Gas Exchange and Chlorophyll Content of 'Tifblue' Rabbiteye and 'Sharpblue' Southern Highbush Blueberry Exposed to Salinity and Supplemental Calcium

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Additional index words. mineral nutrition, salt stress, salinity, photosynthesis, transpiration, stomatal conductance, *Vaccinium ashei*, *Vaccinium corymbosum*

Abstract. 'Tifblue' rabbiteye blueberry (*Vaccinium ashei* Reade) and 'Sharpblue' southern highbush blueberry (primarily *V. corymbosum*) were treated with 0, 25, or 100 mM Na⁺ as Na₂SO₄ or NaCl, and 0, 1, 3, or 10 mM supplemental Ca²⁺ in sand culture in the greenhouse. Greatest stomatal conductance (g) and net assimilation (A) occurred in unsalinized 'Tifblue' plants not given additional Ca²⁺. Stomatal conductance, A, transpiration (E), and xylem water potential (Ψ_w) of 'Tifblue' and 'Sharpblue' plants were all lowered as salinity increased, and these effects were more pronounced with NaCl than with Na₂SO₄. After 63 days, for plants given 100 mM Na⁺ as NaCl, g and net assimilation rate were reduced to only 10% of the unsalinized controls, while for plants salinized with 100 mM Na⁺ as Na₂SO₄, g and A were 35% and 43%, respectively, of unsalinized controls. Leaf necrosis was more extensive on 'Sharpblue' plants given NaCl than on 'Tifblue' plants. Neither Ca²⁺ nor Na⁺ treatments led to severe chlorosis; reductions in leaf chlorophyll content were mainly due to necrosis. The Na⁺ induced reduction in gas exchange was associated with negative Ψ_w , Ca²⁺ deficiency, or a combination of these factors. Additional factors leading to inhibition of gas exchange in NaCl stressed plants include Cl toxicity and leaf necrosis. Calcium supplements were unable to ameliorate NaCl damage in 'Tifblue' or 'Sharpblue' plants, possibly because of the inability of Ca²⁺ to counter Cl entry and toxicity. In contrast, additional Ca²⁺ improved g, A, Ψ_w , and leaf chlorophyll content of 'Tifblue' plants that received Na₂SO₄. For plants treated with 25 mM Na⁺ as Na₂SO₄ and 1 mM Ca²⁺, g was 1.5 to 2.5 times higher than in plants without added Ca²⁺. Low (1 mM) concentrations of Ca²⁺ were more effective in ameliorating the effects of 100 mM Na⁺ as Na₂SO₄ than were 3 or 10 mM Ca²⁺ supplements, possibly because higher Ca²⁺ concentrations damaged the metabolism of the calcifuge blueberry.

Blueberries require high quality irrigation water to thrive. Poor ground water quality has impeded blueberry expansion in the southeastern United States, where sufficient good quality water may not be available throughout the season. Irrigation water in eastern Texas often exceeds 15 mM Na⁺, 6 mM Cl⁻, and 10 mM HCO₃⁻ (Texas Dept. of Health, 1990). Water pH levels >7.0, total bicarbonates (HCO₃⁻) >5 mM, total Na⁺ >2.0 mM, and total Cl⁻ >4.0 mM indicate poor quality water (Haby and Pennington, 1988).

Salinity can adversely affect plant water relations and growth because it leads to increasingly negative water potential (Ψ_w) of xylem sap. Salinity can also adversely affect plant growth and metabolism because of the accumulation of potentially toxic concentrations of Na⁺ and Cl⁻ in the leaf mesophyll of salt-sensitive

plants (Greenway and Munns, 1978). When salt sensitive plants are exposed to high levels of salt, particularly under conditions of poor root aeration, energy dependent processes that normally exclude Na⁺ and Cl⁻ from the xylem fail, and these ions are transported to the shoot (Drew and Dikunwin, 1985; Drew and Läuchli, 1985).

The damaging effects of poor quality water on growth of rabbiteye blueberry have been reported to be due to high concentrations of Na⁺ (Haby et al., 1986). Field studies showed that weight gain of plants irrigated with well water containing 7.83 mM Na⁺ was only 65% of that of plants irrigated with surface water containing 0.23 mM Na⁺ (Haby et al., 1986). Similarly, Bush et al. (1990) compared plants treated with pond and saline well water. After 5 months, 39% of the plants irrigated with well water died, and after 12 months, the mortality was 60%. For 'Tifblue' plants treated with 100 mM NaCl, shoot dry weight was only 57% of that of unsalinized controls (Wright et al., 1992). Additionally, growth of southern highbush blueberries appears to be just as adversely affected by NaCl as does that of rabbiteye blueberries (Wright et al., 1992).

