

Division - Soil Use and Management | Commission - Soil Fertility and Plant Nutrition

Polyol-ester impact on boron foliar absorption and remobilization in cotton and coffee trees

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ABSTRACT: Foliar fertilization can be recommended to treat boron (B) deficiency in coffee and cotton. Considering that B foliar fertilizers with polyol-boron complexes can affect B uptake and mobility differently within the plant, and coffee and cotton have different cuticles and stomata density, a differential response would be expected. We aimed to study the foliar application of boric acid combined with sorbitol on B uptake and translocation in cotton and coffee. Green-house grown plants received B as boric acid and a sorbitol-monoethanolamine complex and were sampled up to 96 h after application. Boron absorption was fast, reaching 60 and 80 % in cotton and coffee 96 h after application, respectively. Uptake rates and total B absorption were similar for the fertilizers. The proportion of B taken up by coffee is greater than by cotton likely because of the greater stomata density in coffee and less likely due to the higher amount of wax in cotton cuticle. Boron remobilization is higher in coffee as compared with cotton. Sorbitol seems to increase B transport in the transpiratory stream of cotton, but impairs remobilization in the phloem since B translocation to roots is decreased in both cotton and coffee.

Keywords: *Coffea arabica*, foliar fertilizer, foliar spray, *Gossypium hirsutum*, micronutrients.

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INTRODUCTION

Boron (B) is the micronutrient most deficient in cotton (Ahmad et al., 2009) and coffee (Brown and Shelp, 1997). In most species, B is not phloem mobile, since it is highly complexed on the cell wall pectin (Brown and Hening, 1994). However, B remobilization was observed in a number of species in which polyols are abundant (Brown and Shelp, 1997). Sugar alcohols (polyhydric alcohols or polyols) present in the phloem sap of these species contain cis-diol groups such as sorbitol and mannitol, which form stable complexes with B and facilitate its remobilization from old to new plant organs such as meristematic tissues, young leaves, and fruits (Brown and Hening, 1994; Shelp et al., 1998; Bielecki and Briggs, 2005). Therefore, B phloem redistribution is associated with the type and abundance of hydroxyl-bearing moieties, such as sugar-alcohols (Liakopoulos et al., 2009; Liu et al., 2015).

In cotton phloem, boron mobility is low, but under mild deficiency (Bogiani et al., 2014) and applied to old leaves (Oliveira et al., 2006), some B can be remobilized from vegetative to reproductive tissues. When applied to young immature leaves of deficient cotton plants, B concentration increases only locally, but there is a positive response to B applied to old leaves, which can be remobilized to younger tissues (Rosolem and Costa, 2000; Oliveira et al., 2006). Recently it has been shown that B applied to cotton leaves is taken up and transported preferentially to young tissues within 24 h, but this is not enough to secure a normal growth (Bogiani et al., 2014). These authors suggested that foliar sprays of B to cotton could be used only to cope with a temporary deficiency, but to warrant full growth and development, B must be available throughout the plant cycle. Although soil applications generally give better results (Silva et al., 1999), the possibility of foliar-applied B mobilization out of the leaves would explain responses observed in acidic soils in Brazil (Carvalho et al., 1996) and calcareous soils of Greece (Dordas, 2006) and India (Kumar et al., 2018). As B has low mobility in cotton, frequent applications would be required to meet plant demand, but successive foliar applications can lead to toxicity problems (Ahmad et al., 2009).

Boron deficiency is common in Brazilian coffee plantations (Malavolta et al., 2001), resulting in decreased root growth, flower abortion, fruit malformation, and low yields (Franco, 1982). Under B deficiency, the vascular tissues of coffee trees are disorganized, and the xylem vessel walls are thinner. Boron deficient coffee leaves have fewer and malformed stomata (Rosolem and Leite, 2007). Coffee was reported to produce mannitol (Brown and Shelp, 1997), and there is evidence of B remobilization from vegetative organs to coffee berries via symplast, both in deficient and well-nourished plants (Konsaeng et al., 2005). Between 33 to 40 % of the B found in coffee berries is taken up during fruit formation, and under field conditions, when B is sprayed on the leaves, about 4 % of the fruit B was derived from the leaf applied fertilizer (Leite et al., 2007). However, there is still controversy as to the best way to apply B to coffee trees. There are reports on better results both to soil and foliar fertilization, however, B leaf spraying results in better nutrient use efficiency (Cong, 2017). Furthermore, an increase in B leaf does not always result in higher yield (Marubayashi et al., 1994; Lima Filho and Malavolta, 1998; Fernandes et al., 2012), and this may be due to poor redistribution of the nutrient.

Mobility or immobility of B in plants directly affects the correction of B deficiency because in immobile-B species foliar-applied B will not be remobilized to deficient regions of the plant. In contrast, in B-mobile plants, foliar sprays of B to functional leaves may be effective at any time. If indigenous polyols facilitate B remobilization, would exogenously applied sugar-alcohols have a similar effect? In a preliminary study, foliar application of B-sorbitol was found to increase foliar uptake of B by soybean, which produces the sugar-alcohol pinitol, but did not improve B redistribution within the plant (Will et al., 2011, 2012). In cotton, it has been observed that foliar B spraying along with a polyol fatty acid ester-based adjuvant was economically superior to both soil and foliar applications without the adjuvant (Roberts et al., 2000).

Foliar absorption of B, despite being considered a fast and target-oriented tool in dealing with deficiencies, is affected by many factors such as the type of the fertilizer, time of application, solution characteristics, relative humidity, number of stomata, type of cuticle, membrane permeability, and leaf age, among others (Fernández et al., 2013). Furthermore, it has been observed that the plant surface chemical composition and structure affect wetting and transport across the surface (Fernández et al., 2013, 2017). The leaf surfaces of cotton and coffee are quite different regarding the amount of waxes (Lichston and Godoy, 2006; Oosterhuis and Weir, 2010). Moreover, the number of stomata in coffee leaves was shown to be 192.2 mm^{-2} (Nascimento et al., 2006) and 87.7 mm^{-2} in cotton (Silva et al., 1989).

