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QUANTIFYING STEM WATER CONTENT IN HARDENED SEEDLINGS OF WOOD SPECIES

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ABSTRACT - The work aimed to compare destructive and non-destructive methodologies in quantifying stem water content in *Nectandra grandiflora* and *Inga sessilis* seedlings hardened with 50 μmol L^{-1} of methyl jasmonate or 20 daily stem bending for eight weeks plus a control treatment. Subsequently to the hardening treatments, seedlings were subjected to water restriction for up to 30 days. Stem water content was evaluated biweekly at zero (T0), 15 (T15) and 30 (T30) days under water restriction using the destructive and non-destructive methods. For *I. sessilis* at T0, both evaluations indicated lower stem water content for the stem bending seedlings, however with the non-destructive method, seedling from the two hardening treatments did not differ from the control. In T15, the treatments were similar according to non-destructive method, while with the destructive method methyl jasmonate ensured greater stem water content. At T30, both quantification methods indicated control seedlings with lower stem water content with both hardening treatments yielding similar results. For *N. grandiflora*, the destructive method did not differ between hardening treatments at T0, while the control seedlings presented lower stem water content with similar results between hardening treatments according to the non-destructive method. At T15, hardening treatments did not differ according to both quantification of water stem methodologies. At thirty days under water restriction, both hardening treatments were efficient in maintaining greater stem water content regardless of the quantification method. Replacing the destructive method with the non-destructive proved to be unfeasible with hardening seedlings of the two species.
Keywords: *Nectandra grandiflora*, *Inga sessilis*, hardening, stem water content.

QUANTIFICAÇÃO DO TEOR DE ÁGUA EM MUDAS DE ESPÉCIES LENHOSAS RUSTIFICADAS

RESUMO - O trabalho objetivou comparar metodologia destrutiva e não destrutiva na quantificação de água do caule em mudas de *Nectandra grandiflora* e *Inga sessilis* rustificadas com metil jasmonato e flexões caulinares, através da pulverização semanal de 50 μmol L^{-1} de metil jasmonato, 20 flexões diárias e um tratamento controle, durante oito semanas. Posteriormente, foram submetidas à restrição hídrica e o teor de umidade foi avaliado quinzenalmente (T0; T15; T30), pelo método destrutivo e não-destrutivo. Para *I. sessilis*, em T0, ambas avaliações apontaram menor teor de umidade para as mudas flexionadas, porém para o método não-destrutivo, não diferiram do controle. No T15, os tratamentos se demonstraram semelhantes conforme não-destrutivo, e de acordo com o destrutivo, metil jasmonato assegurou maior umidade. No T30, ambos métodos indicaram o controle com menor umidade, e as flexionadas não diferiram do metil jasmonato. Para *N. grandiflora*, o método destrutivo não diferiu entre os tratamentos ao T0, enquanto o tratamento controle apresentou menor umidade e as flexionadas não diferiram dos demais, conforme o não-destrutivo. No T15, os tratamentos não diferiram de acordo com ambas metodologias. Aos trinta dias, ambas rustificações foram eficientes em manter maior umidade no caule, independentemente do método alternativo. A substituição do método destrutivo pelo não destrutivo, para ambas espécies, demonstrou-se inviável.
Palavras-chave: *Nectandra grandiflora*, *Inga sessilis*, rustificação, teor de umidade no caule.

INTRODUCTION

Accurate measurements of plant water status are essential for a better understanding of the productivity and management practices of wood species under present, future and diverse environmental conditions. Variations in the water content in the stem are usually related to seasonal precipitation. Stem diameter size fluctuations are particularly associated with variation in bark water content and sapwood swelling and contraction (MALAVASI et al., 2016a).

Changes in the water content of extensible stem tissues are readily reversible, causing diurnal variation attributed to the change in water potential in the xylem. Water can be drawn from the inner woody tissues of the xylem, but cavitation occurs, rather than shrinkage, because this tissue is less elastic. The water stored in the elastic tissues of the stem attenuates the lag between the roots and the shoots, preventing embolisms and ensuring optimal transpiration rates (MALAVASI et al., 2016a).

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Determining the moisture content in wood in a fast, accurate and non-destructive way is a challenge for the forestry sector due to the great influence on industrial processes, especially in moisture contents above the saturation point of the fibers. Therefore, there is a great need to develop technologies aimed at the fast and accurate determination of the water content (moisture) in the stem of seedlings.

