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BORON UPTAKE AND DISTRIBUTION IN FIELD GROWN CITRUS TREES

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BORON UPTAKE AND DISTRIBUTION IN FIELD GROWN CITRUS TREES

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□ *In low fertility tropical soils, boron (B) deficiency impairs fruit production. However, little information is available on the efficiency of nutrient application and use by trees. Therefore, this work verified the effects of soil and foliar applications of boron in a commercial citrus orchard. An experiment was conducted with fertigated 4-year-old 'Valencia' sweet orange trees on 'Swingle' citrumelo rootstock. Boron (isotopically-enriched ^{10}B) was supplied to trees once or twice in the growing season, either dripped in the soil or sprayed on the leaves. Trees were sampled at different periods and separated into different parts for total B contents and $^{10}B/^{11}B$ isotope ratios analyses. Soil B applied via fertigation was more efficient than foliar application for the organs grown after the B fertilization. Recovery of labeled B by fruits was 21% for fertigation and 7% for foliar application. Residual effects of nutrient application in the grove were observed in the year after labeled fertilizer application, which greater proportions derived from the soil supply.*

Keywords: sweet orange, isotope technique, fertigation, foliar spray, plant nutrition

INTRODUCTION

Efficient nutrient management of citrus groves is critical to achieve high yields and crop quality. Along with acidity and limited phosphorus (P) availability of inherently low fertility tropical soils, boron (B) and zinc (Zn) deficiencies impair fruit production of bearing citrus trees in São Paulo (Mattos Jr. et al., 2005). Despite, such importance, little information is available in the literature about the role of B on citrus production. On the contrary, B excesses have been more frequently addressed in areas originating from

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marine rock materials, where its availability is higher in soil, as well as in those irrigated with high B concentration water resulted from anthropogenic additions (Reboll et al., 2000; Papadakis et al., 2004).

The most common practice for supplying B to the citrus consist on foliar sprays during the spring and summer periods, even tough research has demonstrated that B soil application supplies plant nutrient demand as based on correlation established with fruit yield; maximum fruit yield of Pêra sweet orange was observed with soil B (extracted by hot water) of 1 mg dm^{-3} and leaf B of approximately 200 mg kg^{-1} (Quaggio et al., 2003).

Even though foliar application of B appears satisfactory in the field for supplying the demand of citrus trees, there are doubts about the efficiency compared between foliar and soil applications. This arises based on the fact that B from such sprays may be absorbed by leaves or part of it may be rain washed to soil surface and later be taken up by roots (Asad et al., 2003).

Boron presents a particular characteristic among the essential plant mineral elements; its mobility is restricted into the phloem of the majority plant species, even though it might be freely mobile in others that produce significant amounts of suggar-polyols (sorbitol, mannitol and dulcitol) in mature leaves. In this later case, B is readily translocated as consequence of the formation of B-polyol complexes (Brown and Shelp, 1997). These polyols are not documented in citrus (Zimmermann and Ziegler, 1975), rather, sucrose is found in significant concentrations in the phloem of such trees, which does not form the B complexes described above (Marschner, 1995).

Young sweet orange trees grown in nutrient solution enriched with ^{10}B presented 20–35% of their B content in the new parts derived from plant reserves (older leaves, shoots and roots) (Boaretto et al., 2008). In this study, authors demonstrated that B mobility within plants increased with increased ^{10}B contents in the reserve tissues. On the other hand, a trial carried out with young citrus plants in pots filled with organic growing media demonstrated increased B concentration in leaves that received ^{10}B foliar application; in this case, substrate contamination from foliar sprays was prevented (Boaretto et al., 2007). However, in this later study, B content in the leaf flush developed after fertilization did not change significantly and only 3% of ^{10}B taken up by orange leaves were translocated in the plant.

Based on the above discussion, our goals were to determine the efficiency of soil and foliar application of B in a citrus grove, with aid of the stable ^{10}B isotope, and evaluated residual effects of plant absorbed ^{10}B on plant growth in the subsequent year.

