Full Length Research Paper

Effect of deprivation of selected single nutrients on biometric parameters of cedar seedlings (*Acrocarpus fraxinifolius*) grown in nutritive solution

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The purpose of this work was to assess biometric parameters of cedar seedlings (*Acrocarpus fraxinifolius*) associated with visual symptomatology of macronutrients deficiency, growth rate, and content and accumulation of nutrients in aerial parts of seedlings grown in nutritive solution. The trial was established in completely randomized design with seven treatments, four replications, and one plant per pot. Treatments consisted of complete nutrient solution, Hoagland and Arnon, and deprivation of the following selected single nutrients: N, P, K, Ca, Mg and S. Micronutrients were provided to all treatments. The following biometric parameters were assessed 90 days after the transplant: shoot height, root collar diameter, root and shoot dry weight. The Dickson index and the ratio given by roots dry weight and shoot dry weight were obtained, as well as the content and accumulation of nutrients in aerial parts of seedlings. The Sisvar statistical analysis system was run for analysis of variance, and averages were compared by means of Scott-Knott test at 5% significance. Treatments consisting of omissions of macronutrients were found to be limiting factors of plants growth and obtaining of seedlings shoot dry weight in the following order: N > P > K > Ca > Mg > S. Deficiencies of these macronutrients caused visible morphological abnormalities, where common symptoms of deficiency of N, P, K and Ca appeared before symptoms of deficiency of Mg and S. The content of nutrients found in aerial parts of seedlings of *A. fraxinifolius* grown in nutritive solution was greater for treatments consisting of deprivation of N, P and K. The content of nutrients found for treatments consisting of deprivation of Mg and S was not significantly different from that found for the complete nutrients solution.

**Key words:** Nutritional requirements, forest nutrition, Indian cedar.

INTRODUCTION

The increased demand for forest products has led to constant search for novel silvicultural techniques to increase the productivity of forest plantations worldwide. Although, Brazil has the largest area of natural tropical forest in the world, there is a successful experience in introducing species from other countries, such as species
of the genera, Eucalyptus and Pinus. In addition, a new species, *Acrocarpus fraxinifolius*, has recently aroused the interest of Brazilian researchers for its rapid growth (Gonçalves et al., 2012).

*A. fraxinifolius* is a large deciduous emergent tree native to the family, Fabaceae, subfamily Caesalpinioideae. This has been used as shade trees in the coffee plantations in India, as well as for wood production and/or forest enrichment. Besides, this species is found to be the best-suited tree for plantations in badly degraded areas which are not protected from cattle grazing (Gonçalves et al., 2012; Martínez et al., 2006).

A review is provided of the current state of understanding of *A. fraxinifolius*, focusing on silvicultural aspects, plantations management and utilization of the specie (Mishra et al., 2015; Martínez et al., 2006). However, there is no sufficient information regarding the nutritional requirements of the species so far, which can compromise the success of projects of reforestation and recomposition of native areas (Sorreano et al., 2012; Aquino et al., 2013). Thus, considering that fertilization practices are fundamental for the production process of high quality seedlings, and to enable forest plantations to reach adequate growth stages on field (Gonçalves et al., 2012; Mishra et al., 2015), a study on fertilizing of potentially producing species is found necessary.

Nutritional requirements refer to amounts of macronutrients and micronutrients that a given crop takes from soil, fertilizer and air, to attend its requirements, to grow up and produce adequately. The amount of nutrients required is described as function of the existing contents in the plant material, as well as from the total dry matter (Faquin, 1994). Regarding forest species, studies have demonstrated that fertilizing of such species increase productivity, quality and establishment of forest plantations over time (Braga, 1995).

One of the ways used to assess nutritional requirements of plant species is by means of the Minus One Element Technique, which determines soil nutrient deficiencies in actual field or greenhouse conditions, based on the law of minimum. This technique consists of testing a complete nutrient treatment along with treatments based on individual omissions of other crop nutrients (Silva et al., 2016) and provides semiquantitative nutrients-related data that may limit plants development (Malavolta, 1980; Vieira et al., 2008; Locatelli et al., 2007; Silva et al., 2005; Matheus et al., 2011; Silva et al., 2011; Andrade and Boaretto, 2012).

