Full Length Research Paper

**Perennial soybean seeds coated with high doses of boron and zinc**

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The objective of this work was to study combinations of high doses of boron (B) and zinc (Zn) in the recoating of perennial soybean seeds, in order to provide these nutrients to the future plants. The physical, physiological and nutritional characteristics of the coated seeds and initial development of plants in a greenhouse were evaluated. Tests carried out in the laboratory were conducted in a completely randomized design, and the experiments completed in a greenhouse were a randomized block design. The coating with the dose 0.8 kg of H₃BO₃ + 0.8 kg of ZnSO₄.kg⁻¹ of seeds provides the best quality coating. The combination of B and Zn in seed coating reduces the production of shoot dry matter, while the other treatments do not affect the growth variables of the plants. Plants absorb and accumulate the micronutrients added to the coating of the seeds.

**Key words:** Micronutrients, coater, soybean, seeds.

**INTRODUCTION**

The adoption of intercropping of Poaceae and Fabaceae in Brazil is viable in the pasture ecosystem due to the capacity of atmospheric nitrogen fixation. Furthermore, Fabaceae can influence the quality of the pasture, which can increase production of bovine milk and meat, and protect the soil (Macedo et al., 2014).

The Neonotonia wightii is a palatable herbaceous Fabaceae of high nutritional value, intercropping-friendly and of good natural reseeding. These qualities make this plant one of the most important tropical Fabaceae plants in the world, being highly indicated to haymaking, pasture establishment and green fertilization (Barcellos et al., 2008). Gama et al. (2011) verified that perennial soybean is persistent throughout the years, even thriving in adverse conditions, contributing to intercrop productivity. The predominant soils in Brazil are dystrophic latosols, which are naturally devoid of micronutrients, especially zinc and boron. The deficiency of these or any other micronutrient is capable of causing a reduction in the development, growth and hence crop yields, they play important roles in the metabolism of the plant. Thus, treatment of seeds with micronutrients is a viable way to provide them to the plants (Ker, 1997; Prado et al., 2008).

Perennial soybean seeds are small, and can hinder its

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distribution when seeding. Seed coating is a solution that only increases the size and shape of seeds, but provides the possibility of adding needed nutrients, growth regulators, insecticides and fungicides. These plants require only a small amount of such products and this technique allows an effective and safe application of them.

These studies evaluated the coating of perennial soybean seeds with boric acid (B) and zinc sulfate (Zn) at different rates, in order to provide these micronutrients in enough quantity to supply the needs of a plant life cycle.

**MATERIALS AND METHODS**

These experiments were conducted in a laboratory and greenhouse, both belonging to the State University of Northern Rio de Janeiro, (in Portuguese: Universidade Estadual do Norte Fluminense Darcy Ribeiro) from 06/2015 to 09/2015. The commercial perennial soybean seeds went through chemical scarification by being immersed in previously tested concentrated sulfuric acid for 20 min, prior to coating seed for the experiments.

To coat seeds, the methodology used by Xavier et al. (2015) was adapted in a N10 Newpack coater, regulated so the chamber would spin at the speed of 90 rpm and the compressed air that activates the solution would be under 4 bar pressure during 1 s. Then, the hot-air blower was switched on at the temperature of 40°C for 2 min.

As stuffing material for the coating, dolomite lime and activated vegetable charcoal were used in a 3:1 proportion (p/p) and 0.08:1 (p/p), respectively. Elmer’s Glue-All, a polyvinyl acetate (PVA) based glue, diluted in deionized water previously boiled at 70°C, in the proportion of 1:1 (v/v) was used for the adhesive material (Mendonça et al., 2007).

