



## **Nutrient levels and incidence of *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) attack in young *Swietenia macrophylla* King (Meliaceae) plants exposed to lime and boron levels**

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### **Abstract**

The aim of this study was to evaluate the nutrient content and the control of *H. grandella* in *Swietenia macrophylla* seedlings under increasing lime and boron levels. The experimental design was completely randomized in a factorial arrangement ( $4 \times 4 + 1$ ), with additional treatment (control treatment), four levels of lime (0.5, 1.0, 1.5 and 2.0 t ha<sup>-1</sup>) and four levels of boron (1, 2, 3, and 4 mg kg<sup>-1</sup> substrate) as factors. The characteristics were the contents of macro- (N, P, K, Ca, Mg and S) and micronutrients (B, Cu Fe, Mn and Zn) in the dry matter of the stem and leaves, gallery length and attack percentage of *H. grandella*. The nutrient content of mahogany seedlings was positively influenced by lime x boron interaction, most advantageous treatment was 1.5 t ha<sup>-1</sup> lime x 1.0 mg kg<sup>-1</sup> of boron. The two analyzed factors were not significant ( $p < 0.05$ ) in the attack percentage of *H. grandella* on the mahogany seedlings. The interaction between 1.5 t ha<sup>-1</sup> of lime and 4.0 mg kg<sup>-1</sup> boron levels was most advantageous in reducing the gallery length in young plants.

**Key words:** Brazilian mahogany, lime, boric acid, Meliaceae shoot borer,

### **Introduction**

Mahogany (*Swietenia macrophylla* King) is one of the wood species with greatest economic value in tropical America<sup>1</sup>. The biggest barrier to the implementation of mahogany commercial plantations is the Meliaceae shoot borer attack, *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae), which results in deformation of the shaft or excessive branch of the tree, reducing considerably the economic value of the log, thus becoming an extremely limiting factor in the production<sup>2</sup>.

Some studies have shown the beneficial effect of lime on the growth of mahogany seedlings<sup>3-7</sup>. Between the mineral nutrients essential to the plants, boron is less well understood, although in molar terms is required in larger quantities by dicot among all micronutrients<sup>8</sup>. According Dechen and Nachtigall<sup>9</sup>, conditions of excess lime can reduce availability of boron.

Several studies are focused to the nutritional needs and development of mahogany<sup>10-16</sup>, but the research relating to mineral nutrition species of Meliaceae as an efficient mechanism to combat the *H. grandella*<sup>2,17</sup> is almost nonexistent.

Silva *et al.*<sup>17</sup>, evaluating the effect of calcium in the control of *H. grandella* on mahogany seedlings grown in hydroponics, observed that the application in nutrient solution had reduced the gallery length where the larva develops itself and served as a

good prospect for the action on pest control.

Because of their structural function, calcium acts on the maintenance of the physical integrity of the cell wall and boron acts on the cellular development of the plant, influencing physical properties, structural and differentiation of the cell wall<sup>18</sup>. It is suggested that in plants well nurtured in calcium and boron, the resistance to *H. grandella* attack is increased depending on the application amount of nutrients.

The aim of this study was to evaluate the effect of increasing doses of lime and boron on the nutrient content and incidence of *H. grandella* attack on young of mahogany plants.

### **Material and Methods**

**Experiment localization, soil sample and seeds:** The experiment was conducted from June of 2009 to November of 2010 in a greenhouse of the Soil Science Sector of the Federal Rural University of Amazonia (UFRA). In Company Tramontina S.A. farm, located in the city of Aurora do Pará (1°27'S, 48°26'W; 7 m asl), we proceeded to the collection of mahogany arrays seed and substrate of the upper layer (0-0.2 m) of a Yellow Oxisol<sup>19</sup>, medium texture (Table 1).

**Table 1.** Chemical attributes and clay content of soil used as substrate for production of mahogany plants in the Eastern Amazon.

pH	N	P	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup> + Al <sup>3+</sup>	T	t	m	V	OM	Clay
%	mg dm <sup>-3</sup>		cmol <sub>c</sub> dm <sup>-3</sup>							%	g kg <sup>-1</sup>			
5.4	0.27	2.0	0.05	0.05	1.80	0.60	0.40	4.21	6.71	2.9	13.8	37.3	21	230

pH in water (1:2.5), P and K by Mehlich I extraction, Mg and Al extractable by 1 M KCl solution; level of organic matter (OM). T = Cation exchange capacity at pH 7.0; t = Effective cation exchange capacity ; m = Aluminium saturation index; V = Base saturation index. The soil granulometry was determined by the pipette method.