Salinity stress also causes stomatal closure and reduced gas exchange (Dinkelberg and Liidders, 1990; Downton et al., 1990;

Received for publication 20 Apr. 1992. Accepted for publication 3 Nov. 1992. Contribution from the Texas Agricultural Experiment Station (Paper no. TA30664) and supported in part by the Expanded Research Area Fund of the Experiment Station. We would like to thank Fincastle Nursery, La Rue, Texas, and Finch Nursery, Bailey, N.C., for the generous donation of the plants used in this study. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Abbreviations: A, net assimilation; E, transpiration; g, stomatal conductance; PPFD, photosynthetic photon flux density; SAR, sodium adsorption ratio; Ψ_w , xylem water potential.

Drew et al., 1990; Seemann and Critchley, 1985). Salinity inhibits photosynthesis activity (Downton et al., 1990; Drew et al., 1990; Gupta and Berkowitz, 1987), possibly by reducing the activity of ribulose-1,5-bisphosphate carboxylase (Bongi and Loreto, 1989; Seemann and Critchley, 1985; Ziska et al., 1990). Reduced gas exchange may also be due to the lack of chlorophyll synthesis, as we have observed that field-grown plants show severe interveinal chlorosis when subjected to saline well water for long periods.

The benefits of Ca^{2+} application to salt-stressed plants is widely recognized. Improved shoot and/or root growth, as a result of Ca^{2+} application, occurred in bean *Phaseolus vulgaris* L. (LaHaye and Epstein, 1971), cotton *Gossypium hirsutum* L. (Cramer et al., 1988), maize *Zea mays* L. (Maas and Grieve, 1987), and *Citrus sinensis* (L.) Osb. (Ben-Hayyim and Kochba, 1982). Although reports of $\text{Na}^+/\text{Ca}^{2+}$ interactions affecting A are rare, there is evidence that Na^+ can compete with Ca^{2+} for the binding site on the O_2 evolution reaction center in photosystem II (Waggoner et al., 1989).

Plants that have low leaf Ca^{2+} concentrations and thrive in soils low in Ca^{2+} , such as *Vaccinium* species, are regarded as calcifuges (Hope-Simpson, 1938). Despite some research indicating that high soil Ca^{2+} is associated with poor blueberry growth and vigor (Austin et al., 1986; Ballinger et al., 1958), supplemental Ca^{2+} applications were found to improve the growth of 'Tifblue' and 'Brightwell' rabbiteye blueberries under medium and high Na_2SO_4 salinity (Wright et al., 1992). Therefore, the question arises as to whether Ca^{2+} application might improve growth of salinized blueberry by maintaining gas exchange and chlorophyll content.

The following experiments were designed to determine the effect of Na_2SO_4 and NaCl on gas exchange and chlorophyll content of rabbiteye and southern highbush blueberry plants. Sodium sulfate was applied to provide insight into the specific effect of Na^+ , since SO_4^{2-} is absorbed slowly by plants (Marschner, 1986) and is not known to damage blueberries. Salinization with NaCl was an alternative treatment designed to help understand the additional effect of Cl^- on gas exchange and chlorophyll content. Finally, plants were provided with supplemental Ca^{2+} to determine whether it exerted a protective effect on salinized blueberry plants.

Materials and Methods

Na_2SO_4 experiment. One-year-old, dormant 'Tifblue' rabbiteye blueberries were potted in sand and placed in a greenhouse on 15 Mar. 1988. Watering was by drip irrigation. Details of potting method and pretreatment irrigation were reported in Wright et al. (1992).

Treatments on 72 plants began 60 days later and consisted of 0, 25, or 100 mM Na^+ (0, 12.5, or 50 mM Na_2SO_4), arranged factorially with 0, 1, 3, or 10 mM Ca^{2+} , and applied with the remaining essential elements in the nutrient solution. Because of the insolubility of CaSO_4 , only 70% of each Ca^{2+} treatment was CaSO_4 ; the remainder was made up of 20% $\text{Ca}(\text{NO}_3)_2$ and 10% CaCl_2 . All treatments contained equal amounts of NO_3^- , NH_4^+ , and K^+ . Plants received ≈ 400 or 500 ml of experimental solution daily by drip irrigation; this was sufficient for some solution to drain from the containers. Solution pH and electrical conductivity were recorded when solutions were formulated, and each time they were replenished. These values, along with solution composition and sodium adsorption ratio (SAR), are found in Table 1.

