Effect of Phytophthora Root Rot on Na^+ Uptake and Accumulation by Safflower

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ABSTRACT

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Experiments with healthy and *Phytophthora cryptogea* infected safflower plants grown in nutrient solution containing 0, 50, or 100 meq L^{-1} of $NaCl/CaCl_2$ (in a 10:1 equivalent ratio) showed that the concentration of Na^+ in leaf tissue after 35 days was positively correlated ($P < 0.001$) with root rot severity. Relative to healthy controls, foliar Na^+ concentration was significantly higher in plants with ≥40% root infection. Pathogen-induced changes in Na^+ transport were investigated in various accumulation and efflux experiments. After pulse exposures of 1–60 min, the rate of $^{22}Na^+$ accumulation and the final concentration in root and shoot tissues of diseased plants were significantly ($P < 0.01$) higher than in healthy plants. When roots were incubated in $^{22}Na^+$ solutions and then immersed in unlabeled, iso-osmotic solution, healthy roots lost 7–10% of accumulated $^{22}Na^+$ after 10 h, whereas diseased roots had a significantly higher rate of efflux and lost up to 55%. The efflux experiments suggested that increased Na^+ uptake and accumulation by diseased plants was the result of *Phytophthora*-induced changes in root permeability to Na^+.

Additional keywords: biological stress, *Carthamus tinctorius*, root disease, salt stress.

Approximately one-third of the earth's irrigated cropland is considered saline (11), and salinity problems continue to increase. In California, the Imperial, Coachella, and southern San Joaquin valleys are considered critically affected by salinity (1), and most crop plants are regarded as "sensitive" or "moderately sensitive" to soil salts. Many approaches have been taken to try to minimize the impact of salinity on crop productivity, including efforts to breed plants with higher levels of salt tolerance (11,23).

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Most crop plants are categorized as glycophytes (plants that osmotically adjust by producing organic solutes). Glycophytes often are designated "salt excluders," because Na^+ transport to foliar tissues is limited. This limited transport is accomplished by several mechanisms. Roots may preferentially take up K^+, which is less toxic than Na^+. Sodium may be compartmentalized within root vacuoles or accumulated within the cells of organs (i.e., stems) least affected by Na^+ (10). Sodium may be retranslocated from leaves to roots (20) and extruded back to the rhizosphere (16). Substantial evidence indicates that selective transport, compartmentation, and efflux of Na^+ are intrinsic, permease-supported functions of membrane systems (15,20,21).

Root rots caused by *Phytophthora* spp. are economically important throughout the world and cause damage to many agronomic and horticultural crops. One impact of *Phytophthora* infection is the loss of selective permeability by root cell membranes. This has been demonstrated through electron micrographs showing membrane fragmentation (27), a report of increased electrolyte leakage from *P. cinnamomi* infected eucalyptus roots (6), and a demonstration that *Phytophthora* infection increases root respiration through an apparent uncoupling of phosphorylation (5).

Because *Phytophthora* infection causes both physical and physiological damage to root membranes, this pathogen could impair root functions that maintain favorable K^+/Na^+ ratios, compartmentalize Na^+ within cells, or limit Na^+ transfer to aboveground tissues. Thus, although plants can tolerate various levels of root disease without adverse effects, *Phytophthora* infection could predispose plants to salt injury at soil salinity levels that otherwise could be tolerated. Some evidence supports this hypothesis (14), but no thorough, quantitative studies of the impacts of root infection on salt regulation have been done, even though plant roots exposed to salinity can be predisposed to *Phytophthora* infection (2,3,18,19,24,25).

The objective of this study was to quantify the impact of Phytophthora root rot on plant-Na^+ relations. Phytophthora root rot of safflower was used as a model system in these experiments, because safflower is both moderately salt-tolerant and susceptible to Phytophthora root rot.

MATERIALS AND METHODS

Inoculum preparation. An isolate of *Phytophthora cryptogea* Pethybr. & Lafferty (isolate PCR1, obtained from J. M. Duniway, University of California, Davis), which originated from diseased safflower, was used in all experiments. Zoospore inoculum was produced by incubating 1–4-cm^2 pieces of mycelium cut from the surface of 5- to 7-day-old V8 juice agar cultures. These pieces were placed in petri dishes that contained 12–18 ml of sterilized soil extract (18). The soil extract was changed every 3 days. After 7 days, zoospore release was stimulated by chilling the petri dishes in an incubator at 5 C for 1–2 h, followed by rewarming to room temperature (22–26 C) for 30–45 min. Zoospores were separated from mycelial mats by filtration through cheesecloth and were quantified with a hemacytometer.