MATERIALS AND METHODS

The study was conducted in a 4-year-old ‘Valencia’ sweet orange [*Citrus sinensis* (L.) Osbeck] on ‘Swingle’ citrumelo [*Citrus paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf.] commercial grove, beginning in the spring

(September 2003). The commercial area, located in the city of Reginópolis (21°53′ S 49°13′ W), State of São Paulo, Brazil, was planted at 3.0 m × 7.5 m, fertigated with double driplines and presented a light textured ultisol (clay = 150 and sand = 850 g kg^{-1}). Soil chemical analysis at the 0–20 cm depth layer of the experimental area and previously to B fertilization showed: pH [calcium chloride ($CaCl_2$)] = 5.5; P-resin = 12 mg dm^{-3}; exchangeable cations, in mmol$_c$ dm^{-3}, calcium (Ca) = 18; magnesium (Mg) = 13 and potassium (K) = 2.1; and B (hot water) = 0.5 mg dm^{-3}, according to methods described by van Raij et al. (2001).

The treatments consisted in two systems of fertilization (fertigation and leaf sprayed) and two B rates. Leaf sprays were made at the rates of 0.5 kg ha^{-1} once a year = $L_{0.5}$ or the same rate twice a year as 0.5 + 0.5 kg ha^{-1} = $L_{1.0}$. Fertigation treatments were applied at the rates of 1.0 kg ha^{-1} once a year = $S_{1.0}$ or twice a year as 1.0 + 1.0 kg ha^{-1} = $S_{2.0}$. The four treatments were applied to one tree plots during the growing season, using ^{10}B labeled boric acid. A factorial design on complete randomized blocks was assigned to the experiment and treatments were replicated three times. The boron rates were defined with basis on research of Quaggio et al. (2003) and which were expected to result in improved nutrient use by trees.

Nutrient solutions were prepared with boric acid isotopically enriched with 92.73 atom% ^{10}B and were applied either on the leaves or in the soil once as described earlier, in September 2003, during the bloom period, and twice, in September 2003 and January 2004. In order to allow estimation of the ^{10}B natural abundance three additional trees were fertilized with unlabeled boric acid in the citrus grove.

Leaf application of B was done with 0.375 g L^{-1} labeled nutrient solution in the amount of 3.0 L per plant using a backpack sprayer, once ($L_{0.5}$) or twice ($L_{1.0}$). This volume was sufficient to cover the whole foliage with a fine mist and to avoid dripping of solution to the soil surface. The nutrient solution was sprayed at the end of the day and the soil was also covered with a plastic film, during the leaf spread, in order to avoid ground contamination with leaf applied ^{10}B.

The fertigation application of ^{10}B occurred in the wet bulb, using two 2.5 L plastic bottles at each side of trees, with 2.0 L of 0.281 g L^{-1} labeled solution each, totalizing 8 L of fertigation solution each application ($S_{1.0}$ = once; $S_{2.0}$ = twice). These bottles were adjusted to deliver the solution volume within 2–3 days, directly on the drippers placed along the fertigation lines.

Leaf flushes of trees during blooming, before treatment applications (September 2003), were marked in order to identify newer ones along the growing season and subsequently evaluate B uptake and distribution by trees. Thereafter, plant parts were sampled for isotopic analysis in the following period: (i) four months after the first ^{10}B fertilization (at the day before the second ^{10}B application; January 2004): old leaves, 1st leaf flush (represented by complete expanded young leaves present at the time of the first ^{10}B fertilization), 2nd leaf flush (young leaves not yet completely expanded developed after the first ^{10}B fertilization), and young fruits (3–4 cm diameter); and (ii) twelve months after the first ^{10}B fertilization (August 2004), at fruit maturity (total soluble solids/acidity juice ratio ≈ 12): same 1st and 2nd leaf flushes sampled previously, 3rd leaf flush (represented by complete expanded young leaves developed after the second ^{10}B fertilization), and 4th leaf shoot (young leaves not yet completely expanded developed after the second ^{10}B fertilization), mature fruits (8–9 cm diam.) and new flowers. Fruit yield was also evaluated in August 2004, at the same time of the second plant sampling.

The residual effect of ^{10}B on citrus trees during the following growing season was also evaluated based on isotopic analysis of additional samples collected: (i) six months after the first ^{10}B fertilization (March 2004): mature leaves from fruiting terminals according to leaf sampling guidelines for diagnostic of the nutritional status of citrus trees as recommended by Quaggio et al. (2005); (ii) sixteen months after the same period (January, 2005): fruits (2–3 cm diam.); (iii) eighteen months after (March, 2005): mature leaves as recommended by Quaggio et al. (2005) and fruits (5–6 cm diam.); and (iv) twenty-four months after (September 2005): mature fruits (8–9 cm diam.).

