Tree Physiology 8, 47–57

Sodium and chloride distribution in salt-stressed *Prunus salicina*, a deciduous tree species

LEWIS H. ZISKA, THEODORE M. DEJONG, GLEN F. HOFFMAN and RICHARD M. MEAD

[1] *Department of Pomology, University of California, Davis, CA 95616, USA*

[2] *USDA-ARS, Water Management Laboratory, Fresno, CA 92727, USA*

Received February 2, 1990

Summary

Measurements were made over four growing seasons of the Na^+ and Cl^- content of leaves and woody tissues (twigs, branches, trunk and roots) of mature, fruit-bearing *Prunus salicina* Lindl. (on Marianna 2624 rootstock) trees irrigated during the growing season with water containing 3, 14 or 28 mM salt (2/1 molar ratio of NaCl and $CaCl_2$). At the beginning of the study, the trees were 19 years old. Woody tissues of trees irrigated with water containing 14 or 28 mM salt accumulated Na^+ and Cl^-. Leaves of trees irrigated with water containing 14 or 28 mM salt accumulated Cl^-, but not Na^+, unless they had visible symptoms of salt injury. X-Ray microanalysis of leaf mesophyll cells indicated some ability of the cells to sequester Cl^- in the vacuole. The data demonstrate a capacity for ion compartmentation among tissues and cell organelles in mature *Prunus salicina*, which may explain the ability of the species to survive low levels of salinity for several years in the field.

Introduction

In many plant species, functional capacities are seriously impaired when soil water salinity causes the internal concentrations of specific ions to exceed the optimal range for growth and development (Flowers et al. 1977, Greenway and Munns 1980, Lauchli and Epstein 1984). Some plants, however, avoid ion toxicity and maintain normal metabolism under saline conditions by compartmenting potentially damaging ions among tissues and cell organelles (Yeo 1983).

It has been shown that, in halophytic plants, excess Na^+ and Cl^- may be sequestered in leaf cell vacuoles, away from ion-sensitive cytoplasmic enzymes (Osmond and Greenway 1972, Harvey et al. 1981, Yeo 1981). Halophytes may also limit the toxic effect of accumulated ions by regulating ion transport among tissues in relation to plant development (Marschner et al. 1981, Kramer 1983, Yeo 1983).

In non-halophytic plants the extent of ion distribution is unclear. Significant intercellular ion compartmentation has been reported in spinach (Robinson et al. 1983), and there are data suggesting that other salt-sensitive plants have some control over ion uptake and compartmentation (Walker et al. 1981, Lauchli and Epstein 1984, Seemann and Critchley 1985). It should be noted, however, that the high concentrations of salt (100 mM, 20% seawater) used in many studies on salt tolerance could overwhelm any ability that the plants may have to exclude lower concentrations of damaging ions from important metabolic sites.

Little information exists on ion distribution in mature, fruit-bearing, deciduous tree crops. As the competition for available agricultural water intensifies, the quality of irrigation water is likely to decline. Increases in the concentrations of soluble salts (especially Na^+, Cl^- and Ca^{2+}) in the water supplied to tree crops that until now have received high quality water may adversely affect marketable yield. Based on the responses of young trees grown in pots or sand culture, deciduous tree crops have been classified as sensitive to salinity, especially chloride (Bernstein and Hayward 1958, Maas 1986). However, field observations on ion uptake and compartmentation within and among tissues under conditions of low or moderate salinity are essential to characterizing the salt tolerance of these economically important species.

In the present study we examined 19-year-old trees of *Prunus salicina* Lindl. (cv. Santa Rosa plum). The trees exhibited leaf damage and vegetative decline and some were dead. The injury or death was not associated with a seasonal decline in leaf water status (Hoffman et al. 1989, Ziska et al. 1989), but may have been related to ion toxicity. To evaluate organ and cellular ion distribution on a whole plant basis we attempted to determine: (a) the capacity of support structures (trunk and branches) to function as reservoirs of Na^+ and Cl^-; (b) the change in leaf concentrations of Na^+ and Cl^- during the season and between years; and (c) the ability of leaf mesophyll cells to compartmentalize Na^+ and Cl^-.

