Salinity's influence on boron toxicity in broccoli: II. Impacts on boron uptake, uptake mechanisms and tissue ion relations

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ABSTRACT

Limited research has been conducted on the interactive effects of salinity and boron stresses on plants despite their common occurrence in natural systems. The purpose of this research was to determine and quantify the interactive effects of salinity, salt composition and boron on broccoli (*Brassica oleracea* L.) performance, particularly, element accumulation, ion interactions and boron uptake processes. A greenhouse experiment was conducted using a sand tank system where salinity-B treatment solutions were supplemented with a complete nutrient solution. Chloride-dominated salinity and salinity characteristic of California's San Joaquin valley (SJV), or sulfate-dominated, were tested at ECw (electrical conductivity of the irrigation water) levels of 2, 12 and 19 dS m^{-1}. Each salinity treatment consisted of boron treatments of 0.5, 12 and 24 mg L^{-1}. Salinity, regardless of salt composition, reduced shoot boron concentration at very high boron concentration (24 mg L^{-1}). However, increased salinity increased shoot boron concentration when external boron concentration was low (0.5 mg L^{-1}). Tissue Ca, Mg, Na, K, S and Cl concentrations were also affected by salinity level, chloride or sulfate salinity composition, and in some cases by substrate boron concentration. Calcium concentrations in shoots were greater for chloride-treated salinity as compared to SJV salinity-treated plants; magnesium concentrations trended opposite and were greater in those treated with SJV salinity. Chloride and sodium shoot concentrations both increased with salinity. Shoot chloride was greater with chloride substrate salinity and shoot sodium was greater with SJV substrate salinity. Using stable isotope analysis of solutions to separate transpiration from evapotranspiration (ET), we found that boron uptake and accumulation in the shoot was not simply the product of mass flow (solution concentration × cumulative transpiration), and the vast majority of the water lost from the tank system was by transpiration (>90%) regardless of treatment. Under low substrate boron, the levels of boron in broccoli shoots could be not accounted for by simple passive uptake and transport in the transpiration stream, which suggests that some energy-dependent process was also occurring. However, under high boron treatments, broccoli plants exhibited a mechanism that restricted boron uptake, transport and accumulation in the shoot.

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1. Introduction

The vast majority of research on salinity and excess boron stresses on crop performance has been done independent of one another (Maas and Grattan, 1999). Recently, however, there has been some research that addresses the interactive effects of these two abiotic stresses (Nicholaichuk et al., 1988; Ben-Gal and Shani, 2002; Wimmer et al., 2005; Yermiyahu et al., 2008).

Research conducted on the interactions of these two stresses as they affect plant performance is contradictory. When both stresses occur together, studies have shown that salinity may reduce or increase boron's toxic effect. Some studies have shown that increased salinity can increase boron-related toxic effects in crops such as tomato, cucumber and wheat (Alpaslan and Gunes, 2001; Grieve and Poss, 2000; Wimmer et al., 2003, 2005). Conversely, increases in salinity decreased boron toxicity in numerous vegetables, *Prunus* rootstocks, wheat and chickpeas (El-Motaium et al., 1994; Ferreyra et al., 1997; Holloway and Alston, 1992; Yadav et al., 1989). Hypotheses provided to explain these effects include: (1) salinity-induced reduction of evapotranspiration-related boron uptake (Hu and Brown, 1997; Grattan et al., 1996); (2) soil pH changes affecting boron speciation (i.e. B(OH)$_3$ vs.

$B(OH)_4^-$) and soil adsorption processes ultimately affecting boron absorbed by plants (Goldberg et al., 2000); (3) boron solubility and mobility within the plant (Brown and Hu, 1994); (4) indirect effects related to calcium nutrition and membrane integrity (Marsh and Shive, 1941; Minarik and Shive, 1939); (5) other ionic interactions such as sulfate (SO_4^{2-}) reducing boron uptake in plants (Manchanda and Sharma, 1991).

Although high soil-boron can be found in the absence of salinity such as those areas irrigated with high boron well waters (e.g. many geothermal ground waters and other well waters whose parent material is rich in boron minerals), potentially toxic levels of soil-boron can also be associated with saline soils in many regions of the world. One area of particular interest to us is the west side of California's San Joaquin Valley (SJV) where saline-sodic drainage water containing high boron is used for irrigation of salt tolerant crops as a means of reducing drainage volume. Therefore, improved understanding of plant response to high soil-boron concentrations in the presence of salinity could provide insight into better management of crops grown under these harsh conditions.

Salinity, regardless of its composition, reduces crop growth and yield by osmotic effects but the ionic composition of the salt solution can also affect crop performance (Läuchli and Grattan, 2007). Moreover, research directed towards understanding salinity–boron interactions in crops have generated contradictory results, which may be related, in part, to the salt composition used in the study. With wheat as the test crop, Bingham et al. (1987) found no boron interactions with salinity, as did researchers with alfalfa, barley, corn and wheatgrass (Diaz and Grattan, 2009; Mikkelsen et al., 1988; Shani and Hanks, 1993). Conversely, researchers have observed severe leaf injury in wheat associated with excessive boron uptake from chloride-based saline irrigation waters (Grieve and Poss, 2000; Läuchli et al., 2001; Wimmer et al., 2003). Tomato and cucumber showed greater sensitivity to boron with increasing salinity in sodium–chloride environments (Alpaslan and Gunes, 2001). However, many studies found that salinity stress actually increased tolerance to boron toxicity. This response has been documented in several crops (Ferreyra et al., 1997) including eucalyptus (Grattan et al., 1996), pistachio (Ferguson et al., 2002), chickpeas (Yadav et al., 1989) and *Prunus* spp. rootstocks (El-Motaium et al., 1994).

We conducted a comprehensive sand tank study in a greenhouse because of the uncertainties salt solution composition may play on salinity–boron interactions. The purpose of this research was to quantify and evaluate the interactive effects of salinity, including two salt compositions, and boron on broccoli (*Brassica oleracea* L., botrytis group) appearance, boron uptake mechanisms (passive or active), and ion accumulation and interactions within the plant. This paper is written to complement the first paper in this series which addressed interactive effects on yield, biomass distribution among shoot organs, evapotranspiration and water use efficiency (Smith et al., this issue).