Differences in cotton response to foliar sprays of sodium borate, borax, and granubor solution were found non-significant (Kumar et al., 2018), and no difference in B sources was found in coffee, including boric acid (Fernandes et al., 2012). However, it remains unknown if the response to polyol-boron complexes applied to leaves would be affected differently by different cuticle types, as well as what the response of a mannitol-species would be compared with a non-mannitol species as to foliar B uptake and redistribution in the plant. This research aimed to study the effect of foliar application of boric acid combined with sorbitol on B uptake and translocation in cotton and coffee.

MATERIALS AND METHODS

Two experiments, one with cotton and the other with coffee were conducted in a greenhouse in Botucatu, in the state of São Paulo, Brazil, $22^{\circ} 51' \text{ S}$, $48^{\circ} 26' \text{ W}$ Greenwich and altitude 840 m. Cotton seeds were germinated in trays with washed sand. Ten days after emergence, cotton seedlings were transferred to plastic pots. Coffee seeds were germinated in tubes filled with a commercial substrate and transferred to plastic pots when the plantlets had 6-8 pairs of leaves, approximately 60 days after emergence. Two plants of cotton or coffee were grown per 8.0 L pot, filled with 7.0 L of washed sand. Sand was first washed with chloridric acid 0.1 mol L^{-1} , and then with deionized water, to remove organic materials and clay. A nutrient solution (Hoagland and Arnon 1950) with $\text{Ca}(\text{NO}_3)_2$ 5.0 mmol L^{-1} , KNO_3 5.0 mmol L^{-1} , KH_2PO_4 1.0 mmol L^{-1} , MgSO_4 2.0 mmol L^{-1} , H_3BO_3 $45.3 \text{ } \mu\text{mol L}^{-1}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ $9.1 \text{ } \mu\text{mol L}^{-1}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ $0.7 \text{ } \mu\text{mol L}^{-1}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ $0.4 \text{ } \mu\text{mol L}^{-1}$, NaMoO_4 $0.1 \text{ } \mu\text{mol L}^{-1}$, and FeEDTA $20.0 \text{ } \mu\text{mol L}^{-1}$, was used. For the first seven days after transplant, the solution was diluted to 1:10 strength and 1 L was applied per pot. From 7 to 14 days, the solution concentration was increased to 1:5 strength. From 14 days, full strength solution was applied twice a week. From 21 days, boron was not added to nutrient solution. The water content in the pots was monitored daily and kept at around 80 % of the water holding capacity.

The treatments consisted of foliar application of solutions B 1.04 mg L^{-1} , prepared from boric acid (H_3BO_3) and from a B fertilizer with 8.0 % of B, 19 % of sorbitol, and 14 % of monoethanolamine (B-sorbitol). Both boric acid and B-sorbitol were enriched to 99 % of ^{10}B atoms. Using cotton swabs, the solution was carefully applied by brushing both adaxial and abaxial sides of the leaves.

In coffee, the treatments were applied 28 days after plantlet transplant, and the fertilizers were applied to the third and fourth pair of leaves from the plant top, avoiding the contamination of the other leaves. For cotton, the treatments were applied when the first floral bud appeared (35 days after transplant), to leaves in the fifth and sixth nodes. The total quantity of B solution applied to each pot was determined by weighing the cotton swabs with the vials containing the B solution before and after application.

For both, cotton and coffee, the amount of B applied to each plant was different (Table 1), because the fertilizer densities and viscosities were different, and the leaves were not exactly the same size. Considering these differences, the data are presented as a proportion of the amount applied. At 6, 12, 24, and 96 h after application the plants

Table 1. Amount of B applied to cotton and coffee leaves

Boron source	Cotton	Coffee
	mg plant ⁻¹	
Boric acid	0.817 + 0.071	0.385 + 0.092
B-sorbitol	1.289 + 0.314	0.434 + 0.073

were harvested, and cut to separate roots, bottom (region below the nodes where the treatments were applied), third (containing the leaves where the treatments were applied), and top (region above the nodes where the treatments were applied). The samples were washed in deionized water.

Plant samples were dried at 65 °C to a constant weight in an air-forced oven and ground and sieved through a 1.0 mm-sieve for B determination. Sub-samples of 0.2 g were transferred to porcelain crucibles, incinerated at 550 °C for 3 h, and the ash was dissolved in 10 mL of HCl 0.1 mol L⁻¹. Total B was determined by ICP- OES (Inductive Coupled Plasma - Optical Emission Spectrometry) at a wavelength of 249.773 nm. The procedures for calculating the isotopic ratio (¹⁰B/¹¹B), after determining ¹⁰B and ¹¹B by ICP-MS (Inductive Coupled Plasma-Mass Spectrometry) and calculating the concentration of B in the plant were the same as those described by Boaretto et al. (2008), as follows.

The amount of B derived from the fertilizer in the plant tissues was calculated using equation 1.

$$\%B_{DFF} = [(\% \text{ }^{10}\text{B}_{\text{sample}} - \% \text{ }^{10}\text{B}_{\text{natural}})/(\% \text{ }^{10}\text{B}_{\text{fert.}} - \% \text{ }^{10}\text{B}_{\text{natural}})] \times 100 \quad \text{Eq. 1}$$

in which: % ¹⁰B_{sample} = ¹⁰B in the sample; % B_{natural} = ¹⁰B in no treated samples; % ¹⁰B_{fert.} = ¹⁰B atm in the fertilizer (99 % enrichment).