Materials and methods

Plants

A 2.3 ha orchard of plum, *Prunus salicina* Lindl. (cv. Santa Rosa) on *Prunus cerasifera* M.F. Ehrh. (Marianna 2624) rootstock planted in the spring of 1966 and located in the central San Joaquin Valley near Fresno, California, was irrigated with water of three different salinities (3, 14, and 28 mM salt) as described by Ziska et al. (1989) and Hoffman et al. (1989). Irrigation with saline water began in the spring of 1984 and continued throughout the growing season (April–October) of each year of the experiment. Salt water was obtained from a holding reservoir where NaCl and $CaCl_2$ were added in equal amounts on a mass basis. (The approximate molar ratio of applied NaCl to $CaCl_2$ was 2/1.) The 3 mM salt treatment represents normal irrigation water for the site and was used as the control.

Ionic tissue content

In each year of the experiment, fully expanded leaves were sampled at the beginning and end of the growing season (May–September). Samples consisted of 40 leaves from each of two trees in four replications of each salinity treatment. All samples were of mature, disease-free leaves located at the canopy periphery (2.5–3.0 m from ground level). In 1986 and 1987, sampled leaves were separated into two distinct morphological types. Spur leaves were defined as leaves produced early in the season from shoots that grew less than 30 cm. Shoot leaves were defined as fully expanded leaves from shoots that actively grew until mid-July. Shoot leaves had greater leaf

area and were well separated on the shoot. Both leaf types were initiated at the same time early in the growing season. In 1986 and 1987, both leaf types were sampled at approximately monthly intervals throughout the season. Leaf samples were dried for 48 h at 75 °C and then ground in a Wiley mill. Total nitrogen was determined by a modified Kjeldahl technique, chloride by coulombic titration, and Na^+ and K^+ by atomic absorption spectroscopy.

Woody tissue of roots, trunk, large branches, small branches and twigs was sampled on April 2 (before application of salt water) and on September 26, 1987 (near the end of the growing season) from two trees in two replications. Each sample was a composite taken from three different locations on the same tree. Tissue samples were characterized as follows: a) roots, both fibrous and woody, 1–3 mm and 1–2 cm in diameter, respectively, located within 45 cm of the soil surface; b) trunks, 60–80 cm in diameter, near ground level below the bud graft line; c) large branches, 25–30 cm in diameter and 1 m from ground level; d) small branches, 10–15 cm in diameter and 1.8–2.5 m from the ground; e) twigs, 1–3 years in age, 2–8 mm in diameter and 2.8–3.5 m from the ground.

Roots were sampled with a 60-cm soil corer, washed in distilled water to remove soil, and blotted dry. Cross-sectional samples of trunk and branch tissues were obtained with a hand-held auger with a 12-mm diameter bit. These tissues were separated into three categories: a) outer bark to a depth of 15 mm (which was assumed to contain only functional xylem and phloem); b) inner sapwood; and c) heartwood. Sapwood and heartwood were distinguished by color.

All wood samples were dried at 68 °C for 48 h and then ground in a Wiley mill. Ten ml of 2% acetic acid was added to each of the samples, which were then placed in a shaker for 1 h. Chloride was determined by chloridimeter analysis and sodium by atomic absorption spectroscopy.

Cellular distribution

The relative distribution of ions within cells was investigated by X-ray microanalysis (Pitman et al. 1981). For X-ray microanalysis, 2–4 leafy branches (1–2 cm in diameter, 2.5–3.0 m from ground level) from two replications of each treatment were transferred in a cold (0 °C), dark container to a scanning electron microscope laboratory. Undamaged leaves of the 28 mM Cl^--treated sample were separated from leaves showing visible damage as a result of salt stress. For each treatment, three to four small pieces of green leaf (0.5 cm^2) were inserted lengthwise into a copper sample holder containing 1–2 drops of a colloidal graphite slurry and frozen in liquid N_2. The copper holder was then transferred to a work chamber kept at −190 °C and a pressure of 0.1 torr. Transverse sections of the different leaves were prepared by fracturing with a cooled (−190 °C) surgical blade. A carrier was then threaded onto the copper holder and the assembly transferred to the cryostage of a scanning electron microscope (Hitachi S-800 field emission SEM with a KEVEX 3000 X-ray analyzer). Resolution of the cellular contents by scanning electron microscope images was sufficient to allow easy identification of chloroplasts and vacuoles in fully

hydrated cells with no indication of ice crystal formation during the freezing process.

