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ORIGINAL ARTICLE – Silviculture

Nursery and Field Development of *Cordia Trichotoma* Seedlings Subjected to Hardening Practices

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Abstract

Lignin is related to mechanical support, fluidity of water transport by xylem and resistance to pest and pathogen infections. This research measured the concentration of lignin and its correlation with field performance of *Cordia trichotoma* (Vell.) Arrab. ex Steud seedlings submitted to hardening. Seedlings were treated with 20 stem bending for 4 or 8 weeks, application of 50 µmol L-1 of methyl jasmonate for 4 or 8 weeks and a control treatment. In the nursery after treatments, six seedlings were randomly selected for determination of root electrolytes and lignin content from stem and root tissues. Application of jasmonates or stem bending in the nursery promoted an increase in stem lignin concentration but did not induce a greater lignification of root systems. After 450 days of planting, saplings height and diameter increments and percentage survival were calculated along shoot lignin content. Saplings showed no differences in shoot lignin from nursery hardening.

Keywords: Forest species, Jasmonates, Nursery, Tigmomorphogenesis.

1. INTRODUCTION

The limited supply of high-quality seedlings for a wide range of terrestrial wood species is a major reason for the limited success of reforestation project in many countries although seedling production for planting is one of the best options (Martínez-Garza & Howe, 2003). Therefore, a need for further research exists on efficient techniques for production of high-quality seedlings (Bellotto et al., 2009).

Nurseries may apply various hardening techniques such frequency of irrigation and fertilization, exposure to different light levels and root pruning. The purpose of hardening is to "prepare or adapt" seedlings to withstand abiotic stresses (Lima et al., 2014; Oliveira, 2016).

Lignin is not yet accepted as an attribute of seedling quality. However, lignin is known for important roles in terrestrial wood plants (Malavasi et al., 2016). After cellulose, lignin is the most abundant organic molecule in terrestrial wood plants with functional roles such as to provide the rigidity necessary to maintain the verticality of the stem and to ensure the existence of fast water circulation pathways through the xylem. In addition to mechanical support, lignin is related to plant defense against herbivores (Monteiro et al., 2004; Meents et al., 2018).

Lignin content from seedlings of wood species is susceptible to modulation through mechanical stimulus as reported by Dranski et al. (2015) with *Pinus taeda* L. seedlings submitted to stem bending. However, mechanical stimulus is difficult to operate in large-scale nurseries. Therefore, the use of other methods is of interest. Oliveira et al. (2009) reported that jasmonic acid resulted in an increased of lignin in *Eucalyptus urophylla* S. T. Blake.

Jasmonic acid and its methyl ester (methyl jasmonate) are part of a class of hormones known as jasmonates. Jasmonates are synthesized from linolenic acid and its biosynthesis depends on the sequential action of several enzymes (Behr et al., 2019).

In terrestrial plants, this group of hormones exerts effects like those of ethylene and abscisic acid by inducing the expression of genes involved in resistance to pathogens and insects, stimulating the formation of tubers and causing leaf senescence and abscission with the degradation of chlorophyll (Per et al., 2018).

Cordia trichotoma (Vell.) Arrab. ex Steud, popularly known as louro-pardo or ipê-branco is a native Brazilian wood species occurring from Ceará to Rio Grande do Sul states, predominantly present in the Deciduous and Semi-deciduous Seasonal Forests. The species is classified as pioneer with potential use for environmental and productive purposes (Coradin et al., 2011) or a secondary initial (Carvalho, 2003).

Our research aimed to quantify stem and root lignin content of *C. trichotoma* seedlings subjected to hardening treatments with jasmonate and stem bending and to correlate them with seedling /sapling survival and growth after planting.

2. MATERIALS AND METHODS

Seedlings were propagated using 120 cm^3 plugs filled with commercial substrate (Plantmax®) and sterilized sand in a ratio of 4:1 (v.v.) with addition of 1.44 kg m^{-3} of controlled-release fertilizer (Basacote® 18N-5P2O5-9K2O) under 50% shading provided by a shade house located at 24° 55' 83" S e 54º 04' 56"O from September to November 2014. The mean ambient temperature at the time of application of the treatments was 26.3 ° C and the relative humidity was 51%.