The rationalization of plants nutrition management in the seedlings production process may increase the knowledge on interactions between nutrients in higher plants, thus contributing to the productivity of forest plantations (Martínez et al., 2006). When one nutrient is missing or deficient for example, such deficiency causes anomalies due to changes made to the plant metabolism (Epstein and Bloom, 2006). The purpose of this study was to assess the effect of deprivation of selected single nutrients (N, P, K, Ca, Mg and S) on biometric parameters of cedar seedlings (*A. fraxinifolius*) grown in nutritive solution.

**MATERIALS AND METHODS**

The trial was conducted in a greenhouse in the Forest Nursery of the Federal University of Lavras (UFLA) located in Lavras, State of Minas Gerais, Brazil, at 21°14' South, 44°00' West and 919 m elevation. Seeds of *A. fraxinifolius* were collected from mother trees at the Historical Campus of UFLA, cleaned and scarified in the Laboratory of Silviculture of the Department of Forest Sciences, as outlined in Venturin et al. (2014). Then, seeds were sown in 55 cm³ tubes containing vermiculite substrate, and wet using deionized water.

After reaching 5 to 10 cm height, about 30 days after sowing, seedlings were washed with deionized water in bare root and transplanted to a plastic tray containing 20 L of complete nutrient solution, Hoagland and Arnon (1950). The nutrient solution was maintained under constant aeration with compressed air to maintain the air flow and oxygenate the hydroponic nutrient solution. This solution was at 30 and 60% of its ionic force and seedlings were kept for 15 days in each solution as outlined in Marques et al. (2004). After these days, which are described here as the adaptation period, each seedling was transplanted in a 5 L pot and put on a stand under constant aeration. The pH rose to about 5.5 and very little precipitation occurred. Seedlings were then fixed by means of the stem with the help of polystyrene sheets of about 2 cm thick (Silva et al., 2016).

The trial was established in completely randomized design with seven treatments, four replications, and one plant per pot. Treatments consisted of complete nutrient solution Hoagland and Arnon, and omissions of selected single nutrients, namely N, P, K, Ca, Mg and S, based on the law of minimum. Micronutrients were provided to all treatments under study.

Analytical reagent and deionized water were used to prepare nutrient stock solutions. The nutrient solution was changed biweekly, since it was used for a fast-growing forest specie, and because is characterized by high concentration of nutrients (Epstein and Bloom, 2006). Plants were daily monitored and the solution volume was completed using deionized water whenever it was necessary. Seedlings were constantly monitored to diagnose nutrient deficiency symptoms under test and the first common symptoms of each nutrient were, in general, observed on seedlings about 45 days after the transplant, except symptoms of deficiency of N that appeared 20 days earlier.

About 90 days after the transplant, shoot height (H) and root collar diameter (D) were measured. After these assessments, plants were harvested, separated into shoots and roots, and washed in running water and deionized water. Then, plants were dried in a forced air heating system (hothouse) at 65°C temperature (Sorreano et al., 2011). Thus, the plant material was weighted on a
Acrocarpus 2.5

In this study, 5 s ÷ diameter (76.6%), shoot dry weight (98.3%), root dry weight (78.7%), total dry matter (95.6%), ratio given by shoot dry weight divided by root dry weight (91.6%), and Dickson quality index (89.4%) (Table 1). According to Souza and Fernandes (2006), this finding was expected because this nutrient is commonly required in greater amounts and is found to be the most limiting factor for crops growth. Besides, when the N is missing, the synthesis of proteins and nucleic acids is compromised, causing a reduced plants growth (Marschner, 2012). For example, studies have shown that deficiency of N causes a significant reduction of shoot and root dry weight in many crops (Silva et al., 2016; Moretti et al., 2011; Silva et al., 2011; Camacho et al., 2014; Corcioli et al., 2014).