The coating process was divided in portions of 50 g of seeds, each of these portions being put in the chamber inside the coater, along with another portion of stuffing material (initially 6.25 g of dolomite lime). Then the adhesive solution spray was applied three times repeatedly along with another portion of the stuffing material (6.25 g of dolomite lime) which was added over the seeds with another application of adhesive solution. Right after, the air blower (40°C) was used for 2 min. This procedure resulted in the first coating layer. For the following layers, another jet of adhesive solution followed by another portion of stuffing material were applied; then, another jet of adhesive solution, accompanied by the second portion of stuffing material. Finally, another jet of adhesive solution was activated, before the final hot air blowing, which lasted for more 2 min. This procedure was repeated until the stuffing material was over. The portions of activated vegetable charcoal (1 g) were added after the third layer with dolomite lime, making the 4th and 5th layers from the same material. The doses of boric acid (H3BO3) and zinc sulfate (ZnSO4) were added all at once in the sixth coating layer, between portions of dolomite lime and glue. At the end of this process, 14 coating layers were formed.

The tested treatments were: TR1) Uncoated seeds; TR2) Coated seeds, without micronutrients; TR3) 0.8 kg of H3BO3 + 0.8 kg of ZnSO4 kg⁻¹ seed; TR4) 1.0 kg of H3BO3 + 0.9 kg of ZnSO4 kg⁻¹ seed; TR5) 1.2 kg of H3BO3 + 1.0 kg of ZnSO4 kg⁻¹ seed; TR6) 1.4 kg of H3BO3 + 1.1 kg of ZnSO4 kg⁻¹ seed; TR7) 1.6 kg of H3BO3 + 1.2 kg of ZnSO4 kg⁻¹ seed; and TR8) 1.8 kg of H3BO3 + 1.3 kg of ZnSO4 kg⁻¹ seed. All treatments were determined based on the fertilizing regulation for soils, recommended for forage crops, considering a seeding of 2.5 kg of seeds.

After coating, seeds were evaluated regarding physical and physiological features and nutritional value, in laboratory and in a greenhouse.

Using the same criteria established in the rules for seed analysis (Brasil, 2009) the laboratory tests were conducted in completely randomized design, using 4 repetitions of 50 seeds for the germination test, during which the percentage of germination, dead seeds and soaked seeds was determined after 10 days. The water content, the weight of a thousand seeds and the germination speed were also measured.

To determine the maximum diameter (MAD), the minimum diameter (MID) and the contour irregularity of the seeds, four repetitions of 50 seeds from each treatment were used, to be ultimately analysed by the seed analysis equipment Graundeye®, with the results expressed in centimeters (cm).

The emergence test, which took place inside a greenhouse, was put together in plastic trays containing previously washed up sand, where 4 blocks of each treatment including 50 seeds were sown in randomized design. The test ran for 90 days; in the first 30 days, there was a daily counting to determine the emergency speed index (ESI). By the end of the test, 10 plants were selected from the portion and had its aerial parts and roots separated, with the purpose of determining their length with the assistance of a ruler.

Using the Groundeye® equipment, the scientists determined the number of ramifications and the full length of the root, accounting the length of each ramifications. Further on, the aerial part and the root were stowed in paper sacks and taken to a greenhouse with an air circulation of 65°C for 72 h, so they could establish the shoot dry matter (SDM) and the root dry matter (RDM).

To determine the nutrient content in the seeds (in the aerial part and in the roots of plants developed inside the greenhouse) after a 72-h drying period (at the temperature of 65°C) the seeds were macerated and the aerial part and root milled, to only then be stocked in hermetically sealed flasks. For the analysis of the seeds, 4 repetitions of each treatment were used. However, for plant analysis (aerial part and root) there was not enough material for repetitions. Therefore, the results of the analysis were based solely on the material utilized in each treatment. To determine calcium, magnesium, boron and zinc contents the material went through nitric digestion and the analysis of the extract was made using the ICPE-9000.

Data were tested for normality and data did not require any type of transformation. Means were tested using an analysis of variance (F-test) and the Tukey’s range test (at a 5% probability level) with help from the statistical program SAEG.

