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The studies on redistribution of boron in apple trees as a result of leaf boron application

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Abstract

Redistribution of boron (B) within apple trees (Malus domestica Borkh.) as a result of leaf B application was examined. The experiments were carried out in a greenhouse on M.26 apple rootstock and in field conditions on five-year-old 'Elstar' and 'Szampion' apple trees grafted on M.26 rootstock. Boron was applied in a form of boric acid solution. It was found that leaf B application resulted in movement of B to leaves, flowers and apple fruits untreated with B. These results clearly showed that B was retranslocated within apple tree. The retranslocation of B within apple trees took place when leaf B applications were done both in summer and autumn. Additionally, it was observed that B from leaf application had particularly high ability of movement within plant at insufficient B content (0.2μM) in solution. Generally, these data indicated that B within apple trees was transported in phloem, however mechanism of this phenomenon has not been well known yet. Thus, leaf B application of apple trees can be more successful treatment in reduction of B deficiency in plants compared to soil B application.

INTRODUCTION

Nutrient elements have different mobilities in plants. This differential mobility is related to the relative importance of xylem vs. phloem in providing nutrients to developing sinks. The first route involves the primary translocation of minerals by the water stream in the xylem, whereas the second route is secondary translocation or retranslocation in the phloem, away from sites of initial deposition to sites that do not lose water readily (Marschner, 1995). Macronutrients, with the exception for calcium are readily retranslocated. In contrast, the retranslocation of micronutrients is strongly reduced and may depend on a number of factors, including plant nutrient status and external supply of the nutrient (Marschner, 1995).

It has been observed for years that plants grown with an adequate boron (B) supply have B concentrations that decrease from old leaves to young leaves

(Bowen, 1972; Kohl and Oertli, 1961; Michael et al., 1969; Shelp et al., 1992). In addition, B deficiency symptoms occur in meristematic tissues, while B toxicity symptoms appear first in margins of oldest leaves, generally at the end of the transpiration stream (Kłossowski et al., 1978; Shelp et al., 1995). These observations are the basis of the historical classification of B as an immobile element which is transported solely in the xylem. It has recently been demonstrated that B exhibits rapid and significant phloem mobility in species for which sorbitol is a primary photosynthetic product (Brown and Hu, 1996). This hypothesis was verified through the isolation and characterisation of B-polyol complexes from celery (*Apium graveolens*) and peach (*Prunus persica*) (Hu et al., 1997). Apple tree is the species in which sorbitol is a predominant photosynthate (Hanson, 1991). It suggests that B transport within apple tree appears also in phloem. The aim of these investigations were to study redistribution of B within apple tree as a result of foliar B application.

MATERIAL AND METHODS

Experiment 1

The purpose of the examination was to estimate retranslocation of B within apple rootstock as a result of leaf B application by different B status in the nutrient solution.

The object was M.26 apple rootstock grown semi-hydroponically in a greenhouse in 2-L plastic pots filled with perlite and supplied with modified Hoagland solution, pH 5.8 (Hoagland and Arnon, 1950). Boron was supplied at a rate of 0.2; 20 and 40 μM in form of boric acid. The nutrient solutions were applied three times a week. The apple rootstocks grew singly in each pot. The plants were maintained under day/night temperatures of 24-27/13-18 °C and a 16 h light period. Natural lighting was supplemented by high-intensity sodium vapour lamps yielding a quantum flux density at pot level of 150 μmol m^{-2} s^{-1}. After 6 weeks of growth when the rootstocks had developed 9 to 12 mature fully expanded leaves, five leaves from 5 to 9 (counting from bottom) were immersed for 10 s in solution with 30 mM of B in form of boric acid. This treatment was repeated 4, 8 and 12 days later. The rootstocks treated with water were served as the control. Each treatment consisted of 9 replications (9 plants). During the growth of rootstocks the lateral shoots were systematicly removed. After 40 days after the last dipping leaves in boric acid solution or in water, from each plant leaves according to location on the shoot were sampled. The leaves were washed for 1 min. in distilled water and dried at 65 °C for 4 days. Leaf dry weight was recorded and tissue was ground in a mill to pass a 40-mesh screen. The samples were ashed in a muffle furnace at 450 °C for 7 h (Ostrowska et al., 1991). Ash was dissolved in 3M HNO_3 with 1000 mg Li /liter (as a matrix modifier) and analyzed for total B by inductively coupled plasma-mass spectrometer.