In the same context, the treatment consisting of omission of P affected significantly the plants height (63.8%), root collar diameter (73.5%), shoot dry weight (93.2%), root dry weight (81.28%), total dry matter (91.6%), ratio given by shoot dry weight divided by root dry weight (61.6%), and Dickson quality index (89.8%) (Table 1). In general, the deficiency of P limits the plant growth because this macronutrient is found as part of key-molecules of cell metabolism such as ATP and nucleic acids (George et al., 1995). In this study, seedlings grown under deficiency of P showed a reduction in plant size, leaves and root weight, and long roots and few lateral roots. P plays an important role in the synthesis of energy, so that its deficiency may be reflected in reduced plant growth (Taiz and Zaiger, 1998). Similar results were also found in many other studies (Benedetti et al., 2009; Moretti et al., 2011; Vieira et al., 2016).

Regarding the deficiency of K, a significant reduction was found for the following parameters: plants height (44.9%), root collar diameter (60.4%), shoot dry weight (78.7%), root dry weight (79.6%), total dry matter (78.8%), and Dickson quality index (82.5%) (Table 1). These findings were expected since K is commonly required by plants in greater amounts (Niu et al., 2013). In addition, this nutrient is associated with osmoregulation.

### RESULTS AND DISCUSSION

**Growth of seedlings**

Treatments consisting of omissions of selected single macronutrients, namely N, P, K, Ca, Mg and S were found to be the most limiting for all morphological parameters under study in the following order: N > P > K > Ca > Mg > S. In addition, statistically significant differences between treatments were found for all variables under study (Table 1).

The treatment consisting of omission of N showed the major restrictive effect on the seedlings growth. When the N was missing, biometric parameters reduced significantly as compared to the complete nutrient solution as follows: plants height (81.1%), root collar diameter (76.6%), shoot dry weight (98.3%), root dry weight (78.7%), total dry matter (95.6%), ratio given by shoot dry weight divided by root dry weight (91.6%), and Dickson quality index (89.4%) (Table 1). According to Souza and Fernandes (2006), this finding was expected because this nutrient is commonly required in greater amounts and is found to be the most limiting factor for crops growth. Besides, when the N is missing, the synthesis of proteins and nucleic acids is compromised, causing a reduced plants growth (Marschner, 2012). For example, studies have shown that deficiency of N causes a significant reduction of shoot and root dry weight in many crops (Silva et al., 2016; Moretti et al., 2011; Silva et al., 2011; Camacho et al., 2014; Corcioli et al., 2014).

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### Table 1. Biometric parameters obtained for cedar seedlings (Acrocarpus fraxinifolius) grown under nutrients omission, 90 days after the transplant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H (cm)</th>
<th>D (mm)</th>
<th>SDW (g)</th>
<th>RDW (g)</th>
<th>TDM (g)</th>
<th>SDW+RDW</th>
<th>DQI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>87.87 a</td>
<td>16.07 b</td>
<td>78.27 a</td>
<td>12.18 b</td>
<td>90.45 a</td>
<td>6.59 a</td>
<td>7.56 a</td>
</tr>
<tr>
<td>minus N</td>
<td>16.62 d</td>
<td>3.75 d</td>
<td>1.36 b</td>
<td>2.59 c</td>
<td>3.95 b</td>
<td>0.53 b</td>
<td>0.80 c</td>
</tr>
<tr>
<td>minus P</td>
<td>31.75 c</td>
<td>4.25 d</td>
<td>5.34 b</td>
<td>2.28 c</td>
<td>7.63 b</td>
<td>2.53 b</td>
<td>0.77 c</td>
</tr>
<tr>
<td>minus K</td>
<td>48.37 b</td>
<td>6.37 c</td>
<td>16.68 b</td>
<td>2.48 c</td>
<td>19.16 b</td>
<td>6.85 b</td>
<td>1.32 c</td>
</tr>
<tr>
<td>minus Ca</td>
<td>51.50 b</td>
<td>7.37 c</td>
<td>10.35 b</td>
<td>1.48 c</td>
<td>11.83 b</td>
<td>7.57 b</td>
<td>0.85 c</td>
</tr>
<tr>
<td>minus Mg</td>
<td>96.62 a</td>
<td>14.12 b</td>
<td>79.80 a</td>
<td>9.06 b</td>
<td>88.86 a</td>
<td>9.32 a</td>
<td>5.64 b</td>
</tr>
<tr>
<td>minus S</td>
<td>100.42 a</td>
<td>18.37 a</td>
<td>80.02 a</td>
<td>12.76 a</td>
<td>92.78 a</td>
<td>6.34 a</td>
<td>7.91 a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>13.54</td>
<td>13.12</td>
<td>20.36</td>
<td>24.68</td>
<td>18.95</td>
<td>32.01</td>
<td>22.04</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. H = shoot height, D = root collar diameter, SDW = shoot dry weight, RDW = root dry weight, TDM = total dry matter and DQI = Dickson quality index. CV = coefficient of variation.
Nitrogen (N)

