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Research Article

Characterization of Micronutrient Deficiency in Australian Red Cedar (*Toona ciliata* M. Roem var. *australis*)

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The Australian Red Cedar presents a great exploitation potential in Brazil, but works about the nutrient requirements and deficiency characterization in that species are still scarce. The objectives of this work were evaluating the effects of the omission of micronutrients and characterizing the nutrient deficiency symptoms in Australian Red Cedar saplings. The experiment was conducted in a greenhouse for a 90-day period. Australian Red Cedar cuttings were cultivated in pots with a nutrient solution under the missing element technique. The omission of the micronutrients B, Cu, Fe, Mn, and Zn affect negatively the height, diameter, and dry matter yield of the Australian Red Cedar plants. The micronutrient which affected the relative growth of the plants the most was B. Australian Red Cedar plants deficient in micronutrients present several visual symptoms characteristic of the metabolism disorders. The perception of the deficiencies through the visual diagnosis can be useful in the nutrient management of the culture of the Australian Red Cedar.

1. Introduction

With increasing demand for wood and the constant pressure for the purchase of raw materials in a sustainable way, forest enterprises have invested in new alternatives to the cutting of woods of native forests [1–3]. Species of the genus *Eucalyptus* and *Pinus* are the most spread in the commercial plantings [4, 5], but other species have aroused the interest of both foresters and researchers in Brazil [6–9]. The states of Espírito Santo and Minas Gerais already possess sapling-growing nurseries and commercial plantings of Australian Red Cedars. The species has adapted well in the Brazilian edaphoclimatic conditions and has presented a great productive potential. The wood produced is of low density [10] and highly valued in commerce, mainly in the furniture making [11].

For concerning a species recently introduced into the country, works about the nutrient requirements and characterization of deficiencies are still scarce, but indispensable to the management of the cultivations. Among the procedures to diagnose either the micronutrient deficiency or excess in plants, soil and plant analyses, visual evaluation, and knowledge of the history of the cultivated area stand out. In general, the plants present visual symptoms of deficiency when the level of some nutrient required is below that considered sufficient for the species. The deficiency of any micronutrient can cause problems in growth, development, and amount of production. Deficiency is the outcome of the decrease of uptake and concentration of the nutrient in the plant, which along the time becomes more and more perceptive through the visual symptoms [12], which in acute cases can lead the plant to death.

The symptoms of nutrient absence are characteristic in most of the crops [13]; nevertheless, they can be expressed at different extents or be confused with some anomaly exogenous to the plant; therefore, it becomes necessary to characterize it adequately. In that sense, works with the use of nutrient solutions have been conducted for several species, for they allow a more rigorous control of the composition of the solution which is to supply the plants' roots.

So, the aims of the present work were to evaluate the effect of the omission of the micronutrients B, Cu, Fe, Mn, and Zn in the development of Australian Red Cedar saplings and characterize the visual symptoms of deficiencies in plants.

2. Material and Methods

The work was conducted in a greenhouse of the Soil Science Department of the Federal University of Lavras, MG (Universidade Federal de Lavras, MG). Commercial clonal saplings of Australian Red Cedar (*Toona ciliata* var. *australis* (F. Muell.) Bahadur) were grown in 4 dm^3 pots with nutrient solution according to Hoagland and Arnon [14]. The utilized design was the completely randomized with seven treatments and four replicates. The saplings were transferred into the growing solution with all the nutrients at 60 days after cutting. For the adaptation of the plants, the ionic strength of the solution was reduced down to 25% of the original concentration in the first 07 days and 50% in the next week.

After the adaptation period, the saplings were transferred into the growing solution with ionic strength of 100%, which consisted in the treatments: full nutrient solution with nutrient omission; solution without born (−B); without copper (−Cu); without iron (−Fe); without manganese (−Mn); without zinc (−Zn); and without boron and zinc (–B–Zn). For the preparation of the solutions, the following sources were utilized: $NH_4H_2PO_4$, KNO_3, $Ca(NO_2)_3 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$, Fe-EDTA, H_3BO_3, $MnCl_2$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $H_2MoO_4 \cdot H_2O$. The concentration of each nutrient of the growing solution is detailed in Table 1.