As an alternative for the characterization of this material (seedling stem), non-destructive techniques have been gaining ground due to the possibility of reusing the evaluated sample, as well as the speed of application of the method. There are several methods to check the degree of moisture of wood tissues, some are exact, but not immediate; others offer a quick response, but their accuracy is disputed by the literature (NASCIMENTO et al., 2020).

In this sense, alternative methods, also called non-destructive are of fundamental importance in the context of wood science and technology. Studies involving conventional and non-destructive tests and the comparison between the two methods are essential to disseminate them with the ratification of veracity for different species. As an example of non-destructive evaluation, electric meters with resistive and capacitive principles provide immediate answers, but still have questioned accuracy. Thus, in choosing the most appropriate method for a given species, it is necessary to understand how the water is found in the wood and, mainly, to confront different techniques for determining moisture (CRISÓSTOMO et al., 2016).

In addition, water restriction is an important limiting factor to plant production. Among the essential factors, water can be considered one of the most restrictive, and estimates indicate that, with the increase in global warming, the periods of drought in several regions of the planet will gradually intensify.

In addition, forest nurseries are places that are characterized by a high demand for water, which is attributed to the need for temperature regulation by plants (TURCHETTO et al., 2015). Therefore, the development of plants that are drought tolerant is essential for survival, in order to ensure productivity, without burdening the activity by eventual replanting.

Drought susceptibility or tolerance is characterized by several attributes (anatomical, morphological, physiological, and biochemical) expressed simultaneously through various ways, and are related to the severity and rate of imposition of water deficit, phenological stage, nutritional conditions, and evaporative demand of the atmosphere (MATOS et al., 2014). In this context, tolerance to water scarcity has been the scope of studies that indicate the possibility of induction, through practices capable of modulating morphophysiological characteristics, making plants able to establish themselves in the adverse conditions to which they will be inserted. The application of mechanical stimuli and plant regulators are some techniques that can be adopted in the nursery routine to promote hardening (DRANSKI et al., 2015).

According to Jaffe (1973), the alteration in growth patterns as a response to mechanical stimuli is described as tigmomorphogenesis. The mechanical movement resulting from the action of winds, the passage of animals, overlapping branches, the impact of raindrops, among others, induce variable responses between species. However, in wood plants, it is commonly observed a reduction in cell elongation resulting in lower height, and an increase in radial growth, promoting expansion of the stem diameter, attributes related to seedling quality indicators (VOLKWEIS et al., 2014).

The effect of plant regulators such as jasmonic acid and its derivatives can induce seedling hardening. These are endogenous regulators derived from linoleic acid, related to plant senescence, leaf abscission, embryo development and defense mechanism, which act as stress signals, by inducing the expression of genes involved in the resistance of pathogens and insects (COLLI, 2012).

Among the native species of the Atlantic Forest Biome with ecological potential used in plantations for environmental recovery or enrichment of areas, *Nectandra grandiflora* and *Inga sessilis* stand out (CARVALHO, 2010). *Nectandra grandiflora* Nees et Mart., commonly known as canela-amarela, occurs in Semideciduous Forest and Dense Ombrophilous Forest, at altitudes between 600 and 1,300 m. As for its economic importance, its wood can be used in civil construction, but its great potential lies in environmental purposes and in non-timber products, such as phyto-pharmaceuticals (CARVALHO, 2010).

Inga sessilis (Vell.) Mart., popularly known as ingá (ingá-do-brejo, ingá-banana, among others) belongs to the Fabaceae family, with a center of origin in Central America, exclusively considered neotropical, that is, it also occurs in the southern part of North America, Central America and South America. In Brazil it is widely distributed, from the Amazon to the Northeast, also extending to the South region. This species can be used in agroforestry systems, recovery of degraded areas and afforestation, has economic potential, being used in the timber industry, for energy, medicinal, food and canopy purposes, promoting shading for crops such as cocoa and coffee (LORENZI, 2009).

Based on the above, the research aimed to compare a destructive and a non-destructive methodology for the quantification of stem water content in seedlings of *Inga sessilis* and *Nectandra grandiflora* hardened with methyl jasmonate and stem bending subjected to water restriction.

MATERIAL AND METHODS

The experiment was conducted from May to September 2018, in a shade house, covered with 150 µ thick low-density polyethylene and anti-UV film resulting in 20% shading. The protected environment belongs to the State University of Western Paraná (Unioeste), in Marechal Cândido Rondon (PR). It is located in an area with an average altitude of 420 m, latitude of 24º 33' 40" S and longitude of 54º 04' 12" W.