The chlorosis appeared firstly on mature leaves about 25 days after the transplant (Figure 1A). Young leaves appeared already chlorotic, with size drastically reduced. After 90 days, there was a generalized chlorosis in the leaf blade, characteristic of deficiency of N, as well as in all leaves. Similar symptoms were described by Silveira et al. (2002), on hybrids of Eucalyptus grandis × Eucalyptus urophylla.

Phosphorous (P)

Deficiency of P resulted in the shorter and narrow leaf blade. The inhibition of axillary buds caused a reducing growth and branching, resulting in smaller plants (Figure 1B). The leaf blade showed a dark green coloring at first and, then, chlorotic and withered folioles. Symptoms of deficiency of P occur because this macronutrient plays an important role in the metabolism of the energy of plants such as photosynthesis and respiration (Furlani, 2004). Thus, the growth of plants under deficiency of P is retarded (Mengel and Kirkby, 1987). Similar findings were described by Sorreano et al. (2012) for seedlings of Astronium graveolens Jacq. and Enterolobium contortisiliquum (Vell).

Potassium (K)

The deficiency of K was characterized by chlorosis on leaves edges, which developed to necrosis of the whole leaf blade (Figure 1C). The size of seedlings was reduced, either for shoots or root system, and the apical dominance was lost. The pronounced necrosis of the leaf blade occurred as a result of accumulation of chemical compounds coming from metabolic disorders such as accumulation of soluble or free nitrogen compounds. These compounds can be amino acids, amides, ammonia, amines, products that result from the decarboxylation of amino acids such as putrescine and agmatine (Malavolta and Crocomo, 1982; Epstein and Bloom, 2006). The chlorosis on mature leaves followed by the reduction of apical dominance in seedlings of Croton urucurana Baill was also found by Sorreano et al. (2011).

Symptoms of deficiency of macronutrients

Seedlings grown in nutritive solution, with a nutrient missing, showed visual symptoms of deficiency in different moments (Figure 1). However, the first common symptoms of each macronutrient were observed about 45 days after the transplant, except symptoms of deficiency of N that appeared 25 after the transplant. Then, seedlings were kept in the greenhouse for additional 45 days after the transplant for symptoms assessment and further tests.
died, compromising the plant growth, and resulting in smaller plants. The appearing of symptoms on edges of younger leaves and other new tissues was due to the lack of this macronutrient in plant tissues (Malavolta, 2006). A similar growth pattern was described by Silveira et al. (2002) for hybrids of eucalyptus. On the other hand, the deficiency of Ca resulted in the interruption of the production process of new roots, and rottenness of secondary roots, as was also described by Barroso et al. (2005) for seedlings of Tectona grandis and by Muniz and Silva (1995) for seedlings of Aspidosperma polyneuron.

**Magnesium (Mg)**

The deficiency of Mg for seedlings of A. fraxinifolius resulted in chlorosis in spaces between nervures of foliodes of older leaves (Figure 1E). The size and number of foliodes per leaf were reduced in relation to plants of the treatment consisting of the complete nutrient solution. According to Taiz and Zeiger (2013), Mg is one of the main enzymatic activators in the photosynthesis and synthesis of DNA and RNA, and is part of the structure of the molecule of chlorophyll. Thus, its deficiency promotes necrosis at the leaves apex and chloroplasts; and

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**Figure 1.** Symptomatology of deficiency of macronutrients on cedar seedlings (A. fraxinifolius) grown in nutritive solution. The left side of each picture shows plants grown in complete nutrient solution, and the right side shows solution formulated with deficiency of N (A), P (B), K (C) Ca (D), Mg (E) and S (F).
The external concentration of ions is one of factors that affect the absorption of ions by living roots (Marschner, 2012). In this study, by limiting a given nutrient in the solution, the content of such nutrient into plant tissues reduced (Tables 2 and 3).

