



MACRONUTRIENT DEFICIENCY IN *CARINIANA ESTRELLENSIS* (Raddi) KUNTZE: FROM CHANGES IN CELL ULTRASTRUCTURE TO VISUAL SYMPTOMS

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ABSTRACT – The nutritional requirements of native forest species can influence productivity. Thus, the understanding of these requirements enables us to optimize the use of inputs and reduce the environmental impacts in forest restoration projects. The present study aimed to evaluate changes in cellular ultrastructure and the anatomy of leaf laminae as well as observe visual signs of nutrient deficiency in young *Cariniana estrellensis* (Raddi) Kuntze plants, a forest species widely used in ecological restoration projects. The experiment was conducted in a greenhouse in random blocks with three replications of seven treatments using nutrient subtraction (I.e., control [plants grown with all nutrients], -N, -P, -K, -Ca, -Mg, and -S). The plants were harvested 135 days after the beginning of the treatments when the deficiency of the macronutrients resulted in visible abnormalities. Changes in the cell ultrastructure and structure of the chloroplasts, cytoplasm, and stromal lamellae were observed, as well as starch and lipid concentrations in the cytoplasm, intercellular spaces, and parenchymal cells. Changes in the cell ultrastructures, leaf laminae, and visual signs of nutrient deficiency hindered the development of young *C. estrellensis* plants; therefore, forest restoration projects that use this species in soils that require nutritional supplementation may have limited success in the absence of nutritional support.

Keywords: Deficiency symptom; Transmission electron microscopy; Mineral nutrition.

DEFICIÊNCIA DE MACRONUTRIENTE EM CARINIANA ESTRELLENSIS (Raddi) Kuntze: DE MUDANÇAS NAS ULTRASTRUTURAS CELULARES AOS SINTOMAS VISUAIS

RESUMO – Os requisitos nutricionais das espécies florestais nativas podem influenciar a produtividade. Assim, o entendimento desses requisitos nos permite otimizar o uso de insumos e reduzir os impactos ambientais em projetos de restauração florestal. O presente estudo teve como objetivo avaliar mudanças na ultraestrutura celular e na anatomia das lâminas foliares, bem como observar sinais visuais de deficiência de nutrientes em plantas jovens de *Cariniana estrellensis* (Raddi) Kuntze, uma espécie florestal amplamente utilizada em projetos de restauração ecológica. O experimento foi conduzido em casa de vegetação em blocos aleatórios com três repetições de sete tratamentos usando subtração de nutrientes (ou seja, controle [plantas cultivadas com todos os nutrientes], -N, -P, -K, -Ca, -Mg e -S). As plantas foram colhidas 135 dias após o início dos tratamentos, quando a deficiência de macronutrientes resultou em anormalidades visíveis. Foram observadas alterações na ultraestrutura celular e na estrutura dos cloroplastos, citoplasma e lamelas estromais, bem como concentrações de amido e lipídios no citoplasma, espaços intercelulares e células parenquimatosas. Alterações nas ultraestruturas celulares, lâminas foliares e sinais visuais de deficiência de nutrientes impediram o desenvolvimento de plantas jovens de *C. estrellensis*; portanto, projetos de restauração florestal que utilizam essa espécie em solos que requerem suplementação nutricional podem ter sucesso limitado na ausência de suporte nutricional.

Palavras-Chave: Sintoma de deficiência; Microscopia eletrônica de transmissão; Nutrição mineral.



1. INTRODUCTION

Forest restoration in degraded areas has expanded through the integration of strategies aimed at mitigating climate change, soil improvement, and biodiversity conservation. Seedling production and the planting of native tree species are key actions for the implementation of such programs. In many cases, soils in areas intended for forest restoration do not have adequate fertility conditions for native species to develop satisfactorily. Such nutritional deficiency affects the growth, morphology, anatomy, and composition and, consequently, production of the plants (Marschner, 2012). However, few and limited studies have been conducted on native Brazilian forest species.

Knowing the nutritional requirements of native species can facilitate the development of technologies to obtain healthy seedlings intended for revegetation programs, thus enabling them to develop in previously degraded areas. For forest development to succeed, it is important to understand the nutritional requirements of each species (Lima et al., 2000). Determining the physiological stages of development in which various elements are in their highest demand can help in fertilization planning to provide them artificially, thereby correcting any nutritional deficiencies.

Although such information is essential for the success of plant growth (Kramer and Kozłowski, 1960), information regarding the nutritional requirements of forest species is scarce in the literature (Schumacher et al., 2004), even though mineral deficiencies with consequent growth disorders in forest species have been commonly observed (Dreschel and Zech, 1991). To determine the extent to which chemical elements found in plants are essential and their roles in metabolism, experiments have been undertaken using soil and nutrient solutions (Malavolta, 2006; Kerbauy, 2012; Marschner, 2012; Taiz and Zeiger, 2017). For nutritional diagnosis, the omission of nutrients in experimental nutrient solutions has been widely utilized. Although this is an indirect approach, it can provide information on the requirement for fertilizers and can improve the quality of plants (Benedetti et al., 2009).

C. estrellensis (Raddi) (Kuntze), family Lecythidaceae, is an important tree species for heterogeneous ecological reforestation programs, although its nutritional requirements and deficiency symptoms are unknown. It is a semi-deciduous tree in

winter and prefers sunny areas; however, it can also develop in shaded areas in wet and deep soils, which are characteristics of climax forests. It is rare in the *Cerrado* and dry terrains. Although it has ornamental qualities, owing to its large size, it is usually used only in landscaping parks and large gardens. Its wood has great potential for use in the wood industry. It reaches 35–45 m in height and its trunk reaches 90–120 cm in diameter, it blooms from October to December, and the fruits ripen from July to September. Its seeds are consumed and distributed by monkeys (Lorenzi, 2006).

