Plant Physiol. (1990) 93, 864–870
0032-0889/90/93/0864/07/$01.00/0

Received for publication August 25, 1989
Accepted January 24, 1990

Salinity Induced Limitations on Photosynthesis in *Prunus salicina*, a Deciduous Tree Species[1]

Lewis H. Ziska*, Jeffrey R. Seemann, and Theodore M. DeJong

Department of Pomology, University of California, Davis, California 95616 (L.H.Z., T.M.D.); and Department of Biochemistry, University of Nevada, Reno, Nevada 89557 (J.R.S.)

ABSTRACT

The response of photosynthetic CO_2 assimilation to salinization in 19 year old *Prunus salicina* was evaluated under field conditions for a 3 year period. The observed decline in CO_2 assimilation capacity was apparently related to increasing leaf chloride (Cl^-) content, and independent of changes in leaf carbohydrate status. The response of net CO_2 assimilation (A) to leaf intercellular CO_2 partial pressure (C_i) indicated that the reduction in the capacity for A with Cl^- was not the result of decreased stomatal conductance but a consequence of nonstomatal inhibition. The nonstomatal limitations to CO_2 assimilation capacity, as determined by the response of A to C_i and biochemical assay, were related to a decline in the activity of ribulose 1,5-bisphosphate carboxylase (Rubpcase) and the pool size of triose phosphate, ribulose 1,5-bisphosphate (Rubp) and phosphoglycerate with increasing salinity. Lack of agreement between the initial slope of the A to C_i response curve and Rubpcase activity suggests the occurrence of heterogeneous stomatal apertures with the high salinity treatment (28 millimolar). Prolonged exposure to chloride salts appeared to increase the Rubp or Pi regeneration limitation, decrease Rubpcase activity and reduce leaf chlorophyll content. Observed changes in the biochemical components of CO_2 fixation may, in turn, affect total leaf carbohydrates, which also declined with time and salinity. The reduction in Rubpcase activity was apparently a consequence of a reduced Rubpcase protein level rather than either a regulatory or inhibitory effect.

As competition for available irrigation water intensifies, water quality is expected to decline with a subsequent reduction in crop productivity, especially among salt-sensitive species. The decline in productivity observed for many plant species subjected to excess salinity is often associated with a reduction in photosynthetic capacity. Although the factors which limit CO_2 uptake in salt-stressed plants have been investigated for a number of sensitive species (4, 14, 15, 17, 18), the mechanistic pattern of inhibition is unclear. Several studies have demonstrated that the decline in photosynthetic capacity with salt stress is partially the result of decreased stomatal conductance (5, 22, 28, 29). However, salinity has also been shown to inhibit nonstomatal factors associated with the biochemical efficiency of CO_2 assimilation (2, 5, 9, 22, 23). In contrast, other researchers have suggested that salinity reduces growth to a greater extent than photosynthesis, causing a subsequent carbohydrate buildup (either as starch and/or soluble sugars), which can be associated with a reduction in photosynthetic capacity (10, 11). Therefore, an investigation to effectively define limitations to the photosynthetic process as a result of increased salinity requires: (a) determination of the influence of salinity on leaf carbohydrate status and the potential for feedback inhibition, and (b) separation and quantification of the stomatal and nonstomatal factors which affect photosynthetic capacity. However, no study has examined both (a) and (b) for plants exposed to salinity under field conditions.

The experiments reported here were designed to analyze limitations to CO_2 assimilation in mature trees of *Prunus salicina* (L. cv Santa Rosa plum), acclimated to salinity under field conditions. Previous experiments with *P. salicina* in this location have shown that the decline in productivity associated with salinity was not related to a reduction in leaf water content or turgor potential (12, 30). Furthermore, measurements of the ion content of wood and leaf tissue, as well as cellular ion compartmentation, indicated a limited capacity of this species to regulate distribution of toxic ions (Cl^-) (31).

MATERIALS AND METHODS

Plants

A mature orchard (19 years old) of *Prunus salicina* (L. cv Santa Rosa), located in the central San Joaquin Valley near Fresno, CA, was exposed to irrigation water of four different qualities (3, 7, 14, and 28 mM salt) over a period of 3 years. Environmental conditions during the summers in this area are relatively consistent with respect to both light and temperature. The experimental field site consisted of a 2.3 ha orchard, planted in the spring of 1966 with 53 rows and 13 trees per row. The experimental design consisted of the four salinity treatments replicated five times in a randomized block. Each treatment plot contained two rows of trees and five trees per row. One tree row surrounded each plot and these trees served as the experimental border. Tree spacing was 5.6 × 6.4 m. Additional details are given in Ziska *et al.* (30). Micro-sprinkler irrigation of saline water began on March 15, 1984 and continued at regular intervals during the growing season (April through October) to maintain a high leaching fraction and uniform soil distribution of salinity for each year of the experiment. Salt water concentrations were obtained by adding NaCl and $CaCl_2$ in equal portions. Meas-

[1] This research was supported in part by the U.S. Department of Agriculture Research Grant Office under agreement No. 87-CRCR-1-2470 to J.R.S.