Long-term $^{23}Na^+$ accumulation. Nonradioactive Na^+ ($^{23}Na^+$) was used in experiments to quantify Na^+ accumulation in plants exposed to salt and root infection over long periods. These experiments were done in a greenhouse where temperatures varied between 24 C (day) and 18 C (night), and light intensity (measured with a LI-COR model LI-190SB quantum sensor, Li-cor, Lincoln, NE) averaged 1,700 $\mu E\ s^{-1}\ m^{-2}$ at the plant canopy at noon. A 12–14-h photoperiod was maintained by using supplemental lights when necessary.

The safflower (*Carthamus tinctorius* L.) cv. Gila was used in all experiments. Seeds were germinated in trays of vermiculite irrigated with solutions of $NaCl/CaCl_2$ (10:1 equivalent ratio) that had total salt concentrations of 0 (deionized water), 50, or 100 meq L^{-1}. Containers were leached periodically to minimize salt accumulation. Ten days after emergence (DAE), seedlings were transferred to 1.8–2.0-L ceramic crocks containing aerated 50% Hoagland's solution (12). The nutrient solutions were amended with $NaCl/CaCl_2$ (10:1 equivalent ratio) as needed to provide three salt treatments: 0 (unamended nutrient solution), 50, or 100 meq L^{-1}. The electrical conductivities of these solutions were 0.7–1.0, 5.5–6.0, and 9.2–10.7 dS m^{-1}, respectively. Seedlings were assigned to salt treatments corresponding to the germination salt treatments. Two seedlings were placed in each crock, and each crock was considered an individual experimental unit. A total of 24 experimental units were in each salt treatment, and all crocks were arranged randomly on the greenhouse bench.

Five days after plant transfer (15 DAE), some solution was removed so hypocotyls were 3–4 cm above the solution surface, and 0, 500, or 5,000 zoospores were pipetted into each crock. The low solution levels confined infection to the roots and prevented potentially lethal hypocotyl infections. Each salt $\times$ inoculum treatment combination was replicated with eight crocks. Crocks again were arranged in a completely randomized design on a greenhouse bench. Five days after inoculation (20 DAE), all nutrient solutions were replaced with fresh solutions at the same salt concentration and were restored to preinoculation levels. Thereafter, solution levels were maintained with daily additions of deionized water, and solutions were changed every 7 days.

Experiments were terminated 26 days after inoculation (41 DAE). Because the harvest required approximately 2 days to complete, experimental units were harvested randomly. Disease severity for each experimental unit was determined by removing approximately 10 g (fresh wt) of root tissue, which was dispersed in water in a 14-cm-diameter petri dish. The petri dish was placed over a grid, and the total root length and necrotic root length were calculated for each sample (26). These measurements were used to estimate the proportion of necrotic roots in each experimental unit.

The oldest four true leaves were removed from both plants in each experimental unit and combined into a single sample. Leaf samples collected from each experimental unit were air-dried and ground in acid-washed mortars. The samples then were dried to constant weight at 90 C and placed into screw-cap vials. The Na^+ concentration in each sample was determined by boiling 0.20–0.60 g of the dried tissue in 3 ml of concentrated nitric acid until tissue dissolved. The digestant was clarified by dropwise addition of 9 ml of 30% hydrogen peroxide and was boiled for approximately 1 h. Each extract then was diluted to 50 ml with purified water (MilliQ, Millipore Corporation, Concord, CA). Aliquots (1.0 and 0.5 ml) of the extracts were diluted 30- and 60-fold, respectively, and analyzed for Na^+ by atomic absorption spectrophotometry (Perkin Elmer 360, Perkin Elmer, Norwalk, CT). To increase the tissue available for analysis, the entire experiment was repeated once with three plants per experimental unit rather than two.