Fresh sampled plant parts were washed in deionized water, oven-dried at 65°C for 3 days, and then ground to a fine powder to pass through a 10-mesh screen, ashed in a muffle furnace at 550°C for 3 hours; ashes were dissolved in 0.1 mol L^{-1} hydrochloric acid (HCl). Peel, pulp, and seeds of fresh fruits collected in August 2004, were separated before oven-drying. Total B was determined using the colorimetric azomethine-H method (Wolf, 1974). Extracts of plant parts were diluted to approximately 0.1 mg kg^{-1} of B for determination of the isotope ratios (^{10}B/^{11}B) using inductively coupled plasma—mass spectroscopy (ICP-MS; model 3000DV, Perkin Elmer, Wellesley, MA, USA) according to Brown et al. (1992).

Percentage of B in plant parts derived from the labeled fertilizer (% B$_{\mathrm{dff}}$) was calculated by Equation 1.

$$\%\mathrm{B}_{\mathrm{dff}} = [(\mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{samp}} - \mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{uft}})/(\mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{fert}} - \mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{uft}})] * 100 \quad (1)$$

where: % B$_{\mathrm{dff}}$ = percentage of B derived from fertilizer; $\mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{samp}}$ = atoms % of ^{10}B in the sample; $\mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{uft}}$ = atoms % of ^{10}B in unfertilized tissues; $\mathrm{AT}\%^{10}\mathrm{B}_{\mathrm{fert}}$ = atoms % of ^{10}B in fertilizer.

Data were tested for significant differences among treatments using a randomized complete block ANOVA by using the GLM procedure of the SAS system (SAS Institute, Cary, NC, USA). Duncan's multiple range test was performed to compare to compare sets of means.

TABLE 1 January 2004 Leaf and fruit boron concentrations derived from the labeled fertilizer (B_{dff}) in orange trees four months after the first fertilizer application

	B application treatment[1, 2]			
	Soil		Leaf spray	
Plant part	(2.0 kg ha^{-1})	1.0 kg ha^{-1}	(1.0 kg ha^{-1})	0.5 kg ha^{-1}
	B_{dff}, mg kg^{-1}			
Old leaves	23.6 B a	22.4 B a	5.7 A b	8.6 A b
Leaves 1st flush	41.3 A a	46.6 A a	5.4 A b	4.8 A b
Leaves 2nd flush	10.7 B a	15.9 B a	1.7 B b	1.2 B b
Fruits (3–4 cm diam.)	3.0 C a	2.6 C a	0.4 B b	0.4 B b

[1] Boron rates between parenthesis indicate that only half of the total annual amount was applied till the sampling date.

[2] Means followed by different capital letters in the same column, and small letters in the same line, are significantly different at the 0.05 level using Duncan's test.

RESULTS AND DISCUSSION

Boron Derived from the Fertilizer

Boron concentrations (B_{dff}) in tree parts were greater under soil fertigated compared to leaf sprayed treatments after 4-month of the initial application of the labeled fertilizer (Table 1). Despite the fact that at the time of tissue samplings the amount of B applied to the soil in both fertigated treatments (1.0 kg ha^{-1}) represented the double of that applied to the leaves (0.5 kg ha^{-1}) large differences on B_{dff} observed for all tissues (4 to 10-fold in magnitude) within the application method suggested that soil fertigation turned out as more efficient strategy for B management in the citrus grove compared to leaf spray. This assumption was strengthened based on differences for B concentrations found according to leaf tissue age. In this study, B_{dff} in old leaves of fertigated trees were approximately 23 mg kg^{-1}, which average was smaller than that in young mature leaves (1st flush leaves $>$40 mg kg^{-1}) (Table 1).

Based on the fact that B tends to accumulate in leaves carried by the transpiration stream (Eaton and Blair, 1935), such mature, fully expanded, leaves were expected to contribute more significantly with plant transpiration and consequently better benefit, as a sink from B taken up by roots. Similarly, the later discussion applies to the young leaves not expanded (2nd leaf flush) and fruits, in which transpiration was probably less significant too.

Small differences on B_{dff} observed for leaves of trees that received the foliar fertilization were also probably more related to the similarity of leaf area of old and 1st flush leaves in contact with sprayed solution. Therefore, old and young mature leaves presented B_{dff} concentrations of approximately 6 mg kg^{-1}. The lack of effect of foliar B sprays on B level in leaf and fruit of apple trees was related to limited B absorption by plant tissue, which was

TABLE 2 August 2004 leaf and fruit boron concentrations derived from the labeled fertilizer (B_{dff}) in orange trees twelve months after the first ^{10}B fertilizer application

	B application treatment[1]			
	Soil		Leaf spray	
Plant part	2.0 kg ha^{-1}	1.0 kg ha^{-1}	1.0 kg ha^{-1}	0.5 kg ha^{-1}
	Bdff, mg kg^{-1}			
Leaves 1st flush	76.6 A a	54.4 A a	19.1 A b	7.0 A b
Leaves 2nd flush	35.7 B a	19.2 B b	9.1 B bc	1.8 B c
Leaves 3rd flush	26.4 B a	13.9 C b	4.2 C c	2.1 B d
Leaves 4th flush	6.6 C a	3.4 D b	1.6 C b	0.6 C b
Fruits (7–8 cm diam.)	5.2 C a	3.8 D a	1.3 C b	0.5 C c

[1] Means followed by different capital letters in the same column, and small letters in the same line, are significantly different at the 0.05 level using Duncan's test.

possibly because of spur leaf area of apple tree at beginning of the growing season, during spray application, was small (Wojcik et al., 2008). Effects of treatments were also observed for young fruits developed after fertilizer application, with B_{dff} higher for fertigated trees (3 mg kg^{-1}) compared to those sprayed with the nutrient (0.4 mg kg^{-1}) (Table 1).

Boron concentrations (B_{dff}) in fruit samples collected 12-months after initiated the labeled fertilizer applications also indicated that contribution of leaf applied B is small to the fruiting process and supported the discussion about the improved efficiency for soil B supply on citrus orchards (Tables 1 and 2).

Comparisons made between treatments in which total B rates were 1.0 kg ha^{-1} ($S_{1.0}$ and $L_{1.0}$) demonstrated that B_{dff} in leaves and fruits were significantly greater when the micronutrient was applied to the soil. Smaller differences were observed for the youngest leaves marked as the 4th growing flush in the trees; however, in this case, B_{dff} concentrations were lower than 3 mg kg^{-1} (Table 2). Boaretto et al. (2007) verified that less than 9% of the B sprayed to the leaves of citrus trees were taken up, and which event occurred mainly in the first day after foliar application. The effects of B rates were also observed for both soil and leaf spray treatments in which increased B supply determined increased B concentration in plant parts. Differences among leaf tissue age were in accordance with results observed in Table 1.

Uptake of boron by roots was proportional to residual ^{10}B availability in the soil since B_{dff} in the 1st flush of leaves of trees that received a single soil application of the nutrient ($S_{1.0}$) increased from 44 mg kg^{-1} to 54 mg kg^{-1}, 6- to 12-months after the first labeling event, whereas for those which received two applications ($S_{2.0}$), B_{dff} increased to 77 mg kg^{-1} in the same period (Tables 1 and 2). Similar results were observed for B_{dff} in the 2nd flush of leaves and the fruits from trees after soil fertigation. In the case of leaf sprayed trees, increases on B_{dff} in different leaf flushes and fruits for the

$L_{0.5}$ treatment were less pronounced (<2.2 mg kg^{-1}) and resulted probably from B redistribution in the plant, since soil was covered with a plastic film before leaf application to limit root uptake of the nutrient from the labeled solution. The second leaf application ($L_{1.0}$) caused more significant increases in B_{dff} (<13.7 mg kg^{-1}) for all tree parts (Tables 1 and 2).

Based on the total content of B of samples (data not shown) and B_{dff} concentrations in Table 2, about 3% of boron in the 3rd and 4th leaf flushes were redistributed from older tissues which received 0.5 kg ha^{-1} of the nutrient via foliar application ($L_{0.5}$) and 4 to 6% in the same flushes which received 1.0 kg ha^{-1} ($L_{1.0}$). Since polyols do not occur in citrus trees, B-phloem mobility in the plant was limited, and the absorbed B remained mostly where it was applied. These are in line with results reported by Konsaeng et al. (2005) and Boaretto et al. (2007) for citrus species. These later authors demonstrated that only 3.2% of ^{10}B taken up by leaves were retranslocated in young sweet orange plants. On the other hand, in significant phloem B mobility tree species more than 70% of B taken up by leaves are exported to young and meristematic tissues (Hanson, 1991; Shu et al., 1994; Picchioni et al., 1995).

