# DIVISÃO 3 - USO E MANEJO DO SOLO

## Comissão 3.1 - Fertilidade do solo e nutrição de plantas

## COMPARED BORON UPTAKE AND TRANSLOCATION IN COTTON CULTIVARS<sup>(1)</sup>

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#### SUMMARY

The mobility of boron (B), a commonly deficient micronutrient in cotton, has been shown to be low in the plant phloem. Nevertheless, studies have indicated that cotton cultivars can respond differently to B application. A greenhouse experiment was conducted to compare B absorption and mobility in cotton cultivars grown in nutrient solution. Treatments consisted of three cotton cultivars (FMT 701, DP 604BG and FMX 993), and five B rates (0.0, 2.5, 5.0, 10.0, and 20.0  $\mu$ mol L<sup>-1</sup>). Plant growth and development were monitored for four weeks from the appearance of the first square. The time of onset and severity of B deficiency symptoms varied among cotton cultivars. Initial B uptake of cv. DP 604BG was lower than of the other cultivars, but a greater amount of available B in the nutrient solution was required to prevent deficiency symptoms in this cultivar. Boron deficiency impairs cotton growth, with no differences among cultivars, regardless of the time of appearance and intensity of B deficiency symptoms.

Index terms: variety, micronutrient, mobility, remobilization.

## **RESUMO:** ABSORÇÃO E TRANSLOCAÇÃO COMPARADA DE BORO POR CULTIVARES DE ALGODÃO

O boro (B) tem baixa mobilidade no floema das plantas e é reconhecidamente o micronutriente cuja deficiência é mais comum no algodoeiro. Neste trabalho foi estudada a absorção e mobilidade do B em cultivares de algodão. O experimento foi conduzido em casa de vegetação, e as plantas foram cultivadas em solução nutritiva. Os tratamentos foram constituídos de três cultivares de algodão (FMT 701, DP 604BG e FMX 993) e cinco doses de B

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(0,0; 2,5; 5,0; 10,0 e 20,0 μmol L<sup>-1</sup>). As avaliações foram feitas em quatro semanas consecutivas, a partir da primeira semana após emissão do primeiro botão floral. A época de aparecimento e a intensidade de sintomas de deficiência de boro entre cultivares de algodão são diferentes. A cultivar DP604BG é inicialmente menos exigente em B, porém há necessidade de maior disponibilidade desse micronutriente no meio nutritivo para evitar o aparecimento de deficiência. O crescimento do algodoeiro é prejudicado pela carência de B, independentemente das diferenças no aparecimento de sintomas, não havendo diferença entre as cultivares.

Termos de indexação: variedade, micronutriente, mobilidade, remobilização.

#### INTRODUCTION

Boron (B) has been known as an essential nutrient for over 85 years (Miwa & Fugiwara, 2010). It plays a role in cell wall formation (Brown et al., 2002), cell division and elongation (Goldbach et al., 2001; Oliveira et al., 2006), membrane functioning (Tanada, 1983; Zhao & Oosterhuis, 2003), and in carbohydrate metabolism and transport (Zhao & Oosterhuis, 2002). Boron deficiency, which leads to a rapid inhibition of plant growth due to its restricted phloem mobility (Rosolem & Costa, 2000), is the most common micronutrient deficiency in cotton crops around the world (Rosolem et al., 1999; Ahmad et al., 2009). However, some controversy remains about B mobility within cotton plants (Fontes et al., 2008), with regard to the most appropriate rates and application forms of the nutrient. In low B-mobility species, the part of the plant grown during a temporary deficiency shows the characteristic symptoms and growth impairment. However, if B were remobilized within the plant, the organs developed under the same conditions could be supplied with B translocated from other plant parts.

Cotton responds positively to B fertilization (Silva et al., 1995; Howard et al. 2000; Görmüs, 2005; Dordas, 2006) and enhanced knowledge about its mobility in the phloem is an important tool for diagnosing and correcting deficiency as well as toxicity (Brown & Shelp, 1997). Rosolem & Costa (2000) observed that foliar-applied B is not translocated from the application site to other plant parts, indicating restricted mobility of this nutrient in cotton. However, yield increases resulting from foliar applications of B (Carvalho et al., 1996) and B accumulation in shoots of plants under deficiency (Oliveira et al., 2006) suggest that there may be some B translocation within the plant. Although differential responses of cotton cultivars to B were not observed some time ago (Rosolem et al., 1999), modern cultivars could respond differently to B fertilization, as concluded by Fontes et al. (2008). Under field conditions, in the Brazilian cerrado, higher symptom intensity of B deficiency was reported for cv. ITA 90 than cv. Fibermax 966, but yields were not affected by B fertilization (Zancanaro & Tessaro, 2006).