Short-term $^{22}Na^+$ uptake. Short-term uptake and efflux experiments were conducted in growth chambers (Model E8VH, Controlled Environments Ltd., Winnipeg, Manitoba, Canada). All plants were started and established in a "clean chamber" and then transferred to a "containment chamber" for experiments involving radionuclides. Temperatures in both chambers were set to 25 C (day) and 20 C (night), and daylengths were 14 h. Light intensity at the plant canopy was 550 $\mu E\ s^{-1}\ m^{-2}$.

Seeds were germinated in quartz sand irrigated with deionized water. Five days after emergence (5 DAE), 13 seedlings were placed in each of eight plastic tubs containing 3,500 ml of 50% Hoagland's solution. Each tub had a plastic lid into which a series of holes had been drilled. Seedlings were placed in individual styrofoam holders and inserted into the holes in the plastic lid; roots were suspended in the nutrient solution. Five days after transfer (10 DAE), plants in four of the tubs were inoculated by replacement of the nutrient solution with 3,500 ml of soil extract containing 5×10^3 zoospores per milliliter. Plants in the remaining four tubs were treated similarly, but the soil extract did not contain zoospores. After 16 h, solutions in all containers were replaced with fresh 50% Hoagland's solution.

Three days after inoculation (13 DAE), plants were transferred to individual containers (250-ml plastic drinking cups) containing 50% Hoagland's solution amended with $NaCl/CaCl_2$ (10:1

equivalent ratio). To minimize osmotic shock, salt levels were increased in two increments: 25 meq L^{-1} for the first 24 h, followed by exposure to the final 50 meq L^{-1} treatment. During exposure to the 50 meq L^{-1} treatment, plants were paired into experimental units. Each experimental unit consisted of two diseased or two healthy plants. Healthy, noninoculated plants were paired at random. Diseased plants first were assessed visually for root rot severity, and only plants with 30–70% root rot were paired into experimental units. The desired number of plants with the desired level of infection was not always available, so the experimental design was unbalanced.

Radio-labeled solutions were prepared by adding 7.4–11.1 MBq $^{22}Na^+$ (Amersham, Arlington Heights, IL) as aqueous NaCl to 190 ml of half-strength Hoagland's solution amended with an additional 50 meq L^{-1} of $NaCl/CaCl_2$ (10:1). The specific activity of these solutions was 569 ± 14 dpm μM^{-1} Na^+. Healthy and diseased plant pairs were removed from the 50 meq L^{-1} nutrient solutions and immersed in $^{22}Na^+$-amended solutions for 1, 5, 10, 15, 30, or 60 min. When exposure times exceeded 5 min, solutions were aerated. Two to three plant pairs (depending on availability) were used for each exposure duration.