2. Materials and methods

A greenhouse experiment was conducted, using broccoli cv. Seminis PX511018 as the test crop, in a sand tank system at the USDA-ARS, U.S. Salinity Laboratory located on the University of California, Riverside campus. This sand tank apparatus is described in detail in the first paper of this series.

Six sand tanks were not planted but were irrigated like the planted tanks to provide maximum evaporation rates and amounts. These tanks were used as a reference for maximum evaporation using a stable isotope analyses procedure to separate quantities of water lost as evaporation and transpiration (Grattan et al., 2008) in the tanks planted with broccoli. The general

principle is that enrichment of delta ^{18}O in the reservoir water in this closed sand tank system that supplies irrigation water to the sand tanks occurs only by evaporation since this process involves a phase change from liquid to vapor (Gat, 1996). Root water uptake leading to transpiration, on the other hand, does not discriminate with isotopic form of water since water remains in the liquid phase as it moves from the soil water to inside root cells. Therefore water lost from the sand tank system via transpiration will not enrich the $H_2^{18}O$ in the storage reservoirs. Delta ^{18}O (δ_{sample}) is defined below where $^{18}O/^{16}O$ is the isotopic ratio of $H_2^{18}O$ to $H_2^{16}O$ in the water sample or standard mean ocean water (SMOW). The isotopic ratio of standard mean ocean water is the reference that has been adopted to relate isotopic changes in water samples (Gat, 1996):

$$\sigma_{sample} = \frac{^{18}O/^{16}O_{sample} - {}^{18}O/^{16}O_{smow}}{^{18}O/^{16}O_{smow}}$$

Systems losing water primarily by transpiration would not experience large changes in the isotopic signature (δ_{sample}) such that a tank losing all of its water as transpiration would not have any change in the isotopic ratio over time. On the other hand, water lost from the system only as evaporation, the enrichment of the heavy isotope in the reservoir would be equivalent to that observed in the irrigated tanks that were not planted. Therefore, the degree at which a planted tank-reservoir system evaporates water will cause differential enrichment of $H_2^{18}O$ of the solution in the reservoirs.

Cumulative transpiration volumes per plant were estimated from total ET losses by comparing the changes in the isotopic signature in the storage reservoirs over time. Based on this, we were able to determine the percentage of ET that was related to transpiration. With these values expressed on a per plant basis (accounting for the changing plant density in the tanks), we then multiplied the cumulative transpiration volumes by the soil solution concentrations to determine how much total boron would accumulate in the plant by a simply passive process.

This exercise was done so that the total shoot boron content (mg B shoot^{-1}) could be compared to the potential cumulative boron (mg B shoot^{-1}), i.e. liters of transpired water × solution boron concentration. Water samples were collected on 5 May, 9 May, and 21 May during which time the system refill was turned off. To minimize variance, the reservoir water was cycled in the sand tank system for several hours prior to water sample collection in the reservoirs so that the isotopic ratio in the sand water was the same as that as the water in the reservoir. This comparison allows us to determine if boron accumulation in the shoot is a completely passive and non-restrictive transport process to the shoot, or whether there is some energy-dependent process allowing the shoot to accumulate more boron than can be accounted for by a simple passive process. This is an important component of the study since boron uptake by the plant at adequate and high substrate concentrations is thought to occur largely by a non-metabolic process (Hu and Brown, 1997) and its transport to the shoot is thought to be passively controlled by the transpiration stream (Marschner, 1995).

The irrigation treatments consisted of three targeted salinity levels representing non-saline (2.0 dS m^{-1}), moderately saline (11 dS m^{-1}) and saline (18 dS m^{-1}) conditions. The actual measured EC levels over the season were 2.4, 11.8 and 19.1 dS m^{-1}, which took into account the nutrient salts and the mineral ions present in Riverside municipal tap water. Based on soil–water (ECsw – ECe[1]) relations for most mineral soils at 'field capacity' these respective saline treatments translate into average rootzone

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[1] ECsw and ECe refer to the electrical conductivity of the soil water and saturated soil extract, respectively.

Table 1
Ionic compositions of waters for the various salinity treatments. SJV refers to San Joaquin Valley or sulfate-dominated water and Cl is chloride-dominated water.

Salt type	ECw (dS m^{-1})	Ca (mmol$_c$ L^{-1})	Mg (mmol$_c$ L^{-1})	Na (mmol$_c$ L^{-1})	K (mmol$_c$ L^{-1})	Cl (mmol$_c$ L^{-1})	SO$_4$ (mmol$_c$ L^{-1})
SJV	2	5.3	3.0	4.3	3.0	2.3	6.0
SJV	12	25.6	19.4	76.8	3.0	37.4	79.6
SJV	19	29.6	32.6	134.3	3.0	65.5	127.0
Cl	2	5.2	3.0	6.0	3.0	11.0	4.1
Cl	12	47.7	6.0	48.5	3.0	102.2	2.0
Cl	19	81.2	6.0	85.4	3.0	170.0	2.0

salinities (ECe) of 1.2, 5.9 and 9.5 dS m^{-1} (Ayers and Westcot, 1985). Each salinity level was comprised of either chloride-dominated salts (Cl) or synthetic saline drainage water with an ion composition typical to that found in shallow, saline water tables in the western San Joaquin Valley (SJV) (Table 1). We also refer to this treatment as sulfate-dominated.

Each of these salinity treatments were tested at three boron concentrations ranging from low, such as that found in solution cultures (i.e. 0.5 mg L^{-1}), to high (12 mg L^{-1}) and very high B concentrations (24 mg L^{-1}) that can be found in drainage water from western SJV. Irrigation waters were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) three times during the experiment to confirm that target ion concentrations were maintained.