X-Ray microanalyses of chloroplasts and vacuoles were made under the following conditions: accelerating voltage 10 kV; beam area 2–2.4 μm^2 raster; specimen temperature −190 °C; count rate 500 s^{-1}; incident angle 45°; working distance 15 mm. For an accelerating voltage of 10 kV, an estimated spatial resolution of 4–5 μm can be obtained (Pitman et al. 1981). Peak height to background (P/B) ratios of chloroplast/cytoplasm areas were determined with the beam rastered on an exposed or partially embedded chloroplast. Resolution of these chloroplasts (4–5 μm in diameter) was such that contamination by vacuolar or cell wall components was estimated to be less than 10%.

Results

Leaf ion content

A continuous increase in Cl^- concentration in spur leaves of trees grown in the presence of 28 mM salt was noted during the growing season of each year of the experiment (Figures 1 and 2). Visible symptoms of salt damage (i.e., leaves exhibiting chlorosis or necrosis) were not observed in the 28 mM salt treatment until the end of the 1986 season. At the end of the 1987 season, however, 90–100% of leaves collected from trees in the 28 mM treatment exhibited severe leaf damage. At the same time, leaf damage was first observed in trees growing in the presence of 14 mM salt.

Early in the season, Cl^- accumulated at different rates in spur and shoot leaves in the 28 mM treatment (Figure 2). By September however, Cl^- concentrations were similar in both leaf types (Figure 2). A similar pattern was observed in the 14 and 28 mM treatments in 1987 (data not shown). At 28 mM salt, a steady increase in leaf Cl^- was noted in both leaf types throughout the growing season (Figure 2). In contrast to Cl^-, a large increase in leaf sodium was noted only in the last two years of the experiment in the 28 mM treatment (Table 1). A 10-fold increase in leaf Na^+ was observed following visible symptoms of leaf damage (Table 2).

Although shoot leaves had a 20% higher leaf nitrogen concentration than spur leaves throughout the season regardless of treatment, neither leaf nitrogen nor potassium declined as a function of increased salinity in either leaf type (data not shown).

Ion content of woody tissues

In trees irrigated with saline water, woody tissues accumulated Cl^- (Figure 3). Chloride concentration increased with increasing salinity of the irrigation water in each type of woody tissue sampled (Figure 3). At high salinity, seasonal increases in Cl^- were also noted in each type of woody tissue.

Sodium was also retained in woody tissue as salinity increased (Figure 4). Given the 3/1 ratio of applied Cl^- to Na^+ ion, it appears that Na^+ was retained in the roots, trunk, and branches to a greater extent than Cl^- (cf. Figures 3 and 4). As Na^+ did not

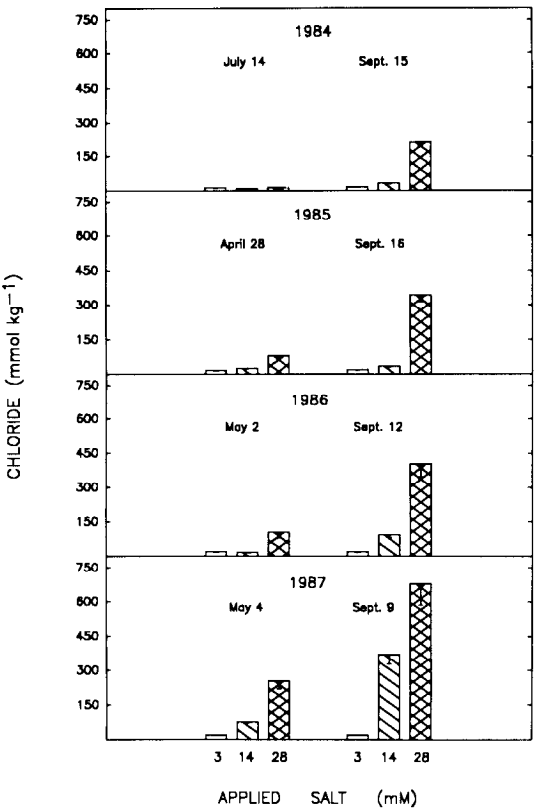

Figure 1. Cumulative seasonal increase in chloride concentration (mmol kg^{-1} dry weight) of spur leaves of *Prunus salicina* irrigated during the growing season with water containing 3 mM (control), 14 mM or 28 mM salt from 1984–1987. Vertical bars = SE. Visible symptoms of foliar injury were first observed in the 28 mM treatment at the end of the 1986 growing season.

accumulate in the leaves before symptoms of injury were visible, woody tissue appears to have been the primary repository of Na^+. The observed distribution of Na^+ between woody tissue and leaves is characteristic of a plant that excludes Na^+ from the leaves.

The concentrations of Cl^- and Na^+ in trunk and branches shown in Figures 3 and 4 are for the outermost 15 mm of tissue only, i.e., phloem and functional xylem. It was in this outer portion of the trunk and branches that Na^+ and Cl^- accumulated to the highest concentrations during irrigation with saline water. By comparison, the inner sapwood and heartwood accumulated concentrations of Na^+ and Cl^- that were only 25–35% and 5–10% as high, respectively. Although the relative volumes of woody tissue in each component of the tree were not determined, it is clear that the trunk and branches provided a much greater volume of tissue for ion retention than the small roots and twigs. Thus, despite higher concentrations of Na^+ and Cl^- in roots and twigs than in trunks and branches, the greatest quantity of these ions must have been in the trunk and branches.

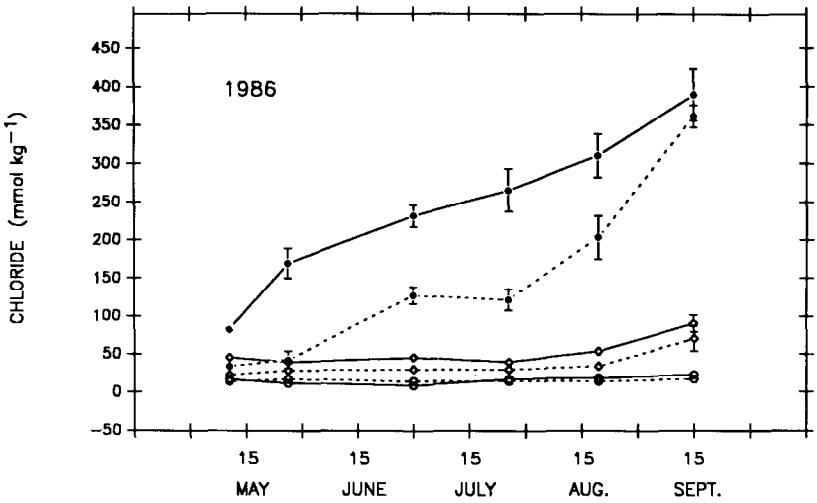

Figure 2. Cumulative increase during the 1986 season in chloride concentration (mmol kg^{-1} dry weight) for spur leaves (solid line) and shoot leaves (dashed line) of *Prunus salicina* irrigated with water containing 3 mM (O, control), 14 mM (◇) or 28 mM (●) salt. Spur leaves and shoot leaves are described in Materials and methods.

Table 1. Average Na^+ content (mmol kg^{-1} dry weight) of spur leaves of *Prunus salicina* grown at three different salinities. Measurements were taken in September of each year of the experiment.

Salt conc.	Year			
(mM)	1984	1985	1986	1987
3	*4.3* ± 0.1	4.0 ± 0.2	4.3 ± 0.2	4.1 ± 0.3
14	4.4 ± 0.1	4.3 ± 0.1	4.1 ± 0.3	39.1 ± 8.3
28	8.7 ± 0.3	17.4 ± 4.3	56.5 ± 8.6	196.6 ± 24.1

Table 2. Changes in Cl^- and Na^+ concentrations (mmol kg^{-1} dry weight) as a function of the presence of (+) or absence (−) of foliar damage, integrated over all salinity treatments for September 1986. (+/−) represents the highest Cl^- or Na^+ concentration found in the leaf that did not exhibit foliar damage.

Damage	Cl^-	Na^+
(−)	84.6 ± 14.3	4.3 ± 0.4
(+)	516.2 ± 53.6	82.6 ± 24.1
(+/−)	290.5 ± 55.4	8.7 ± 2.1

X-Ray microanalysis

The peak to background ratio (P/B) of each element (Cl^- or Na^+) from X-ray spectra of the vacuole (V) and chloroplast (C) is shown in Figure 5. For leaves that had no visible symptoms of injury, vacuole Cl^- increased more than chloroplast Cl^- with

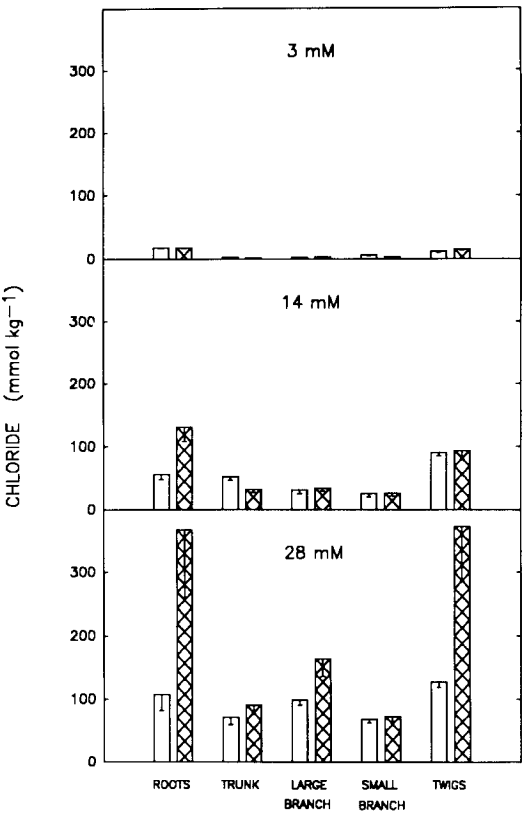

Figure 3. The mean concentration of chloride (mmol kg^{-1} dry weight) in woody tissues of *Prunus salicina* irrigated with water containing 3, 14 or 28 mM salt. Observations were made at the beginning (open bars, April 2) and end (shaded bars, September 26) of the growing season in 1987. Vertical bars = SE.

increased salinity (Figure 5a). However, large increases in both vacuole and chloroplast Cl^- were noted in leaves with visible foliar damage. Visible injury symptoms occurred before senescence. The data indicate a limited ability to compartmentalize Cl^- within the vacuole. The capacity for intracellular compartmentation of Cl^- declined with the appearance of visible symptoms of foliar damage.

Sodium did not demonstrate the large increases in P/B ratios seen for Cl^- (Figure 5b). Under control conditions, the vacuole P/B ratio was higher than the chloroplast for Na^+ (Figure 5b). In leaves with visible symptoms of injury, Na^+ in the chloroplast was the same as in the vacuole.

Chloroplast and vacuole P/B ratios for K^+ are shown in Figure 6. Potassium uptake appeared to balance the large increase in Cl^- observed in visibly damaged leaves.

Discussion

Although seasonal accumulation of leaf Cl^- is typical of tree and vine crops subjected

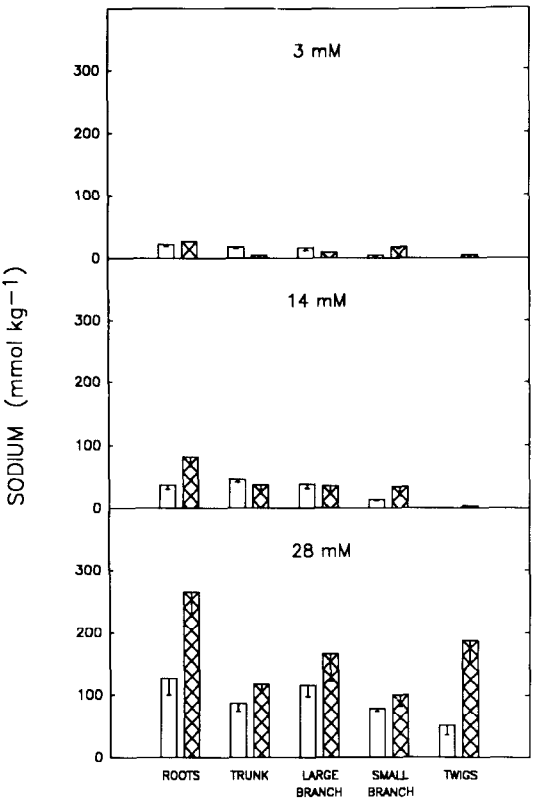

Figure 4. The mean concentration of sodium (mmol kg^{-1} dry weight) in woody tissues of *Prunus salicina* irrigated with water containing 3, 14 or 28 mM salt during the 1987 growing season. Observations were made at the beginning (open bars, April 2) and end (shaded bars, September 26) of the growing season in 1987. Vertical bars = SE.

to salinity (Wadleigh et al. 1951, Downton 1977, Walker et al. 1981), the concentration of Cl^- in leaves of mature, fruit-bearing *Prunus salicina* exposed to 28 mM (and eventually in trees subjected to 14 mM) salt increased from one season to the next. In addition, at high salinity, dissimilar leaf types in the same tree started each growing season with large differences in Cl^- concentration. These differences might be explained by retention of Cl^- in the wood during the winter, and by differences in the concentration of Cl^- in woody tissue of shoots producing spur leaves and shoot leaves.

In mature trees, wood represents a large proportion of plant volume. For mature *Prunus salicina*, wood thus provides a large volume of tissue for the accumulation of Na^+ and Cl^- when trees are salinized. Although woody tissue did not prevent the accumulation of Cl^- in the leaves, it apparently reduced or limited the total amount of Cl^- that the leaves took up. If Cl^- taken up by the trees during the season had not accumulated in the trunk and branches, it could have caused far greater increases in Cl^- concentrations in the leaves. The differences between leaf and woody tissues in

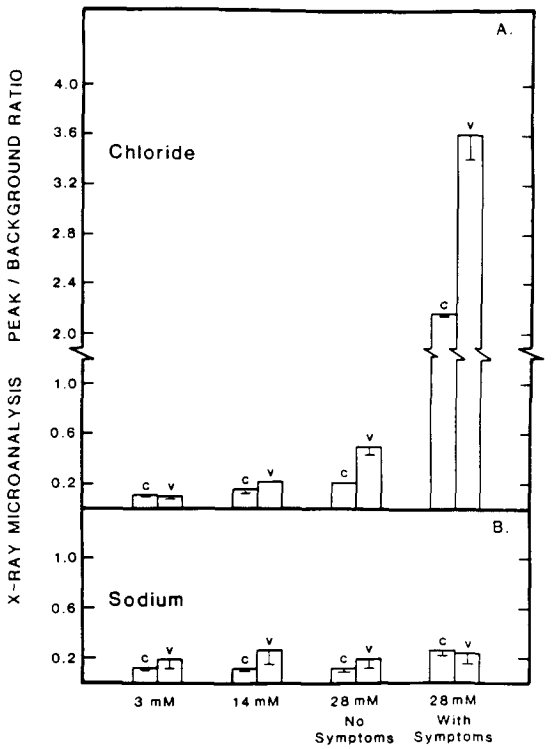

Figure 5. X-Ray microanalysis peak to background (P/B) ratios for chloride (A) and sodium (B) contained in mesophyll-chloroplast/cytoplasm and mesophyll vacuoles of leaf cells of *Prunus salicina* irrigated with water containing 3, 14 or 28 mM salt. Leaves with and without visible symptoms of injury were analyzed separately. Vertical bars = SE. The P/B vacuole to chloroplast ratios with increasing salinity are 0.82, 1.4, 2.4, and 1.7 for chloride, and 1.5, 2.3, 1.7 and 0.92 for sodium.