The climate is characterized, according to Köppen (IAPAR, 2018) as *Cfa*, humid mesothermic subtropical with hot summers, winter with infrequent frosts and annual rainfall between 1,600 and 1,800 mm.

Seedlings of *Inga sessilis* and *Nectandra grandiflora* were acquired at 80 days after sowing from a commercial nursery located in Foz do Iguaçu (PR) under coordinates of 25º 32' 49" S and 54º 35' 18" W and altitude 174 m. The seedlings were produced in polypropylene tube containers with 120 cm^3 capacity, filled with commercial Humusfértil® substrate and incorporation of slow-release fertilizer ($15N\text{-}8P_2O_5\text{-}12K_2O$), in the proportion of 1.44 kg m^{-3} of substrate. Seedlings had a mean height and diameter of 16.07 cm/ 3.04 mm and 19.03 cm/ 3.61 mm, respectively. Seedlings were acclimatized at the experimental site for 30 days, remaining in prolipopylene trays, with a capacity of 96 tubes, under daily micro-sprinkler irrigation and receiving weekly fertilization of 2.0 mL of nutrient solution (Table 1).

TABLE 1 - Composition of the nutrient solution for fertilization of *Inga sessilis* and *Nectandra grandiflora* seedlings.

Nutrient solution	Quantity (mL L^{-1})
KH_2PO_4	1.0
$MgSO_4$	2.0
KNO_3	5.0
$Ca(NO_3)_2\ 4H_2O$	5.0
Complete Micro	1.0
Fe-EDTA	1.0

Subsequently, the imposition of hardening treatments for eight weeks began (CADORIN et al., 2015) with deionized water + nonionic surfactant applied weekly (control treatment), spraying with 50 μmol L^{-1} methyl jasmonate + deionized water + nonionic surfactant, applied weekly and twenty stem bending daily (tigmomorphogenesis). The non-ionic surfactant used was Agral (Syngenta®), following the manufacturer's dose recommendations and applications performed with a manual knapsack sprayer, up to the point of leaf runoff, with an approximate volume of 7.0 mL per seedling.

The mechanical stimuli were carried out following the methodology of Volkweis et al. (2014), with the help of a structure built from adaptations of a model proposed by Jacobs and Landis (2009), which is based on a metal apparatus on bearings and a PVC pipe, which collides with the stem of the seedlings, in the lower third of the foliage. The stem bending consisted of twenty round-trip movements, always at the same time in the afternoon, at a speed of 0.10 m sec^{-1}.

Then, for the imposition of water restriction, seedlings were transplanted to polypropylene pots with a capacity of 5 L, filled with dry sand, sieved in a 2 mm mesh which remaining under complete water restriction, in a shade house for a period of 30 days.

The destructive method, known as gravimetric or conventional, is the difference in masses before and after drying in an oven, according to the standard established by ABNT NBR 14929 (ABNT, 2017), while the non-destructive method used a digital wood moisture measuring device (Homis® model 118). The non-destructive method, known as resistive or alternative consists of fixing two metal pins, at a depth of 1/3 of the thickness of the material, taking care not to perforate completely and go through the stem and exposed. The results of this evaluation consisted of averages of measurements carried out in the basal, median and apical parts of the seedling stem.

The experimental design for each species was an entirely randomized, comprising three treatments (jasmonate, stem bending and control), seven replications and three experimental units (seedlings) for each replication. The data were analyzed for the normality of the residuals and homogeneity of the variances by the Shapiro-Wilk and Bartlet tests respectively, and then submitted to analysis of variance. When the means of the treatments were significantly different ($p<0.05$), Tukey's test was performed at 5% probability of error. Additionally, Pearson's correlation was calculated with the aid of Sisvar 5.3 software (FERREIRA, 2011).

RESULTS AND DISCUSSION

The variations in temperature and relative humidity of the air inside the shade house during the experiment are shown in Figure 1. The acclimatization of the seedlings took place during the months of May and June. The treatments were applied in June and July and the imposition of water deficit, as well as the evaluations, were carried out in August and September.

Nectandra grandiflora Nees and Mart.

According to the destructive method used at T0 (before water restriction) there was no difference between the hardening treatments ($p>0.05$), with an average stem moisture of 63.2%. For the resistive apparatus (non-destructive method), the treatments diverge from each other ($p<0.05$), manifesting higher stem water content in seedlings sprayed with methyl jasmonate compared to the control (Table 2); however, both treatments did not differ with stem bending seedlings.