Broccoli was planted on 4 February 2003, at a density of 60 plants per tank and salinization began 16 days later when plants had approximately two leaves. Salinization was delayed in order for the plants to become well established and to correct micronutrient deficiencies that were detected shortly after emergence. The plant density was also reduced to 30 plants per tank at this time (February 21st) and further thinned to 18 plants per tank on March 28th. The crop was harvested on April 24th (mid-season sample (MSS)) and thinned to a final density of 12 plants per tank. At this time, total shoot biomass was determined and plants were saved for ion analyses. The remainder of the plants were harvested at maturity on May 21st (late-season sample (LSS)—90 days after salinization). At that time, broccoli shoots were divided into heads, stems, young leaves (most recent fully expanded leaves and younger), mid-stem leaves and bottom leaves (all remaining leaves). Immature heads, from salt-stressed treatments, were given the opportunity to mature and these heads were harvested on May 29th. Tissues from both harvests were analyzed for mineral ion content. Samples were washed in deionized water, dried in a forced-air oven at 70 °C for 72 h, then ground to pass a 20-mesh screen. Total-S, total-P, Na, Ca, Mg, K, were determined on nitric-perchloric acid digests of the tissues by inductively coupled plasma optical emission spectrometry (ICP-OES). Chloride was determined on nitric-acetic acid extracts by coulometric–amperometric titration. For B analysis, ground plant samples were ashed, dissolved in nitric acid and analyzed by ICP-OES. ANOVA and surface regression model analysis were performed on the data.

During the first few weeks of the study, the pH of the solution was adjusted to 6.0 every 2 days using sulfuric acid. We found that the pH of the solutions rose to about 8.0 over the 2-day period. Between 28 February and 15 April 2003, the pH of the solutions was adjusted to 6.0 approximately every 2 weeks. Thereafter the pH was not adjusted and remained about 8.0 for most of the experimental time.

Ambient daytime air temperatures in the greenhouse during the experiment ranged from 14 to 37 °C (mean = 28 °C); nighttime temperatures ranged from 12 to 30 °C (mean = 21 °C). Relative humidity ranged from 41.2% to 47.7% with a mean of 44.6% during the day and 45.3% during the night.

3. Results and discussion

3.1. Tissue ion accumulation

Extensive ion analyses were conducted to evaluate the influence of salinity on ion uptake and distribution within broccoli shoots. Whole shoots were analyzed mid-season (April 24, 2003), whereas the final May 20th harvest included various aged leaves, stems and heads.

Shoot tissue that was sampled on April 24th exhibited interesting ion relations and interactions. At very high solution boron concentrations (24 mg L^{-1}), shoot boron concentration decreased by nearly half as salinity increased from 2 to 19 dS m^{-1} 1 (Fig. 1). This reduction in tissue boron occurred under both salt compositions. Interestingly at low solution boron, the behavior was just the opposite. An increase in salinity increased shoot boron concentration. Again this was true regardless of the salt composition. At high concentrations of boron (12 mg L^{-1}), an increase in salinity from 2 to 12 reduced shoot boron but further increases in salinity increased shoot B. Examining the data from another perspective, under non-saline conditions, increasing boron from 0.5 to 24 mg L^{-1} increased shoot boron concentration by nearly 9 times whereas the same increase in boron at high salinity only doubled the concentration. Increased salinity was also found to decrease tissue boron concentration in wheat, chickpea, eucalyptus, Prunus spp. rootstocks (Manchanda and Sharma, 1991;
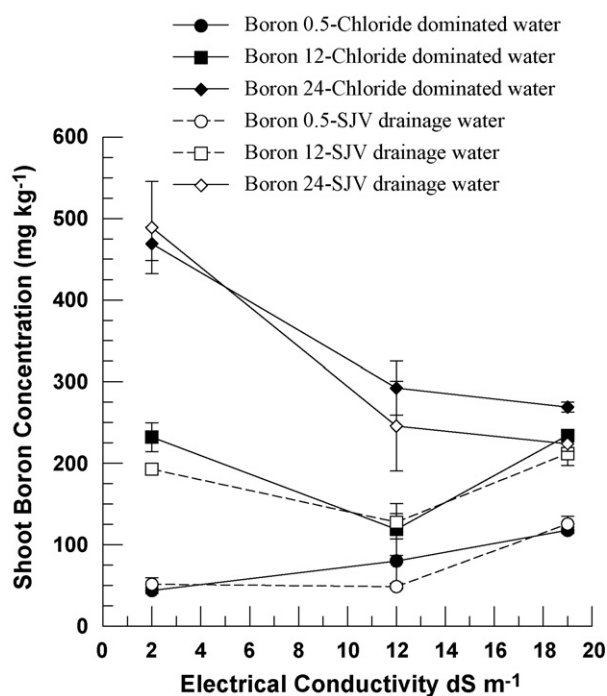

