Labeled Sodium ($^{22}\text{Na}^+$) Uptake and Translocation in Rabbiteye Blueberry Exposed to Sodium Chloride and Supplemental Calcium

Glenn C. Wright¹

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

Kim D. Patter²

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

Malcolm C. Drew³

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

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Abstract. 'Tifblue' rabbiteye blueberry (*Vaccinium ashei* Reade) plants grown in complete nutrient solution were supplied with 25 mM NaCl and with either 0, 3, or 10 mM supplemental Ca^{2+} added as a mixture of the sulfate, nitrate, and chloride salts. Uptake and partitioning of $^{22}\text{Na}^+$ into leaves, stems, and roots from labeled nutrient solutions and subsequent translocation in the absence of additional labeled Na^+ (pulse-chase experiment) was determined. Plants were harvested at intervals following the uptake period. At 28 days, plants supplied with 10 mM Ca^{2+} accumulated 35% to 68% more $^{22}\text{Na}^+$ in the 3rd-18th leaves from the apex and in herbaceous stems than plants not supplied with Ca^{2+} . Leaf Na^+ concentrations followed a similar trend. There was a preferential translocation of Na^+ to the shoots compared to the other plant parts, which accounted for 30% of the total plant Na^+ immediately following the uptake period and 15% at 28 days. Blueberry plants supplied with 3 mM Ca^{2+} did not have greater leaf or stem $^{22}\text{Na}^+$ concentrations or total Na^+ content than plants not supplied with Ca^{2+} . The $^{22}\text{Na}^+$ content did not decrease with 3 mM Ca^{2+} treatments. It is proposed that the failure of a high level (10 mM) of Ca^{2+} to protect against Na^+ uptake and translocation to the herbaceous shoots is due to metabolic dysfunction. Abnormally high levels of Ca^{2+} in the cytoplasm may lead to an inability to sequester or exclude Na^+ .

The damaging effects of saline water on growth of rabbiteye blueberry are increasingly recognized. There is appreciable retardation of shoot and root growth, leaf necrosis, and leaf gas exchange inhibition in the presence of medium (25 mM) and high (100 mM) external Na^+ concentrations (Wright et al., 1992; Wright et al., 1993). Similar results were obtained with highbush blueberry (*Vaccinium corymbosum* L.) plants after exposure to NaCl and Na_2SO_4 (Muralitharan et al., 1992). Such results are compatible with those of Spiers (1983), who found that rabbiteye blueberries readily absorbed Na^+ when exposed to high salinity and suggested that they lack a mechanism to regulate Na^+ uptake.

Sodium exclusion mechanisms in most Na^+ -sensitive plants rely on relatively high Ca^{2+} concentrations in the rooting medium (Marschner, 1986). The ability of high external concentrations of Ca^{2+} to limit Na^+ influx into roots and inhibit Na^+ transport from roots to shoots was first established in bean (LaHaye and Epstein, 1971) and later in cotton (Cramer et al., 1987). In contrast, our work with rabbiteye blueberry showed that low (1 mM) concentrations of Ca^{2+} were sometimes more effective in ameliorating salinity damage than higher Ca^{2+} concentrations (10 mM) when

Na_2SO_4 was the Na^+ source (Wright et al., 1992; Wright et al., 1993; Wright et al., 1993). It seems likely that such amelioration may be due at least in part to Ca^{2+} -induced reductions in Na^+ absorption and/or translocation.

Our earlier work also showed that Ca^{2+} was not an effective ameliorator of salinity damage when NaCl was the Na^+ source. In all of those studies, Na_2SO_4 and NaCl were applied. Na_2SO_4 was applied with the assumption that the SO_4^{2-} , which is known to be absorbed relatively 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, we assumed that all Na_2SO_4 response was due to the specific effect of Na^+ . Comparison with experiments using NaCl provided an indication of the additional effect of Cl^- . We attributed the differences in the response of rabbiteye blueberry to the two Na^+ sources to the addition of Cl^- , and we suggested that the ameliorative effects of Ca^{2+} upon Na^+ were masked by the deleterious effects of the Cl^- .