The experiment was conducted for 90 days; in that period, the development in height and diameter of the saplings as well as the appearance and evolution of the visual symptoms of nutrient deficiency in the plants were evaluated. At the end of the experimental period, the plants were packed into paper bags, taken to the laboratory and separated into stem, leaves (including petioles), and roots. All the materials were dried in an forced air circulation oven at 65°C till it reached constant mass, its being weighted afterwards for determination of the dry matter of the shoot and roots.

The data were surveyed statistically with the aid of the SISVAR program [15]. The means were compared through the Scott-Knott test at 5% of probability. The relative growth index of the plants with omission of micronutrients in relation to the full nutrient solution-grown plants according to the following formula was computed [16]:

$$\text{CR (\%)} = 100 * \frac{\text{MST treatment with omission}}{\text{MST full treatment}}. \quad (1)$$

3. Results and Discussion

3.1. Height and Diameter Growth. The omission of the micronutrients Fe, Zn, and B and the omission in joint with B and Zn affected significantly the height of the Australian Red Cedar saplings. There was a reduction of the diameter growth of the plants mainly with omission of B, the lack of the elements contributed to the diameter reduction in the following order: = −B and −Zn > −Fe = −Cu > −Zn > −Mn (Figure 1).

Table 1: Nutrients concentration ($mg\,dm^{-3}$) in growing solution according to Hoagland and Arnon [14].

N	P	K	Ca	Mg	S	B	Cu	Mn	Mo	Zn
					$mg\,dm^{-3}$					
210	31	234	160	48	64	0,5	0,02	0,5	0,01	0,05

Boron was the nutrient most limiting to the development of plants (Figure 1); this was found both in the treatment without born and in the treatment without B and Zn. At 65 days of growing in nutrient solution without born, the interruption in the height and diameter growth of the plants was verified; afterwards, the plants died. The functions of B in the plant have not been completely elucidated by research [12]. The literature reports the involvement of B in the structure of organic compounds (phenols, organic acids, and amino acids), in the enzyme regulation and in uracil biosynthesis. B deficiency inhibits the activity of the enzymes glucose-6-phosphate dehydrogenase, AIA oxidase, polyphenol oxidase, and RNAse [17, 18].

The plants submitted to the Zn treatment presented both height and diameter 17% as small as the full treatment; this can be related to the function of enzymatic activator played by Zn in plants [12]. Thus, the absence of Zn brought about a decrease of the leaf expansion and stem elongation; hence, there was a shortening of the internodes. The symptoms observed can have occurred due to the decrease of the synthesis of RNA, proteins, and AIA which need Zn for their formation [19].

There was no difference in the plants' height grown in nutrient solution without Cu relative to the full treatment; nevertheless, Cu omission affected the diameter growth of the Australian Red Cedar. This can have direct implications in the volume of wood produced by the cedar. The literature reports the function of copper in the plant as a structural component of proteins and processes such as photosynthesis, respiration, and hormonal regulation; so, Cu deficiency affects cell division and decreases the development and vigor of the plant [18, 19].

The plants cultivated in nutrient solution with omission of Fe presented less height and diameter than the plants grown with all the nutrients (full treatment). This can be explained by the functions of this nutrient as a component of a series of enzymes and by its involvement in electron transport via cytochromes and ferredoxin [20]. In this way, Fe-deficient plants have photosynthesis hindered and, hence, less energy supply to keep their development [18].

Mn omission did not affect the height and diameter growth of the plants during the evaluated period. Apparently, the Australian Red Cedar presents poor nutrient requirement in manganese. These results agreed with those obtained by other authors who studied the effect of the Mn omission in forest species. Da Silva et al. [21] did not find the influence of Mn omission upon the height and diameter growth of Mogno saplings till the 120 days. Other authors as de Wallau et al. [22] also found no response to Mn supply in saplings of

