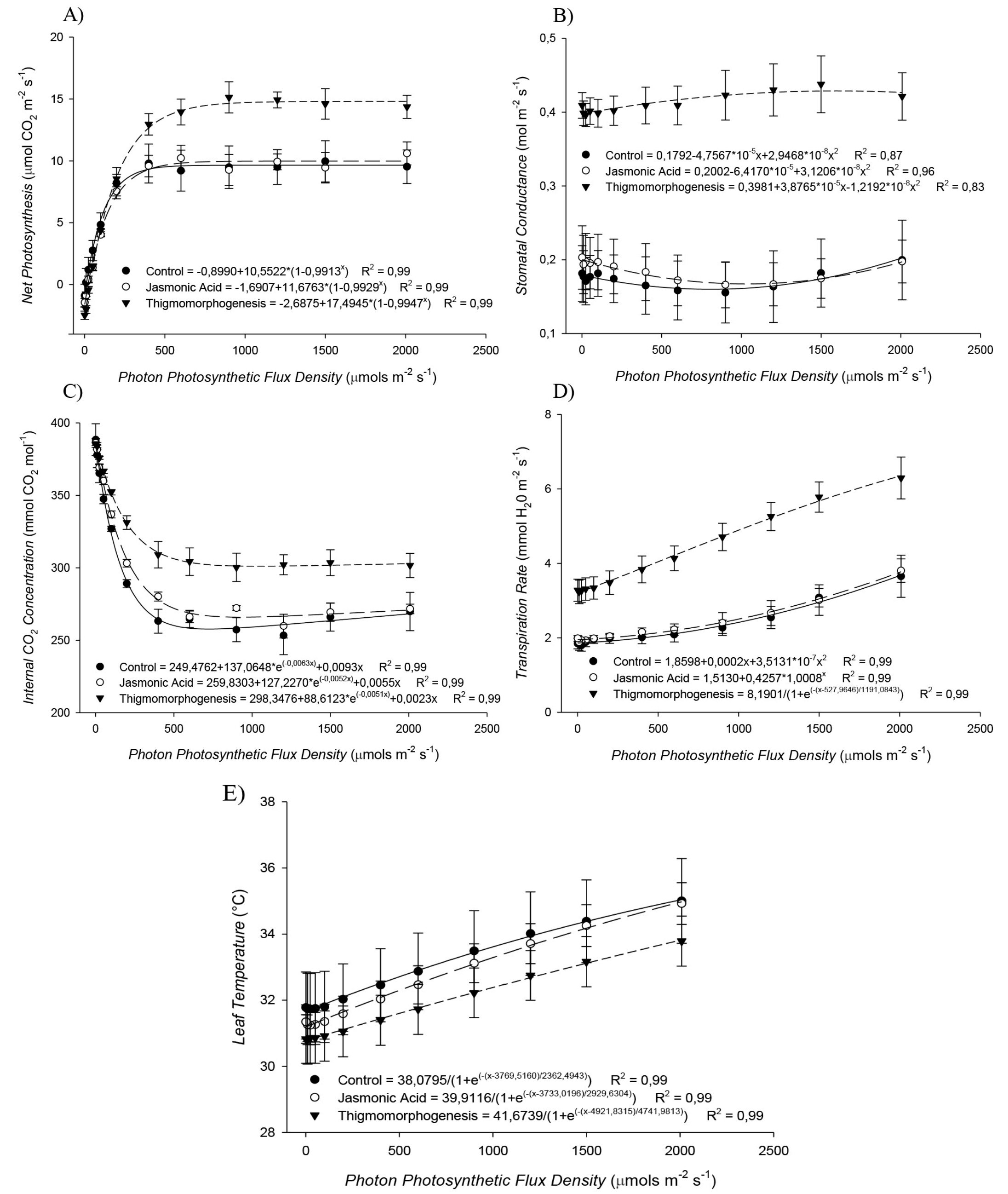
Different results as a function of the application of the JA derivative were reported by Heijari *et al.* (2005) who investigated the methyl jasmonate (MJ) potential in inducing defense reactions in two-year-old *Pinus sylvestris* L. seedlings. The authors found that the highest dose (100 mmol L^{-1}) reduced ($P = 0.027$) net photosynthesis in relation to control seedlings. However, the same authors did not observe effect on stomatal conductance between treatments. Suhita *et al.* (2003) observed that stomatal opening in *Nicotiana glauca* Graham plants was suppressed by the presence of MJ.