**RESULTS AND DISCUSSION**

As for the quality of the coating, the highest TSW and maximum diameter were reached in the combination of smaller doses of micronutrients of 0.8 kg of H3BO3 kg⁻¹ of seeds + 0.8 kg of ZnSO4 kg⁻¹ of seeds (TR3), which enhanced the seed mass twice if compared to the uncoated seeds (TR1) (Figure 1). Thus, the first goal of the coating process, which is to enhance and modify the shape and density of the seed was reached, what should provide greater accuracy in sowing and in the application of chemicals (Mendonça et al., 2007). However, the use of larger doses of micronutrients ended up being harmful to the increase of the weight of the seeds, because as the doses grew larger, the adhesiveness of the coating material reduced. It was also noted that the coating process was not efficient when it came to correct the irregularity of seed contour, considering that there was not a significant difference in this variable (p<0.05) between treatments. The results can be associated with
the nutritional content of the seeds, in which the combinations of B and Zn increased, causing significant reduction of dolomite lime (Ca and Mg) adhesiveness and of the micronutrients B and Zn, consequently influencing the quality of the coating.

The low adhesiveness of the material added to the coating layer may be linked to the chemical scarification to which the seeds were submitted earlier. It is believed that the sulfuric acid ($H_2SO_4$) responsible for taking down the impermeable barrier of the seed coat turned its surface smooth, not permitting a better coating. However, this hypothesis needs to be verified.

The granulometry of the material used throughout the coating process may also have influenced its quality. The zinc sulphate has bigger, heavier particles, while the dolomite lime and the charcoal both have finer textures; boric acid sits in the middle. During the coating process materials of finer granulometry should be preferred in the layers closer to the nucleus, so an enhancement of the seed’s contact surface and weight is assured (Silva and Nakagawa, 1998). This way, it is believed that the methodology utilized in this work favoured the adherence of zinc sulphate, which was not capable of keeping the surface of the layers even, by virtue of its crystalized shape, thus reducing the adhesiveness of the other materials (Figure 2).

Another factor related to the quality of the coating is the water content of the seeds after they are coated, and in Figure 3 it is noted a certain reduction of humidity in the coated seeds, showing that the utilized materials did not absorb water. It also became clear that the drying made inside the coater during the coating was efficient, providing the removal of the water applied through the glue on the formation of the coating layers, avoiding absorption by the seed.

The coating of seeds with or without micronutrients was harmful to the normal seedlings formation and to the germination speed, being this effect progressive as the combinations of B and Zn were enhanced (Figures 4 and 5). A similar result was found by Pessoa et al. (2000), who observed an increase of heterogeneity and a delay in germination, and still a low initial development of plants when tested increasing doses of B in corn seeds. Yagi et al. (2006) also verified that the application of zinc sulfate in sorghum seeds in a dose of 28.56 g.kg of seeds resulted in a minor percentage of germination. However, Tavares et al. (2013) and Pletsch et al. (2014) using in the recoating of seeds doses of commercial products that possessed 780 g.L⁻¹ of Zn in wheat seeds and 780 g.L⁻¹ of Zn in canola seeds, respectively, observed that the treatments provided benefits for the germination and initial establishment of plants. Nonetheless, Ohse (2000), in irrigated rice seeds, observed that the combination of 0.67 g.kg⁻¹ of zinc sulfate and 0.065 g.kg⁻¹ of boric acid did not affect the germination, although it increased the number of abnormal seedlings, not being indicated by the author due to the diminishment caused in the seed strength.

However, it is a belief that negative results for germination are connected to the methodology used to execute the test recommended for non-coated seeds, when the seeds are disposed in the germination box,
which benefits the salt concentration present in the recoating after being put in a soluble state, altering the hydric potential of the substrate, creating an inhospitable condition for the seeds, on the higher doses of B + Zn (Figure 4) (Xavier, 2015).

As a consequence of substrate hydric potential alteration, there is a significant number in TR3 (41%), TR4 (39%) and TR5 (37.5%) of seeds which did not conclude the germination process, seeds that soaked but did not complete the third step of germination with the radicle development (Marcos Filho, 2015).

In addition to these conditions influencing negatively the germination, recoated seeds soak more slowly due to the necessity of rupture of more than one physical barrier to initiate the germination process (Derré et al., 2013), influencing directly the IVG (Figure 5).