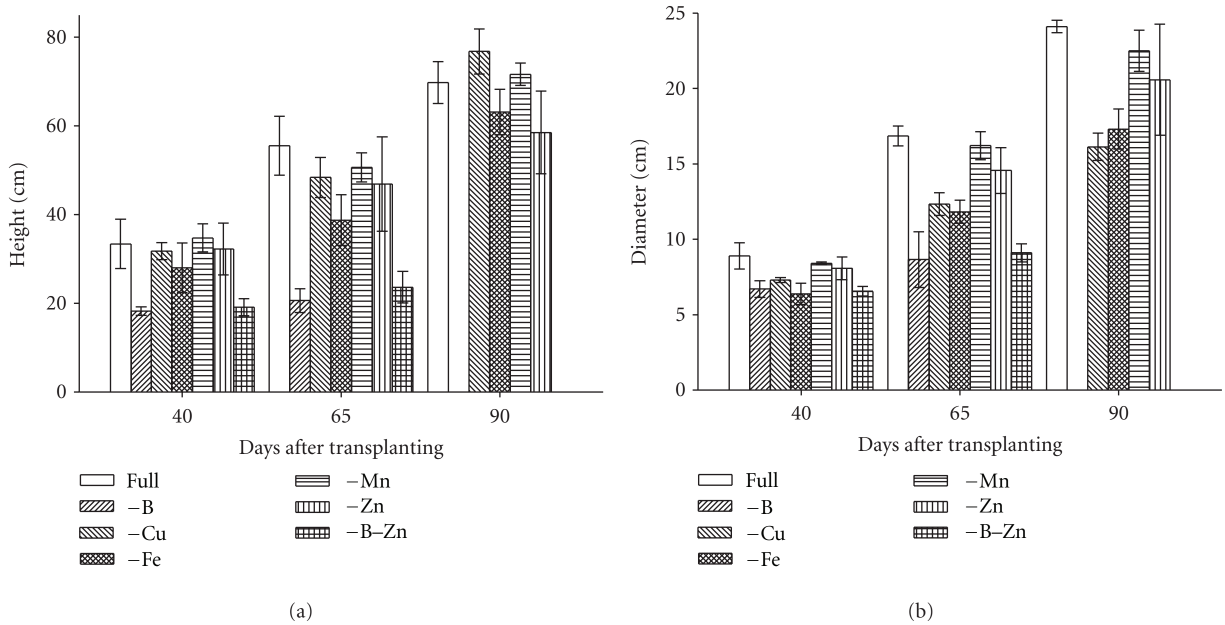


Figure 1: Height (a) and diameter (b) of the root collar of the Australian Red Cedar on the basis of the omission of Cu, Fe, Mn, B, and Zn, evaluated at 40, 65, and 90 days after transplanting.

forest essences in the early phase of development, though Mn is the micronutrient most required by the crops and performs several functions in the plant [12]. The poor response to Mn omission may be related to the adequate Mg supply, since those elements may replace one another in the activation of a great deal of enzymes [18].

Moretti et al. [23] observed no differences in the height and diameter of the plants under the omission of micronutrients in studying the effect of the micronutrient deficiency in Australian Red Cedar grown in pots with Typical Red Latosol. The authors ascribed the result to the possible supply of micronutrients by soil and to the redistribution of some elements by the plant.

3.2. Yield of Total Dry Matter Yield (MST), Shoot Dry Matter (MSPA), and Root Dry Matter (MSR). The omissions of the evaluated micronutrients influenced total dry matter yield (MST). MST yield was according to the following decreasing order: full > −Mn > −Zn > −Cu = Fe > −B = −Zn e −B (Figure 2).

Boron was the nutrient which limited the most the growth of the Australian Red Cedar; hence, the plants of the treatments where the omission of this nutrient yielded less amount of dry matter, stressing the increased born requirement of the species. B deficiency may have caused a poor formation of the tissue of the vascular cambium vascular, which is related to the formation of conducting vessels, causing collapse of the phloem and xylem. So, there was reduction in the growth of the root owing to the supply in photosynthates and of the shoot on the grounds of the poor water-and-nutrient-absorbing capacity [24]. Similar result was found by Locatelli et al. [25] who evaluated the development of the Spanish cedar (*Cedrela odorata* L.)

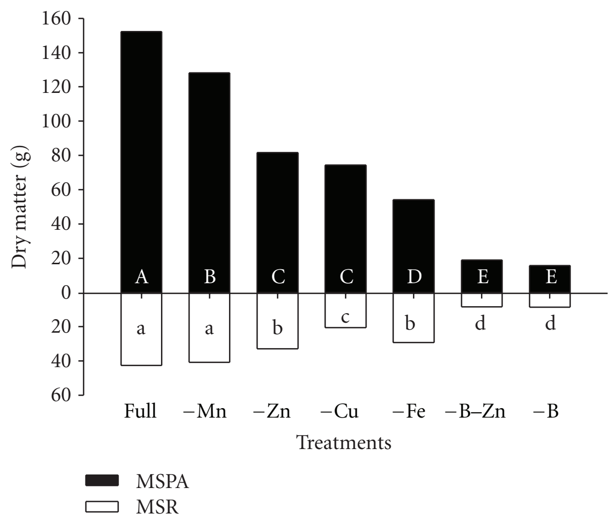


Figure 2: Total dry matter yield (g) of the Australian Red Cedar plants after 90 days in pot with a nutrient solution. Data followed by equal letters do not differ from one another by the Scott Knott test at 5% of probability. Statistical analyses for means at 90 days.

