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

Glenn C. Wright
Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133

Kim D. Patten
Texas A&M University Agricultural Research and Extension Center at Overton, P.O. Drawer E, Overton, TX 75684

Malcolm C. Drew
Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133

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 NaSO₄ or NaCl, and 0, 1, 3, or 10 mM supplemental Ca in sand culture in the greenhouse. Greatest stomatal conductance (gₛ) and net assimilation (A) occurred in unsalinized ‘Tifblue’ plants not given additional Ca. Stomatal conductance, A, transpiration (E), and xylem water potential (Ψₛ) of ‘Tifblue’ and ‘Sharpblue’ plants were all lowered as salinity increased, and these effects were more pronounced with NaCl than with NaSO₄. After 63 days, for plants given 100 mM Na as NaCl, gₛ and net assimilation rate were reduced to only 10% of the unsalinized controls, while for plants salinized with 100 mM Na as NaSO₄, gₛ 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 Ca 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 Ψₛ, 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 gₛ, A, Ψₛ, and leaf chlorophyll content of ‘Tifblue’ plants that received NaSO₄. For plants treated with 25 mM Na as NaSO₄ and 1 mM Ca²⁺, gₛ 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 NaSO₄ 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 (Ψₛ) 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 Dikumwin, 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 Liiders, 1990; Downton et al., 1990;...
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’ application to salt-stressed plants is widely recognized. Improved shoot and/or root growth, as a result of Ca’ 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 Na’/Ca’ interactions affecting A are rare, there is evidence that Na’ can compete with Ca’ for the binding site on the O₂ evolution reaction center in photosystem II (Waggoner et al., 1989).

Plants that have low leaf Ca’ concentrations and thrive in soils low in Ca’, such as Vaccinium species, are regarded as calcifuges (Hope-Simpson, 1938). Despite some research indicating that high soil Ca’ is associated with poor blueberry growth and vigor (Austin et al., 1986; Ballinger et al., 1958), supplemental Ca’ applications were found to improve the growth of ‘Tifblue’ and ‘Brightwell’ rabbiteye blueberries under medium and high Na₂SO₄ salinity (Wright et al., 1992). Therefore, the question arises as to whether Ca’ application might improve growth of salinized blueberry by maintaining gas exchange and chlorophyll content.

The following experiments were designed to determine the effect of Na₂SO₄ 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 SO₄²⁻ 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’ to determine whether it exerted a protective effect on salinized blueberry plants.

Materials and Methods

Na₂SO₄ 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 mmoles Na’ (0, 12.5, or 50 mmoles Na₂SO₄), arranged factorially with 0, 1, 3, or 10 mmoles Ca’, and applied with the remaining essential elements in the nutrient solution. Because of the insolubility of CaSO₄, only 70% of each Ca’ treatment was CaSO₄; the remainder was made up of 20% Ca(NO₃)₂ and 10% CaCl₂. All treatments contained equal amounts of NO₃, NH₄, 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 h 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 ≈ 700 umol·m⁻²·s⁻¹; therefore, all subsequent measurements were taken at PPFD above the light saturation point, usually above 1000 mol·m⁻²·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 ≈ 300 ml-min⁻¹. Air temperature within the growth chamber was at 27°C, and relative humidity was maintained at ≈ 50%. Leaf temperature ranged from 22 to 32°C. External CO₂ applied to the leaf was ≈ 340 µbar-bar’ (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 Na₂SO₄ and Ca’ treatments.

Predawn Ψ was measured 62 days from the start of salt treatments, between 0100 and 0600 h, using a pressure chamber (Soil Moisture Corp., Santa Barbara, Calif.). Ψ 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 cm² disks were removed from each leaf. Chlorophyll was extracted using N, N-dimethylformamide, and chlorophyll concentration, and each disk was calculated from the equation developed by Moran (1982): Extractable chlorophyll (mg liter⁻¹) = (20.27 × A₆64) + (7.04 × A₆66), where A₆₆₄ and A₆₆₆ refer, respectively, to absorbance 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 (mg·g⁻¹ fresh weight) = -722.182 + (71.81 × SPAD reading) R² = 0.85.

The design was a randomized complete block. Experiments were 4 (Ca’ levels) × 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 Na₂SO₄, 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...
those solutions containing 0 and 10 mM Ca\(^{2+}\) because sufficient plants were not available to test all Ca\(^{2+}\)-levels.

Gas exchange and 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 Na\(_2\)SO\(_4\) 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 Ca\(^{2+}\) are given graphically (Figs. 1 to 6).

