Salinity and Supplemental Calcium Influence Growth of Rabbiteye and Southern Highbush Blueberry

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Abstract. 'Tifblue' and 'Brightwell' rabbiteye blueberries (*Vaccinium ashei* Reade.) were subjected to 0, 25, or 100 mM Na⁺, as Na₂SO₄ or NaCl, and 0, 1, 3, or 10 mM supplemental Ca²⁺, primarily as CaSO₄, in an irrigated sand culture in the greenhouse. Additionally, the effect of NaCl on 'Sharpblue' southern highbush blueberry (primarily *V. corymbosum* L.) was examined. For unsalinized plants, fastest growth occurred in plants not receiving supplemental Ca²⁺. Root and shoot growth were depressed as salinity increased in plants lacking additional Ca²⁺. With 100 mM Na⁺ as Na₂SO₄, 'Tifblue' root and shoot dry weight increases were only 37% and 25%, respectively, of the increase of unsalinized controls, while with 100 mM Na⁺ as NaCl, the corresponding shoot and root dry weight increases were only 38% and 43%, respectively. 'Brightwell' plants reacted similarly to 'Tifblue' in salinity treatments with Na₂SO₄ and NaCl, but 'Sharpblue' plants were more severely affected by 100 mM NaCl than were the rabbiteye cultivars. In no case did addition of Ca²⁺ have any ameliorative effect on either the dry weight of roots of plants exposed to 25 or 100 mM NaCl or on the shoot growth of plants exposed to NaCl. The inability of Ca²⁺ to counter Cl⁻ entry or toxicity may account for the lack of amelioration. In contrast, additional Ca²⁺ did improve shoot growth of plants exposed to Na₂SO₄. For 'Tifblue' plants supplied with 25 mM Na⁺ as Na₂SO₄, growth increased by almost 25% in the presence of 10 mM Ca²⁺, while for 'Tifblue' plants treated with 100 mM Na⁺ as Na₂SO₄, growth was more than three times greater in plants supplied with 1 mM Ca than in those not given any Ca²⁺. Growth increase was primarily due to increased leaf area and number. Low (1 mM) concentrations of Ca²⁺ were more effective in ameliorating the effects of 100 mM Na⁺ as Na₂SO₄ than were 3- and 10-mM Ca²⁺ supplements, possibly because higher Ca²⁺ additions lead to metabolic damage in these calcifuge *Vaccinium* species.

Rabbiteye and southern highbush blueberries require good quality water to thrive and produce well. Many regions of the southeastern United States lack sufficient good quality water throughout the growing season. Saline ground water has emerged as a major difficulty limiting the expansion of the industry. Water pH < 7.0, total Na⁺ < 2.0 mM, total HCO₃⁻ < 1.5 mM, and total Cl⁻ < 4.0 mM are indicators of good quality water (Haby and Pennington, 1988). Although precipitation and surface water often meet these requirements and are preferred, these sources are often inadequate and lower quality water must be used for irrigation. Irrigation water in East Texas often exceeds 15 mM Na⁺, 6 mM Cl⁻, and 10 mM HCO₃⁻ (Texas Dept. of Health, 1990).

Haby et al. (1986) suggested that the deleterious effects of poor quality water [pH = 8.7, EC = 0.695 dS·m⁻¹, and so-

dium adsorption ratio (SAR) = 29.71 on rabbiteye blueberries were due to high levels of Na⁺. Furthermore, Patten et al. (1988) showed that Na⁺ concentrations in the root zone may be two to four times higher than in the irrigation water, due to surface evaporation. Plants irrigated with such water exhibited significantly less growth than those irrigated with surface or rain water. Spiers (1983) noted that rabbiteye blueberries took up Na⁺ rapidly, and that Na⁺ was detrimental to their growth. We found no reports of the response of rabbiteye blueberry to other components of saline irrigation water, such as Cl⁻, nor of the response of southern highbush blueberry to salinity.

The beneficial effect of supplemental Ca²⁺ on growth of salt-stressed plants is widely recognized. LaHaye and Epstein (1969) showed that adequate Ca²⁺ was a requirement for shoot and root growth in salt-stressed bean (*Phaseolus vulgaris* L.). Similar results have been reported in shoots and/or roots of maize (*Zea mays* L.) (Cramer et al., 1988; Maas and Grieve, 1987). In bean and cotton (*Gossypium hirsutum* L.), adequate Ca²⁺ can exclude or limit Na⁺ uptake into roots and Na⁺ translocation from roots to shoots (Cramer et al., 1987; LaHaye and Epstein, 1971).