The analysis of variance showed that, in the evaluation carried out on the 15th day of water restriction, the hardening treatments did not differ from each other ($p>0.05$) under both quantification methodologies with mean values of stem moisture of 33.3% and 27.2%, for the destructive and the non-destructive method, respectively.

The differences between the hardening treatments ($p<0.05$), according to the gravimetric method, suggested that the highest moisture content on the 30th day under water restriction was detected in mechanically stimulated seedlings (stem bending) which confers less resistance to the passage of water through vessels (GANN, 2019).

The hardening treatments did not differ from each other (p>0.05) with the use of a non-destructive method suggesting that it was inefficient to quantify stem water content. Even though the method of determining humidity by electric resistive instruments presented immediate results, enabling the subsequent use of the material, the negative correlation between the two methodologies (Table 3) refutes the accuracy of the method, making it impossible to replace the gravimetric (traditional) method.

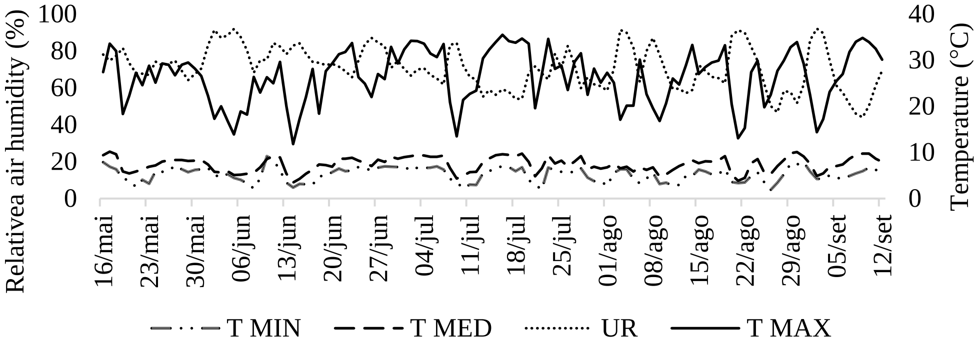

FIGURE 1 - Average temperature (ºC) and relative air humidity (%) inside the greenhouse, during the experimental period.

TABLE 2 - Stem water content (%) determined by destructive and non-destructive methodologies of *Ectandra grandiflora* seedlings as a function of hardening and days under water restriction.

Hardening Treatments	Days under water restriction		
	0	30	
	Methodologies		
	Non-destructive	Destructive	Non-destructive
Tigmomorphogenesis	27.00* ab	61.96 a	27.06 a
Control	26.60 b	49.30 ab	27.06 a
Metil Jasmanato	27.30 a	43.80 b	27.48 a

*Means followed by the same letter in the column do not differ statistically from each other, by Tukey's test, at a 5% probability of error.

TABLE 3 - Pearson's correlation between the methodologies tested for quantification of stem water content from *Nectandra grandiflora* seedlings as a function of days under water restriction.

Methodologies	Days under water restriction					
	0		15		30	
	Methodologies					
	Destructive	Non-destructive	Destructive	Non-destructive	Destructive	Non-destructive
Destructive	1.00		1.00		1.00	
Non-destructive	-0.23	1.00	-0.33	1.00	-0.16	1.00
	p=0.3 ns		p=0.1 ns		p=0.5 ns	

ns = not significant.

***Inga sessilis* (Vell.) Mart.**

In the destructive evaluation carried out at the beginning of the water restriction period, a difference was detected between the imposed hardening treatments (p<0.05). Seedlings submitted to stem bending corresponded to the lowest mean stem water content (Table 4). In seedlings with the application of jasmonate no divergences (p>0.05) were detected compared to control seedlings presenting averages of 37.8 and 42.4%, respectively.

The destructive (traditional) method results indicated that at 15 days of water restriction, methyl jasmonate was efficient in maintaining higher stem water content in relation to the other treatment. The hardening treatments did not result in differences (p>0.05) when the non-destructive method was used.

At thirty days of water restriction, both evaluation methods indicated that the lowest stem water was quantified in control seedlings, while hardening (chemical or mechanical) ensured higher stem water content (Table 5). Those results quantified in hardened seedlings may be related to morphometric changes, especially in root dry mass, or even to the lignin content (DRANSKI et al., 2015; HEBERLE et al., 2018). Lignin is a fundamental component in water transport and is possibly associated with resistance to water stress in wood species (MALAVASI et al., 2016b).

The reduction of the permeability and porosity of the cells, due to the deposition of lignin in the cell wall increases the rigidity and allows greater fluidity in the conduction of water and salts. In this way, it confers greater plant resistance to embolism, which is a cause of seedling mortality under conditions of severe water restriction (HERBETTE et al., 2015).