Contents of P, K, Ca and Mg increased in the treatment consisting of omission of N for shoots of seedlings of *A. fraxinifolius* as compared to the complete nutrient solution (Table 2). The greatest content of P occurred probably as effect of the concentration of P absorbed to the lesser dry weight produced by plants. High concentrations may be related to the reduced growth that promoted the effect of N found in the dry weight. Therefore, data suggest that N is a significantly limiting factor for the growth of seedlings of *A. fraxinifolius*.

Treatment with omission of P resulted in the minor content of nutrients found in the shoots, probably because of the lower plants growth that reduced production of dry matter, with effect in the accumulation of P (Table 2). In addition, this lesser accumulation may be associated with the fact that phosphorus is a major nutrient, meaning that it is frequently deficient for crop production and it is found in every living plant cell.

### Table 2. Content of macronutrients (g.kg⁻¹) of shoots of cedar seedlings (*A. fraxinifolius*) grown under nutrients omission, 90 days after the transplant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>23.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus N</td>
<td>11.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus P</td>
<td>32.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus K</td>
<td>31.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus Ca</td>
<td>39.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus Mg</td>
<td>29.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus S</td>
<td>25.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. CV = Coefficient of variation.

### Table 3. Accumulation of macronutrients (g.plant⁻¹) of shoots of cedar seedlings (*A. fraxinifolius*) grown under nutrients omission, 90 days after the transplant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>1822.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>403.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>533.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus N</td>
<td>15.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus P</td>
<td>172.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus K</td>
<td>527.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>minus Ca</td>
<td>406.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.4&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>minus Mg</td>
<td>2105.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>170.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>539.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>minus S</td>
<td>2067.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>420.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>523.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CV (%)</td>
<td>24.14</td>
<td>33.52</td>
<td>34.82</td>
<td>39.99</td>
<td>36.87</td>
<td>48.25</td>
</tr>
</tbody>
</table>

*Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. CV = Coefficient of variation.

Symptoms of deficiency of Mg were also described by Mendonça et al. (1999) for seedlings of *Myracrodruon urundeuva*.

**Sulphur (S)**

Symptoms of deficiency of S were the last to appear. At first, small whitish spots appeared on leaf blades (Figure 1F). Then, these spots became chlorotic and covered almost all young and old leaves. Deficiency of S resulted in small sized leaves, with edges and tips of folioles rolled up; however, the plant size did not reduce. Similar results were found by Wallau et al. (2008) for seedlings of *Swietenia macrophylla*, and Sarcinelli et al. (2004) for seedlings of *Acacia holosericea* grown in nutritive solutions.

**Concentration of macronutrients**

The concentration of macronutrients (Table 2) of shoots of cedar seedlings (*A. fraxinifolius*) grown under nutrients omission, 90 days after the transplant.

The external concentration of ions is one of factors that affect the absorption of ions by living roots (Marschner, 2012). In this study, by limiting a given nutrient in the solution, the content of such nutrient into plant tissues reduced (Tables 2 and 3).

Contents of P, K, Ca and Mg increased in the treatment consisting of omission of N for shoots of seedlings of *A. fraxinifolius* as compared to the complete nutrient solution (Table 2). The greatest content of P occurred probably as effect of the concentration of P absorbed to the lesser dry weight produced by plants. High concentrations may be related to the reduced growth that promoted the effect of N found in the dry weight. Therefore, data suggest that N is a significantly limiting factor for the growth of seedlings of *A. fraxinifolius*.

Treatment with omission of P resulted in the minor content of nutrients found in the shoots, probably because of the lower plants growth that reduced production of dry matter, with effect in the accumulation of P (Table 2). In addition, this lesser accumulation may be associated with the fact that phosphorus is a major nutrient, meaning that it is frequently deficient for crop production and it is found in every living plant cell.
involved in several key plant functions, including energetic metabolism.