Fig. 1. Shoot boron concentrations (mid-season sampling, MSS) as influenced by chloride or sulfate salinity and substrate boron concentration.
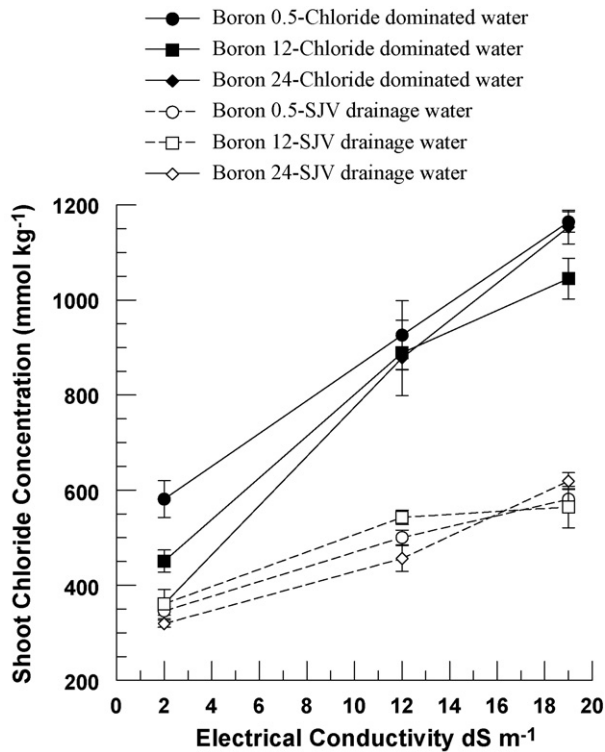
Fig. 2. Shoot chloride concentrations (mid-season sampling, MSS) as influenced by chloride or sulfate salinity and substrate boron concentration.
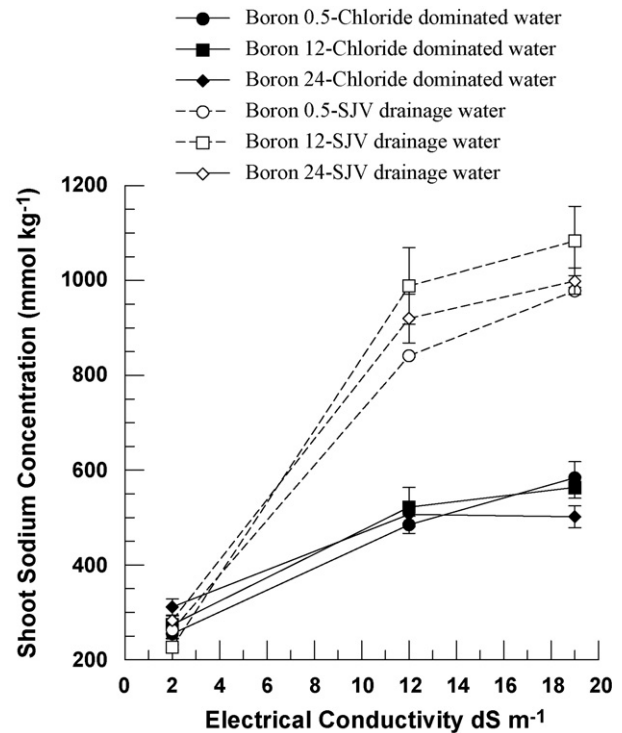
Bingham et al., 1987; Yadav et al., 1989; Grattan et al., 1996; El-Motaium et al., 1994).

Shoot chloride increased in a somewhat linear manner as salinity increased (Fig. 2). The slopes of these relationships under the chloride-dominated treatments were at least twice that in the SJV treatments. This is not surprising considering that at any particular iso-electric solution, solution Cl concentrations in the Cl-dominated treatments were more than twice that in the sulfate-dominated treatments (Table 1). Under the SJV salt composition treatments, shoot Cl concentration was not influenced by substrate boron. However, in the chloride-dominated treatment, an increase in solution boron concentration at low salinity ($2 \, dS \, m^{-1}$) decreased shoot Cl. There was no significant difference in shoot Cl among plants treated with different levels of boron at intermediate salinity but at high salinity, plants treated with low and very high boron accumulated more Cl in their shoots than did those treated with $12 \, mg \, L^{-1}$ of B. Work by others has also shown that boron affects tissue chloride concentrations and vice versa (Yermiyahu et al., 2008). In their study, the investigators suggested that this interaction could be a key factor in understanding salinity-B relations.

Like Cl, shoot Na concentrations increased with increased salinity but this increase was curvilinear, not linear (Fig. 3). Unlike shoot Cl, shoot Na concentration was considerably higher in SJV-treated plants than those treated with Cl-dominated waters and these differences were related to differences in the ionic composition of the two different solutions (Table 1). The SJV solutions had higher Na and lower Cl than the Cl-dominated solutions. The effect of substrate boron on shoot Na concentration was not significant.

Shoot K concentrations behaved similarly to those reported in other studies where salinity is the sole stress-causing factor (Grattan and Grieve, 1999). As salinity increased, shoot K concentrations decreased (Fig. 4), presumably due to the increased Na concentration in the soil solution (Table 1). Such Na-induced

Fig. 3. Shoot sodium concentrations (MSS) as influenced by chloride or sulfate salinity and substrate boron concentration.

Fig. 4. Shoot potassium concentrations (mid-season sampling, MSS) as influenced by chloride or sulfate salinity and substrate boron concentration.

reductions in tissue K are a common observation (Grattan and Grieve, 1999). Also, those iso-electric solutions with higher Na (i.e. the SJV-dominated solutions), decreased shoot K proportionally more than those that were Cl-dominated. For example, as salinity increased from 2 to 19 $dS \, m^{-1}$, shoot K decreased by about 10–20% in the Cl-dominated treatments whereas it decreased by nearly

Fig. 5. Shoot magnesium concentrations (mid-season sampling, MSS) as influenced by chloride or sulfate salinity and substrate boron concentration.
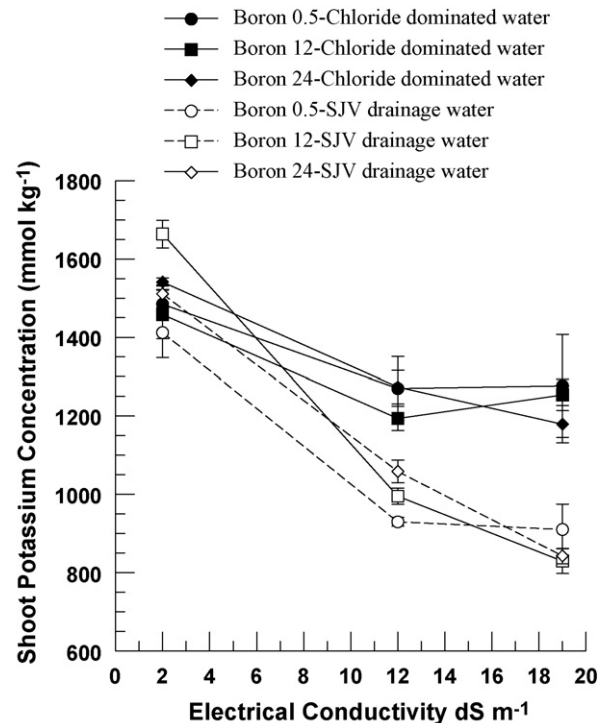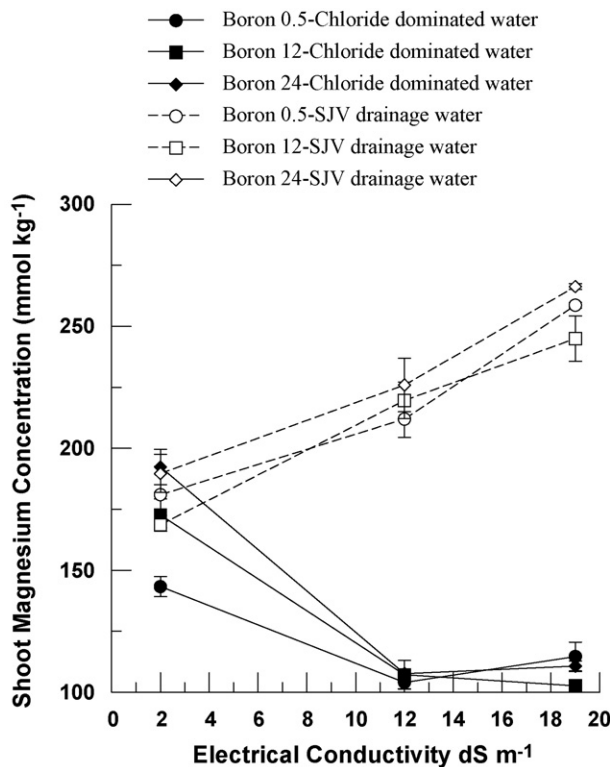