The mineral nutrition of native species has been widely studied in recent years (Sorreano, 2006; Andrade, 2010; Viéguas et al., 2012; Valeri et al., 2014; Carlos et al., 2015; Sousa et al., 2018). However, there are no studies related to the mineral nutrition of this native species or how nutritional deficiency can affect the cell ultrastructure and visual symptoms.

The present study aimed to evaluate the changes in the cell ultrastructure and leaf lamina anatomy as well as the visual symptoms of young *C. estrellensis* plants grown under deficient nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) conditions.

2. MATERIAL AND METHODS

The experiment was conducted in the greenhouse of the Laboratory of Mineral Nutrition of Plants of the Center for Nuclear Energy in Agriculture, University of São Paulo, in the city of Piracicaba/SP, located at 22°42'30" S latitude, 47°38'00" W longitude (SIRGAS 2000). *C. estrellensis* seedlings were purchased from a nursery growing native species and were transported in rigid PVC plastic tubes containing 120 mL commercial substrate (Silva and Stein, 2008), 60 days after seeding. Prior to the nutrient omission treatments, a complete solution with equal mineral concentrations was used as proposed by Johnson et al. (1957). However, the *C. estrellensis* plants did not develop well, showing symptoms of nutrient toxicity, including burning of the lamina and leaf lamina edges. Thus, the *C. estrellensis* seedlings used in the experiment remained for 21 days in the greenhouse in a nutrient solution described by Johnson et al. (1957), with 50% of the concentration of the original solution of macronutrients. Micronutrients were also added as described by Johnson et al. (1957). After this procedure, the plants were subjected to diagnosis by subtraction for 135 days. Weekly nutrient

solutions were replaced and descriptions of the visual symptoms were taken.

The experiment was performed based on a randomized block design with three replicates per of seven treatments using nutrient subtraction (I.e., control [plants grown with all nutrients], -N, -P, -K, -Ca, -Mg, and -S). Each replicate included a single plant in a vessel with 2 L of nutrient solution. A total of 21 plants were included in the study. The volume of the nutrient solution was maintained daily with aerated deionized water. The nutrient solutions were refreshed when the electrical conductivity of the nutrient solution decreased to 70% of the initial electrical conductivity or every 21 days.

For anatomical evaluation by light microscopy and ultrastructural evaluation by transmission electron microscopy, samples were collected from the middle region of the leaf lamina (1 × 2 mm). Semi-thin (120 nm) and ultrathin (60 to 90 nm thick) sections were prepared according to Reynolds (1963) with minor modifications. Drops of 2.5% uranyl acetate were placed on a glass slide and samples were added face-down. A copper screen was placed on top and the sample was allowed to air-dry for 12 minutes in a dark space. The copper screen containing the affixed sample was then washed three times in distilled water and dried. Drops of lead citrate were added and the sample was placed on a new glass slide with NaOH tablets to remove any moisture and dried for a further 12 minutes in the dark. The samples were washed three times in distilled water and dried again. Then, the samples were examined under a Zeiss EM-900 transmission electron microscope operating at 50 kV.

To determine the nutrient content, each of the young *C. estrellensis* plants was separated into stems, roots, and leaves at the end of the experiment. Each tissue was

washed with deionized water and dried in a greenhouse with forced air circulation at a temperature of 60 °C to a constant mass. All parts were then ground in a mill and the macromicronutrients were extracted and analyzed according to the methodology described by Sarruge and Haag (1974). Nutrient content was compared using analysis of variance (Pimentel-Gomes, 1990).

3. RESULTS

Macronutrient deficiency in young *C. estrellensis* plants caused decreases in nutrient levels in different parts of the plant as well as alterations in the ultrastructure, organization, and overall structure of the leaf lamina cells. This caused overt anatomical changes in the plant tissues and visible signs of nutrient distress. Damage to organelles and other cell structures can compromise several physiological functions (photosynthesis, transpiration, and respiration) of *C. estrellensis* and affect biomass production.

3.1. Nutrient content of leaves, stems, and roots

To direct nutritional management for optimal production, plant tissue analysis was performed to provide information on plant nutritional status (Smith and Loneragan, 1997).

The deficiency of one nutrient leads to a lower level of the same nutrient in the leaves, stems, and roots. However, there may also be lower levels of other nutrients. In the control group, all macronutrient levels were in the normal range, demonstrating that the measured effects were caused by the deficiency of the respective nutrient minerals (Table 1).

During the analysis of the macronutrient levels in the leaves, stems, and roots, we found all had lower

Table 1 – Macronutrient contents in organs of young *Cariniana estrellensis* plants.

Tabela 1 – Teores de macronutrientes em partes de plantas jovens de *Cariniana estrellensis*.