Results divergent from our experiment were reported by Gonçalves, Sousa and Velini, (2015) when studying hybrid eucalyptus seedlings (*Eucalyptus grandis* vs. *Eucalyptus urophylla*) under water stress. The above mentioned authors reported a transpiration reduction of control treatment in relation to JA and MJ treatments, showing that the application of those compounds had influenced perception and signaling resulting in greater efficiency of stomatal closure and transpiration rate. Pereira *et al.* (2006) evaluating the possible stress effects on *Eucalyptus urograndis* seedlings detected an inverse relation between transpiration and stomatal closure.

Higher transpiration values allow cooling of leaf temperature reducing potential damages to the photosynthetic apparatus and losses in the photosynthetic effectiveness (MILBURN, 1979; ENDRES *et al.*, 2010; TAIZ; ZEIGER, 2013). Results from Lima *et al.* (2014) showed that water stress induced stomatal closure resulting in leaf heating in seedlings of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos. In addition, Endres *et al.* (2010) reported that leaf temperature increase in response to stress could be explained by the latent heat loss reduction through transpiration. JA seedlings initially reduced values of PPFD in relation to the control seedlings under low radiation. This tendency may indicate difficulties of the photosynthetic apparatus in the conversion of light energy into ATP under external JA since part of the unused energy is lost as heat.

Figure 1 – Net assimilation rate (A), internal CO_2 concentration (B), stomatal conductance (C), transpiration rate (D) and leaf temperature (E) in Eucalyptus seedlings Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) subjected to pulverization of jasmonic acid (8.0 µmol L^{-1}) and thigmomorphogenesis (40 bendings) as a function of the photosynthetic active photon flux density.

Figura 1 – Taxa de assimilação líquida (A), condutância estomática (B), concentração interna de CO_2 (C), taxa de transpiração (D) e temperatura foliar (E) em mudas de eucalipto Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) submetidas à pulverização de ácido jasmônico (8,0 µmol L^{-1}) e tigmomorfogênese (40 flexões caulinares) em função da densidade de fluxo de fótons fotossinteticamente ativos.