under the omission of micronutrients and found that boron deficiency was the one which limited dry matter yield the most.

As well as for the height growth, out of the micronutrients omitted, Mn was that which interfered in total dry matter yield the least. In a study conducted on mahogany (*Swietenia macrophylla* King) under the micronutrient omission, Da Silva et al. [21] also found that manganese omission does not interfere in dry matter yield, the result being ascribed to the poor requirement of the crop for the nutrient.

The shoot dry matter yield (MSPA) was influenced by the omission of the micronutrients Mn, Zn, Cu, Fe, and B and the joint omission of B and Zn (Figure 2). The greatest shoot dry matter yield was observed in the full treatment. The best performance of the complete treatment as to the MSPA yield as well as the high quality observed of the plants prove that the balance in the macro- and micronutrient supply is essential to the excellent plant development [13]. The influence of the nutrient omission on the shoot dry matter yield decreases in the following order: –B–Zn = −B > Fe > Cu = Zn > Mn.

Root dry matter yield (MSR) was influenced by the omission of the micronutrients Zn, Fe, Cu, and B and joint omission of B–Zn (Figure 2). The highest values of MSR were obtained in the treatments full and under manganese omission (−Mn), reinforcing the poor manganese requirement hypothesis of the species. Increased reduction in the root dry matter yield in relation to the complete treatment was found, respectively, with the omission of B (−B) and joint of B and Zn (–B–Zn), followed by the Cu omission and at last of either Fe or Zn.

The poor performance of the sapling under the joint omission of B and Zn occurred mainly in the absence of B (Figure 2). That can be proved by the nonexistence of significant difference in dry matter yield both of the shoot and of the root, among the plants cultivated under B omission and joint of B and Zn. The lowest MSPA and MSR yield in the treatment under boron omission may be related to a poorer integrity of membranes of the root tips with consequent malformation of cells, earliness of differentiation, and death of meristematic tissues [26, 27].

3.3. Relative Growth (CR). According to Sánchez [28], nutritional deficiency is regarded as severe only when the dry matter yield decreases 40% in relation to the full treatment. So, one can consider as a severe deficiency the ones found in the treatments −B, –B–Zn, −Fe, −Cu, and −Zn (Figure 3) with CR of 12%, 14%, 43%, 49%, and 59%, respectively. The treatment −Mn was the one which approached the most of the full treatment (Figure 3), CR of 87%, not being considered a severe deficiency.

3.4. Visual Symptoms of Deficiency. B deficiency symptoms were the first to be observed at 20 days after the start of the treatments (Figure 4). The young leaves presented themselves deformed (wrinkled) and smaller than the leaves of the plants of the complete treatment, with subsequent occurrence of fall of the first pairs of leaves, but persistence of the mature leaves. The death of the apical bud and exudation close to the axillary buds is also observed. In the most advanced stage of symptomatology, death of the terminal stem cuttings and of branches with secondary growth of lateral buds along the stem occurred. Those plants presented still leathery leaves with suberization and slits along the veins. On the stem, there was formation of protuberances and exudation of gum stressing the collapse and generalized death of cells as a final result of the deficiency. Silveira [29] also found the same symptoms in hybrid clone of *Eucalyptus grandis* with *Eucalyptus urophylla* in nutrient solutions.