Treatment	N	- N	P	- P	K	- K	Ca	- Ca	Mg	- Mg.	S	- S
Organ												
Leaf	31 [±] 4 [*]	9 [±] 2	6.0 [±] 1,5	0.4 [±] 0.15	21 [±] 3	1 ^{±0.2}	21 ^{±2}	4 ^{±0.5}	4.0 [±] 0.7	1.6 [±] 0.2	4.9 [±] 0.5	1.3 [±] 0.4
Stem	20 ^{±3}	7 [±] 2	7.0 [±] 1.9	1.0 ^{±0.2}	20 ^{±3}	11 ^{±1}	15 ^{±1}	2 ^{±0.3}	3.6 [±] 0.4	2.9 [±] 0.4	3.6 ^{±0.2}	1.6 [±] 0.1
Root	16 ^{±2}	7 ^{±2}	6.0 ^{±2}	1.0 ^{±0.2}	22 ^{±2}	8 ^{±1} 2	18 [±]	7 ^{±0.8}	3.8 [±] 0.3	1.7 [±] 0.3	4.7 [±] 0.2	3.1 ^{±0.2}

Standard deviation from the mean.
Desvio Padrão da média.

levels in the treatments with a deficiency of one of the nutrients.

3.2. Changes in cell ultrastructure and leaf lamina tissues

Comparative analysis of cell ultrastructures and leaf lamina tissues among plants with macronutritional deficiency showed alterations in chloroplasts, with disorganization of the thylakoid structure (*granum*) and absence of stromal lamellae in treatments deficient in N (Figure 1B), P (Figure 1C), and S (Figure 1G). A decrease in chloroplast size was also observed in the P-deficient treatment (Figure 1C). However, an increase in the size and number of chloroplasts in the K-deficient treatment was observed (Figure 1D). Mg deficiency disrupted chloroplast membranes (Figure 1F).

Deficiencies in N (Figure 1B), Ca (Figure 1E), and Mg (Figure 1F) caused an accumulation of starch granules in the chloroplasts. When K was deficient (Figure 1D), no starch granules were observed in the vacuoles. The thin cross sections revealed an increase in the number of starch granules in N-deficient leaf lamina cells (Figure 2B) compared to the control treatment (Figure 2A). Meanwhile, an increase in lipid granules was observed in chloroplasts that were deficient in N (Figure 1A), P (Figure 1C), Ca (Figure 1E), and S (Figure 1G).

3.3. Visual symptoms of nutrient deficiency

Leaf chlorosis occurred in treatments with N deficiency (initially in new leaves and then in old leaves, Figures 3B and 3I), P deficiency (new leaves, Figure 3D), Ca deficiency (old leaves, Figure 3K), Mg deficiency (interveinal chlorosis in old leaves, Figures 3L, 3N, and 3O), and S deficiency (new leaves, Figure 3G). P deficiency also limited root development (Figure 3U). K deficiency caused the deformation of new leaves (Figure 3F), wrinkling and chlorosis of new and old leaves (Figures 3F and 3J), and petiole collapse (Figure 3T); thus, causing the abscission of old and intermediate leaves from 90 days after the start of treatment.

The collapse of the petiole and abscission of old leaves also occurred in plants with Ca deficiency (Figures 3R and 3S). White and necrotic spots on older leaves were observed under Mg deficiency. Newly deformed, filiform, and wrinkled leaves, which deformed the shoot apex, were produced in plants with S deficiency (Figure 3C, 3G, and 3H).



Figure 1 – Ultrathin cross-sections of *C. estrellensis* leaf lamina under complete nutrition (A), with N deficiency (B), with P deficiency (C), with K deficiency (D), with Ca deficiency (E), with Mg deficiency (F), and with S deficiency (G). Abbreviations: pc = cell wall, cl = chloroplast, ga = starch granules, ti = thylakoid, pe = peroxisome, v = vacuole, lm = middle lamella. Bars = 2 μ m.

Figura 1 – Seções ultrafinas da lâmina foliar de *C. estrellensis* sob nutrição completa (A), com deficiência de N (B), com deficiência de P (C), com deficiência de K (D), com deficiência de Ca (E), com deficiência de Mg (F) e com deficiência de S (G). Abreviaturas: pc = parede celular, cl = cloroplasto, ga = grânulos de amido, ti = tilacóide, pe = peroxissomo, v = vacúolo, lm = lamela média. Barra = 2 μ m.

4. DISCUSSION

4.1. Changes in cell ultrastructure and leaf lamina tissues

N deficiency in *C. estrellensis* causes disorganization of the thylakoid structure and increases starch and lipid granules inside the chloroplasts, which

