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Articles

Physiological changes induced by hardening in seedlings of eucalyptus as a function of growth stages

Alterações fisiológicas induzidas pela rustificação em mudas de eucalipto em função dos estádios de crescimento

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ABSTRACT

Hardening appears as an interesting strategy to improve the quality of seedlings in forest nurseries, favoring their survival in the field. Thus, the objective of this research was to show the physiological alterations resulting from the application of methyl jasmonate and stem flexion in three stages of growth in *Eucalyptus urograndis* seedlings. The experiment was carried out in Marechal Cândido Rondon, Paraná, and *Eucalyptus urograndis* seedlings were used. Treatments consisted of weekly applications of methyl jasmonate (MeJA), daily imposition of stem flexion and a control treatment. Analyzes included quantification of flexural stiffness, lignin content in stems and roots, phenolic compounds in leaves and roots, electrolyte leakage in roots and field survival. In seedlings of *Eucalyptus urograndis* at 100 days after emergence (DAE) and at the three distances used to evaluate flexural rigidity, the highest averages were observed in seedlings submitted to chemical and mechanical treatments, coinciding with the increase in lignin content in the stem. In the roots, after quantifying the extravasation of electrolytes in Stage I, the means decreased with the application of treatments. In the field phase, there were no statistically significant differences between seedling survival assessments, quantified every 15 days, for a period of 90 days after planting. However, seedlings in Stage I were lost because they were too young and could not tolerate the stressful conditions observed in the field.

Keywords: Growth; Lignin; Physiologically; Stem flexibility

RESUMO

A rustificação surge como uma estratégia interessante a fim de melhorar a qualidade das mudas nos viveiros florestais, melhorando a sobrevivência a campo. Assim, o objetivo da pesquisa foi evidenciar alterações fisiológicas resultantes da aplicação de metil jasmonato e flexões caulinares nos três estádios de crescimento em mudas de *Eucalyptus urograndis*. O experimento foi conduzido em Marechal Cândido Rondon, no Estado do Paraná, e foram utilizadas mudas de *Eucalyptus urograndis. O*s tratamentos foram constituídos de aplicações semanais de metil jasmonato (MeJA) e imposição diária de flexões caulinares, além do tratamento controle. As análises consistiram na quantificação da rigidez flexural, lignina nos caules e raízes, compostos fenólicos nas folhas e raízes, perda de eletrólitos nas raízes e sobrevivência a campo. Em *Eucalyptus urograndis* com 100 dias após a emergência (DAE) e nas 3 distâncias utilizadas para avaliação da rigidez flexural, as maiores médias foram observadas em mudas submetidas aos tratamentos químico e mecânico coincidindo com o aumento do teor de lignina no caule. Nas raízes, após a quantificação da perda de eletrólitos, no estádio I, houve redução das médias com a aplicação dos tratamentos. Na fase de campo, não houve diferença significativa estatística entre as avaliações morfométricas a campo e a sobrevivência de mudas, quantificadas a cada 15 dias, até completarem 180 dias do plantio. Ainda, as mudas pertencentes ao estádio I foram perdidas, visto que estas eram muitos jovens e não toleraram as condições estressantes.

Palavras-chave: Lignina; Metil jasmonato; Rigidez flexural; Rustificação

1 INTRODUCTION

Terrestrial wood species are faced with numerous stresses from germination to the final stages of life. There are protocols, namely hardening, to attenuate the adversities found in the initial stages of plant growth. The hardening process in seedlings of wood species has been an option to acclimatize young plants to environmental conditions, without drastically harming their growth and development. These processes or practices can be introduced into nursery routines, changing the morphometric parameters and thus modulating the desirable characteristics for seedling.

Among the processes or practices of hardening, the literature lists suspension of fertilization, frequency of irrigation, alteration of luminosity and application of plant regulators or mechanical stimuli (Jacobs; Landis, 2009; Volkweis; Dranski; Oro; Malavasi; Malavasi, 2014; Dranski; Malavasi; Malavasi, 2015; Cadorin; Malavasi; Malavasi; Dranski; Coutinho, 2021).

Jasmonates are a group of phytohormones that act in the regulation, defense, and signaling on plants. This phytohormone is derived from 12-oxo-phyto dienoic acid, via linolenic acid and synthesized in peroxisomes. There are several substances derived from these compounds and they can be named development regulators, in the case of jasmonic acid. The main compounds in the volatile form are methyl jasmonate and cis-jasmonate which can be quickly transferred between cells, plant structures or between plants (Wasternack; Hause, 2013).

Jasmonates and salicylates can be applied to promote hardening of seedlings from wood species improving their performance (i.e. survival and growth) after outplanting (Lima; Malavasi; Lopes; Dranski; Malavasi; Borsoi, 2020; Cadorin; Malavasi; Malavasi; Dranski; Coutinho, 2021).