However, saline water in the blueberry-growing areas of Texas does not contain Na_2SO_4 , but may contain NaCl. Thus, we consider NaCl to be a more appropriate Na^+ source in this study. Using $^{22}\text{NaCl}$, Na^+ uptake and translocation patterns would still be revealed and a realistic picture of the interaction between Na^+ and Ca^{2+} would be gained. Therefore, the aim of this study was to determine the effects of high and low external concentrations of Ca^{2+} on the uptake and translocation of Na^+ , using $^{22}\text{Na}^+$ as a tracer, in NaCl salinized rabbiteye blueberry plants.

Materials and Methods

Experiments were conducted from May to August 1988 in College Station, Texas. Dormant, bare-root, 'Tifblue' rabbiteye blueberry plants were grown in a greenhouse in individual contain-

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¹Assistant research scientist. Present address: Univ. of Arizona, Yuma Mesa Agricultural Center, Route 1, Box 40M, Somerton, AZ 85350.

²Associate horticulturist. Present address: Washington State Univ.-Long Beach Research and Extension Unit, Route 1, Box 570 Pioneer Rd., Long Beach, WA 98631.

³Professor of horticultural sciences.

ers filled with 325 ml of aerated complete nutrient solution. Greenhouse temperatures were maintained at $32 \pm 2^\circ\text{C}$ during the day and $22 \pm 2^\circ\text{C}$ at night. Each container consisted of a two-part threaded lid on a glass jar. A 2.5 cm-diameter hole was drilled in each lid, and the plant was supported in the jar with a foam rubber collar inserted around the stem and into the hole. An aeration tube extended into each jar. Containers of nutrient solution were shielded from light using aluminum foil. The solution consisted of 0.5 mM $(\text{NH}_4)_2\text{SO}_4$, 0.1 mM KH_2PO_4 , 0.72 mM K_2SO_4 , 0.5 mM CaSO_4 , 0.65 mM MgSO_4 , 0.125 mM ethylene diaminetetraacetic acid iron (III), monosodium salt (FeNa-EDTA), and the remaining micronutrients. Solutions were changed or additional solution was added at weekly intervals to return the volume to 325 ml. The pH of the solution was 5.1. Plants were shoot and root pruned to 15 and 10 cm respectively at planting to stimulate growth. When new root and new shoot growth were extensive (usually 3 to 4 weeks), the experiments were initiated.

The first experiment consisted of measuring the uptake of $^{22}\text{Na}^+$ from labeled nutrient solutions containing 25 mM NaCl, with and without 10 mM Ca^{2+} , and subsequent translocation in the absence of additional labeled Na^+ (i.e., pulse-chase experiments). A subsequent experiment was identical, except uptake was measured from solutions containing 25 mM NaCl, with and without 3 mM Ca^{2+} . The solutions containing 0 mM Ca^{2+} consisted of 4 mM $(\text{NH}_4)_2\text{SO}_4$, 4 mM KNO_3 , 0.1 mM KH_2PO_4 , 0.65 mM MgSO_4 , 0.125 mM FeNa-EDTA, micronutrients, and 25 mM NaCl. Solution pH was 5.0. The solutions containing 3 mM Ca^{2+} consisted of 4 mM $(\text{NH}_4)_2\text{SO}_4$, 2.8 mM KNO_3 , 1.2 mM K_2SO_4 , 0.1 mM KH_2PO_4 , 0.65 mM MgSO_4 , 2.1 mM CaSO_4 , 0.6 mM $\text{Ca}(\text{NO}_3)_2$, 0.3 mM CaCl_2 , 0.125 mM FeNa-EDTA, the remaining micronutrients, and 25 mM NaCl. Solution pH was 4.9. The solutions containing 10 mM Ca^{2+} consisted of 4 mM $(\text{NH}_4)_2\text{SO}_4$, 4 mM KNO_3 , 0.1 mM KH_2PO_4 , 0.65 mM MgSO_4 , 7 mM CaSO_4 , 2 mM $\text{Ca}(\text{NO}_3)_2$, 1 mM CaCl_2 , 0.125 mM FeNa-EDTA, micronutrients, and 25 mM NaCl. Solution pH was 5.1.