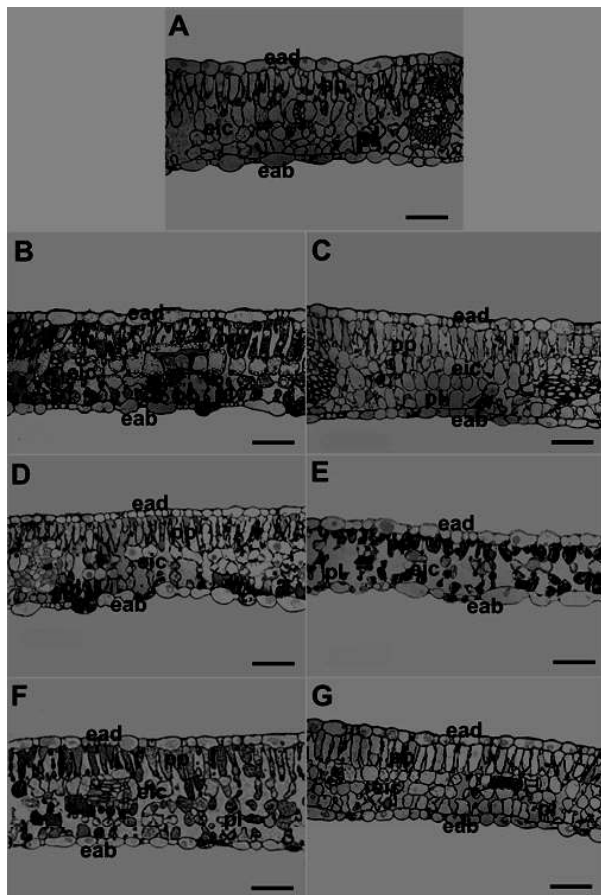


Figure 2 – Thin cross-sections of *C. estrellensis* leaf lamina under complete nutrition (A), with N deficiency (B), with P deficiency (C), with K deficiency (D), with Ca deficiency (E), with Mg deficiency (F), and S deficiency (G). Abbreviations: ead = adaxial epidermis, eab = abaxial epidermis, pp = palisade parenchyma, pl = sponge parenchyma, eic = intercellular space. Bars = 50 μm.

Figura 2 – Seções finas da lâmina foliar de *C. estrellensis* sob nutrição completa (A), com deficiência de N (B), com deficiência de P (C), com deficiência de K (D), com deficiência de Ca (E), com deficiência de Mg (F) e deficiência de S (G). Abreviações: ead = epiderme adaxial, eab = epiderme abaxial, pp = parênquima paliçada, pl = parênquima esponjoso, eic = espaço intercelular. Barras = 50 μm.

are similar symptoms to those found in cotton (Malavolta et al., 2004), *Ceiba speciosa* (A. St.-Hil.) Ravenna (Sorreano, 2006), *Zea mays* L. (Hall et al., 1972), and *Hevea* sp. (Hamzah and Gómez, 1979). A lack of N forming nitrogenous compounds that combine with carbohydrates to produce amino acids and proteins can prompt the accumulation of starch and lipids (Malavolta et al., 2004).

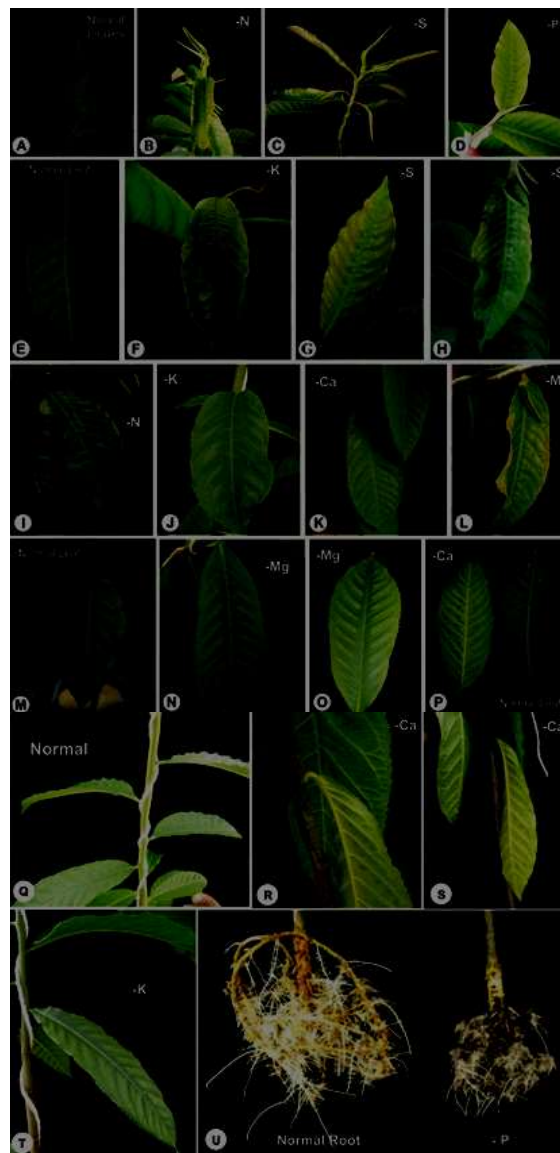


Figure 3 – Young *C. estrellensis* normal leaves (A and E) and leaves deficient in potassium (F), sulfur (C, G, and H), nitrogen (B), and phosphorus (D). Old *C. estrellensis* normal leaves (M) and those deficient in potassium (J), nitrogen (I), calcium (K), and magnesium (L, N, and O). Normal old leaves with calcium deficiency (P). Normal *C. estrellensis* leaves (Q), versus those deficient in Ca (R and S) and K (T). Normal root of *C. estrellensis* and with P deficiency (U).

Figura 3 – Folhas novas normais de *C. Estrellensis* (A e E) e folhas deficientes em potássio (F), enxofre (C, G e H), nitrogênio (B) e fósforo (D). Folhas velha normais de *C. Estrellensis* (M) e deficientes em potássio (J), nitrogênio (I), cálcio (K) e magnésio (L, N e O). Folhas velhas normais com deficiência de cálcio (P). Folhas normais de *C. Estrellensis* (Q), versus aquelas deficientes em Ca (R e S) e K (T). Raiz normal de *C. Estrellensis* e com deficiência de P (U).

P deficiency has also been shown to cause the disorganization of thylakoid structures in the leaf chloroplasts of *Z. mays* (Hall et al., 1972) and *Gossypium hirsutum* L. (Zhao et al., 2001), and a decrease in the size of chloroplasts in *Ceiba speciosa* (Sorreano, 2006). In *C. estrellensis* leaf laminae, we found that P deficiency caused a decrease in the number of starch granules inside the cells as well as in the intercellular spaces. P is a structural component of nucleotides, phospholipids, co-enzymes, phosphoproteins, and nucleic acids, and its deficiency results in a shortage of adenosine triphosphate energy storage molecules that maintain photosynthetic processes (Mengel and Kirkby, 2001; Malavolta, 2006; Taiz and Zeiger, 2017). This may explain the disorganizations observed in the chloroplasts as well as the small number of starch granules and the decrease in intercellular spaces.