#### Na\(_2\)SO\(_4\) experiment

Stomatal conductance (g\(_s\)) decreased linearly with increasing Na\(_2\)SO\(_4\) concentrations in the external solution and with time of exposure to salinity (Table 2). The Ca\(^{2+}\) × Na\(^{+}\) treatment interaction also led to significant differences in g\(_s\) (Fig. 1). For unsalinized plants, g\(_s\) was not affected by Ca\(^{2+}\)-treatment until day 49, when plants supplied with 10 mM Ca\(^{2+}\) 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 Na\(_2\)SO\(_4\), Ca\(^{2+}\) 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 Ca\(^{2+}\) addition, with somewhat smaller increases in g\(_s\) with 3 and 10 mM Ca\(^{2+}\), compared to plants not supplied with Ca\(^{2+}\).

Changes in leaf transpiration rate (E) in response to Na\(_2\)SO\(_4\) and Ca\(^{2+}\) 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, Ca\(^{2+}\) did not lead to improved assimilation (Fig. 2A). After 63 days, unsalinized plants treated with 10 mM Ca\(^{2+}\) exhibited a 50% drop in A compared to plants not receiving Ca\(^{2+}\). However, Ca treatments were beneficial to salinized plants (Fig. 2B and 2C). With 12.5 mM Na\(^{+}\), plants receiving supplemental Ca\(^{2+}\) 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 Ca\(^{2+}\). For example, after 35 days of treatment, A of plants supplied with 1 mM Ca\(^{2+}\) was 1.5 to 2 times higher than that of plants not treated with Ca\(^{2+}\).

#### Table 2. Influence of Na\(_2\)SO\(_4\) and Ca\(^{2+}\) 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 (mmol 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 2          | 0.180                          | 4.53            | 7.65              |
| 25 3          | 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**

| Ca\(^{2+}\) | NS | 0.0441 | 0.0249 |
| Linear      | NS | 0.0451 | 0.0822 |
| Quadratic   | NS | 0.0001 | 0.0001 |
| Na\(^{+}\)  | 0.0001 | 0.0001 | 0.0001 |
| Time\(^{3}\) | 0.0001 | 0.0001 | 0.0001 |
| Na\(^{+}\) × time | 0.0011 | 0.0002 | 0.0092 |
| Ca\(^{2+}\) × Na\(^{+}\) | 0.0029 | 0.0007 | 0.0088 |

**Probability of a significant F value, NS = nonsignificant. Both quadratic and cubic Na\(^{+}\) contrasts were nonsignificant.**

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

| Solution | Na\(^{+}\) (mM) | Ca\(^{2+}\) (mM) | CaSO\(_4\) (mM) | Na\(_2\)SO\(_4\) (mM) | K\(_2\)SO\(_4\) (mM) | pH | E.C. (dS m\(^{-1}\)) |
|----------|----------------|-----------------|----------------|------------------|------------------|----|------------------|
| 0        | 0              | 0               | 0              | 0               | 4.0              | 6.1 | 1.22             |
| 0        | 1              | 0.7             | 0.2            | 0.1             | 3.6              | 4.0 | 5.4              |
| 0        | 3              | 2.1             | 0.6            | 0.3             | 2.8              | 4.0 | 5.4              |
| 0        | 10             | 7.0             | 2.0            | 1.0             | 0.0              | 4.0 | 5.4              |
| 25       | 0              | 0.0             | 0.0            | 0.0             | 4.0              | 0.0 | 31.0             |
| 25       | 1              | 0.7             | 0.2            | 0.1             | 3.6              | 4.0 | 19.4             |
| 25       | 3              | 2.1             | 0.6            | 0.3             | 2.8              | 4.0 | 13.0             |
| 25       | 10             | 7.0             | 2.0            | 1.0             | 0.0              | 4.0 | 7.6              |
| 100      | 0              | 0.0             | 0.0            | 0.0             | 4.0              | 124.0| 6.0             |
| 100      | 1              | 0.7             | 0.2            | 0.1             | 3.6              | 4.0 | 77.8             |
| 100      | 3              | 2.1             | 0.6            | 0.3             | 2.8              | 4.0 | 52.3             |
| 100      | 10             | 7.0             | 2.0            | 1.0             | 0.0              | 4.0 | 30.6             |

### Notes

1. Solutions contained 0.1 mM KH\(_2\)PO\(_4\), 0.65 mM MgSO\(_4\), 0.125 mM FeNa\((O\(_2\)CCH\(_3\))\(_2\))\(_2\), 0.076 mM ZnSO\(_4\), and 10.9 μM MnSO\(_4\), 1.2 μM H\(_3\)BO\(_3\), 0.2 μM CaSO\(_4\), 15 μM (NH\(_4\))\(_2\)MoO\(_4\). SAR = sodium adsorption ratio.

2. Total Na\(^{+}\) as Na\(_2\)SO\(_4\) or NaCl.

3. pH and E.C. values are means of at least three replications.
Xylem water potential ($\Psi_w$) 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$_2$SO$_4$, Ca$^{2+}$ treatments did not improve $\Psi_w$. However, for plants treated with 100 mM Na$^+$, $\Psi_w$ 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$_2$SO$_4$. 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$\text{\textsubscript{s}}$ of 'Tifblue' plants subject to 100 mM Na$^+$ as NaCl was only 10% of unsalinized controls (Fig. 4). The g$\text{\textsubscript{s}}$ of unsalinized controls was unaffected by Ca$^{2+}$ treatment. Similarly, Ca$^{2+}$ had little apparent influence on g$\text{\textsubscript{s}}$ of plants subject to 25 mM Na$^+$ until day 49, after which time, plants supplied with 1 mM Ca$^{2+}$ had significantly higher g$\text{\textsubscript{s}}$ than plants not given Ca$^{2+}$. For plants subjected to 100 mM Na$^+$ treatment, g$\text{\textsubscript{s}}$ decreased to a very low value (0.01 to 0.02 mol·m$^{-2}$·s$^{-1}$) by day 7. At day 35 and 49, g$\text{\textsubscript{s}}$ 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$\text{\textsubscript{s}}$ (Table 4).

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

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**Table 3.** Influence of Na$_2$SO$_4$ or NaCl and Ca$^{2+}$ treatments on xylem water potential ($\Psi_w$) of 'Tifblue' and 'Sharblue' cultivars of southern highbush blueberry plants.

| Treatment (mM) | Na$_2$SO$_4$ | NaCl | NaCl |
|---------------|--------------|------|------|
|               | 'Tifblue'    | 'Tifblue' | 'Sharblue' |
| Na$^+$ Ca$^{2+}$ |              |      |      |
| 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**

Ca$^{2+}$: NS 0.0144 NS
Na$^+$: 0.0001 0.0001 0.0001
Linear: 0.0001 0.0001 0.0001
Ca$^{2+}$ × Na$^+$: 0.0020 0.0207 NS

*Probability of a significant F value, NS = nonsignificant. Linear Ca$^{2+}$ was nonsignificant.*
Fig. 3. Leaf chlorophyll concentration of ‘Tifblue’ rabbiteye blueberries treated with Na$_2$SO$_4$ 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 ($\Psi_w$) of ‘Tifblue’ plants also decreased linearly with increasing NaCl (Table 3). For plants salinized with 100 mM Na$^+$, $\Psi_w$ 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 $\Psi_w$ (-0.17 MPa) than in the controls (-0.29 MPa). Calcium treatments did not influence $\Psi_w$ 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 ‘Sharpsblue’ 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$_2$SO$_4$ or NaCl to ‘Tifblue’ or ‘Sharpsblue’ 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 ≈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$_2$SO$_4$ was still 35% of that of unsalinized controls.

One factor leading to stomatal closure in blueberry under high Na$^+$ concentrations may be the lower $\Psi_w$ (Table 3). Regardless of Na$^+$ source, $\Psi_w$ 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$_s$ (mol·m$^{-2}$·s$^{-1}$) | E (mmol·m$^{-2}$·s$^{-1}$) | A (µmol·m$^{-2}$·s$^{-1}$) |
|---------------|-------------------------------|-----------------|-----------------|
| Na$^+$ Ca$^{2+}$ |                               |                 |                 |
| 0             | 0.194                         | 4.24            | 7.77            |
| 0             | 0.161                         | 3.75            | 5.92            |
| 0             | 0.167                         | 3.99            | 6.52            |
| 0             | 0.101                         | 2.53            | 4.06            |
| 0             | 0.105                         | 2.88            | 5.08            |
| 0             | 0.125                         | 3.27            | 5.62            |
| 0             | 0.105                         | 2.93            | 5.01            |
| 0             | 0.025                         | 0.70            | 1.33            |
| 0             | 0.035                         | 1.15            | 1.91            |
| 0             | 0.039                         | 1.24            | 2.28            |
| 0             | 0.057                         | 0.70            | 2.18            |

| Source $^a$ | Na$^+$ | Linear | Time $^b$ | Linear | Quadratic | Na$^+$ × time | Na$^+$ × Ca$^{2+}$ |
|-------------|--------|--------|-----------|--------|-----------|---------------|-------------------|
| NS          | 0.0001 | 0.0001 | 0.0001    | 0.0001 | 0.0001    | 0.0001        | 0.0001            |
| NS          | 0.0001 | 0.0001 | 0.0001    | 0.0001 | 0.0001    | 0.0001        | 0.0001            |
| NS          | 0.0001 | 0.0001 | 0.0001    | 0.0001 | 0.0001    | 0.0001        | 0.0001            |

$^a$Probability of a significant F value, NS = nonsignificant.