Mechanical hardening or thigmomorphogenesis according to Jaffe (1973) is a stimulus that promotes changes in plants that simulates stressful conditions in the field. This technique could act to increase plant tolerance, improving post-planting survival through low-intensity controlled stresses (Volkweis; Dranski; Oro; Malavasi; Malavasi, 2014; Dranski; Malavasi; Malavasi, 2015; Lima; Malavasi; Lopes; Dranski; Malavasi; Borsoi, 2020; Cadorin; Malavasi; Malavasi; Dranski; Coutinho, 2021).

The hybrid *Eucalyptus urophylla* x *E. grandis* or *Eucalyptus urograndis*, when under ideal water, nutritional and climatic conditions present favored primary and secondary growth in addition to increasing basal area, trunk volume, and plant biomass (Fernandes; Florêncio; Faria, 2012).

Dispatching seedlings for planting are important, as it will confirm whether the practices carried out in the forest nursery were efficient and improved post-planting survival (Araújo; Navroski; Schorn, 2018). In this way, seedlings submitted to chemical or mechanical hardening will change their physiological or morphometric characteristics through controlled disturbances aiming to improve tolerance to stressful conditions in the field.

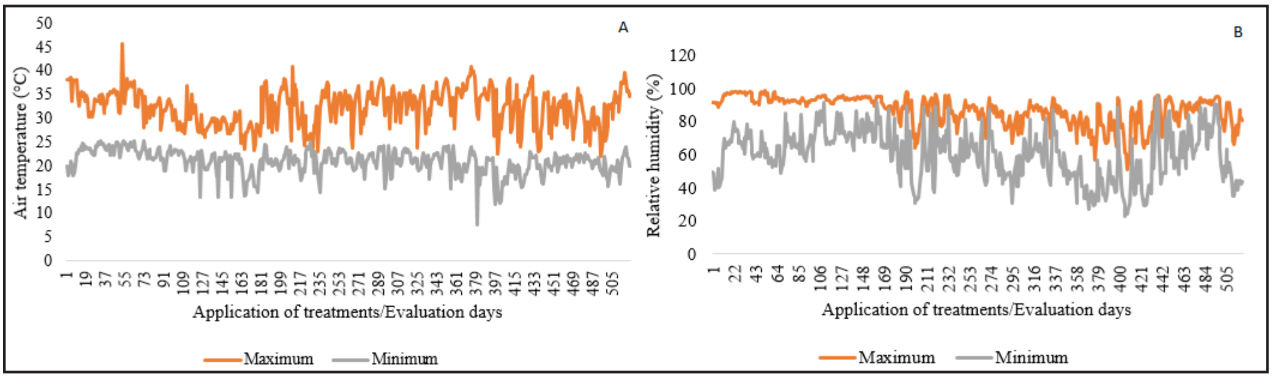
According to the above arguments, the objective of the research was to quantify physiological changes resulting from the application of methyl jasmonate and stem bending as a function of growth stages in seedlings of *Eucalyptus urograndis* and their post-planting survival.

2 MATERIALS AND METHODS

The research was conducted in Marechal Cândido Rondon-PR with geographic coordinates of 24°33'24" S, longitude of 54°05'67" W and altitude of 420 m. According to the climatological classification of IAPAR and Köppen, the climate of the region is Cfa subtropical with average temperatures between 22 and 23°C, good distribution of rainfall throughout the year characterized by hot summers (Alvares; Stape; Sentelhas; Gonçalves; Sparovek, 2013).

Relative humidity and air temperature measurements (Figure 1) were obtained daily with a datalogger (KlimaLogg Smart®) throughout the experiment.

Figure 1 – Air temperature and humidity obtained in a protected environment during the experiment with *Eucalyptus urograndis* seedlings



Source: Authors (2022)

We used seedlings of *Eucalyptus urograndis* produced from seeds bought from the Instituto de Pesquisas e Estudos Florestais (IPEF) and propagated in a shade house with a 150 micron thick anti-UV low-density polyethylene film, equivalent to

20% shading. Seeds were collected in Bofete-SP from a seed production area (APS) F3 generation in 2018.

Seedlings were produced with 120 cm^3 plastic plugs that received approximately 5 seeds per container. After two weeks, germinants were thinned keeping the central one with greater height and well-formed protophylls. The commercial substrate used was Humusfertil® vermicompost based on pine bark, sand and vermiculite, with electrical conductivity of 1.5 mS cm^{-1}; density of 480 kg m^{-3}; pH of 6.5; maximum moisture and retention capacity (CRA) by mass/mass equal to 60%.

Until beginning of hardening, seedlings were fertilized with a nutrient solution and fertilizer concentrations were 50.2 g of Osmocote® (slow-release fertilizer) plus 50.6 g of urea diluted in 2 L of water. Finally, 80.4 g of NPK (10-15-15) was added to the solution diluted in 2 L of water. The solutions were prepared separately, but the application was simultaneously every 15 days. The amount used was 20 mL in a volume of 10 L of water. Fertilization was interrupted as soon as hardening treatments began, in January 2020.

