

STEM BENDING AND MACRONUTRIENT ALLOCATION FROM MUTAMBA SEEDLINGS

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ABSTRACT - Seedling mineral nutrition affects the allocation of biomass to the various components above and below ground. Hardening or acclimatization via mechanical treatment has resulted in differentiated nutritional allocation among plant tissues. *Guazuma ulmifolia* Lamarck seedlings 90 days after sowing (DAS) were subjected to four daily frequencies of stem bending (zero, 10, 20 or 40 flexions) and evaluated for stress and macronutrient allocation. The tested treatments did not show stress induction in the seedlings when measured by the PER test but increased the calcium and magnesium contents of the stems. The use of 10 daily stem bending resulted in an increase in nitrogen concentration in stem tissues, while phosphorus concentration increased in both stem and roots. The imposition of 10 or 20 daily stem bending provided a reduction in the potassium concentration in the roots, as well as an increase in the calcium and magnesium contents in the stem.

Keywords: *Guazuma ulmifolia* Lamarck, electrolyte loss test, assessment of seedling stress, nursery techniques.

FLEXÕES CAULINARES E A ALOCAÇÃO DE MACRONUTRIENTES EM MUDAS DE MUTAMBA

RESUMO - A nutrição mineral vegetal afeta a alocação de biomassa nos vários componentes acima e abaixo do solo. A rusticificação ou aclimação via tratamento mecânico tem resultado em alocação nutricional diferenciada entre os tecidos vegetais. Mudas de *Guazuma ulmifolia* Lamarck com 90 dias após a semeadura (DAS) foram submetidas a quatro frequências diárias de flexões caulinares (zero, 10, 20 ou 40 flexões) e avaliadas quanto ao estresse e a alocação de macronutrientes. Os tratamentos testados não evidenciaram indução de estresse nas mudas quando aferido pelo teste PER mas aumentaram os teores de cálcio e magnésio do caule. O uso de 10 flexões caulinares diárias resultou em aumento da concentração de nitrogênio nos tecidos do caule, enquanto a concentração de fósforo aumentou tanto no caule como nas raízes. A imposição de 10 ou 20 flexões caulinares diárias proporcionaram redução da concentração de potássio nas raízes, mas não influenciaram a concentração deste nutriente no caule.

Palavras-chave: *Guazuma ulmifolia* Lamarck, avaliação do estresse, técnicas de viveiro, teste da perda de eletrólitos.

INTRODUCTION

The study of nutrient allocation in the tissues of tree species is important for understanding their life histories and their success in dispersal as they influence their competitive capacity (LARCHER, 2006). Inadequate mineral nutrition during seedling production has been identified as limiting the growth and success of establishing forest stands (OLIET et al., 2009). Plant mineral nutrition generally affects biomass allocation in the various components above and below ground to maintain metabolism and survival (QI et al., 2019). The allocation of nutrients in terrestrial plants has been described through the preferential distribution of biomass in its various organs.

Mechanical manipulations on nursery seedlings generally induce morphometric changes resulting from turgor pressure in the longitudinal direction, with greater radial rather than longitudinal growth, as this alters the orientation of microtubules and microfibrils of cellulose in

the primary cell wall and cytoplasm (LOPEZ et al., 2014). Growth patterns correlate with post-planting survival and mortality (VOLKWEIS et al., 2020).

The induction of hardening or acclimatization in the production of seedlings of tree species aims to alter plant growth patterns (SAMPATHKUMAR et al., 2014). The hardening or acclimatization process is characterized using practices in the nursery with the purpose of increasing tolerance to post-planting abiotic stresses (HEBERLE et al., 2018) through mechanical manipulations or the application of plant growth regulators. Hardening or acclimation induces physiological, anatomical, or morphological adjustments of the plant that improve performance or survival in response to environmental changes or stressors. The mechanical strength of the stem depends on carbohydrates, including non-structural and structural carbohydrates, mainly lignin and cellulose (ZHANG et al., 2014). The induction of hardening or acclimation via mechanical

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treatment (i.e. stem bending) can induce stress resulting in differentiated nutritional allocation between shoot and root tissues (PUIJALON et al., 2007).