It was used equation 2 to calculate B content in the tissues.

$$B_{DFF} \text{ (mg kg}^{-1}\text{)} = [\%B_{\text{dff}} \times B \text{ (mg kg}^{-1}\text{)}]/100 \quad \text{Eq. 2}$$

Data from each experiment were analyzed separately. Following tests for homogeneity and normality, data was subjected to analysis of variance (ANOVA), following a split plot design in randomized complete blocks, with four replications. Means were compared by orthogonal linear contrasts (p<0.05) using the GLM procedure in SAS software (version 9.4, SAS Inst., North Carolina, U.S.). The data was adjusted to exponential equations using SigmaPlot software (version 14, Systat Software Inc., San Jose, California, U.S.).

RESULTS

Considering that the amount of B applied to each plant was different owing to differences in fertilizer density and viscosity, and the leaves were not exactly the same size, data are presented and discussed as a proportion of the amount applied. This is usual in experiments using stable isotopes, including B (Leite et al., 2007).

Boron absorption was relatively fast, since more than 50 % of the amount applied was taken up in 24 h, for both cotton and coffee (Figure 1). After 24 h, the absorption rate decreased; and 96 h after the application, the total absorption reached approximately 60 and 80 % of the amount applied to cotton and coffee, respectively. The absorption rates, as well as the amounts of boron taken up were similar for the two sources of the nutrient.

Despite the fast absorption, most of the B taken up was found in the region of application (middle third) in cotton (Figure 2) and coffee (Figure 3). Leaf applied B to cotton represented

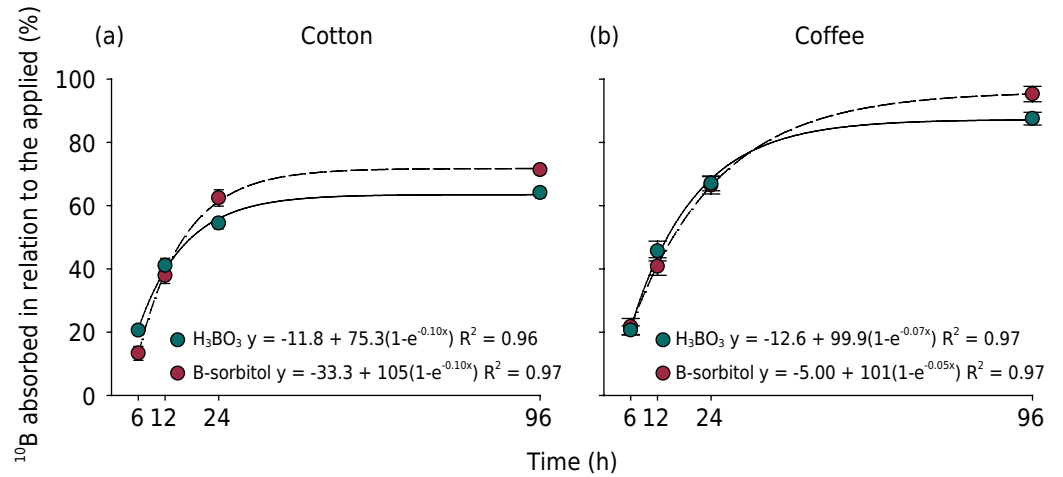


Figure 1. Percentage of ^{10}B taken up in relation to the amount applied to cotton (a) and coffee (b), as a function of time after application of B as boric acid (H_3BO_3) and boric acid with sorbitol. Vertical bars represent the standard error ($n = 4$).