Hardening treatments were control (without hardening), chemical (100 μmol L^{-1} of methyl jasmonate) and mechanical (through pendulum movement of the seedling stem). The dose of metyl jasmonate has been set based on pre-tests carried out before the conduction of the research definitive, in a protected environment, such as all other experiments conducted.

The design used was a completely randomized consisting of three treatments and seven replications (30 seedlings each) totaling 630 seedlings per growth stage of *Eucalyptus urograndis*. The hardening periods followed the results of Rocha (2022) and were defined at 70, 100 and 130 days after emergence (DAE).

Chemical hardening occurred weekly, while mechanical hardening was performed daily, for 4 weeks, at stages determined via pre-test (70, 100 and 130 days after emergence). The period of seedling production and application of hardening treatments occurred from November 2019 to February 2020.

Chemical hardening used methyl jasmonate solution at a concentration of 100 μmol L^{-1}, deionized water and non-ionic surfactant (Agral-Syngenta®) at a proportion of 30 mL in 100 L of water. The non-ionic surfactant was used to increase the absorption spectrum of the solution applied to the leaves, maximizing the use of the product. The solution was applied with a manual sprayer between 06:00 and 06:30 pm. The amount applied was 100 L ha^{-1} following recommendations from application technology. Mechanical hardening consisted of 20 stem bending at a constant speed of 0.10 m s^{-1} applied from 06:00 pm according to Volkweis, Dranski, Oro, Malavasi and Malavasi (2014) and Jacobs and Landis (2009).

Throughout the research the necessary cultural treatments (i.e.weeding) were carried out. Micro-sprinkler irrigation in 5 daily 10-minute irrigation shifts in the summer (07:00am; 10:00am; 13:00pm; 04:00pm and 07:00pm) and in 3 daily 10-minute shifts during the winter (09:00 am; 01:00pm and 05:00pm) resulted in a water table equal to 4 mm.

After the end of the treatments (approximately 5 weeks after initiation of treatment), we quantified flexural stiffness (Lima; Malavasi; Lopes; Dranski; Malavasi; Borsoi, 2020), lignin content in the stems and roots (Van Soest, 1994), phenolic compounds (PC) in the leaves and roots (Georgé; Brat; Alter; Amiot, 2005) and loss of electrolytes in the roots (Wilner, 1995).

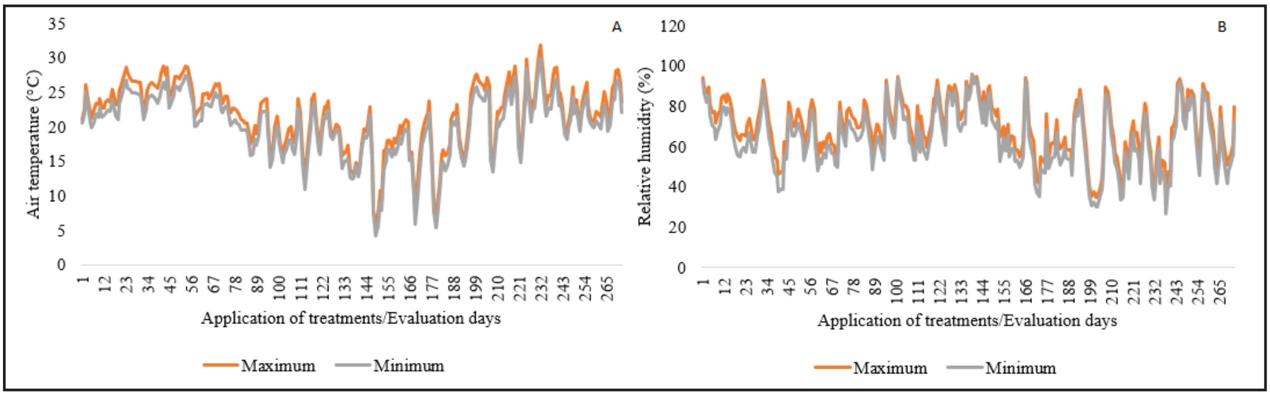
Flexural stiffness of stems was determined in 63 seedlings from each growth stage randomly selected with an equipment adapted according to Lima, Malavasi, Lopes, Dranski, Malavasi and Borsoi (2020). From the measurement of mass and distance, the data were expressed in Newton (N cm^{-1}).

After hardening treatments, part of the randomly selected seedlings was outplanted in an area with coordinates of 24°53’17,98” S and 54°02’56,71” W, 363 m of altitude to quantify survival and growth.

To quantify the morphometric parameters, analyzes were performed every 15 days, up to 90 days. While, survival was evaluated up to 180 days. This difference occurred because in a certain phase, due to biotic and abiotic stresses, some seedlings were lost, such as, they lost their leaves and the evaluation was interrupted.

The experimental design was in a randomized block design, consisting of 3 treatments (control, methyl jasmonate, and stem bending), 3 blocks and 3 replications totaling 27 seedlings per growth stage. Climatic data from the field trial (Figure 2) were obtained from the meteorological station near by accessing the data history on the website of the National Institute of Meteorology (INMET).