**Treatments and experimental design:** The experimental design was completely randomized with different numbers of repetitions, in a factorial arrangement ( $|4 \times 4|+1$ ), with control treatment, the factors being: a) four lime levels (0.5, 1.0, 1.5 and 2.0 t ha<sup>-1</sup>) and b) four boron levels (1, 2, 3 and 4 mg kg<sup>-1</sup>). The liming agent used was the limestone (total relative neutralization power 96%) and boron rates were applied using boric acid (H<sub>3</sub>BO<sub>3</sub>).

**Experimental conditions:** The substrate was incubated for a period of 30 days, keeping the moisture content close to the field capacity. Seedlings were transplanted at 35 days of age, one young plant pot<sup>-1</sup> (replicate). Throughout the trial period, the soil moisture was maintained close to 60% of field capacity. Irrigation was done using distilled water, proceeding to daily monitoring for this control<sup>20</sup>. The temperature inside of the greenhouse presented the average value of 30 °C, with a minimum of 23°C and a maximum of 36 °C.

**Fertilization:** The basic fertilization was performed 60 days after transplanting, using urea solutions (CH<sub>4</sub>N<sub>2</sub>O), potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) and sodium phosphate (NaH<sub>2</sub>PO<sub>4</sub>) in the NPK ratio of 200-500-300 kg ha<sup>-1</sup><sup>10</sup>. The nitrogen fertilizer was divided into two applications<sup>21</sup>, 60 and 105 days after transplanting, respectively. A single application of micronutrients and boron doses was performed 105 days after transplanting. The following reagents were used as sources of micronutrients: Cu (CuSO<sub>4</sub>), Fe (FeCl<sub>3</sub>.6H<sub>2</sub>O), Mn (MnCl<sub>2</sub>.4H<sub>2</sub>O), Mo (Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O) and Zn (ZnSO<sub>4</sub>.7H<sub>2</sub>O). The concentrations (substrate mg kg<sup>-1</sup>) were: 1.5 of Cu, 5.0 of Fe, 5.0 of Mn, 0.15 of Mo, and 5.0 to Zn. The evaluations were realized eight months after planting, when the plants were in condition to be transplanted to the field.

**Plant tissue analysis:** Plant tissue samples were analyzed according to Malavolta *et al.*<sup>22</sup>. The plant material was collected and separated into stems and leaves (shoot), placed in previously identified paper bags and taken to the forced ventilation oven at 70 °C until constant weight. Then the samples were crushed (Wiley mill type) and chemical analyses were performed to obtain the concentrations of nutrients in the stems and leaves. The nutrient content was obtained from the average of five replicates.

**Determination of nutrient contents:** Samples of leaves and stems were solubilized with nitric perchloric acid solution to extract the elements P, K, Ca, Mg, S, Cu, Fe, Mn and Zn. P was determined by colorimetry, K by flame photometry, Ca and Mg by atomic

absorption, S by turbidimetry and Cu, Fe, Mn and Zn by atomic emission spectrometry with plasma induction (EEA-ICP). Nitrogen was determined by sulfuric acid digestion and semi-micro Kjeldahl method. B was extracted by dry solubilization means and determined by colorimetry (azomethine-H).

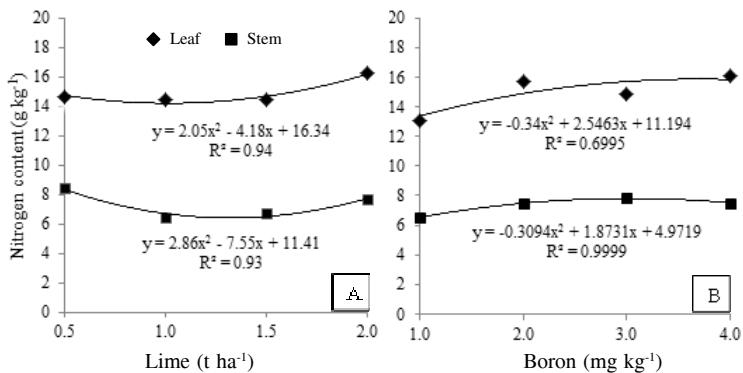
**Production and inoculation of *H. grandella* eggs:** The *H. grandella* eggs were placed at 255 days after planting the seedlings with newly launched shoots, between 16:30 h and 17:00 h, which is the period under natural conditions to egg-laying<sup>23</sup>. The eggs produced in the Entomology Laboratory of UFRA, according to the methodology proposed by Almeida<sup>24</sup>, were placed in the region near the apical meristem (two eggs.plant<sup>-1</sup>) with the aid of an entomological pin, trying to simulate the natural condition to egg-laying. The hatched eggs were observed after 24 hours. The damage caused in the apical meristem during the period from three to ten days was characterized by exudation of gum and release of sawdust, indicating the effective shoot borer attack.