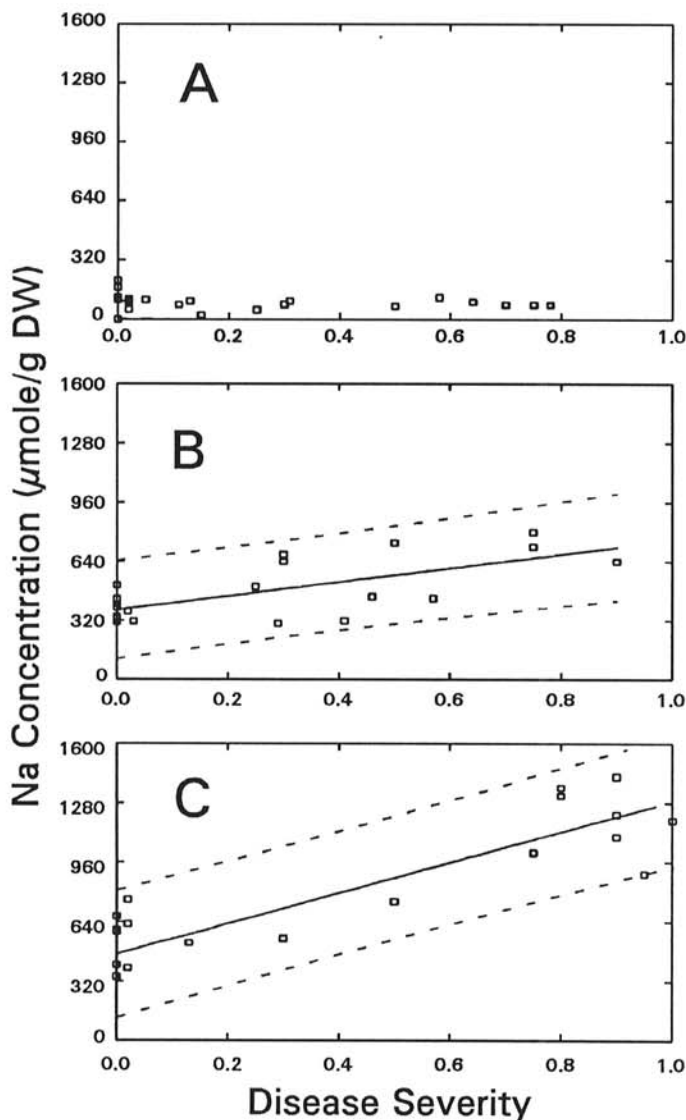

Fig. 1. Influence of Phytophthora root rot severity on sodium concentration (μmol g^{-1} dry wt) in safflower. Disease severity shown as the fraction of the root system infected. Plants were continuously exposed to **A,** 0; **B,** 50; or **C,** 100 meq L^{-1} of $NaCl + CaCl_2$ in hydroponic culture. Solid lines are regession lines, and dotted lines represent 95% confidence intervals for the regression lines. Regression parameters are presented in Table 1.

After exposure, roots were immersed in cold (2–5 C), nonlabeled 50 meq L^{-1} salt solution to rinse $^{22}Na^+$ from the root free-space. Roots were rinsed twice, blotted on absorbent paper pads, and separated into roots and shoots by cutting above and below the foam holder. A 2.5-cm portion of the hypocotyl (the tissue within the foam holder) was discarded. Root and shoot fresh weights were determined for each plant in each experimental unit. Root and shoot tissues were placed in separate glass scintillation vials, dried for 1 h at 100 C, ashed for 4 h at 550 C, and suspended in 1 ml of distilled water and 15 ml of scintillation cocktail (Scintiverse E, Fisher Scientific, Springfield, NJ). Sample activity was measured in a liquid scintillation counter (model LS100C, Beckman, Irvine, CA) after ashes had settled. Accumulation and net influx rates were calculated for shoots and roots on a per fresh weight of root basis. The entire experiment was conducted four times.

$^{22}Na^+$ efflux. Seeds were germinated in sand and transferred to a tub containing 50% Hoagland's solution as described above. Five to seven days after transfer to the tub (10–12 DAE), nine to 12 plants were placed individually in 30-ml syringe barrels held upright in a ringstand and filled with aerated nutrient solution. A stopcock valve was mounted on the syringe tip to prevent solution drainage when closed and to allow rapid solution extraction under vacuum when opened. Syringe barrels were wrapped in aluminum foil to block light from the roots and prevent algal growth in the solutions. Solution volumes in the syringe containers were relatively small, so distilled, deionized water was added to the containers as needed (every 1–2 h during the light cycle) to maintain the original solution volume.

Three to four days after plants were transferred to the syringe barrels (14–16 DAE), six to eight plants were selected at random and inoculated by replacing the nutrient solution with 20 ml of fresh solution containing 5×10^3 zoospores per milliliter. After 16 h, these solutions were drained and replaced with fresh 50% Hoagland's solution. Three days later (17–19 DAE), the nutrient solution in all containers was replaced with salt-amended Hoagland's solution. Plants were exposed to a 25 meq L^{-1} solution containing 3.7 MBq $^{22}Na^+$ for 24 h, followed by exposure to a 50 meq L^{-1} solution containing 7.4 MBq $^{22}Na^+$. The specific activity of these solutions was 6,159 ± 11 dpm μM^{-1} Na^+.