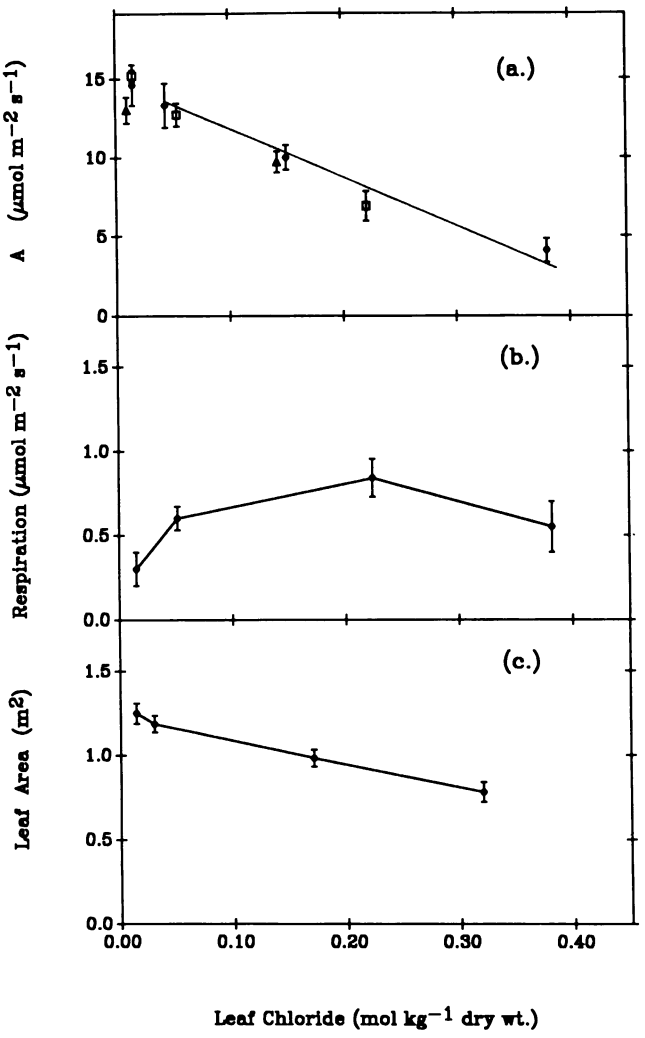

Figure 1. Relationship between leaf chloride and (a.) net CO_2 assimilation for intact leaves of *P. salicina* grown at 3 and 28 mM (1985, △); 3, 14, and 28 mM (1986, □); and 3, 7, 14, and 28 mM salt (1987, ●); and (b.) dark CO_2 respiration for intact leaves of *P. salicina* at 3, 7, 14, and 28 mM salt in 1987 (●). Gas exchange values in (a) and (b) were averaged for all sampling dates for a given year; (c), Cumulative leaf area for 500 leaves treatment^{-1} in *P. salicina* at 3, 7, 14, and 28 mM salt in 1987 (●). Vertical bars = SE.

urements used in these experiments were conducted on trees grown at 3, 14, and 28 mM salt ± 10%. Measurements were also taken on the 7 mM treatment in 1987. The 3 mM treatment represents the normal irrigation water quality for this area and was used as a control.

All leaves used for analysis or measurement in this experiment were obtained from 1 to 2 branchlets on two trees in two replications of each salinity treatment. Each branchlet consisted of 10 to 20 leaves and was tagged at the beginning of the experiment in 1984. These same branchlets were used throughout the experiment. Gas exchange measurements and biochemical analysis were made using adjacent leaves. Fresh and dry weight of leaves were determined following gas exchange measurements. Leaf area was determined using a leaf area meter (LiCOR LI 3000, Lincoln, NE) for a subsample of 500 leaves treatment^{-1} (100 leaves tree^{-1} × 5 replications) and in single leaves immediately after a gas exchange measurement. All leaves were disease free and located 3 to 4 m in height in the peripheral, sunlit portion of the tree canopy.

Carbohydrate Determination

Leaves were sampled mid-day (1300–1500 PST) on May 21 and 22 and August 5 and 6, 1987. Upon sampling, leaf tissue was immediately frozen in liquid N_2 and transferred to the laboratory for freeze drying. Freeze-dried leaf tissue (0.5–1.0 g) for each sampling date was bulked for analysis and ground in a Wiley mill. Soluble and insoluble carbohydrates were separated by extraction in boiling 80% ethanol for 6 h.