Considering that differences may exist in B uptake and redistribution within cotton cultivars, this study investigated the absorption and distribution of this micronutrient in cotton and its effects on plant growth as affected by B availability in the nutrient solution.

#### MATERIAL AND METHODS

Three cotton cultivars were grown in a greenhouse in Botucatu, São Paulo, Brazil, in 4.0 L plastic pots with modified Hoagland & Arnon nutrient solution (Hoagland & Arnon, 1950). Cotton cultivars FMT 701 (medium to late maturity), FMX 993 (late maturity) and DP 604BG (transgenic and late maturity) were grown in solutions containing 0.0, 2.5, 5.0, 10.0 and 20.0  $\mu$ mol L<sup>-1</sup> B. The seeds (without pesticide treatment) were pre-germinated in trays containing washed sand, as described in Brazil (2009). Following emergence, the seedlings were selected for uniformity and two were planted per pot. During the first week, the nutrient solution was diluted to 1:10, the next week the dilution was 1:5. and after another week. the original solution was used until the end of the experiment. Boron rates were maintained constant. The nutrient solution was continuously aerated and monitored every 2 days. Lost water was replaced and the pH maintained at  $5.0 \pm 0.5$  by adding 0.1 mol L<sup>-1</sup> NaOH or 0.1 mol L<sup>1</sup> HCl. Deionized water was used throughout the experiment to prepare stock solutions, nutrient solution, and to wash the material (all plastic). As the plants grew, they were staked and tied to prevent lodging.

Four sets of pots were prepared and each one was harvested at weekly intervals, starting 31 days after plant emergence or at B1 (first square) stage, as described by Marur & Ruano (2001). At each harvest, plant shoots were separated in leaves, reproductive structures and stems. The number of reproductive and vegetative branches, number of internodes, plant height and stem diameter were determined. Plant tissue was dried at 65 °C in a forced air oven for three days, and then weighed and ground. Plant samples were digested according to Malavolta et al. (1989) and B was analyzed in an Inductively Coupled Plasma-Spethophotometer-ICP at a wavelength of 249.773 nm.

The experiment was arranged in a  $3 \times 5$  factorial design in complete randomized blocks with four replications. ANOVA was performed, and treatment means were compared using LSD (p < 0.05) using

SISVAR 5.3 (Ferreira, 2011) and polynomial regressions were fit to B responses using Sigmaplot 10.0.

#### **RESULTS AND DISCUSSION**

#### **Deficiency symptoms**

Boron deficiency appeared five days after transplanting and increased with time in plants grown without B in the nutrient solution. The symptoms were similar among cultivars and were first noticed in roots and later in the shoots. After 15 days, root growth was completely inhibited, roots were thicker, and there was an excessive emission of lateral roots not exceeding a length of 1.0 cm. The interruption or inhibition of root cell elongation is the most immediate response to B deficiency, and may start about three hours after supply interruption, with total inhibition within 24 h (Marschner, 1995). Boron deficiency symptoms in cotton shoots were first noticed and developed with higher intensity in cv. DP 604BG. Stem cracks were observed 23 days after emergence (DAE) in cv. DP 604BG and from 30 to 33 DAE in cv. FMT 701 and FMX 993. The petioles became twisted and lumpy; the leaf surface was smaller and deformed, and the stems showed dark green spots rings with corresponding internal necrosis.

For plants grown in the 2.5  $\mu$ mol L<sup>-1</sup> B solutions, the first deficiency symptoms (dark green rings on the petioles of new leaves) also appeared on DP 604BG seedlings, 25 DAE. This symptom was noticed 30 DAE on cv. FMT 701 and FMX 993. At this time, cv. DP 604BG began to show B deficiency symptoms in the treatment with 5.0  $\mu$ mol L<sup>-1</sup> B. The deficiency symptoms were present in all cultivars grown in solutions with B rates up to 5.0  $\mu$ mol L<sup>-1</sup> 38 DAE, but were most severe in DP 604BG.

The first symptoms of B deficiency in plants grown in the 10.0  $\mu$ mol L<sup>-1</sup> B solution appeared 52 DAE in cv. DP 604BG. At this time, dark green rings appeared on the stems of all plants grown in 2.5  $\mu$ mol L<sup>-1</sup> B; however, the rings on DP 604BG were larger, darker, and when cut lengthwise, there was a dark brown spot in the inner part of the lesion. On the other cultivars, the brown spot was lighter.