Pearson correlation resulted in a low correlation value at all the three periods of water restriction assessment with a mean of 51.66%. (Table 6). The results from the alternative method (i.e.Holmis®) did not express the values obtained through the gravimetric method and, therefore, it is not indicated as appropriate.

Wood tissue offers a high resistance to the passage of electric current. However, the variation in moisture content alters its characteristic as an electrical insulator, establishing an inversely proportional relationship, in which the higher the moisture content, the lower its resistance. This characteristic can be considered intrinsic to almost all types of wood (GANN, 2019).

TABLE 4 - Stem water content (%) determined by destructive and non-destructive methodologies from *Inga sessilis* seedlings as a function of hardening and days under water restriction.

Treatments	Days under water restriction			
	T0		T15	
	Methodologies			
	Destructive	Non-destructive	Destructive	Non-destructive
Tigmomorphogenesis	28.9 b*	27.3 b	55.9 b	28.5 a
Control	42.4 a	27.9 ab	58.3 b	28.5 a
Metil Jasmanato	37.8 a	28.3 a	77.7 a	29.0 a

*Means followed by the same letter in the column do not differ statistically from each other, by Tukey's test, at 5% probability of error.

TABLE 5 - Stem water content (%) determined by destructive and non-destructive methodologies from *Inga sessilis* seedlings as a function of hardening 30 days under water restriction.

	Methodologies	
	Destructive	Non-destructive
Tigmomorphogenesis	44.1 a*	26.9 a
Control	32.1 b	25.9 b
Metil Jasmanato	43.7 a	26.8 a

*Means followed by the same letter in the column do not differ statistically from each other, by Tukey's test, at 5% probability of error.

TABLE 6 - Pearson's correlation between methodologies for determining stem water content from *Inga sessilis* seedlings as a function day under water restriction.

Methodologies	Days under water restriction					
	0		15		30	
	Methodologies					
	Destructive	Non-destructive	Destructive	Non-destructive	Destructive	Non-destructive
Destructive	1.0		1.0		1,0	
Non-destructive	0.5	1.0	0.5	1.0	0.5	1.0
	p=0.3*		p=0.01*		p=0.02*	

*Significant at 5% probability of error.

To determine the moisture content based on its resistivity, it is necessary to use devices that are characterized by the presence of needles, also called electrodes, which must be introduced into the wood tissue. Also, the needles must penetrate approximately ¼ inch of the thickness of the sample to minimize issues related to the moisture gradient. However, it should be considered that the resistance to the passage of electric current varies according to the species, temperature, the position of the electrodes towards the fibers and the depth of penetration of these into the wood tissue.

Batista et al. (2011) found better correlations of the treatments which used electrodes corresponding to ½ inch of the thickness of the pieces. The same authors reported that with regard to orientation, the perpendicular insertion of the electrodes to the fibers showed greater convergence to the traditional method of moisture quantification. However, due to the smaller diameter, the use of seedlings as test material does not allow any other orientation to fix the electrode that is not parallel to the fibers, which may contribute to the low compatibility with the gravimetric method, since the authors cited obtained negative correlations for this direction.

Donato et al. (2014) observed a low correlation between capacitive and resistive methods, and the ABNT gravimetric method. Those authors found that, although the capacitive electric meter was closer to the average humidity obtained by the ABNT method than the resistive meter, the latter had a greater correlation, due to the greater homogeneity of its results. It should be noted that those meters were developed to measure moisture content in sawn wood, and no record was found in the literature of their use for wood logs or seedlings.

Because these are different methods and developed for different purposes, it is expected that they will present divergent results. Therefore, Donato et al. (2014) established a correction factor and a regression model and observed good adjustments for the resistive method of moisture determination, emphasizing the second method presented as the most adequate, which in addition to correcting the difference between the alternative and the traditional method, also corrects the mean difference between the values obtained by the difference in diameter, being the two parameters of regression.

In addition, the reliability range of portable meters was defined by Teixeira et al. (2013) as between 6-30%. Considering that the higher values of humidity, it is possible to justify the divergence between the gravimetric and resistive methods. Therefore, it is relevant to determine, through new studies, equations to correlate the results for better management aiming at the production of seedlings with satisfactory performance under water stress for greater effectiveness in the establishment of forest stands.

CONCLUSION

The determination of stem water content from *N. grandiflora* as well as I. *sessilis* seedlings by a resistive method did not present results that can replace the traditional gravimetric method.

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