A 2 mL aliquot of the ethanol extract containing soluble carbohydrates was eluted through an ion exchange column (16), (with two 1 mL washes of distilled water) to separate phenolics and organic acids. The eluted volume was evaporated to dryness and the residue dissolved in 5 mL of deionized water. A 1-mL aliquot of this solution was added to 0.2 mL of an internal standard (0.013 M trehalose), and the solution was freeze dried to concentrate available sugars. The solution was sonicated for 20 min following addition of a silating compound, 1 mL heptane and 0.5 mL deionized water, then centrifuged for 5 min at 5000*g*. The supernatant was removed and dried with N_2 gas before addition of 100 mL heptane. An aliquot was then injected into a gas chromatograph (Shimadzu Corp., Tokyo, Japan) to identify and quantify individual sugars.

The dried, insoluble powder containing insoluble carbohydrates was used for starch determination. For each 200 mg sample, 1 mL saturated $Ca(OH)_2$ was added and the mixture autoclaved for 20 min. A 1.5 mL aliquot of Na^+-acetate (4.6 pH) was added and the mixture sonicated for 5 min. The mixture was then incubated overnight at 55°C with 5 mL of α-amylase (1 mg mL^{-1}, Sigma Chemical). The following morning the solution was centrifuged for 5 min at 5000*g* and the amount of glucose in the supernatant determined spectrophotometrically by a glucose diagnostic test (Sigma Chemical).

Gas Exchange

A mobile gas exchange laboratory (3) was used to determine leaf gas exchange in the field for each year of the experiment.

Table I. *Average Total Soluble and Insoluble Carbohydrates ± SE in Leaves of P. salicina Grown at Four Different Salinities in 1987*

Applied Salt Concn.	Total Soluble Carbohydrates		Total Insoluble Carbohydrates	
	5/21–22	8/5–6	5/21–22	8/5–6
mM	% dry wt			
3	13.9 ± 1.6	12.0 ± 0.3	15.9 ± 2.0	11.0 ± 3.5
7	13.2 ± 1.2	12.9 ± 0.6	15.5 ± 3.5	8.4 ± 0.9
14	12.0 ± 2.2	9.8 ± 1.5	14.3 ± 1.3	6.3 ± 2.0
28	11.2 ± 4.5	6.5 ± 1.1	22.5 ± 4.9	3.8 ± 1.1

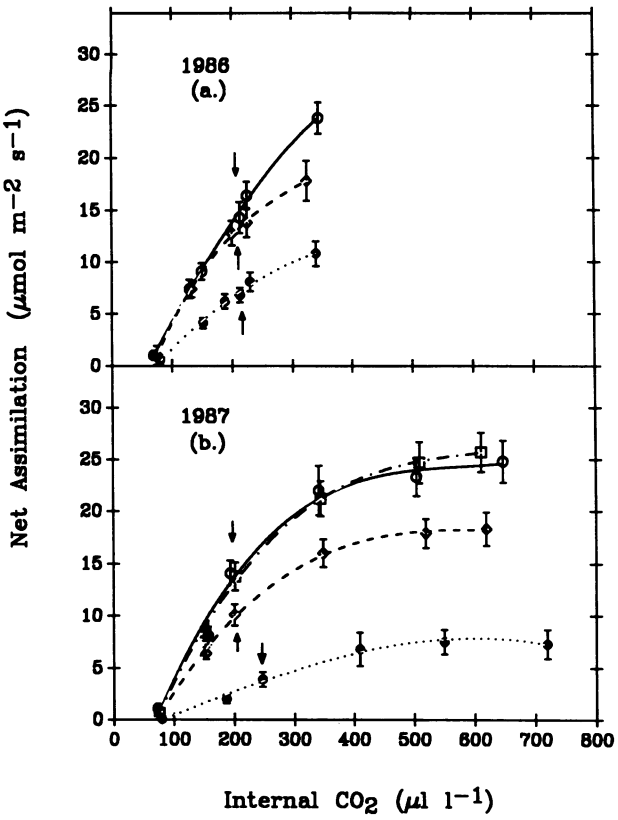

Figure 2. Response of net CO_2 assimilation (A) to changes in leaf internal CO_2 (C_i) for intact leaves of *P. salicina* exposed to (a.) 3 (○), 14 (◇), and 28 mM (●) salt in 1986, and (b.) 3 (○), 7 (□), 14 (◇), and 28 mM (●) salt in 1987. A and Ci values were averaged for all sampling dates during 1987. Vertical bars = SE. The arrows indicate the photosynthetic rate and C_i obtained at ambient conditions (350 ppm) of CO_2. Additional details on gas exchange are given in text.