When seedlings reached a mean height of 15.0 cm and a mean stem diameter of 3.22 mm we imposed treatments: (CONTROL) deionized water plus nonionic surfactant applied weekly; (TIG4) 20 daily stem bending for 4 weeks plus deionized water and nonionic surfactant applied weekly; (TIG8) 20 daily stem bending for 8 weeks plus deionized water and non-ionic surfactant applied weekly; (MJ4) 50 μmol L^{-1} of methyl jasmonate plus deionized water and non-ionic surfactant applied to seedling leaves weekly for 4 weeks; (MJ8) 50 μmol L^{-1} of methyl jasmonate plus deionized water and non-ionic surfactant applied to seedling leaves weekly for 8 weeks.

Application of stem bending and methyl jasmonate for 4 weeks occurred in the last weeks in relation to treatments that lasted for 8 weeks. During the first 4 weeks, the untreated seedlings remained under daily sprinkler irrigation of 10 mm.

Stem bending followed Volkweis et al. (2014) while application of the growth regulator was performed once a week by foliar sprays of methyl jasmonate at a concentration of 50 μmol L^{-1}, with a volume equivalent to 7 mL per seedling applied with a manual sprayer.

After treatments, six seedlings were randomly selected for determination of root tissue electrolytes according to Wilner (1960) and quantification of lignin content from stem and root tissues according to Van Soest (1994) and calculated with Equation 1:

$$\text{Lignin (\%)} = \frac{[(Res_{As} - Res_{c}) * (100/2 * MA)]}{MA} \quad (1)$$

where RESAS is dry biomass resulting from sulfuric acid digestion, RESc is dry biomass resulting from carbonization and MA is initial dry biomass. At the same time, four seedlings were randomly selected for the determination of root growth potential according to Ritchie et al. (2010).

Field trials comprised the random selection of four seedlings which were outplanted in a 3 x 2 m spacing with 100 g per planting role of 5N-25P2O5-25K2O following a randomized block design in an area located at 26º 13 '46 "S, 52º 40'14" W and altitude of 760 m in November of 2014. The climate at the planting site is classified as Cfa - humid subtropical mesothermic climate (IAPAR, 2000) and soil as a dystrophic Red Latosol (EMBRAPA, 2006). Cultivation involved weeding as needed.

Soil at planting site had the following attributes in the 0-20 cm layer: MO: 26.8 g dm^{-3}, P(Mehlich): 1.97 mg dm^{-3}, K (Mehlich): 0.13 cmoL dm^{-3}, pH CaCl2: 5.3 H^+Al: 2.26 cmoL dm^{-3}, Ca: 5.0 cmoL dm^{-3}, Mg: 2.30 cmoL dm^{-3}, SB: 7.43 cmoL dm^{-3} and V: 68.48%.

After 450 days of planting, saplings had an average height of 141 cm and were harvested at 30 cm from the soil surface. Approximately 10 cm of the stem above the harvest cut was used for lignin quantification. At that time, the increments in height and stem diameter at the soil level and the percentage of seedling/sapling survival were calculated.

Data from nursery and field trial were examined regarding normality of residue distribution and homogeneity of the variances by the Shapiro-Wilk and Bartlett tests, respectively and submitted to analysis of variance. Treatment means were compared by Tukey at 5% of error probability.

Correlation between variables from the nursery and those after 450 days of planting yielded multicollinearity based on the condition number (NC) and the variance inflation factor (VIF). Later, a path analysis was performed, in which survival rate, height increase and diameter at the soil level were considered as predictor variables. For the statistical analysis, we used GENES program (Cruz, 2001).

3. RESULTS AND DISCUSSION

Seedings of *C.trichotoma* submitted to stem bending and application of methyl jasmonate for eight weeks had a higher stem lignin content compared to control seedlings (Table 1).

Table 1. Lignin content in stems of *C.trichotoma* seedlings as a function of hardening treatments.