Na^+ concentration suggest a functional role of wood in preventing Na^+ accumulation in the leaf. The findings that Na^+ was excluded from the leaves and that leaf Na^+ concentrations increased after leaf damage occurred, suggest that Na^+, in addition to Cl^-, may be toxic to fruit crops. The mechanism by which Na^+ is excluded from the leaf is unclear. For annual plants, exchange of K^+ for Na^+ in roots or stems, or both, has been suggested as a mechanism controlling the rate of Na^+ transport to the leaf (Lauchli 1984).

The patterns of ion distribution between woody tissues and leaves observed in this experiment contrast with those reported earlier for young *Prunus salicina* trees on Marianna rootstock, which showed almost no uptake or retention of either Cl^- or Na^+ after exposure to 50 mM salt for 3 years (Bernstein et al. 1956). The data presented here support the suggestion of Bernstein (1980) that sensitivity to salinization in perennial plants increases with age. If true, information on the response of young, deciduous fruit trees may not be indicative of either the tissue ion distribution or yield response of mature, field-grown trees.

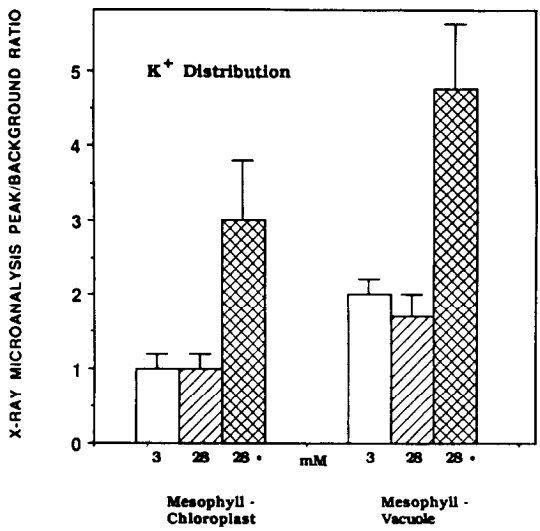

Figure 6. X-Ray microanalysis peak to background (P/B) ratios for K^+ contained in mesophyll-chloroplast/cytoplasm and mesophyll vacuoles of leaf cells of *Prunus salicina* irrigated with water containing 3 mM salt (open bars), or 28 mM salt. Leaves with (shaded bars) and without (cross-hatched bars) visible symptoms of injury were analyzed separately.

When ion concentrations reach damaging levels in the leaf, their subcellular distribution within photosynthetically active cells can determine which metabolic processes are affected (Jennings 1976). In this experiment, the ability of leaf mesophyll cells to sequester Cl^- in the vacuoles, away from the chloroplasts and cytoplasm, is consistent with the results obtained by Robinson et al. (1983) for spinach. Unlike spinach, however, the relatively high P/B ratios in *Prunus* chloroplasts suggest a moderate increase in chloroplast Cl^- with increased salinity, and a subsequent decline in compartmental efficiency.

Little change in the relative amounts of vacuolar and chloroplast Na^+ was noted as irrigation water salinity increased to 28 mM. When visible symptoms of injury occurred, however, a relative 2.6-fold increase in chloroplast Na^+ was observed compared to the controls. Lack of compartmentation of Na^+ (and Cl^- when visible damage occurs) may be related to an increase in the ratio of Na^+/Ca^{+2}, with possible consequences for membrane permeability (Lahaye and Epstein 1971, Greenway and Munns 1980, Cramer et al. 1985).

Although the effects of irrigation water salinity on ion partitioning among tissues and among cell compartments have been discussed separately, the two sets of responses should be not be viewed in isolation, but as aspects of the response of an integrated plant system. The duration of salinity exposure encountered by perennial glycophytes in the field may necessitate ion compartmentation at the organ, tissue or cell level to allow survival with substantial amounts of absorbed Na^+ and Cl^-. The results from this experiment and the recent experiments of Binzel et al. (1988) suggest that the ability to regulate ion transport and to control ion distribution at the

cellular level may also be features of non-halophytic plants.

References

Bernstein, L. 1980. Salt tolerance of fruit crops. USDA Agric. Information Bull. No. 292, 8 p.

Bernstein, L., J.W. Brown and H.E. Hayward. 1956. The influence of rootstock on growth and salt accumulation in stone-fruit trees and almonds. Proc. Amer. Soc. Hort. Sci. 68:86–96.

Bernstein, L. and H.E. Hayward. 1958. Physiology of salt tolerance. Ann. Rev. Plant Physiol. 9:25–46.

Binzel, M.L., F.D. Hess, R.A. Bressan and P.M. Hasegawa. 1988. Intracellular compartmentation of ions in salt-adapted tobacco cells. Plant Physiol. 86:607–614.

Cramer, G.R., A. Lauchli and V.S. Polito. 1985. Displacement of Ca by Na from the plasmalemma of root cells: a primary response to salt stress? Plant Physiol. 79:207–211.

Downton, W.J.S. 1977. Photosynthesis in salt stressed grapevines. Aust. J. Plant Physiol. 4:183–192.

Flowers, T.J., P.F. Troke and A.R. Yeo. 1977. The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol. 28:89–121.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in non-halophytes. Ann. Rev. Plant Physiol. 31:149–190.

Harvey, D.M.R., J.L. Hall, T.J. Flowers and B. Kent. 1981. Quantitative ion localization within *Suaeda maritima* leaf mesophyll cells. Planta 151:555–560.

Hoffman, G.J., P.B. Catlin, R.M. Mead, R.S. Johnson, L.E. Francois and D. Goldhammer. 1989. Yield and foliar injury responses of mature plum trees to salinity. Irrig. Sci. 10:215–229.

Jennings, D.H. 1976. The effect of sodium chloride on higher plants. Biol. Rev. 51:453–486.

Kramer, D. 1986. The possible role of transfer cells in the adaptation of plants to salinity. Physiol. Plant. 58:549–555.

Lahaye, P.A. and E. Epstein. 1971. Calcium and salt toleration by bean plants. Physiol. Plant. 25:213–218.

Lauchli, A. 1984. *In* Salinity Tolerance in Plants: Strategies for Crop Improvement. Eds. R.C. Staples and G.A. Toenniesson. Wiley, New York, pp 171–187.

Lauchli, A. and E. Epstein. 1984. Mechanisms of salt tolerance in plants. Calif. Agric. 38(10):18–20.

Maas, E.V. 1986. Salt tolerance of plants. Appl. Agric. Res. 1:12–26.

Marschner, H., A. Kylin and P.J.C. Kuiper. 1981. Differences in salt tolerance of three sugar beet genotypes. Physiol. Plant. 69:234–238.

Osmond, C.B. and H. Greenway. 1972. Salt responses of carboxylation enzymes from species differing in salt tolerance. Plant Physiol. 49:260–263.

Pitman, M.G., A. Lauchli and R. Stelzer. 1981. Ion distribution in roots of barley seedlings measured by electron probe X-ray microanalysis. Plant Physiol. 68:673–679.

Robinson, S.P., W.J.S. Downton and J.A. Millhouse. 1983. Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. Plant Physiol. 73:238–242.

Seemann, J.R. and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta 164:151–162.

Wadleigh, C.H., H.E. Hayward and A.D. Ayers. 1951. First year growth of stone fruit trees on saline substrates. Proc. Amer. Soc. Hort. Sci. 57:31–36.

Walker, R.R., E. Torokfalvy, N.S. Scott and P.E. Kriedemann. 1981. An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. Aust. J. Plant Physiol. 8:359–374.

Yeo, A.R. 1981. Salt tolerance in the halophyte *Suaeda maritima* L. Dum.: evaluation of the effect of salinity upon growth. J. Exp. Bot. 31:1171–1183.

Yeo, A.R. 1983. Salinity resistance: physiologies and prices. Physiol. Plant. 58:214–222.

Ziska, L.H., R.B. Hutmacher, G.J. Hoffman and T.M. DeJong. 1989. Evaluation of salinity induced osmotic stress for a mature field grown glycophyte, *Prunus salicina*. Physiol. Plant. 77:141–149.