Fig. 6. Shoot calcium concentrations (mid-season sampling, MSS) as influenced by chloride or sulfate salinity and substrate boron concentration.

half in the sulfate-dominated treatments. This result was probably the result of the higher Na concentrations in the SJV waters compared to the Cl-dominated system. Regardless of salt composition, there was no significant difference in shoot K concentration among treatments with different substrate boron concentrations. In no case, however was tissue concentrations critically low as to cause a potassium deficiency.

Shoot Mg concentrations were also affected by both salinity and salt composition treatment (Fig. 5). In sulfate-dominated treatments, increased salinity in concert with increasing substrate Mg, increased shoot Mg in a linear manner. Substrate boron did not influence this relationship. However, in the Cl-dominated treatments, increased salinity decreased shoot Mg but not to tissue levels that would be considered deficient. Substrate boron did not have a significant effect on salt-stressed plants but at low salinity, as boron increased to 12 or 24 mg L^{-1}, shoot Mg increased over 20%.

Shoot Ca concentrations (Fig. 6) were lower in plants grown in iso-electric solutions that were sulfate-dominated as compared to those that were in Cl-dominated solutions. In those plants, increased salinity decreased shoot Ca, a response routinely observed in other cruciferous vegetable crops irrigated with SJV waters (Grieve et al., 2001). Interestingly at high salinity, shoot Ca concentration was actually higher when substrate B was increased to 24 mg L^{-1}. In the Cl-dominated treatments, increased salinity did not have much effect on shoot Ca.

Ion distribution within the broccoli plants is beyond the scope of this paper. A detailed analyses of ion distribution in broccoli under these abiotic stresses will be described in different paper (Grieve et al., unpublished data) highlighting that broccoli is characteristic of a B-immobile plant. Boron-immobile plants do not readily re-mobilize boron in the phloem once it is accumulated in the leaf, therefore concentrations of boron are highest in older leaves, and particularly leaf margins, and become less in younger tissue (Hu and Brown, 1997).
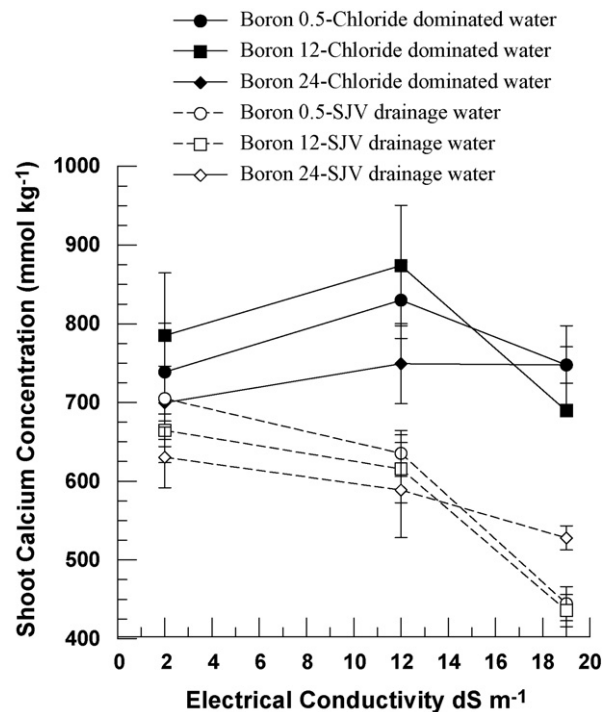
Comparison of broccoli total shoot ion concentrations between the two sample dates of April 24 (or mid-season sample (MSS)) and May 20, 2003 (or late-season sample (LSS)) yielded similar trends on the subject of ion shoot concentrations.

Shoot boron concentration increased with increasing substrate boron at MSS (Fig. 2) and LSS (Table 2), regardless of whether it was Cl-dominated or had a composition characteristic of California's SJV. Tissue boron concentrations at the low substrate boron concentration remained similar between the two sample dates but the 12 and 24 mg kg^{-1} boron treatments were higher in the LSS. Increasing soil solution salinity decreased shoot boron concentrations with the exceptions of low boron (0.5 mg L^{-1}) for both salt types and at both sample dates, where there were consistent significant increases between the 2 and 19 dS m^{-1} levels. For the SJV-treated plants at low boron, an increase in salinity actually increased boron.