Gypsum (CaSO_4) was chosen as the major Ca^{2+} source in all these solutions. However, because of the low solubility of CaSO_4 , it was not possible to provide 100% of the Ca^{2+} as CaSO_4 in the 10 mM Ca^{2+} solution. Therefore, only 70% of the Ca^{2+} was provided as CaSO_4 , 20% as $\text{Ca}(\text{NO}_3)_2$, and 10% as CaCl_2 . The same percentages were maintained in the 3 mM Ca^{2+} solution. All solutions had equimolar levels of NH_4^+ , NO_3^- , PO_4^{3-} , and K^+ .

Plants were moved to a growth chamber and pretreated with a nutrient solution containing unlabeled 12.5 mM Na^+ and 0 mM Ca^{2+} for 12 h, under the conditions of the experiment, before exposure to the labeled solution. Plants were then transferred to the appropriate radioactive nutrient solution, containing 3.6 $\mu\text{Ci/liter}$ $^{22}\text{Na}^+$ supplied as $^{22}\text{NaCl}$ in aqueous solution (Amersham Corp., Arlington Heights, Ill.). Conditions in the growth chamber were temperature $26 \pm 2^\circ\text{C}$, light intensity $550 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a 16-h photoperiod, and a relative humidity of 50%. Exposure to the labeled solution began immediately after the start of the light period. After 24 h, the plants were transferred into the corresponding unlabeled solution, and moved back into the greenhouse for the duration of the experiment.

Plants were harvested immediately following return to the greenhouse (hereafter referred to as day 0) and 1, 2, 4, 7, 14, and 28 days following the end of the uptake period. Unlabeled nutrient solutions were replenished every 7 days. Harvested roots were separated from shoots, rinsed for 2 min in distilled water, blotted dry, and weighed. Leaves were detached from woody and herbaceous stems, and each shoot component was weighed separately. Leaves were further subdivided into categories based on leaf position on the stem, including the growing tip and first two leaves

below the apex (hereafter referred to as growing tip), the 3rd-6th leaves below the apex, the 7th-12th leaves, the 13th-18th leaves, and the 19th-24th leaves. Roots, stems, and leaves were dried at 60°C for a minimum of 48 h, then ashed at 575°C for 18 h and subsequently counted for $^{22}\text{Na}^+$ using an automatic gamma counter (LKB Compugamma, Gaithersburg, Md.). Appropriate corrections were made for background radiation.

Root $^{22}\text{Na}^+$ efflux was determined by removing a 1 -ml sample of the unlabeled nutrient solution from the plants to be harvested on day 28 at 1, 2, 4, 7, 14, 21, and 28 days following the end of the uptake period. These samples were counted in the same manner as reported above.

Dried, ashed plant samples were then dissolved in 1 N HNO_3 , and total Na^+ was determined using an inductively coupled plasma emission spectrophotometer (Allied Research Laboratories, Sunland, Calif.).

Uptake, efflux, and translocation data are expressed as $\mu\text{mol } ^{22}\text{Na}^+$ and refer to the ions obtained from the labeled solution calculated from the tissue counts and the initial specific radioactivities in the uptake solution. The experiments were factorial, with Ca^{2+} treatments and harvest date as the factors. There were six single-plant replications. Experimental design was randomized complete block. Data were analyzed using the General Factorial ANOVA of the SPSS for Windows software package (SPSS, Chicago).

Results

After a few days of treatment, plants subjected to 25 mM NaCl ceased growing, and later some of them began to exhibit visible damage. A tan leaf necrosis, usually manifesting itself after 14 days of treatment, was the most distinctive response. Necrosis appeared on the older leaves first, usually at the point of the leaf blade, or occasionally at the margins. Necrosis progressed basipetally toward the petiole. Soon after the leaf blade died, the petiole died. In severe cases, the young leaves would appear partially necrotic by day 28 and some of the roots would appear necrotic as well. There was no visible difference in the extent of the necrosis across the treatments. We attribute the necrosis to excessive Cl^- , because a similar symptom appeared in some rabbiteye blueberry plants subjected to 100 mM NaCl in our previous work and did not appear in plants subject to 100 mM Na_2SO_4 (Wright et al., 1992).