Guazuma ulmifolia Lamarck is a tree species used in energy plantations to obtain charcoal as well as in the revegetation of degraded areas (PAIVA SOBRINHO; SIQUEIRA, 2008). The species is common on the edges of "cerradão" and even in the Pantanal or on the banks of small watercourses. Additionally, the species has a wide range of potential uses, mainly linked to pharmacological and medicinal purposes due to its therapeutic properties (SILVA et al., 2020). The species is also characterized by having active principles used to control parasites, such as *Leishmania brasiliensis*, *L. infantum* and *Trypanosoma cruzi* (CALIXTO JÚNIOR et al., 2016) and as an alternative treatment against the HIV virus in Brazil (GOUVEIA, 2018) and Venezuela (SINGER, 2018).

Given the above, the experiment aimed to quantify the effect of the frequency of stem bending on the allocation of macronutrients during the propagation of mutamba.

MATERIALS AND METHODS

The experiment was conducted in a shade house (50%) using mutamba (*Guazuma ulmifolia* Lamarck) seedlings, produced with seeds collected at the nursery in Foz do Iguaçu-PR with coordinates of 24° 33' 22" S and 54° 03' 24" W. The seedlings resulted from sowing and procedures used in that nursery using commercial substrate Humusfértil Florestal®, composed of pine bark, sand, substrate, vermicompost and vermiculite with the addition of Osmocote® 14-14-14, accompanied by fertilization with NPK 10-10-10 every 15 days, for three months after sowing.

Seedlings 90 days after sowing (DAS) were subjected to four daily frequencies of stem bending (zero, 10, 20 or 40 bendings) according to Volkweis et al. (2014). The treatments were applied for 30 days in which all seedlings received irrigation until saturation once a day in the morning. Before applying the treatments, the morphometry of the seedlings was characterized in terms of height and stem diameter, with which the robustness index (relationship between height and stem diameter) was calculated, and the number of leaves tallied. The chlorophyll index was determined in the leaves with a chlorophyll meter (SPAD 502 Minolta®).

After the application of hardening treatments, the variables evaluated in seedlings with 120 days DAS included the extrusion of electrolytes from root tissues (PER), the levels and contents of N, P, K, Ca and Mg in stem and root tissues. The determination of the PER test followed the methodology of Wilner (1955) while that of macronutrients used sulfuric digestion for N and nitro-perchloric digestion for K, Ca, Mg and P described by Lana et al. (2010). Additionally, the biomass of aerial and root tissues was determined by drying in an oven with air circulation at 65°C for 72h. The experiment followed a

randomized block design with four frequencies of daily stem flexion (zero, 10, 20 or 40 flexions), containing 5 repetitions of 7 seedlings each, totaling 280 seedlings.

The data were checked for normality of residual distribution using the Shapiro-Wilk test, and homogeneity of variances using the Brown-Forsythe test and subjected to analysis of variance with Sigma Plot 12.0 (SIGMAPLOT, 2011). When there were significant differences, the treatment means were compared using the Tukey test, with a 5% probability of error.

RESULTS AND DISCUSSION

The morphometric analysis of the seedlings before treatments indicated an average height of 20.1 cm, with a stem diameter of 2.8 mm, containing 6 leaves, an average of 28.8 SPAD units and a robustness index of 7.2 indicative of slender seedlings (AVELINO et al., 2021). Seedling robustness index reveals the growth balance between shoot and root tissues. The lower the value of the index the better the quality of the seedling with a greater probability of survival and establishment after planting (CARGNELUTTI FILHO et al., 2018).

After 30 days of stem bending treatments, it was observed that *G. ulmifolia* seedlings had a reduction in the accumulation of biomass in the root system with 40 daily bendings, while lower bending intensities did not alter the biomass allocation (Figure 1a). Stem tissues displayed a significant reduction of biomass in relation to control seedlings (zero stem bending) with the lowest values measured in seedlings that received 10 and 20 daily bendings (Figure 1b).

The analysis of the stressful effect of treatments with stem bending was estimated by the electrolyte extrusion test (PER) from root tissues. The interpretation of the results indicated an increase in electrolyte extrusion ($P < 0.05$) in seedlings subjected to a frequency of 40 daily stem bendings (35.1%) compared to those obtained in seedlings from the control treatment (21.0%) signaling increased stress. The PER test values detected in seedlings subjected to frequencies of 10 (28.3%) and 20 (23.3%) daily stem bendings suggest that those treatments did not induce stress, as they were similar to that observed in the control treatment. The PER test estimates the integrity and selectivity in cellular membranes of the root system (RADAGLOU et al., 2007). The test is characterized by being a quick indicator of seedling stressful conditions (EARNSHAW, 1993).

The nitrogen (N) content in the stem of mutamba seedlings (Table 1) increased ($P < 0.05$) compared to that quantified in control seedlings (0.41 g Kg^{-1}) with the application of 10 (0.65 g Kg^{-1}) and 40 daily bendings (0.57 g Kg^{-1}). However, when analyzing the content in the element, it is only observed that with the frequency of 20 stem bendings there was a reduction of the nutrient element. Therefore, mutamba seedlings subjected to 10 and 40 bendings concentrated more N to the detriment of the lower biomass allocated in the stem tissues.

Regarding seedling root system, it was observed that N was significantly lower only with 20 bendings,

indicating that the stress suffered resulted in lower absorption of N, given that root biomass was not significantly changed at this frequency; furthermore, the N content detected was significantly lower than the control treatment. Additionally, the low content observed in stem

tissues corroborates this behavior. For seedlings exposed to 40 daily bendings, a lower root biomass was observed, which resulted in a lower N content in the organ without, however, differences in the content measured in the root system.

TABLE 1 - Content (T) and concentration (C) of nitrogen (N) in the stem (C) and root (R) of mutamba seedlings as a function of the frequency of stem bending.

Frequência de flexões caulinares	TNC	TNR	CNC	CNR
	----- g Kg ⁻¹ -----		----- g per plant -----	
0	0.41 b*	0.69 a	0.46 a	0.65 a
10	0.65 a	0.66 a	0.43 a	0.63 a
20	0.40 b	0.50 b	0.30 b	0.46 b
40	0.57 a	0.66 a	0.47 a	0.50 b
CV (%)	6.8	5.0	6.9	5.2
W _p -valor	0.28	0.91	0.14	0.97
B-F _p -valor	0.27	0.07	0.45	0.11

*Means followed by the same lowercase letter in the column do not differ significantly from each other using the Tukey test, at a 5% probability of error. CV: coefficient of variation; Wp-value: the probability of significance of normal distribution of experimental errors; B-Fp-value: the probability of significance of homogeneity of experimental error variances.

Nitrogen (N) is considered an essential element for terrestrial plants, as it is present in the composition of the most important biomolecules such as ATP, NADH, NADPH, chlorophyll, proteins, and numerous enzymes. Nitrogen is the mineral nutrient proportionally most required by terrestrial plants (GIRACCA; NUNES, 2016). Due to the greater investment in the growth of the aerial

part (BRONDANI et al., 2008) it is common for there to be greater nutritional demand for that plant system. Of all the nutritional elements in higher plants, in general, nitrogen is the one found in the highest concentrations. In higher plants, nitrogen is part of proteins, nucleic acids, and other important cellular constituents, including membranes and several plant hormones.

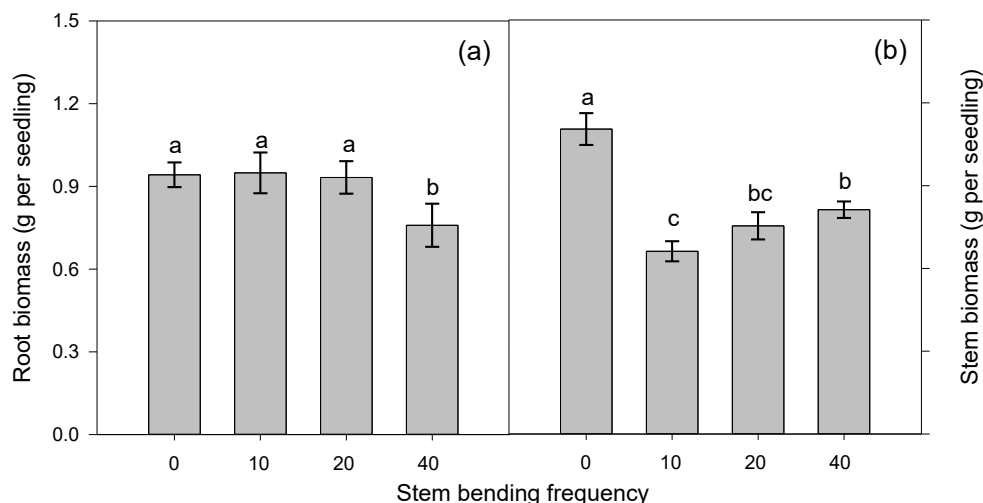


FIGURE 1 - Accumulation of aboveground (a) and belowground (b) biomass of mutamba seedlings as a function of stem bending frequency. *Averages not followed by the same lowercase letter differ significantly from each other, using the Tukey test, at a 5% probability of error.

With phosphorus (Table 2), it is noted that when applying the frequency of 10 stem bending there is a greater content and accumulation of the element in the root tissues, while in the stem tissues, despite there being a higher content of the element, the content is relatively low given the lower biomass. It is also noteworthy that in seedlings subjected to 20 daily stem bendings, the element is preferentially allocated to the root system, considering

that in aerial tissues the content is significantly lower in relation to other treatments.

The lack of phosphorus (P) in most tropical soils is a limiting factor for the growth of exotic and native woody species (FURTINI NETO et al., 1999). Phosphorus is an essential element in plant metabolism, fundamental in transferring energy from cell respiration and photosynthesis. Phosphorus deficiency in substrates causes

irregular growth, both in the aerial part and in the root system, damaging the quality of the seedlings (GOMES; PAIVA, 2012). Plants invest in different defense strategies against stressors triggered in the stress restitution phase involving anabolism such as biosynthesis for physical barriers or repellency responses (CHEN, 2008).

Mutamba is a species with high pharmacological potential and among its identified compounds, associated with defense metabolism, the most representative are the contents of terpenoids, flavonoids, phenolic compounds, tannins and alkaloids, compounds that are associated with

the plant defense response (PRAHASTUTI et al., 2020; RAFI et al., 2020). These compounds, like terpenoids, require phosphorus in their constitution, in the mevalonic acid biosynthetic pathway (TAIZ et al., 2017). Therefore, the mechanical stimulus induced by 10 daily stem bendings can signal the activation of that route causing phosphorus to be required in greater concentration. Increasing the frequency of bendings resulted in a decreased demand indicating exhaustion of the injury, leaving the plant that is most susceptible and will culminate in metabolic collapse.

TABLE 2 - Content (T) and concentration (C) of phosphorus (P) in the stem (C) and root (R) of mutamba seedlings as a function of the frequency of stem bending.

Frequência de flexões caulinares	TPC ----- g Kg ⁻¹ -----	TPR ----- g Kg ⁻¹ -----	CPC ----- g per plant -----	CPR ----- g per plant -----
0	2.03 c*	1.59 b	2.24 a	1.50 b
10	2.84 a	2.05 a	1.88 c	1.95 a
20	1.87 d	1.49 b	1.41 d	1.39 b
40	2.45 b	1.61 b	1.99 b	1.22 c
CV (%)	2.8	3.5	2.6	3.4
W _p -valor	0.47	0.40	0.52	0.13
B-F _p -valor	0.12	0.41	0.12	0.41

*Means followed by the same lowercase letter in the column do not differ significantly from each other using the Tukey test, at a 5% probability of error. CV: coefficient of variation; W_p-value: the probability of significance of normal distribution of experimental errors; B-F_p-value: the probability of significance of homogeneity of experimental error variances.

The potassium content in stem tissues was not affected by the frequencies of stem flexures (P>0.05). However, given the lower biomass observed in seedlings that suffered mechanical injury, the contents were significantly lower compared to the control treatment (Table 3). Meanwhile, in the root system, the frequencies of 10 and 20 daily stem bending's resulted in a lower content in that organ. It is also noteworthy that even though they had statistically the same biomass, the contents were lower than that of the control treatment.

The lower potassium (K) values may be associated with the integrity of the biological membranes of the root system measured by the PER test. The greater extravasation with the increase in the frequencies of stem bending indicated that nutrient was not fully absorbed by the plant cells, but was acting to maintain the electrochemical potential of the biological membranes of the root system, in the active transport of nutrients such as calcium and magnesium, as well as acting synergistically in the absorption of phosphate in the root system.

TABLE 3 - Content (T) and concentration (C) of potassium (K) in the stem (C) and root (R) of mutamba seedlings as a function of the frequency of stem bending.

Frequência de Flexões caulinares	TKC ----- g Kg ⁻¹ -----	TKR ----- g Kg ⁻¹ -----	CKC ----- g por plant -----	CKR ----- g por plant -----
0	12.12 a*	20.19 a	13.39 a	18.72 a
10	8.23 a	11.59 b	5.45 c	11.56 bc
20	11.64 a	8.28 b	8.78 b	7.98 c
40	10.89 a	17.3 a	8.85 b	12.99 b
CV (%)	26.5	17.4	29.4	17.2
W _p -valor	0.41	0.27	0.58	0.41
B-F _p -valor	0.71	0.96	0.66	0.92

*Means followed by the same lowercase letter in the column do not differ significantly from each other using the Tukey test, at a 5% probability of error. CV: coefficient of variation; W_p-value: the probability of significance of normal distribution of experimental errors; B-F_p-value: the probability of significance of homogeneity of experimental error variances.

The absence of K can result in restricted photosynthesis, chlorosis followed by necrosis and decreased growth according to Raji (1991). The K element has no structural function but is associated with greater resistance of plants when subjected to adverse conditions, such as low water availability and extreme temperatures,

due to its function in opening and closing stomata (KERBAUY, 2004). Therefore, K deficiency reduces plant tolerance to water deficit via prevention and adaptation to stress (TRÄNKNER et al., 2018).

Of all mineral nutrients, potassium (K) plays a particularly critical role in plant growth and metabolism

contributing to survival under various abiotic stresses. K is an essential nutrient and the most abundant cation of higher terrestrial plants. The concentration of K⁺ within the cytoplasm has been consistently found to be between 100 and 200 mM (SHABALA; POTTOSIN, 2010) while the apoplastic K⁺ concentration can vary between 10 and 200 or even reach up to 500 mM (WHITE et al., 2010). The main functions that K⁺ performs in higher terrestrial plants include enzymatic activation, active participation in the establishment of cellular turgor and the maintenance of cellular electro-neutrality, in addition to being involved in photosynthesis, carbohydrate transport, protein synthesis, cell expansion and stomatal movement (TAIZ et al., 2017).

The calcium (Ca) content in the stem (Table 4)

increased with 20 stem bending's (9.07 g Kg⁻¹) when contrasted with the content determined in control seedlings (6.70 g Kg⁻¹). However, even with an increase in the Ca content in the stem tissues, there seems to be a concentrating effect of the element, as the content was statistically equal to that observed in control seedlings while the content observed in the root system was similar to that observed in dry biomass root. Calcium is considered a basic element in the acid-base balance of vegetables, whose alteration impairs or reduces growth. Additionally, it changes the shape of plant tissues, reducing root formation and delaying both flowering and fruiting, constituting an important component of cell wall integrity.

TABLE 4 - Content (T) and concentration (C) of calcium (Ca) in the stem (C) and root (R) of mutamba seedlings as a function of the frequency of stem bending.

Frequência de flexões caulinares	TcaC	TCaR	CCaC	CCaR
	----- g Kg ⁻¹ -----		----- g per plant -----	
0	6.70 b*	6.02 a	7.41 a	5.67 ab
10	8.34 ab	6.48 a	5.52 b	6.15 a
20	9.07 a	6.83 a	6.83 a	6.38 a
40	8.68 ab	6.90 a	7.06 a	5.24 b
CV (%)	11.2	6.4	12.1	6.6
W _{p-valor}	0.92	0.95	0.46	0.82
B-F _{p-valor}	0.08	0.96	0.09	0.97

*Means followed by the same lowercase letter in the column do not differ significantly from each other using the Tukey test, at a 5% probability of error. CV: coefficient of variation; Wp-value: the probability of significance of normal distribution of experimental errors; B-Fp-value: the probability of significance of homogeneity of experimental error variances.

The magnesium (Mg) content in the stems of mutamba seedlings increased (Table 5) with all frequencies of stem bending (average of 2.42 g Kg⁻¹) in relation to the value obtained from the stems of control seedlings (1.76 g kg⁻¹). With increasing concentration and given the lower biomass, the Mg content in the stems did not change significantly. The magnesium (Mg) content in the root system reduced with 20 and 40 stem bendings and appears to be associated with the phosphorus content

observed in the root system. Mg acts as a phosphorus carrier in the plant, that is, there would be greater absorption of phosphorus in the presence of magnesium given its importance in phosphorylation reactions (LIMA et al., 2018). According to Souza et al. (2009), the omission and low requirement of Mg in the initial propagation phase contributed to the formation of pink cedar seedlings with larger stem diameters than control seedlings.

TABELA 5 - Content (T) and concentration (C) of magnesium (Mg) in the stem (C) and root (R) of mutamba seedlings as a function of the frequency of stem bending.

Frequência de flexões caulinares	TMgC	TMgR	CMgC	CMgR
	----- g Kg ⁻¹ -----		----- g per plant -----	
0	1.76 b*	1.95 a	1.94 ^a	1.84 a
10	2.58 a	1.89 a	1.71 ^a	1.80 ab
20	2.47 a	1.32 b	1.86 ^a	1.23 c
40	2.20 a	1.79 ab	1.79 ^a	1.36 bc
CV (%)	8.2	13.0	8.3	13.2
W _{p-valor}	0.15	0.73	0.14	0.45
B-F _{p-valor}	0.86	0.58	0.81	0.55

*Means followed by the same lowercase letter in the column do not differ significantly from each other using the Tukey test, at a 5% probability of error. CV: coefficient of variation; Wp-value: the probability of significance of normal distribution of experimental errors; B-Fp-value: the probability of significance of homogeneity of experimental error variances.

Increasing evidence suggests that mineral nutrients play a critical role in the resistance of terrestrial plants to stresses (MARSCHNER, 2012). The production of resistant seedlings, capable of surviving and tolerating

post-planting abiotic adversities is one of the possible alternatives to minimize post-planting mortality, especially water deficit.

The cultural and silvicultural practices used in

nurseries have a strong influence on the performance of seedlings immediately after planting in the field (RIIKONEN; LUORANEN, 2018). Several authors indicated that mechanical stimuli, irrigation cycles and application of plant growth regulators presented beneficial results in the adaptation of seedlings of tree species to post-planting stress (VILLAR-SALVADOR et al., 2004; JACOBS; LANDIS, 2009; CADORIN et al., 2021).

Hardening or acclimatization techniques imposed on the seedlings in the present experiment did not result in stress assessed indirectly by the results of the PER test. The PER test consists of quantifying ions extravasated through the cell membrane into the solution and can even be non-destructive, as it uses a sample of roots. Under stress conditions, cell membranes lose their selective permeability and consequently, their ability to retain ions. Therefore, the quantification of ions that leak through the cell membranes of root tissues estimates their cellular integrity conditions (FERNANDES; SOUZA, 2006; DRANSKI et al., 2017).

CONCLUSIONS

The PER test involving the extrusion of electrolytes from root tissues did not detect stress in seedlings subjected to 10 and 20 stem bendings.

Induction of 10 daily stem bendings resulted in an increase in nitrogen concentration in stem tissues, while phosphorus concentration increased in both stem and roots.

Seedling hardening treatments via stem bending resulted in an increase of calcium and magnesium content in the stem without influencing those nutrients in the roots. The application of 10 or 20 daily stem bendings resulted in a reduction in the concentration of potassium in the roots but did not influence the concentration in the stem.

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