The MSS and LSS displayed chloride concentrations that were much higher in plants from chloride-dominated treatments than those in sulfate-dominated treatments. In both sulfate and Cl-dominated treatments, LSS shoot Cl increased with increased salinity to 12 dS m^{-1}. Further increases in salinity did not increase shoot Cl further. Interestingly, as boron increased at low salinity levels at the MSS, tissue Cl decreased, regardless of salt type. However, the LSS shoot chlorides increased with increasing substrate boron in the chloride-dominated conditions and remained stable in the sulfate salt conditions. This interactive effect with chloride and boron was discussed by Yermiyahu et al. (2008). LSS total shoot chloride levels increased with increasing salinity and were greater in concentration within the shoots of the chloride salt than the sulfate salt conditions by approximately 25–100%.

Shoot calcium concentrations from the late-season sample were significantly higher in plants treated with chloride-based salinity than those treated with the SJV-dominated salts at 12 and 19 dS m^{-1} salinity levels but not in the low salinity condition of 2 dS m^{-1} (statistics not shown). This was not surprising based on the differences in salt compositions (see Table 1). LSS shoot

Table 2
Ion concentrations in whole broccoli shoots sampled May 20, 2003 (LSS). Means with the same upper case letter within columns indicate no significant ($P > 0.05$) salinity difference at a particular boron concentration. Similarly, means with same lower case letters across rows are not statistically significant ($P > 0.05$) for boron at a particular salinity level. These statistics do not compare salt types.

ECw (dS m^{-1})	B 0.5 mg L^{-1} chloride	B 12 mg L^{-1} chloride	B 24 mg L^{-1} chloride	B 0.5 mg L^{-1} sulfate	B 12 mg L^{-1} sulfate	B 24 mg L^{-1} sulfate
Ca (mmol kg^{-1} dry wt)						
2	435 A a	501 A a	481 A a	474 A a	484 A a	511 A a
12	640 B a	620 B a	598 B a	513 A a	460 A a	512 A a
19	663 B a	652 B a	584 B b	425 A a	398 B a	384 B a
Mg (mmol kg^{-1} dry wt)						
2	106 A a	130 A b	152 A c	141 A a	139 A a	188 A b
12	89 B a	97 B b	95 B ab	181 B a	189 B a	199 A a
19	94 B a	93 B a	85 C b	236 C a	240 C a	232 B a
Na (mmol kg^{-1} dry wt)						
2	123 A a	132 A a	135 A a	132 A b	111 A a	182 A c
12	280 B a	316 B ab	348 B b	464 B a	474 B a	565 B a
19	343 C a	407 C b	426 C b	653 C ab	613 C a	688 C b
K (mmol kg^{-1} dry wt)						
2	1241 A a	1297 A a	1295 A a	1316 A a	1369 A a	1342 A a
12	1216 A a	1255 A ab	1349 A b	1028 B a	1059 B a	1051 B a
19	1050 B a	1111 B a	1061 B a	972 B a	963 C a	884 C b
P (mmol kg^{-1} dry wt)						
2	144 A a	136 A a	149 A a	145 A a	169 A a	139 A a
12	109 B ab	111 B a	104 B b	118 C ab	125 B a	112 B b
19	107 B a	111 B a	101 B a	130 B a	112 C b	138 A a
S (mmol kg^{-1} dry wt)						
2	331 A a	411 A b	440 A b	371 A a	407 A b	430 A b
12	311 AB a	335 B b	338 B b	439 B a	432 A a	458 A a
19	294 B ab	311 B a	286 C b	408 C a	421 A a	429 A a
Cl (mmol kg^{-1} dry wt)						
2	494 A a	691 A b	652 A b	392 A a	413 A a	428 A a
12	789 B a	969 B b	1058 B b	531 B a	486 B a	502 B a
19	885 B a	1133 C b	1099 B b	556 B a	396 A b	526 B a
B (mg kg^{-1} dry wt)						
2	50 A a	403 A b	526 A c	67 A a	394 A b	586 A c
12	53 A a	214 C b	456 B c	114 B a	189 B b	392 B c
19	53 A a	282 B b	441 B c	100 B a	174 B b	288 B c

calcium levels were also lower than the MSS levels for both salt types by approximately 20% but showed slight shoot calcium concentration increases with increased salinity levels (Table 2).

Shoot magnesium concentration was affected by the composition of the salt solution. Magnesium concentrations were higher in plants treated with SJV water compositions than they were in plants treated with chloride-dominated waters. LSS shoot magnesium concentrations were considerably lower than calcium concentrations and increased with increasing sulfate salinity but decreased with increased chloride salinity. This same pattern was observed with whole shoots at the earlier sampling.

Increasing salinity of both salt composition treatments substantially increased sodium concentration in broccoli shoots. Sodium levels were higher in shoots treated with sulfate salinity than chloride solutions. This is not too surprising since the sodium concentration was higher in the SJV treatment compared to the Cl-dominated treatment at corresponding salinity levels. Also, increased substrate boron inconsistently increased shoot Na concentrations in plants in both salt types and all salinity levels.

Total shoot K concentration in broccoli was substantially higher than any other cation. Potassium accumulation in shoots sampled late-season was greater in plants treated with chloride-dominated than sulfate-dominated salinity. However, this relationship was limited to moderate and high salinity in LSS shoots and not significant for the low salinity condition of 2 dS m^{-1}. Increasing salinity decreased LSS shoot potassium concentration in both salt types. This result is consistent with other studies (Grattan and Grieve, 1999) where increasing sodium reduced potassium uptake.

Potassium concentration in broccoli shoots were unaffected by changes in solution boron concentration.

Shoot S concentrations in SJV salt-treated plants were generally higher than those treated with Cl salts, particularly at moderate and high salinity levels. In Cl-dominated treatments, increased salinity reduced shoot S concentration. Conversely, in the SJV treatments and at low substrate boron, increased salinity increased shoot S concentration.

The data indicate that this salinity increased reduction of shoot boron concentration when substrate boron is high (12 mg L^{-1}) and extremely high (24 mg L^{-1}) is the primary explanation of why increased boron in the presence of salinity is less damaging on growth and yield than the combined effects of salinity and boron (see paper I in this series). In a recent review by Takano et al. (2008) they describe two kinds of boron transporters in the root cell membranes: (1) a boric acid channel important for boron uptake and (2) a boric acid/borate exporter that is considered to be involved with boron transport into the xylem of the root that partially controls the amount of B transported to the shoot. Reid (2007) concluded that these boron exporters regulate tissue boron concentrations and are key factors in determining B tolerance in plants.