Concentrations of B_{dff} in mature fruits, 12-mo after first labeled fertilizer application, varied from 0.5 mg kg^{-1} to 5.2 mg kg^{-1}, with greater values associated with soil fertigation at the highest nutrient rate ($S_{2.0}$) and, on the contrary, smaller values associated with leaf application at the lowest rate ($L_{0.5}$) (Table 2). Fruit yield of trees in the field did not varied in response to applied treatments in 2004, which average was 27 ± 9 t ha^{-1} of fresh fruits. Based on production of dry mass and total B content (18 mg kg^{-1}) of mature fruits in the same year, the amount of the nutrient exported with fruit harvest on an area basis was 82 g ha^{-1}, whereas the same for the labeled B was 17.2 g ha^{-1} for the $S_{1.0}$ (Fruit B_{dff} = 3.8 mg kg^{-1}) and 5.9 g ha^{-1} for the $L_{1.0}$ (fruit B_{dff} = 1.3 mg kg^{-1}) treatments. Therefore, fertilization efficiency, based on nutrient distribution in plants and at same nutrient rate, was 21.1% for soil fertigation and 7.2% for foliar application.

Wojcik et al. (2008) demonstrated that higher concentration of leaf B and increased root growth of apple trees was observed with use of soil B compared to either foliar application or control without B. Even though foliar B sprays had no effect on B status in leaf and fruit tissues, increased flower B concentration and fruit set determined increased fruit yield. In this case, foliar B sprays were applied four times per season when conditions of soil B shortage were probable on the contrary of the fertigation used in our study.

Greater concentration of total B in fruits was found in the peel (>20 mg kg^{-1}) (Figure 1), mostly because its transport is primarily driven by transpiration stream (Brown and Shelp, 1997). Furthermore, B is reported to crosslink structurally complex pectic polysaccharides in cell walls confering stability to cell structure and consequently adequate fruit development. This

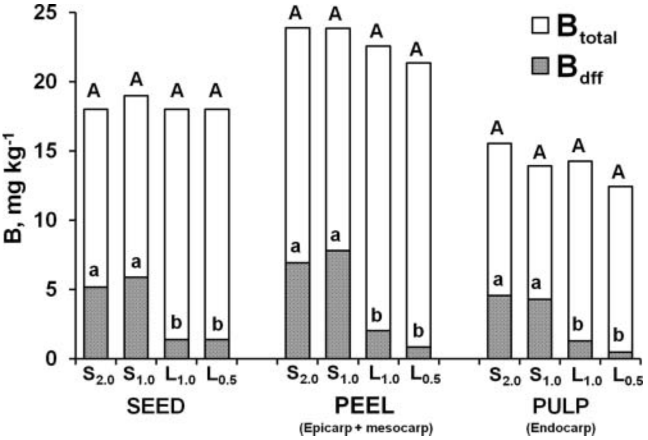

FIGURE 1 Concentrations of boron (total—B_{total} or derived from the fertilizer—B_{dff}) in mature orange fruits parts (seeds, pulp and peel) twelve months after ^{10}B labeled fertilizer application. August, 2004. Legends: S = soil application, L = leaf spray; subscript numbers indicate boron rate in kg ha^{-1}. Columns with different capital letters (total B) or small letters (B_{dff}) are significantly different at the 0.05 level using Duncan's test.

characteristic might also explain the differences observed with seeds and pulp. The evaluation of B_{dff} demonstrated again that soil fertigation was more effective on supplying fruit demand compared to leaf spray since reported values were up to 4-fold greater for $S_{1.0}$ treatments compared to $L_{1.0}$.

Residual Effect of Boron Fertilization

The residual effect of the labeled boron fertilization in the citrus grove was evident with analysis of leaf samples recommended for the nutritional diagnostic of citrus conducted for two seasons (Figure 2). Trends observed for the B_{dff} concentrations in recent mature leaves of trees in 2004 repeated one year later in 2005, with soil fertigated treatments promoting greater boron concentration in plant tissue. No differences in B concetrations of leaves for the $S_{1.0}$ and $S_{2.0}$ treatments were observed what suggest that B transport in the soil below the root zone might decrease the nutrient availability to plants (Shorrocks, 1997). In this case, split applications in the field have to be considered in soils prone to prevent significant leaching losses. In 2005, B_{dff} in leaves for soil treatments were about 20 mg kg^{-1}, which represented approximately 10% of total B concentration in the tissue. On the other hand, less than 2% were observed for leaf sprayed treatments, which were directly related to smaller quantities absorbed by trees in the previous year.