This work’s seed treatment goal was to transform them into seeds rich in B and Zn nutrients, to subsequently be made available for plants. However, the combination of 1.6 kg of H₃BO₃ kg⁻¹ of seeds + 1.2 kg of ZnSO₄ kg⁻¹ of seeds (TR7) and 1.8 kg of H₂BO₃ kg⁻¹ of seeds + 1.3 kg of ZnSO₄ kg⁻¹ (TR8) was toxic for seeds, providing a bigger percentage of dead seeds in the germination box (Figure 4).

As apposed from the lab results, when seeds were sowed in sand and disposed in a greenhouse, there was an increase in the percentage of emerged seedlings compared to the percentage of germination (Figure 4). It is possible to relate this fact to the condition to which the seeds were submitted, the greenhouse expresses in a
more realistic way the field conditions, perforated trays that permit the leaching of salt and humidity excess, strengthened by the necessity of more intense irrigation, as reported by Abreu et al. (2001) and also because it is a test with longer duration (90 days). However, the effects of high doses of fertilizers are also felt in the greenhouse in the highest dose of B and Zn added to the recoating (TR8), when a significant diminishing of E and ESI was noticed (Figures 4 and 5).

Based on the evaluation done on plants, the combination of 1.8 kg of H$_3$BO$_3$ and 1.3 kg of ZnSO$_4$ kg$^{-1}$ of seeds (TR8) has shown itself harmful to the production of dry mass, this being the only treatment which differed from the control treatment (TR1) (Table 1). Thus, it is noticed that with the exception of the highest dose of B and Zn in the coating, there is no harm in the coating with B and Zn to the growth of plants related to control (TR1). These results agree with Araújo and Silva (2012), whose work with cotton trees highlights the direct connection between B and the plant development; and Funguetto et al. (2010) who highlight the Zn participation in different metabolic routes which provide growth, therefore
parameters due to its importance in the root dry mass. The toxicity symptoms of Zn in plants include accumulation of B on the aerial part were the same when its supply rate is higher than its absorption, coherent with the situation found in this work (Figure 6).

According to Marschner (1995), Zn accumulation in roots accumulated in the aerial part, as well as in the inhibition of radicular lengthening (Marschner, 1995), which was not observed in a significant way on the Table 2. Perennial soybean plants developed in the greenhouse have shown an additional accumulation of B and Zn micronutrients when originated from coated seeds, accumulated in the aerial part, as well as in the roots. According to Marschner (1995), Zn accumulation in roots may have been caused by the accumulation of nutrients on its proximities, which is more common when the transport of nutrient happens by diffusion and it occurs when its supply rate is higher than its absorption, coherent with the situation found in this work (Figure 6).

The treatments which were prominent by the accumulation of B on the aerial part were the same treatments with bigger accumulation of Zn, also on the aerial part: TR4, TR7 and TR8, which presented a gain compared to the control treatment (TR1), of 66.55;

### Table 1. Shoot Dry Mass (SDM) and Height (PHT) of perennial soybean plants (Neonotonia wightii cv. Common) 90 days after sowing in greenhouse sand.

<table>
<thead>
<tr>
<th>Treatment (kg.kg⁻¹ of seeds)</th>
<th>SDM (g/pl)</th>
<th>PHT (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncased</td>
<td>0.13a</td>
<td>1.20ab</td>
</tr>
<tr>
<td>Without micronutrients</td>
<td>0.12ab</td>
<td>1.21ab</td>
</tr>
<tr>
<td>0.8 H₂BO₃ + 0.8 ZnSO₄ (¹)</td>
<td>0.11ab</td>
<td>1.23a</td>
</tr>
<tr>
<td>1.0 H₂BO₃ + 0.9 ZnSO₄</td>
<td>0.12ab</td>
<td>1.27ab</td>
</tr>
<tr>
<td>1.2 H₂BO₃ + 1.0 ZnSO₄</td>
<td>0.13ab</td>
<td>1.09ab</td>
</tr>
<tr>
<td>1.4 H₂BO₃ + 1.1 ZnSO₄</td>
<td>0.11ab</td>
<td>1.09ab</td>
</tr>
<tr>
<td>1.6 H₂BO₃ + 1.2 ZnSO₄</td>
<td>0.10bc</td>
<td>1.04b</td>
</tr>
<tr>
<td>1.8 H₂BO₃ + 1.3 ZnSO₄</td>
<td>0.08c</td>
<td>1.03c</td>
</tr>
<tr>
<td>Average</td>
<td>0.16</td>
<td>1.14</td>
</tr>
<tr>
<td>CV (%)</td>
<td>36.62</td>
<td>7.22</td>
</tr>
</tbody>
</table>

*Average followed by the same letter do not differ statistically among themselves by Tukey test (p<0.05).