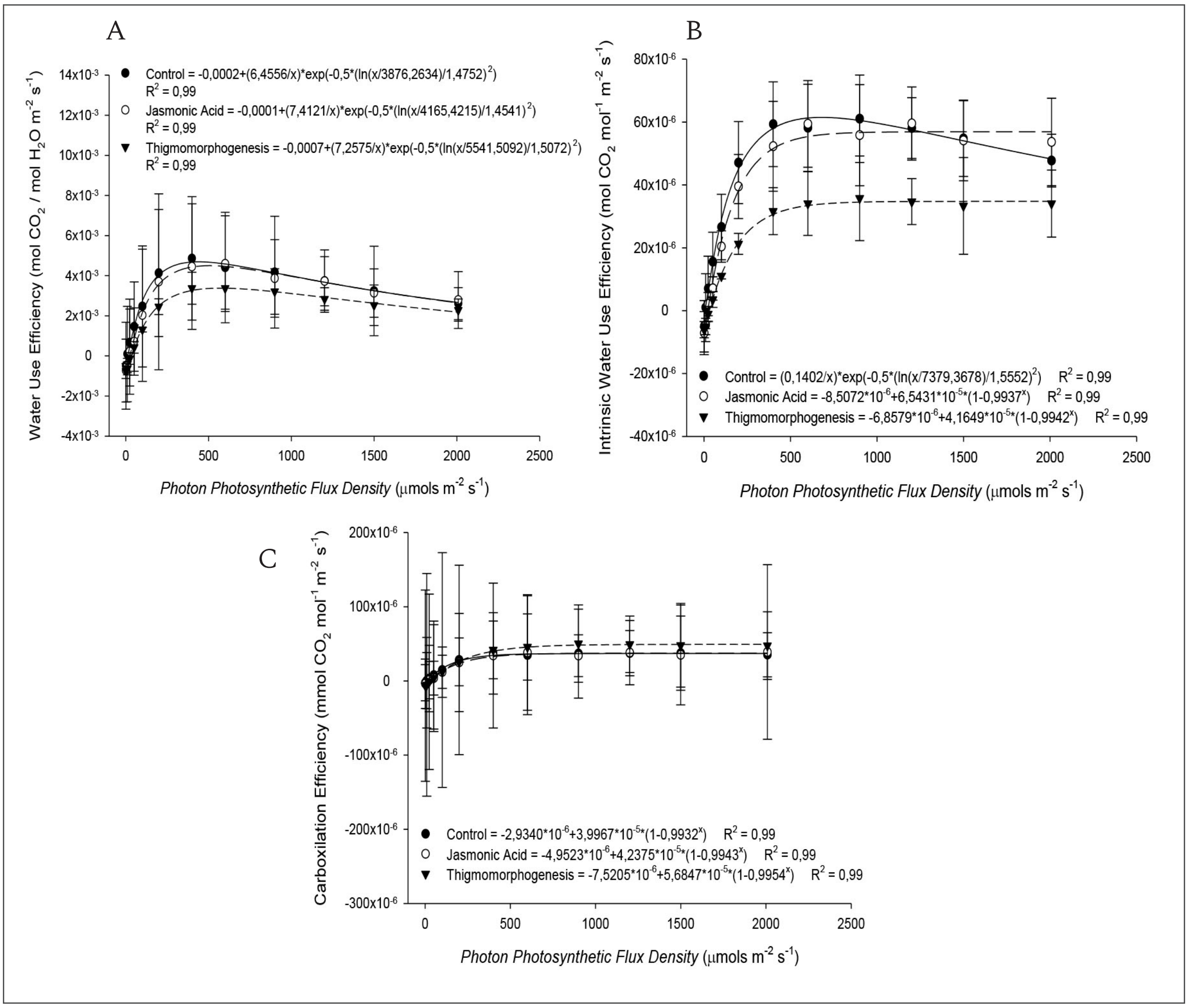


Source: Authors (2018)
Fonte: Autores (2018)

Regarding photosynthetic efficiency, in spite of expressing high values of net CO_2 assimilation, stem bending reduced water efficiency by 31.85% when compared to other treatments (Figure 2a). Mechanically disturbed seedlings assimilate fewer CO_2 molecules per molecule of transpiration of water when compared to control and JA seedlings (Figure 2b). Consequently, due to the lower efficiency in water use, eucalyptus seedlings subjected to stem bending needed to open their stomatals wide in order to assimilate the same amount of CO_2 than the other seedlings.

Figure 2 – Efficiency of water use (A/E) (A), intrinsic water use efficiency (A/gs) (B) and carboxylation efficiency (A/Ci) (C) of seedlings of eucalyptus Cl 1528 (Eucalyptus urophylla x Eucalyptus grandis) subjected to pulverization of jasmonic acid and thigmomorphogenesis as a function of photosynthetic active photon flux density.

Figura 2 – Eficiência do uso da água (*A/E*) (A), eficiência intrínseca do uso da água (*A/gs*) (B) e eficiência de carboxilação (*A/Ci*) (C) de mudas de eucalipto Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) submetidas à pulverização de ácido jasmônico e tigmomorfogênese (40 flexões caulinares) em função da densidade de fluxo de fótons fotossinteticamente ativos.



Source: Authors (2018)
Fonte: Autores (2018)

Under adequate water availability, mechanically bended seedlings showed greater carbon sequestration, whereas, in situations of restricted water availability seeddling treated with jasmonic acid and control seedlings could improve utilization of water by controlling the stomatal opening.

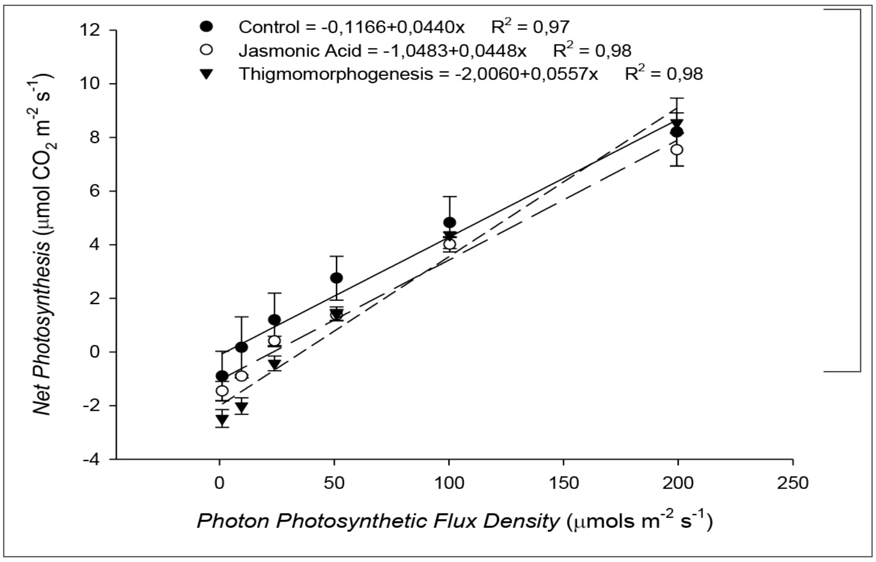
Chamber substomatic CO_2 concentration in seedlings submitted to stem bending showed higher values when compared to JA and control seedlings (Figure 2c). Despite the greater CO_2 availability in the substomatal chamber (*Ci*), there was also an increase in Calvin cycle in relation to the other treatments, collaborating in photosynthetic efficiency. Such trend can be explained by leaf nitrogen evaluated through SPAD readings from mechanically disturbed seedlings ($p < 0.05$). SPAD units were 28.82, 28.00 and 32.52 for control, JA and mechanically perturbed seedlings. It is important to highlight that from 30 to 50% of plant nitrogen is for rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) synthesis (SINCLAIR; HORIE, 1989; MARSCHNER, 2011). Therefore, seedlings submitted to stem bending showed higher content of the above enzyme per unit leaf area.