Figure 2 – Air temperature and humidity obtained in the field during the experiment with *Eucalyptus urograndis* seedlings in 2021



Source: Authors (2022)

Eucalyptus seedlings were planted according to each hardening treatment which lasted from February 28 to October 31 of 2021. The difference in dates between the production of seedlings and planting in the field occurred because the field experiment was lost three times and had to be repeated, therefore, new seedlings had to be produced, increasing the period between the initial analysis of the seedlings and the expedition the field.

The planting holes were approximately 27 cm in diameter and 60 cm in depth. The planting spacing was 1 m between rows and 1 m between seedlings. The soil in the planting area is classified as typical dystroferric RED LATOSOL with a very clayey texture.

The cultural practices throughout the field phase involved hand weeding according to their growth. We irrigated after planting for the first 20 days every 3 days to make the transition from the nursery phase to the planting site less stressful. The amount of water per seedling was 2 L in the canopy projection area. In addition, we controlled ants using either mechanical or chemical methods.

The results obtained were tested to confirm the existence of statistical assumptions, including normality and homogeneity of data. Upon confirmation, the data was submitted to an analysis of variance. If detected significance between treatments, the results were split and tested using comparison of means by Tukey test at 0.05 probability. Furthermore, the statistical program used was GENES (CRUZ, 2013).

3 RESULTS AND DISCUSSION

The highest values of flexural stiffness were quantified in seedlings with 100 DAE submitted to hardening treatments (Table 1). Because it is a mechanical stimulus, the stem bending changes the cell wall of the stems and even the roots due to cell and anatomical differentiation (Monteiro; Pereira; Abreu, 2012). Substances linked to the structural part of the plants (lignin) can be synthesized, increasing the stiffness of the stem and consequently increasing the force that should be applied (Vanholme; Demedts; Morreel; Ralph; Boerjan, 2010).

Table 1 – Flexural stiffness in stems of *Eucalyptus urograndis* seedlings as a function of hardening methods and growth stages

	Stage I (70DAE)			**Stage II (100DAE)**			**Stage III (130DAE)**		
	1 cm	**2 cm**	**3 cm**	**1 cm**	**2 cm**	**3 cm**	**1 cm**	**2 cm**	**3 cm**
Cont		***		0.1842b	0.2262b	0.2291b	0.3726	0.6199	0.5099
MeJA		0.3432a		0.3691 a	0.3046	0.5357	0.4451		
		0.4462 a							
Flexural stiffness		0.3103a		0.3299 a	0.3937	0.5590	0.5218		
		0.4210 a							
Fcal		***		9.45 **	11.86 **	4.64 *	1.67 ns	0.25 ns	0.78 ns
Dms		0.0986		0.1212	0.1299	0.7478	0.1691		
		0.1261							
CV (%)		25.87		28.7	26.66	40.16	25.17		
		25.37							

Source: Authors (2022)

In where: *** little stiff stems, it was not possible to perform the analysis of flexural stiffness/ ** significant at 1%.

