Revista Brasileira de Ciência do Solo
ISSN: 0100-0683
revista@sbcs.org.br
Sociedade Brasileira de Ciência do Solo
Brasil

Rosolem, Ciro Antonio; Leite, Vagner Maximino
COFFEE LEAF AND STEM ANATOMY UNDER BORON DEFICIENCY
Revista Brasileira de Ciência do Solo, vol. 31, núm. 3, 2007, pp. 477-483
Sociedade Brasileira de Ciência do Solo
Viçosa, Brasil

SEÇÃO IV - FERTILIDADE DO SOLO E NUTRIÇÃO DE PLANTAS

COFFEE LEAF AND STEM ANATOMY UNDER BORON DEFICIENCY[1]

Ciro Antonio Rosolem[2] & Vagner Maximino Leite[3]

SUMMARY

Boron deficiency in coffee is widely spread in Brazilian plantations, but responses to B fertilizer have been erratic, depending on the year, form and time of application and B source. A better understanding of the effects of B on plant physiology and anatomy is important to establish a rational fertilization program since B translocation within the plant may be affected by plant anatomy. In this experiment, coffee plantlets of two varieties were grown in nutrient solutions with B levels of 0.0 (deficient), 5.0 µM (adequate) and 25.0 µM (high). At the first symptoms of deficiency, leaves were evaluated, the cell walls separated and assessed for B and Ca concentrations. Scanning electron micrographs were taken of cuts of young leaves and branch tips. The response of both coffee varieties to B was similar and toxicity symptoms were not observed. Boron concentrations in the cell walls increased with B solution while Ca concentrations were unaffected. The Ca/B ratio decreased with the increase of B in the nutrient solution. In deficiency of B, vascular tissues were disorganized and xylem walls thinner. B-deficient leaves had fewer and deformed stomata.

Index terms: boron deficiency, Ca/B ratio, coffee nutrition, stomata.

RESUMO: *ANATOMIA DE RAMOS E FOLHAS DE CAFEEIRO SOB DEFICIÊNCIA DE BORO*

A deficiência de B é muito comum nos cafezais brasileiros, mas as respostas do cafeeiro ao B têm sido erráticas, dependendo do ano, do modo e época de aplicação e, ainda, da fonte de B empregada. Um melhor entendimento dos efeitos do B na fisiologia e anatomia

---

[1] Parte da Tese de Doutorado do segundo autor. Financiado parcialmente pela FAPESP. Recebido para publicação em maio de 2005 e aprovado em fevereiro de 2007.

[2] Professor Titular do Departamento de Produção Vegetal, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista – UNESP. Caixa Postal 237, CEP 18603-970 Botucatu (SP). Bolsista do CNPq. E-mail: rosolem@fca.unesp.br

[3] Professor da Faculdade de Agronomia e Engenharia Florestal de Garça. Rua das Flores 740, Caixa Postal 321 CEP 17400-000 Garça (SP). E-mail: vleite@fca.unesp.br

do cafeeiro é importante para o desenvolvimento de um programa racional de adubação boratada, uma vez que a anatomia da planta pode influenciar a translocação do nutriente. Neste experimento, plantas de dois cultivares foram cultivadas em soluções nutritivas com 0,0 (deficiente), 5,0 (adequado) e 25,0 µM (alto) de B. Quando os primeiros sintomas de deficiência apareceram, as folhas foram cortadas e tiveram suas paredes celulares isoladas e analisadas quanto aos teores de B e Ca. Cortes foram feitos em folhas novas e no ápice de ponteiros e fotografados em microscópio eletrônico de varredura. A resposta dos dois cultivares ao B foi semelhante, não tendo sido observados sintomas de toxidez. O teor de B nas paredes celulares foi aumentado com o incremento da concentração desse elemento na solução, enquanto o teor de Ca não foi afetado. A relação Ca/B decresceu com o aumento da concentração de B na solução. Com deficiência de B, os tecidos vasculares foram desorganizados e as paredes do xilema ficaram mais finas. Folhas de café com deficiência deste nutriente apresentaram menos estômatos, os quais se encontravam.

Termos de indexação: deficiência de boro, relação Ca/B, nutrição do cafeeiro, estômatos.

INTRODUCTION

Coffee is one of the most B-sensitive and responsive species (Brown & Shelp, 1997). Boron deficiency is widespread in Brazilian plantations (Malavolta, 1986; Malavolta et al., 2001), resulting in a reduced root system, flower abortion, fruit malformation and consequently low yields (Franco, 1982). Corrêa et al. (1986) reported higher B concentrations in coffee leaves than in stems and fruits. Moreover, it was observed that fruit-bearing branches had more B than branches without fruits and that B concentration in the plant was highest during the rainy months, indicating B transport through the xylem (Chaves, 1982). Boron fertilizer is recommended in Brazil when soil B (hot water extraction) is below 0.06 mg dm^{-3} (Raij, 1996) or leaf B content below 60 mg kg^{-1} (Malavolta et al., 2001), but responses of coffee trees to B fertilizer have been erratic, depending on the year, way and time of application and B source (Santinato et al., 1991; Almeida & Matiello, 1996).

Lima Filho & Malavolta (1998) found a positive relation between coffee branch length and B leaf concentrations, whereas dry matter yield was not related to B levels in the nutrient medium. Moreover, Marubayashi et al. (1994) observed that B concentrations in the leaves were not related to coffee yields when boric acid was applied to coffee leaves. Santinato et al. (1991) sprayed B onto coffee leaves immediately after a weak flush of flowers (September) and before main flowering (October) and observed an increase in coffee yields due to higher fruit retention (30 % superior to the control), while in the second year, when coffee yields were lower, there was no response to foliar applied B.

Boron deficiency in coffee first becomes apparent in newly developed leaves, which is typical of immobile nutrients (Brown & Hu, 1994). Leaf B concentrations generally increase as plant and leaf age. In extreme situations, older leaves can be affected by B toxicity and the younger ones by B deficiency (Oertly, 1994). This can be a problem in managing B fertilization in coffee, because a recently matured leaf sampled for foliar diagnosis may not be representative, since a significant portion of B in the leaf is provided by the xylem. Boron concentration of a mature leaf can therefore not adequately reflect the B status of growing tissues for which a constant B supply is most critical (Brown & Shelp, 1997).

When B availability is low, over 90 % of the total B in plant tissues is bound in the cell walls (Matoh et al.; 1992). Brown & Hu (1994) and Matsunaga & Nagata (1996) also determined that most of the plant B is strongly complexed in the pectin fraction of the cell walls. As a result of the critical role of B in expanding tissues and its limited mobility, it has to be supplied continually throughout the plant life cycle, usually through the roots (Brown & Hu, 1994). If it is withdrawn from the nutrient medium of a plant that does not translocate B in the phloem, even for a short period, the deficiency causes reduced growth (Dell & Huang, 1997). In faba bean (*Vica faba*), the xylem of plants exposed to B deficiency for more than 24 hours was deformed (Robertson & Loughman, 1974). Boron deficiency in cotton leads to necrosis of the xylem tissue. Moreover, in B-deficient cotton plants, the xylem vessel walls were thicker and vessels were fewer, with an irregular perimeter (Sanfuentes, 1966). Hypertrophy and size modification of the cortex cells and poor differentiation of the vascular tissues in B-deficient common bean were reported by Moraes-Dallaqua (1992), and thinned cell walls were observed in tomato roots (Kouchi & Kumazawa, 1976).

Cotton shoot dry matter yields, plant height and flower and fruit set were reduced by a temporary B deficiency, which could not be prevented by foliar application of B (Rosolem & Costa, 2000). The authors hypothesized that temporary B deficiency in a plant that does not retranslocate B causes permanent damage, once growth was not fully recovered after replacing B in the nutrient solution. However, this effect could be prevented with B application to mature

cotton leaves during B shortage in the solution, probably by preventing xylem malformation during the deficiency period (Robertson & Loughman, 1974).

Considering the lack of relationship between B concentrations in leaves and fruits (Marubayashi et al., 1994; Lima Filho & Malavolta, 1988) and the inconsistent responses to B fertilization (Santinato et al., 1991; Almeida & Matiello, 1996), as well as a likely effect of B on xylem formation (Rosolem & Costa, 2000), a temporary deficiency could impair B translocation and redistribution thereafter.

A better understanding of the effects of B on plant anatomy and physiology is important in the development of a rational fertilization program. The objective of this paper was to study the effects of B deficiency on the leaf anatomy of two coffee varieties.

MATERIAL AND METHODS

Coffee plantlets were grown from seeds of two representative Brazilian varieties (Catuai and Mundo Novo) in 12 L pots filled with washed sand and nutrient solutions (Hoagland & Arnon, 1950) with 0.0 (deficient), 5.0 μmol L^{-1} (adequate) and 25.0 μmol L^{-1} (high) B. The pots were irrigated with nutrient solution twice a week. Two plants were grown per pot. When the first deficiency symptoms appeared, the 8th branch from the plant apex was marked and leaves were collected from the 4th node from the branch apex. The two leaves from the node were collected. One of the leaves was used to determine B in the tissue, using dry ashing and azomethine-H (Malavolta et al., 1997). The other leaf from the same node was used for cell wall analysis. Cell walls were isolated (Kobayashi & Matoh, 1997) and assessed for B and Ca concentrations. The leaves for cell wall isolation were wrapped in plastic bags and covered with TRIS-HCl/sucrose pH 7.0 and deep-frozen at -18 °C. The leaves, thawed 24 h before processing, were ground with the proper solutions. Cell walls were separated by filtration, using an Erlenmeyer, a vacuum pump and two filters: a 25 mesh plastic filter and a fiberglass filter. The filtered material was collected and washed thrice with ethanol (99.5 %). Then the material was immersed in a 98.5 % chloroform – 99.5 % ethanol solution (2:1) for 14 hours, filtered again and dried. Boron was assessed in the leaves and for Ca analysis the cell walls were wet-digested and Ca was determined by Atomic Absorption (Malavolta et al., 1997).

Simultaneously the eight most recently expanded deficient and non-deficient leaves were sampled and cuts were made in leaves and stems using a microtome and scanning electron micrographs were taken. No leaves with toxicity symptoms were found. The samples were covered with 2.5 % glutaraldehyde diluted in phosphate buffer and stored in the refrigerator. They were washed thrice in NaCl (9 g L^{-1}) for 10 min to remove glutaraldehyde and treated with osmium tetroxide (10 g L^{-1}) for 2 hours. Then they were washed again with NaCl. After this, they were dehydrated in 50, 70, 80 and 90 % alcohol (15 min each) and washed four times in 100 % alcohol. Then the samples were fixed with carbon glue, the alcohol eliminated and they were saturated with liquid CO_2 and coated with gold. The readings were made in a scanning electronic microscope (LEO Electron Microscopy Ltd, Model 435 VIP, with visualization in high vacuum). Pictures were taken on Neopan ACROS 100, 120 mm film and developed on Kodachrome 2 RC photographic paper.

The experimental design was a 2 x 3 factorial in complete randomized blocks, with six replications. Means were compared using LSD ($P < 0.05$).

RESULTS AND DISCUSSION

The xylem vessels of non-deficient coffee branch tips were continuous and relatively straight (Figure 1a). On the contrary, in B-deficient plants the xylem vessels were tortuous and there were discontinuities. The same was true for the main and secondary veins of the leaves (Figures 2, 3). The vascular bundles in B deficient leaf veins were randomly distributed, unlike non-deficient plants. Besides, the xylem vessel walls were thinner in B-deficient solution. A very similar effect was observed in the secondary leaf veins (Figure 3). Thinner cell walls were reported for tomato roots as well (Kouchi & Kumazawa, 1976). Oliveira et al. (2003) reported that in cotton there was a decrease in the number of vascular bundles set in a continuous ring. The xylem elements lacked differentiation, the medulla was smaller and the cells had different shapes. These results possibly occurred as a consequence of the effect of B on cell division and differentiation (Cakmak & Homheld, 1997).

In some of the pictures the xylem vessels seem to have been ruptured rather than cut by the microtome blade. This may be due to a lower resistance of the tissues caused by the thinned cell walls or by reduced lignification of the tissues. The thinned walls can be explained by the dependence of xylem walls on lignin synthesis (Brett & Waldron, 1996) and on pectin deposition, which can both be impaired by B deficiency (Pilbean & Kirby, 1983).

Oertly (1994) observed that B concentration was variable and that the transpiration rate affected B transport within the leaf. In our experiment the stomata amount and shape were different in leaves of deficient plants (Figure 4). In non-deficient leaves or a leaf region where B deficiency symptoms were not apparent there were many more stomata and their distribution in the leaf was more uniform than in

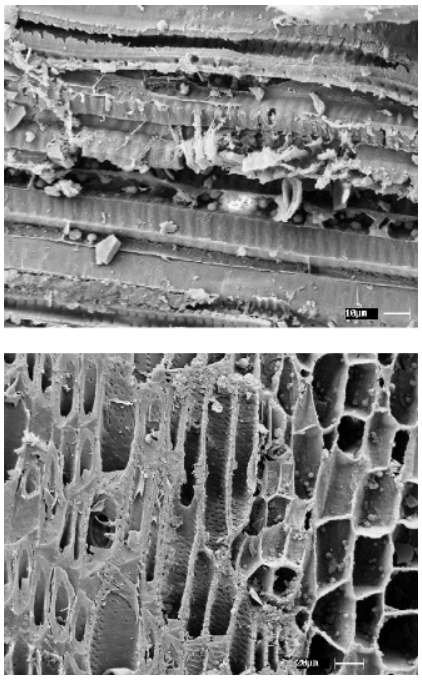

Figure 1. Vascular bundles in coffee tree stems grown in adequate (top) and deficient bottom B solutions. Observe xylem and phloem vessel discontinuities in deficient plants.

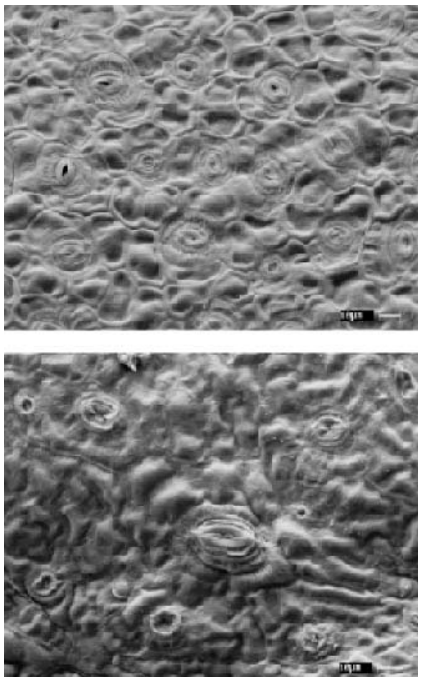

Figure 3. Abaxial leaf surfaces of coffee trees grown in sufficient (top) and deficient (bottom) B solutions.

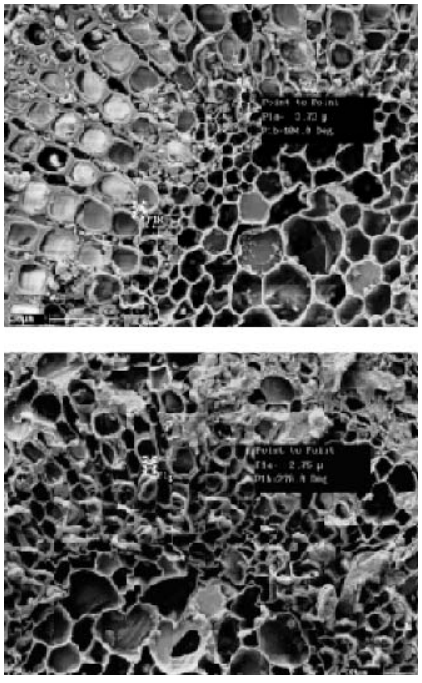

Figure 2. Central vein of leaves from coffee trees grown in adequate top) and deficient (bottom) B solutions. Xylem vessels are disorganized and the walls thinner in deficient plants.

deficient leaf regions or in deficient leaves. These alterations can affect the transpiration rate, nutrient absorption and transport, and eventually leaf and plant growth.

Total B concentrations in coffee leaves were higher in cv. Mundo Novo than Catuaí (Table 1). In comparison with the adequate levels of 60 to 80 mg kg^{-1} (Malavolta et al., 2001), the values observed were relatively low. However, only plants in the deficient nutrient solutions presented B deficiency symptoms. Conversely, the toxicity level reported by Malavolta et al., (2001), of over 200 mg kg^{-1}, was far higher than the B concentrations in this experiment. Considering B and Ca in the cell walls, the response to B deficiency was similar for both cultivars. Boron concentrations in the cell walls increased with B solution, while calcium concentrations were not affected and Ca/B ratio decreased with the increase in B solution (Table 2). Boron concentrations in the cell walls were higher for Mundo Novo, but the Ca/B ratio was higher for Catuaí. Considering that Ca/B in the wall constitution should be constant because of their role in RG-II (Rhamnogalacturam) synthesis (Power & Woods, 1997), it can be inferred that low B supply was not enough for the establishment of a normal cell wall, leading to cell wall and vessel mal-formation, with abnormal thickness and distribution. On the other hand, the excess B in solution further decreased Ca/B ratios, showing that this ratio is not constant in the cell walls. In this case, part of this B would be

available for translocation, because it is probably not bound to the cell wall pectin chains. The variation in Ca/B ratio seems to depend on the species and/or cultivar.

Boron concentrations in the cell walls were 4 to 6 times higher than in leaves, showing that a major proportion of the nutrient was bound to cell walls. Furthermore, no relationship of this ratio to B levels in the nutrient solution was observed. According to Matoh et al. (1992) and Brown & Hu (1994) most of the total B in plant tissues is bound in the cell walls when B availability is low, complexed in the pectin fraction of the cell walls. Hu & Brown (1994) reported that with an increase in B supply there was a relative decrease in the B bound to cell walls. According to Dannel et al. (2002), the subcellular compartmentation of B still remains a matter of controversy, but it is unequivocally clear that B is present in all subcellular compartments (apoplasm, cell wall, cytosol and vacuole). The relative distribution of B among these compartments can vary considerably, and depends on plant species and experimental conditions.

The results of this experiment show that, if there is some translocation of B to growing tissues in coffee trees, it is not enough to prevent tissue malformation. It has been shown that B applied to mature leaves can be retranslocated to growing points in some fruit trees containing sugar-alcohols (Hanson, 1991). However, it is accepted that once B has been incorporated into cell walls it is effectively removed from circulation and not available to support new growth (Shorrocks, 1997). Here, B was incorporated into cell walls, probably bound to Ca, and was unavailable for the regular formation of new vascular tissue (Figures 2 and 4).

Rosolem & Costa (2000) observed that a temporary B deficiency seems to cause a permanent damage to cotton plants, once there was no full growth recovery when B was replaced in nutrient solution. This was attributed to xylem malformation in the deficiency period (Robertson & Loughman, 1974) what seems to be the case in this experiment with coffee as well. That deficient plants fail to resume elongation is due either to a lack of incorporation of B into the cell wall or to a requirement for B early in the development that conditions later cell wall expansion (Jiao et al., 2005). Thus, it is possible to infer that after a B deficiency coffee plants can not fully recover growth,

Table 1. Boron concentrations in coffee leaves as affected by cultivar and B in the nutrient solution

Boron level in nutrient solution	Cultivar	
	Mundo Novo	Catuaí
	mg kg^{-1}	
Deficient (0.0)	18.5	9.8
Adequate (5.0 µmol L^{-1})	19.4	19.4
High (25.0 µmol L^{-1})	34.0	23.9
LSD ($P < 0.05$)	2.9	

Table 2. Boron, Calcium and Ca/B ratio in leaf cell walls of coffee trees as affected by B deficiency and cultivar

Boron level in nutrient solution	Cultivar					
	Mundo Novo			Catuaí		
	B	Ca	Ca/B	B	Ca	Ca/B
	mg kg^{-1}			mg kg^{-1}		
Deficient (0.0)	90	23	256	56	23	411
Adequate (5.0 µmol L^{-1})	121	24	198	65	25	384
High (25.0 µmol L^{-1})	165	28	170	101	31	307
LSD ($P < 0.05$)	8.0	9.1	25	8.0	9.1	25

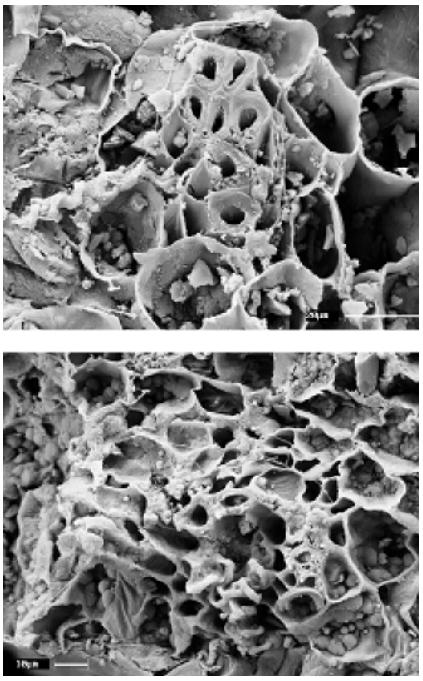

Figure 4. Secondary leaf veins of coffee grown in adequate top) and deficient (bottom) B nutrient solutions. Note disorganization of xylem vessels in deficient plants.

because the vascular system is impaired, and the damage, even of temporary deficiency, is permanent. Boron deficiency in coffee impairs normal xylem formation and alters stomata number, anatomy and distribution. This can eventually result in lower B translocation within the plant.

CONCLUSIONS

1. The response of the two coffee varieties Mundo Novo and Catuaí to B was similar and toxicity symptoms were not observed.

2. Boron concentrations in the cell walls increased with B solution while Ca concentrations were unaffected. The Ca/B rate in the leaf cell walls decreased with the increase of B level in the nutrient solution.

3. Under B deficiency vascular tissues were disorganized and xylem walls were thinner. Deficient regions of coffee leaves showed less and deformed stomata.

LITERATURE CITED

ALMEIDA, S.R. & MATIELLO, J.B. New sources and ways of application of B in coffee. In: CONGRESSO BRASILEIRO DE PESQUISAS CAFEEIRAS, 22., Águas de Lindóia, 1996. Anais. Rio de Janeiro, Instituto Brasileiro do Café, 1996. p.83-84.

BRETT, C.T. & WALDRON, K.W. Cell architecture and the skeletal role of the cell wall. In:BRETT, C.T. & WALDRON, K.W. Physiology and biochemistry of plant cell walls. London, Chapman & Hall, 1996. p.44-74.

BROWN, P.H. & HU, H. Boron uptake by sunflower, squash and cultured tobacco cells. Plant Physiol., 91:435-441, 1994.

BROWN, P.H. & SHELP, B.J. Boron mobility in plants. Plant Soil, 193: 85-101, 1997.

CAKMAK, I. & ROMHELD, V. Boron deficiency-induced impairments of cellular functions in plant. Plant Soil, 193:71-83, 1997.

CHAVES, J.C.D. Concentração de nutrientes nos frutos e folhas e exportação de nutrientes pela colheita durante um ciclo produtivo de cafeeiro. Piracicaba, Escola Superior de Agricultura Luiz de Queiroz, 1982. 131p. (Tese de Mestrado)

CORRÊA J.B., GARCIA, A.W.R. & COSTA, P.C. Extração de nutrientes pelos caffeiros Mundo Novo e Catuaí. In: CONGRESSO BRASILEIRO DE PESQUISAS CAFEEIRAS, 13., São Loureço, 1986. Rio de Janeiro, IBC/GERCA, 1986. p.35-41.

DANNEL, F.; PFEFFER, H. & ROMHELD, V. Update on boron in higher plants - Uptake, primary translocation and compartmentation. Plant Biol., 4:193-204, 2002.

FRANCO, C.M. Micronutrientes na cultura do cafeeiro. In: FRANCO, C.M. Micronutrientes. Campinas, Fundação Cargill, 1982. p.75-90.

HANSON, E.J. Movement of boron out of tree fruit leaves. Hortscience, 26:271-273, 1991.

HOAGLAND, D.R. & ARNON, I. The water culture method for growing plants without soil. S.l., 1950. (Circular, California Agricultural Experiment Stations 347)

HU, H. & BROWN, P.H. Localization of boron in cell walls of squash and tobacco and its association with pectin. Plant Physiol., 105:681-689, 1994.

JIAO, X.Y.; ZHU, Y.G.; JARVIS, B.C.; QUICK, W.P. & CHRISTIE, P. Effects of boron on leaf expansion and intercellular airspaces in mung bean in solution culture. J. Plant Nutr., 28:351-361, 2005.

KOBAYASHI, M. & MATOH, T. In vitro reconstitution of the boron-polysacharide complex purified from cultured tobacco BY-2 cells. In: BELL, R.W. & REKARSEM, B., eds. Boron in soils and plants. Dordrecht, Kluwer Publication, 1997. p.237-241.

KOBAYASHI, M.; NAKAGAWA, H.; ASAKA, T. & MATOH, T. Borate-rhamnogalacturonan II bonding reinforced by Ca retains pectic polysaccharides in higher-plant cell walls. Plant Physiol., 119:199-203, 1999.

KOUCHI, H. & KUMAZAWA, K. Anatomical responses of root tips to boron deficiency. III. Effect of boron deficiency on sub-cellular structure of root tips, particularly on cell wall and its related organelles. Soil Sci. Plant Nutr., 22:53-71, 1976.

LIMA FILHO, O.F. & MALAVOLTA, E. Evaluation of extraction procedures on determination of critical soil and foliar level of boron and zinc in coffee plants. Comm. Soil Sci. Plant Anal., 29:825-833, 1998.

MALAVOLTA, E Nutrição, adubação e calagem. In: RENA, A.; MALAVOLTA, E.; ROCHA, M. & YAMADA, T., eds. Cultura do cafeeiro: Fatores que afetam a produtividade. Piracicaba, Potafós, 1986. p.165-274.