Experiment 2

The purpose of the examination was to estimate redistribution of B within apple tree grown in field conditions as a result of leaf B application applied in summer.

The study was carried out in 1997 in Dąbrowice Experimental Station belonging to Research Institute of Polomogy, Skierniewice. The object were five-year-old 'Elstar' apple trees grafted on M.26 rootstock and planted on the soil with 0.36 mg of hot-water extractable B content. 21, 52, 83 and 114 days after bloom (6 June, 7 July, 7 August and 8 September, respectively) the apple trees were sprayed with B at a rate of 0.3 kg ha^{-1} in form of boric acid using 1000 L of water. Control trees were sprayed with water. In all terms of apple tree sprays selected tops of one-year-old shoots (including the youngest five leaves) and fruitlets were covered with polyethylene bags. The bags from the shoot tops and the fruitlets were removed 15 minute after each spraying. During spraying with B two sides of tree rows were covered with polyethylene sheeting. The experiment was set in a block design with three replicates consisted of 5 trees each.

The following measurements were done:

—Boron concentration in the leaves and the fruitlets covered with the polyethylene bags during spraying with B or water.These measurements were performed 14 days after B spraying. Prior to the fruitlets sample preparation to analysis, seeds and stem were removed and then 2 quarters of apple were cut out from two opposite sides. Further preparation of fruit samples was done identically as leaf samples in the experiment 1.

Experiment 3

The aim of the study was to examine the B redistribution within apple tree grown in field conditions as a result of autumn leaf B application.

The study was carried out in 1996 in field conditions in Dąbrowice Experimental Station. The object were five-year-old 'Elstar' and 'Szampion' apple trees grafted on M.26 rootstock and grown on sandy-loam soil with 0.45 mg of hot-water extractable B content. On 2 October the apple trees were sprayed with B at a rate of 0.9 kg ha^{-1} in form of boric acid at 1000 L of water. Trees sprayed with water served as the control. Between the plots there were two trees as protective belts. During spraying with B two sides of tree rows were covered with polyethylene sheeting that were removed about 10 minute after the treatment.The study was set in a block design with 3 replicates consisted of 20 trees each.

The following measurements were taken:

1. Leaf B concentration after 9 and 24 hours and 3, 9 and 24 days after B spraying. The leaves without no symptoms of infection by fungal pathogens were collected from middle of one-year-old shoots located at outside zone of the tree crown. From each tree four leaves were sampled. The preparation the leaf samples and determination of B concentration were done identically as in the examination 1.
2. Boron concentration in flowers, spur leaves, leaves from one-year-old shoots and fruit in the next year after fall B spraying.
   - Collection of flowers was done when 10% of flowers were in the stage of full bloom. The flowers came from the spur shoots located at outside zone of the tree crown. Flowers were sampled without the pedicles and with the petals and the sepals. Five flowers from each trees were collected.
   - Spur leaves were sampled two weeks after bloom, from the spur shoots with fruits, located at outside zone of the tree crown. Three leaves from each tree were sampled.

– Leaves from one-year-old shoots were collected on 29 July from the middle of shoot located at outside zone of the tree crown. Five leaves from each tree were sampled.

–Apple fruits were collected at the harvest time, from outside part of the tree crown. Five apples from each tree were sampled. The preparation of flower, leaf and fruit samples for mineral analysis was performed identically as in the experiment 1.

Data from experiment 1, 2 and 3 were statistically evaluated with an analysis of variance. Duncan's „t" test was employed to calculate the significance of differences at $P \leq 0.05$.