**Determination of attack percentage and gallery length:** The attack percentage was calculated from the arithmetic mean of three plants in each of the 17 treatments. The gallery length (GL) was measured through the stem cross-section, with the aid of a knife measuring with a caliper. It was considered as gallery the inside part of the stem near to the apex, which was empty due to the consumption of the meristem by the shoot borer, and the arithmetic mean was calculated from four replicates per treatment.

**Data analysis:** Data were submitted to analysis of variance (ANOVA) with differentiated factor, considering the additional treatment, and the significance level determined by the F test and the means were compared by Tukey test at 5% probability. When there was the significance of the factorial ANOVA F test, the variables were submitted to ANOVA of regressions, aiming to set a model (linear or quadratic) considering the nutrient content data as dependent variables of lime and boron levels applied. The Assistat program, version 7.5 beta<sup>25</sup> and Microsoft Office Excel 2007 were used for the statistical analysis.

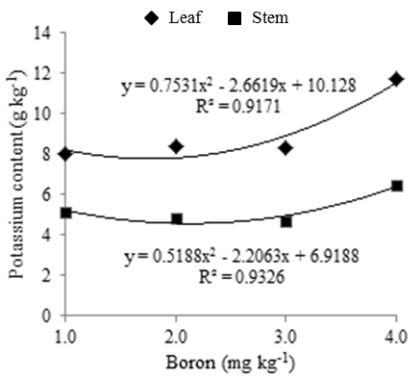
## Results and Discussion

**Nutrient contents in mahogany seedlings:** The N content in dry matter of the stem and leaves showed a quadratic behavior, with increasing doses of lime. There was a decrease in the content of N in the dry matter and from the dose of 1.5 t ha<sup>-1</sup> there was an increasing trend (Fig. 1A). Neves *et al.*<sup>26</sup> observed similar behavior evaluating *Spondias tuberosa* plants under liming effect and



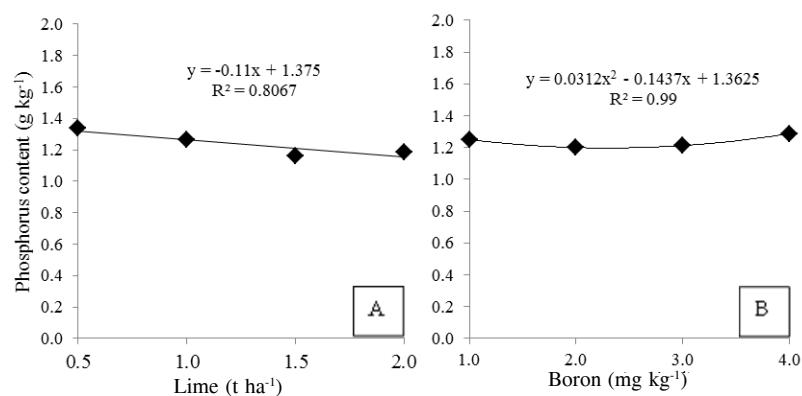
**Figure 1.** Nitrogen contents in leaf and stem of mahogany plants in function of lime (A) and boron (B) levels.

attributed this behavior to the dilution effect caused by increased saturation of soil bases. The fertilization with boron influenced the stem and leaf concentrations of N and K, which showed a quadratic behavior (Figs 1B and 2).



**Figure 2.** Potassium contents in leaf and stem of mahogany plants in function of boron level.

The P content in its turn had a negative linear behavior as a function of liming and quadratic due to the boron (Fig. 3A-B). Tucci *et al.*<sup>27</sup> found no significant effects of liming on the absorption of N, P and K, while Ca, Mg and S responded positively to lime substrate for production of *Ochroma lagopus* seedlings. Since Silva *et al.*<sup>3</sup>, Tucci *et al.*<sup>4</sup> and Silva *et al.*<sup>5</sup> concluded that liming has fundamental importance for the initial growth of mahogany seedlings, it positively affects the absorption of N, P, K, Ca and Mg. Viégas *et al.*<sup>16</sup> highlights the order of N>P>Ca>Mg>K>S to the more restrictive elements to dry matter production



