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Effect of Vapor Gard and Triisopropanolamine on Boron Absorption by Kiwifruit Plants

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ABSTRACT

Kiwifruit plants were grown in sand/perlite mixtures and irrigated with nutrient solutions containing 0.025, 0.2, and 0.5 mM boron (B) combined with 0, 1.5, 3, and 6 mM triisopropanolamine (TIPA). Growth parameters and B concentration of various plant parts were investigated. By increasing TIPA concentration from 1.5 to 6 mM in solutions containing 0.2 mM B, the concentration of leaf B decreased. However, even the highest TIPA concentration did not decrease B concentration of leaves when its concentration in solution was 0.5 mM. The accumulation of B in leaves and its relatively low

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concentration in roots and shoots indicates that kiwifruit lacks a B exclusion mechanism. Furthermore, the occurrence of higher B concentrations in mature leaves in comparison to younger ones is evidence of phloem B immobility of kiwifruit plants. In another experiment, the nutrient solutions contained three B levels (0.025, 0.1, 0.2 mM) and plants sprayed with the antitranspirant compound “vapor gard” with aqueous solutions containing 5, 10, and 15% “vapor gard.” Moderate B toxicity symptoms were exhibited by plants irrigated with solutions containing 0.1 mM B. However, when plants sprayed with the antitranspirant compound no visual symptoms of B toxicity were exhibited. Severe B toxicity symptoms were exhibited by plants irrigated with solutions containing 0.2 mM B even when they were sprayed with “vapor gard.” As the concentration of “vapor gard” increased from 5 to 15%, the shoot height of plants, the mean shoot fresh weight and the mean leaf fresh weight decreased for all B treatments. When plants irrigated with solutions containing 0.1 and 0.2 mM B, the spray with “vapor gard” (10 and 15%) resulted in a decrease of B concentration of leaves.

Key Words: Boron mobility; Boron toxicity; Boron detoxification; Nutrient solutions; Transpiration rate.

INTRODUCTION

Among the nutrient disorders of kiwifruit in Greece, B toxicity appeared in the last few years due to high B concentration of irrigation water, up to 3 mg L^{-1} . Boron toxicity is a nutritional disorder, which decreases plant growth and productivity in arid and semiarid environments throughout the world.^[1]

Kiwifruit is a very sensitive plant to excess B, demanding irrigation water containing less than 0.5 mg B L^{-1} .^[2] Its sensitivity to excess B is associated with its high transpiration rate^[3] and also to its inefficiency to control B absorption and/or transport.^[4] The range of concentrations between B deficiency and toxicity for kiwifruit is very narrow^[5] and application of B can be extremely toxic at concentrations only slightly above optimum.^[6]

It is generally accepted that higher plants do not have an effective mechanism for regulating B uptake.^[7] Although there is little evidence supporting active B absorption, B uptake in higher plants is probably a passive process acting in response to external boric acid concentration, membrane permeability, and transpiration rates.^[8]

The objectives of the present research were a) to study B absorption and mobilization after being incorporated into a complex organic



molecule (triisopropanolamine) and its effects on plant growth, b) to study B absorption and mobilization by kiwifruit plants after being sprayed with the antitranspirant compound “vapor gard.”

MATERIALS AND METHODS

First Experiment

Two years-old kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* cv. Hayward] plants uniform in macroscopic characteristics were planted in plastic containers containing 3 L of sand-perlite medium (1:1). The experimental plants were maintained in a growth room at $22 \pm 1^\circ\text{C}$, 16 h light and 8 h dark period and photosynthetic photon flux $550 \mu\text{mol m}^{-2} \text{s}^{-1}$. The plants were irrigated daily with 0.2 L of modified Hoagland's nutrient solutions.^[9] Macro-nutrients were supplied at half strength and micronutrients except B at full strength. Boron was supplied at three concentrations (0.025, 0.2, and 0.5 mM) and TIPA (triisopropanolamine: 1,1',1''-nitrilo-tri-2-propanol) at four levels (0, 1.5, 3, and 6 mM). Therefore, 12 treatments were included in the experiment. In each treatment 7 plants (replicates) were included. The nutrient solutions were prepared and allowed to stand 24 h at 24°C before use; this time was sufficient to allow esterification of boric acid by TIPA, to reach approximately 85% equilibrium.^[10] The pH of the nutrient solutions remained nearly constant at pH 6.5 during the entire growth period. Fresh nutrient solutions were furnished every 15 days. After 8 weeks of growth, when B toxicity symptoms were clearly visible in certain treatments, the plants were harvested and growth parameters were measured such as: shoot growth, number of leaves and mean fresh weight of leaves, shoots, and roots. Samples for chemical analysis were separated into: 1 year-old shoots, 2 year-old shoots, roots, upper leaves, and lower leaves. The samples were initially washed with tap water and afterwards twice with distilled water, dried in a forced draft oven at 68°C for 72 h and ground in a mill to pass a 30-mesh screen. Boron was determined by the azomethine-H method.^[11]

The experimental design used was the completely randomized one with 7 replications and two factors (B and TIPA concentrations). Differences between means were evaluated by Duncan's Multiple Range Test at $P \leq 0.05$.



Second Experiment

In a second parallel experiment kiwifruit plants were irrigated with nutrient solutions containing 0.025, 0.1, and 0.2 mM B. The two higher B concentrations were chosen in order to induce different degrees of B toxicity. Plants were sprayed twice with the antitranspirant compound “vapor gard” (pinolene, 96%) at 5, 10 and 15% concentrations. The first spray was performed at the initiation of the experiment and the second spray was one month later. All other experimental conditions were similar to those of the first experiment.

After 8 weeks of growth the following growth parameters were measured: shoot height, mean shoot fresh weight, number of leaves and mean leaf fresh weight. Samples for chemical analysis were separated and analyzed as in the first experiment. The statistical analysis performed was similar to that of the first experiment.

RESULTS AND DISCUSSION

Severe B toxicity symptoms were exhibited by plants irrigated with solutions containing 0.5 mM B independently of inclusion in solution of TIPA. This is in agreement with the results of other authors.^[12,13] The first sign of B toxicity was a yellow-green interveinal and marginal chlorosis, which developed on the older leaves and progressed to the younger ones. Various degrees of moderate B toxicity symptoms were exhibited by plants irrigated with solutions containing 0.2 mM B and 0, 1.5, and 3 mM TIPA. However, addition of 6 mM TIPA corrected boron toxicity symptoms.

Boron concentration in solution affected shoot growth and mean shoot fresh weight of kiwifruit plants. Plants produced the longest shoots with 0.025 mM B (Table 1). As B concentration in solution increased from 0.025 to 0.5 mM, shoot length and mean shoot fresh weight of plants decreased. The effect of excess B is a reduction in growth and yield of plants.^[14]

In plants irrigated with solutions containing 0.025 and 0.2 mM B, the addition of 6 mM TIPA had as a result the higher shoot growth of plants in comparison to those where no TIPA was added. However, the highest TIPA concentration had no beneficial effect on growth when 0.5 mM B was supplied.

Concerning the number of produced leaves per plant, B concentration of 0.025 mM produced more leaves in comparison to the rest of the tested B concentrations. As B concentration in solution increased, the number of



Table 1. Effects of B (mM) and TIPA (mM) of the nutrient solution on growth parameters of kiwifruit plants.

B (mM)	TIPA (mM)	Shoot growth (cm)	Mean shoot fresh weight (g)	Number of leaves	Mean leaf fresh weight (g)
0.025	0	160 b ^a	19.1 ab	16 ab	3.8 a
0.025	1.5	167 ab	20.2 a	17 a	3.6 ab
0.025	3	169 ab	18.1 ab	17 a	3.83 a
0.025	6	179 a	19.3 ab	17 a	3.73 a
0.2	0	141 c	16.4 bc	10 c	3.23 abc
0.2	1.5	151 bc	16.1 bc	13 abc	3.13 abc
0.2	3	159 b	17.6 b	13 abc	3.23 abc
0.2	6	169 ab	18.1 ab	15 ab	3.27 abc
0.5	0	102 de	12.2 d	8 d	3.13 abc
0.5	1.5	109 de	11.3 d	10 cd	2.98 bc
0.5	3	100 de	13.1 d	12 bc	3.08 abc
0.5	6	110 de	13.4 d	12 bc	2.58 c

^aMeans within columns followed by the same letter are not significantly different (Duncan's multiple range test, 5%).

produced leaves per plant decreased (Table 1). Inclusion of 6 mM TIPA in the nutrient solutions containing 0.2 and 0.5 mM B resulted in the formation of greater number of leaves in comparison to those when no TIPA was added. Plants produced the greater leaf plus shoot weight with 0.025 mM B in solution (Table 1). Inclusion of TIPA in solution increased leaf plus shoot weight only when B concentration was 0.2 mM.

Boron forms borate-ester cross-links with pectin in the cell wall and the breaking and reformation of those borate-ester bonds may control the expansion of cell wall.^[15] At high concentrations of B (0.5 mM) the restriction of growth observed was due to the decrease of photosynthetic rate and to a decrease of water use efficiency.^[16] Furthermore, due to B toxicity the formation of zones of dead tissue between the veins had a negative effect on plant growth.

The increase of B concentration from 0.025 to 0.5 mM in the nutrient solution, resulted in an increase of B concentration of 1 year-old shoots and 2 year-old shoots and roots (Table 2). Inclusion of 6 mM TIPA in solutions containing 0.2 and 0.5 mM B resulted in a decrease of B concentration of roots in comparison to those when no TIPA was added. The same effect was observed for 1 year-old shoots when the B concentration in solutions was 0.2 mM.



Table 2. Effects of B (mM) and TIPA (mM) of the nutrient solution on B concentration of shoots and roots of kiwifruit plants.

B (mM)	TIPA (mM)	1 year-old shoots ($\mu\text{g g}^{-1}$ d.w.)	2 year-old shoots ($\mu\text{g g}^{-1}$ d.w.)	Roots ($\mu\text{g g}^{-1}$ d.w.)
0.025	0	16 efgh ^a	11 ef	44 g
0.025	1.5	12 gh	11 ef	28 gh
0.025	3	12 gh	12 ef	30 gh
0.025	6	13 fgh	9 f	25 h
0.2	0	28 b	19 bcde	100 d
0.2	1.5	25 bc	16 cdeh	87 de
0.2	3	21 bcd	18 bcde	90 de
0.2	6	20 cde	16 cdeh	67 f
0.5	0	42 a	24 ab	187 a
0.5	1.5	39 ab	25 ab	169 b
0.5	3	37 ab	28 a	159 b
0.5	6	39 ab	22 abc	134 c

^aMeans within columns followed by the same letter are not significantly different (Duncan's multiple range test, 5%).

Boron concentration of lower leaves was significantly higher than that of upper leaves for the two higher B concentrations in solution (Fig. 1). The accumulation of B in leaves and its relatively low concentration in roots and shoots indicate that kiwifruit lacks a B exclusion mechanism. Furthermore, the occurrence of higher B concentrations in mature leaves in comparison to younger ones is evidence of B phloem immobility. In kiwifruit, the highest B accumulation occurred in leaf tip and in leaf margin.^[13] Therefore, kiwifruit represents a striking example of species with B immobility. Other species, where B has limited phloem mobility are walnut (*Juglans regia* L.) and pistachio (*Pistacia vera* L.).^[17] On the contrary, high B concentrations in apical tissues indicate phloem B mobility.^[18] Boron is phloem mobile in many species where sorbitol, mannitol, or dulcitol are the primary photosynthates since these polyols can effectively complex B. Examples of apparent B mobility were reported for pear (*Pyrus communis* L.), apple (*Malus domestica* Borkh.), cherry (*Prunus avium* L.), and apricot (*Prunus armeniaca* L.).^[19] The mobility of B in polyol transporting species influences B diagnosis and correction. For species that do not remobilize B within the plant (including kiwifruit), the B concentration of a developed leaf may not reflect the B status of growing tissues, for which a constant B supply is a critical factor. Since tissue expansion is one of the



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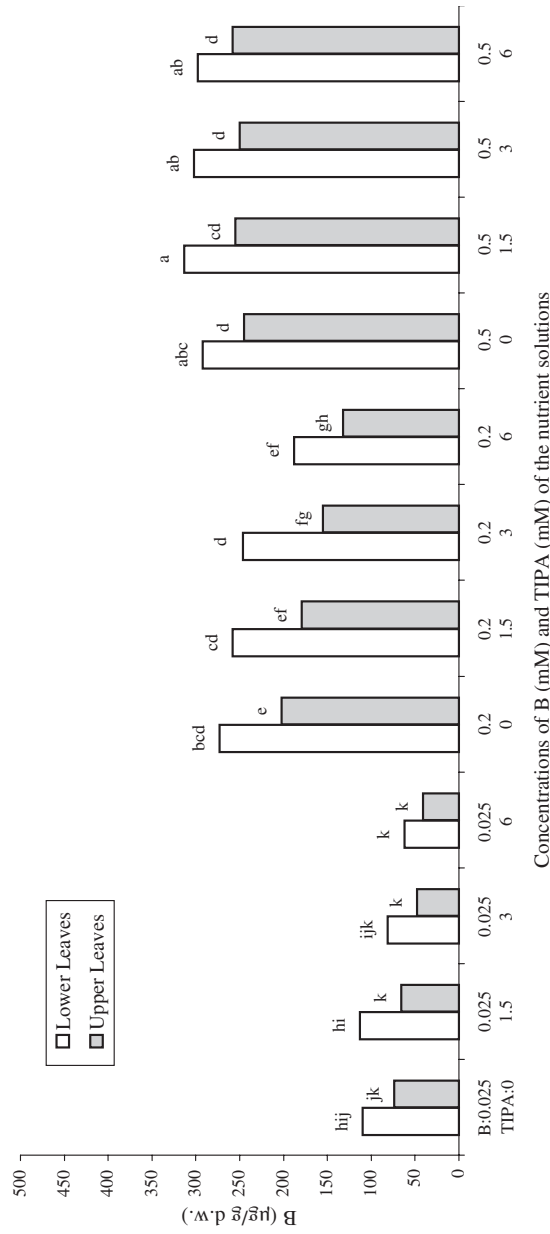


Figure 1. Effect of B (mM) and TIPA (mM) of the nutrient solutions on B concentration ($\mu\text{g g}^{-1}$ d.w.) of kiwifruit leaves.



first processes influenced by B deficiency, B deficiency diagnosis must reflect current availability and this can be achieved by sampling growing tissues.^[17]

By increasing TIPA concentration from 1.5 to 6 mM in solutions containing 0.2 mM B, the concentration of B in lower and upper leaves decreased. However, even the highest TIPA treatment did not decrease B concentration of leaves, when B concentration in solution was 0.5 mM (Fig. 1).

Boric acid is a weak acid in aqueous solution. In neutral or slightly acid soils, frequently occurring in many areas, B exists mainly as undissociated boric acid.^[20] Boron absorption can be best explained as a passive diffusion of free boric acid into the cell, followed by a rapid formation of B-complexes within the cytoplasm and the cell wall. The decline in free boric acid within the cell by the formation of B-complexes allows further absorption of B from the external solution and results in tissue B concentration that can exceed the free boric acid concentration of the uptake solution.^[8]

Boric acid and TIPA in aqueous solutions undergo triple esterification followed by the formation of a trans-annular bond between the nitrogen and boron atoms. Since TIPA contains three equivalent asymmetric centers, it exists as two diastereoisomers and two diastereoisomers of TIPA borate result upon esterification with boric acid. These two esterification reactions occur simultaneously, but proceed at different rates and approach different equilibria.^[10,21] Boron in the resulting complex with TIPA is not toxic to plants, even when it is absorbed by the plants. The degree of protection against B toxicity that TIPA provides through this reaction is equal to the fraction of the excess B that is predicted to be esterified by the equilibrium constant for the esterification reaction. Since this constant is not large, the molar ratio of TIPA to boric acid must be large before protection against B toxicity becomes significant. The amount of TIPA required is large enough, that its use for B detoxication in practical agriculture is not economical. Furthermore, TIPA borate is degraded microbially fast enough, thus there is a limitation for using TIPA in the soil.^[10]

Severe and moderate B toxicity symptoms were exhibited by plants irrigated with solutions containing 0.2 and 0.1 mM B respectively, and were not sprayed with "vapor gard." When plants sprayed with "vapor gard" (5, 10, 15%) and irrigated with solutions containing 0.1 mM B, no visual symptoms of B toxicity were exhibited. Moderate B toxicity symptoms were exhibited by plants irrigated with solutions containing 0.2 mM B even if they were sprayed with "vapor gard." However, when plants sprayed with 15% "vapor gard" scorch of the shoot apical meristem



and burst of axillary buds was observed. Furthermore, discoloration of leaves was observed with the appearance of light green areas on the leaf blade.

Plants produced the longest shoots and the highest shoot fresh weight with 0.025 mM B in the nutrient solution (Table 3). As the concentration of “vapor gard” increased from 5 to 15%, the shoot height of plants, the mean shoot fresh weight and the mean leaf fresh weight decreased for all B treatments.

Concerning the number of leaves per plant, fewer leaves were produced when B concentration of the solution was 0.5 mM and plants sprayed with 10% “vapor gard” in comparison to plants irrigated with solutions containing 0.025 mM B and sprayed with solutions containing 0–5% “vapor gard” (Table 3).

The increase of B concentration from 0.025 to 0.2 mM in the nutrient solution resulted in an increase of B concentration of one year-old shoots, two year-old shoots and roots (Table 4). Spray of kiwifruit plants with the antitranspirant compound “vapor gard” did not significantly affect B concentration of shoots and roots (Table 4).

By increasing B concentration of the nutrient solution from 0.025 to 0.2 mM, B concentration of upper and lower leaves increased (Fig. 2). When plants irrigated with solutions containing 0.1 and 0.2 mM B, the

Table 3. Effects of B (mM) of the nutrient solution and “vapor gard” (%) on growth parameters of kiwifruit plants.

B (mM)	Vapor gard (%)	Shoot height (cm)	Mean shoot fresh weight (g)	Mean leaf fresh weight (g)	Number of leaves
0.025	0	220 a ^a	49 a	2.99 a	26 a
0.025	5	171 b	33.1 d	2.15 b	26 a
0.025	10	88 e	19.06 f	1.67 cde	23 ab
0.025	15	85 ef	27.88 e	1.88 bc	23 ab
0.1	0	206 a	44 b	2.82 a	21 ab
0.1	5	155 c	24.2 ef	1.32 f	25 ab
0.1	10	81 ef	23.57 ef	1.73 cd	22 ab
0.1	15	80 ef	20 f	1.42 ef	21 ab
0.2	0	178 b	38.4 c	2.74 a	22 ab
0.2	5	110 d	22.79 ef	1.66 cde	23 ab
0.2	10	77 ef	11.72 g	1.40 ef	18 b
0.2	15	70 f	21.66 def	1.46 def	20 ab

^aMeans within columns followed by the same letter are not significantly different (Duncan’s multiple range test, 5%).



Table 4. Effects of B (mM) of the nutrient solution and “vapor gard” (%) on B concentration of shoots and roots of kiwifruit plants.

B (mM)	Vapor gard (%)	1 year-old shoots ($\mu\text{g g}^{-1}$ d.w.)	2 year-old shoots ($\mu\text{g g}^{-1}$ d.w.)	Roots ($\mu\text{g g}^{-1}$ d.w.)
0.025	0	13 de ^a	12 bc	18 e
0.025	5	12 e	8 c	23 e
0.025	10	12 e	10 c	18 e
0.025	15	14 cde	9 c	22 e
0.1	0	23 b	16 b	36 d
0.1	5	19 bcd	13 bc	30 d
0.1	10	20 bc	18 b	45 cd
0.1	15	24 ab	16 b	32 d
0.2	0	27 ab	18 b	54 b
0.2	5	30 a	24 ab	67 a
0.2	10	28 ab	18 b	53 b
0.2	15	30 a	28 a	59 b

^aMeans within columns followed by the same letter are not significantly different (Duncan’s multiple range test, 5%).

spray with “vapor gard” (10 and 15%) resulted in a decrease of B concentration of leaves (Fig. 2). Finally, B concentration of lower leaves was higher than that of upper leaves.

Boron absorption by plant roots is affected by various soil and climatic conditions. Important soil factors influencing B absorption from solution include the initial B content of the soil, the pH of the soil, the amount and type of minerals in the soil, the kind of exchangeable ions present in the soil, the soil organic matter content and the wetting and drying cycles.^[1] More specifically, low B availability to the plants would be expected in high pH, low organic matter and coarse textured soils. The most important climatic condition governing B absorption seems to be the transpiration rate. Thus, relative humidity, temperature, and light intensity will affect B absorption. In particular, reduced humidity, increased temperature which intensifies water deficits and high light intensity increases transpiration, resulting in an increase in B absorption.^[8] Vapor pressure deficit is determined by temperature and relative humidity and exerts strong influence on transpiration rate.^[22] In the present study, transpiration rate of kiwifruit plants was expected to be similar for all treatments, since the plants were grown under identical conditions. The decrease of growth of kiwifruit plants when sprayed with



