J. Plant Nutr. Soil Sci. 2003, *166,* 529–532 **DOI:** 10.1002/jpln.200320264

Boron toxicity in kiwifruit plants (*Actinidia deliciosa*), treated with nitrate, ammonium, and a mixture of both

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Accepted 10 June 2003

Summary – Zusammenfassung

The objective of this research was to study the effects of nitrogen (N) forms (NO_3^-, 2.6 mM; NH_4^+,2.6 mM; NO_3^-, 1 mM + NH_4^+, 1.6 mM) on the growth and mineral composition of kiwifruit plants exposed to three boron (B) levels (0.025, 0.1, 0.3 mM). The kiwifruit plants were grown in a 1:1 sand : perlite mixture and irrigated daily with nutrient solutions. Shoot height, mean shoot dry weight, the number of leaves, mean leaf dry weight, and N concentration of NH_4-treated plants were significantly higher compared to the NO_3^- treatment at all B levels. The concentration of 0.3 mM B significantly reduced shoot height for all N treatments. Boron toxicity symptoms appeared 14 days after starting the experiment, when plants were treated with 0.1 and/or 0.3 mM B. The nitrate supply reduced the B concentration of roots, but B levels of different leaf parts were hardly affected by the N form. Furthermore, the NH_4-N form significantly reduced the Mg concentration of the leaves.

Bor-Toxizität bei Kiwi (*Actinidia deliciosa*) in Abhängigkeit von der Ernährung mit verschiedenen N-Formen

Ziel der Untersuchungen war es, den Einfluss unterschiedlicher Stickstoffformen auf die Ausprägung von Bor-Toxizität in Kiwi-Pflanzen festzustellen. Die Stickstoff-Konzentration in der Nährlösung betrug 2,6 mM, angeboten entweder in Form von NO_3^- oder NH_4^+, bzw. als eine Mischung beider N-Formen (1,0 mM NO_3^- + 1,6 mM NH_4^+). Drei Bor-Konzentrationen wurden getestet: 0,025, 0,1 und 0,3 mM B. Die Kiwi-Pflanzen wurden in einem Sand-Perlite-Gemisch angebaut und täglich mit 250 ml Nährlösung bewässert.

Die NH_4^+-Ernährung wirkte sich im Vergleich zur NO_3^- -Ernährung positiv auf das Pflanzenwachstum aus. Das zeigte sich in einer höheren Masse und Länge der Sprosse sowie einer höheren Blattzahl, die zudem eine höhere mittlere Blattmasse aufwiesen. Die Konzentration von 0,3 mM Bor reduzierte die Sprossmasse signifikant im Vergleich zu den Varianten mit geringerem Bor-Angebot in der Nährlösung. Bereits 14 Tage nach Versuchsbeginn zeigten die Pflanzen in den Varianten mit 0,3 und 0,1 mM Bor in der Nährlösung erste Symptome von Bor-Toxizität. Die Bor-Konzentration in den Wurzeln der mit Nitrat ernährten Pflanzen war geringer als bei den mit NH_4^+ ernährten Pflanzen. Die N-Form hatte dagegen keinen nennenswerten Einfluss auf die Bor-Gehalte in den Blättern. Auf die N-Gehalte in den Blätter wirkte sich NH_4^+-Ernährung allerdings positiv aus.

Key words: boron toxicity / boron absorption / kiwifruit / nitrogen PNSS P02/64P

1 Introduction

Among the nutrient disorders of kiwifruit in Greece, in certain areas B toxicity has gained importance over the last few years, due to the high B concentration in the irrigation water (up to 0.28 mM). Nitrogen fertilization of kiwifruit in such areas is also of great importance in order to maintain high productivity. The ratio in absorption of anions and cations by plants may cause substantial shifts in the pH level in the rhizosphere. Nitrogen may be supplied either as cation (NH_4^+) or as anion (NO_3^-) and the ratio of these two forms in the nutrient solution may exert significant pH shifts in the solution with time (*Jeong* and *Lee*, 1996; *Wojcik*, 2000). A pH increase in the rhizosphere when NO_3^- is absorbed may decrease B availability to the roots. The opposite is to be expected for a pH decrease when NH_4^+ is absorbed, which may lead to B toxicity. The various N forms of the fertilizers may promote (*Wojcik*, 2000), reduce (*Bar* et al., 1997), or not affect B absorption and/or B toxicity (*Jeong* and *Lee*, 1996). However, no information is available concerning the B concentration of kiwifruit plants in relation to the N form applied.

In areas where the B concentration in irrigation water is high, the need to use N forms for kiwifruit fertilization, which may reduce the B concentration of plant tissues, led us to study the relationship between the N form (NO_3, NH_4, NO_3/NH_4) and B concentration and their effects on the growth and mineral composition in young kiwifruit plants.

2 Materials and methods

One year-old kiwifruit (*Actinidia deliciosa)* plants [(A. Chev.) C. F. Liang et A. R. Ferguson var. *deliciosa* cv. Hayward] uniform in size and macroscopic characteristics were planted in plastic containers containing 3 l of a 1:1 sand : perlite inert medium. The experimental plants were grown in a growth room at 22±1 °C and light intensity of 550 μmol m^{-2} s^{-1} (16 hours light and 8 hours dark period). These plants were irrigated daily with 250 ml of modified Hoaglands nutrient solutions (*Hoagland* and *Arnon*, 1950).

Every week an adequate quantity of distilled water was supplied to each plastic container, allowing some drainage for salt leaching from the planting medium. The treatments included three B concentrations (0.025, 0.1, and 0.3 mM) combined with three N sources (NO_3^-, 2.6 mM; NH_4^+, 2.6 mM;

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 1436-8730/03/0408-529

Table 1: The composition of the experimental nutrient solutions.
Tabelle 1: Die Zusammensetzung der im Versuch verwendeten Nährlösung.

Solution	KH_2PO_4 (mM)	$MgSO_4 \cdot 7H_20$ (mM)	$Ca(NO_3)_2 \cdot 4H_2O$ (mM)	KNO_3 (mM)	KCl (mM)	$CaCl_2$ (mM)	NH_4Cl (mM)
NO_3^-–0.025 mM B	0.4	0.25	1.0	0.6	–	–	–
NO_3^-–0.1 mM B	0.4	0.25	1.0	0.6	–	–	–
NO_3^-–0.3 mM B	0.4	0.25	1.0	0.6	–	–	–
NH_4^+–0.025 mM B	0.4	0.25	–	–	0.6	1.0	2.6
NH_4^+–0.1 mM B	0.4	0.25	–	–	0.6	1.0	2.6
NH_4^+–0.3 mM B	0.4	0.25	–	–	0.6	1.0	2.6
NO_3^-/NH_4^+–0.025 mM B	0.4	0.25	0.5	–	0.5	0.5	1.6
NO_3^-/NH_4^+–0.1 mM B	0.4	0.25	0.5	–	0.5	0.5	1.6
NO_3^-/NH_4^+–0.3 mM B	0.4	0.25	0.5	–	0.5	0.5	1.6

and NO_3^-/NH_4^+, 1:1.6 mM). The two highest B concentrations (0.1 and 0.3 mM) were employed in order to induce B toxicity symptoms in kiwifruit plants. Nine nutrient solutions were used at pH 5.9 (Tab. 1).

Each treatment included six replicates (plants). Fifty days after the initiation of the experiment, growth and weight of shoots and the number and weight of leaves were measured. Thereafter, the plants were harvested and separated into leaves, shoots, and roots. The leaves were divided into petioles, leaf margin (5 mm) and the remaining part of the blade, in order to follow the nutrient distribution throughout the leaves. All plant samples were washed once with tap water and twice with distilled water, dried in a forced-air oven at 70 °C for three days and ground in a mill. Samples were dry ashed and analyzed. Nitrogen was determined by the Kjeldahl method. Regarding B concentration measurements, 0.5 g of each sample was dry ashed in a muffle furnace. Well-glazed porcelain crucibles were used, which are suitable ashing vessels. The plant material ashed at 500 °C for 6 hours, which proved satisfactory for most plant tissues, to avoid B loss by volatilization (*Wikner*, 1986). The ash was dissolved in 0.1 N HCl, and B was determined by the Azomethine-H method (*Wolf*, 1971). For Mg determination, another 0.5 g of each sample was dry ashed for 6 hours at 550 °C, dissolved in 3 ml 6 N HCl and diluted with deionized water. Mg determination was performed by atomic absorption spectroscopy (Perkin-Elmer 2340).

The experiment was laid out completely randomized. Differences between means were evaluated by Duncan's multiple range test ($P = 0.05$).

3 Results

3.1 Growth parameters

Shoot length and dry weight of those kiwifruit plants treated with NH_4-N was greater than the respective values of plants treated with nitrate or mixed N forms (Tab. 2). Furthermore, the shoot length of plants irrigated with the nutrient solutions containing 0.3 mM B was significantly lower than that of the other two B treatments, for all N sources. Regarding the number of leaves and the leaf dry weight (d.w.), more leaves with a significantly higher dry weight were formed in the NH_4^+-treated plants than in the NO_3^--treated plants (Tab. 2). In the treatments containing 0.3 mM B, the mean leaf d.w. of plants was significantly lower than that of the control, for all the N treatments.

Table 2: Growth of kiwifruit plants (*Actinidia deliciosa*) treated with nitrate, ammonium, and a mixture of both and exposed to different boron concentrations in the nutrient solution (means of six plants).
Tabelle 2: Wachstum von Kiwi-Pflanzen (*Actinidia deliciosa*) in Abhängigkeit von der Stickstoffform und der Bor–Konzentration in der Nährlösung (Mittelwerte von sechs Pflanzen).

B (mM)	N source	Shoot height (cm)	Mean shoot dry weight (g)	Number of leaves	Mean leaf dry weight (g)
0.025	NO_3^-	26 ef	6.72 cd	15 bcd	1.02 d
0.1	NO_3^-	26 ef	6.67 cde	13 cd	0.99 d
0.3	NO_3^-	16 g	5.91 e	12 d	0.61 e
0.025	NH_4^+	95 a	13.54 a	22 a	1.70 a
0.1	NH_4^+	54 b	9.29 b	20 ab	1.55 abc
0.3	NH_4^+	34 de	7.82 bcd	19 abc	1.16 bcd
0.025	NO_3^-/NH_4^+	50 bc	8.99 b	18 abcd	1.64 ab
0.1	NO_3^-/NH_4^+	40 d	8.18 bc	15 bcd	1.54 abc
0.3	NO_3^-/NH_4^+	23 ef	6.11 de	13 cd	1.24 cd

Means in the same column followed by the same letter are not significantly different (Duncan's multiple range test, 5 %).

3.2 Nutrient concentrations

The root N concentration of the NO_3-treated plants was considerably lower than the values in the other two N forms (Tab. 3). The increase of B concentration in the nutrient solution did not significantly influence the N concentration of roots with all N forms. The inclusion of NH_4-N in the nutrient solution significantly increased the N concentration of the annual

Table 3: Nitrogen and boron concentrations in roots and shoots of one year old kiwifruit plants (*Actinidia deliciosa*) treated with nitrate, ammonium, and a mixture of both and exposed to different boron concentrations in the nutrient solution (means of six plants).
Tabelle 3: Stickstoff- und Bor-Konzentrationen in den Wurzeln und Sprossen von einjährigen Kiwi-Pflanzen (*Actinidia deliciosa*) in Abhängigkeit von der Stickstoffform und der Bor-Konzentration in der Nährlösung (Mittelwerte von sechs Pflanzen).

B (mM)	N (source)	Roots N (mg (g d.w.)$^{-1}$)	1 year old shoots N (mg (g d.w.)$^{-1}$)	Roots B (μg (g d.w.)$^{-1}$)	1 year old shoots B (μg (g d.w.)$^{-1}$)
0.025	NO_3^-	10.05 c	6.7 cd	19 f	17 de
0.1	NO_3^-	9.4 c	5.6 d	35 ef	31 b
0.3	NO_3^-	8.8 c	5.3 d	83 bc	47 a
0.025	NH_4^+	10.50 ab	8.6 bc	47 e	10 f
0.1	NH_4^+	10.59 a	9.4 a	68 cd	20 cde
0.3	NH_4^+	10.61 a	9.1 abc	144 a	25 c
0.025	NO_3^-/NH_4^+	10.47 ab	7.2 bcd	40 e	15 ef
0.1	NO_3^-/NH_4^+	10.34 b	7.5 bcd	53 de	22 cd
0.3	NO_3^-/NH_4^+	10.57 ab	8.6 bc	97 b	25 c

Means in the same column followed by the same letter are not significantly different (Duncan's multiple range test, 5 %).

shoots in comparison to the NO_3-N form (Tab. 3). The increase of the B concentration in the nutrient solutions significantly increased root B concentration (Tab. 3). However, the supply of NO_3-N in the nutrient solution significantly reduced the B concentration of roots and increased the B concentration of new shoots, especially at B concentrations of 0.1 and 0.3 mM.

Yet, the inclusion of the three different N forms in the nutrient solutions did not significantly influence the B concentration of the various leaf parts (Tab. 4). For each N form, the increase of B concentration in the nutrient solution resulted in a significant increase of B concentration in the leaf margin and the remaining part of the leaf blade. The maximum B concentration was recorded in the leaf margins, followed by the remaining part of the blades, and the minimum was recorded in the petioles (Tab. 4).

The N concentration of the various parts of leaves was greater in the NH_4-treated plants than in the NO_3-N treated ones (Tab. 5). Also, the lowest N concentrations were measured in the petioles, for all the treatments.

Ammonium-N treated plants showed a decrease of Mg concentration in the petioles and in the remaining part of the blade, when compared to the NO_3-treated ones (Tab. 6). Higher Mg concentrations were measured in the petioles, than in the other parts of leaves, for all the treatments.

Table 4: Boron concentration in the leaves of one year old kiwifruit plants (*Actinidia deliciosa*) treated with nitrate, ammonium, and a mixture of both and exposed to different boron concentrations in the nutrient solution (means of six plants).
Tabelle 4: Bor-Gehalte in den Blättern von einjährigen Kiwi-Pflanzen (*Actinidia deliciosa*) in Abhängigkeit von der Stickstoffform und der Bor-Konzentration in der Nährlösung (Mittelwerte von sechs Pflanzen).

B (mM)	N–source	B in petioles (μg (g d.w.)$^{-1}$)	B in remaining part of blade (μg (g d.w.)$^{-1}$)	B in leaf margin (μg (g d.w.)$^{-1}$)
0.025	NO_3^-	21 d	71 d	179 e
0.1	NO_3^-	25 cd	189 b	253 c
0.3	NO_3^-	53 a	266 a	292 a
0.025	NH_4^+	17 d	160 c	179 e
0.1	NH_4^+	22 d	170 bc	252 c
0.3	NH_4^+	38 b	254 a	285 ab
0.025	NO_3^-/NH_4^+	21 d	85 d	205 d
0.1	NO_3^-/NH_4^+	31 bc	168 bc	266 bc
0.3	NO_3^-/NH_4^+	50 a	254 a	296 a

Means in the same column followed by the same letter are not significantly different (Duncan's multiple range test, 5 %).

Table 5: Nitrogen concentration in the leaves of one year old kiwifruit plants (*Actinidia deliciosa*) treated with nitrate, ammonium, and a mixture of both and exposed to different boron concentrations in the nutrient solution (means of six plants).
Tabelle 5: Stickstoff-Gehalte in den Blättern von einjährigen Kiwi-Pflanzen (*Actinidia deliciosa*) in Abhängigkeit von der Stickstoffform und der Bor-Konzentration in der Nährlösung (Mittelwerte von sechs Pflanzen).

B (mM)	N–source	N in petioles (mg (g d.w.)$^{-1}$)	N in remaining part of blade (mg (g d.w.)$^{-1}$)	N in leaf margin (mg (g d.w.)$^{-1}$)
0.025	NO_3^-	7.0 b	16.0 d	16.8 de
0.1	NO_3^-	5.6 c	14.5 de	21.4 ab
0.3	NO_3^-	5.5 c	13.0 e	14.6 e
0.025	NH_4^+	9.9 a	22.1 b	22.7 ab
0.1	NH_4^+	10.2 a	24.9 a	18.8 cd
0.3	NH_4^+	10.4 a	22.3 b	23.2 a
0.025	NO_3^-/NH_4^+	10.0 a	19.5 c	20.4 bc
0.1	NO_3^-/NH_4^+	7.6 b	20.4 bc	21.3 ab
0.3	NO_3^-/NH_4^+	10.2 a	21.3 bc	22.1 ab

Means in the same column followed by the same letter are not significantly different (Duncan's multiple range test, 5 %).

Table 6: Magnesium concentration in the leaves of one year old kiwifruit plants (*Actinidia deliciosa*) treated with nitrate, ammonium, and a mixture of both and exposed to different boron concentrations in the nutrient solution (means of six plants).

Tabelle 6: Magnesium-Gehalte in den Blättern von einjährigen Kiwi-Pflanzen (*Actinidia deliciosa*) in Abhängigkeit von der Stickstoffform und der Bor-Konzentration in der Nährlösung (Mittelwerte von sechs Pflanzen).

B (mM)	N source	Mg in petioles (mg (g d.w.)$^{-1}$)	Mg in remaining part of blade (mg (g d.w.)$^{-1}$)	Mg in leaf margin (mg (g d.w.)$^{-1}$)
0.025	NO_3^-	24.7 a	17.0 ab	5.7 ab
0.1	NO_3^-	24.9 a	16.0 b	5.2 ab
0.3	NO_3^-	25.7 a	18.0 a	6.6 a
0.025	NH_4^+	10.0 c	8.3 d	4.9 b
0.1	NH_4^+	13.5 b	12.2 c	5.3 ab
0.3	NH_4^+	8.9 c	4.5 e	4.5 b
0.025	NO_3^-/NH_4^+	9.9 c	5.5 e	4.9 ab
0.1	NO_3^-/NH_4^+	13.3 b	5.6 e	5.2 ab
0.3	NO_3^-/NH_4^+	14.5 b	5.7 e	5.5 ab

Means in the same column followed by the same letter are not significantly different (Duncan's multiple range test, 5 %).

4 Discussion

The effect of excess B concentration in plant tissues is a reduction in growth and yield (*Gupta*, 1993; *Nable* et al., 1997). The observed growth decline of kiwifruit plants due to B toxicity could be ascribed to the decreased photosynthetic rate and water use efficiency and also to a loss in the leaf area (*Sotiropoulos* et al., 2002).

Ammonium nutrition resulted in a faster vegetative growth of kiwifruit plants due to direct incorporation of NH^+_4 into amino acids, while NO_3^--N has to be reduced initially to NH^+_4 before being incorporated into proteins by plants (*Hayness*, 1990). The data of the present study indicate that the N concentration of kiwifruit plants was greater when N was supplied as NH_4^+ or as NH_4^+/NO_3^- than as NO_3^-. The significantly higher N absorption from NH_4-N or from mixed N sources is well documented in wheat (*Triticum aestivum* L.) (*Criddle* et al., 1988), whereas N yield efficiency in endive (*Cichorium endivia* L.) was increased when N was supplied in a mixed form (*Elia* et al. 1997).

With regard to the B distribution in leaves, the data confirm previous results (*Sotiropoulos* et al., 1997), i.e., the leaf margin is the most appropriate indicator for the diagnosis of B toxicity. Furthermore, the antagonistic effect of NH_4-N on Mg absorption has been well-documented (*Simonne* et al., 1993; *Jeong* and *Lee*, 1996).

With the NH_4-N form, more B was accumulated in the roots than with NO_3-N. However, in the leaves no differences in B accumulation were recorded between the NH_4-N and NO_3-N forms. These results suggest that in areas with high B concentration in irrigation water the NH_4-N form in low concentration (2.6 mM) is more efficient for growth, without promoting B toxicity to leaves. Hence, under such circumstances the NO_3^- and NH_4^+ forms may be used equally well in kiwifruit, without the appearance of B toxicity.

Our hypothesis that the NH_4-N form may promote high B concentration in leaves and B toxicity proved to be invalid, since the pH value of the substrate solution was more or less constant and identical with the pH value (5.9) of the nutrient solutions we used for irrigation.

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