After 59 days, B deficiency symptoms with slightly deformed petals and smaller, yellowish bracts appeared on the flower buds of DP 604BG plants grown in solutions with 2.5  $\mu$ mol L<sup>-1</sup> B, similar to symptoms reported by Rosolem & Bastos (1997). At the dose of 10.0  $\mu$ mol L<sup>-1</sup> B, all cultivars showed B deficiency, which was again more intense in cv. DP 604BG.

No B-deficiency symptoms were observed on plants grown in the 20.0  $\mu mol \ L^{-1} \ B$  nutrient solution.

### Plant growth

The cv. FMX 993 was taller and showed greater number of internodes than the other cultivars up to two weeks after B1 (first square), but this effect disappeared from the third week onwards (Table 1). Cotton growth

Table 1. Plant height, number of internodes and baron (B) accumulated in cotton roots, stems, leaves, reproductive structures and total B in the plant, obtained in four harvests at weekly intervals starting at the first square (B1), as affected by B rates (0.0, 2.5, 5.0, 10.0 and 20.0  $\mu$ mol L<sup>-1</sup>) in the nutrient solution

Cultivar	Week			
	$1^{\mathrm{st}}$	$2^{\mathrm{nd}}$	$3^{\mathrm{rd}}$	$4^{\mathrm{th}}$
Plant height, cm				
FMT 701	44.9	54.5	54.2	61.1
$\mathrm{DP}~604~\mathrm{BG}$	45.0	53.1	55.5	61.2
FMX 993	48.2	56.4	56.1	62.8
LSD	2.5*	2.7*	$3.6^{\rm ns}$	$4.7^{\mathrm{ns}}$
Number of internodes				
FMT 701	8.5	10.2	9.9	10.4
$\mathrm{DP}~604~\mathrm{BG}$	8.6	10.1	9.9	10.4
FMX 993	9.0	10.6	10.3	10.9
LSD	0.4*	0.3**	$0.5^{ m ns}$	$0.5^{\rm ns}$
B accumulated in roots, mg/plant				
FMT 701	0.201	0.127	0.066	0.060
$\mathrm{DP}~604~\mathrm{BG}$	0.177	0.127	0.058	0.059
FMX 993	0.176	0.080	0.046	0.062
LSD	0.019*	0.024**	0.008**	$0.008^{\mathrm{ns}}$
B accumulated in stems, mg/plant				
FMT 701	0.199	0.201	0.153	0.207
$\mathrm{DP}~604~\mathrm{BG}$	0.171	0.184	0.158	0.217
FMX 993	0.211	0.166	0.166	0.189
LSD	0.022**	$0.021^{\rm ns}$	$0.021^{\rm ns}$	$0.029^{\rm ns}$
B accumulated in leaves, mg/plant				
FMT 701	0.244	0.262	0.240	0.298
$\mathrm{DP}~604~\mathrm{BG}$	0.197	0.236	0.258	0.269
FMX 993	0.208	0.307	0.307	0.313
LSD	0.034*	0.034**	0.055*	$0.056^{\rm ns}$
B accumulated in reproductive structures, mg/plant				
FMT 701	-	0.019	0.026	0.035
$\mathrm{DP}~604~\mathrm{BG}$	-	0.017	0.024	0.036
FMX 993	-	0.023	0.025	0.044
LSD	-	0.005*	$0.005^{\mathrm{ns}}$	0.008*
Total B accumulated in plant, mg/plant				
FMT 701	0.645	0.606	0.480	0.593
$\mathrm{DP}~604~\mathrm{BG}$	0.545	0.559	0.542	0.618
FMX 993	0.595	0.615	0.491	0.556
LSD	0.038**	0.046*	$0.074^{\rm ns}$	$0.069^{\rm ns}$

LSD = Least Significant Difference (p < 0.05); \* and \*\*: significant at 5 and 1 %, respectively, <sup>ns</sup>: no significant.

decreased when grown without B in the nutrient solution. Plant height was increased by B levels up to 6.74, 6.54, 5.87 and 6.14  $\mu$ mol L<sup>-1</sup>, with no further significant response up to 20.0  $\mu$ mol L<sup>-1</sup> (Figure 1a). Plant height and number of internodes on the main stem were not affected by the interaction of B rates

The number of internodes showed a significant increase with B rates to 2.5  $\mu$ mol L<sup>-1</sup> and then a smaller increase up to 7.03, 5.43, 5.80 and 5.80  $\mu$ mol L<sup>-1</sup> (Figure 1b). Boron deficiency results in plant growth inhibition by affecting cell division and elongation and damaging the cell wall structure of the meristematic tissues (Marschner, 1995). In addition, it decreases essential carbohydrate synthesis and transport within the plant (Zhao & Oosterhuis, 2002). Although B is important for cotton growth (Rosolem & Costa, 2000), the level between 5.87 and 6.74  $\mu$ mol L<sup>-1</sup> in the nutrient solution was enough and adequate for the plants to express their maximum growth (Figure 1a).

In the four evaluations made after B1, no significant differences were observed in stem diameter between cultivars, and the responses to B followed the same trend as for height, with the maximum stem diameter at the dose of 7.11, 6.79, 6.13 and 6.41  $\mu$ mol L<sup>-1</sup> for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week, respectively (Figure 1c). Under severe B deficiency, cotton plant growth is affected as a whole because there are few malformed and/or disrupted xylem vessels, with thicker walls (Oliveira et al., 2006), and this anomaly reduces assimilate translocation from leaves to the plant meristems and fruits (Zhao & Oosterhuis, 2002). When B deficiency is less severe, the stems are thickened because of the changes between source and sink favor vegetative growth (Rosolem et al., 2001). Therefore, cotton plants under mild deficiency of B showed main stem diameter similar or even greater than those with adequate B supply (Figure 1c). Generally cotton cultivars responded similarly to B; however, at 20.0 µmol L<sup>-1</sup>, the stem diameter of FMX 993 was greater than of the other cultivars (Figure 1d).

There was no significant difference in dry matter yields of root, stems and leaves among cotton cultivars. In contrast, Rosolem et al. (2012) observed a difference in dry matter production of roots and shoots between cultivars FMT 701 and FMT 523, FMX 993 and FMX 910 grown in normal and deficient B solutions.

Root growth was inhibited when there was no B in the solution (Figure 1e). Root dry matter production was highest at 7.41, 6.45, 6.26 and 7.34  $\mu$ mol L<sup>-1</sup> for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week, respectively, another indication that this B level in the nutrient solution was adequate for plant growth. However, it is worth mentioning that at this dose, the typical B deficiency symptoms on the cotton petioles, i.e. dark green rings and pith necrosis, as described by Rosolem et al. (2001), were easily noticed.

Generally, leaves, stem and toots dry-matter yields increased with B rates up to 6.0  $\mu$ mol L<sup>-1</sup> (Figure f,g),

and there was no interaction between B solution levels and cultivars in leaf and shoot dry matter production. When solution B was increased to 20  $\mu$ mol L<sup>-1</sup>, leaf dry matter production was decreased, a possible B toxicity symptom.

Interestingly, some B deficiency symptoms were observed in plants grown in 10.0  $\mu$ mol L<sup>-1</sup> B solutions, but plant height, number of nodes and dry matter yields were not affected. Likewise, Oliveira et al. (2006) stated that plant height was the most sensitive parameter when measuring cotton response to B application.

#### B uptake and distribution within the plant

Boron accumulation in cotton roots increased with B rates up to 20.0  $\mu$ mol L<sup>-1</sup> (Figure 2a). For three weeks, after the first square (B1), cv. FMT 701 always showed the highest B accumulation in roots, and cv. FMX 993 the lowest B, but cv. DP 604BG is equal FMT 701 (Table 1). The presence of B in plants grown without B in the nutrient solution is probably due to nutrient reserves from the seeds.

There was no difference among cultivars in the amount of B accumulated in stems, except for the first week after B1, when the cv. FMX 993 and FMT 701 showed higher values than DP 604BG (Table 1). Boron uptake by cotton cultivars followed the same pattern as dry matter accumulation (Figure 2b). Boron accumulation in the cotton stems, roots and reproductive structures was not affected by the interaction of B doses with cultivars. The substantial B accumulation in leaves can be explained by the low mobility of the nutrient in the cotton phloem (Rosolem & Costa, 2000), because the leaves have a high transpiration rate, the main driving force of B transport within the plant (Ahmed et al., 2008), decreasing the chance of differences among cultivars. The amount of B accumulated in the leaves increased exponentially with B rates in the nutrient solution (Figure 2c). For the second week after B1, there was increased amount of B in leaves of the three cotton cultivars, being higher for the FMX 993, and lowest for the DP604BG (Figure 2e).