Net assimilation, E, and g, were measured using an ADC portable system (Analytical Development Co., Hoddesdon, Herts., United Kingdom). Calculations used to obtain the above values were as described by von Caemmerer and Farquhar (1981) and

Parkinson and Allen (1985). Plants were transferred, within ≈ 3 min, from the greenhouse to a nearby growth chamber, and gas exchange measurements were made immediately. All measurements were made between 1000 and 1500 HR CDT on the most recent fully expanded leaves. One leaf was measured on each of the 72 plants; thus six leaves were measured per treatment.

The ADC equipment allowed for continuous measurements on the target leaf, which was inserted into a 620 mm² leaf cuvette. Preliminary measurements indicated that the light saturation point for blueberry was at a photosynthetic photon flux density (PPFD) of $\approx 700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; therefore, all subsequent measurements were taken at PPFD above the light saturation point, usually above 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

A photo incandescent flood lamp provided supplementary light when necessary. Air was drawn from outside the growth chamber and supplied to the leaf cuvette at $\approx 300 \text{ ml}\cdot\text{min}^{-1}$. Air temperature within the growth chamber was at 27°C, and relative humidity was maintained at $\approx 50\%$. Leaf temperature ranged from 22 to 32°C. External CO_2 applied to the leaf was $\approx 340 \mu\text{bar}\cdot\text{bar}^{-1}$ (partial pressure). Leaves chosen for measurement were allowed to stabilize for 3 min before measurements were taken. Gas exchange was measured 21, 35, 49, and 63 days following initiation of Na_2SO_4 and Ca^{2+} treatments.

Predawn Ψ was measured 62 days from the start of salt treatments, between 0100 and 0600 HR, using a pressure chamber (Soil Moisture Corp., Santa Barbara, Calif.). Ψ_w was determined on two fully expanded leaves to give two sub samples per plant.

Leaf chlorophyll was determined using a SPAD-501 portable chlorophyll meter (Minolta Corp., Ramsey, N.J.), which nondestructively estimates chlorophyll a and b concentration in relative units. Four leaves were measured per plant. Leaves with incremental chlorophyll levels (determined by SPAD-501 readings) were then harvested to construct a standard curve for quantification of chlorophyll content. Five 1.0 cm² disks were removed from each leaf. Chlorophyll was extracted using N, N-dimethyl-formamide, and chlorophyll concentration, and each disk was calculated from the equation developed by Moran (1982): Extractable chlorophyll ($\text{mg}\cdot\text{liter}^{-1}$) = $(20.27 \times A_{647}) + (7.04 \times A_{664})$, where A_{647} and A_{664} refer, respectively, to adsorbance at wavelengths of 647 and 664 nm measured in a spectrophotometer. Adsorbance of leaf extracts was measured using a Varian DMS-100 UV Visible Spectrophotometer (Varian Techtron, Mulgrave, Victoria, Australia). Chlorophyll concentration was expressed on a leaf weight basis, and the standard curve was calculated using the regression equation: Total chlorophyll ($\text{mg}\cdot\text{g}^{-1}$ fresh weight) = $-722.182 + (71.81 \times \text{SPAD reading})$ $R^2 = 0.85$.

The design was a randomized complete block. Experiments were 4 (Ca^{2+} levels) $\times$ 3 (Na^+ levels) factorial with six single-plant replications. Data were analyzed using the General Linear Model procedure (PROC GLM) of the Statistical Analysis Service (SAS) software package (SAS, Cary, N.C.).

NaCl experiment. Dormant, bare-root 'Tifblue' rabbiteye blueberries and 'Sharpblue' southern highbush blueberries were planted in sand on 15 Mar. 1989, placed in the greenhouse, and watered by drip irrigation. The potting method and pretreatment irrigation regime is reported in Wright et al. (1992).

Plants were maintained with the complete nutrient solution until 15 May 1989, when treatments commenced. All plants received NaCl , rather than Na_2SO_4 , as the Na^+ source in 1989, but otherwise the compositions of the 12 nutrient solutions were no different from those of 1988. Solution pH and electrical conductivity were again measured when the solutions were formulated and replenished (Table 1). 'Sharpblue' plants were only treated with

Table 1. Composition, sodium adsorption ratio (SAR), pH, and electrical conductivity of nutrient solutions.²