**Figure 3.** Phosphorus content in leaf of mahogany plants in function of lime (A) and boron (B) levels

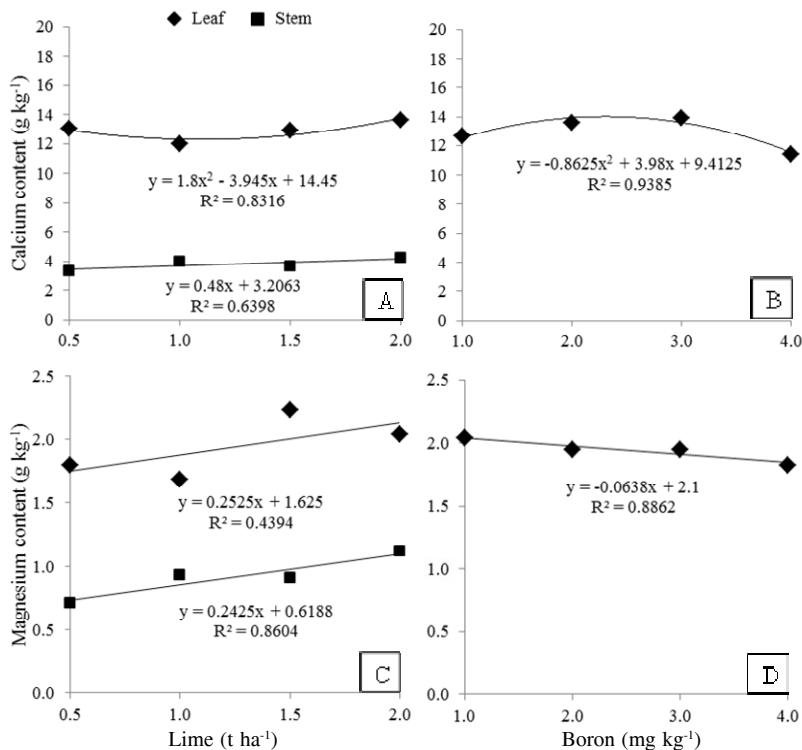
of mahogany plants, which demonstrate that N, P and Ca are essential for mahogany growth.

Pedroso *et al.*<sup>6</sup> and Fontes *et al.*<sup>7</sup> observed an increase in dry matter production of the aerial part of mahogany plants by applying lime to the ground, which may be related to greater absorption of macronutrients.

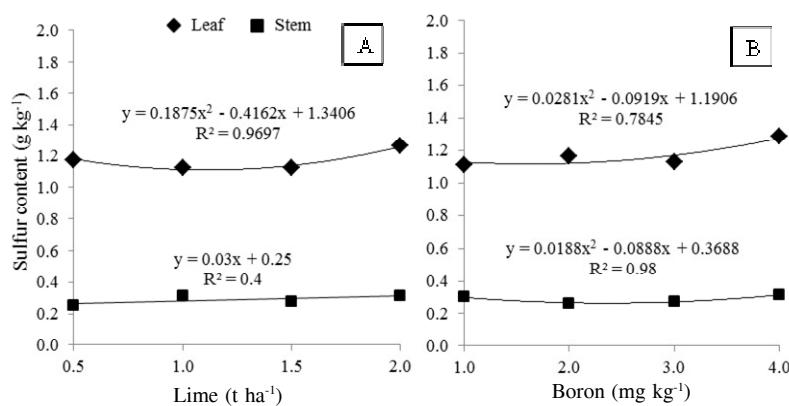
Liming, besides increasing the pH of the soil, provides calcium and magnesium, increasing the availability of these nutrients for absorption by crops<sup>28</sup>. The increase in base saturation provided by liming, promoted the increase of Ca and Mg in the leaf and the stem of mahogany plants, this can be attributed to the increase of exchangeable element content of these nutrients in the soil<sup>29</sup>. The response, based on lime to the calcium contents on the leaves was quadratic, while the Ca content in stem and Mg in stem and leaf were positive linear (Fig. 4A, C). The Mg absorption increases by providing lime to the soil, according to Neves *et al.*<sup>26</sup> working with seedlings of *S. tuberosa* and Silva *et al.*<sup>5</sup> in mahogany seedlings. Silva *et al.*<sup>17</sup> observed that the mahogany seedlings grown hydroponically, increased their calcium content in the stem dry matter with increasing doses of the element in nutrient solution. The increasing doses of boron negatively affected the Ca and Mg content in the leaves of mahogany seedlings, with quadratic and linear behavior, respectively (Fig. 4B and D). According to Dechen and Nachtigall<sup>9</sup>, the boron alters absorption and metabolism of cations, especially calcium, because the boric acid found in soil solution forms complexes with calcium, and generate an acidic environment in the rhizosphere, which may affect the availability of calcium and magnesium.