### Table 2. Root Length (RL), Root Dry Mass (RDM), Ramification Number (RN) and Total Root Size (TRS) of perennial soybean plants (Neonotonia wightii cv. Common) 90 days after sowing in the greenhouse sand.

<table>
<thead>
<tr>
<th>Treatment (kg.kg⁻¹ of seeds)</th>
<th>RL (cm)</th>
<th>RDM (g/pl)</th>
<th>RN</th>
<th>TRS (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncased</td>
<td>10.97a</td>
<td>0.36ab</td>
<td>74.41a</td>
<td>89.43a</td>
</tr>
<tr>
<td>Without micronutrients</td>
<td>11.29a</td>
<td>0.38a</td>
<td>81.08a</td>
<td>97.16a</td>
</tr>
<tr>
<td>0.8 H₂BO₃ + 0.8 ZnSO₄ (¹)</td>
<td>10.86a</td>
<td>0.27ab</td>
<td>75.88a</td>
<td>88.07a</td>
</tr>
<tr>
<td>1.0 H₂BO₃ + 0.9 ZnSO₄</td>
<td>10.77a</td>
<td>0.29ab</td>
<td>76.25a</td>
<td>89.64a</td>
</tr>
<tr>
<td>1.2 H₂BO₃ + 1.0 ZnSO₄</td>
<td>10.61a</td>
<td>0.29ab</td>
<td>73.79a</td>
<td>91.11a</td>
</tr>
<tr>
<td>1.4 H₂BO₃ + 1.1 ZnSO₄</td>
<td>10.43a</td>
<td>0.28ab</td>
<td>78.03a</td>
<td>94.41a</td>
</tr>
<tr>
<td>1.6 H₂BO₃ + 1.2 ZnSO₄</td>
<td>10.29a</td>
<td>0.26b</td>
<td>67.00a</td>
<td>82.51a</td>
</tr>
<tr>
<td>1.8 H₂BO₃ + 1.3 ZnSO₄</td>
<td>9.96b</td>
<td>0.26b</td>
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<td>97.86a</td>
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<tr>
<td>Average</td>
<td>10.65</td>
<td>0.30</td>
<td>75.25</td>
<td>91.27</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.77</td>
<td>16.63</td>
<td>13.5</td>
<td>13.67</td>
</tr>
</tbody>
</table>

* Average followed by the same letter do not differ statistically among themselves by Tukey test (0.05%).

influencing the plant’s active photosynthetic area. Nevertheless, Albuquerque et al. (2010) report the negative influence of Zn to the plants growth when it is found in high amounts in the environment.

Regarding the roots production, which are important for the inhibition of radicular lengthening (Marschner, 1995), which was not observed in a significant way on the Table 2. Perennial soybean plants developed in the greenhouse have shown an additional accumulation of B and Zn micronutrients when originated from coated seeds, accumulated in the aerial part, as well as in the roots. According to Marschner (1995), Zn accumulation in roots may have been caused by the accumulation of nutrients on its proximities, which is more common when the transport of nutrient happens by diffusion and it occurs when its supply rate is higher than its absorption, coherent with the situation found in this work (Figure 6).