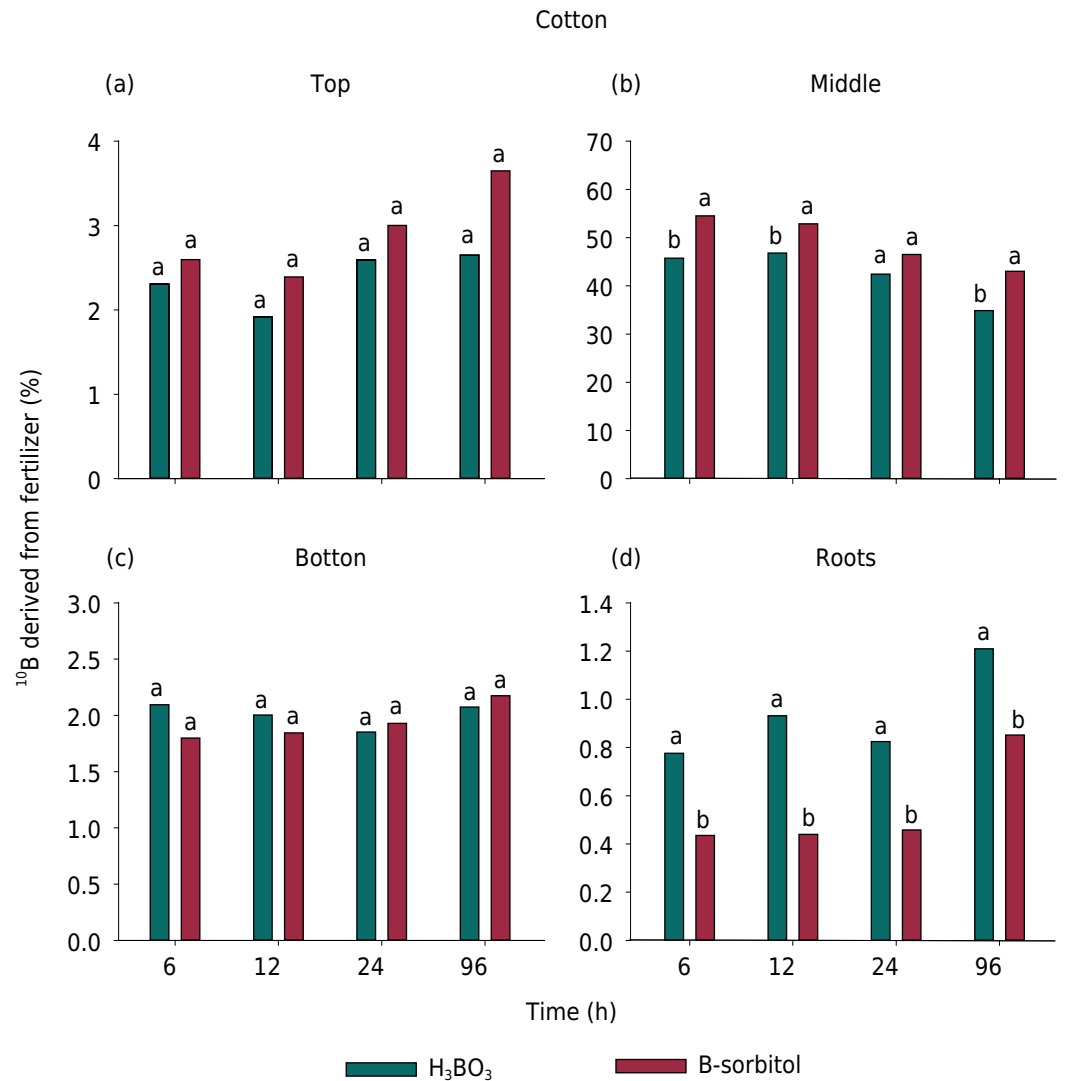


Figure 2. Percentage of ^{10}B derived from fertilizer in the top (a), middle (b), bottom (c), and roots (d) of cotton, as a function of time after application of B as boric acid (H_3BO_3) and boric acid with sorbitol. Each plant part includes stems, leaves, and reproductive structures. Leaves of the middle were treated with B. Different letters indicate significant differences between treatments by orthogonal linear contrasts ($p < 0.05$) for each time.

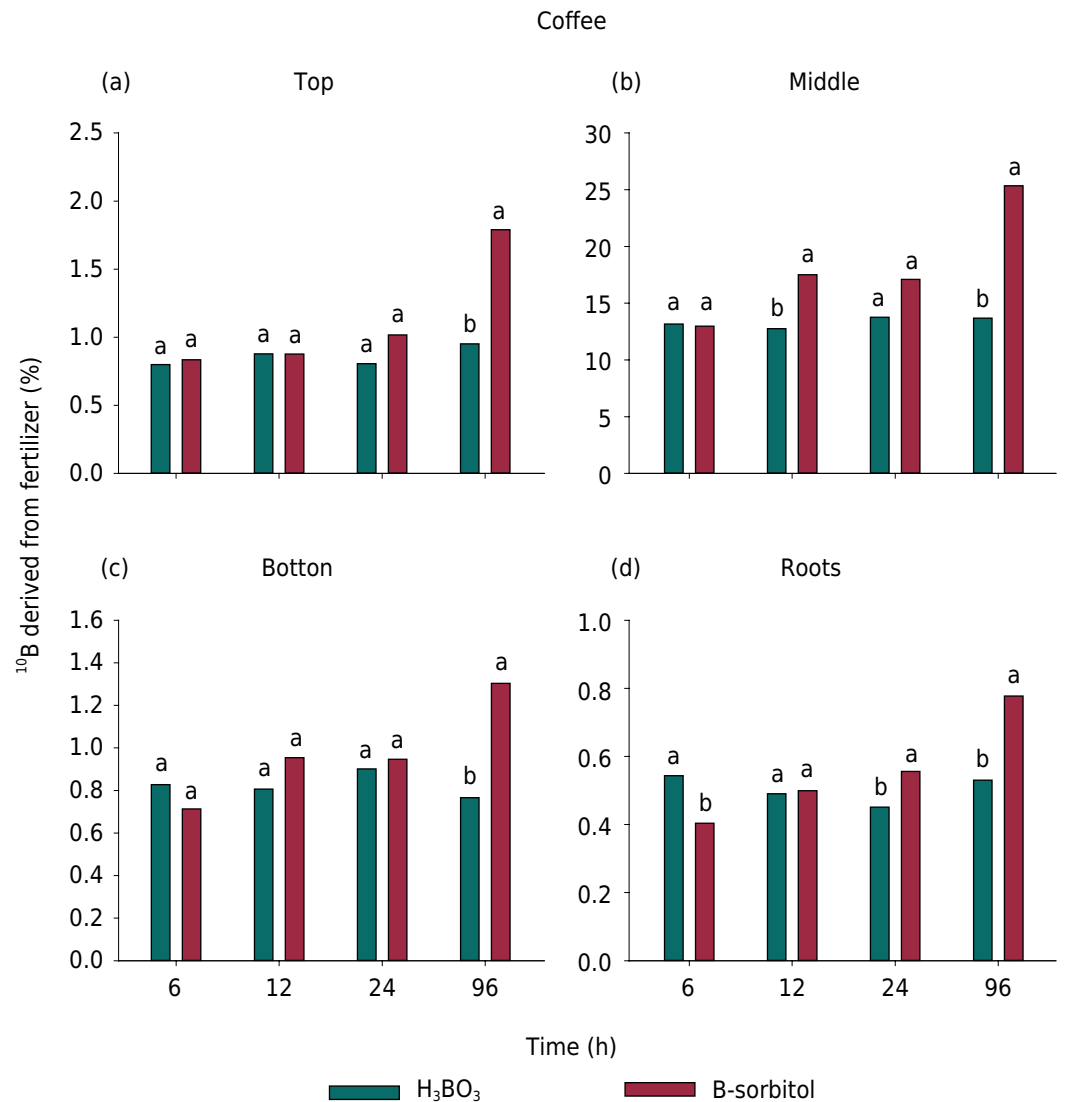


Figure 3. Percentage of ¹⁰B derived from fertilizer in the top (a), middle (b), bottom (c), and roots (d) of coffee, as a function of time after application of B as boric acid (H₃BO₃) and boric acid with sorbitol. Each plant part includes stems and leaves. Leaves of the middle were treated with B. Different letters indicate significant differences between treatments by orthogonal linear contrasts ($p < 0.05$), for each time.

a small amount of the nutrient found above (less than 4.0 %) or below the treated (less than 2.0 %) portions of the plant, regardless of the source used (Figure 2). Although there was a trend of increasing B derived from the fertilizer (B_{diff}) in the upper portion of the plant with time, and a concomitant decrease in the region that received the application, the difference was significant only 96 h after application, in the applied region, showing that the applied B was mobilized out of this plant portion. Although B_{diff} in roots accounted for less than 1.4 % of the total, the amount derived from pure boric acid was higher than from the B-sorbitol fertilizer (Figure 2d). In the middle third of the plant region in which B was applied, the percentage of B derived from the B-sorbitol fertilizer was higher than that of boric acid after 6, 12, and 96 h of application.