Total Na ⁺	Total Ca ²⁺	(mM)							pH ^x		E.C.	
		CaSO ₄	Ca(NO ₃) ₂	CaCl ₂	KNO ₃	(NH ₄) ₂ SO ₄	K ₂ SO ₄	SAR	Na ₂ SO ₄	NaCl	Na ₂ SO ₄	NaCl
											(dS·m ⁻¹)	
0	0	0.0	0.0	0.0	4.0	4.0	0.0	0.0	6.1	5.4	1.22	1.18
0	1	0.7	0.2	0.1	3.6	4.0	0.4	0.0	5.4	5.3	1.30	1.38
0	3	2.1	0.6	0.3	2.8	4.0	1.2	0.0	5.2	5.0	1.62	1.81
0	10	7.0	2.0	1.0	0.0	4.0	4.0	0.0	5.1	5.1	2.37	2.49
25	0	0.0	0.0	0.0	4.0	4.0	0.0	31.0	5.5	6.4	3.23	3.80
25	1	0.7	0.2	0.1	3.6	4.0	0.4	19.4	5.6	5.0	3.34	3.75
25	3	2.1	0.6	0.3	2.8	4.0	1.2	13.0	6.1	5.1	3.72	3.80
25	10	7.0	2.0	1.0	0.0	4.0	4.0	7.6	6.0	4.9	4.00	4.53
100	0	0.0	0.0	0.0	4.0	4.0	0.0	124.0	6.7	5.3	8.87	8.80
100	1	0.7	0.2	0.1	3.6	4.0	0.4	77.8	6.0	5.4	8.08	9.35
100	3	2.1	0.6	0.3	2.8	4.0	1.2	52.3	6.2	5.0	8.92	9.55
100	10	7.0	2.0	1.0	0.0	4.0	4.0	30.6	5.9	5.1	8.72	8.48

²Additionally, solutions contained 0.1 mM KH₂PO₄, 0.65 mM MgSO₄, 0.125 mM FeNa (O₂CCH₂)₂NCH₂CH₂N(CH₂CO₂)₂, 10.9 μM MnSO₄, 1.2 μM H₃BO₃, 0.2 μM CuSO₄, 0.076 μM ZnSO₄, and 15 μM (NH₄)₆Mo₇O₂₄. SAR = sodium adsorption ratio.

³Total Na⁺ as Na₂SO₄ or as NaCl.

^xpH and E.C. values are means of at least three replications.

those solutions containing 0 and 10 mM Ca²⁺ because sufficient plants were not available to test all Ca²⁺ levels.

Gas exchange and Ψ_w measurement protocol was the same as that of the 1988 experiment. Gas exchange was measured immediately before salt treatment (time = 0) and 7, 21, 35, 49, and 63 days after treatment. Xylem water potential was measured just 30 days after the salt treatments commenced because leaf necrosis on high NaCl treatments was becoming apparent.

Leaf chlorophyll concentration was again determined using the SPAD-501 portable chlorophyll meter as described above, and was expressed on a leaf weight basis.

The design and data analysis were the same as described previously. The experiment involving 'Sharpblue' was a 2 × 3 factorial with four replications for a total of 24 plants

Results

Blueberry cultivars responded differently to Na₂SO₄ and NaCl treatment, so that data could not be averaged over both cultivars. The principal effects of salinity on gas exchange are described below (Tables 2 to 5), and interactions between salt concentration and Ca²⁺ are given graphically (Figs. 1 to 6).

Na₂SO₄ experiment. Stomatal conductance (g_s) decreased linearly with increasing Na₂SO₄ concentrations in the external solution and with time of exposure to salinity (Table 2). The Ca²⁺ × Na⁺ treatment interaction also led to significant differences in g_s (Fig. 1). For unsalinized plants, g_s was not affected by Ca²⁺ treatment until day 49, when plants supplied with 10 mM Ca²⁺ had significantly lower g_s, and at 63 days when g_s of those same plants was halved. In contrast, with 25 mM Na⁺ as Na₂SO₄, Ca²⁺ addition did not reduce g_s. For plants treated with 100 mM Na⁺, g_s was 1.5 to 2.5 times higher with 1 mM Ca²⁺ addition, with somewhat smaller increases in g_s with 3 and 10 mM Ca²⁺, compared to plants not supplied with Ca²⁺.

Changes in leaf transpiration rate (E) in response to Na₂SO₄ and Ca²⁺ were very similar to the response of g_s (Table 2).