MALAVOLTA, E.; ROSIAS, F.G.; OLIVEIRA, S.A; HEINRICHS, R.; CASALE, H.; BRAGANÇA, S.M. & GUIMARÃES, P.G. Cacau, café, chá, fumo e mate. In: FERREIRA, M.E.; CRUZ, M.C.P.; RAIJ, B. van & ABREU, C.A. Micronutrientes e elementos tóxicos na agricultura. Jaboticabal, CNPq/FAPESP/ Potafos, 2001. p.425-458.

MALAVOLTA, E.; VITTI, G.C. & OLIVEIRA, S.A. Avaliação do estado nutricional das plantas. Piracicaba, Potafos, 1997. 319p.

MARUBAYASHI, O.M.; PEDROSO, P.A.C.; VITTI, G.C. & COSTA, W.M. Efeito de fontes e formas de aplicação de boro e zinco na cultura do cafeeiro. Científica, 22:289-299, 1994.

MATOH, T.; ISHIGAKI, K.I.; MIZUTAMI, M.; MATSUNAGa, W. & TAKABE, K. Boron nutrition of cultured tobacco BY-2 cells I. Requirements for an intracellular localization of boron and selection of cells that tolerate low levels of boron. Plant Physiol., 33:1135-1141, 1992.

MATSUNAGA, T. & NAGATA, T. In vivo 11B NMR observation of plant tissue. Anal. Sci., 11:889-892, 1996.

MORAES-DALLAQUA, M.E. Alterações em órgãos vegetativos do feijoeiro (*Pahseolus vulgaris* L., cv. Carioca) causadas por níveis de B na solução nutritiva. Rio Claro, Universidade Estadual Paulista, 1992. 69p. (Tese de Doutorado)

OERTLY, J.J. NO-Homogeneity of boron distribution in plants and consequences for foliar diagnosis. Comm. Soil Sci. Plant Anal., 25:1133-1147, 1994.

OLIVEIRA, R.H.; ROSOLEM, C.A. & MORAES-DALLAQUA, M.H. Alterações na anatomia do algodoeiro induzidas pela deficiência de boro. In: CONGRESSO BRASILEIRO DE CIÊNCIA DO SOLO, 24., Ribeirão Preto, 2003. Anais. Ribeirão Preto, SBCS/UNESP, 2003. CD-ROM

PILBEAN, D.J. & KIRBY, E.A. The physiological role of boron in plants. J. Plant Nutr., 6:563-582, 1983.

POWER, P.P. & WOODS, W.G. The chemistry of boron and its speciation in plants. Plant Soil, 193:1-13, 1997.

RAIJ, B.van; CANTARELLA, H.; QUAGGIO, J.A. & FURLANI, A.M.C. Recomendações de adubação e calagem para o Estado de São Paulo. 2.ed. Campinas, Instituto Agronômico e Fundação IAC, 1996. 285p. (Boletim Técnico, 100)

ROBERTSON, G.A. & LOUGHMAN, B.C. Response to boron deficiency: a comparison with responses produced by chemical methods of retarding root elongation. New Phytol., 73:821-832, 1974.

ROSOLEM C.A. & COSTA, A. Cotton growth and boron distribution in the plants as affected by a temporary deficiency of boron. J. Plant Nutr., 23:815-825, 2000.

SANFUENTES, J.R. Concentração crítica de B em algodoeiro *(Gossypium hirsutum* L., cv. IAC 12*)*. Piracicaba, Escola Superior de Agricultura Luiz de Queiroz, 1966. 40p. (Tese de Mestrado)

SANTINATO, R.; SENA, C.A.; SILVA, A.A. & CAMARGO, R.P. Efeitos de P, Ca e B via foliar no pegamento de florada e frutificação do cafeeiro. In: CONGRESSO BRASILEIRO DE PESQUISA CAFEEIRA, 17., Campos do Jordão, 1991. Anais. Rio de Janeiro, IBC, 1991. p.36-38.

SHORROCKS, V. The occurrence and correction of boron deficiency. Plant Soil, 193:121-148, 1997.