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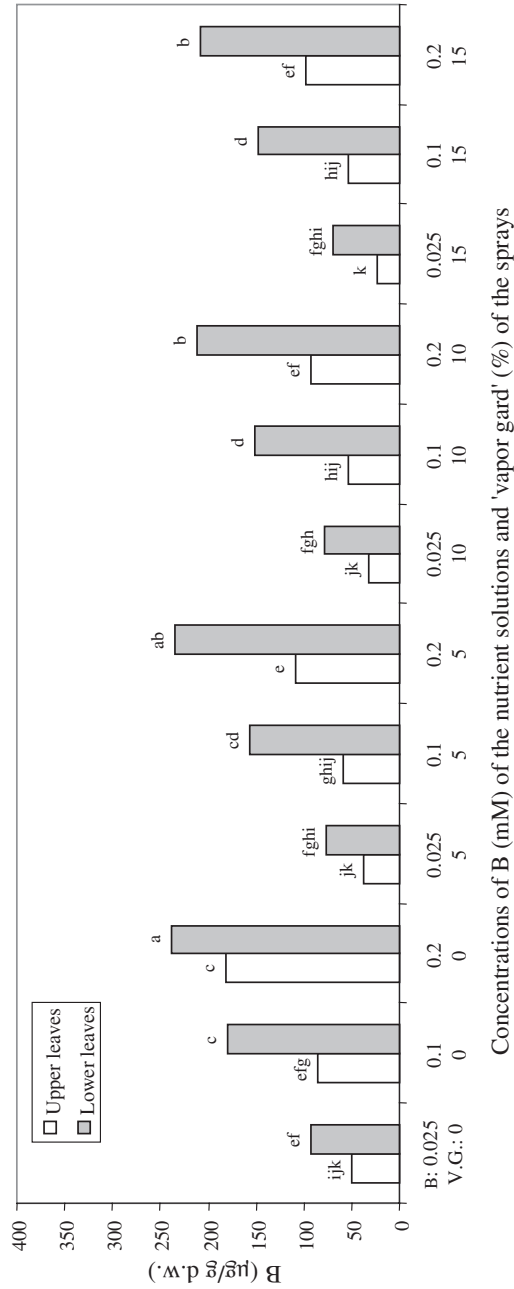


Figure 2. Effect of B (mM) of the nutrient solutions and “vapor gard” (%) of the sprays on B concentration ($\mu\text{g g}^{-1}$ d.w.) of kiwifruit leaves.



“vapor gard” indicates that the concentrations 10 and 15% affected adversely plant metabolism.

CONCLUSIONS

Plants produced the longest shoots with 0.025 mM B in the nutrient solution. As B concentration in solution increased from 0.025 to 0.5 mM, shoot length and mean shoot fresh weight of plants decreased. In plants irrigated with solutions containing 0.025 and 0.2 mM B, the addition of 6 mM TIPA resulted in the highest shoot growth of plants. However, the highest TIPA concentration had no beneficial effect on growth, when 0.5 mM B was supplied. By increasing TIPA concentration from 1.5 to 6 mM in solutions containing 0.2 mM B, the concentration of leaf B decreased. However, even the highest TIPA addition did not decrease B concentration of leaves, when B concentration in solution was 0.5 mM. The data indicate that TIPA protected plants against the injurious effects of high B, especially when B concentration in solution was 0.2 mM. The amount of TIPA required is large enough that its use for B detoxification in practical agriculture is not economical. The accumulation of B in leaves and its relatively low concentration in roots and shoots indicate that kiwifruit plants lack a B exclusion mechanism. Furthermore, the occurrence of higher B concentrations in mature leaves in comparison to younger ones is evidence of B phloem immobility in kiwifruit. When plants sprayed with “vapor gard” (5, 10, 15%) and irrigated with solutions containing 0.1 mM B, no visual symptoms of B toxicity were exhibited. When plants sprayed with 15% “vapor gard,” toxic symptoms appeared in plants such as: scorch of the shoot apical meristem, which resulted in burst of axillary buds. When plants irrigated with solutions containing 0.1 and 0.2 mM B, the spray with “vapor gard” (10 and 15%) resulted in a decrease of B concentration of leaves. For kiwifruit (which do not retranslocate B within the plant), the B concentration of a developed leaf may not reflect the B status of growing tissues for which a constant B supply is critical. Since tissue expansion is one of the first processes influenced by B deficiency, B deficiency diagnosis must reflect current availability and this can be achieved by sampling young leaves.

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