Attaran *et al.* (2014) analyzing the effect of JA on the photosynthetic efficiency in leaves of *Arabidopsis thaliana* (L.) Heynh.did not observe a decrease of photosynthesis.

Based on the fact that high CO_2 concentrations can overcome stomatal limitations in photosynthesis, Attaran *et al.* (2014) tested the exposure of plants treated with JA under elevated CO_2 levels and verified an induction of photosystem II (PSII) decrease in early morning. However, after the supplementation of plants with high CO_2 during a period of 2 hours (starting 1 h before dawn), such PSII effect was alleviated. Those results help to explain the responses found in this study, since seedlings under mechanical stress showed a smaller amount of leaf area, but with higher SPAD unit in relation to the control and JA seedlings.

Figure 3 – Net assimilation rate of CO2 in seedlings of Eucalyptus Cl 1528 (Eucalyptus urophylla x Eucalyptus grandis) submitted to jasmonic acid pulverization and thigmomorphogenesis as a function of the photosynthetic active photon flux density between 0 and 200 μmol photons m^{-2} s^{-1}.

Figura 3 – Taxa de assimilação líquida de CO2 em mudas de eucalipto Cl 1528 (Eucalyptus urophylla x Eucalyptus grandis) submetidas à pulverização de ácido jasmônico e tigmomorfogênese (40 flexões caulinares) em função da densidade de fluxo de fótons fotossinteticamente ativos entre 0 e 200 μmol photons m^{-2} s^{-1}.



Source: Authors (2018)
Fonte: Autores (2018)

Hristova and Popova (2002) found a different result when studying barley seedlings (*Hordeum vulgare* L.) sprayed with MJ and observed a decrease in chlorophyll content, in the net photosynthesis and leaf transpiration rate in relation to the control treatment.

Using low PPFD values (0 µmol m^{-2} s^{-1} to 200 µmol m^{-2} s^{-1}), linear equations were set (Figure 3) which indicated the apparent quantum efficiency Φ promoted by the treatments (Table 1). In other words, the inverse relationship of the constant of the linear equation shows the capacity of application and use of reducing power and ATP in the Calvin cycle (MACHADO *et al.*, 2005). It mean that 17.95 µmol $photons^{-1}$ being required for fixation of 1 µmol CO_2 for seedlings submitted to stem bendings, 22.32 µmol $photons^{-1}$ µmol CO_2^{-1} in the seedlings submitted to JA and 22.72 µmol $photons^{-1}$ µmol CO_2^{-1} in control seedlings respectively, evidencing an increase in the photophosphorylation efficiency promoted by mechanical disturbances.

Results from this study are different from those of Ballaré (2009) and Meldau, Erb and Baldwin (2012). Both papers reported that JA signaling by the plant effectively decouples when photosynthesis increases, and that JA acts as a signal to re-orient the biosynthetic capacity of the plant defense metabolism. Recent studies of nitrogen flow dynamics in response to stress induced by herbivorous insects support this idea (ULLMANN-ZEUNERT *et al.*, 2013). Other studies have shown that (induced stress) performed with herbivory simulation can redirect the carbon allocation from the leaves to the roots (SCHWACHTJE *et al.*, 2006; FERRIERI *et al.*, 2013; DRANSKI *et al.*, 2016). Carbon partitioning translocating to underground tissues may represent a plant strategy to protect energy consumption and better tolerate abiotic stress (SCHWACHTJE *et al.*, 2006). Perhaps the amount of JA to which the plants have been submitted in this work were not sufficient to express photosynthetic reactions other than the control treatment, or even, such absence of difference in behavior is related to the genetic factor of the hybrid.

Several authors have reported that JA and its derivatives exert distinct biological activities when applied to plant tissues (BLECHERT *et al.*, 1999; MIERSCH *et al.*, 1999; STINTZI *et al.*, 2001; RIBOT *et al.*, 2008; WANG *et al.*, 2008). JA applied exogenously does not target a single specific cell type. It is often applied to non-physiological concentrations and is subject to unknown metabolic paths, necessitating additional approaches to determine the specific in vivo role of JA and its derivatives.

Table 1 – Apparent quantum efficiency (Φ) and light compensation point (Γ) obtained from net assimilation rate (A) values between 0 µmol m^{-2} s^{-1} and 200 µmol m^{-2} s^{-1} density of photosynthetically active photons in Eucalyptus Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) seedlings as a function of treatments.