In coffee, the percentage of B_{diff} was also low (less than 2.0 %) in all parts of the plant that have not received foliar application (Figure 3). However, the proportion of B derived from B-sorbitol was higher than that derived from boric acid 96 h after application in the upper and lower third of the plants. The percentage of B from boric acid was higher than that from the B-sorbitol in coffee roots 6 h from application, but from 24 h after application, B-sorbitol resulted in a higher percentage of B_{diff} .

When analyzing the amount of B that was remobilized relative to that applied to cotton leaves (Figure 4), it was noted that the mobilization, although very small, was virtually concomitant with the absorption, occurring up to approximately 30 h after the application. Approximately 50 % of the applied B was still in the region of the plant that received the foliar application, at least up to 96 h after the application. From 4 to 5 % of the nutrient applied was found in the upper, and therefore newer parts of the plants; from 5 to 6 % were in the lower third of the cotton canopy; and 4 to 5 % in the roots. Again, in this case, there was no difference between the sources of B.

In the coffee trees, approximately 50 % of the B applied to the leaves was also in the plant middle third, the region where B was applied, up to 96 h from the application. A little more than 15 % was transported to the upper third of the plant, approximately 15 % to the lower third, and approximately 10 % to the roots (Figure 5). In the case of coffee, there was more B from B-sorbitol in the applied region than when the source was boric acid. However, in the other parts of the plant, the difference was not significant.

When analyzing the amount of B that was mobilized in relation to the amount taken up by cotton over time (Figure 6), 96 h after application, some of the foliar-applied B was found in the upper third and in the roots. Translocation to the upper plant part was higher with B-sorbitol than boric acid, but there was more B from boric acid than from B-sorbitol in the roots.

In the case of coffee, more B of boric acid was found in the younger plant parts and in the roots 96 h after application (Figure 7).

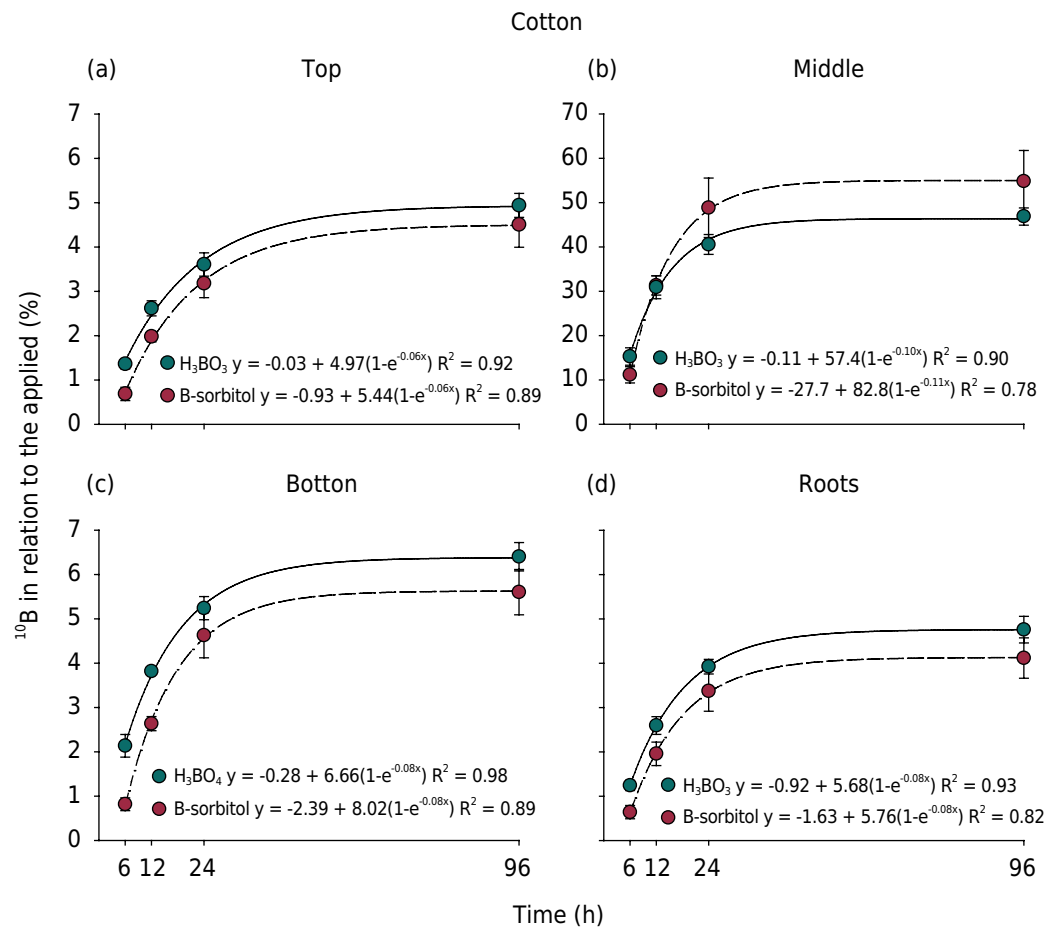


Figure 4. Percentage of ^{10}B in relation to the applied, in the top (a), middle (b), bottom (c), and roots (d) of cotton as a function of time after application of B as boric acid (H_3BO_3) and boric acid with sorbitol. Each plant part includes stems, leaves, and reproductive structures. Leaves of the middle were treated with B. Vertical bars represent the standard error ($n = 4$).