On day 0, roots and woody stems accumulated large amounts of $^{22}\text{Na}^+$, which was not immediately translocated to the herbaceous tissue and was not apparent in the tissue on subsequent days. Determinations of $^{22}\text{Na}^+$ root efflux into the hydroponic solution showed a large peak on day 1. We attribute these phenomena to be the loss of $^{22}\text{Na}^+$ from the apoplast and not a true indication of $^{22}\text{Na}^+$ uptake or efflux. Only small amounts of $^{22}\text{Na}^+$ were effluxed from roots to the unlabeled solution after day 1, indicating little or no efflux of $^{22}\text{Na}^+$ by blueberry roots (data not presented). Total $^{22}\text{Na}^+$ loss from roots to the external solution accounted for <5% of the total $^{22}\text{Na}^+$ taken up. Because of the artificial efflux peak on day 1, root and woody shoot $^{22}\text{Na}^+$ concentrations for day 0 are not included in the statistical analysis.

Plants supplied with 10 mM Ca^{2+} accumulated significantly more $^{22}\text{Na}^+$ in the 3rd-18th leaves and in herbaceous stems than plants not supplied with Ca^{2+} (Table 1). Calcium supplementation led to 35% to 68% more $^{22}\text{Na}^+$ in these plant parts. These results suggest that there had been preferential translocation to the mature leaves in the presence of high levels of Ca^{2+} , but not to the growing tip. Calcium treatments had no other effect upon the $^{22}\text{Na}^+$ content of the shoot or root or upon total $^{22}\text{Na}^+$ content. The presence of 10

mM Ca²⁺ did not inhibit ²²Na⁺ uptake by roots, because total ²²Na⁺ content was not significantly influenced by Ca²⁺ levels. There were no interactions between Ca²⁺ and treatment duration.

Although root ²²Na⁺ content appeared to decline with time, this was not statistically significant over the duration of the experiment (Table 1). The ²²Na⁺ in woody stems was greatest after 2 days, but ²²Na⁺ content decreased progressively thereafter, ultimately by 53%. Herbaceous stem ²²Na⁺ content did not increase with time. Growing tips differed little in their ²²Na⁺ content with time, but the ²²Na⁺ content of leaves 3-6 and 13-18 increased by ≈ 150% over the duration of the experiment. There was no significant change in ²²Na⁺ content of leaves 7-12 and 19-24 with time.

Effects of Ca²⁺ on total Na⁺ content (Table 2) was similar to the effects of Ca²⁺ on ²²Na⁺ uptake and partitioning. Treatments with 10 mM Ca²⁺ led to significantly greater levels of Na⁺ in the herbaceous stems and leaves 3-12 and significantly less Na⁺ in the roots. There were significant interactions between Ca²⁺ and treatment duration upon Na⁺ content of the tip, leaves 7-12, and woody stems. A 10 mM Ca²⁺ supplementation led to greater Na⁺ content of the growing tip and woody stem only at 14 days and to greater Na⁺

content of leaves 7-12 from 14 days onward. Similarly, 10 mM Ca²⁺ addition also led to greater total plant Na⁺ content only at 14 days (Fig. 1).

Total Na⁺ content of shoots and roots increased throughout the experiment. Final Na⁺ content of roots and woody stems at 28 days was 6 to 7 times greater than the original content, while final Na⁺ content of herbaceous stems and leaves increased 12 to 13 times. There appeared to be a preferential translocation of Na⁺ to the herbaceous tissue. At day 0, herbaceous stems and leaves accounted for 30% of the total Na⁺ accumulation, whereas 28 days later Na⁺ in herbaceous tissue represented 45% of the total.

Blueberry plants supplied with 3 mM Ca²⁺ did not have greater leaf ²²Na⁺ content than those plants not supplied with Ca²⁺ (Table 3). The ²²Na⁺ content of all herbaceous growth, except the growing tip, increased over the 28-day duration of the experiment, while ²²Na⁺ content of woody stems and roots decreased.

Furthermore, 3 mM Ca²⁺ supplementation led to no significant main effects on total leaf, stem, or root Na⁺ content (Table 4). The Ca²⁺ × treatment duration interactions indicated that 3 mM Ca²⁺ supplements led to less Na⁺ in leaves 7- 18 at 28 days. Shoot and

Table 1. Influence of 25 mM NaCl and 0 and 10 mM Ca²⁺ treatments on ²²Na⁺ uptake and translocation in ‘Tifblue’ rabbiteye blueberry plants.