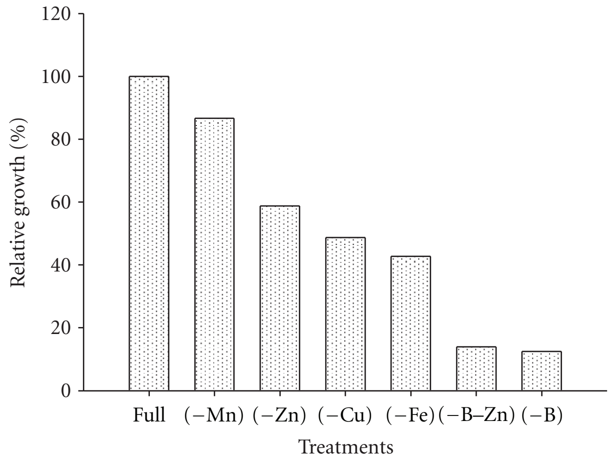
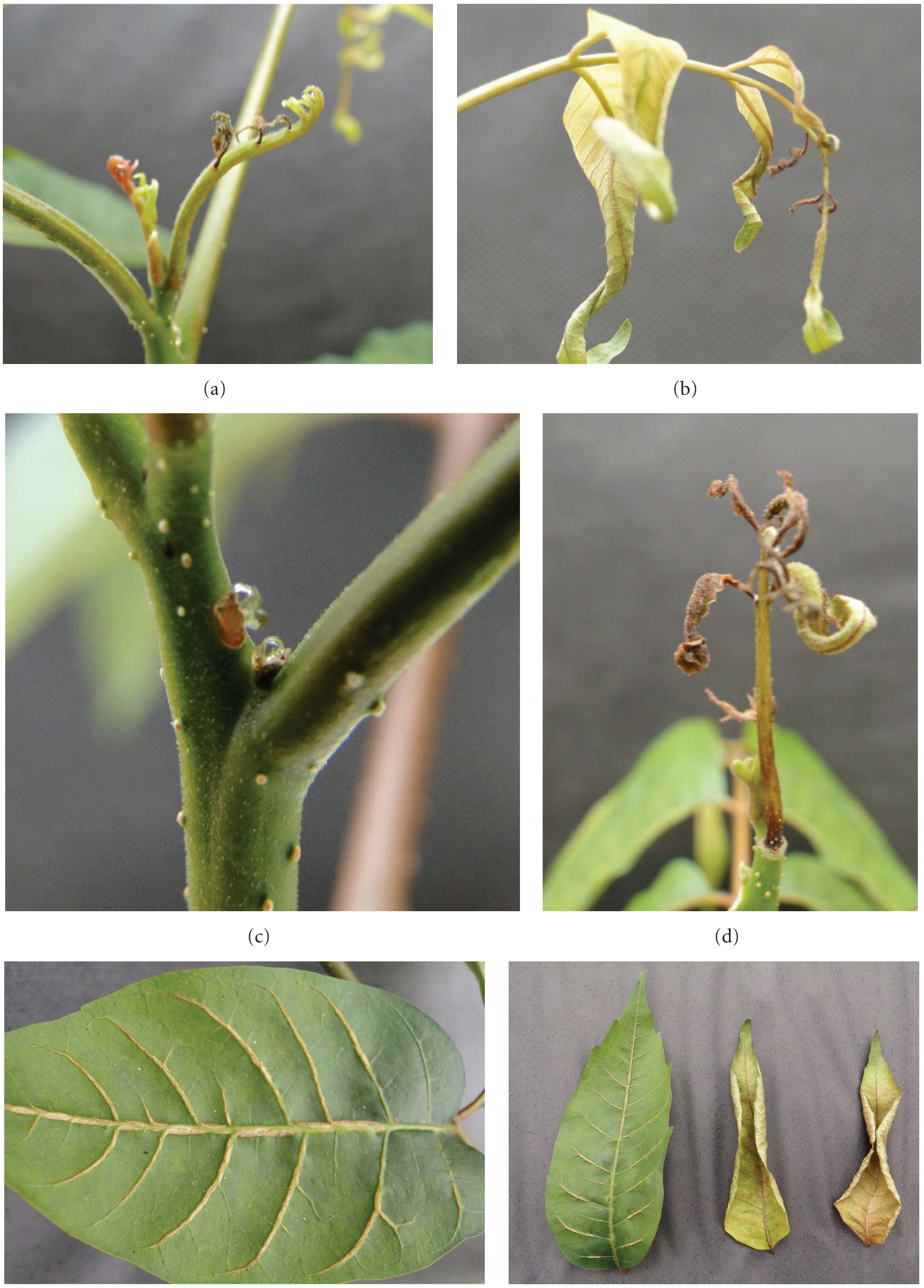


Figure 3: Relative growth (%) of Australian Red Cedar plants in relation to total dry matter yield of the full treatment.