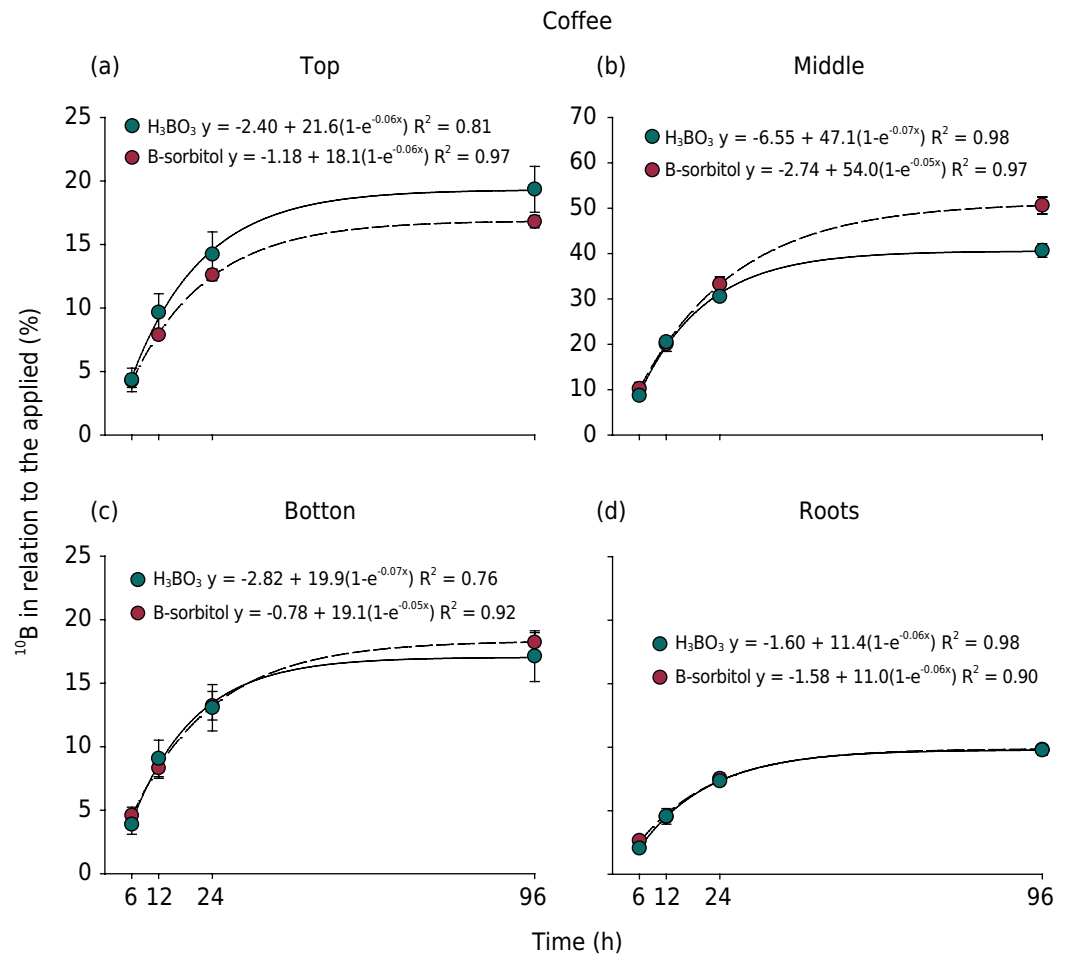


Figure 5. Percentage of ^{10}B in relation to the applied, found in the top (a), middle (b), bottom (c), and roots (d) of coffee as a function of time after application of B as boric acid (H_3BO_3) and boric acid with sorbitol. Each plant part includes stems and leaves. Leaves of the middle were treated with B. Vertical bars represent the standard error ($n = 4$).

DISCUSSION

Foliar absorption of B was fast for cotton and coffee, despite the very different leaf surface characteristics and different numbers of stomata of these species. Bogiani et al. (2014) had already shown that foliar absorption of B by cotton is rapid, generally occurring within the first 24 h after application. However, total absorption reached over 80 % of the applied amount in coffee, but only around 60 % in cotton (Figure 1). Solutes applied to leaves may be taken up via cuticle, cuticle imperfections, through stomata, the base of trichomes or even by specialized cells (Fernández et al., 2013). The cuticular and stomatal pathways have been studied to a certain extent, and there is no consensus that the penetration of foliar fertilizers decreases with increased cuticle wax content (Fernández and Eichert, 2009). Also, there seems to be little argument on the importance of the stomatal pathway, since the more stomata, the higher the uptake (Shu et al., 1994; Will et al., 2012). Stomata density in cotton is much lower than in coffee (Silva et al., 1989; Nascimento et al., 2006) and this could explain the difference in B absorption in this experiment. However, cotton leaf cuticle has $91.7 \mu\text{g cm}^{-2}$ of wax (Bondada et al., 1996), while $70.3 \mu\text{g cm}^{-2}$ was found on coffee leaves (Lichston and Godoy, 2006), on average, which could have also had an effect on B absorption by each species. Furthermore, the chemical composition of the leaf and its physical structure are also important factors to be considered (Fernández et al., 2013).