Treatment ^a	Root	Woody stem	Herbaceous stem	Growing tip	3rd–6th leaves	7th–12th leaves	13th–18th leaves	19th–24th leaves	Total
²² Na ⁺ content (μmol)									
Ca ²⁺									
0 mM	6.73	5.53	2.06	0.74	1.46	2.60	2.06	1.13	21.97
10 mM	5.26	4.71	2.79	0.74	2.44	4.38	3.03	1.85	24.73
	NS	NS	**	NS	***	***	***	NS	NS
Duration (days)									
0 ^b			4.01	0.25	2.07	3.06	1.94	0.68	
1	6.24	5.51	2.70	0.74	1.72	2.61	1.48	0.92	21.67
2	8.45	6.67	2.53	0.58	2.04	3.05	2.75	0.79	26.17
4	8.21	5.93	2.31	0.56	1.23	2.62	1.48	1.41	23.42
7	6.19	5.81	2.62	0.67	1.74	3.84	2.79	3.25	25.94
14	3.94	3.67	2.20	0.93	1.93	3.78	3.20	1.66	21.51
28	2.96	3.15	2.22	0.97	3.09	5.05	3.66	0.91	21.41
	NS	**	NS	NS	**	NS	***	NS	NS

^aTreatments include 25 mM NaCl and the Ca²⁺ concentration indicated.

^bValues for root, woody stem, and total ²²Na⁺ content at day 0 are not shown as they are artifacts (see discussion).

NS, *, **, ***Nonsignificant and significant F test with *P* = 0.10, 0.05, or 0.01, respectively.

Table 2. Influence of 25 mM NaCl and 0 and 10 mM Ca²⁺ treatments on total Na⁺ content in ‘Tifblue’ rabbiteye blueberry plants.

Treatment ^a	Root	Woody stem	Herbaceous stem	Growing tip	3rd–6th leaves	7th–12th leaves	13th–18th leaves	19th–24th leaves	Total ^b
Na ⁺ content (μmol)									
Ca ²⁺									
0 mM	1308	1300	312	285	306	337	292	230	4091
10 mM	1211	1301	330	279	317	391	346	273	4140
	*	NS	*	NS	*	**	NS	NS	NS
Duration (days)									
0	442	431	81	53	61	66	65	55	1175
7	623	641	134	108	118	151	135	141	1936
14	1364	1355	308	263	298	372	346	298	4291
28	2613	2703	745	681	786	842	736	626	9060
	***	***	***	***	***	***	***	***	***
Interaction significance									
Ca ²⁺ × day	NS	***	NS	*	NS	**	NS	NS	***

^aTreatments include 25 mM NaCl and the Ca²⁺ concentration indicated.

^bTotal Na⁺ contents include roots, stems, growing tips, and leaves 3-24.

NS, *, **, ***Nonsignificant and significant F test with *P* = 0.10, 0.05, or 0.01, respectively.

root Na^+ content increased throughout the duration of the experiment. Leaf and herbaceous stem Na^+ contents accounted for ~25% of the total plant Na^+ at the commencement of the experiment, and 45% of the total 28 days later.

Discussion

Most crop species that can be characterized as glycophytes avoid salinity damage by salt exclusion at the roots (Greenway and

Munns, 1980). Sodium exclusion can be effective at low external concentrations, but when salinity is high or when roots lack O_2 for respiration, Na^+ exclusion fails and appreciable amounts reach the leaves (Drew and Dikunwin, 1985; Drew and Läuchli, 1985). In some species, considerable amounts of Na^+ are translocated from the leaves back to the roots, and out to the external solution. Our research on rabbiteye blueberry suggests that efflux of Na^+ taken up in the first 36 h from roots back to the solution is minor; instead a large portion of Na^+ taken up into the roots of the blueberry plant

Fig. 1. Effect of Ca^{2+} supplements and treatment duration on total plant Na^+ content. The bars show SE.

Table 3. Influence of 25 mM NaCl and 0 and 3 mM Ca^{2+} treatments on $^{22}\text{Na}^+$ uptake and translocation in 'Tifblue' rabbiteye blueberry plants.