Boron undergoes unidirectional transport in the xylem, via transpiration stream, being practically still in the phloem. In this way, Boron accumulates in the older leaves and the symptoms occur in the younger leaves or organs with less transpiration stream [19]. As regards the cell wall, it is proved that B-deficient plants have walls resistant than the nondeficient plants [30]. That explains the fact that the younger leaves became both deformed and small (Figure 4).

The plants of the treatment −Mn presented normal appearance till the first two months after the omission of the nutrient (Figure 5). After that period, a slight alteration in the coloration of the leaves and bending of the leaflets was noticed, but without evolution of the symptom. de Wallau et al. [22] reported a darker green coloration of the leaves of mahogany saplings in nutrient solution, but with size of the plants similar to that of the treatment in full nutrient solutions. When there is severe Mn deficiency, a break of the chloroplast structure occurs which cannot be reversed. Mn is fundamental in the electron chain transport during photosynthesis as soon as deficiency of that nutrient takes place; the reaction light during photosynthesis is seriously damaged as well as all the other reactions associated with electron transport. Chloroplasts are the cell organelles most sensitive to Mn deficiency, which brings about the disorganization of the lamellar system and consequently more visible symptoms of chlorosis [31].

Under Cu omission, the first symptoms at 60 days after the start of the treatment were observed (Figure 6). Deficiency symptoms were found in the youngest leaves, due to the poor mobility of Cu in the plant, with manifestation of wilt and bluish punctuations on leaves on the medium part of the plants. With evolution of the symptoms, chlorosis followed by necrosis with fall of leaves and death of the petiole was observed. According to Marques et al. [32], copper deficiency can be characterized by bluish-green and flaccid leaves with their increased sizes depending on the species. The appearance of wilted leaf in the Cu-deficient



(a) (b)

(c) (d)

(e) (f)

Figure 4: Symptoms of boron deficiency in Australian Red Cedar seedlings. Death and fall of young leaves ((a) and (b)), gum exudation (c), death of apical bud (d), slits along the leaf veins (e), and progress of the symptom in the leaf (f).

plants may be explained by the role of the micronutrient in tissue lignification. Cu deficiency decreases the activity of polyphenol oxidase, ascorbate oxidase, and diamine oxidase, important enzymes in the pathway of transformation of phenols into lignin [31].

In spite of the iron deficiency being little common in the Brazilian soils, its characterization is of vital importance, since in sapling production poor supply of this nutrient can occur, harming the plant's development. In addition to the poor height and diameter of the plants in relation to the plants of the full treatment, Fe omission (Figure 7) provoked alterations in the coloration of leaves. At first, the Fe-deficient plants presented young leaves with interneval chlorosis forming a fine reticulate (green fine network of veins on yellow background), that symptom progressed to the other leaves and caused a generalized chlorosis on the upper