The time that foliar fertilizers remain in the liquid phase is crucial for uptake, and it is affected by the relative humidity and the deliquescence relative humidity (DRH) of the

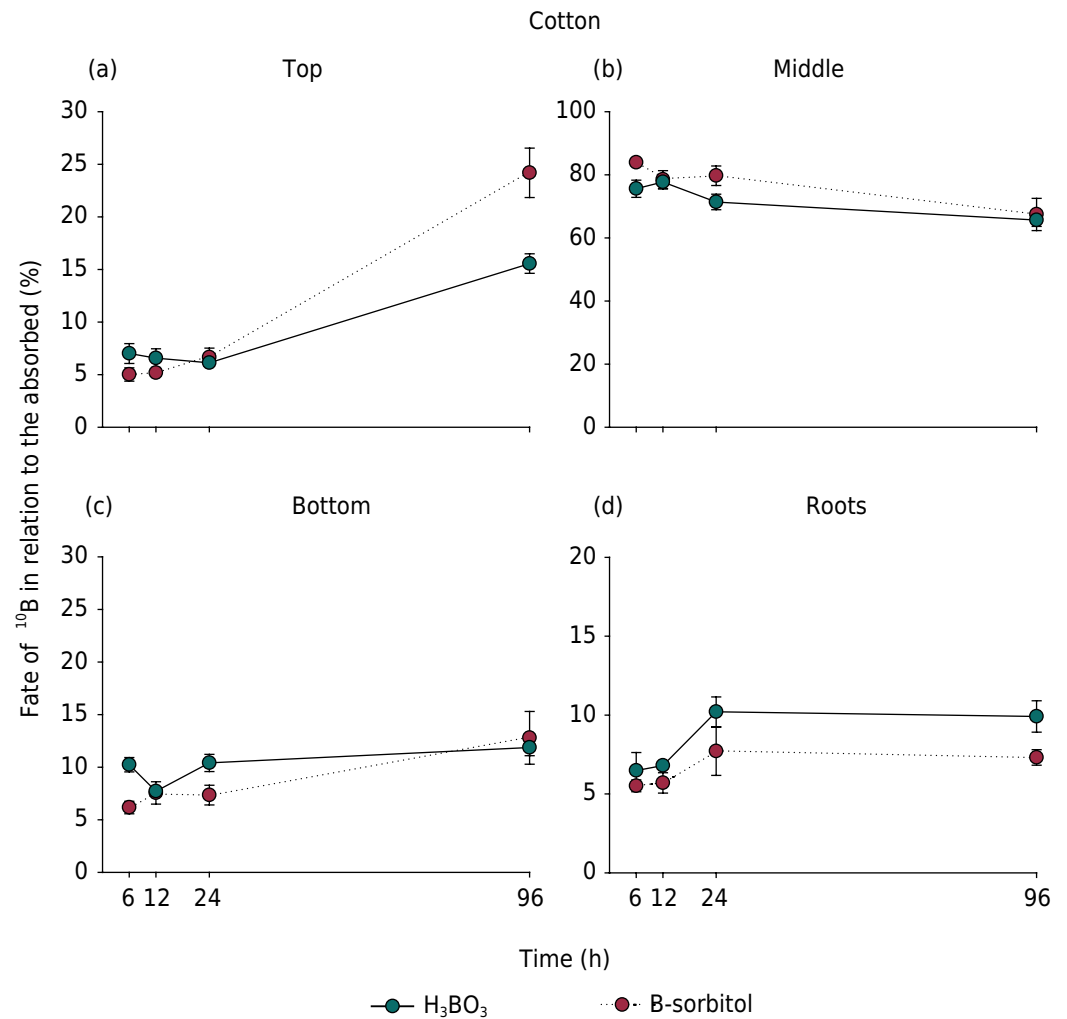


Figure 6. Fate of ^{10}B in relation to the absorbed, in the top (a), middle (b), bottom (c), and roots (d) of cotton, as a function of time after application of B as boric acid (H_3BO_3) and boric acid with sorbitol. Each plant part includes stems, leaves, and reproductive structures. Leaves of the middle were treated with B. Vertical bars represent the standard error (n = 4).

foliar-applied solution, or the minimal water-vapor pressure in which salts absorb water from the air and dissolve (Burkhardt and Eiden, 1994). Boric acid has a DRH of 98 %, an indication that it will be in solution in almost moisture-saturated air. Sorbitol has a DRH of 69 %, and should decrease the solution DRH when mixed to boric acid (Salameh et al., 2006). Hence, it was expected that sorbitol would improve boron uptake. In soybeans, B sources combined with sorbitol or mannitol applied via foliage increased in 18 to 25 % the B uptake compared with boric acid, but the absorption was only 32 % of the applied (Will et al., 2011). In our experiment, when the amount of B absorbed was compared with that applied to the leaves, in cotton and coffee plants, no significant differences were observed between B sources (Figure 1). The difference from results observed with soybean may be due to differences in cuticle wax thickness and nature.

There is evidence that some non-sugar-alcohol-producing plants can transport B to young tissues under low B supply (Tanaka and Fujiwara, 2008), and it has already been reported that there is small remobilization of B to the newer parts of cotton plants (Rosolem and Costa, 2000; Bogiani et al., 2014). However, in the present case, the remobilization of the applied nutrient combined with sorbitol was greater than when B was applied as boric acid (Figure 6), supporting early findings that B phloem redistribution is associated with the type and abundance of hydroxyl-bearing moieties, such as sugar-alcohols (Liakopoulos et al., 2009; Liu et al., 2015).