The sulfur contents in leaf and stem were influenced both by liming, and by fertilization with boron (Fig. 5A and B). The sulfur is component of amino acids (cysteine, cystine and methionine) and proteins, as well as N, also being a constituent of other compounds such as lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, adenosine 5'-phosphosulfate and 32-phosphoadenosine<sup>30</sup>. Perhaps the greatest concentration of protein in the leaves, the addition of limestone, in virtue to favor the multiplication of microorganisms and facilitate greater mineralization of organic matter, has favored the absorption and translocation of S to the leaves, unlike what happened in the stem, which showed lower levels of aluminium than those observed in the leaves. Vitti *et al.*<sup>31</sup> comment that the S ( $\text{SO}_4^{2-}$ ) is absorbed by the roots in small amounts, and the transport occurs mainly by the xylem vessels, predominantly toward the base to the plant apex<sup>22</sup>. Silva *et al.*<sup>5</sup> found, testing different sources of P in the presence and absence of liming, that the absorption of S is not affected by the addition of corrective. Viegas *et al.*<sup>16</sup> verifies that S is the least required macronutrient in mahogany culture.

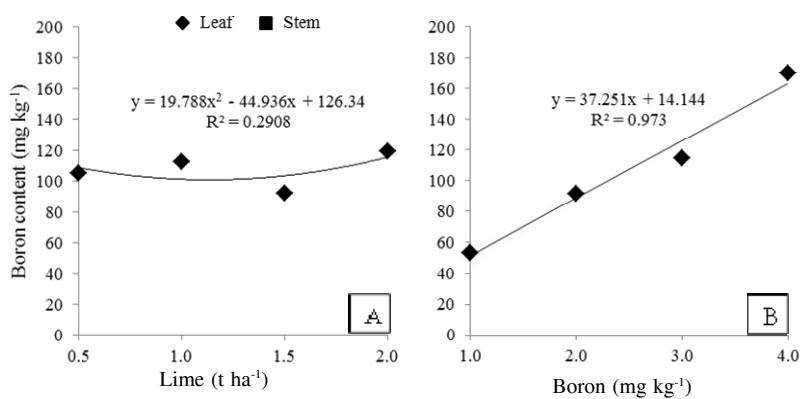
Liming significantly influenced the stem and foliar contents of all micronutrients in mahogany seedlings, except Cu, which showed stem average content of 5.58 mg kg<sup>-1</sup>. The fertilization with boron significantly influenced the shoot contents of all micronutrients, while foliar Mn and Zn were not affected by these treatments. By evaluation of mahogany seedlings grown in Oxisol, Silva *et al.*<sup>3</sup> observed that liming positively affected the absorption of Fe and Mn due



**Figure 4.** Calcium and magnesium contents in leaf and stem of mahogany plants in function of lime (A and C) and boron (B and D) levels.



**Figure 5.** Sulfur contents in leaf and stem of mahogany plants in function of lime (A) and boron (B) levels.



**Figure 6.** Boron contents in leaf and stem of mahogany plants in function of lime (A) and boron (B and C) levels.

to increased soil pH, whereas the absorption of Cu and Zn was not affected.

The B content in leaves was higher at a dose of 2.0 t ha<sup>-1</sup> lime, with an average of 119.5 mg kg<sup>-1</sup> (Fig. 6A). Neves *et al.*<sup>26</sup>, increasing the base saturation of different soils, found an increase in boron levels in the shoots of various grasses and seedlings of *S. tuberosa*, respectively, as well as decrease in Fe, Mn and Zn. The boron content in the leaf increased linearly with the boron levels (Fig. 6B), as found by Moreira *et al.*<sup>32</sup> and Ramos *et al.*<sup>33</sup> in eucalyptus. The boron content in stem presented quadratic behavior under increasing of boron levels, reducing the levels until the dose of 3 mg kg<sup>-1</sup>, 10.93 to 9.38 mg kg<sup>-1</sup>, reaching maximum value (11.44 mg kg<sup>-1</sup>) of boron at the highest dose, corresponding to 4 mg kg<sup>-1</sup> of boron applied to the substrate (Fig. 6C).

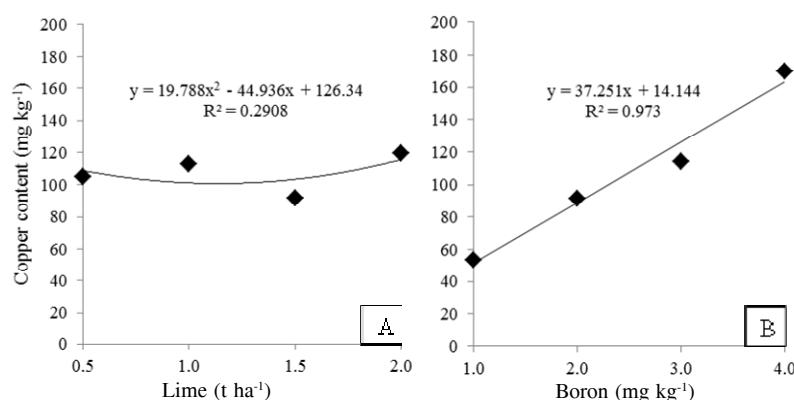
The Cu content of the leaf increased linearly by increasing of boron levels in the soil (Fig. 7B), while the leaf content of this micronutrient, under the action of lime, best fittes to a quadratic equation (Fig. 7A). Fe levels in leaves and stem presented quadratic behavior in function of B addition (Fig. 8B), as observed in the Fe content in leaves as function of lime levels (Fig. 8A). The concentrations of Mn (stem and leaf) and Zn (stem) have been reduced with increasing of lime levels (Figs 8C and 9A), confirming the results obtained by Neves *et al.*<sup>26</sup>.

According to Raij<sup>28</sup>, liming promotes a decrease in the availability of cationic micronutrients in the soil, among them are Mn and Zn. As a consequence, mahogany seedlings showed lower levels in the stem and leaves. The stem levels of Mn, were reduced from the dose with 2.0 mg kg<sup>-1</sup>, with a significant quadratic response (Fig. 8D), while Zn showed negative linear behavior as a function of boron level (Fig. 9B).

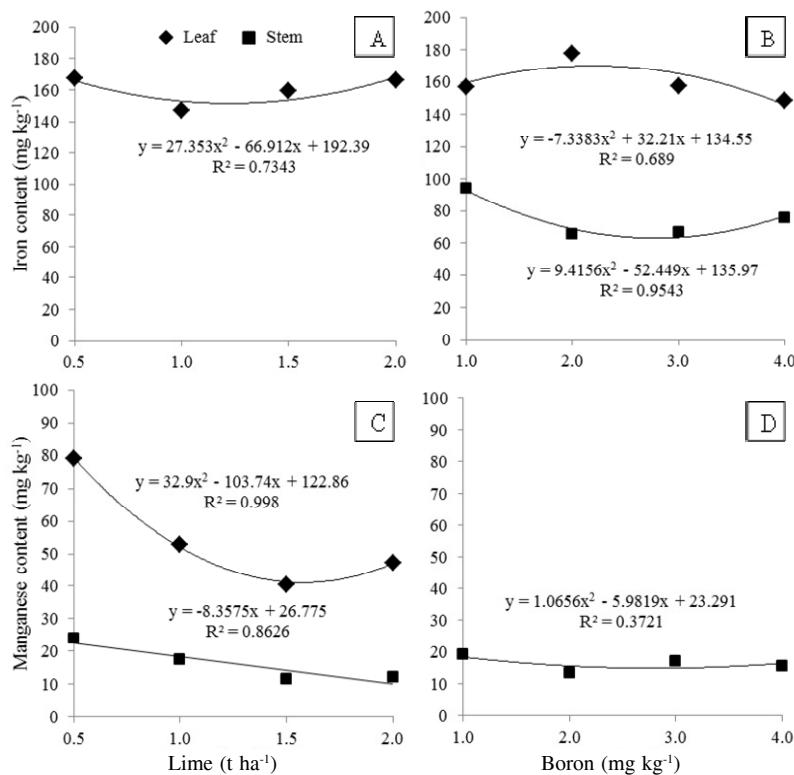
Moreira *et al.*<sup>32</sup> found that foliar Mn and Zn were affected by increasing doses of boron applied to the soil, presenting quadratic effect in rubber seedlings, also there was increase in foliar Zn with the addition boric acid in soil. According Lima Filho and Malavolta<sup>34</sup>, boron has similarity with Zn absorption

and transport to a long distance by plants, and the mass flow and diffusion primarily responsible for providing these two nutrients in the soil solution to the roots<sup>22</sup>.