Mg deficiency caused changes in the chloroplasts, with both starch granulation and membrane rupture. Similar observations have been made in *Phaseolus vulgaris* L. (Thomson and Weier, 1962), *Zea mays* (Hall et al., 1972), *Hevea* sp. (Hamzah and Gómez, 1979), *Ceiba speciosa* (Sorreano, 2006), and *Ricinus communis* L. cultivar *Iris* (Lavres Junior et al., 2009). Mg is a key component of the chlorophyll structure, together with N and other elements (Taiz and Zeiger, 2017). Meanwhile, leaf laminae in *C. estrellensis* with Mg deficiency showed no changes compared to the control. Other signs of nutrient deficiency included low cytoplasmic volume under K deficiency, which has also been observed by Hamzah and Gómez (1979) in *Hevea* sp. Leaves, and increased intercellular spaces and deep changes in parenchymal cells associated with Ca and S deficiency. Inadequate K has previously been observed to affect cell expansion, promoting tissue compaction, and resulting in a reduction in leaf thickness, chloroplast degradation, and deformation in cell ultrastructures (Dickison, 2000); however, this was not observed in *C. estrellensis*.

The presence of intercellular spaces and the destructuring of the middle lamella under Ca deficiency have also been observed in *Ceiba speciosa* leaves (Sorreano, 2006). This occurs because Ca is a component of pectates that form the cell wall and middle lamella (Taiz and Zeiger, 2017) as well as the plasma membrane (Kirkby and Pilbeam, 1984). Ca is essential for membrane stability (Marinos, 1962) and cell wall rigidity (Christiansen and Foy, 1979). The degeneration of the cytoplasm in the tissue and disintegration of the

plasma membrane have been observed in potato sprouts with Ca deficiency, resulting in an overall reduction in growth (Hecht-Buchholz, 1979).

The formation of intercellular space, caused by the disorganization of cell ultrastructures, has been explained by Marinos (1962), who studied the submicroscopic aspects of Ca deficiencies in the shoot apex of barley and observed that the first indisputable signs of structural abnormalities appear when the nuclear membrane and plasma and vacuolar membranes are ruptured. The disorganization of other structures such as the Golgi complex and mitochondria also occurs, whereas plastids are more persistent, although eventually they also disintegrate. As Ca deficiency progresses, darkened spots on the cell walls and gaps may appear, indicating a weakening of their structure and formation of intercellular spaces (Marinos, 1962).

In sections of *C. estrellensis* leaves with S deficiency, chloroplasts with thylakoid disorganization and an absence of stromal lamellae were observed. Similar modifications have also been observed in S-deficient *Ceiba speciosa* leaves (Sorreano, 2006). The disorganization of chloroplasts owing to S deficiency begins with a decrease in protein synthesis based on S-containing amino acids, which leads to the accumulation of starch granules (Haneklaus et al., 2006; Malavolta and Moraes, 2007). In *C. estrellensis* leaves, S deficiency also led to a decrease in intercellular spaces owing to an increase in parenchymal cell size.

4.2 Visual symptoms of nutrient deficiency

N deficiency is the most likely cause and characteristic sign of uniform chlorosis in dicotyledon leaves (Raij, 1991; Malavolta, 2006; Taiz and Zeiger, 2017). Leaf chlorosis caused by a lack of N has been observed in other forest species, such as *Amburana acreana* (Ducke) A. C. Smith (Vieira et al., 2011), *Croton urucurana* Baill (Sorreano et al., 2011), *Swietenia macrophylla* King (Wallau et al., 2008), *Eucalyptus citriodora* Hook (Maffeis et al., 2000), *Bombacopsis glabra* (Pasq.) A. Robyns (Camacho et al., 2014), and *Schizolobium amazonicum* Herb. (Leite et al., 2017). Uniform chlorosis in N-deficient plant leaves is associated with decreased chlorophyll synthesis or the decomposition of proteins into simpler compounds (Kramer and Kozłowski, 1960; Fasabi, 1996; Malavolta et al., 1997). When the supply is inadequate, the N of old leaves is mobilized and redistributed to the younger

organs and leaves, causing chlorosis in the old leaves. However, in the present study, leaf chlorosis occurred in treatments with N deficiency, initially in new leaves and then in old leaves.

Chlorosis in new leaves caused by P deficiency has been observed in *Croton urucurana*, *Cyathorexylum myrianthum* Cham., *Acacia polyphylla* DC., *Lonchocarpus muehlbergianus* Hassl., *Ceiba speciosa*, and *Astronium graveolens* Jacq. (Sorreano, 2006), as well as in *Paubrasilia echinate* Lam. (Valeri et al., 2014). P deficiency causes a reduction in the growth of *Azadirachta indica* A. Juss (Silva et al., 2011), *Swietenia macrophylla* (Viégas et al., 2012), and *Handroanthus ochraceus* (Cham) Mattos. (Vieira et al., 2016) as well as causing longer main roots with fewer lateral roots in *Toona ciliata* M. Roem var. *australis* (Moretti et al., 2011) and *Acrocarpus fraxinifolius* Wight & Arn (Munguambe et al., 2017).