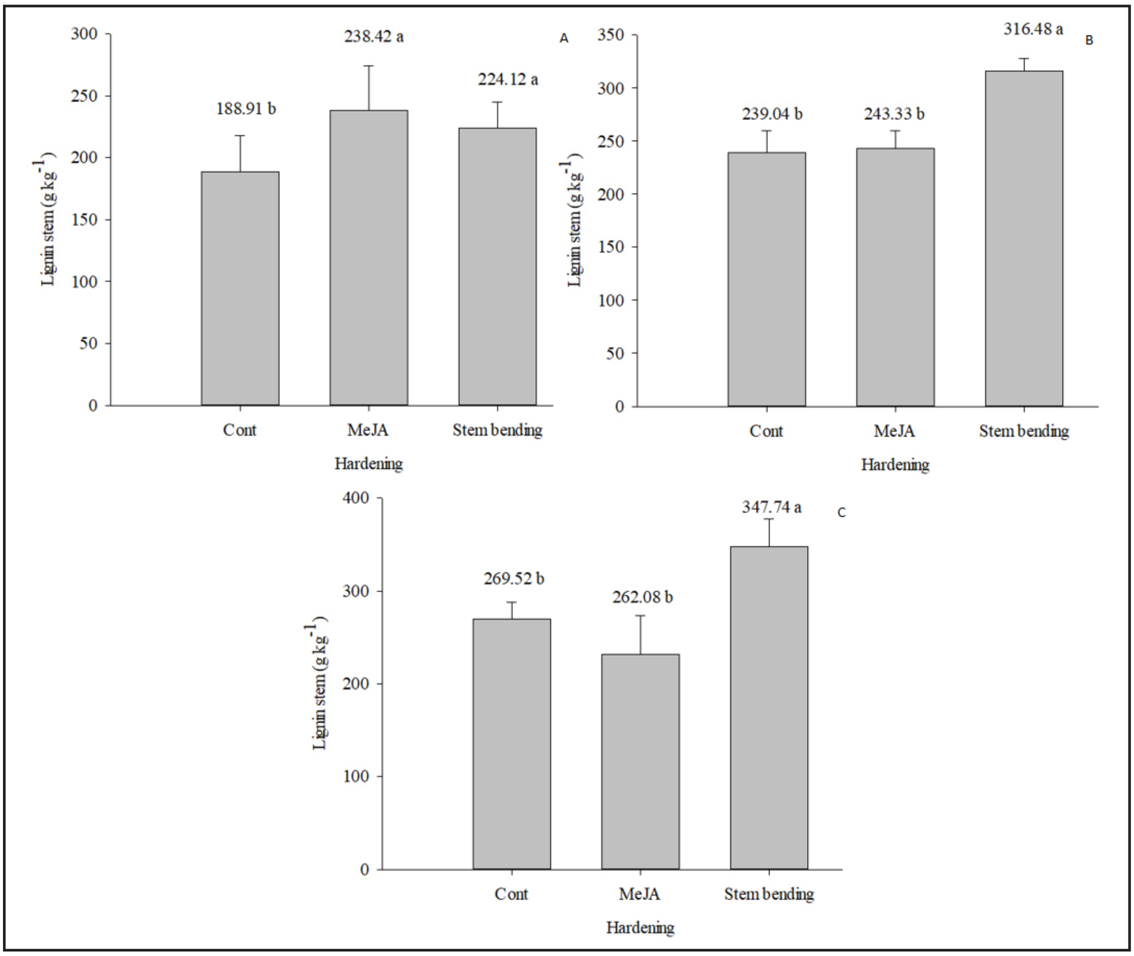
Plant growth regulators, especially from the jasmonate group, may also be responsible for morphophysiological changes, proving their effectiveness against abiotic stresses (Sanchez, 2008; Martin; Leblanc-Fournier; Julien; Moulia; Coutand, 2010). Eucalyptus also has more flexible stems, but no less resistant; as seedlings age the resistance to the stem bending movement also increases. Lima, Malavasi, Lopes, Dranski, Malavasi and Borsoi (2020) observed similar responses where the highest values resulted from treatments with 8.0 μmol L^{-1} of jasmonic acid and in seedlings that received 20 daily stem bending with means between 0.2885 and 0.3005 N cm^{-1} in seedlings of eucalyptus clone 1528.

Growth stage ii seedlings (100 dae) showed a reduction in stem stiffness compared to the 2 cm distance (table 1). According to Rankenberg, Geldhof, Veen, Holsteens, Poel and Sasidharan (2021) younger seedlings show greater plasticity when compared to seedlings with advanced growth stages.

Lignification is a plant response to stress in general. Some plant species can form lignin through the phenylpropanoid pathway. Lignin is a structural component that promotes greater support, elasticity, and rigidity to the stem and roots (Malavasi; Malavasi, 2016).

Quantification of lignin in aboveground tissues in eucalyptus seedlings with 70 DAE showed that the hardening treatments increased the content of the macromolecule in question (Figure 3A). Oliveira, Abreu and Pereira (2009) treated *Eucalyptus urophylla* seedlings with jasmonic acid at concentrations of 1 and 2 μmol L^{-1} which resulted in changes in lignin content. In the same work mentioned above, the non-hardened seedlings presented an average for the lignin content of 26.43%, while the hardened seedlings presented average results of 41.56%. The above authors as well as Sanchez (2008) justified that the effect of activating the pathway of precursors (phenylpropanoid) linked to lignin synthesis by the plant regulator signal the synthesis of general and specific phenolic compounds, as occurs in the lignin molecule.

Figure 3 – Lignin content in *Eucalyptus urograndis* seedling stems as a function of hardening methods and growth stages at 70 (stage I) (A), 100 (stage II) (B) and 130 (stage III) (C) days after emergency



Source: Authors (2022)

In where: The means followed by the same letter do not differ statistically from each other by the Tukey test at 1 and 5% probability.

Seedlings of growth stages II and III showed the highest levels of lignin when treated with stem bending with increases of 32.4 and 29.2 % respectively when compared to control seedlings (Figures 3B and 3C). The lignification process is much more intense in older plants (Rankenberg; Geldhof; Veen; Holsteens; Poel; Sasidharan, 2021) because, in addition to being a defense strategy, it is a natural process of the development of lignified plants. Therefore, it would be predictable that in the more advanced stages, the lignin values would be higher. Still, it is essential to mention that this transition of growth phases in some plants can be more subtle than in others, mainly in species with slow growth which would influence the stress responses. However, Braz, Oliveira, Rosado, Vidaurre, Paes, Tomazello Filho and Loiola (2014) studying *Eucalyptus urophylla* x *E. grandis* over 35 months showed a reduction in insoluble lignin with values close to 31.33% with plant age growing in a region classified as sub-humid hot tropical climate (AW).

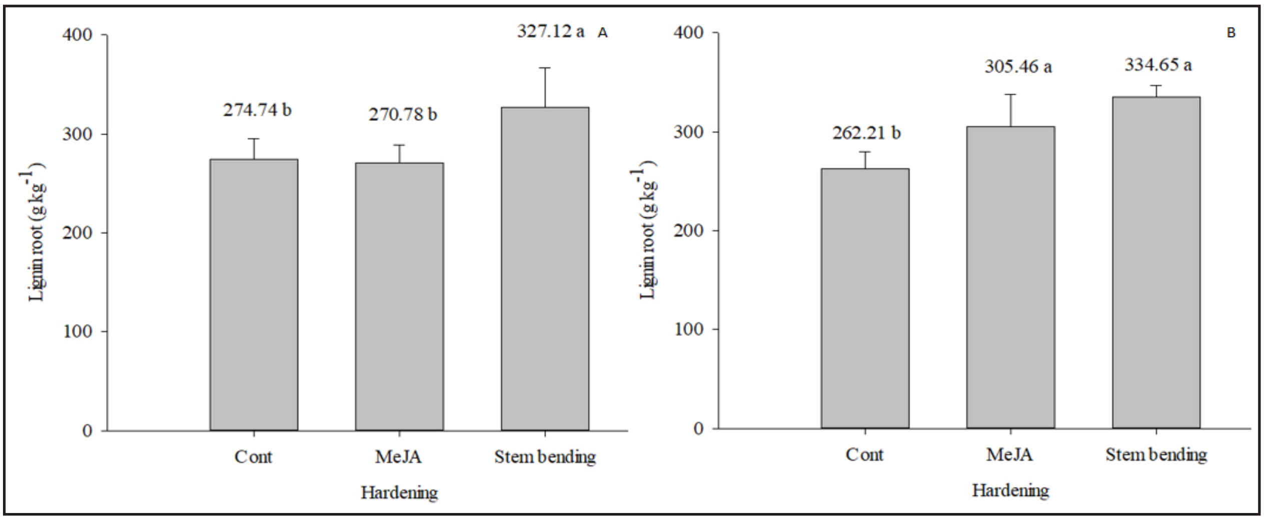
In general younger plants are less lignified. However, when subjected to disturbances regardless of the origin plants start to produce secondary substances to induced defense, such as lignin. Stems and roots will differentiate and distinctly and independently will increase lignin, as those organs may be more drastically affected depending on the type of stress. However, the variation will depend on the types of treatment and the environmental conditions of cultivation (Novaes; Kirst; Chiang; Winter-Sederoff; Sederoff, 2010).

In *Eucalyptus urograndis* seedlings, the levels of lignin in the roots did not show a significant difference in stage I (P>0.05) in relation to the treatments. However, in the other growth phases, II and III, the data differed statistically (P>0.05). Dranski, Malavasi and Malavasi (2015) reported that, after imposing mechanical disturbances on stems of *Pinus taeda* seedlings, there was a 32% increase in the lignin content in the roots.

In these results, it is possible to observe that, in stage II, the highest concentration of lignin in the roots was observed in mechanically rustified seedlings, corroborating the theory that seedlings under stress activate secondary metabolic routes that will act to protect them. Also, lignification is a plant response to stress in general, and some species have the ability to form lignin, from the phenylpropanoid pathway. In addition, lignin is a structural component that promotes greater support, elasticity and rigidity to the stem and roots. The presence of this substance contributed to the evolution of plants, such as the transition of plants from the aquatic to the terrestrial environment (Jung, 2004; Boerjan; Ralph; Baucher, 2003; Monteiro; Pereira; Abreu, 2012).

Evidences that corroborate the results found in the present study are described by Aoyama, Matsumura, Tsutsumi and Nishida (2001) and Seifert and Blaukopf (2010) who argued that lignin can be increased if mechanical stimuli are used in angiosperm seedlings, altering other structures linked to the cell wall, its composition and conformation. Lignin has an already recognized function in the integrity of the cell wall, as well as in the transport of water in the soil-plant-atmosphere continuum.

Figure 4 – Lignin content in roots of *Eucalyptus urograndis* seedlings as a function of hardening methods and growth stages at 100 (stage II) (A) and 130 (stage III) (B) days after emergency



Source: Authors (2022)

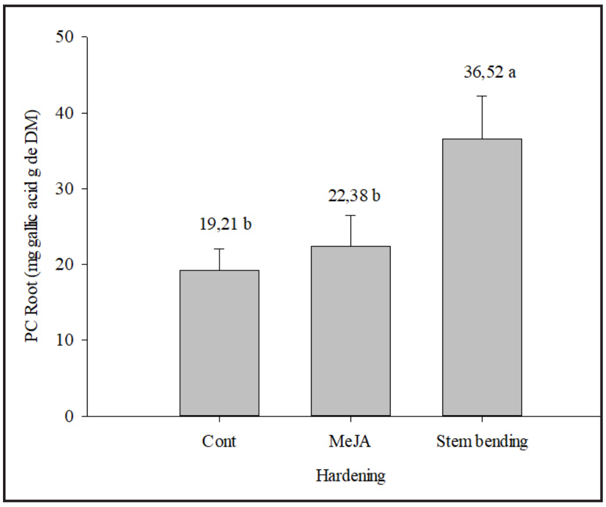
In where: The means followed by the same letter do not differ statistically from each other by the Tukey test at 1 and 5% probability.

Leaf concentration of phenolic compounds (PC) in *Eucalyptus urograndis* seedlings was not altered (P>0.05) as result of hardening treatments and growth stages. The average values of CF in seedlings of growth stage I were 25.76; 21.67; and 22.84 mg of gallic acid per g of dry biomass (DM) in the control, MeJA and stem bending treatments, respectively. In seedlings of growth stage II, the average values were 20.81; 17.85; and 22.21 mg gallic acid per g of DM while from seedlings of growth stage III the averages were 16.19; 15.65; and 13.30 mg gallic acid per g of DM in the same order as described above, respectively.

This result can be influenced by several factors, including climate, therefore, the changes that occurred were not due to differences between treatments, but to external and internal conditions related to young *Eucalyptus urograndis* plants. In addition, the concentrations of these compounds are usually very sensitive to environmental variations and, therefore, their alteration may be a consequence of the imposition of treatments, as well as variations in the environment in which the plants are being grown (Gobbo-Neto; Lopes, 2007; Ramakrishna; Ravishankar, 2011; Ncube; Finnie; Staden, 2012).

PC from the roots from growth stages I and III seedlings showed no difference (P>0.05) between the imposed hardening treatments. CF values were 14.80; 13.34; and 15.11 mg of gallic acid per g of DM in control, chemically and mechanically hardened seedlings, respectively. In hardened and control seedlings from growth stage III CF values were 9.62; 11.47; and 12.21 mg of gallic acid g DM, in the same sequence as above mentioned. Phenolic compounds in the roots of control seedlings from growth stage II was lower than those quantified from seedlings hardened with stem bending demonstrating that at this growth phase the defense system of eucalyptus seedlings was activated (Figure 5).

Figure 5 – Concentration of phenolic compounds in *Eucalyptus urograndis* seedling roots as a function of hardening methods and growth stage at 100 (stage II) days after emergency



Source: Authors (2022)

In where: The means followed by the same letter do not differ statistically from each other by the Tukey test at 1 and 5% probability.

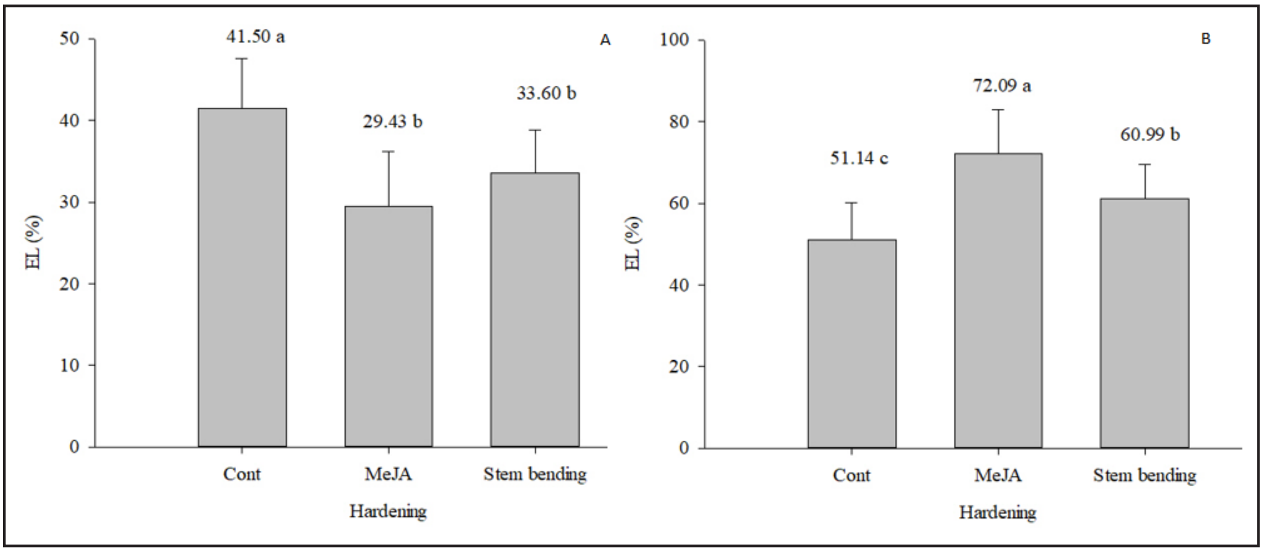
The increase in PC concentrations serve as a warning and signaling in stressful situations. The concentrations of these compounds tend to be very sensitive to environmental variations and, therefore, their variation may be a consequence of hardening, as well as any variations in the environment in which plants are being cultivated (Ramakrishna; Ravishankar, 2011).