The treatments which were prominent by the accumulation of B on the aerial part were the same treatments with bigger accumulation of Zn, also on the aerial part: TR4, TR7 and TR8, which presented a gain compared to the control treatment (TR1), of 66.55;

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Levels of boron and zinc in the aerial part and root in perennial soybean plants (*Neonotonia wightii* cv. Common) 90 days after sowing seeds in the greenhouse sand. Treatments (Kg.Kg\(^{-1}\) of seeds): TR1: Uncoated; TR2: Without micronutrients; TR3: 0.8 H\(_3\)BO\(_3\) + 0.8 ZnSO\(_4\); TR4: 1.0 H\(_3\)BO\(_3\) + 0.9 ZnSO\(_4\); TR5: 1.2 H\(_3\)BO\(_3\) + 1.0 ZnSO\(_4\); TR6: 1.4 H\(_3\)BO\(_3\) + 1.1 ZnSO\(_4\); TR7: 1.6 H\(_3\)BO\(_3\) + 1.2 ZnSO\(_4\); TR8: 1.8 H\(_3\)BO\(_3\) + 1.3 ZnSO\(_4\).

47.33; 66.00 mg of B per kg of the plant dry mass and 205.43; 133.91; 319.8 mg of Zn per kg of the plant dry mass, respectively (Figure 6). On these same treatments, different from the others, it is always observed a higher concentration of Zn than of B; and the maximum absorption of Zn, regarding TR8, gets to be two times bigger than the absorption of B in the same treatment, confirming that the nutritional interactions interfere on the plant mineral composition, one element being able to stimulate or inhibit the absorption of another element (Araújo and Silva, 2012).

Thus, on the results of other treatments, when absorption of B absorption was higher, the capacity of absorption of Zn was decreased. Ohse (2000), in his work, noticed a possible antagonistic effect between Zn and B, as well as between B and Cu; however, Hosseini et al. (2007) reported a significant interaction between B and Zn on corn plants growth, with a synergetic effect among these nutrients. Lefebre et al. (2002) report that the Zn response in function of the concentration of B depends on the analyzed organ, because when analyzing tobacco plants the effect on leaves was the opposite of what was found on the root; in other words, the decrease of Zn levels in function of the increase of concentrations of B. Ziaeyan and Rajaie (2009) also observed the diminishing of Zn concentration in corn leaves with an increase of B concentration. The different responses can occur based on the organs and species analyzed.

On the basis of the levels of B and Zn on the aerial part of the plants, the minimum level was 137.85 and 98.2 mg.kg\(^{-1}\) and the maximum was of 204.40 and 418 mg.kg\(^{-1}\), respectively (Figure 6). In Yamada’s work (2004), the adequate level of micronutrients in the foliar analysis was listed for some crops in the cerrado region (Brazilian savanna). Among them, it is the perennial soybean that is considered an adequate amount of 30-50 mg.kg\(^{-1}\) of B and 20-50 mg.kg\(^{-1}\) of Zn. However, Fageria (2000a, b), regarding beans and soybean plants, which are from the same family, observed as ideal levels for these plants an amount of 10 - 55 mg.kg\(^{-1}\) of B and 21 - 35 mg.kg\(^{-1}\) of Zn.
There are few data with toxicity values for micronutrients, but Fageria (1992) reported values referring to the toxicity of zinc in the aerial part of annual crops, with higher levels than 400 mg kg\(^{-1}\) of dry mass. The outcomes of the nutritional analysis performed in this work were superior to those reported by these authors, including the control treatment (TR1). Still, it is not possible to compare these results directly, due to the different environment conditions in which the plants were developed and due to the evaluation criteria used by each author to perform the analysis; Besides, \( \text{Fabaceae} \) species differ in nutritional efficiency, being identified variations on the absorption efficiency, translocation and utilization of macro and micronutrients related to the species and/or cultivars (Vieira, 2013).

**Conclusions**

1) The coating with a dosage of 0.8 kg of \( \text{H}_3\text{BO}_3 \) + 0.8 kg of \( \text{ZnSO}_4 \) kg\(^{-1}\) seed (TR3) provides greater weight and diameter of the seeds. All treatments increase the minimum diameter and decrease the humidity of seeds, but they are not capable of changing their irregularity of contour.

2) The combination of 1.8 kg of \( \text{H}_3\text{BO}_3 \) + 1.3 kg of \( \text{ZnSO}_4 \) kg\(^{-1}\) seed (TR8) decreases shoot dry mass production, whereas other treatments do not affect plant growth variables.

3) Perennial soybean plants absorb and accumulate micronutrients added to the seed coating.

**Conflict of Interests**

The authors have not declared any conflict of interests.

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