Leaf-atmosphere differential measurements of CO_2 were made with an ADC MK III gas analyzer. Intact individual leaves were placed inside a cylindrical, well-stirred cuvette (3). All gas exchange measurements were made at full sunlight which varied between 1700 and 2000 μmol m^{-2} s^{-1}. Leaf temperatures were maintained at 32°C ± 2.0°C by circulating water from a water bath through a heat exchanger at the bottom of the cuvette. The vapor pressure deficit for the cuvette was maintained at ≤2.0 kPa by humidifying the gas stream and then dehumidifying to a known dewpoint temperature in a glass condenser placed within a water bath. Vapor pressure was measured with a RH sensor maintained at a known temperature.

For the determination of the response of A to C_i,[2] leaves were exposed to a range of CO_2 concentrations by supplying the assimilation cuvette with air from CO_2 tanks. Tanks with absolute concentrations of 85, 238, 310, 350 (ambient), 384, and 561 ppm CO_2 in air were used in 1986. In 1987 a broader range of CO_2 concentrations was used; 85, 238, 350 (ambient), 579, 850, and 1055 ppm CO_2. The response of A to C_i in various leaves was determined over a 2 to 3 week period in late May to early June and again in mid to late July in 1986 and 1987. Dark respiration was determined at ambient CO_2 for the same time period and treatments in 1987 by covering the chamber with aluminum foil until a steady state respiration rate was obtained. In 1985 CO_2 assimilation was determined at ambient conditions over a 1 week period in early June and for an additional 1 week period in late July. Although measurements of gas exchange were taken at two separate times during each season, the consistency of the CO_2 assimilation results (and for biochemical analysis as well) allowed pooling of all data within a given year.

Net photosynthesis, C_i, and transpiration were calculated from CO_2 and water vapor flux measurements as outlined by von Caemmerer and Farquhar (27). All CO_2 assimilation data were calculated on a leaf area basis.

Biochemical Determinations

A hand-held, copper-headed freeze clamp cooled to liquid N_2 temperature was used to punch out circular leaf discs in the field. This freeze-clamp technique is designed to rapidly stop the metabolism of an actively photosynthesizing leaf (<250 ms to 0°C following interruption of light). Leaf discs were collected in early June and late July 1987 from the 3, 7, 14, and 28 mM treatments following gas exchange measurements. Leaf segments were placed in 4 mL plastic containers and stored in liquid N_2 for biochemical analysis.

One-half of each leaf disc (3 cm^2) was extracted in ice-cold, CO_2-free 100 mM Bicine (pH 7.8), 5 mM $MgCl_2$, 0.1 mM EDTA, 2% PVP, and 10 mM DTT. The extract was quickly centrifuged and an aliquot of the supernatant assayed for 30 s at 25°C for Rubpcase activity. The assay buffer has been described previously (21). The procedure from extraction to assay required 3 to 4 min. The activity measured represents the *in vivo* activity (initial) of Rubpcase (21). An additional aliquot of this same extract was reassayed after incubation at 10 mM $NaHCO_3$ and 20 mM $MgCl_2$ for 10 min at 25°C. This activity represents the fully activated (total) amount of Rubpcase. All activities were corrected for the slower rate (5%) of fixation of $^{14}CO_2$ compared with $^{12}CO_2$.

Metabolite pool sizes were determined on the correspond-

Table II. *Average Percent Reduction in CO_2 Assimilation Due to Stomatal Limitation as Determined by A C_i Analysis for Leaves of P. salicina Grown at Different Salinities*

Applied Salt Concn.	Stomatal Limitation	
	1986	1987
mM	%	
3	39	34
7	NA[a]	35
14	28	37
28	37	31

[a] Data not available.

[2] Abbreviations: C_i, intercellular CO_2 partial pressure; PGA, P-glycerate; Rubp, ribulose 1,5-bisphosphate; Rubpcase, Rubp carboxylase (EC 4.1.1.39); A, net photosynthetic CO_2 assimilation; G1-P, glucose 1-P; F6-P, fructose 6-P; TP, triose P; FBP, fructose 1,6-bisP; g_s, stomatal conductance to water vapor.