Treatment ^a	Root	Woody stem	Herbaceous stem	Growing tip	3rd-6th leaves	7th-12th leaves	13th-18th leaves	19th-24th leaves	Total
<i>²²Na⁺ content (μmol)</i>									
Ca^{2+}									
0 mM	6.96	9.51	3.80	1.98	2.90	4.88	2.70	1.81	33.86
3 mM	6.01	7.62	3.29	1.96	3.76	4.81	2.53	1.74	31.59
	NS	*	NS	NS	NS	NS	NS	NS	NS
Duration (days)									
0 ^y			5.60	0.76	1.59	2.23	1.33	0.31	
1	7.32	7.98	2.48	0.43	1.13	2.80	1.25	2.36	25.06
2	7.44	14.01	6.21	1.83	2.51	3.23	2.66	1.70	38.89
4	7.57	11.10	3.14	2.60	3.34	4.73	1.94	1.31	36.28
7	8.99	8.54	3.10	2.90	3.65	4.26	2.47	1.10	34.52
14	4.80	4.02	2.37	1.41	4.40	5.22	2.65	1.05	25.76
28	2.79	5.76	3.96	2.68	4.97	8.85	4.75	3.13	35.85
	*	***	**	NS	**	***	***	***	**

^aTreatments include 25 mM NaCl and the Ca^{2+} concentration indicated.

^yValues for root, woody stem, and total $^{22}\text{Na}^+$ content for day 0 are not shown as they are artifacts (see discussion).

NS, *, **, ***Nonsignificant and significant F test with $P = 0.10, 0.05, \text{ or } 0.01$, respectively.

Table 4. Influence of 25 mM NaCl and 0 and 3 mM Ca²⁺ treatments on total Na⁺ content in 'Tifblue' rabbiteye blueberry plants.

Treatment ^a	Root	Woody stem	Herbaceous stem	Growing tip	3rd-6th Leaves	7th-12th Leaves	13th-18th Leaves	19th-24th Leaves	Total ^b
<i>Na⁺ content (μmol)</i>									
Ca ²⁺									
0 mM	1259	1253	352	265	307	307	320	353	4215
3 mM	1213	1228	357	255	351	223	235	263	4016
	NS	NS	NS	NS	NS	NS	NS	NS	NS
Duration (days)									
0	647	624	73	64	68	66	63	63	1627
7	772	716	173	146	175	191	158	140	2396
14	1185	1201	269	236	284	323	258	265	3909
28	2290	2368	864	578	770	460	602	658	8315
	***	***	***	***	***	***	***	***	***
Interaction significance									
Ca ²⁺ × day	NS	NS	NS	NS	NS	**	*	NS	NS

^aTreatments include 25 mM NaCl and the Ca²⁺ concentration indicated.^bTotal Na⁺ contents include roots, stems, growing tips, and leaves 3–24.NS, *, **, ***Nonsignificant and significant F test with *P* = 0.10, 0.05, or 0.01, respectively.

was translocated to the herbaceous tissue within 24 h. Once in the herbaceous tissue, Na⁺ was not distributed evenly but accumulated in the mature leaves to a greater extent than in the younger tissue (Tables 1 and 2). Yeo and Flowers (1982) noted a similar distribution pattern in leaves of rice and suggested that the ion concentration gradient formed between younger leaves and older ones allows for the maintenance of sublethal Na⁺ concentrations in the younger leaves. Such a gradient may have been formed in leaves of blueberries not subject to 10 mM Ca²⁺.

Our previous studies showed amelioration of 25 mM Na₂SO₄ salinity in rabbiteye blueberries supplied with 10 mM Ca²⁺ (Wright et al., 1992; Wright et al., 1993), however, amelioration did not occur in NaCl salinized plants. We have attributed this difference to the toxic effect of Cl⁻. These results suggest that the effects of Cl⁻ may only be partially responsible for the lack of amelioration.