It is uncertain exactly why excess boron is toxic to plants but high tissue concentrations of boron could disrupt metabolism by binding to ribose moieties of ATP, NADPH or NADH in cells (Takano et al., 2008). However, tissue boron concentration in itself appears to be a poor indicator of boron toxicity (Nable et al., 1997; Wimmer et al., 2003). Therefore there may be more complex interactions

Table 3
Percent evaporation and transpiration, cumulative transpiration volumes, change in delta $^{18}O/^{16}O$ between initial and final measurements in each of the salinity and boron treatment solutions. % Evap = change in delta ($^{18}O/^{16}O/L$) treatment/change in delta ($^{18}O/^{16}O/L$) evaporation × 100.

Salt type	ECw (dS m^{-1})	Boron (mg L^{-1})	Change in delta $^{18}O/^{16}O$	Water use (L)	Change in delta $^{18}O/^{16}O/L$	Evaporation (%)	Transpiration (%)
Cl$^-$	2	0.5	−0.13	76.4	−0.0017	5	95
Cl$^-$	12	0.5	−0.18	53.2	−0.0034	9	91
Cl$^-$	19	0.5	−0.12	39.9	−0.0030	8	92
Cl$^-$	2	12	−0.06	59.8	−0.0010	3	97
Cl$^-$	12	12	−0.18	46.5	−0.0038	10	90
Cl$^-$	19	12	−0.11	33.2	−0.0033	8	92
Cl$^-$	2	24	−0.09	53.2	−0.0017	5	95
Cl$^-$	12	24	−0.12	46.5	−0.0026	7	93
Cl$^-$	19	24	−0.11	36.6	−0.0029	8	92
SO$_4$$^{2-}$	2	0.5	−0.13	73.1	−0.0018	5	95
SO$_4$$^{2-}$	12	0.5	−0.13	59.8	−0.0022	6	94
SO$_4$$^{2-}$	19	0.5	−0.10	39.9	−0.0025	7	93
SO$_4$$^{2-}$	2	12	−0.10	59.8	−0.0017	5	95
SO$_4$$^{2-}$	12	12	−0.14	49.9	−0.0028	8	92
SO$_4$$^{2-}$	19	12	−0.14	39.9	−0.0035	9	91
SO$_4$$^{2-}$	2	24	−0.12	46.5	−0.0026	7	93
SO$_4$$^{2-}$	12	24	−0.11	49.9	−0.0022	6	94
SO$_4$$^{2-}$	19	24	−0.09	36.6	−0.0025	7	93
Evaporation sand tank			−0.61	16.6	−0.0367	100	0

that occur at the biochemical level that prevent or promote toxic effects.

In addition to reduced tissue B concentration, there may be some interactions with certain nutrients such as Ca that may be playing an important role. For example increases SJV salinity reduced shoot Ca concentration and since this ion is known to have low mobility in the plant (Marschner, 1995), deficiencies may be expressed particularly in newly developing tissues.

3.2. Is boron uptake and accumulation exclusively passive?

Although the mechanism of boron uptake by plants is not fully understood, it was once believed that the passive diffusion of boric acid [B(OH)$_3$] across the lipid bilayer is the primary and perhaps only mechanism where boron passes across root–cell membrane (Hu and Brown, 1997). Moreover, the transport of boron from the root to the shoot was believed to be a passive process via mass flow within the transpiration stream (Marschner, 1995; Nable et al., 1990; Yu and Bell, 1998). However, more recent reviews (Brown et al., 2002; Dannel et al., 2002; Läuchli, 2002; Takano et al., 2008) suggest that boron uptake is more complicated and can be regulated using a combination of mechanisms including: (1) passive diffusion across the lipid bilayer; (2) facilitated transport through major intrinsic proteins; (3) active transport through a high affinity uptake system. The later mechanism only occurs when boron concentrations in the soil solution are low (Dannel et al., 2002). In addition, there has also been evidence of a B(OH)$_3$/B(OH)$_4$$^-$ exporter that mediates loading of boron into the xylem (Takano et al., 2008).

Using the stable isotope technique described above, we were able to separate the fraction of water lost from the frequently irrigated sand tank system as evaporation versus that lost as transpiration. Regardless of treatment, evaporation accounted for only 2.5–10% of the cumulative ET (Table 3). This observation is consistent with claims by others (Allen et al., 1998) suggesting that under full or near full canopy cover, over 90% of the water lost as ET from the soil–water system is from transpiration. Evaporation for the low salinity treatments averaged 4.7% as compared to moderate and high salinity with evaporation of 7.5%. This 60% increase in evaporation was consistent with experimental observations of smaller plant size, leaf area, and greater sand-surface exposure. Evaporation rates were not influenced by boron concentration or salt type and both were similar to the overall evaporation average of about 7%.

In our analyses, we compare boron content in the shoot (mg B shoot^{-1}) in relation to what the boron content should be if boron was absorbed and transported passively in the transpiration stream. We assumed that the B concentration in the storage reservoir was equivalent to that in the soil water, which is a good assumption for coarse sand that is irrigated several times per day. We then compared those estimates to the actual shoot boron content. The total shoot boron content (mg shoot^{-1}) was determined by summing the dry biomass of particular shoot organs times their respective boron concentrations (mg kg^{-1} dry wt). A summary of the analysis is shown in Table 4.

Evaluation of the data reveals several interesting relationships. If the actual/passive ratio (far right hand column) is equal to 1.0, this suggests that passive uptake and transport processes alone account for the boron that accumulated in broccoli shoots. If the ratio is greater than one, this would suggest some energy-dependent or active process is also occurring. If the ratio is less than one, this would suggest that some restrictive uptake and/or transport process is occurring.

Data from the low boron–low salinity treatments indicate that the plant actually accumulated 30–50% more boron than that estimated based on transpiration–concentration passive boron accumulation. This would indicate that some energy-dependent boron uptake/transport process is occurring. Data from two salinity treatments at low boron concentration suggest that the plant is accumulating 2–3 times more boron than can be accounted for by simple passive processes. Evidence of an active boron uptake process has been presented by other researchers particularly when solution boron concentrations are low (Dannel et al., 2002; Pfeffer et al., 1999; Takano et al., 2008). Therefore it is possible that when solution boron concentrations are low, the plant has some energy-dependent mechanism that is activated allowing the plant to accumulate boron against a concentration gradient. On the other hand, there are two other salinity treatments at low boron concentration that suggest the shoot accumulation is primarily a passive process.

Conversely, plants grown under high boron treatments have actually accumulated 6–80 times less boron than that predicted based on transpiration–concentration passive uptake. This suggests that the plant has a restricted boron accumulation mechanism under very high solution boron concentrations. A similar response was seen with rice (Yu and Bell, 1998) where a 10-fold difference between the predicted mass flow boron uptake and

Table 4
Broccoli cumulative transpiration volumes, transpiration-based predicted boron uptake, and actual boron uptake with salt solution types, salinity and boron treatment solutions. Transpiration losses per plant are time weighted based on the changing plant density in the tank.

Salt type	ECw (dS m^{-1})	Boron (mg L^{-1})	Transpiration (L plant^{-1})	Transpiration-based boron uptake (mg plant^{-1})	Actual boron uptake (mg plant^{-1})	Actual to passive ratio
Cl$^-$	2	0.5	24.9	12.5	16.2	1.3
Cl$^-$	12	0.5	20.1	10.1	32.7	3.2
Cl$^-$	19	0.5	15.1	7.5	6.1	0.8
Cl$^-$	2	12	25.4	305.2	17.2	0.06
Cl$^-$	12	12	17.5	210.5	12.8	0.06
Cl$^-$	19	12	14.1	168.7	5.4	0.03
Cl$^-$	2	24	17.4	417.4	23.1	0.06
Cl$^-$	12	24	17.5	421.0	12.7	0.03
Cl$^-$	19	24	13.3	318.5	14.6	0.05
SO$_4^{2-}$	2	0.5	26.8	13.4	20.7	1.5
SO$_4^{2-}$	12	0.5	21.6	10.8	10.5	0.97
SO$_4^{2-}$	19	0.5	15.6	7.8	18.9	2.4
SO$_4^{2-}$	2	12	21.4	256.9	22.3	0.09
SO$_4^{2-}$	12	12	17.8	214.0	33.9	0.16
SO$_4^{2-}$	19	12	14.3	172.0	17.6	0.10
SO$_4^{2-}$	2	24	16.8	402.9	4.6	0.01
SO$_4^{2-}$	12	24	18.3	439.8	11.2	0.03
SO$_4^{2-}$	19	24	15.1	362.8	49.5	0.14

actual boron uptake occurred. Although root tissue was not measured, it is unlikely that the faction of 'unaccounted for' boron would be all sequestered in that organ. For example if the root biomass was 50% of the shoot mass, the accumulated boron in the roots would have to be 12–160 times greater to account for the difference between predicted and actual accumulated boron. Boron concentration in roots are typically lower than those found in shoots (Kaur et al., 2006).

Rather it is more likely the plant has a mechanism, yet not fully identified, that regulates against excessive boron accumulation under high solution boron concentrations. The mechanism may be an energy-dependent pumping process against the boron concentration gradient generating a net efflux of boron (Reid, 2007). Another metabolic regulating mechanism that may explain reduced boron uptake amid high soil-boron concentration involves known boron exporters in the pericycle that are responsible for regulation and loading of boron into the xylem as part of the transpiration stream distribution within the plant. Takano et al. (2005) identified a transport mechanism which catalyzes the loading of boron from parenchyma tissue into xylem tissue in roots that was expressed in toxic boron conditions and was also degraded in high boron levels. These different B transporters in cells may explain the differences in shoot boron accumulation found in this study. Plants sense and respond to different boron concentrations in the soil solution regulating transporter expression (Takano et al., 2008). It is possible that under low boron supply, the boric acid channel is stimulated allowing B transport against the concentration gradient while at high external boron concentrations, the B exporter is stimulated, reducing the amount of B accumulated by the plant.

Non-metabolic processes may also be occurring. For example Hu and Brown (1997) suggested that B uptake is controlled largely by the formation of non-exchangeable boron complexes in cell water and cytoplasm. A remaining possible explanation of the restrictive boron uptake and transport process exists and it may be a barrier to boron transport, operating at a still unknown location within the plant.

There were no statistically significant interactions of salinity level, boron content (mg plant^{-1}) or salt type with respect to actual boron uptake in the broccoli plants. Boron contents were statistically significant with respect to effects on the passive to actual ratio. Therefore our data indicate that boron uptake is not a simple passive process but broccoli has some energy-dependent mechanism at low boron and a restrictive mechanism at higher

concentrations. These mechanisms have yet to be identified but should be studied more closely in future work.

4. Conclusions

The shoot and head biomass of broccoli plants were reduced by increased boron but proportionately less under saline conditions (Smith et al., this issue). This occurred whether the salinizing solution was dominated by Cl or sulfate salts. Under high and very high substrate boron concentrations, the shoot boron concentration was also reduced by increased salinity suggesting that this may be a factor why the combined effects from salt and boron stresses were not additive and that boron injury is less than when salinity is present.

In addition, this study also indicates that transpiration accounts for the vast majority (>90%) of the ET and boron uptake and accumulation in the shoot is not a simple passive process. Boron accumulation at low B was more than accounted for by passive process suggesting some active uptake process is operating at these low concentrations. At high and very high B concentrations, the opposite occurs suggesting there is a restrictive B uptake mechanism operating. These mechanisms operate in the presence or absence of salinity. It is likely that substrate B concentration controls transporters/exporters at the membrane level. In addition to these mechanisms, passive B uptake may also be occurring and could account for the major pathway under adequate and not excessive substrate boron concentrations.

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