Hardening treatments	Lignin (%)
CONTROL	10.28 b
TIG4	11.77 ab
TIG8	12.43 a
MJ4	11.45 b
MJ8	12.45 a
CV(%)	7.55

Means followed by the same lowercase letter in the column do not differ statistically from each other by the Tukey test at 5% error probability.

TIG4 = Stem bending for four weeks; TIG 8 = Stem bending for eight weeks; MJA = methyl jasmonate applications for four weeks; MJ8 = methyl jasmonate applications for eight weeks.

No differences (P>0.05) were detected in root lignin from treated seedlings (μ = 10.7%) compared to control seedlings (μ = 12%). The results differ from those from Dranski et al. (2015) who reported a 32% increase in lignin content of roots from treated seedlings compared to control seedlings of *Pinus taeda* L.

On the other hand, the results of the present research are like those from Heberle et al. (2018) who found an increase in the lignin content of the stems tissues but did not observe a difference in the root tissues of *Cordia americana* (L.) Gottshling & J.E.Mill seedlings with the application of jasmonic acid. The same authors argued that concentrations of jasmonic acid used (0.5, 1 and 1.5 μmol L^{-1}) may have been too low to stimulate root lignification.

C.trichotoma seedlings did not show differences (P>0.05) in stem lignin content as a function of treatments after 450 days of outplanting with a mean value of 12.7%. Those results indicated that both chemical or mechanical hardening resulted in phenotypic modifications at nursery but did not last long. After 450 days of outplanting, *C. trichotoma* seedlings showed a height increment of 120.7 cm and stem diameter increment of 17.37 mm at ground level.

The direct and indirect effects of root lignin concentrations, electrolyte leakage and root growth potential on the out planted seedlings (Table 2) revealed that the condition number which consists of the ratio between the highest and the lowest eigenvalue of the correlation matrix (Montgomery & Peck 1981) was below 100 and inflation factor of variance below 10, indicating collinearity and weak multicollinearity according to Cruz (2006). Therefore, the variables under study are not highly correlated.

Table 2. Correlation coefficient and path analysis coefficients of *C.trichotoma* seedlings.

Variables	Ways of association	Track coefficient					
		Direct effect			Indirect effect		
		SOB	IH	ID	SOB	IH	ID
SL	Direct effect	2.11	2.08	1.08			
	Indirect via RL				0.02	0.05	0.08
	Indirect via PER				-1.10	-1.21	-0.43
	Indirect via PRR				-0.73	-0.40	-0.01
	Total correlation coefficient				0.30	0.53	0.72
RL	Direct effect	0.14	0.45	0.64			
	Indirect via SL				0.25	0.25	0.13
	Indirect via PER				-0.03	-0.03	-0.01
	Indirect via PRR				0.02	0.01	0.01
	Total (correlation coefficient)				0.38	0.68	0.76
PER	Direct effect	1.18	1.30	0.46			
	Indirect via SL				-1.96	-1.93	-1.00
	Indirect via RL				-0.01	-0.01	-0.01
	Indirect via PRR				0.60	0.32	0.01
	Total (correlation coefficient)				-0.18	-0.31	-0.55

Table 2. Continued...

Variables	Ways of association	Track coefficient					
		Direct effect			Indirect effect		
		SOB	IH	ID	SOB	IH	ID
PRR	Direct effect	-1.16	-0.63	-0.01			
	Indirect via SL				1.32	1.30	0.67
	Indirect via RL				-0.01	-0.01	-0.01
	Indirect via PER				-0.61	-0.68	-0.24
	Total (correlation coefficient)				-0.46	-0.01	0.41
	Determination coefficient		0.99	0.99	0.99		
	Condition number (NC)		4.1	69.6	2.80		
	Inflation factor of variance (FIV)		<1.0	2.0	<1.0		

In which: (SL) stem tissue lignin; (RL) root tissue lignin; (PER) loss of root electrolytes; (PRR) potential of root growth; (SOB) survival; (IH) height increment; (ID) stem diameter increment.