**Induction of resistance:** The two factors evaluated (calcium and boron) were not significant ( $p<0.05$ ) in the percentage of *H. grandella* attack on the mahogany seedlings. The control treatment did not differ from factorial treatment for this variable and the average attack percentage was 61%. The lowest attack percentage (22%) was obtained from the interaction of treatment with 1.5 kg ha<sup>-1</sup> of lime and 4.0 mg kg<sup>-1</sup> of boron (Fig. 10). Silva *et al.*<sup>17</sup> evaluated the effect of calcium in the control of *H. grandella* on mahogany seedlings in hydroponic system, observed that plants with 189 and 211 days of age had increased resistance to shoot borer, with the doses of 160, 240 and 320 mg L<sup>-1</sup> calcium



**Figure 7.** Copper content in leaf of mahogany plants in function of lime (A) and boron (B) levels.



**Figure 8.** Iron (A and B) and manganese (C and D) contents in leaf and stem of mahogany plants in function of lime and boron levels.

concentration in solution, the attack percentage of 20, 60 and 80%, respectively.

The gallery length showed significance ( $p<0.05$ ) with boron levels. However, there was no significant difference between the lowest (1.0 mg kg<sup>-1</sup>) and highest dose (4.0 mg kg<sup>-1</sup>) (Fig. 11).

There was a significant interaction between the two factors evaluated in gallery length of *H. grandella* ( $p<0.01$ ). Analyzing the deployment of lime versus boron interaction, the average of gallery length was not different at levels of 1.0 and 2.0 t ha<sup>-1</sup> of lime (Fig. 12). From the boron level of 2.0 mg kg<sup>-1</sup>, the means obtained with 0.5 t ha<sup>-1</sup> of lime had an increasing trend. Only at levels of 3.0 and 4.0 mg kg<sup>-1</sup> of boron, the average gallery length showed difference between the lime levels. Significant reduction in the shoot borer gallery length of *H. grandella* was observed with the lime level of 1.5 t ha<sup>-1</sup> interacting with boron level equal to 4 mg kg<sup>-1</sup>.

The reduction of the gallery length in mahogany seedlings using increasing doses calcium may be related to the increased calcium content in dry matter stem<sup>17</sup>. Ohashi *et al.*<sup>2</sup> observed positive effect of fertilization with boron and soil amendment with dolomitic limestone and gypsum in mahogany resistance of *H. grandella* attack.

## Conclusions

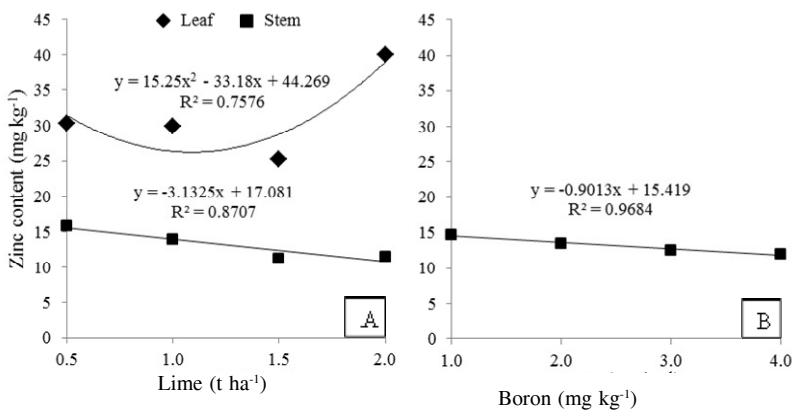
The nutrient contents of mahogany seedlings were positively influenced by lime x boron interaction, most advantageous treatment being 1.5 t ha<sup>-1</sup> lime x 1.0 mg kg<sup>-1</sup> of boron. The interaction between 1.5 t ha<sup>-1</sup> of lime and 4 mg kg<sup>-1</sup> of boron levels was most advantageous in reducing shoot borer gallery length in mahogany seedlings. The mineral nutrition of plants may be a viable alternative when combined with other techniques described in the integrated pest management in order to reduce the damage caused by the Meliaceae shoot borer attack.