Water flows more efficiently through some parts of the plant than others. However, ions need carrier proteins (channels or ion pumps) to be transported from the extracellular to the intracellular medium. Thus, electrolytes tend to remain in cells if plants are not exposed to conditions that are configured as stresses. However, amid adversities, membranes can lose their selectivity, resulting in leakage of cellular content and, consequently, ions. Such damage to the plasma membrane may or may not be reversible; in the first case, there will only be a modification of the membrane, without however, leading to disintegration, while in the second case, depending on the intensity, the membrane can be ruptured and result in cell death. Therefore, by evaluating the loss of electrolytes, it is possible to infer the condition of the plant and how a given treatment will be stimulating it (Santarém, 2017). Thus, the higher the values, the greater the stress caused by the treatments.

In samples from growth stage I the highest value of the electrolyte loss test was quantified in control seedlings. Seedlings treated with both hardening treatments showed a reduction in ion concentrations. That result reinforces that hardened seedlings can present a complex and efficient signaling system that is converted into defensive responses, such as the conservation of membrane integrity and less electrolyte extravasation in the intracellular environment (Figure 6A).

Similar results were reported by Volkweis, Dranski, Oro, Malavasi and Malavasi (2014) who associated the increase in the number of stem bending to the reduction of electrolyte loss in roots of *Maytenus ilicifolia* [(Schrad.) Planch.] seedlings. In *Eucalyptus* seedlings from growth state I hardening with methyl jasmonate was the one that resulted in less ion extravasation and consequently greater membrane integrity (Figure 6A).

Figure 6 – Loss of electrolytes (LE) in *Eucalyptus urograndis* seedlings as a function of hardening methods in seedlings and growth stages at 70 (stage I) (A) and 130 (stage III) (B) days after emergence



Source: Authors (2022)

In where: The means followed by the same letter do not differ statistically from each other by the Tukey test at 1 and 5% probability.

In seedlings from growth stage III the opposite result was observed depending on the hardening treatments (Figure 6B). Although the stressful condition caused by the release of ions is an inconvenience, it can also be a beneficial strategy. The objective of hardening is to use controlled stress to improve seedling tolerance (Telewski; Jaffe, 1986).

The evaluation of *Eucalyptus urograndis* seedlings from stage I in the field up to 30 days did not result in mortality. However, after that period, the seedlings from the control treatment and those submitted to mechanical disturbance had a survival rate of 55.6% in relation to the total number of plants taken to the field per treatment, while with the seedlings that received the chemical treatment it was 33.4%.

From 60 days after planting, there was a total loss of plants belonging to stage I, caused by low air temperature, reaching a minimum temperature of 4.2°C (Appendix B - Figure 3) and ant attack. As for air temperature, between the months of June and July (Figure 2) there were three severe frosts, which were extremely harmful, especially in younger plants, resulting in their death.

Thus, the rains were irregular and poorly distributed over the months which caused the combination of two dangerous stresses, water restriction and extreme temperatures. In addition to these, leaf-cutting ants (*Atta laevigata*) were one of the most common and constant problems with eucalyptus seedlings throughout the experiment.

With the seedlings of stages II and III, the hardening treatments did not influence the post-planting survival, since they survived despite the stresses (the same ones described above) and the percentage of survival in both was equal to 100%.

Similar to the results of the present research, Cadorin, Malavasi, Coutinho, Dranski and Malavasi (2015) studying the effect of chemical and mechanical hardening treatments on seedlings of *Cordia trichotoma* (Vell.) Arrab. ex Steud reported no relationship of those with seedling survival at 90 and 180 days after planting, with a survival percentage of 97 and 95%, respectively. Del Campo, Navarro and Ceacero (2010) observed that quality attributes, mainly morphometric of seedlings were only correlated with survival in *Quecus ilex* subsp. *Ballota* (Desf.) Samp. when evaluated for one year under intense water restriction conditions.

Increments of morphometrics variables after 90 days of outplanting did not result in differences (P>0.05) as a function of hardening treatments. Height increments were 63.04, 56.88 and 54.66 cm in seedlings of growth stage II (100 DAE) from control, MeJA and stem bending treatments while diameter increments were 9.55, 8.09, and 8.13 mm, respectively. With seedlings from growth stage III (130 DAE) height increments were 38.55, 35.41, and 34.67 cm with diameter increments of 6.17, 6.52, and 5.92 mm from control, MeJA and stem bending treatments, respectively. Increments of height and diameter from seedling with 130 DAE were smaller than seedlings with 100 DAE because growth was affected by seasonal stresses.

4 CONCLUSIONS

Eucalyptus urograndis seedlings hardened with stem bending increased flexural stiffness when compared to control seedlings. Hardening with 100 μmol L^{-1} methyl jasmonate and stem bending showed similar results in stem and root lignin content and loss of root cell electrolytes. Therefore, both hardening methods could be chosen by nursery managers depending on the availability of products and equipment. Outplanting *Eucaliptus urograndis* seedlings with 70 DAE should be avoided as they are more susceptible to biotic and abiotic stresses. Seedlings with 100 DAE and 130 DAE showed 100% post-planting survival under the conditions of the research.

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