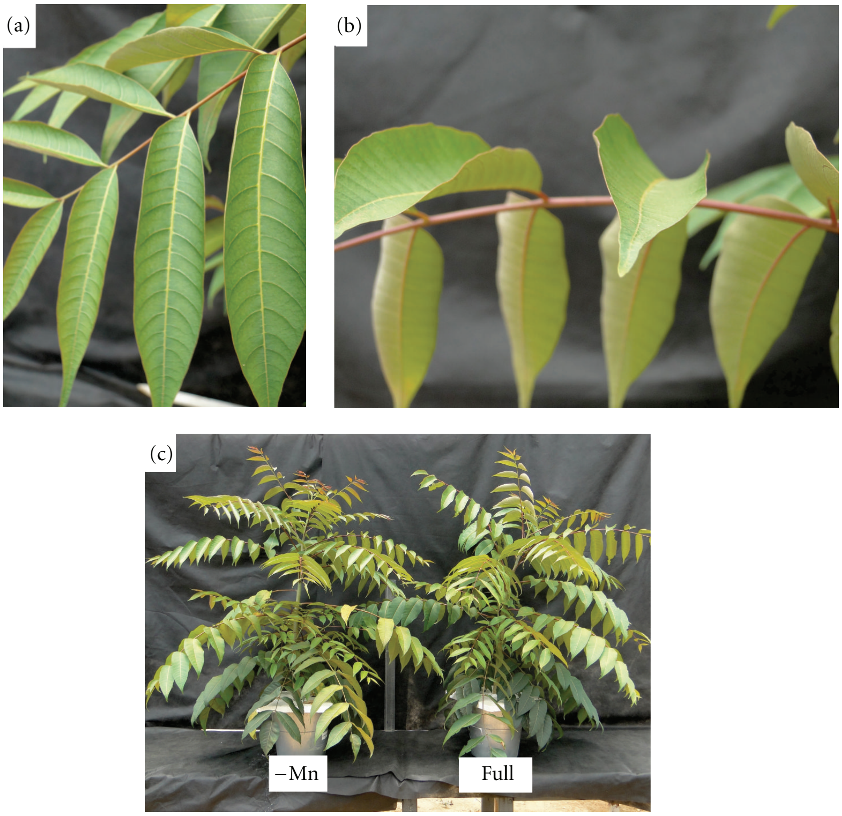


Figure 5: Symptoms of manganese deficiency in Australian Red Cedar seedlings. Bent leaves ((a) and (b)), and young leaves lighter in relation to the plants in the full treatment (c).

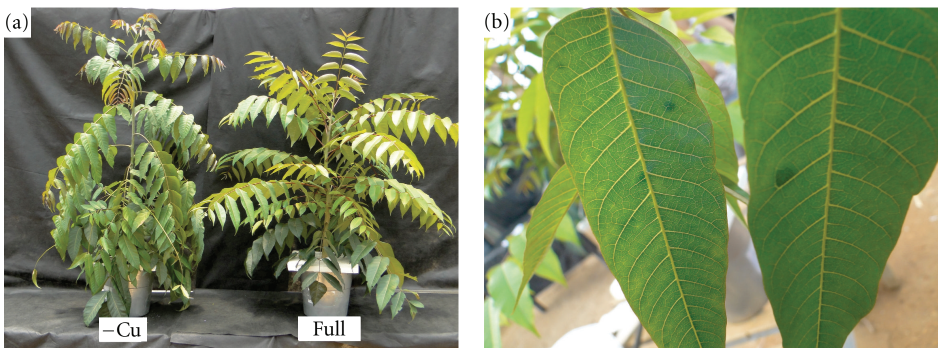


Figure 6: Symptoms of Cu deficiency in Australian Red Cedar seedlings. Wilt of leaves (a) and green-bluish punctuations on the leaflets (b).

half of the plants. Similar symptoms were reported by de Wallau et al. [22] on mahogany plants. Fe deficiency affects initially the development and function of the chloroplast. The electron transport chain during the photosynthesis in the thylakoid membrane of chloroplasts contains several heme groups containing iron and iron-sulfur clusters. Under iron deficiency, there is a decrease of chlorophyll and other pigments which entrap light [31]; therefore, the Fe-deficient Australian Red Cedar plants presented chlorosis on the leaflets of the young leaves.

The plants with Zn omission presented deficiency symptoms on the young leaves at 30 days after the start of the treatment. Internerval chlorosis was found, which differed from that observed in the absence of iron for forming a thick reticulate. In addition to veins, a narrow strip of the green tissue formed along the same ones (Figure 8). Leaves of reduced sized, narrow, and more marked lanceolate in shape were observed. Shortening of the internodes was verified and as a consequence, poor growth in relation to the plants of the full treatment. The inhibition of internode elongation of the plants is related to the poorer production of indoleacetic acid (AIA) which is responsible for plant growth. Tryptophan, precursor of AIA biosynthesis, requires Zn in its formation; so, when Zn deficiency occurs, less AIA is synthesized.