Vaccinium spp. including blueberries, are regarded as calcifuges, plants that have low leaf Ca²⁺ concentrations and thrive in soils low in Ca²⁺ (Hope-Simpson, 1938). However, the response of blueberries in the field to Ca²⁺ additions appears to be variable. Some research has indicated that high soil Ca²⁺ is associated with poor blueberry growth and vigor (Austin et al., 1986; Ballinger et al., 1958), while other work found that Ca²⁺

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addition was not harmful (Spiers, 1979). The question therefore arises as to whether Ca^{2+} might offer any effective protection against salinity in a calcifuge species like blueberry.

The aim of our research was to determine the extent of Na^+ and Cl^- damage at particular external salt concentrations on the growth of rabbiteye and southern highbush blueberry. Sodium sulfate was applied with the assumption that the SO_4^{2-} , which is known to be absorbed slowly by roots of higher plants (Marschner, 1986), is relatively noninjurious to blueberries. Since we found no indication of SO_4^{2-} toxicity to blueberries in the literature, any damage would be due to the specific effect of Na^+ . Comparison with experiments using NaCl would then provide an indication of the additional effect of Cl^- . A further objective was to evaluate the ability of supplemental Ca^{2+} to ameliorate salinity damage.

Materials and Methods

Sodium sulfate experiment. Dormant-potted 'Tifblue' and 'Brightwell' rabbiteye blueberries were received on 15 Mar. 1988. Shoots were pruned back to 40 cm to stimulate growth, flower buds were removed, and roots were rinsed with distilled water to remove as much peatmoss/shredded pine bark mulch potting medium as possible. Plants were transplanted into sterilized 5500-ml pots containing silica propping agent [man-made sand with a particle size between 0.635 and 1.270 mm (B.J. Titan, Bryan, Texas)] lined with fiberglass insulation (to prevent loss of medium) and placed in the greenhouse.

Plants were irrigated daily with ≈ 400 ml of a complete nutrient solution consisting of (in mM) 0.5 $(\text{NH}_4)_2\text{SO}_4$, 0.1 KH_2PO_4 , 0.72 K_2SO_4 , 0.5 CaSO_4 , 0.65 MgSO_4 , 0.125 Fe as ethylenediaminetetraacetic acid iron (III) monosodium salt, and (in μM) 7.6×10^{-3} ZnSO_4 , 2.0×10^{-2} CuSO_4 , 10.9 MnSO_4 , 1.2 H_3BO_3 and 1.34×10^{-1} $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$. Solution was supplied automatically via trickle irrigation. When tensiometers indicated that the xylem water potential (ψ^w) of the medium was more negative than -0.2 MPa, the irrigation rate was increased to ≈ 500 ml·day $^{-1}$. Plants were maintained with the complete nutrient solution for 60 days, until new shoot and root growth was extensive.

Six plants of each cultivar were destructively harvested before experimental treatments were applied, to measure initial plant weight. Shoot and root fresh weight of each plant was determined, and shoot and root dry weights were also recorded after drying at 60°C for 48 h. Dry weights for all six plants of each cultivar were pooled, and average shoot and root dry weight was determined. These data were later used in the calculation of the shoot and root dry weight increase over the duration of the experiment.

Experimental treatments on 144 plants, began on 15 May 1988 and consisted of 0, 25, or 100 mM Na^+ (0, 12.5, or 50 mM Na_2SO_4) and 0, 1, 3, or 10 mM Ca^{2+} applied with the remaining essential elements in the nutrient solution. Calcium and Na^+ levels were crossed, forming 12 total treatments. Because of the low solubility of CaSO_4 , only 70% of each Ca^{2+} treatment was CaSO_4 , the remainder was made up of 20% from $\text{Ca}(\text{NO}_3)_2$ and 10% from CaCl_2 . All treatments contained equal amounts of NO_3^- , NH_4^+ , and K^+ . Plants received ≈ 400 or 500 ml experimental solution daily as described; this was sufficient for some solution to drain from the containers. Solution pH and electrical conductivity (EC, corrected to 25°C) were recorded when solutions were initially prepared and then again each time they were replenished. These values, along with solution composition and SAR are found in Table 1.

Plants were destructively harvested on 30 July 1988 following 76 days of treatment. Leaves and stems were separated from roots; and roots were rinsed with distilled water to remove as much silica as possible without causing damage, then were blotted dry. Root fresh weight was not determined because large amounts of silica remained within the root ball following washing (silica was easily removed following drying). Leaf and stem fresh weights for each plant were recorded, and a 20-leaf sample was removed at random from the total leaf mass of each plant. Fresh weight and total leaf area of the sample were determined, then the sample was reincorporated into the total leaf mass for the individual plant. Estimated total leaf count was calculated using the formula:

$$\text{Est. total leaf count} = \frac{(\text{Sample leaf count}) \times (\text{Total leaf fresh weight})}{\text{Sample leaf fresh weight}}$$

Plants were dried at 60°C for 48 h, then leaf, stem, and root dry weights were determined. Shoot : root ratio, leaf fresh weight : dry weight ratio, and specific leaf area (a measure of relative thickness) were also calculated.

The design was randomized complete block. Experiments involving 'Tifblue' and 'Brightwell' were 4 (Ca^{2+} levels) $\times$ 3 (Na^+ levels) $\times$ 2 (cultivars) factorial with six replications, for a total of 144 plants. Data were analyzed using the General Linear Model procedure (PROC GLM) of the Statistical Analysis Service software package (SAS Inc., Cary, N.C.).

Sodium chloride experiment. Dormant, bare-root 'Tifblue' and 'Brightwell' rabbiteye blueberries and 'Sharpblue' southern highbush blueberries were planted on 15 Mar. 1989. The rabbiteye blueberries were handled in the same manner as in 1988, while the 'Sharpblue' plants were transplanted into sterilized 2750-ml pots containing the fiberglass insulation and silica propping agent as described above. 'Sharpblue' plants were pruned to a height of 20 cm. The irrigation regime was the same as that of 1988.

Six 'Tifblue' and six 'Brightwell' plants were again removed in 1989, before treatment. Leaf, stem, and root fresh and dry weights were measured and estimated total leaf count was determined as in 1988. Insufficient plant material prevented pre-treatment sampling of 'Sharpblue'.

Plants were maintained with the complete nutrient solution until 15 May 1989, when treatments commenced. All plants received 0, 25, or 100 mM NaCl , rather than Na_2SO_4 , as the Na^+ source, but otherwise the compositions of the 12 nutrient solutions were the same as those of 1988 (Table 1). Solution pH and EC were again measured when the solutions were made and replenished. 'Sharpblue' plants were only treated with those solutions containing 0 or 10 mM Ca^{2+} because insufficient plants were available to test all Ca^{2+} levels. Plants were destructively harvested on 17 July 1989, following 63 days of treatment. Harvest protocol was the same as that of the 1988 experiment.

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

Results

The blueberry cultivars responded differently to salinization with Na_2SO_4 and NaCl . Furthermore, each cultivar performed differently with salinization, so that data could not be averaged over all cultivars. The principal effects of salinity on growth are described below (Tables 2-5), and interactions between salt concentration and Ca^{2+} are given graphically (Figs. 1 and 2).

Table 1. Composition, sodium adsorption ratio (SAR), pH, and electrical conductivity of nutrient solutions for blueberries.^a

Total Na ⁺ (mm)	Total Ca ²⁺ (mm)	CaSO ₄ (mm)	Ca(NO ₃) ₂ (mm)	CaCl ₂ (mm)	KNO ₃ (mm)	(NH ₄) ₂ SO ₄ (mm)	K ₂ SO ₄ (mm)	SAR	pH ^x		EC (dS·m ⁻¹)	
									Na ₂ SO ₄	NaCl	Na ₂ SO ₄	NaCl
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

^aAdditionally, solutions contained 0.1 mM K H₂P O₄, 0.65 mM M g S O₄, 0.125 mM FeNa(O₂CCH₃)₂NCH₂CH₂N(CH₂CO₂)₂, 10.9 μM MnSO₄, 1.2 μM H₃BO₃, 0.2 μM CuSO₄, 0.076 μM ZnSO₄, and 15 μM (NH₄)₆Mo₇O₂₄.

Total Na⁺ as Na₂SO₄ or as NaCl.

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

Sodium sulfate experiment. Dry weight increase of roots and shoots of both blueberry cultivars decreased linearly as Na⁺ concentration in the nutrient solution increased (Table 2), although only a small inhibition was found at 25 mM Na⁺. For ‘Tifblue’ plants, shoot and root dry weight increase of plants subject to 100 mM Na⁺ was only 37% and 25%, respectively, of the increase of unsalinized controls. The Na⁺ effect on ‘Brightwell’ was more severe. Dry weight increase of ‘Brightwell’ shoots and roots of plants treated with 100 mM Na⁺ was only 18% and 15%, respectively, of the increase of unsalinized controls.

The Ca²⁺ treatments had no significant main effect on the

dry weight increase of ‘Tifblue’ shoots, but an interaction between the Ca²⁺ and Na₂SO₄ treatment effects was significant at *P* = 0.07 (Fig. 1A). The greatest shoot dry weight increase was with controls receiving no additional Ca²⁺. For plants treated with 25 mM Na⁺, those receiving 10 mM Ca²⁺ additions grew almost 25% more than those not supplied with additional Ca²⁺. For plants treated with 100 mM Na⁺, those that received 1 mM Ca²⁺ produced more than three times more growth than plants not receiving Ca²⁺. Salinity was most detrimental to dry weight increase when there was no supplemental Ca²⁺ (Fig. 1A).

Calcium treatments affected growth of ‘Brightwell’ shoots, being significant at close to *P* = 0.10, but there was no sig-

Table 2. Effect of Na₂SO₄ or NaCl and Ca²⁺ treatments on dry weight increase of rabbiteye blueberry plants.

Ion and concn (mm)	Dry wt increase (g/plant)							
	1988 Na ₂ SO ₄ study				1989 NaCl study			
	Tifblue		Brightwell		Tifblue		Brightwell	
	Shoots ^z	Roots ^y	Shoots	Roots	Shoots	Roots	Shoots	Roots
Na ⁺								
0	59.1	17.9	48.5	17.8	44.4	12.9	50.0	18.2
25	55.3	16.5	41.7	16.0	40.5	13.1	39.3	15.0
100	21.7	4.5	8.6	2.6	17.1	5.5	22.0	7.5
Ca ²⁺								
0	41.7	12.5	26.8	9.0	36.2	11.1	37.5	13.6
1	50.0	14.3	34.5	12.9	33.5	9.0	39.2	14.3
3	39.0	9.0	35.4	12.9	32.8	10.7	38.2	15.4
10	47.2	14.7	34.0	13.4	33.1	10.8	33.2	11.5
Source ^x								
Na ⁺	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Ca ²⁺	NS	NS	0.1036	NS	NS	NS	NS	NS
Ca ²⁺ × Na ⁺	0.0639	NS	NS	NS	NS	NS	NS	NS
Contrast ^w								
Linear Na ⁺	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

^zShoot dry weight increase = [(leaf dry weight + stem dry weight) - shoot dry weight before treatment initiation].

^yRoot dry weight increase = [root dry weight - root dry weight before treatment initiation].

^xProbability of significant F value, NS = nonsignificant.

^wBoth linear and quadratic Ca²⁺ contrasts were nonsignificant.

Fig. 1. Shoot dry weight increase of 'Tifblue' (A) and 'Brightwell' (B) rabbiteye blueberries treated with Na₂SO₄ and Ca²⁺. Vertical bars indicate SE.

nificant Ca²⁺ × Na₂SO₄ interaction (Fig 1B). Plants receiving no Ca²⁺ supplement grew the least, regardless of the level of Na⁺ supplied. For 'Brightwell' plants, 1 mM Ca²⁺ was no more effective in alleviating Na⁺ inhibition of growth than lack of Ca²⁺ in the solution or higher concentrations.

Calcium treatments had no significant effects on the dry weight increase of 'Tifblue' or 'Brightwell' roots (Table 2).

All measured growth characteristics of 'Tifblue', except the shoot : root ratio, declined linearly in response to increased Na⁺ levels (Table 3). Specific leaf area was not affected by Ca²⁺ or Na⁺ treatments (data not shown). Leaf and stem fresh weight, dry weight of leaf, stem, and root, and estimated leaf count declined slightly in plants supplied with 25 mM Na⁺ compared with unsalinized controls, and an appreciably greater reduction took place at 100 mM Na⁺ (Table 3).

The Na⁺ × Ca²⁺ interaction had a significant effect on leaf dry weight (Fig. 2) and leaf area (data not shown). For unsalinized plants, leaf dry weight at the end of the experiment was highest without supplementary Ca²⁺. However, for salinized plants, both attributes showed a distinct response to supplementary Ca²⁺, particularly with 1 mM Ca²⁺ and 100 mM Na⁺, when leaf dry weight was more than three times higher and leaf area was ≈50% higher, both compared with the 0 mM Ca²⁺ treat-

ments. Estimated leaf count was highest in plants treated with 1 mM Ca²⁺ (Table 3). A gray leaf necrosis appeared on older leaves of some plants treated with 100 mM Na⁺ and was more extensive on plants not supplied with Ca²⁺. The lower leaf fresh weight : dry weight ratio of plants subject to 100 mM Na⁺ was at least partially because of the tissue necrosis. 'Tifblue' stem fresh and dry weights and root dry weight were not affected by Ca²⁺ treatment.

'Brightwell' plants responded to Na₂SO₄ salinity in essentially the same way as did 'Tifblue' plants (data not shown). Specific leaf area was not affected by Na⁺ or Ca²⁺ treatments. There were decreases in leaf and stem fresh weights, leaf stem and root dry weights, estimated leaf count, leaf fresh weight : dry weight ratio, shoot : root ratio and average leaf area with increasing Na⁺ concentrations in the external solution. 'Brightwell' plants treated with any concentration of Ca²⁺ showed improved leaf fresh weight, dry weight, and estimated leaf count. Like 'Tifblue', when 'Brightwell' plants were subjected to 25 and 100 mM Na⁺, supplemental Ca²⁺ led to a larger average leaf area than in those not supplied with Ca²⁺ (data not shown).

Sodium chloride experiment. The Ca²⁺ × Na⁺ interaction effect on shoot and root dry weight increase was not significant, but shoot and root dry weight increase was slowed as Na⁺ increased in the external solution (Table 2), and the relationship between dry weight increase and external Na⁺ concentration was linear. For 'Tifblue' plants, shoot and root dry weight increase of plants subject to 100 mM NaCl was only 38% and 43%, respectively, of the increase of unsalinized controls.

The inhibitory effect of Na⁺ on dry weight increase of 'Brightwell' was similar to the effect on 'Tifblue'. Dry weight increase of 'Brightwell' shoots and roots exposed to 100 mM Na⁺ was 44% and 42%, respectively, of the dry weight increase of the controls.

Calcium treatments had no significant influence on the dry weight increase of 'Tifblue' or 'Brightwell' shoots and roots exposed to Na⁺.

All 'Tifblue' growth measurements decreased linearly as Na⁺ increased in the external solution (Table 4). Treatment with 1 mM Ca²⁺ led to increased leaf fresh weight : dry weight and shoot : root ratios and to larger average leaf area. A tan leaf necrosis that appeared on plants provided 100 mM Na⁺ as NaCl was more extensive than on plants treated with 100 mM Na⁺ as Na₂SO₄. Calcium treatments had no significant influence on the other growth parameters.

For 'Brightwell' plants, growth was also retarded by Na⁺ in the external solution: leaf and stem fresh weight, estimated leaf count, leaf, stem, and root dry weight, leaf fresh weight : dry weight ratio, shoot : root ratio, and average leaf area all decreased (data not shown), and a negative linear relationship was found between each characteristic and Na⁺ concentration. Calcium treatments had no influence on growth characteristics, nor were there any significant Ca²⁺ × Na⁺ interactions. Tan leaf necrosis also appeared on 100 mM Na⁺-treated plants.

For 'Sharpblue' plants, increasing Na⁺ concentrations in the external solution caused decreases in all characteristics measured (Table 5). Estimated leaf count of plants given 100 mM Na⁺ was less than half the leaf count of those plants not under Na⁺ treatment. Leaf dry weight and leaf area of the 100 mM NaCl-salinized plants were only 21% and 29%, respectively, of leaf dry weight and leaf area of the nontreated plants, and root dry weight of the 100-mm plants was only 26% of the control plants. Tan leaf necrosis appeared on some plants treated with 25 mM Na⁺, and was more severe on plants supplied with 100

Table 3. Influence of Na₂SO₄ and Ca²⁺ treatments on growth parameters of 'Tifblue' rabbiteye blueberry plants.

Ion and concn (mM)	Fresh wt ^z (g)		Estimated leaf count	Dry wt (g)			Leaf fresh wt : dry wt ratio	Shoot : root ratio	Avg leaf area (cm ²)
	Leaf	Stem		Leaf	Stem	Root			
Na ⁺ (Na ₂ SO ₄)									
0	112.3	63.7	319	41.1	24.0	23.8	2.7	3.0	12.7
25	106.7	60.7	300	38.3	22.9	22.4	2.8	2.9	12.9
100	39.4	26.1	136	16.6	11.2	10.4	2.3	2.9	9.8
Ca ²⁺									
0	76.8	46.0	216	29.3	18.3	18.4	2.5	2.6	10.8
1	97.4	54.6	284	35.3	20.6	20.2	2.7	3.0	12.6
3	78.5	43.0	241	28.5	16.5	14.9	2.7	3.2	11.4
10	84.6	53.3	246	32.5	20.7	20.6	2.6	2.9	12.1
Source ^y									
Na ⁺	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	NS	0.0001
Ca ²⁺	0.0893	NS	0.0868	NS	NS	NS	0.0001	NS	0.0437
Ca ²⁺ × Na ₂ SO ₄	0.0889	NS	NS	0.0528	NS	NS	0.0228	NS	0.0006
Contrast ^x									
Linear Na ⁺	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	NS	0.0001
Quadratic Ca ²⁺	NS	NS	NS	NS	NS	0.1029	0.0005	0.0371	NS

^zAll fresh and dry weight values are reported as grams per plant.^yProbability of a significant F value, NS = nonsignificant.^xLinear Ca²⁺ contrasts were nonsignificant. Fig. 2. Leaf dry weight of 'Tifblue' rabbiteye blueberries treated with Na₂SO₄ and Ca²⁺. Vertical bars indicate SE.

mM Na⁺. One-third of these plants died. Calcium treatments had no ameliorative effect on salinity damage to 'Sharpblue' plants.

Discussion

Salinization with Na₂SO₄ or NaCl retarded vegetative growth of 'Tifblue', 'Brightwell', and 'Sharpblue' blueberries, although plants appeared to tolerate the lower concentration (25 vs. 100 mM Na⁺). Supplemental Ca²⁺ appeared to offset salinity damage to shoots when plants were treated with Na⁺ as Na₂SO₄, but not with Na⁺ as NaCl.

Sodium or NaCl application depressed root growth. Root growth was not improved by Ca treatments, regardless of Na⁺ concentration, a result that contrasts with studies indicating Ca²⁺ amelioration of NaCl inhibited root growth, using comparable concentrations of Na⁺, in species as diverse as cotton (Cramer et al., 1987) barley (*Hordeum vulgare* L.) (Cramer et al., 1989), and bean (LaHaye and Epstein, 1971).

Effects of the higher concentration of Na⁺ as NaCl on growth were more severe than the effects of the higher concentration of Na₂SO₄ at equimolar concentrations of Na⁺. The inhibition of growth reported in our experiments, ≈50% for 'Tifblue' plants at 100 mM Na⁺ as NaCl is greater than the 33% decrease in dry weight of 'Tifblue' in sand culture, under high salinity, reported by Spiers (1983). In Spiers' experiment, the highest salt concentration used was only ≈ 1/20th (5.21 mM NaCl) of the highest level used in this experiment, but the experiment lasted almost 16 months.

Growth reduction was often followed by leaf necrosis, beginning at the tip and margins, then advancing basipetally. Ehlig (1965) noted severe necrosis on *Rubus* spp. treated with NaCl and attributed injury primarily to Cl⁻ because Cl⁻ accumulated in the shoots faster than Na⁺. Similarly, Cl⁻ damage was judged to be more severe than Na⁺ damage in diverse other species (Bongi and Loreto, 1989; Downton, 1977; Manchanda and Sharma, 1989). Ballinger (1962) found that 104 mM Cl⁻ produced only limited foliar tip yellowing and occasional browning in highbush blueberry. But in our case, it is likely that Cl⁻ was more damaging to rabbiteye and southern highbush blueberry shoots than was Na⁺, because leaf necrosis was more severe on NaCl-treated plants than on plants supplied with Na⁺ as Na₂SO₄.

Other studies indicate that the detrimental effects of NaCl on plants may be overcome, at least in part, by CaSO₄ application. LaHaye and Epstein (1971) found with brittle wax bean that dry weights of stem and leaves of NaCl-stressed plants increased as the CaSO₄ concentration of the solution was raised from 1 to 10 mM. Similar results have been reported on *Leucaena leucocephala* (Hansen and Munns, 1988), and sour orange (*Citrus aurantium* L.) (Zekri and Parsons, 1990). The presence of Ca²⁺ is necessary to maintain K⁺/Na⁺ selectivity, to maintain the Ca²⁺ status of developing leaves, and to prevent the harmful displacement of Ca²⁺ by Na⁺ from the cell membranes and intercellular pools (Lauchli, 1990). Adequate Ca²⁺ is necessary to maintain cell growth. Zhong and Lauchli (1988) reported that 150 mM NaCl severely inhibits the incorporation of glucose into cellulose, an inhibition that is reversed by 10 mM Ca²⁺ supplementation. However, in our experiments, supplemental Ca²⁺

Table 4. Influence of NaCl and Ca²⁺ treatments on growth characteristics of 'Tifblue' rabbiteye blueberry plants.

Ion and concn (mM)	Fresh wt ^z (g)		Estimated leaf count	Dry wt (g)			Leaf fresh wt : dry wt ratio	Shoot : root ratio	Avg leaf area (cm ²)
	Leaf	Stem		Leaf	Stem	Root			
Na ⁺ (NaCl)									
0	87.9	56.8	409	28.6	22.4	16.7	3.1	3.1	10.0
25	73.8	54.4	383	25.5	21.6	16.9	2.8	2.9	8.8
100	29.2	29.0	223	10.4	13.2	9.3	2.7	2.6	5.9
Ca ²⁺									
0	65.7	48.4	348	22.7	20.2	14.9	2.8	2.8	8.0
1	66.6	44.8	337	21.2	18.9	12.8	3.1	3.3	8.9
3	62.7	46.4	365	21.6	17.8	14.5	2.8	2.7	7.5
10	59.0	46.8	301	20.5	19.2	14.6	2.8	2.7	8.4
Source ^y									
Na ⁺	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Ca ²⁺	NS	NS	NS	NS	NS	NS	0.0612	0.0658	NS
Ca ²⁺ × NaCl	0.0881	NS	NS	NS	NS	NS	NS	NS	NS
Contrast ^x									
Linear Na ⁺	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

^zAll fresh and dry weight values are reported as grams per plant.

^yProbability of a significant F value, NS = nonsignificant.

^xBoth linear and quadratic Ca²⁺ contrasts were nonsignificant.

Table 5. Influence of NaCl and Ca²⁺ treatments on growth characteristics of 'Sharpblue' southern highbush blueberry plants.

Ion and concn (mM)	Fresh wt ^z (g)		Estimated leaf count	Dry wt (g)			Leaf fresh wt : dry wt ratio	Shoot : root ratio	Avg leaf area (cm ²)
	Leaf	Stem		Leaf	Stem	Root			
Na ⁺ (NaCl)									
0	59.9	26.0	177	20.2	8.7	12.9	2.9	2.3	18.4
25	41.4	19.0	124	12.9	6.8	8.4	3.2	2.5	16.6
100	8.9	7.2	72	4.2	3.2	3.3	1.2	2.0	5.4
Ca ²⁺									
0	36.0	16.6	141	11.5	6.2	7.6	2.7	2.4	12.8
10	35.5	17.4	124	12.7	6.1	8.4	2.1	2.1	13.7
Source ^y									
Na ⁺	0.0001	0.0002	0.0440	0.0001	0.0067	0.0004	0.0001	NS	0.0002
Ca ²⁺	NS	NS	NS	NS	NS	NS	0.0779	NS	NS
Contrast									
Linear Na ⁺	0.0001	0.0001	0.0556	0.0001	0.0022	0.0002	0.0001	NS	0.0001

^zAll fresh and dry weight values are reported as grams per plant.

^yProbability of a significant F value, NS = nonsignificant. The Ca²⁺ × Na⁺ interaction was nonsignificant.

could not ameliorate the detrimental effects of 100 mM Na⁺ as NaCl on blueberry growth.

The inability of Ca²⁺ to limit NaCl salinity damage may be related to the specific ion effects of NaCl salinity. Since SO₄²⁻ is not known to be damaging to blueberries, the shoot growth depression caused by Na₂SO₄ salinity may be due chiefly to Na⁺, and the greater depression caused by NaCl salinity, relative to Na₂SO₄, may be due at last partially to the added effect of the Cl⁻. Therefore, a plausible explanation of the inability of Ca²⁺ treatments to offset NaCl salinity damage is that Ca²⁺ cannot restrict the entry of Cl⁻ or overcome its effects in Cl⁻-sensitive species. In fact, Brown et al. (1953) proposed that Ca²⁺ facilitates Cl⁻ uptake in various *Prunus* spp., all of which are sensitive to high levels of Cl⁻, but Ehlig (1965) did not find that Ca²⁺ enhanced Cl⁻ uptake in raspberries (*Rubus idaeus* L.). This question remains to be investigated in blueberry.

Additionally, the higher Ca²⁺ treatments may have been detrimental to unsalinized blueberries because excessive Ca²⁺ has been shown to limit growth by reducing cell wall expansion in some species. Hansen and Munns (1988) found that for *Leucaena leucocephala*, high CaSO₄ levels depressed growth of unsalinized plants. Eklund and Eliasson (1990) demonstrated that cellulose deposition in spruce [*Picea abies* (L.) Karst.] was inhibited by high Ca²⁺. Calcium supplements decreased the plastic extensibility of soybean [*Glycine max* (L.) Merr.] hypocotyl cell walls (Virk and Cleland, 1988). Calcium may also directly inhibit cell wall growth by displacing H⁺ from the Donnan Free Space near the cell wall, thus inhibiting enzymes responsible for wall loosening that have acidic pH optima (Cleland et al., 1990). Furthermore, high Ca²⁺ may adversely affect cell metabolism in calcifuge species, such as blueberries, which are efficient Ca²⁺ accumulators, as high Ca²⁺ disrupts metabolic processes in other species (Brauer et al., 1990). Calcium levels within the cytoplasm must remain

low to avoid precipitation of inorganic phosphorus, competition with Mg^{2+} and inadvertent activation or inactivation of enzyme systems (Marschner, 1986).

In contrast to its ineffective protection from NaCl salinity, and possible detrimental effects on unsalinized plants, Ca^{2+} was often able to ameliorate the detrimental effects of Na^+ as Na_2SO_4 on blueberry shoot growth. In 'Tifblue' plants this resulted mainly from greater leaf fresh and dry weight, probably because of greater leaf area and the emergence of new leaves on Ca^{2+} -treated plants. Under Na_2SO_4 salinization (25 mM Na^+), 3 and 10 mM Ca^{2+} appeared to be optimal for growth of blueberries. These treatments were equivalent to $Na^+ : Ca^{2+}$ ratios of 8.3:1 and 2.5:1. While little work has been done using Na_2SO_4 , other research indicates that growth of plants exposed to NaCl is least affected by salinity under similar $Na^+ : Ca^{2+}$ ratios; growth of NaCl-treated sour orange was optimum at a ratio of 8:1 (Zekri and Parsons, 1990), and dry weight of NaCl-treated bean with a $Na^+ : Ca^{2+}$ ratio of 16.7:1 almost equalled the dry weight of control plants (LaHaye and Epstein, 1971). With 50 mM Na_2SO_4 , the optimum Ca^{2+} concentration was 1 mM. This contrasts with barley, where Cramer et al. (1989) found that shoot growth of NaCl-stressed plants was optimal at 10 mM Ca^{2+} . A similar positive response to high Ca^{2+} for plants salinized with NaCl was previously reported with *L. leucocephala* (Hansen and Munns, 1988), sour orange (Zekri and Parsons, 1990), and bean (LaHaye and Epstein, 1971), but none of the above species are calcifuge.

Why might 'Tifblue' blueberry react differently than other species to high and low supplemental Ca^{2+} concentrations under high Na_2SO_4 salinity? At Ca^{2+} concentrations > 1 mM, an ameliorative effect in relation to Na^+ may be offset by the detrimental effect of Ca^{2+} on the metabolism of the calcifuge blueberry. Such a harmful effect may be exacerbated because of the presence of high Na^+ . This relationship suggests that management of Ca^{2+} in blueberry plants exposed to salinity requires the maintenance of low but appreciable amounts of Ca^{2+} in the rooting zone.

The ameliorative effects of Ca^{2+} appear to be cultivar specific, since no single Ca^{2+} treatment led to significantly improved 'Brightwell' shoot growth at high Na^+ as Na_2SO_4 concentrations, and Ca^{2+} had no ameliorative effect on 'Sharpblue' growth. Thus, 'Brightwell' and 'Sharpblue' may be more sensitive to high concentrations of Na_2SO_4 than 'Tifblue', or, alternatively, the former cultivars may be more sensitive to excess Ca^{2+} .

In conclusion, rabbiteye and southern highbush blueberries are sensitive to a high level (100 mM Na^+) of salinity, which causes severe depression of growth of shoots and roots, whereas there was much less effect at 25 mM Na^+ . Root growth was depressed more than shoot growth, regardless of the Na^+ salt applied. Supplemental Ca^{2+} did not ameliorate NaCl salinity damage to shoots on any cultivar, possibly because of the inability of Ca^{2+} to counter the toxic effects of Cl^- . This lack of effect suggests that blueberries are especially sensitive to Cl^- . Under moderate Na_2SO_4 salinity treatment (25 mM Na^+), supplemental Ca^{2+} (3 or 10 mM) successfully ameliorated salinity damage by improving leaf growth. Under high Na_2SO_4 salinity treatment (100 mM Na^+), 1 mM Ca^{2+} produced optimum leaf growth, possibly because application of higher concentrations of Ca^{2+} were detrimental to the metabolism of the calcifuge blueberry.

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