The failure of high (10 mM) concentrations of Ca²⁺ to alleviate the damaging effects of NaCl salinity in blueberry (see also Wright et al., 1992; Wright et al., 1993; Wright et al., 1994) is in direct contrast to investigations using other plant species. Calcium supplements, ranging from 0.1 to 30 mM, have been shown to counter the effects of greater applications of NaCl to bean (LaHaye and Epstein, 1969), barley (Rains and Epstein, 1967), maize, (Baligar, 1985), and cotton (Cramer et al., 1987). Our results indicate that 10 mM supplemental Ca²⁺ was not only ineffective in reducing Na⁺ content in leaves of 'Tifblue' plants treated with 25 mM NaCl, but led to greater ²²Na⁺ translocation to and increased total Na⁺ content in the mature leaves. This result may explain some of the results found previously. Conversely, supplementation with 3 mM Ca²⁺, which has been shown to potentially alleviate some of the effects of Na₂SO₄ salinity in some rabbiteye blueberry cultivars (Wright et al., 1992; Wright et al., 1993) while not causing a reduction of the Na⁺ content, did not lead to greater ²²Na⁺ translocation to the mature leaves or to greater Na⁺ content.

Any explanation for the inability of high supplemental Ca²⁺ concentrations to ameliorate Na⁺ damage must take into account the special metabolism of the blueberry. Because blueberries are calcifuges and are adapted to sandy, acidic soils with low Ca²⁺ concentrations, they are efficient in their Ca²⁺ uptake and their requirement for external Ca²⁺ is low. However, when a calcifuge is exposed to high soil Ca²⁺, it cannot regulate Ca²⁺ influx, thus, it accumulates excessive amounts (Ghorbal and Salsac, 1979; Salsac,

1980; Wacquant and Bouab, 1982).

Electrochemical potential gradients across the plasmalemma strongly favor passive entry of Ca²⁺ (Macklon, 1975). However, free cytosolic Ca²⁺ is very low (0.1 to 1 μM) (Haiech et al., 1986; Poovaiah, 1988; Gilroy et al., 1989). Low cytoplasmic Ca²⁺ concentrations are maintained by active efflux across the plasmalemma or into a cell organelle, such as the vacuole or the endoplasmic reticulum. Calcium efflux is via a Ca²⁺ ATPase, but there is evidence that there is very little Ca²⁺ ATPase activity in calcifuge species such as oat (Kylin and Sommarin, 1986; Monestiez et al., 1982) and lupine (Monestiez et al., 1982), so regulation of intracellular Ca²⁺ may be inadequate when external concentrations are high.

Among others, the role of Ca²⁺ as a second messenger requires that free Ca²⁺ be maintained at very low concentrations in the cytosol (Gilroy and Trewavas, 1990). Lauchli (1990) has proposed a cellular model by which Ca²⁺ as a second messenger plays a role in the regulation of plant response to salt stress. Challenge by Na⁺ would cause elevated Ca²⁺ concentrations in the cytosol, then changes in gene expression, metabolism, growth, and development. Sodium would likely be transported across the tonoplast and sequestered in the vacuole, or would be excluded from the cell. Under high salt stress, the model predicts that Ca²⁺ influx following stimulus would be inhibited, Ca²⁺ efflux and Na⁺ influx stimulated, and K⁺ leakage increased. In the case of salinized blueberry supplemented with high levels of Ca²⁺, these difficulties would be compounded by an inability to achieve a low Ca²⁺ resting level once the stimulus is removed. Failure of the second messenger system may be the cause of the inability of the blueberry to maintain a low, tissue Na⁺ concentration.

In conclusion, Ca²⁺ did not assist exclusion of Na⁺ by roots of blueberry exposed to NaCl. Instead, 10 mM Ca²⁺ led to a greater uptake of ²²Na⁺ into mature leaves. We suggest that in the calcifuge blueberry, high Ca²⁺ exacerbates the detrimental effects of Na⁺ on cell metabolism. Our results indicate that tissue Na⁺ levels are not increased when blueberry plants are subject to 3 mM Ca²⁺ levels. Plants supplied with 3 mM Ca²⁺ exhibited no additional uptake of ²²Na⁺, perhaps because of displacement of Na⁺ from exchange sites in the stem xylem walls by Ca²⁺. However, 3 mM Ca²⁺ did not reduce Na⁺ uptake or translocation. Levels as low as 1 mM may be necessary for maintenance of normal growth (Wright et al., 1992).

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