RESULTS AND DISCUSSION

Experiment 1

In general, leaf B concentration of apple rootstock was positively correlated to B content in medium (Tables 1, 2, 3). Thus, the lowest leaf B concentration was found in solution with 0.2 mM of B (13-16 mg B kg^{-1} d.wt.) whereas the highest when solution of 40 mM of B was applied (31-35mg kg^{-1}) to the root system. These data suggest that B uptake by the rootstocks was passive as was shown by Bingham et al. (1970) and Brown and Hu (1997). In apple rootstocks grown in the nutrient solution with the lowest B content and untreated with exogenous B it was observed typical B deficiency symptoms in young leaves (26-29 leaf position on shoot) include deformed leaves with 'wavy' leaf margins, pale green colour and reduced leaf size. Boron concentration in these leaves was 13 mg kg^{-1} d.wt. (Table 1). There were no symptoms of B deficiency in the older leaves although leaf B concentration was low and ranged from 15 to 16 mg kg^{-1} d.wt. (Table 1). A lack of visible differences in B concentration between mature and apical leaves indicates indirectly that B was partly mobile in phloem of apple rootstock. According to Brown and Shelp (1997) occurrence of symptoms of B deficiency in growing leaf tissue is not evidence against B retranslocation within plant. The authors state that the growing tissues have higher requirement for B and are more sensitive to B shortage than mature tissues at similar B concentration in those tissues. No visual symptoms of B deficiency was also observed in leaves of rootstocks grown in the medium with 20 and 40 µM of B. Boron concentration in rootstock leaves at supplied 20 and 40 µM of B ranged 25-29 and 31-35 mg kg^{-1} d.wt., respectively (Table 2, 3).

Table 1

Effect of dipping leaves number 5-9 in boric acid solution on leaf boron concentration of M.26 apple rootstock grown in the solution with 0.2 µM of B

Treatment	Leaf B concentration [mg B kg^{-1} d.wt.]						
	Leaf number						
	1-4	5-9	10-13	14-17	18-21	22-25	26-29
Dipping leaves	21b	33b	25b	24b	25b	23b	25b
Control	16a	15a	16a	15a	15a	16a	13a

Notice: Means marked with the same letters in column do not significantly differ at a=0.05

Table 2
Effect of dipping leaves number 5-9 in boric acid solution on leaf boron concentration of M.26 apple rootstock grown in the solution with 20 μM of B

Treatment	Leaf B concentration [mg B kg^{-1} d.wt.]						
	Leaf number						
	1-4	5-9	10-13	14-17	18-21	22-25	26-29
Dipping leaves	32b	47b	34b	37b	35b	39b	38b
Control	25a	26a	28a	25a	27a	29a	29a

Notice: Means marked with the same letters in column do not significantly differ at $\alpha=0.05$

Table 3
Effect of dipping leaves number 5-9 in boric acid solution on leaf boron concentration of M.26 apple rootstock grown in the solution with 40 μM of B

Treatment	Leaf B concentration [mg B kg^{-1} d.wt]						
	Leaf number						
	1-4	5-9	10-13	14-17	18-21	22-25	26-29
Dipping leaves	41b	54b	45b	46b	41b	43b	42b
Control	32a	35a	32a	31a	35a	32a	31a

Notice: Means marked with the same letters in column do not significantly differ at $\alpha=0.05$

Generally, immersion of leaves number 5-9 in boric acid solution significantly increased B concentration in leaves located both over and below the treated leaves compared to the control ones (Table 1, 2, 3). These results clearly showed that B in apple rootstock was mobile. Brown and Hu (1996) proposed that the occurrence of significant phloem mobility of B is species dependent. Based upon the movement of isotopic B and a review of existing chemical data, Makkee et al. (1985) hypothesised that the apparent mobility of B in *Malus* species is a consequence of the use of sorbitol as a primary translocated photosynthate. It is worth to note that ability of B to retranslocation within apple rootstock was clearly related to B status in plant. In our study on average the increase of leaf B concentration at 0.2, 20 and 40 μM of B in nutrient solution were 67, 38 and 36%, respectively compared to the control ones. These results show that B retranslocation within plant was the highest in case of the lowest B status in plant. It is difficult to explain this phenomenon, because in the world literature there is no information about ability of B movement within plant in relation to plant B status.

Experiment 2

Generally, B sprays of 'Elstar' apple trees in all terms caused significant increase of B concentration in the fruitlets and the leaves covered with the plastic bags (Table 4). These data clearly showed that B was retranslocated in phloem to these organs. Also, Brown and Shelp (1997) emphasize that young leaves and fruits are strong sink tissues for B movement in fruit crop. It was found that the increase of B concentration in top leaves as a result of leaf B application in June, July, August and September were 43, 45, 43 and 35%, respectively. The lower increase of leaf B concentration in September compared to earlier terms of B sprays probably results from the lower activity of leaf tissues. According to Marschner (1995) growing

Table 5
Effect of autumn boron spray (2 October) of 'Elstar' and 'Szampion' apple trees on leaf boron concentration

Treatments	Leaf B concentration [mg B kg^{-1} d.wt.]									
	Time after leaf B application									
	9h		1d		3d		9d		24d	
	E*	S*	E	S	E	S	E	S	E	S
Leaf B application	44.1b	46.1b	44.5b	46.5b	39.8b	41.0b	32.5b	33.5a	28.1a	29.5a
Control	25.0a	26.2a	26.1a	26.3a	26.5a	26.2a	27.1a	27.8a	27.0a	28.5a

* E- Elstar cultivar ; S- Szampion cultivar

Notice: Means marked with the same letters in column do not significantly differ at α=0.05

Table 6
Effect of autumn boron spray of 'Elstar' and 'Szampion' apple trees on boron concentration in selected plant organs in the next year

Treatments	B concentration [mg B kg^{-1} d.wt.]							
	Flowers		Spur leaves		Leaves from current shoots		Fruit	
	E*	S*	E	S	E	S	E	S
Leaf B application	39.0b	37.3b	30.0a	34.1b	30.0b	29.8b	18.9b	18.2b
Control	30.0a	28.1a	23.3a	28.5a	24.7a	25.5a	14.4a	13.7a

* E- Elstar cultivar ; S- Szampion cultivar

Notice: Means marked with the same letters in column do not significantly differ at α=0.05

In the next year after autumn B spray of apple trees, B concentration in the flowers, the spur leaves, the leaves from one-year-old shoots and in the apple fruits were significantly higher compared to these organs from trees unsprayed with B (Table 6). This effect was observed in both cultivars. On average for both cultivars the increase of B concentration in the flowers, the spur leaves, the leaves from one-year-old shoots and apples were 31, 24, 19 and 32%, respectively. Thus, exported B from leaves as a result of autumn B spray was utilized in the next year by leaf, flower and fruit tissues. It is worth to note that the highest increase of B concentration was found in flower and apple tissues. It is evidence that phloem rather than xylem is predominant source of B for developing sinks and that B is mobile in phloem in apple tree. The results of this study show that autumn B spray of apple trees may be successful treatment in increase of fruit set and yield as was shown by Hanson et al., (1985) because B plays an important role in pollen germination and growth of pollen tube (Rerkasem, 1996).

CONCLUSIONS

The relative mobility of foliage-applied B within apple tree obtained in this examination indicates that leaf B application both in summer and in autumn may be an effective solution to transient plant B deficiencies and would have particular value as a means of supplying rapidly growing tissue such as flowers, young leaves and fruits. It seems that particularly leaf B application in autumn is succesful treatment because it gives possibility of B increase in the flowers which could increase fruit set and yield of apple trees. Leaf B application of apple trees should have much importance on sandy soils with low pH (< 4.5) and low content of organic matter (< 1.3g C $100g^{-1}$) because in this conditions usually there is low B content (< 0.5 mg hot water-extractable B per kg). In Poland leaf B application of apple trees could be successful treatments in increasing yield and improving fruit quality because ca. 70% of soils have low B content (Grześkowiak, 1996). However, the studies on terms of leaf B application of apple trees are necessary.

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Badania nad redystrybucją boru w jabłoni pod wpływem opryskiwania borem

Streszczenie

Celem badań było określenie redystrybucji boru w jabłoni pod wpływem opryskiwania tym mikroelementem. Badania przeprowadzono w szklarni na podkładce M.26 oraz w warunkach polowych na pięcioletnich jabłoniach odmiany Elstar i Szampion szczepionych na podkładce M.26. Opryskiwania borem wykonywane były w formie roztworu kwasu borowego. Wyniki badań wykazały, że bor podlegał redystrybucji w jabłoni pod wpływem opryskiwania tym mikroelementem. Retranslokacja boru w jabłoni zachodziła zarówno przy opryskiwaniu borem w okresie letnim jak i jesiennym. Stwierdzono także, że przy niedostatecznej zawartości boru w roztworze odżywczym (0.2mM B) retranslokacja boru w jabłoni zachodziła szczególnie intensywnie. Jakkolwiek w badaniach tych stwierdzono redystrybucję boru w jabłoni to jednak mechanizm tego zjawiska jest nadal mało poznany. Biorąc pod uwagę uzyskane wyniki badań, opryskiwanie jabłoni borem może być bardziej efektywnym zabiegiem w ograniczaniu niedoboru boru w roślinie w porównaniu do nawożenia doglebowego tym mikroelementem.