## Acknowledgments

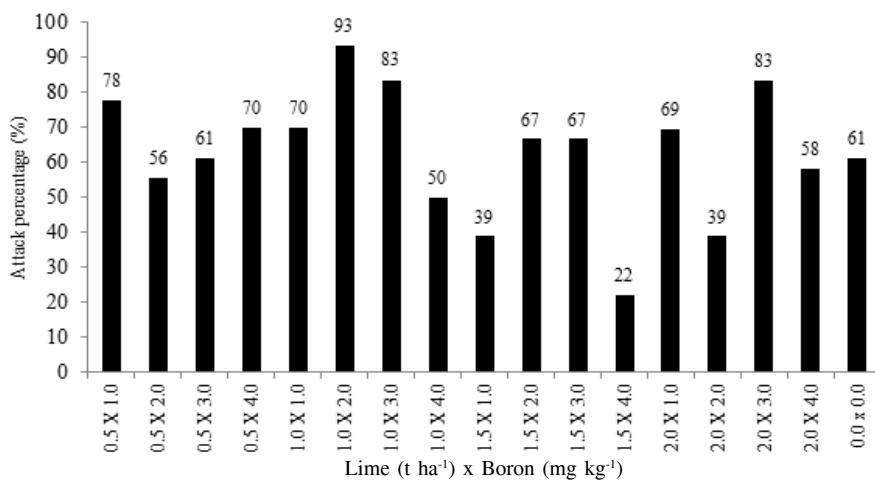
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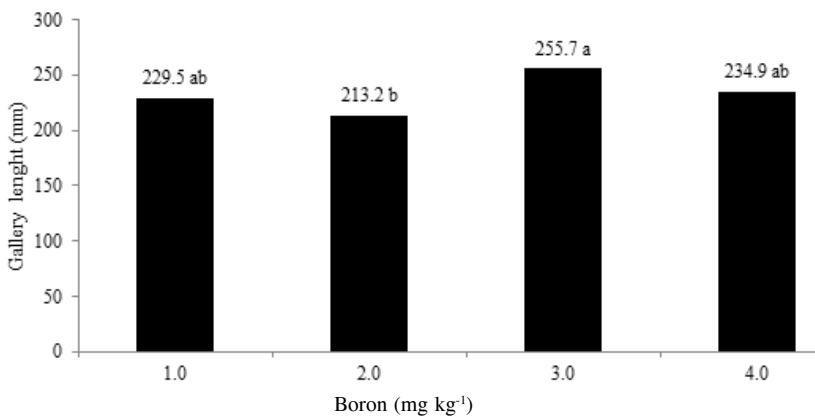
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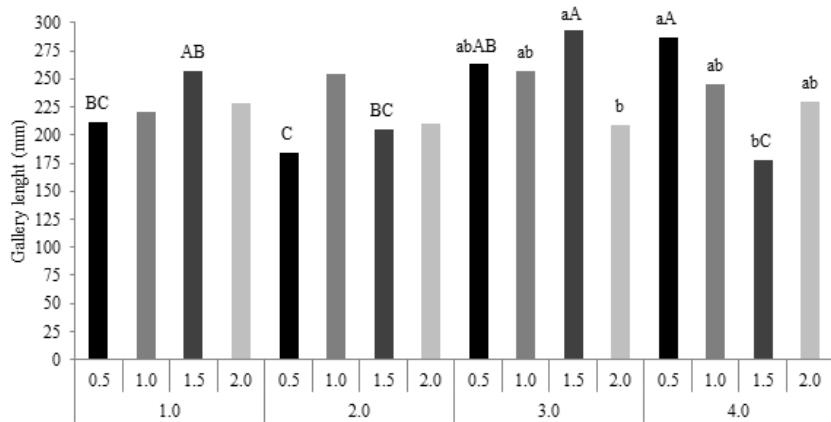
**Figure 9.** Zinc contents in leaf and stem of mahogany plants in function of lime (A) and boron (B) levels.



**Figure 10.** Attack percentage of *H. grandella* shoot borer in mahogany plants in function of lime and boron levels.



**Figure 11.** Gallery length of *H. grandella* shoot borer in mahogany plants under increasing of boron levels.



Interaction of Lime (0.5, 1.0, 1.5 and 2.0 t ha<sup>-1</sup>) x Boron (1, 2, 3 and 4mg kg<sup>-1</sup>)

**Figure 12.** Unfolding of interaction and mean of gallery length of *H. grandella* in mahogany plants under increasing of lime and boron levels. (Lower case letters compare effect of lime within each dose of boron and boron capital letters compare effect within each dose of lime by Tukey test at 5% probability).