Tabela 1 – Eficiência quântica aparente (Φ) e ponto de compensação luminoso (Γ) obtidos a partir dos valores da taxa de assimilação líquida (A) entre 0 µmol m^{-2} s^{-1} até 200 µmol m^{-2} s^{-1} de densidade de fótons fotossinteticamente ativos, em mudas de eucalipto Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) em função dos tratamentos.

Treatment	Φ (µmol CO_2 µmol photons)	Γ (µmol m^{-2} s^{-1})
Control	0.0438 b	5.0192
Jasmonic Acid	0.0447 ab	23.4366
Thigmomorphogenesis	0.0555 a	35.8772

Different letters represent difference by the Tukey test at 5% of error probability.

It is well established that JA levels vary widely depending on the stage of development and the biotic and abiotic conditions in which plants are submitted (CREELMAN; MULLET, 1997; WASTERNACK, 2007). Our results lead us to believe that 8.0 μmol L^{-1} of exogenously applied JA was not detrimental to the organism physiology, since the responses were similar to that of the control treatment. However, mechanical stimuli (stem bendings) simulating a stress induction were enough to expose a higher photosynthetic rate, greater stomatal opening, resulting in greater CO_2 assimilation, probably in response to a smaller leaf area and higher concentration of the SPAD unit.

Authors have been reporting that mechanical damage results in an increase in the rapid JA accumulation of up to 10-fold (within 5 minutes) after tissue injury (CHUNG *et al.*, 2008; GLAUSER *et al.*, 2008; KOO *et al.*, 2009). However, JA level can be altered by mechanically induced damage leading to an increased JA concentration in the plant, it seems likely that the jasmonic acid dose used in this work was not sufficient for *Eucalyptus* seedlings to present responses similar to those caused by mechanical stress. Thus, it is evident the need to quantify the JA concentration after the mechanical stress induction by stem bending.

Despite higher Φ values, mechanically stressed seedlings showed the highest light compensation point (Γ), followed by JA seedlings (Table 1). On the contrary, control seedlings showed the lowest Γ values. These proportions, although not show statistical differences, indicate the tendency that in spite of the photosynthetic increments promoted by the mechanical disturbance and jasmonic acid, they show greater respiration in the dark, due to the need of energy expenditure to recover any lesions at the cellular level caused by the stem bendings and increment of the secondary metabolism for structuring intermediate compounds promoted by the jasmonic acid.

Conclusion

JA dose applied to Eucalyptus Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) seedlings was insufficient to change the photosynthetic responses in relation to the control treatment.

Mechanical stress increased maximum photosynthetic capacity and maximal CO_2 assimilation. Therefore, they showed higher transpiration rate and lower leaf temperature in Eucalyptus Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) seedlings.

Induced mechanical stimuli in Eucalyptus Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) seedlings, despite high values of photosynthesis, resulted in a reduction in water use efficiency, that is, lower photosynthetic efficiency when compared to other treatments.

Eucalyptus Cl 1528 (*Eucalyptus urophylla* x *Eucalyptus grandis*) seedlings submitted to mechanical stimuli and JA showed greater respiration in the dark.

References

ASSIS, T. F.; MAFIA, R. G. Hibridação e clonagem. *In:* BORÉM, A. (Ed.). **Biotecnologia Florestal**. Viçosa, MG: Universidade Federal de Viçosa, 2007. p. 93-121.

ASSOCIAÇÃO BRASILEIRA DE CELULOSE E PAPEL. **Desempenho do setor em 2006 e projeções para 2007**. São Paulo: BRACELPA, 2008. 8 p. Disponível em: <http://www.bracelpa.org.br/bra/estatisticas/pdf/anual/desempenho_2006.pdf>. Acesso em: 16 jan. 2016.

ATTARAN, E. *et al.* Temporal dynamics of growth and photosynthesis suppression in response to jasmonate signaling. **Plant Physiology**, Rockville, v. 165, p. 1302-1314, 2014.

BALLARÉ, C. L. Illuminated behaviour: phytochrome as a key regulator of light foraging and plant anti-herbivore defence. **Plant, Cell and Environment**, Oxford, v. 32, p. 713-725, 2009.

BLECHERT, S. *et al.* Structure–activity analyses reveal the existence of two separate groups of active octadecanoids in elicitation of the tendril-coiling response of *Bryonia dioica* Jacq. **Planta**, [S.l.], v. 207, p. 470-479, 1999.

BORCIONI, E.; NEGRELLE, R. R. B. Aplicação de análogo de brassinosteroide (Biobras 16®) sobre a germinação e crescimento in vitro de embriões zigóticos e aclimatização de plântulas de bocaiúva. **Ciência Rural**, Santa Maria, v. 42, n. 2, p. 270-275, fev. 2012.