Net assimilation rate (A) was also reduced linearly as Na⁺ in the external solution increased (Table 2). For unsalinized plants, Ca²⁺ did not lead to improved assimilation (Fig. 2A). After 63 days, unsalinized plants treated with 10 mM Ca²⁺ exhibited a 50% drop in A compared to plants not receiving Ca²⁺. However, Ca treat-

ments were beneficial to salinized plants (Fig. 2B and 2C). With 12.5 mM Na⁺, plants receiving supplemental Ca²⁺ had significantly higher A after 35 days of treatment. For plants supplied with 50 mM Na⁺, A was usually significantly higher when plants were supplied with Ca²⁺. For example, after 35 days of treatment, A of plants supplied with 1 mM Ca²⁺ was 1.5 to 2 times higher than that of plants not treated with Ca²⁺.

Table 2. Influence of Na₂SO₄ and Ca²⁺ treatments on stomatal conductance (g_s), transpiration (E), and net assimilation (A) of 'Tifblue' rabbiteye blueberry plants.

Treatment (mM)		g _s (mmol·m ⁻² ·s ⁻¹)	E (mol·m ⁻² ·s ⁻¹)	A (μmol·m ⁻² ·s ⁻¹)
Na ⁺	Ca ²⁺			
0	0	0.213	4.96	9.00
0	1	0.187	4.56	8.47
0	3	0.207	4.73	8.79
0	10	0.154	3.95	7.67
25	0	0.148	3.90	6.87
25	1	0.165	4.25	8.16
25	3	0.180	4.53	7.65
25	10	0.184	4.61	8.95
100	0	0.066	2.05	3.80
100	1	0.125	3.57	6.55
100	3	0.090	2.93	6.14
100	10	0.103	3.13	6.68
Source ^c				
Ca ²⁺		NS	0.0441	0.0249
Linear		NS	NS	0.0822
Quadratic		NS	0.0451	NS
Na ⁺		0.0001	0.0001	0.0001
Linear		0.0001	0.0001	0.0001
Time ^b		0.0001	0.0001	0.0001
Linear		0.0001	0.0001	0.0593
Quadratic		0.0001	0.0001	0.0001
Na ⁺ × time		0.0011	0.0002	0.0092
Ca ²⁺ × Na ⁺		0.0029	0.0007	0.0088

²Probability of a significant F value, NS = nonsignificant. Both quadratic and cubic Na⁺ contrasts were nonsignificant.

³Days after salt application (treatment duration).

Fig. 1. Stomatal conductance of 'Tifblue' rabbiteye blueberries treated with Na_2SO_4 and Ca^{2+} . Vertical bars indicate SE.

Fig. 2. Net assimilation rate of 'Tifblue' rabbiteye blueberries treated with Na_2SO_4 and Ca^{2+} . Vertical bars indicate SE.

Xylem water potential (Ψ_w) also declined in response to increasing Na^+ in the external solution (Table 3). For unsalinized plants and those plants treated with 25 mM Na^+ as Na_2SO_4 , Ca^{2+} treatments did not improve Ψ_w . However, for plants treated with 100 mM Na^+ , Ψ_w was at least 15% higher when any level of Ca^{2+} was applied. Leaf chlorophyll concentration increased with time (Fig. 3). Calcium concentration did not consistently affect leaf chlorophyll concentration of unsalinized plants or plants treated with 25 mM Na^+ as Na_2SO_4 . However, for plants supplied with 100 mM Na^+ , Ca^{2+} supplementation led to visibly higher leaf chlorophyll concentrations after 42 days of treatment.

NaCl experiment. Stomatal conductance of 'Tifblue' plants decreased linearly with increasing concentrations and duration of Na^+ treatment (Table 4). After 63 days, g_s of 'Tifblue' plants subject to 100 mM Na^+ as NaCl was only 10% of unsalinized controls (Fig. 4). The g_s of unsalinized controls was unaffected by Ca^{2+} treatment. Similarly, Ca^{2+} had little apparent influence on g_s of plants subject to 25 mM Na^+ until day 49, after which time, plants supplied with 1 mM Ca^{2+} had significantly higher g_s than plants not given Ca^{2+} . For plants subjected to 100 mM Na^+ treatment, g_s decreased to a very low value (0.01 to 0.02 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) by day 7. At day 35 and 49, g_s was significantly higher in plants given additional Ca^{2+} , which paralleled a small rise in A.

Effects of NaCl salinity and Ca^{2+} on 'Tifblue' E were similar to the effects on g (Table 4).

Net assimilation of 'Tifblue' plants decreased linearly as the concentration of NaCl in the external solution increased (Table 4).

Table 3. Influence of Na_2SO_4 or NaCl and Ca^{2+} treatments on xylem water potential (Ψ_w) of rabbiteye and southern highbush blueberry plants.

Treatment (mM)	Na^+ Ca^{2+}	Ψ_w (MPa)		
		Na_2SO_4 'Tifblue'	NaCl 'Tifblue'	NaCl 'Sharpblue'
0	0	-0.226	-0.187	-0.190
0	1	-0.300	-0.178	---
0	3	-0.282	-0.178	---
0	10	-0.320	-0.190	-0.242
25	0	-0.365	-0.298	-0.360
25	1	-0.446	-0.236	---
25	3	-0.450	-0.171	---
25	10	-0.408	-0.241	-0.357
100	0	-0.611	-0.398	-0.543
100	1	-0.484	-0.416	---
100	3	-0.520	-0.385	---
100	10	-0.520	-0.371	-0.540
Source ^a				
Ca^{2+}		NS	0.0144	NS
Na^+		0.0001	0.0001	0.0001
Linear		0.0001	0.0001	0.0001
$\text{Ca}^{2+} \times \text{Na}^+$		0.0020	0.0207	NS

^aProbability of a significant F value, NS = nonsignificant. Linear Ca^{2+} was nonsignificant.

Fig. 3. Leaf chlorophyll concentration of 'Tifblue' rabbiteye blueberries treated with Na_2SO_4 and Ca^{2+} . Vertical bars indicate SE.

For plants treated with 100 mM Na^+ , A was only 10% to 20% of that of unsalinized controls after 63 days of treatment. There was only slight ameliorative effect of Ca^{2+} when NaCl was applied. For unsalinized 'Tifblue' plants, Ca^{2+} had no influence on A, while for plants receiving 25 mM NaCl, Ca^{2+} supplementation had improved net A only at 49 days after treatment initiation (Fig. 5). For plants treated with 100 mM NaCl, Ca^{2+} treatments did not improve A, which dropped within the first 7 days of treatment, and rose only slightly thereafter.

Xylem water potential (Ψ_w) of 'Tifblue' plants also decreased linearly with increasing NaCl (Table 3). For plants salinized with 100 mM Na^+ , Ψ_w decreased to about -0.39 MPa. Calcium treatments were most effective in plants salinized with 25 mM NaCl, where 3 mM Ca^{2+} led to a higher Ψ_w (-0.17 MPa) than in the controls (-0.29 MPa). Calcium treatments did not influence Ψ_w of plants under 0 or 100 mM NaCl salinity.

Leaf chlorophyll of 'Tifblue' plants was seldom influenced by Ca^{2+} and NaCl treatment (data not shown). For unsalinized plants, Ca^{2+} treatments had no consistent effect on leaf chlorophyll concentration. With 25 mM Na^+ as NaCl, the highest chlorophyll concentration was in the leaves of plants supplied with 3 mM Ca^{2+} , while at 100 mM NaCl salinity those that received 1 mM Ca^{2+} additions often had the most chlorophyll. In no case did we observe severe chlorosis resembling that which we have observed in field-grown blueberries irrigated with saline well water.

Stomatal conductance, A, and E of 'Sharpblue' southern high-bush plants were severely inhibited by NaCl (Table 5). Calcium

had no ameliorative effect of NaCl-induced reductions in gas exchange. In contrast, both Ca^{2+} and NaCl treatments had significant influences on leaf chlorophyll concentration (Fig. 6). For unsalinized controls, plants supplied with 10 mM Ca^{2+} had higher concentrations than plants without added Ca^{2+} . For plants supplied with 25 mM NaCl, Ca^{2+} treatment had little influence on leaf chlorophyll concentration. After 35 days of 100 mM NaCl salinity, plants given 10 mM Ca^{2+} had lower leaf chlorophyll concentrations, compared to plants given no additional Ca^{2+} . However, this reduction was more due to extensive leaf necrosis in the 10 mM Ca^{2+} treated plants than to leaf chlorosis.

Discussion

High (100 mM) Na^+ supplied as Na_2SO_4 or NaCl to 'Tifblue' or 'Sharpblue' blueberries reduced g_s (Tables 2, 4, and 5). After 21 days of 100 mM NaCl salinization, g_s decreased by 90%. In contrast, g_s of cucumber (*Cucumis sativus* L.) dropped by only $\approx 50\%$ after 28 days of 50 mM NaCl salinization (Drew et al., 1990). Also, Bongi and Loreto (1989) noted that g_s of olive (*Olea europaea* L.) dropped significantly in the presence of 250 mM NaCl (dilute seawater). In our study, reduction in g_s was also dependent on the Na^+ salt applied. For example, after 63 days, conductance of plants subject to 100 mM Na^+ as Na_2SO_4 was still 35% of that of unsalinized controls.

One factor leading to stomatal closure in blueberry under high Na^+ concentrations may be the lower Ψ_w (Table 3). Regardless of Na^+ source, Ψ_w decreased as Na^+ concentration in the external solution increased. A loss of leaf turgor could cause stomatal closure. In other studies, the rise in leaf ABA concentration and an associated closure of stomates has been well documented (Zeevart

Table 4. Influence of NaCl and Ca^{2+} treatments on stomatal conductance (g_s), transpiration (E), and net assimilation (A) of 'Tifblue' rabbiteye blueberry plants.

Treatment (mM)		g_s ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	E ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	A ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
Na^+	Ca^{2+}			
0	0	0.205	4.34	7.77
0	1	0.194	4.24	4.24
0	3	0.161	3.75	5.92
0	10	0.167	3.90	6.52
25	0	0.101	2.53	4.06
25	1	0.105	2.88	5.08
25	3	0.125	3.27	5.62
25	10	0.105	2.93	5.01
100	0	0.025	0.70	1.33
100	1	0.035	1.15	1.91
100	3	0.039	1.24	2.28
100	10	0.057	0.70	2.18
Source ²				
Ca^{2+}		NS	NS	NS
Na^+		0.0001	0.0001	0.0001
Linear		0.0001	0.0001	0.0001
Time ³		0.0001	0.0001	0.0001
Linear		NS	0.0001	0.0001
Quadratic		0.0001	0.0001	0.0001
$\text{Na}^+ \times \text{time}$		0.0001	0.0001	0.0001
$\text{Ca}^{2+} \times \text{Na}^+$		0.0001	0.0826	NS

²Probability of a significant F value, NS = nonsignificant.

³Days after salt application (treatment duration).

Fig. 4. Stomatal conductance of 'Tifblue' rabbiteye blueberries treated with NaCl and Ca^{2+} . Vertical bars indicate SE.

and Creelman, 1988). A further source of ABA to leaves may be the roots in contact with medium of low water potential (Zhang et al., 1987).

Like g_s , A also decreased as salt concentrations in the irrigation solution increased (Figs. 2 and 5). Stomatal closure often limits net A, but that is not always the case. With salinized cucumber (Drew et al., 1990), bell pepper (*Capsicum annuum* L.) (Bethke and Drew, 1992), rice (*Oryza sativa* L.) (Yeo et al., 1985), and *Phaseolus vulgaris* L. (Seemann and Critchley, 1985), partial closure of stomata did not restrict CO_2 entry into the leaf enough to reduce internal CO_2 concentration. Inhibition of A was attributed in all these cases to do damage to the photosynthetic apparatus of the chloroplasts, and such damage is possibly a major factor in the decreases in A found in our studies.

Calcium deficiency may also contribute to impaired net A under high salinity treatments. Leaf Ca^{2+} levels of plants treated with high levels of Na_2SO_4 and NaCl were 0.11% and 0.15%, respectively (Wright et al., unpublished data). These concentrations border on the deficient (Patten, 1988). Leaf Na^+ concentrations of these plants were 0.14% and 1.1%, respectively. Lynch and Läuchli (1985) suggest that high salinity reduces Ca^{2+} transport by interfering with active loading of Ca^{2+} into xylem vessels. Sodium would then compete with fewer Ca^{2+} ions for a binding site on the O_2 evolution reaction center in photosystem II (Waggoner et al., 1989).

Fig. 5. Net assimilation rate of 'Tifblue' rabbiteye blueberries treated with NaCl and Ca^{2+} . Vertical bars indicate SE.

Table 5. Influence of NaCl and Ca^{2+} treatments on stomatal conductance (g_s), transpiration (E), and net assimilation (A) of 'Sharpblue' southern highbush blueberry plants.

Treatment (mM)	g_s ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	E ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	A ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
Na^+ Ca^{2+}			
0 0	0.139	3.22	4.49
0 10	0.135	3.10	4.24
25 0	0.103	2.68	4.10
25 10	0.077	2.09	3.28
100 0	0.034	0.57	0.87
100 10	0.025	0.78	1.12
Source ²			
Ca^{2+}	NS	NS	NS
Na^+	0.0001	0.0001	0.0001
Linear	0.0001	0.0001	0.0001
Time ³	0.0003	0.0160	0.0248
Linear	0.0001	0.0368	NS
Quadratic	0.0707	0.0398	0.0267
$\text{Na}^+ \times \text{time}$	0.0001	NS	0.0510

²Probability of a significant F value, NS = nonsignificant. $\text{Ca}^{2+} \times \text{time}$ interaction, $\text{Ca}^{2+} \times \text{Na}^+$ interaction, and $\text{Ca}^{2+} \times \text{Na}^+ \times \text{time}$ interaction were not significant.

³Days after salt application (treatment duration).

As noted above with g_s , effects of NaCl salinity on A were often more damaging than were the effects of Na_2SO_4 ; assimilation of plants given 100 mM NaCl was only 28% of the A of unsalinized controls, while A of plants given 50 mM Na_2SO_4 was 69% of unsalinized controls. Calcium supplementation was usually less effective with NaCl than with Na_2SO_4 . This result suggests that the harmful consequences of NaCl salinity are due not only to the damaging action of Na^+ , as discussed previously, but also to the negative effects of Cl^- .

Many fruit plants are sensitive to high concentrations of Cl^- (Brown et al., 1953; Ehlig, 1965). Rabbit-eye and southern highbush blueberry growth appeared to be reduced by high concentrations of Cl^- (Wright et al., 1992). Net assimilation of grapevine (*Vitis vinifera* L.) was inhibited under high leaf Cl^- concentrations (Downton, 1977). Chloride-induced damage to RuBP carboxylase may be the primary cause of diminished A (Bongi and Loreto, 1989; Seemann and Critchley, 1985). Ziska et al. (1990) found that prolonged exposure to Cl^- led to reduced RuBP activity and RuBP carboxylase regeneration in plum (*Prunus salicina* Lindl.). It is likely that salinity-induced decreases in blueberry growth are due to damage to the photosynthetic mechanism.

In general, Ca^{2+} supplementation ameliorated gas exchange of only the salinized 'Tifblue' rabbiteye blueberry. Low Ca^{2+} treatments (1 mM) were most effective in plants treated with Na_2SO_4 . Blueberries are efficient Ca^{2+} accumulators, thus they may be damaged by high levels of Ca^{2+} . Calcium levels within the cytoplasm must remain low to avoid precipitation of inorganic P,

competition with Mg^{2+} , and/or uncontrolled activation or inactivation of enzyme systems (Marschner, 1986).

Reduced root Na^+ influx in the presence of adequate Ca^{2+} may account for some of the ameliorative effects of Ca^{2+} on cotton (Cramer et al., 1987). Further, LaHaye and Epstein (1971) reported that Ca^{2+} reduced Na^+ translocation from bean roots to shoots. Similar phenomena may occur in rabbiteye blueberries, leading to the improved A, E, and g_s found here. The inability of Ca^{2+} to counter NaCl-induced inhibition of gas exchange of 'Sharpblue' southern highbush blueberry (Table 5) indicates that this cultivar may be more sensitive to supplemental Ca^{2+} , or more sensitive to NaCl, and that the detrimental effects of Cl^- may mask any putative amelioration from Ca^{2+} of Na^+ uptake.

Diminished gas exchange could also be caused by the degradation of chlorophyll, but, in this study, there was little difference in leaf chlorophyll concentration due to Na^+ or Ca^{2+} treatments (Figs. 3 and 6). Although leaf chlorophyll concentration of 'Tifblue' plants subjected to 100 mM Na^+ was often less than that of plants subjected to 0 or 25 mM Na^+ , this decrease was due predominantly to leaf necrosis, rather than chlorosis. 'Sharpblue' also exhibited more extensive necrosis than did 'Tifblue' when subject to 25 and 100 mM NaCl, but little chlorosis appeared. Chlorosis never approached the severity found in field plants irrigated with saline water, suggesting that Na^+ and Cl^- are not responsible for such damage. Bicarbonates may lead to decreased Fe in the cell (Mengel and Geurtzen, 1986) and may be the cause of the chlorosis noted in the field.

In summary, 100 mM Na^+ treatments led to decreases in g_s , A, and E. NaCl salinity led to greater reductions in gas exchange than did Na_2SO_4 salinity, presumably due to the additional effects of Cl^- . Low (1 mM) Ca^{2+} supplements were often able to ameliorate the salinity damage, but Ca^{2+} was more beneficial when applied to plants in which salinity was induced with Na_2SO_4 than with NaCl. Leaf chlorophyll concentration was not greatly diminished by Na^+ or improved by Ca^{2+} ; decreases were due more to leaf necrosis than to leaf chlorosis. The cultivar 'Sharpblue' was more sensitive to NaCl salinity than was 'Tifblue'.

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Fig. 6. Leaf chlorophyll concentration of 'Sharpblue' southern highbush blueberries treated NaCl and Ca^{2+} . Vertical bars indicate SE.

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