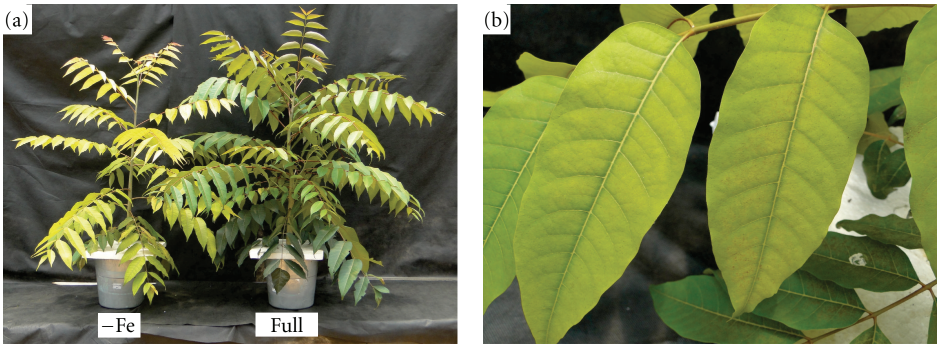


Figure 7: Symptoms of Fe deficiency on Australian Red Cedar seedlings. Chlorosis, smaller size and number of leaves in the treatment with Fe omission in relation to the full treatment (a) fine reticulate on the leaflets (b).

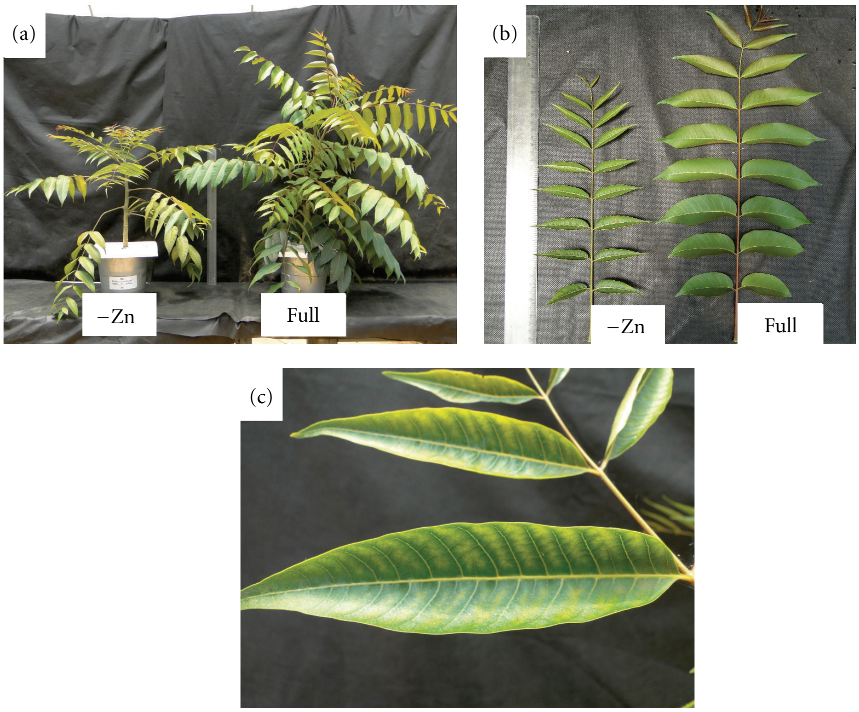


Figure 8: Symptoms of Zn deficiency on Australian Red Cedar seedlings. Fall of leaflets and smaller size of the plant (a), lanceolate leaf (b) interveinal chlorosis (c).

In addition, enzyme superoxide dismutase contains Zn and plays an important role in removing superoxide radicals (O_2); in that way, under Zn deficiency, O_2 generation is increased and there is increased oxidation of AIA by the greater activity of peroxidase or of free O_2 radicals [12, 31].

Zn deficiency also interferes in photosynthesis, since Zn is part of the enzyme carbonic anhydrase localized in the cytoplasm and in the chloroplasts, which can make CO_2 transfer to photosynthetic fixation easy. When Zn deficiency occurs, the activity of carbonic anhydrase decreases which brings about less photoassimilate production and consequently poorer formation of carbon skeletons for the formation of amino acids and proteins [18, 31].

4. Conclusions

The omissions of the micronutrients B, Cu, Fe, Mn, and Zn affects negatively the height, diameter, and dry matter yield of the Australian Red Cedar plants, seeing that the micronutrient which harmed the most the relative growth of the plants was B.

The main deficiency symptoms of the Australian Red Cedar cultivated without Mn were bending of the leaves upwards and a slight chlorosis. The plants cultivated without Cu presented leaf wilting and the presence of blue points on the young leaves. There was a reduction in the internodes and lanceolate-shaped leaves in the plants cultivated without

Zn. And Fe absence brought about plants' growth delay and chlorosis of the young leaves. B deficiency caused shriveling of the young leaves and morphological alterations both in the shoot and in the root.

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