CADORIN, D. A. *et al.* Metil jasmonato e flexões caulinares na rustificação e crescimento inicial de mudas de *Cordia trichotoma*. **CERNE**, Lavras, v. 21, n. 4, p. 657-664, 2015.

CAMPOS, M. K. F. *et al.* Drought tolerance and antioxidant enzymatic activity in transgenic 'Swingle' citrumelo plants over-accumulating proline. **Environmental and Experimental Botany**, Oxford, v. 72, n. 2, p. 242-250, 2011.

CARVALHO, A. M.; NAHUZ, M. A. R. Valorização da madeira do híbrido *Eucalyptus grandis* x *Eucalyptu surophylla* através da produção conjunta de madeira serrada em pequenas dimensões, celulose e lenha. **Scientia Forestalis**, Piracicaba, n. 59, p. 61-76, 2001.

CAVIGLIONE, J. H. *et al.* **Cartas climáticas do Paraná**. Londrina: IAPAR, 2000. CD-ROM.

CHAVARRIA, G.; SANTOS, H. P. Plant water relations: absorption, transport and control mechanisms. *In:* MONTANARO, G.; DICHIO, B. (Org.). **Advances in selected plant physiology aspects**. Rijeka: Intech, 2012. v. 1. p. 105-132.

CHINI, A. *et al.* The JAZ family of repressors is the missing link in jasmonate signalling. **Nature**, London, v. 448, p. 666-671, 2007.

CHUNG, H. S. *et al.* Regulation and function of Arabidopsis JASMONATE ZIM-domain genes in response to wounding and herbivory. **Plant Physiology**, Rockeville, v. 146, p. 952-964, 2008.

CHUNG, H. S.; HOWE, G. A. A critical role for the TIFY motif in repression of jasmonate signaling by a stabilized splice variant of the JASMONATE ZIM domain protein JAZ10 in Arabidopsis. **The Plant Cell**, Rockville, v. 21, p. 131-145, 2009.

CRAMER, G. R. Abiotic stress and plant responses from the whole vine to the genes. **Australian Journal of Grape and Wine Research**, [S.l.], v. 16 p. 86-93, 2010.

CREELMAN, R. A.; MULLET, J. E. Biosynthesis and action of jasmonates in plants. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo Alto, v. 48, p. 355-381, 1997.

DEUNER, C. *et al.* Ácido jasmónico como promotor de resistência em plantas. **Revista de Ciências Agrárias**, Lisboa, v. 38, n. 3, p. 275-281, 2015.

DRANSKI, J. A. L.; MALAVASI, U. C.; MALAVASI, M. M. Relationship between lignin content and quality of *Pinus taeda* seedlings. **Revista Árvore**, Viçosa, MG, v. 39, p. 905-913, 2015.

DRANSKI, J. A. L. *et al.* Desenvolvimento inicial de mudas de pinhão manso depende da intensidade de desfolha. **Magistra**, Cruz das Almas, v. 28, n. 2, p. 700-709, abr./jun. 2016.

ENDRES, L. *et al.* Gas exchange alteration caused by water deficit during the bean reproductive stage. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 14, p. 11-16, 2010.

FERRIERI, A. P. *et al.* Temporal changes in allocation and partitioning of new carbon as 11C elicited by simulated herbivory suggest that roots shape aboveground responses in Arabidopsis. **Plant Physiology**, Rockville, v. 161, p. 692-704, 2013.

FRANCISCO, A. A. *et al.* Reguladores e teores endógenos de poliaminas durante o desenvolvimento de taro cultivado in vitro. **Ciência Rural**, Santa Maria, v. 38, n. 5, p. 1251-1257, ago. 2008.

FRANK, S.; ROSS, S. **Plant Physiology**. 4th ed. California: Wadsworth, 1991.

GLAUSER, G. *et al.* Spatial and temporal dynamics of jasmonate synthesis and accumulation in Arabidopsis in response to wounding. **Journal of Biological Chemistry**, North Bethesda, v. 283, p. 16400-16407, 2008.

GONÇALVES, K. S.; SOUSA, A. P.; VELINI, E. D. S. Aplicação de reguladores vegetais e de fosfito de potássio em mudas de eucalipto submetidas à deficiência hídrica. **Irriga**, Botucatu, v. 20, n. 2, p. 273-285, 2015.

HEIJARI, J. *et al.* Application of methyl jasmonate reduces growth but increases chemical defence and resistance against *Hylobius abietis* in Scots pine seedlings. The Netherlands Entomological Society. **Entomologia Experimentalis et Applicata**, [S.l.], v. 115, p. 117-124, 2005.