Similar to *C. estrellensis* under K deficient conditions, Sorreano et al. (2011) noted that the first symptoms of K deficiency in *Croton urucurana* were chlorosis in older leaves, followed by necrosis of the leaf margins and tips, and a decrease in apical dominance at 90 days after the start of the treatment. Chlorosis in older leaves under K deficiency was also observed in *Amburana acreana* (Vieira et al., 2011), *Swietenia macrophylla* (Wallau et al., 2008), *Handroanthus ochraceus* (Vieira et al., 2016), and *Schizolobium amazonicum* (Leite et al., 2017). The loss of old leaves after 90 days of K deficiency also occurred in *Khaya ivorensis* A. Chev. (Corcioli et al., 2014).

The collapse of the petiole and chlorosis in old leaves due to Ca deficiency in *C. estrellensis* has been observed previously in other species such as *Croton urucurana* (Sorreano et al., 2011), and *Spondias tuberosa* Arr. Câm (Gonçalves et al., 2006), and hybrid clones of *Eucalyptus grandis* W. Hill ex Maiden with *Eucalyptus urophylla* S.T. Blake (Silveira et al., 2002). Ca deficiency affects the activity of hormones and enzymes, including those that regulate the senescence and abscission of leaves (Marschner, 2012).

Interveinal chlorosis is a characteristic symptom of Mg deficiency that occurs first in older leaves owing to its mobility (Marschner, 2012). This chlorosis pattern occurs because the chlorophyll in the veins remains unchanged for long periods compared to the chlorophyll in the cells between the veins (Vitti et al.,

2006). Symptoms of Mg deficiency similar to those described in *C. estrellensis* have also been reported in *Schinus terebinthifolius* Raddi (Andrade and Boaretto, 2012), *Croton urucurana* (Sorreano et al., 2011), *Eucalyptus* spp. (Silveira et al., 2002), *Gmelina arborea* Roxb. (Haag et al., 1981), *Toona ciliata* M. Roem. (Moretti et al., 2011), *Azadirachta indica* (Silva et al., 2011), *Swietenia macrophylla* (Viégas et al., 2012), and *Bombacopsis glabra* (Camacho et al., 2014).

The chlorosis in new leaves caused by S deficiency in *C. estrellensis* has been described in other species such as *Schinus terebinthifolius* (Andrade and Boaretto, 2012), *Toona ciliata* (Moretti et al., 2011), *Swietenia macrophylla* (Wallau et al., 2008), *Spondias tuberosa* (Goncalves et al., 2006), *Tectona grandis* L. f. (Barroso et al., 2005), and *Schizolobium amazonicum* (Leite et al., 2017).

Deficiencies of N, P, K, Ca, Mg, and S are detrimental to the development of *C. Estrellensis*; therefore, ecological forest restoration projects using these plants in soils that require nutritional supplementation may have their success compromised if there is no nutritional complementation.

5. CONCLUSION

N, P, K, Ca, Mg, and S deficiencies caused a decrease in their respective content in the leaves, stems, and roots of *C. estrellensis*. Decreased levels of these nutrients cause malformations in cell ultrastructures, such as changes in the amount and shape of chloroplasts, starch granules, and lipids, which hindered the formation of leaf lamina cells and tissues and, consequently, led to the appearance of characteristic visual symptoms.

6. REFERENCES

- Andrade MLF. Deficiência nutricional em três espécies florestais nativas brasileiras. [dissertação]. Piracicaba, SP: Universidade de São Paulo; 2010.
- Andrade MLF, Boaretto AE. Deficiência nutricional em plantas jovens de aroeira-pimenteira (*Schinus terebinthifolius* Raddi). *Scientia Forestalis*. 2012;40(95):383-92.
- Barroso DG, Figueiredo FAMMA, Pereira RC, Mendonça AVR, Silva LC. Diagnóstico de deficiências de macronutrientes em mudas de teca. *Revista Árvore*. 2005;29(5):671-79. doi: 10.1590/

S0100-67622005000500002

Benedetti EL, Wink C, Santin D, Sereda F, Roveda LF, Serrat BM. Crescimento e sintomas em mudas de espinheira-santa com omissão de nitrogênio, fósforo e potássio. *Revista Floresta*. 2009;39(2):335-43.

Camacho MA, Camara AP, Zardin AR. Diagnose visual de deficiência de nutrientes em mudas de *Bombacopsis glabra*. *Revista Cerne*. 2014;20(3):427-31. doi:doi.org/10.1590/01047760201420031304

Carlos L, Venturin N, Farias ES, Venturin RP, Macedo RLG. Growth and mineral nutrition in seedlings of jacarandá-da-bahia subjected to nutrient deprivation. *Revista Floresta*. 2015;45(1):107-16.

Christiansen MN, Foy CD. Fate and function of calcium in tissue. *Communications in Soil Science and Plant Analysis*. 1979;10(1-2):427-42.

Corcioli G, Borges JD, Jesus RP. Sintomas de deficiência nutricional de macronutrientes em mudas de *Khaya ivorensis* cultivadas em solução nutritiva. *Pesquisa Florestal Brasileira*. 2014;34(78):159-64. doi:10.4336/2014.pfb.34.78.641

Dickison WC. *Integrative plant anatomy*. California: Academic Press; 2000. ISBN 9780122151705.

Dreschel P, Zech W. Foliar nutrient levels of broad leaved tropical trees: a tabular review. *Plant and Soil*. 1991;131:29-46.

Fasabi JAV. Carência de macro e micronutrientes em plantas de malva (*Urena lobata*), variedade br-01. [dissertação]. Belém, PA: Faculdade de Ciências Agrárias do Pará; 1996.

Gonçalves EO, Paiva HN, Neves JCL, Gomes JM. Crescimento de mudas de *sansão-do-campo* (*Mimosa caesalpiniaefolia* Benth.) sob diferentes doses de macronutrientes. *Scientia Forestalis*. 2010;38(88):599-09.

Gonçalves FC, Neves OSC, Carvalho JG. Deficiência nutricional em mudas de umbuzeiro decorrente da omissão de macronutrientes. *Pesquisa Agropecuária Brasileira*. 2006;41(6):1053-57. doi:10.1590/S0100-204X2006000600023

Haag HP, Gonçalves AN, Tenório Z, Tenório NA. Distúrbios nutricionais em *Gmelina arborea*. *O Solo*.

1981;73(2):33-38.

Hall JD, Barr R, Al-Abbas AH, Crane FL. The ultrastructure of chloroplasts in mineral-deficient maize leaves. *Plant Physiology*. 1972;50(3):404-09. doi:10.1104/pp.50.3.404

Hamzah S, Gómez JB. Ultrastructure of mineral deficient leaves of *Hevea*. 1. Effects of macronutrients deficiencies. *Journal of the Rubber Research Institute of Malaysia*. 1979;27(3):132-42.

Haneklaus S, Bloem E, Schnug E. Disease control by sulphur induced resistance. *Aspects of Applied Biology*. 2006;79:221-24.

Hecht-Buchholz CH. Calcium deficiency and plant ultrastructure. *Communications in Soil Science and Plant Analysis*. 1979;10(1-2):67-81. doi:10.1080/00103627909366879

Johnson CM, Stout PR, Broyer TC, Carlton AB. Comparative chlorine requirements of different plant species. *Plant and Soil*. 1957;8(4):337-53. doi:10.1007/BF01666323

Kerbauy GB. *Fisiologia Vegetal*. 2.ed. Rio de Janeiro: Guanabara Koogan; 2012.

Kirkby EA, Pilbeam DJ. Calcium as a plant nutrient. *Plant, Cell and Environment*. 1984;7(6):397-05. doi:10.1111/j.1365-3040.1984.tb01429.x

Kramer PJ, Kozlowski TT. *Physiology of trees*. New York: McGraw-Hill; 1960. ISBN-13 978-0070353510.

Lavres Junior J, Nogueira TAR, Cabral CP, Malavolta E. Deficiências de macronutrientes no crescimento e na produção da mamoneira cultivar Iris. *Revista Brasileira de Ciências Agrárias*. 2009;4(4):405-13. doi:10.5039/agraria.v4i4a6

Leite FAP, Weber OLS, Scaramuzza JF. Estudo comparativo de metodologias de preparo de soluções nutritivas no crescimento de mudas de paricá. *Ambiência*. 2017;13(2):273-92. doi:10.5935/ambiencia.2017.02.08

Lima JPC, Mello Filho JA, Freire LR, Vieira F. Absorção de nitrogênio para *Schizolobium parahyba* (VELL.) BLAKE, em fase de viveiro em três ambientes. *Floresta e Ambiente*. 2000;7(1):11-18.

- Lorenzi H. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. 2.ed. Nova Odessa: Platarum; 2006. v.3. ISBN 9788586714504.
- Maffeis AR, Silveira RLVA, Brito JO. Reflexos das deficiências de macronutrientes e boro no crescimento de plantas, produção e qualidade de óleo essencial em *Eucalyptus citriodora*. *Scientia Forestalis*. 2000;57:87-98.
- Malavolta E, Vitti GC, Oliveira SA. Avaliação do estado nutricional das plantas: princípios e aplicações. 2.ed. Ver. Atual. Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato; 1997.
- Malavolta E, Nogueira NGL, Heinrichs R, Higashi EN, Rodríguez V, Guerra E, et al. Evaluation of Nutritional Status of the Cotton Plant with Respect to Nitrogen. *Communications in Soil Science and Plant Analysis*. 2004;35(7-8):1007-19. doi:10.1081/CSS-120030577
- Malavolta E. Manual de nutrição mineral de plantas. São Paulo: Agronômica Ceres; 2006. ISBN: 9788531800474.
- Malavolta E, Moraes MF. Fundamentos do nitrogênio e do enxofre na nutrição mineral das plantas cultivadas. In: Yamada T, Abdalla SRS, Vitti GC, org. Nitrogênio e enxofre na agricultura brasileira. Piracicaba: International Plant Nutrition Institute; 2007. p.189-249. ISBN: 97885985036.
- Marinos NG. Studies on Submicroscopic Aspects of Mineral Deficiencies. 1. Calcium Deficiency in the Shoot Apex of Barley. *American Journal of Botany*. 1962;49(8):834-41. doi:10.1002/j.1537-2197.1962.tb15016.x
- Marschner P, ed. Mineral nutrition of higher plants. 3.ed. London: Academic Press; 2012. ISBN 0081014317.
- Mengel K, Kirkby EA. Principles of plant nutrition. 5.ed. Dordrecht: Kluwer Academic Publishers; 2001. ISBN 9781402000089.
- Moretti BS, Furtini Neto AE, Pinto SIC, Furtini IV, Magalhães CAS. Crescimento e nutrição mineral de mudas de cedro australiano (*Toona ciliata*) sob omissão de nutrientes. *Revista Cerne*. 2011;17(4):453-63. doi:10.1590/S0104-77602011000400003
- Munguambe JF, Venturin N, Silva MLS, Carlos L, Silva DSN, Farias ES, et al. Effect of deprivation of selected single nutrients on biometric parameters of cedar seedlings (*Acrocarpus fraxinifolius*) grown in nutritive solution. *African Journal of Agricultural Research*. 2017;12(39):2886-94. doi:10.5897/AJAR2017.12384
- Pimentel-Gomes F. Curso de estatística experimental. 13.ed. Piracicaba: Nobel; 1990.
- Raij BV. Fertilidade do solo e adubação. São Paulo: Agronômica Ceres; Piracicaba: Potafos; 1991.
- Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*. 1963;17(1):208-12. doi:10.1083/jcb.17.1.208
- Sarruge JR, Haag HP. Análises químicas em plantas. Piracicaba: ESALQ, Departamento de Química; 1974.
- Schumacher MV, Ceconi DE, Santana CA. Influência de diferentes doses de fósforo no crescimento de mudas de angico-vermelho (*Parapiptadenia rigida* (Benth) Brenan). *Revista Árvore*. 2004;28(1):149-55. doi:10.1590/S0100-67622004000100019
- Silva PHM, Stein LM. Produção de mudas e recomendações de adubação no viveiro para pequenos produtores. Piracicaba: Instituto de Pesquisas e Estudos Florestais; 2008. [accessed in 26 jul 2019.]. Available at: <http://www.ipef.br/silvicultura/producaomudas.asp>
- Silva RCB, Scaramuzza WLMP, Scaramuzza JF. Sintomas de deficiências nutricionais e matéria seca em plantas de nim, cultivadas em solução nutritiva. *Revista Cerne*. 2011;17(1):17-22. doi:10.1590/S0104-77602011000100003
- Silveira RLVA, Moreira A, Takashi EN, Sgarbi F, Branco EF. Sintomas de deficiência de macronutrientes e de boro em clones híbridos de *Eucalyptus grandis* com *eucalyptus urophylla*. *Revista Cerne*. 2002;8(2):107-16.
- Smith FW, Loneragan JF. Interpretation of plant