$^b$Days after salt application (treatment duration).
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, 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 CO₂ entry into the leaf enough to reduce internal CO₂ concentration. Inhibition of A was attributed in all these cases to 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 Ca²⁺ levels of plants treated with high levels of Na₂SO₄ 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 Ca²⁺ transport by interfering with active loading of Ca²⁺ into xylem vessels. Sodium would then compete with fewer Ca²⁺ ions for a binding site on the O₂ evolution reaction center in photosystem II (Waggoner et al., 1989).

### Table 5. Influence of NaCl and Ca²⁺ treatments on stomatal conductance (g.), transpiration (E), and net assimilation (A) of ‘Sharpblue’ southern highbush blueberry plants.

| Treatment (mM) | g (mol·m⁻²·s⁻¹) | E (mmol·m⁻²·s⁻¹) | A (µmol·m⁻²·s⁻¹) |
|---------------|----------------|----------------|-----------------|
| Na⁺ Ca²⁺     |                |                |                 |
| 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            |

Source²

| Na⁺ | NS | NS | NS |
|-----|----|----|----|
| Linear | 0.0001 | 0.0001 | 0.0001 |
| Time² | 0.00003 | 0.0160 | 0.0248 |
| Linear | 0.0001 | 0.0368 | NS |
| Quadratic | 0.0707 | 0.0398 | 0.0267 |
| Na⁺ × time | 0.0001 | NS | 0.0510 |

²Probability of a significant F value, NS = nonsignificant. Ca²⁺ × time interaction, Ca²⁺ × Na⁺ interaction, and Ca²⁺ × Na⁺ × time interaction were not significant.

²Days after salt application (treatment duration).
As noted above with g, effects of NaCl salinity on A were often more damaging than were the effects of Na₂SO₄; assimilation of plants given 100 mM NaCl was only 28% of the A of unsalinated controls, while A of plants given 50 mM Na₂SO₄ was 69% of unsalinated controls. Calcium supplementation was usually less effective with NaCl than with Na₂SO₄. 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, Ca²⁺ supplementation ameliorated gas exchange of only the salinized ‘Tifblue’ rabbit-eye blueberry. Low Ca²⁺ treatments (1 mM) were most effective in plants treated with Na₂SO₄. Blueberries are efficient Ca²⁺ accumulators, thus they may be damaged by high levels of Ca²⁺. Calcium levels within the cytoplasm must remain low to avoid precipitation of inorganic P, competition with Mg²⁺, and/or uncontrolled activation or inactivation of enzyme systems (Marschner, 1986).

Reduced root Na⁺ influx in the presence of adequate Ca²⁺ may account for some of the ameliorative effects of Ca²⁺ on cotton (Cramer et al., 1987). Further, LaHaye and Epstein (1971) reported that Ca²⁺ reduced Na⁺ translocation from bean roots to shoots. Similar phenomena may occur in rabbit-eye blueberries, leading to the improved A, E, and g found here. The inability of Ca²⁺ to counter NaCl-induced inhibition of gas exchange of ‘Sharpleblue’ southern highbush blueberry (Table 5) indicates that this cultivar may be more sensitive to supplemental Ca²⁺, or more sensitive to NaCl, and that the detrimental effects of Cl⁻ may mask any putative amelioration from Ca²⁺ 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 Ca²⁺ 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. ‘Sharpleblue’ 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, A, and E. NaCl salinity led to greater reductions in gas exchange than did Na₂SO₄ salinity, presumably due to the additional effects of Cl⁻. Low (1 mM) Ca²⁺ supplements were often able to ameliorate the salinity damage, but Ca²⁺ was more beneficial when applied to plants in which salinity was induced with Na₂SO₄ than with NaCl. Leaf chlorophyll concentration was not greatly diminished by Na⁺ or improved by Ca²⁺; decreases were due more to leaf necrosis than to leaf chlorosis. The cultivar ‘Sharpleblue’ was more sensitive to NaCl salinity than was ‘Tifblue’.

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