Furthermore, approximately 30% of the B_{dff} in flowers in 2005 originated from the previous year too, when the labeled fertilizer was applied to the soil (Figure 3). This proportion was represented by the combined contribution

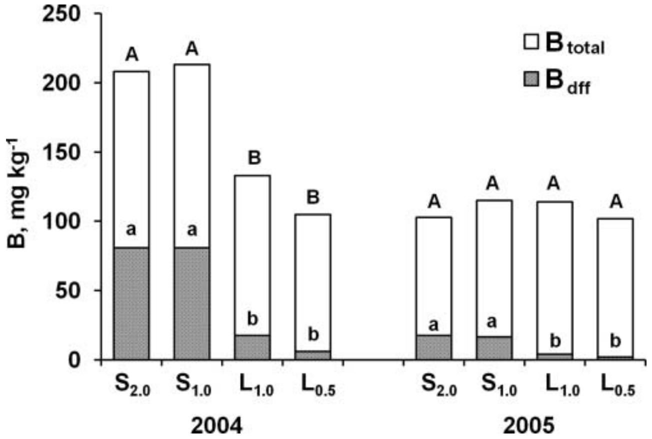

FIGURE 2 Concentrations of boron (total—B_{total} or derived from the fertilizer—B_{dff}) in recent mature leaves collected from fruiting terminals in orange trees six and eighteen months after ^{10}B labeled fertilized application. March 2004 and 2005, respectively. Legends: S = soil application, L = leaf spray; subscript numbers indicate boron rate in kg ha^{-1}. Columns with different capital letters (total B) or small letters (B_{dff}) are significantly different at the 0.05 level using Duncan's test.

of labeled B still available in the soil (nutrient in soil solution, linked to organic matter or recicled from decaying roots) and boron remobilized from older tissues. If transpiration rates of flowers are lower than other tissues, the active transport of boron shall explain this effect since reproductive organs represent stronger sinks for nutrient load. Boaretto et al. (2008) estimated that 30–35% of total boron in the leaves of new flush were remobilizated from plant reserve. Therefore, it is possible to assume that 65–70% of B in

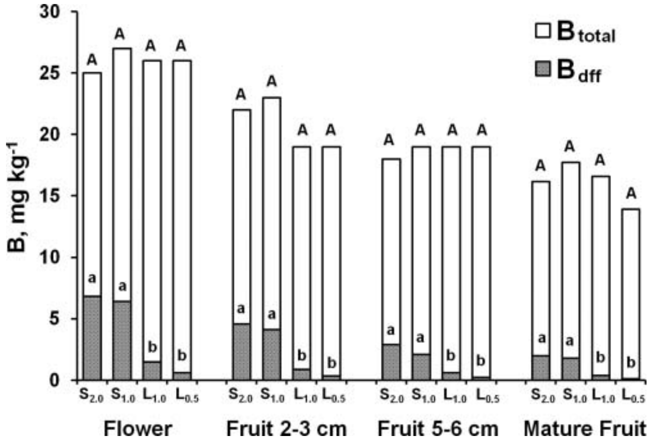

FIGURE 3 Residual boron concentrations (total—B_{total} or derived from the fertilizer—B_{dff}) in flowers and fruits in the subsequent growing season (eighteen months) after ^{10}B labeled fertilized application. Legends: S = soil application, L = leaf spray; subscript numbers indicate boron rate in kg ha^{-1}. Columns with different capital letters (total B) or small letters (B_{dff}) are significantly different at the 0.05 level using Duncan's test.

flowers in this experiment were originated from soil. Similar contribution was observed for young fruits (2–3 cm in diameter).

Concentrations of total B and B_{dff} in fruits during growth decreased mostly because of dilution effect caused by increased fruit mass along the season. Furthermore, nutrient delivery to fruits is more significant at the early stages of fruit maturation when other processes than cell elongation, characteristic of fruit enlargement, occur.

CONCLUSIONS

Soil boron fertilization applied via fertigation to citrus trees represented a more efficient strategy than foliar fertilization for nutrient management of citrus groves. Recovery of labeled B by fruits was 21% for soil fertigation and 7% for foliar application.

Recent mature tissues represented the most important sinks of applied B, and from which redistribution via phloem was not large. Residual effects of nutrient application in the citrus grove were observed in the year after labeled fertilizer application, which greater proportions derived from the soil supply.

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