The omission of K increased the content of P and S in the shoot as compared to the complete nutrient solution (Table 2). A significant content of S was found in this treatment. Besides, although N and P are potential limiting factors for seedlings of A. fraxinifolius, for example, the content of S found in treatments formulated with N and P missing was lesser than that found in the treatment formulated with potassium missing.

The treatment formulated with Ca omission resulted in significant contents of all nutrients under study in shoots as compared to the complete nutrient solution (Table 2). In addition, there was an accumulation effect of Ca due to the low biomass production recorded in this treatment. In the same context, similar results were found in many other studies (Mendonça et al., 1999; Marques et al., 2004; Barroso et al., 2005). Thus, the existing interaction effect between Ca, Mg and K was also found in this study. The increase of the content of Ca and Mg in the shoot is, according to Malavolta (1980) Marschner (1995) and Barroso et al. (2005), related to the deficiency of K, then, favoring the absorption of Ca and Mg by plants, and vice versa.

The treatment formulated with Mg missing reduced the content of Ca in shoots of seedlings of A. fraxinifolius as compared to the complete nutrient solution (Table 2). The reduced content of Ca in this treatment is described as result of the existing antagonism between these two nutrients (Mg and Ca), that is, Mg has a damaging effect on the Ca availability for plants uptake, and excessive amounts of Ca reduces the uptake of cationic macronutrients such as Mg (Epstein, 1975; Malavolta et al., 1997; Mendonça et al., 1999; Barroso et al., 2005).

In the treatment formulated with S omission, a reduction in the concentration of Ca in shoots when compared with the complete nutrient solution was found (Table 2). However, this treatment was characterized by the increase of biomass in the shoot, which was described as result of the dilution effect, from which, the reduction of the content of Ca did not affect the plant growth and accumulation of dry biomass.

Regarding the accumulation of nutrients in the shoot of seedlings of A. fraxinifolius (Table 3), a pattern similar to that described for dry mass obtained from shoots of the same seedlings was found (Table 2). However, treatments consisting of the complete nutrient solution, minus S and minus Mg provided a greater accumulation of nutrients for the majority of nutrients under study. In addition, by limiting a given nutrient in the solution, in general, the content of such nutrient into plant tissues reduced (Table 3).

The treatment formulated with N omission showed lesser accumulation of nutrients in the shoot of seedlings of A. fraxinifolius as compared to the complete nutrient solution (Table 3), probably due to the low-growing effect recorded in the trial. This result shows that the content of this macronutrient in the sample was highly affected by the amount of dry weight obtained per treatment. The accumulation of nutrients depends on the content of dry weight, thus, the accumulation of N in the shoot showed a direct proportionality with the total dry matter produced per plant.

Table 3 shows that the content of nutrients found in the treatment formulated with P omission was relatively low, especially as compared to the complete nutrient solution. This treatment (minus P) resulted in lower accumulation of nutrients in shoots of seedlings of A. fraxinifolius, this described by the low-growing effect and a pronounced reduction in the total dry matter produced per plant.

Therefore, as these macronutrients (N, P, K, Ca, Mg and S) are found to be essential elements for plant growth, there is a pre-determined ratio of them that is required by the plant system, depending on its life cycle, environment and its genotypic characteristics. Amounts found in this study show that the ratio of these macronutrients is more critical than the actual concentration of the individual elements, and macronutrients balancing is an important indicator of a synergistic and/or antagonistic relationships between them; which determines the effective uptake and utilization of a given macronutrient by plants.

Conclusions

Formulated treatments with deficiency of selected single nutrients N, P, K and Ca limited plants growth (shoot height and root collar diameter) and accumulation of shoot dry weight of cedar seedlings (A. fraxinifolius) in the following order: N > P > K > Ca > Mg > S. The deficiency of these macronutrients caused visible morphological abnormalities. However, common symptoms of deficiency of N, P, K and Ca appeared before symptoms of deficiency of Mg and S. The content of nutrients found in aerial parts of seedlings of A. fraxinifolius grown in nutritive solution was greater in treatments consisting of deprivation of N, P and K than in treatments consisting of deprivation of Mg and S. There was no statistically significant difference between treatments consisting of deprivation of Mg and S and the complete nutrients solution.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES