

## Effect of excess boron on summer and winter squash

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### Abstract

The boron tolerance of two summer squash cultivars (*Cucurbita pepo* L. 'Aristocrat Zucchini' and 'Peter Pan Scallop') and one winter squash cultivar (*Cucurbita moschata* Poir. 'Butter boy') was determined in large, outdoor sand cultures. Boron treatments were imposed by irrigation with culture solutions that contained 1.0, 3.0, 6.0, 9.0, 12.0, or 15.0 mg B L<sup>-1</sup>. Relative fruit yields of 'Zucchini', 'Scallop', and 'Butter boy' were reduced 5.2%, 9.8%, and 4.3% with each unit (mg L<sup>-1</sup>) increase in soil solution B ( $B_{sw}$ ) > 2.7, 4.9, and 1.0 mg B L<sup>-1</sup>, respectively. Reduced yields of all cultivars were attributed to a reduction in fruit number and not fruit size. Boron concentrations in leaves and fruit were directly correlated to  $B_{sw}$ .

### Introduction

Although B is an essential constituent of the soil solution for normal plant growth, the difference between adequate and toxic concentrations may be only a few milligrams per liter (Eaton, 1944). Consequently, excess applications of B-containing fertilizers (Skinner et al., 1923) or the use of irrigation waters pumped from wells with high B contents (Eaton 1939) can result in toxic levels of B in the soil solution.

Injury to crop plants caused by high B concentration in the soil solution was first reported by Kelley and Brown (1928). Subsequent studies by Eaton (1944) have become the B tolerance standards for most agricultural crops throughout the world. Unfortunately, his tolerance classification for many crops was based on the occurrence of leaf injury and not on the yield decline of the marketable product. Recent studies (Francois, 1984, 1986, 1988, 1989) have shown that when the product is harvested for anything other than

the leaves, this classification is not a reliable indicator for B tolerance.

Other than limited studies on metabolic processes (Lovatt and Bates, 1984) and vegetative growth (El-Sheikh et al., 1971), there is a lack of information on the effects of excess B on squash. Therefore, this study was initiated to determine the B tolerance of squash as measured by yield of the marketable fruit.

### Methods

Twenty-four sand tanks (2.08 × 0.86 × 0.84 m deep) containing a coarse river sand were used in these tests. The sand was washed to remove fine soil particles that could act as adsorption sites for B in the irrigation waters. Each sand tank was irrigated from a 1365-liter reservoir that contained nutrient solutions with various B concentrations. Irrigation waters were surface-applied three times each day, with the sand being satu-

rated completely with each irrigation. The solutions were collected in corrugated polyethylene tile lines located in the bottom of each sand tank and returned to the reservoirs by gravity flow.

The irrigation waters contained the following nutrient salts per liter: 2.0 mM  $\text{Ca}(\text{NO}_3)_2$ , 1.5 mM KCl, 1.0 mM  $\text{MgSO}_4$ , 0.5 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.5 mg Fe as chelated sodium ferric diethylenetriamine pentaacetate, 0.25 mg Mn as  $\text{MnCl}_2$ , 0.025 mg Zn as  $\text{ZnSO}_4$ , 0.01 mg Cu as  $\text{CuSO}_4$ , and 0.005 mg Mo as  $\text{MoO}_3$ . The B treatments, added as  $\text{H}_3\text{BO}_3$  to the irrigation solutions prior to planting, were 1.0, 3.0, 6.0, 9.0, 12.0, and 15.0 mg  $\text{B L}^{-1}$  for both studies. These concentrations were selected on the basis of data previously reported by Maas (1986). Before adding the  $\text{H}_3\text{BO}_3$ , a sample of the irrigation solution was taken from each reservoir to determine B levels resulting from impurities in the nutrient salts and the tap water used to fill the reservoirs. The amount of  $\text{H}_3\text{BO}_3$  added to each reservoir was adjusted to obtain the desired treatment levels. Irrigation solution samples were taken monthly to monitor and maintain desired B levels. Solution pH varied between 7.5 and 7.8. The experimental design in both studies consisted of six treatments replicated four times in a randomized complete block.

Since the washed sand used in this study possessed negligible adsorption capacity, the B concentration in the irrigation water ( $B_{\text{iw}}$ ) and in the soil water ( $B_{\text{sw}}$ ) were identical. Therefore, since plants respond to the B concentration in the soil water (Bingham et al., 1981; Hatcher et al., 1959), all data presented in this study are given in terms of that parameter. For comparison with soil conditions, the relationship that exists between  $B_{\text{iw}}$  and  $B_{\text{sw}}$  at various leaching fractions has been previously presented (Francois, 1984).

Mature, fully expanded leaves and mature fruit were sampled from all cultivars for elemental analysis. Leaves and fruit were washed, dried at 70°C, and finely ground in a blender. Boron was determined colorimetrically by the Azomethine-H method (John et al., 1975) after the sample material was digested by dry-ashing with CaO. Nitric-perchloric acid digests of the dried ground samples were analyzed for P by molybdovanadate-yellow colorimetry (Kitson

and Mellon, 1944) and for Ca, Mg and K by atomic absorption spectrophotometry.

Calculated coefficients for unequally spaced treatments were used to determine single-degree-of-freedom comparisons.

### *Summer squash*

Two summer squash cultivars, 'Aristocrat Zucchini' and 'Peter Pan Scallop', were planted in each sand tank on 30 April 1986. Two weeks after planting the stand was thinned to 6 plants of each cultivar spaced  $\approx 0.3$  m apart.

Leaves and fruit were sampled for elemental analysis from each cultivar on 12 June and 27 June, respectively. The leaf blades were removed from the petioles and the petioles were discarded.

Initial fruit harvest for 'Scallop' occurred on 14 June and for 'Zucchini' on 20 June, with subsequent harvests every 2–3 days thereafter. The fruit were picked when the 'Zucchini' were about 0.2 m in length and the 'Scallop' were 0.1 m in diameter (Sackett, 1975). Fruit number and total weight were determined for each harvest day. Final harvest for both cultivars occurred on 6 August. After the final harvest, the top growth was removed and air dried to determine vegetative growth.

### *Winter squash*

A winter squash cultivar, 'Butter boy' was planted on 5 May 1987. After emergence, the stand was thinned to 5 plants per sand tank. Final spacing between plants was  $\approx 0.3$  m apart.

On 8 July,  $\approx 5$  weeks before harvest, leaf blades were sampled for elemental analysis.

Fruit was harvested on 12 August, when the fruit skin was sufficiently hard to resist denting by the thumbnail (Sackett, 1975). Total fruit weight and number were determined. Top growth was removed on 13 August, air dried, and weighed.

Fruit samples were taken at harvest for elemental analysis. In contrast to the summer squash samples, the skin on the fruit was removed prior to drying.

## Results and discussion

### Summer squash

Within two weeks after planting, at which time the first true leaves on both cultivars were fully emerged, a chlorosis was noted on the margins of leaves of all plants growing with 6 mg B L<sup>-1</sup> or higher. As the plants matured on the 9, 12, and 15 mg B L<sup>-1</sup> treatments, the chlorotic margins of the older leaves became progressively necrotic. The extent of this chlorosis and/or necrosis was correlated directly to the B<sub>sw</sub> concentration. At no time during the study were leaf injury symptoms observed on plants growing with B<sub>sw</sub> at 1 or 3 mg L<sup>-1</sup>. The leaf injury symptoms and the lowest B level at which injury occurred in this study

agree with those previously reported by El-Sheikh et al. (1971) for summer squash.

Flowering started 40 and 47 days after planting for 'Scallop' and 'Zucchini', respectively. This initial flowering occurred only on those plants growing at 1 and 3 mg B L<sup>-1</sup>. At the four higher B<sub>sw</sub> treatments, a significant delay in flowering occurred with both cultivars. High substrate levels of boron have been previously implicated with delayed flowering of cowpea [*Vigna unguiculata* (L.) Walp] (Francois, 1989) and some fruit trees (Cibes et al., 1955; Crandall et al., 1981). This delay in flowering subsequently caused a delay in reaching harvestable fruit size on the higher B<sub>sw</sub> treatments. By 1 July, nearly 50% of the 'Zucchini' fruit had been harvested from the 1 mg B L<sup>-1</sup> treatment, whereas <20%

Table 1. Vegetative growth and fruit yield of 'Zucchini' and 'Scallop' squash cultivars grown at six B concentrations in the soil solution

Soil water B (mg L <sup>-1</sup> )	Vegetative growth (dry wt/plant, g)	Fruit			
		Total wt (g/plant)	No. per plant	Average wt (g)	
<i>Zucchini</i>					
1.0	182	1573	4.9	321	
3.0	171	1611	4.8	336	
6.0	148	1135	3.4	334	
9.0	160	1133	3.2	354	
12.0	163	924	2.6	355	
15.0	167	490	1.4	350	
<i>Scallop</i>					
1.0	162	2056	6.6	312	
3.0	180	1043	7.4	276	
6.0	138	1610	5.6	288	
9.0	181	1671	5.9	283	
12.0	194	435	1.6	272	
15.0	157	13	0.1	—	
Significance					
	df	Analysis of variance			
<i>Zucchini</i>					
Treatment	5	NS	***	***	*
Linear <sup>a</sup>	1	NS	***	***	**
Quadratic <sup>a</sup>	1	*	NS	NS	NS
<i>Scallop</i>					
Treatment	5	NS	***	***	NS
Linear <sup>a</sup>	1	NS	***	***	*
Quadratic <sup>a</sup>	1	NS	**	***	NS

<sup>a</sup> Single-degree-of-freedom comparison.

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $p = 0.05$ , 0.01, or 0.005, respectively.

had been harvested from the 12 and 15 mg B L<sup>-1</sup> treatments. The 'Scallop' showed a similar harvest delay pattern.

Both cultivars showed a significant reduction in total fruit weight with  $B_{sw} > 3.0$  mg B L<sup>-1</sup> (Table 1). Since individual fruit weight for 'Zucchini' increased slightly and 'Scallop' was not significantly affected by increased levels of  $B_{sw}$ , the reduction in total fruit weight was accounted for primarily by the reduced number of fruit harvested per plant. This reduction in fruit number may be accounted for by poor pollination. Marschner (1986) has reported that leakage of sugar from pollen decreases with increasing external boron concentration. If this occurs with squash, the flowers may become less attractive to pollinating insects.

While poor pollination may primarily account for the poor fruit set with 'Zucchini', an additional phenomena occurred which significantly reduced the number of 'Scallop' fruit at the higher  $B_{sw}$  levels. At 12.0 and 15.0 mg B L<sup>-1</sup> the 'Scallop' fruit set, grew to about 30 mm in diameter, became chlorotic, and then abscised. This premature abortion occurred with 86% and 99% of the fruit set on the 12.0 and 15.0 mg B L<sup>-1</sup> treatments, respectively. High levels of  $B_{sw}$  have been previously reported to cause abortion of young fruit on some fruit trees (Ballinger et al., 1966; Oberly and Boynton, 1966). Over the  $B_{sw}$  range tested, none of the 'Zucchini' fruit aborted.

To determine the B tolerance, fruit weight of each cultivar was statistically analyzed with a piecewise linear response model (Francois, 1984; Maas and Hoffman, 1977; van Genuchten and Hoffman, 1984). The 'Scallop' data indicate that each unit increase in  $B_{sw} > 4.9$  mg B L<sup>-1</sup> (threshold) resulted in a 9.8% decrease in relative yield (Fig. 1, top). For 'Zucchini', each unit increase in  $B_{sw} > 2.7$  mg B L<sup>-1</sup> (threshold) reduced relative yield 5.2% (Fig. 1, bottom). Although the analyses show that 'Scallop' had a significantly higher threshold than 'Zucchini', the yield decline above the threshold was significantly greater for 'Scallop'. Therefore, a 50% yield decline occurred at  $B_{sw}$  concentrations of ~10 and 12 mg B L<sup>-1</sup> for 'Scallop' and 'Zucchini', respectively. Relative yield for any given  $B_{sw}$  concentration exceeding the thresholds

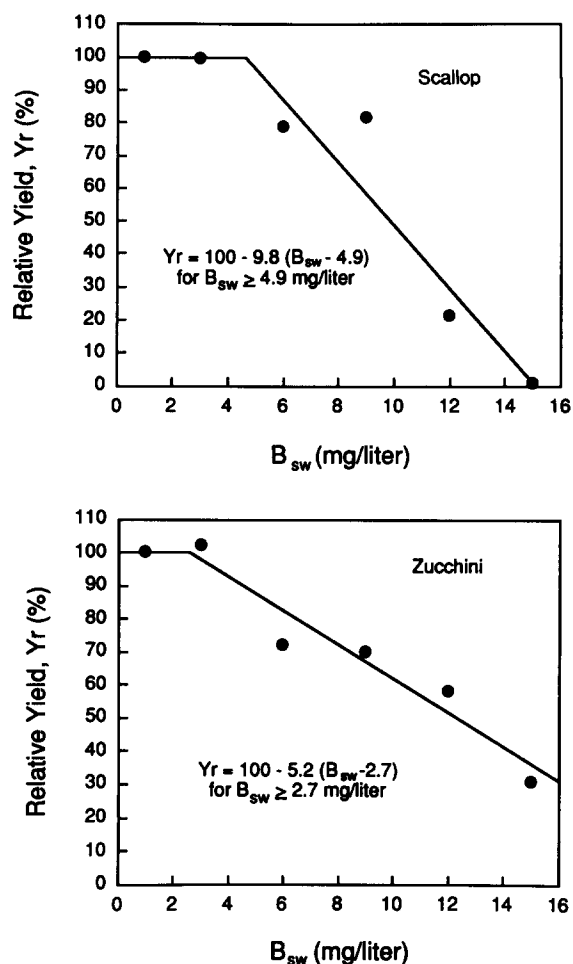


Fig. 1. Relative yield (Yr) of 'Scallop' (top) and 'Zucchini' (bottom) squash as influenced by B concentration in the soil solution.

can be calculated with the equations presented in Figure 1. Based upon the thresholds, the B tolerance categories established by Maas (1986) would classify 'Scallop' as tolerant and 'Zucchini' as moderately tolerant to  $B_{sw}$ .

In contrast to fruit yield, vegetative growth of both cultivars was not significantly reduced over the  $B_{sw}$  range tested (Table 1).

Boron concentration in the fruit of both cultivars showed a significant linear increase with increased  $B_{sw}$  (Table 2). Marketable fruit sampled from the 12.0 mg B L<sup>-1</sup> treated plants contained ~5 times more B than fruit sampled from the 1.0 mg B L<sup>-1</sup>-treated plants. Potassium concentration which increased significantly in 'Zucchini' fruit, remained relatively unchanged in

Table 2. Mineral concentration in fruit of 'Zucchini' and 'Scallop' squash cultivars grown at six B concentrations in the soil solution

Soil water B (mg L <sup>-1</sup> )	B (mg kg <sup>-1</sup> dry wt)	Ca (mmol kg <sup>-1</sup> dry wt)	K	Mg	P	
<i>Zucchini</i>						
1.0	40	53.3	1550	96	156	
3.0	71	56.6	1767	106	176	
6.0	122	56.4	1790	102	173	
9.0	164	58.0	1730	102	167	
12.0	210	58.7	1678	96	156	
15.0	247	60.0	1882	92	164	
<i>Scallop</i>						
1.0	51	84.6	1162	102	150	
3.0	71	82.9	1149	114	157	
6.0	144	80.4	1316	112	156	
9.0	200	88.5	1194	110	153	
12.0	231	82.2	1153	96	173	
15.0	—	—	—	—	—	
12.0 <sup>b</sup>	308	195.8	1751	223	92	
15.0 <sup>b</sup>	374	228.2	1718	231	82	
<b>Significance</b>						
	<b>df</b>	<b>Analysis of variance</b>				
<i>Zucchini</i>						
Treatment	5	***	NS	***	NS	NS
Linear <sup>a</sup>	1	***	NS	***	NS	NS
Quadratic <sup>a</sup>	1	NS	NS	NS	NS	NS
<i>Scallop</i>						
Treatment	4	***	NS	NS	NS	NS
Linear <sup>a</sup>	1	***	NS	NS	NS	NS
Quadratic <sup>a</sup>	1	NS	NS	*	***	NS
Treatment <sup>a,b</sup>	5	***	***	***	***	***
Linear <sup>a,b</sup>	1	***	***	***	***	***
Quadratic <sup>a,b</sup>	1	*	***	*	***	***

<sup>a</sup> Single-degree-of-freedom comparison.

<sup>b</sup> Aborted scallop fruit.

NS, \*, \*\*\* Nonsignificant or significant at  $p = 0.05$ , or  $0.005$ , respectively.

fully grown 'Scallop' fruit. Increased levels of  $B_{sw}$  had no significant affect on Ca, Mg, and P concentrations in the fruit of either cultivar.

The aborted 'Scallop' fruit showed significant changes in mineral concentration for all ions tested. Boron, Ca, K, and Mg concentrations were significantly higher, while P concentration was significantly lower, than the concentrations found in fully mature fruit. Higher mineral concentrations may be attributed to smaller sized fruit with a lower water content. There appears to be a direct correlation between the high B content and the high concentration of the other

ions. A correlation for B and Ca has been previously reported by Dixon et al. (1973), where boron sprays have been shown to increase calcium transport into apples (*Malus* sp.).

At nutritional levels, B normally plays a stabilizing role for plant membranes (Pilbeam and Kirkby, 1983). However, the excessive amounts of Ca, K, and Mg found in the aborted fruit in this study indicate a breakdown in the regulatory aspect of the membranes when B within the plant is excessive.

Since B uptake and accumulation is influenced by the transpiration rate (Raven, 1980), the

concentration of B in the high transpiring leaf tissue was significantly greater than the B concentration in the low transpiring fruit tissue (Table 3). At  $B_{sw}$  greater than  $1.0 \text{ mg L}^{-1}$ , the leaves contained  $\sim 4$  times more B than did the fruit. Increased B concentration in 'Scallop' leaves was associated with a significant decrease in Ca and Mg content and a significant increase in K and P. While these ions tended to follow the same general pattern in 'Zucchini' leaves, the concentrations were not significantly different over the  $B_{sw}$  range tested.

#### Winter squash

All plants grown at  $9 \text{ mg B L}^{-1}$  or higher developed an irregular chlorosis along the leaf margins about 14 days after planting. At harvest,

the earlier chlorosis on the older leaves had developed into necrosis, ranging from 1 mm wide on the  $9.0 \text{ mg B L}^{-1}$  treated plants to 5 mm wide on the  $15 \text{ mg B L}^{-1}$ -treated plants. At the same time, a barely discernible marginal chlorosis had developed on the older leaves of plants grown at  $6.0 \text{ mg B L}^{-1}$ .

Total fruit weight harvested per plant was significantly reduced as  $B_{sw}$  concentration increased (Table 4). Like summer squash, the reduction in yield was attributed primarily to the reduction in fruit number and not to reduced individual fruit weight.

Piecewise linear regression analysis (Francois, 1984; Maas and Hoffman, 1977; Van Genuchten and Hoffman, 1984) for total weight showed that each unit increase in  $B_{sw}$  above  $1.0 \text{ mg B L}^{-1}$  (threshold) decreased yield 4.3% (Fig. 2). The

Table 3. Mineral concentration in leaves of 'Zucchini' and 'Scallop' squash cultivars grown at six B concentrations in the soil solution

Soil water B ( $\text{mg L}^{-1}$ )	B ( $\text{mg kg}^{-1}$ dry wt)	Ca ( $\text{mmol kg}^{-1}$ dry wt)	K	Mg	P
<i>Zucchini</i>					
1.0	96	1028	990	306	113
3.0	243	923	930	292	127
6.0	573	1118	862	349	98
9.0	763	1009	916	318	124
12.0	920	976	845	316	120
15.0	1048	811	880	303	116
<i>Scallop</i>					
1.0	111	1546	684	428	104
3.0	292	1262	651	412	130
6.0	660	1709	610	514	108
9.0	701	1198	744	390	136
12.0	972	1261	709	428	145
15.0	885	1036	732	386	157

Significance	df	Analysis of variance				
<i>Zucchini</i>						
Treatment	5	***	NS	NS	NS	NS
Linear <sup>a</sup>	1	***	NS	NS	NS	NS
Quadratic <sup>a</sup>	1	***	NS	NS	NS	NS
<i>Scallop</i>						
Treatment	5	***	***	*	***	***
Linear <sup>a</sup>	1	***	***	*	NS	***
Quadratic <sup>a</sup>	1	***	NS	NS	*	NS

<sup>a</sup> Single-degree-of-freedom comparison.

NS, \*, \*\*\* Nonsignificant or significant at  $p = 0.05$  or  $0.005$ , respectively.

Table 4. Vegetative growth and fruit yield of 'Butter boy' winter squash grown at six B concentrations in the soil solution

Soil water B (mg L <sup>-1</sup> )	Vegetative growth (dry wt/plant, g)	Fruit		
		Total wt (g/plant)	No. per plant	Average wt (g)
1.0	94	1303	2.32	561
3.0	114	1121	2.15	523
6.0	88	929	1.91	485
9.0	98	992	1.89	524
12.0	80	781	1.56	501
15.0	59	458	1.07	426

Significance	df	Analysis of variance		
Treatment	5	NS	*	NS
Linear <sup>a</sup>	1	**	***	NS
Quadratic <sup>a</sup>	1	NS	NS	NS

<sup>a</sup> Single-degree-of-freedom comparison.

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $p = 0.05, 0.01, \text{ or } 0.005$ , respectively.

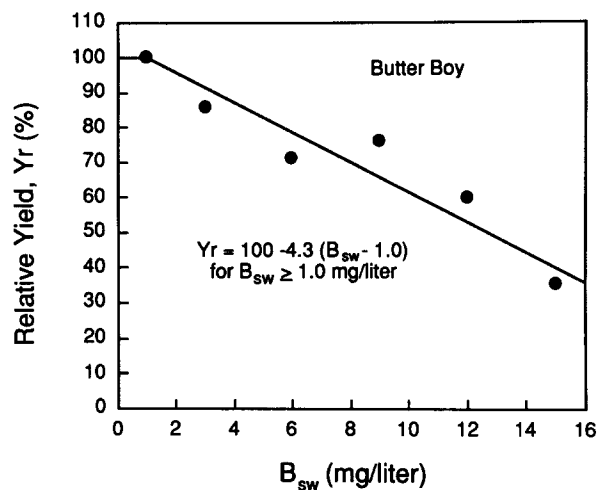


Fig. 2. Relative yield (Yr) of 'Butter boy' squash as influenced by B concentration in the soil solution.

equation presented in the figure indicates that a 25% reduction in weight would occur at a  $B_{sw}$  of 6.8 mg B L<sup>-1</sup>. This species of winter squash, therefore, would be classified as moderately sensitive to  $B_{sw}$  (Maas, 1986).

Like summer squash, the B concentrations in the leaf and fruit tissue were related to the  $B_{sw}$  concentration (Table 5). Boron accumulation in the leaves was about 10 times greater than in the fruit with each unit increase in  $B_{sw}$  above 1.0 mg L<sup>-1</sup>.

Table 5. Boron concentration in leaves and fruit of 'Butter boy' winter squash grown at six B concentrations in the soil solution

Soil water B (mg L <sup>-1</sup> )	Leaves (mg kg <sup>-1</sup> dry wt)	Fruit (mg kg <sup>-1</sup> dry wt)
1.0	112	24
3.0	268	39
6.0	465	83
9.0	914	77
12.0	1025	109
15.0	1224	137

Significance	df	Analysis of variance	
Treatment	5	***	***
Linear <sup>a</sup>	1	***	***
Quadratic <sup>a</sup>	1	NS	NS

<sup>a</sup> Single-degree-of-freedom comparison.

NS, \*\*\* Nonsignificant or significant  $p = 0.005$ , respectively.

Increased B in the soil solution and subsequent B accumulation in the leaves and fruit did not significantly affect the concentrations of Ca, K, Mg, or P within the plant. Over the  $B_{sw}$  range tested, Ca, K, Mg, and P averaged 1040, 798, 329, and 124 mmol kg<sup>-1</sup> dry weight in leaves and 26, 750, 38, and 57 mmol kg<sup>-1</sup> dry weight in fruit, respectively. The ion concentrations in the fruit of this squash species were significantly lower than the two summer squash species.

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