HRISTOVA, V. A.; POPOVA, L. P. Treatment with methyl jasmonate alleviates the effects of paraquat on photosynthesis in barley plants. **Photosynthetica**, Czech Republic, v. 40, p. 567-574, 2002.

JACOBS, D. F.; LANDIS, T. D. Hardening. *In:* DUMROESE, R. K.; LUNA, T.; LANDIS, T. D. (Ed.). **Nursery manual for native plants**: Guide for tribal nurseries. Washington: United States Department of Agriculture, Forest Service, 2009. v. 1. p. 217-228.

JAFFE, M. J. Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation with special reference to *Bryoniadioica*. **Planta**, [S.l.], v. 114, n. 2, p. 143-156, 1973.

KERBAUY, G. B. **Fisiologia Vegetal**. 2. ed. Rio de Janeiro: Guanabara Koogan, 2012.

KERN, A. K. *et al.* Mechanical perturbation affects conductivity, mechanical properties and aboveground biomass of hybrid poplars. **Tree Physiology**, Oxford, v. 25, n. 10, p. 1243-1251, 2005.

KOO, A. J. K. *et al.* A rapid wound signal activates systemic synthesis of bioactive jasmonates in Arabidopsis. **The Plant Journal**, Oxford, v. 59, p. 974-986, 2009.

LIBERATO, M. A. R. *et al.* Leaf water potential, gas exchange and chlorophyll *a* fluorescence in acariquara seedlings (*Minquartia guianensis* Aubl.) under water stress and recovery. **Brazilian Journal of Plant Physiology**, Londrina, v. 18, p. 315-323, 2006.

LIMA, P. R. *et al.* Avaliação morfofisiológica em mudas de *Handroanthus impetiginosus* (Mart. ex DC.) Mattos durante a rustificação. **Floresta e Ambiente**, v. 21, n. 3, p. 316-326, 2014.

LOBATO, A. K. S. *et al.* Biochemical and physiological behavior of *Vigna unguiculata* (L.) Walp. under water stress during the vegetative phase. **Asian Journal of Plant Sciences**, Pakistan, v. 7, n. 1, p. 44-49, 2008.

LOPEZ, F. B.; CHAUHAN, Y. S.; JOHANSEN, C. Effects of timing of drought stress on leaf area development an canopy light interception of short-duration pigeon pea. **Journal of Agronomy and Crop Science**, California, v. 178, n. 1, p. 1-7, 2008.

LORENZO, O.; SOLANO, R. Molecular players regulating the jasmonate signalling network. Curr. Opin. **Plant Biology**, London, v. 8, p. 532-540, 2005.

MACHADO, E. C. *et al.* Respostas da fotossíntese de três espécies de citros a fatores ambientais. **Pesquisa Agropecuária Brasileira**, Brasília, v. 40, p. 1161-1170, 2005.

MARENCO, R. A.; LOPES, N. F. **Fisiologia vegetal**: fotossíntese, respiração, relações hídricas e nutrição mineral. 3. ed. Viçosa, MG: Editora UFV, 2009. 486 p.

MARSCHNER, H. **Mineral nutrition of higher plants**. 3rd Edition. San Diego: Academic Press, 2011. 672 p.

MARTÍN, F. J. P. **Respuestas inducidas por ácido abscísico y ácido salicílico en las simbiosis de judía y alfalfa en estrés salino**. 2009. 395 f. Tese (Doutorado em Ciencias Biológicas) – Universidade de Granada, Granada, 2009.

MCCORMICK, A. J.; CRAMER, M.; WATT, D. A. Changes in photosynthetic rates and gene expression of leaves during a source-sink perturbation in sugarcane. **Annals of Botany**, Oxford, v. 101, n. 1, p. 89-102, 2008. MELDAU, S.; ERB, M.; BALDWIN, I. T. Defence on demand: mechanisms behind optimal defence patterns. **Annals of Botany**, Oxford, v. 110, p. 1503-1514, 2012.

MIERSCH, O. *et al.* Structure–activity relations of substituted, deleted or stereospecifically altered jasmonic acid in gene expression of barley leaves. **Phytochemistry**, Amsterdam, v. 50, p. 353-361, 1999.

MILBURN J. Water flow in plants. London: Longman, 1979.

MORA, A. L.; GARCIA, C. H. **A cultura do eucalipto no Brasil**. São Paulo: Sociedade Brasileira de Silvicultura, 2000. 112 p.

ORO, P. *et al.* Aplicação de regulador vegetal na aclimatação de mudas de *Cariniana estrellensi.* **Cultivando o Saber**, Cascavel, v. 5, n. 4, p. 103-112, 2011.

PEREIRA, M. R. R. *et al.* Comportamento fisiológico e morfológico de clones de *Eucalyptus urograndis* submetidos a diferentes níveis de água no solo. **Irriga**, Botucatu, v. 11, n. 4, p. 518-531, 2006.