Boron accumulation in the reproductive structures of cv. FMX 993 was generally higher than in the other cultivars (Table 1). In the second and fourth week after B1, the amount of B accumulated in the reproductive structures was increased with solution B up to 10.0  $\mu$ mol L<sup>-1</sup>, but in the third week the response was linear up to 20.0  $\mu$ mol L<sup>-1</sup> (Figure 3d).

Total B accumulated in DP 604BG plants during the first two weeks after B1 was lower than the amounts accumulated by cv. FMT 701 and FMX 993, but this difference disappeared after the second week (Table 1). Consequently, it can be inferred that B requirements of FMT 701 and FMX 993 are higher early in the growing cycle. DP 604BG, in spite of requiring less B, showed B deficiencies earlier than the other cultivars and the symptoms were more

and cotton cultivars.



Figure 1. Plant height (a), number of internodes (b) and stem diameter (c) of cotton plants, averaged over three cultivars, as affected by solution B and weeks after the first square stage (B1); and stem diameter of three cotton cultivars at the second week after B1 (d), as affected by B rates in the nutrient solution. LSD = Least Significant Difference; \* and \*\* significant at 5 and 1 %, respectively. ●, ▼, ■, ♦ = 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week, respectively. ●, ▼, ■ = FMT 701, DP 604BG and FMX 993, respectively.

intense at similar B levels in the nutrient solution. Therefore, this cultivar may be less efficient in acquiring B from the solution. A difference in growth was expected, but did not occur. Total B accumulation increased up to 20.0  $\mu$ mol L<sup>-1</sup> of B in the nutrient solution (Figures 3a, b).

The relative B distribution within the plants was calculated every week (Figure 4). In the third week,

cv. FMX 993 and FMT 701 showed similar trends, but at low B availability (up to  $5.0 \ \mu mol \ L^{-1}$ ), the percentage of B was lower in the reproductive structures of DP 604BG. This is an indication that DP 604BG is not more efficient in allocating B to the reproductive structures.

At low B availability, the proportion of B retained in the roots was higher, and was decreased with



Figure 2. Boron accumulated in root (a), stem (b), leaves (c) and reproductive structures (d) of cotton plants, measured in four harvests in weekly intervals starting at B1, as affected by B rates in the nutrient solution. When there was no B in solution all the reproductive structures shed and there was B accumulation at zero B (d). \* and \*\* significant at 5 and 1 %, respectively. ●, ▼, ■, ♦=1st, 2nd, 3rd and 4th week, respectively. ●, ▼, ■ = FMT 701, DP 604BG and FMX 993, respectively.



Figure 3. Total boron accumulated in cotton, measured in each of four harvests at weekly intervals starting at B1 (a), and total B accumulated in plants of three cotton cultivars in the third week after B1 (b), as affected by B rates in the nutrient solution. LSD = Least Significant Difference; \* and \*\* significant at 5 and 1 %, respectively. •, ▼, ■, • = 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week, respectively. •, ▼, ■ = FMT 701, DP 604BG and FMX 993, respectively.

increased B availability in the nutrient solution. As B availability increased, B was accumulated mainly in cotton leaves, probably because transpiration rates are higher in the leaves (Figure 4). It has been observed that B concentrations in the leaf blades of B deficient cotton plants decreased compared with control plants under normal B supply. However, B deficiency decreased the proportion of the nutrient in leaves and fruits and increased the proportion of B retained in roots and stems (Zhao & Oosterhuis, 2002). In this experiment, when B concentration in the solution was low but still sufficient to avoid fruit abortion, the plants tended to accumulate a higher proportion of B in the reproductive structures.

#### CONCLUSIONS

1. The time of onset and severity of B deficiency symptoms varies among cotton cultivars.

2. Cultivar DP 604BG requires less boron up to the second week after the first square, but needs more B available in solution to avoid B deficiency than the other cultivars.

3. Cotton growth is decreased by boron deficiency, with no differences among cultivars, in spite of the differences in symptom intensity.

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Treatment (Cultivar - rate)

Figure 4. Boron distribution in vegetative and reproductive tissues of cotton cultivars (FMT 701, DP 604 BG, FMX 993), three weeks after B1, as affected by boron doses (0.0, 2.5, 5.0, 10.0, and 20.0 μmol L<sup>-1</sup>) in the nutrient solution.

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