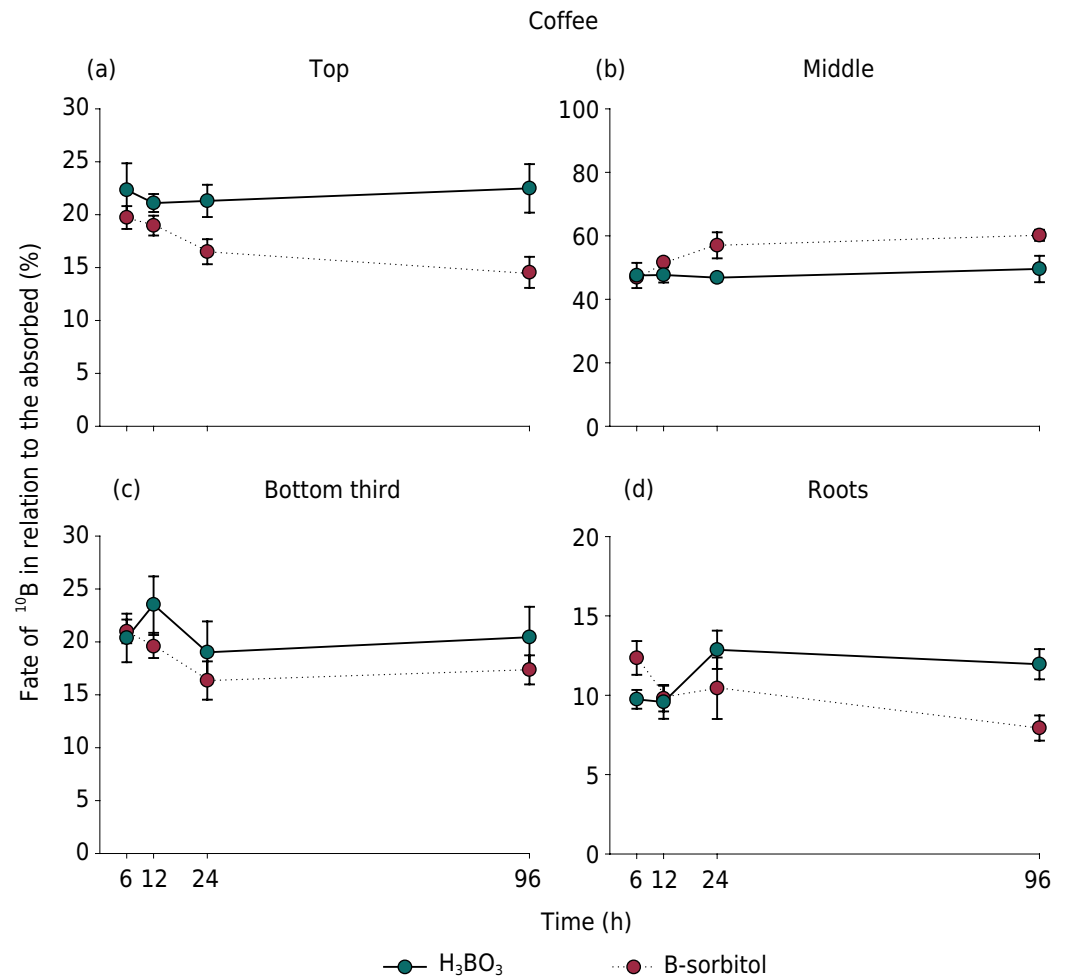


Figure 7. Fate of ^{10}B in relation to the absorbed, in the top (a), middle (b), bottom (c), and roots (d) of coffee, as a function of time after application of B as boric acid (H_3BO_3) and boric acid with sorbitol. Each plant part includes stems and leaves. Leaves of the middle were treated with B. Vertical bars represent the standard error (n = 4).

Leite et al. (2007) showed that B can be mobilized in coffee trees, in a field experiment. In this pot experiment, approximately 15 % of the B applied to coffee leaves was found in shoot parts where the fertilizer was not applied, and approximately 9 % was found in roots (Figure 5), irrespective of B source, which is much higher than in cotton (Figure 4). This may be a consequence of the greater B uptake by coffee, and also due to the natural mannitol content (Brown and Shelp, 1997).

In cotton and coffee, B_{off} accounted for 2 to 3 % in the regions of the plants where B was not applied. This result is similar to that obtained by Leite et al. (2007), who also found less than 3 % of the B in the leaves of coffee, which had come from boric acid applied to the leaves. While B_{off} was generally low in plants, when looking at the amount of B translocated as related to the absorbed, a little over 10 % was found in the bottom third of cotton while around 20 % was found in the bottom part of coffee trees. In cotton roots, there were 8 to 10 % of the B taken up, and in coffee it was from 8 to 12 %. It is noteworthy that in cotton, the addition of polyol enhanced B movement to the upper part of the plant, as discussed, but this was not observed in coffee, a mannitol-plant.



CONCLUSION



Independently of the B source, the proportion of foliar applied B taken up by coffee trees is greater than by cotton, which could be a result of the greater amount of wax in



cotton cuticles, and B remobilization is higher in coffee compared with cotton. The use of a solution of boric acid with 10 % of sorbitol and 14 % of monoethanolamine does not affect on B absorption. Boron mobilization in the transpiratory stream of cotton increases when the spraying solution has sorbitol and monoethanolamine, but not in coffee, a mannitol-bearing plant. However, there is evidence that B from the sorbitol/mannitol B source is less mobile in the phloem than B from boric acid, since its translocation to roots was lower in both cotton and coffee.




AUTHOR CONTRIBUTIONS

Conceptualization:  [Ciro Antonio Rosolem](#) (lead).



Methodology:  [Ciro Antonio Rosolem](#) (lead) and  [Danilo Silva Almeida](#) (supporting).

Validation:  [Danilo Silva Almeida](#) (lead) and  [Ciro Antonio Rosolem](#) (supporting).




Formal analysis:  [Caio Vilela Cruz](#) (lead) and  [Danilo Silva Almeida](#) (supporting).



Investigation:  [Danilo Silva Almeida](#) (lead),  [Ciro Antonio Rosolem](#) (supporting), and  [Caio Vilela Cruz](#) (supporting).


Resources:  [Ciro Antonio Rosolem](#) (lead).

Data curation:  [Danilo Silva Almeida](#) (lead) and  [Ciro Antonio Rosolem](#) (supporting).

Writing - original draft:  [Ciro Antonio Rosolem](#) (lead).

Writing - review and editing:  [Ciro Antonio Rosolem](#) (lead),  [Danilo Silva Almeida](#), and  [Caio Vilela Cruz](#) (supporting).

Visualization:  [Ciro Antonio Rosolem](#) (lead) and  [Danilo Silva Almeida](#) (supporting).

Supervision:  [Ciro Antonio Rosolem](#) (lead).

Project administration:  [Ciro Antonio Rosolem](#) (lead).

Funding acquisition:  [Ciro Antonio Rosolem](#) (lead).

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