POLIZEL, A. M. *et al.* Molecular, anatomical and physiological properties of a genetically modified soybean line transformed with rd29A:AtDREB1A for the improvement of drought tolerance. **Genetics and molecular Research: GMR**, Ribeirão Preto, v. 10, n. 4, p. 3641–56, jan. 2011.

PORTAL FLORESTAL. **Clone – AEC 1528 – Super Clone**. [2016].

Available *in:* <http://www.portalflorestal.com.br/portfolio/mudas-de-eucalipto-clonado-a-venda-clone-aec-1528-super-clone/>. Access *in:* 08 nov. 2016.

PRADO, C. H. B. A.; MORAES, J. A. P. V. Photosynthetic capacity and specific leaf mass in twenty woody species of Cerrado vegetation under field conditions. **Photosynthetica**, Czech Republic, v. 33, p. 103-112, 1997.

RIBOT, C. *et al.* Induction of the Arabidopsis PHO1; H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. **Plant Physiology**, Rockville, v. 147, p. 696-706, 2008.

SANCHEZ, F. Jasmonatos: compuestos de alto valor para la agricultura: actividad biológica y ruta biosintética del ácido jasmónico en plantas. **Revista ICIDCA**, La Habana, v. 42, n. 1-3, p. 51-59, 2008.

SCHWACHTJE, J. *et al.* SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. **Proceedings of the National Academy of Sciences**, Washington, v. 103, p. 12935-12940, 2006.

SINCLAIR, T. R.; HORIE, T. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. **Crop Science**, Madison, v. 29, p. 90-98, 1989.

SILVA, M. A. *et al.* Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. **Brazilian Journal of Plant Physiology**, Piracicaba, v. 19, n. 3, p. 193-201, 2007.

SKIRYCZ, A.; INZE, D. More from less: plant growth under limited water. **Current Opinion in Biotechnology**, Amsterdam, v. 21, p. 197-203, 2010.

SOARES, A. M. S.; MACHADO, O. L. T. Defesa de plantas: sinalização química e espécies reativas de oxigênio. **Revista Trópica – Ciências Agrárias e Biológicas**, Chapadinha, v. 1, n. 1, p. 9-19, 2007.

STINTZI, A. *et al.* Plant defense in the absence of jasmonic acid: the role of cyclopentenones. **Proceedings of the National Academy of Sciences**, Washington, v. 98, p. 12837-12842, 2001.

SUHITA, D. *et al.* Different signaling pathways involved during the suppression of stomatal opening by methyl jasmonate or abscisic acid. **Plant Science**, Amsterdam, v. 164, p. 481-488, 2003.

TAIZ, L. *et al.* **Fisiologia e desenvolvimento vegetal**. 6. ed. Porto Alegre: Artmed, 2017. 888 p.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 5. ed. Porto Alegre: Artmed, 2013. 954 p.

THINES, B. *et al.* JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. **Nature**, London, v. 448, p. 661-665, 2007.

ULLMANN-ZEUNERT, L. *et al.* Quantification of growthdefense trade-offs in a common currency: nitrogen required for phenolamide biosynthesis is not derived from ribulose-1,5-bisphosphate carboxylase/oxygenase turnover. **The Plant journal**, Oxford, v. 75, p. 417-429, 2013.

VOLKWEIS, R. C. *et al.* Efeito da tigmomorfogênese na morfometria de mudas de *Maytenus ilicifolia* (Schrad.) Planch. **Ciência Florestal**, Santa Maria, v. 24, n. 2, p. 339- 342, 2014.

WANG, L. *et al.* Comparisons of LOX3- and JAR4/6-silenced plants reveal that JA and JA-AA conjugates play diferente roles in herbivore resistance of *Nicotiana attenuata*. **Plant Physiology**, Rockville, v. 146, p. 904-915, 2008.

WASTERNACK, C. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. **Annals of Botany**, Oxford, v. 100, p. 681-697, 2007.

YAN, Y. *et al.* A downstream mediator in the growth repression limb of the jasmonate pathway. **The Plant Cell**, Rockville, v. 19, p. 2470-2483, 2007.

YASUDA, M. *et al.* Antagonistic interaction between systemic acquired resistance and the abscisic acid–mediated abiotic stress response in *Arabidopsis*. **The Plant Cell**, Rockville, v. 20, p. 1678-1692, 2008.

ZHANG, S. *et al.* Temperature dependent gas exchange and stomatal/non-stomatal limitation to CO_2 assimilation of *Quercus liaotungensis* under midday higher irradiance. **Photosynthetica**, Prague, v. 39, p. 383-388, 2001.