- analysis: concepts and principles. In: Reuter DJ, Robinson JB, editors. *Plant analysis: an interpretation manual*. Collingwood: Commonwealth Scientific and Industrial Research Organization; 1997. p. 1-33. ISBN 9780643101265.
- Sorreano MCM. Avaliação da exigência nutricional na fase inicial de crescimento de espécies florestais nativas. [tese]. Piracicaba, SP: Escola Superior de Agricultura “Luiz de Queiroz” - Universidade de São Paulo; 2006.
- Sorreano MCM, Malavolta E, Silva DH, Cabral CP, Rodrigues RR. Deficiência de macronutrientes em mudas de sangra d’água (*Croton urucurana*, Baill.). *Revista Cerne*. 2011;17(3):347-52. doi:10.1590/S0104-77602011000300008
- Sousa FF, Braga RM, Venturin N, Macedo RLG, Carlos L, Venturin RP. Exigências nutricionais de mudas de *Dipteryx alata* sob limitação nutricional. *Revista Ciência Florestal*. 2018;28(1):102-14. doi:10.5902/1980509831586
- Taiz L, Zeiger E. *Fisiologia e desenvolvimento vegetal*. 6. ed. Porto Alegre: Artmed; 2017. ISBN 9788582713662.
- Thomson WW, Weier TE. The fine structure of chloroplasts from mineral-deficient leaves of *Phaseolus vulgaris*. *American Journal Botany*. 1962;49(10):1047-55. doi:10.1002/j.1537-2197.1962.tb15045.x
- Valeri SV, Pizaia LGE, Sá AFL, Cruz MCP. Efeitos da omissão de nutrientes em plantas de *Caesalpinia echinata*. *Revista Cerne*. 2014;20(1):73-80. doi:10.1590/S0104-77602014000100010
- Viégas IJM, Lobato AKS, Rodrigues MFS, Cunha RLM, Frazão DAC, Oliveira Neto CF, et al. Visual symptoms and growth parameters linked to deficiency of macronutrients in young *Swietenia macrophylla* plants. *International Journal of Food, Agriculture and Environment*. 2012;10(1):937-40. doi:10.1234/4.2012.2834
- Vieira CR, Weber OLS, Scaramuzza JF, Costa AC, Souza TR. Descrição de sintomas visuais em função das deficiências de macronutrientes em mudas de cerejeira (*Amburana acreana*). *Revista Floresta*. 2011;41(4):789-96. doi:10.5380/rf.v41i4.25343
- Vieira CR, Weber OLS, Scaramuzza JF. Omissão de macronutrientes no crescimento inicial de *Tabebuia ochracea*. *Revista Ambiência*. 2016;12(4):869-83. doi:10.5935/ambiencia.2016.04.08
- Vitti GC, Lima E, Cicarone F. Cálcio, magnésio e enxofre. In: Fernandes MS, ed. *Nutrição Mineral de Plantas*. Viçosa: Sociedade Brasileira de Ciência do Solo; 2006. p. 299-325. ISBN 8586504025.
- Wallau RLR, Borges AR, Almeida DR, Camargos SL. Sintomas de deficiências nutricionais em mudas de mogno cultivadas em solução nutritiva. *Revista Cerne*. 2008;14(4):304-10.
- Zhao D, Oosterhuis DM, Bednarz CW. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. *Photosynthetica*. 2001;39:103-09. doi:10.1023/A:1012404204910