Similarly with our data, daily stem bending for sixty days of *P.taeda* seedlings increased shoot lignin by 16% (Dranski et al., 2015). The increase in lignin concentration of physically stimulated seedlings may be related to the action of enzymes involved in its biosynthesis. Lignin is a phenolic compound derived from the shikimic acid route and formed basically by the polymerization of three different monolignols: coniferyl, coumaryl and sinapyl (Taiz et al., 2017). Lignification encompasses the biosynthesis of those monolignols in the cytoplasmic compartments and subsequent polymerization in the cell wall. Lignification depends on several enzymes both in the formation of their precursors and in their polymerization (Monteiro et al., 2004). The activity of phenylalani e ammonia-lyase can be influenced by several factors such as levels of hormones, luminosity, and injury and infection by pathogens.

Ramos et al. (2012) inclined seedlings of *Pinus radiata* D. Don and *Pinus pinaster* Ait. at 45° and detected increased transcription activity of genes for phenylalanine ammonia-lyase biosynthesis by about two-fold compared to non-tilted seedlings. *Bryonia dioica* Jacq. showed an increase in the activities of phenylalanine ammonia-lyase and peroxidase and in the lignin content in response to friction in the internodes (Jaegher et al., 1985). Lignin concentration of aerial tissues in mechanically stimulated. *C.trichotoma* seedlings corroborate results reported in the literature.

Heberle et al. (2018) tested 1 and 1,5 μmol L^{-1} concentrations of jasmonic acid in *Handroanthus impetiginosus* (Mart ex. DC.) Mattos seedlings and obtained a 23.65% increase in the lignin content of aerial tissues compared to control seedlings. Oliveira et al. (2009) carried out seven applications of 1 μmol L^{-1} of jasmonic acid in *Eucalyptus urophylla* S. T. Blake seedlings at 10-day intervals and obtained an increase in lignin content of 41.56%. The above authors credited the increase in lignin content to a possible activation of the phenylpropanoid pathway.

Jasmonates stimulate the production of plant defense compounds and activate expression genes for enzymes such as peroxidases, polyphenoloxidases, and phenylalanine ammonia lyase. The exogenous application of those regulators may stimulate changes in plant metabolism (Sánches, 2008). Andrade et al. (2013) sprayed 0.1 mmol L^{-1} of jasmonic acid on leaves of *Solanum lycopersicum* L. and observed increased peroxidase activity compared to control treatment plants sprayed with distilled water.

Thus, our results indicated benefits of a growth regulator for seedling hardening as a promoter of lignification of aerial tissues in *C.trichotoma* as result of its been easily applied in nurseries in relation to mechanical stimulus.

During seedling field trial there was no extreme values for air temperature and precipitation at the planting site (Figure 1). Del Campo et al. (2009) reported that seedling quality attributes only correlated with survival in *Quecus ilex* subsp. Ballota (Desf.) Samp. when planted in a year with severe water restriction. Under favorable conditions, the above authors did not detect a correlation between survival and seedling quality attributes, indicating that the expression of seedling quality may be conditioned to adverse environmental situations.

Although stem lignin content showed a weak total correlation with seedling survival among all the correlated characteristics in Table 2 it had the greatest positive direct effect (> 1). The same was detected with height and stem diameter at the soil level 450 days after outplanting (Table 2). Dranski et al. (2015) found a strong positive correlation between the direct effect of lignin content of stem tissues from *P. taeda* seedlings mechanically stimulated with stem volume (R = 0.88) and with seedling height (R = 0.93) 90 days after planting.