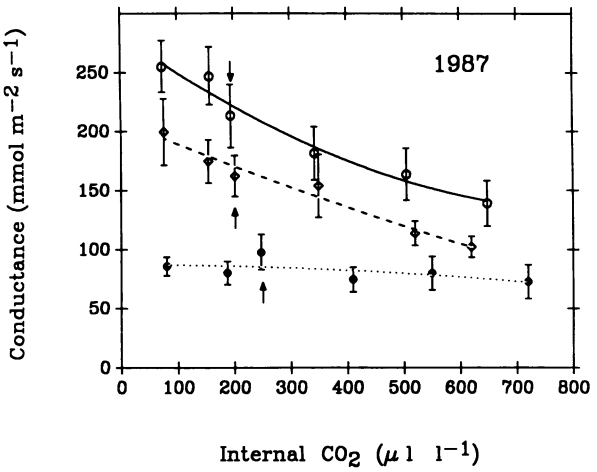

Figure 3. Changes in the response of leaf conductance to variations in intercellular CO_2 in leaves of *P. salicina* exposed to 3, 14, and 28 mM salt in 1987. Symbols are same as Figure 1b. Arrows indicate the C_i present at an ambient CO_2 concentration of 350 ppm. Vertical bars = SE.

ing half of the leaf disc stored in liquid N_2. Rubp, PGA, TP, Glc 6-P, Fru 6-P, and Fru 1,6-bisP levels were determined as described by Seemann and Sharkey (23). Chl content was determined as described by Arnon (1). Leaf chloride content was determined by chloridimeter analysis as described previously (31).

RESULTS AND DISCUSSION

Whole leaf photosynthetic CO_2 assimilation declined linearly when leaf chloride levels increased above 0.07 mol kg^{-1} dry weight (Fig. 1a). The reduction in assimilation was associated with increases in leaf Cl^- from 0.07 to 0.40 mol kg^{-1} dry weight. Increases in leaf Na^+ were not observed until Cl^- had exceeded 0.30 mol kg^{-1} dry weight (31). The sensitivity of leaf gas exchange to Cl^- was relatively consistent for each year of the experiment (Fig. 1a). The observed response of photosynthesis to leaf Cl^- in *Prunus salicina* is typical of other Na^+ excluding glycophytes (22).

A small portion of the decline in CO_2 assimilation (about 10%) may be attributed to a rise in leaf dark respiration associated with increasing leaf Cl^- levels (Fig. 1b). The increase in leaf dark respiration may, in turn, be related to the energy required for ion compartmentation and/or osmotic adjustment. A similar response of maintenance respiration to increased salinity (including the decline in respiration at the highest Cl^- concentrations) has been observed for a number of species (19).

Increased chloride concentration, in addition to reducing whole leaf assimilation, also reduced leaf expansion with a subsequent decline in leaf area and, presumably, whole plant photosynthesis (Fig. 1c). No significant increase in the leaf fresh weight to dry weight ratio was observed even between the 3 and 28 mM treatments (ratios of 3.06 ± 0.071 and 3.14 ± 0.092, respectively), suggesting no dilution of leaf salt concentration on a fresh weight basis with increasing salinity. In this experiment, stomatal control of water loss maintained RWC and ψ_L at or near control levels (29).

The decline in leaf area was not associated with an increase in stored carbon assimilate, however. Mean soluble and insoluble leaf carbohydrate content generally decreased with increasing salinity (Table I). This result is consistent with the decline in sucrose and starch reported by Downton (4) for salinized grape-vine cuttings. No increase in individual sugars was observed in *Prunus salicina* as a function of salinity (data not shown). Therefore, there is no evidence for carbohydrate feedback inhibition of CO_2 uptake in these experiments.

The response of assimilation (A) to C_i (Fig. 2) can be used to estimate the degree of stomatal limitation to net CO_2 uptake by comparing the assimilation rate at $C_i = C_a$ (350 ppm, infinite stomatal conductance) with assimilation at the operating C_i (see arrows in Fig. 2) (7, 13). This type of analysis indicates that the stomatal limitation was approximately 37% for the control plants and 34% for the 28 mM treatment when averaged for 1986 and 1987 (Table II). Similarly, if stomatal conductance (g_s) is examined over a range of C_i's for each salinity treatment in 1987, differences in the stomatal aperture at the operating C_i and at $C_i = C_a$ indicate no significant increases in the stomatal limitation of photosynthesis with increased salinity (17 and 22% stomatal limitation for the 3 and 28 mM treatment, respectively, Fig. 3). These results suggest that the decline in assimilation in response to salinity was not necessarily a consequence of an increased stomatal limitation on photosynthesis, but rather a result of a decline in the biochemical capacity for CO_2 assimilation.

The initial slope of the A to C_i response (Fig. 2, a and b)

Table III. *Comparison of the Initial Slope of the A to C_i Curve ± SE, Initial and Total Rubpcase Activity ± SE for Leaves of P. salicina Exposed to Four Different Salinities in 1987*

Data presented are the average of both sampling dates (June, July).

Applied Salt Concn.	Initial Slope A/C_i	% of Control	Initial Activity Rubpcase	% of Control	Total Activity Rubpcase	% of Control	Initial / Total (×100)
mM	μmol m^{-2} s^{-1}/ ppm CO_2		μmol m^{-2} s^{-1}		μmol m^{-2} s^{-1}		pc Act.
3	0.104 ± 0.007	(100)	18.9 ± 1.2	(100)	29.0 ± 1.8	(100)	65
7	0.100 ± 0.008	(96)	17.5 ± 1.3	(93)	25.8 ± 1.7	(87)	69
14	0.076 ± 0.004	(73)	13.6 ± 4.5	(72)	21.2 ± 1.9	(72)	64
28	0.023 ± 0.003	(22)	10.2 ± 0.7	(54)	16.1 ± 1.1	(55)	65

can be related to the *in vivo* specific activity of Rubpcase (20, 27). The initial slope of the response of A to C_i decreased with increased levels of salt and time of exposure (2a *versus* 2b). The decline in the initial linear response in 1987 (Fig. 2b) was consistent with a decrease in both the initial and total activity of Rubpcase except at the highest level of salt (Table III). The measurement of the response of A to C_i made *in situ* and the *in vivo* measurement provide independent estimates of the decline in Rubpcase activity associated with salinization.

The degree of carbamylation of the enzyme (initial activity/total activity × 100) was unaffected by any of the salt treatments (Table III), indicating that regulation of Rubpcase activity was unaffected by salinity treatment. Since the activation state of Rubpcase was not altered by salinity treatment, the observed reductions in total activity of Rubpcase probably reflect a reduction in the absolute amount of enzyme present. We conclude that the reduction in leaf CO_2 assimilation resulting from salinization of *P. salicina* was a consequence of a decline in the amount of Rubpcase per unit leaf area.

The correlation between *in vivo* and *in situ* estimates of Rubpcase activity was less evident at high salinity (28 mM) (Table III). While the initial slope of the response of A to C_i had declined to 22% of control levels, the total Rubpcase activity had decreased to only 55% of the control level (Table III). An overestimation of C_i and an underestimation of the initial response of A to C_i is likely if the distribution of stomatal pore size across the leaf surface was not uniform. Sharkey and Seemann (25) have recently demonstrated that mild water stress in *Phaseolus vulgaris* results in a nonhomogeneous distribution of stomatal apertures. Similarly, in *Plantago maritima* exposed to salt concentrations of 200 and 350 mol m^{-3} NaCl also resulted in nonuniform stomatal closure after 2 to 5 week period (8). Nonuniform stomatal closure has also been observed recently in ABA-treated leaves of a number of species (6, 24, 26). For ABA treated grapevine and sunflower, measured C_i determined from fluorescence measurements during photosynthesis were 30 to 80% of the C_i values obtained from gas exchange data (6). This is consistent with the ≈50% reduction in C_i necessary to adjust the initial A/C_i slope of *Prunus* at 28 mM salt so that it corresponds to the measured Rubpcase activity (Table III; Fig. 2b).

As the response of A to C_i increases beyond the initial slope region, A should be determined by the capacity to regenerate either Pi or Rubp (27). In this experiment, regeneration capacity appeared to decline both as a function of increased

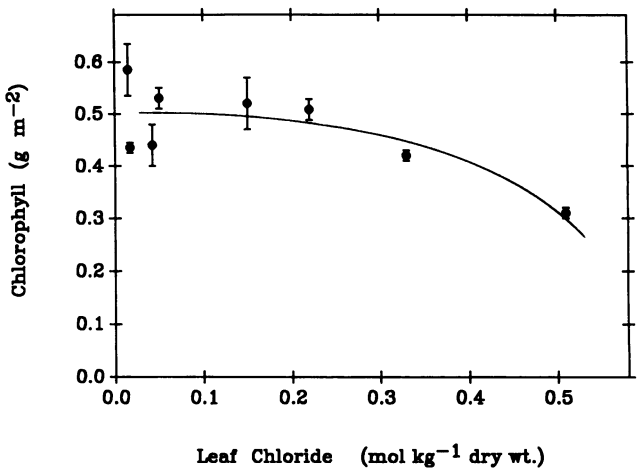

Figure 4. Relationship between internal leaf chloride and Chl concentration (on a leaf area basis) for leaves of *P. salicina* in 1987. Vertical bars = SE.

salinity and time of exposure (Fig. 2, a and b). Although Rubp pool size was reduced as a function of salinity (Table IV), Rubp pool size relative to total ribulose 1,5-bisphosphate carboxylase/oxygenase activity actually increased (2.57 *versus* 3.01 for the 3 and 28 mM salt treatments, respectively). This indicates that Rubp may have been consumed at a slower rate in relation to pool size in the salt-stressed plants.

PGA and TP levels also declined with increased salinity (Table IV). Other metabolites measured in this experiment showed no consistent change with increased salinization (Table IV). Chl *a/b* ratios remained constant at 3.8. However, leaf Chl content did decline after leaf chloride exceeded 0.25 mol Kg^{-1} dry weight (Fig. 4).

Although identification of biochemical changes indicates the nature of the nonstomatal reduction in photosynthesis, the observed sequences of these biochemical changes may also be used to establish the order of limitations to the photosynthetic process in *Prunus salicina* with increasing Cl^-. In 1986, the decline in photosynthesis for the 14 mM treatment (Fig. 1A) indicates a reduction in Rubp or Pi regeneration capacity (Fig. 2a). The following year a decline in Rubpcase activity, PGA and Rubp pool size were noted for this same treatment (Fig. 2b; Table IV). Reductions in the biochemical components of CO_2 assimilation may also be

Table IV. *Average Metabolite Pool Sizes of Key Intermediates in the Calvin Cycle (±SE) for Leaves of P. salicina Grown at Four Different Salinities in 1987*

Data presented are the average of both sampling dates (June, July).

Applied Salt Concn.	Metabolite					
	Rubp	PGA	TP	FBP	F6P	G6P
mM	μmol m^{-2}					
3	74.6 ± 8.6	33.4 ± 6.8	21.7 ± 2.1	8.3 ± 1.3	31.9 ± 4.2	51.0 ± 6.9
7	77.4 ± 3.6	30.0 ± 13.1	17.3 ± 3.8	6.9 ± 1.5	22.7 ± 4.0	40.0 ± 7.3
14	58.1 ± 4.0	24.7 ± 4.0	16.4 ± 3.3	7.5 ± 1.4	16.8 ± 2.0	28.9 ± 2.6
28	48.5 ± 6.2	21.0 ± 6.5	13.5 ± 2.5	9.1 ± 2.7	23.0 ± 3.6	37.0 ± 9.9

related to a net reduction in soluble and insoluble carbohydrates with increased salinity and time of exposure (Table I). Such changes in total carbohydrates may have negative consequences in regard to carbohydrate utilization and may partially explain the observed reductions in growth and reproduction reported previously for this experiment (12). Eventually, if leaf chloride levels exceeded 0.25 mol Kg^{-1} dry weight, a reduction in Chl content (Fig. 3), a loss of effective ion compartmentation, and visual leaf damage were observed (31).

Many of the specific effects of Cl^- on the functioning of the photosynthetic apparatus remain to be determined. Seemann and Sharkey (23) have suggested that the salinity-induced reduction in Rubp regeneration capacity may be associated with a chloride effect on the capacity for ATP formation. The reduction in the amount of Rubpcase protein (and probably other proteins) may imply an effect of salt at the level of transcription, translation or gene regulation. An additional factor may involve ion effects on the conformational structure (and ultimately function) of enzymatic proteins.

CONCLUSIONS

This investigation, indicated that the CO_2 assimilation capacity of leaves of salt-treated *Prunus salicina* grown in the field are highly sensitive to changes in leaf Cl^- content. This sensitivity is apparently not a result of carbohydrate feedback inhibition (10, 11), but is a direct effect of Cl^- on nonstomatal components of photosynthesis. Analysis of the response of A to C_i *in situ* and measurement of Calvin cycle intermediates *in vivo* demonstrate that these nonstomatal factors are related to reductions in Rubpcase activity and levels of triosephosphate and phosphoglycerate. Of the nonstomatal factors studied, the initial response to excess leaf Cl^- suggests a decline in Rubp or Pi regeneration capacity followed by a decline in the initial activity of Rubpcase. In this experiment, nonhomogeneous stomatal closure appeared to be associated only with the most extreme salinity treatment. Changes in the biochemical capacity of net CO_2 uptake may reduce total carbohydrate content with subsequent effects on carbohydrate utilization. Additional details concerning the mechanism of Cl^- toxicity on the photosynthetic process in the field grown plants however, merit further attention.

LITERATURE CITED

1. **Arnon DI** (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15
2. **Ball MC, Farquhar GD** (1984) Photosynthetic and stomatal response of the grey mangrove, *Avicennia marina* to transient salinity conditions. Plant Physiol **74:** 7–11
3. **DeJong TM** (1986) Fruit effects on photosynthesis in *Prunus persica*. Physiol Plant **66:** 149–153
4. **Downton WJS** (1977) Photosynthesis in salt stressed grapevines. Aust J Plant Physiol **4:** 183–192
5. **Downton WJS, Grant WJR, Robinson SP** (1985) Photosynthetic and stomatal responses of Spinach leaves to salt stress. Plant Physiol **77:** 85–88
6. **Downton WJS, Loveys BR, Grant WJR** (1988) Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. New Phytol **108:** 263–266
7. **Farquhar GD, Sharkey TD** (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol **33:** 317–345
8. **Flanagan LB, Jefferies RL** (1989) Effect of increased salinity on CO_2 assimilation and the $\delta^{13}C$ valves of leaves of *Plantago maritima* L. developed at low and high NaCl levels. Planta **178:** 377–384
9. **Gale J, Kohl HC, Hagan RM** (1967) Changes in the water balance and photosynthesis of onion, bean and cotton plants under saline conditions. Physiol Plant **20:** 408–420
10. **Gauch HG, Eaton FM** (1942) Effect of saline substrate on hourly levels of carbohydrates and inorganic constituents of barley plants. Plant Physiol **17:** 347–365
11. **Greenway H, Munns R** (1980) Mechanisms of salt tolerance in non-halophytes. Annu Rev Plant Physiol **31:** 149–190
12. **Hoffman GJ, Catlin PB, Mead RM, Johnson RS, Francois LE, Goldhamer D** (1988) Initial response of mature plum trees to salinity. Irrig Sci **10:** 215–229
13. **Hutmacher RB, Krieg DR** (1983) Photosynthetic rate control in cotton: stomatal and nonstomatal factors. Plant Physiol **73:** 658–661
14. **Kingsbury RW, Epstein E, Pearcy RW** (1984) Physiological responses to salinity in selected lines of wheat. Plant Physiol **74:** 417–423
15. **Longstreth DJ, Nobel PS** (1979) Salinity effects on leaf anatomy: consequences for photosynthesis. Plant Physiol **63:** 700–703
16. **Redgwell RJ** (1980) Fractionation of plant extracts using ion-exchange sephadex. Anal Biochem **107:** 44–50
17. **Richardson SG, McCree KJ** (1985) Carbon balance and water relations of Sorghum exposed to salt and water stress. Plant Physiol **79:** 1015–1020
18. **Robinson SP, Downton WJS, Millhouse JA** (1983) Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. Plant Physiol **73:** 238–242
19. **Schwarz M, Gale J** (1981) Maintenance respiration and carbon balance of plants at low levels of sodium chloride salinity. J Exp Bot **32:** 933–941
20. **Seemann JR, Berry JA** (1982) Interspecific differences in the kinetic properties of Rubpcase protein. Carnegie Inst Wash Year Book **81:** 78–83
21. **Seemann JR, Badger MR, Berry JA** (1984) Variations in the specific activity of ribulose 1,5-bisphosphate carboxylase between species utilizing differing photosynthetic pathways. Plant Physiol **74:** 791–794
22. **Seemann JR, Critchley C** (1985) Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris*. Planta **164:** 151–162
23. **Seemann JR, Sharkey TD** (1986) Salinity and nitrogen effects on photosynthesis, ribulose 1,5-bisphosphate carboxylase and metabolite pool sizes in *Phaseolus vulgaris*. Plant Physiol **32:** 555–560
24. **Seemann JR, Sharkey TD** (1987) The effect of abscisic acid and other inhibitors on photosynthetic capacity and the biochemistry of CO_2 assimilation. Plant Physiol **84:** 969–700
25. **Sharkey TD, Seemann JR** (1988) Mild water stress effects on carbon-reduction-cycle intermediates, Rubp carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. Plant Physiol **89:** 1060–1065
26. **Terashima I, Wong SC, Osmond CB, Farquhar GD** (1988) Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. Plant Cell Physiol **29:** 385–394

27. **Von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387
28. **Walker RR, Torokfalvy E, Scott NE, Kriedemann PE** (1981) An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. Aust J Plant Physiol **8:** 359–374
29. **Walker RR, Torokfalvy E, Downton WJS** (1982) Photosynthetic responses of the citrus varieties Rangpur Lime and Etrog Citron to salt treatment. Aust J Plant Physiol **9:** 783–790
30. **Ziska LH, Hutmacher RB, Hoffman GJ, DeJong TM** (1988) Evaluation of salinity induced osmotic stress for a mature, field grown glycophyte, *Prunus salicina*. Physiol Plant **77:** 141–149
31. **Ziska LH, DeJong TM, Hoffman GJ, Mead RM** (1990) Distribution and accumulation of Na and Cl in cells, leaf and wood for *Prunus salicina* in situ. Tree Physiol (in press)