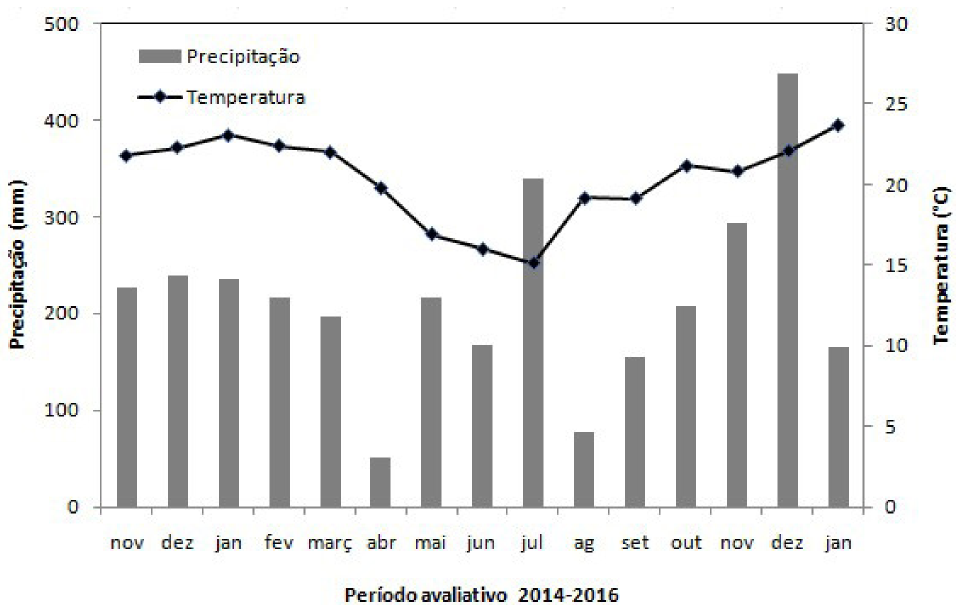

Figure 1. Monthly accumulated precipitation and average air temperature at Pato Branco/PR 2014-2016. Source: Instituto Tecnológico Simepar.

In tropical and subtropical climate conditions, where water availability is seasonal and often restricted, tolerance to xylem embolism is extremely important. Embolism occurs in severe conditions of water restriction, which consequently culminates in increased tension in the xylem and the formation of air bubbles. This fact negatively interferes with the transport of water, decreasing the rates of growth and survival of plants (Matinez-Vilalta & Piñol, 2003; Lens et al., 2013).

Lignin is a key constituent of the tissue in water transport and appears to be related to resistance to water stress in forest plants (Malavasi et al., 2016). Its deposition on cell wall confers greater fluidity in the conduction of water and salts, mainly by reducing the permeability and porosity of the cells and increasing their stiffness. Thus, it provides greater resistance of the plants to the embolism, which is the cause of mortality of seedlings under conditions of severe water restriction (Voelker et al., 2011; Herbette et al., 2015).

In addition to the resistance to embolism, juvenile lignification of the tissues confers resistance to pathogen infection and pest attack (Peltier et al., 2009; Taiz et al., 2017) which may be related to higher survival after planting.

The highest trace coefficients (with height and diameter increase) found for indirect effects on seedling survival (Table 2) were loss of electrolytes from root tissues via stem lignin content. The loss of root electrolytes, although having direct positive effects, had an indirect negative influence. The electrolyte test from root tissues has been cited as a tool to evaluate seedling quality (Ritchie et al., 2010). In the present research, chemically or physically hardened *C.trichotoma* seedlings presented lower extravasation of root tissue electrolytes compared to control seedlings.

We suggest that further research on the topic be conducted under harsher environment conditions.

4. CONCLUSIONS

1. Stem bending for eight weeks at the nursery promoted an increase of stem lignin content in *Cordia trichotoma* seedlings.
2. Application of stem bending and methyl jasmonate for 4 or 8 weeks did not induce greater lignification of root systems on seedlings.
3. After 450 days of planting, *C.trichotoma* seedlings showed no differences in the content of stem lignin induced by nursery hardening treatments under the tested field conditions.
4. Path analysis indicated that lignin content of the stems had a direct positive effect on increases in height, stem diameter and survival of *C.trichotoma* seedlings.

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João Alexandre Lopes Dranski: Formal analysis (Supporting), Investigation (Supporting), Methodology (Equal), Writing – review & editing (Equal).
Pablo Wenderson Ribeiro Coutinho: Data curation (Equal), Investigation (Equal), Writing – review & editing (Equal).
Marlene de Matos Malavasi: Conceptualization (Equal), Supervision (Equal), Writing – review & editing (Equal).

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