At the conclusion of the salt uptake period, each syringe barrel was drained of the $^{22}Na^+$-labeled solution, and root systems were rinsed three times with nonlabeled salt solution (50 meq L^{-1}) to remove $^{22}Na^+$ from the root free-space. This ensured that any further loss of $^{22}Na^+$ to the bathing solution would reflect symplastic movement. Each syringe was drained rapidly through the tip by using a vacuum. The syringes were refilled with nonlabeled salt solution, which was repeatedly drained and replaced at 1, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 540, and 600 min to determine the amount and rate of $^{22}Na^+$ loss from roots. At each sample time, the entire volume of solution was drawn off, after which the syringe container was quickly rinsed once with fresh solution before being refilled. To prevent any physical damage to roots, plants were not disturbed at any time during the $^{22}Na^+$ loading and sampling periods.

TABLE 1. Parameter estimates for dummy variable regression[a]

Variable[b]	df	Parameter estimate[c]	P
I 50	1	373.7	0.0001
dI 100	1	146.8	0.0283
Slope 50	1	303.5	0.0045
dSlope 100	1	428.5	0.0023

[a] R-square = 0.7885.

[b] I 50 = intercept for 50 meg L^{-1} treatments; dI100 = change in intercept for 100 meg L^{-1} treatments; Slope 50 = accumulation of sodium as a function of disease severity for 50 meg L^{-1} treatments; dSlope 100 = change in sodium accumulation as a function of disease severity for 100 meg L^{-1} treatments.

[c] Measured in μmol g^{-1} dry weight.

After the 600-min sample was collected, plants were removed from the syringe containers, and roots were blotted on absorbent pads. Roots and shoots were severed and weighed separately. Taproots and subsamples of the lateral roots were excised from each root mass and floated in a small volume of 50% Hoagland's solution in a 120-mm-diameter petri dish. The dish was laid on a grid, and the roots were photographed for later determination of infection severity. Each photographed root sample was recombined with the corresponding parent root mass in a scintillation vial, and all root and shoot tissues were dried, ashed, and analyzed for activity as described above.

In addition to plant tissues, a 10-ml aliquot of each collected wash solution was evaporated to dryness at approximately 90 C. Residual material was redissolved in 1 ml of distilled water and 15 ml of scintillation cocktail. The count rates in all tissue and solution samples were translated into sodium concentration, and the relative Na^+ content in roots, cumulative efflux, and net efflux rates were calculated on a per fresh weight of root basis. All the Na^+ in the plants was assumed to have come from the $^{22}Na^+$-labeled solutions and, therefore, to be proportional to the radioactivity.

This experiment was done three times. The experimental design was unbalanced, because the success of inoculations or resulting disease severity were not predictable at the outset. Some inoculated plants exhibited only trace symptoms, whereas some developed very severe infections. Such plants were excluded from the efflux measurements. The results from all three experiments were combined for analysis.

Statistical analysis. Data were analyzed with SAS system software versions 6.02 and 6.03 (SAS Institute Inc., Cary, NC). Relationships between long-term $^{23}Na^+$ accumulation and root rot severity were characterized with linear regression. Because mean square errors were similar for respective salt treatments, data from both experiments were combined for analysis. Slope and intercepts of significant models were compared by using dummy variable regression (22), which was also used to test for significant differences between experiments. Regression coefficients were reported with the 50 meq L^{-1} treatment as the base comparison, and the change from the base comparison was reported for the 100 meq L^{-1} treatment. Only significant experimental parameters are reported.

Regression analysis was performed with logarithmic transformations when necessary and with linear regression. Degree of polynomial fit was determined by a sequential sum-of-squares significant at the 0.05 level. In analysis of variance of the short-term $^{22}Na^+$ uptake, inoculum and time were treated as fixed variables and experimental replication as a random variable. Because the design was unbalanced, estimated mean squares were calculated by SAS, and error degrees of freedom were determined by the Satterthwaite approximation (13).

Efflux data were the result of repeated samples taken on the same experimental units over a period of time. First-order autocorrelation was not calculated on this series, because sample size was less than 50 (4). To avoid potential errors associated with first-order autocorrelation, analyses were performed on the first samples and the sums of cumulative efflux. Treatment period (short-term accumulation and efflux experiments) and disease severity (all experiments) were considered independent variables. Sodium concentrations in stem and leaf tissue were considered dependent variables.

RESULTS

Long-term $^{23}Na^+$ accumulation. Although the 100 meq L^{-1} solution reduced germination and caused some stunting, all seedlings were at a similar developmental stage when transferred into solution culture (i.e., elongation of the second pair of true leaves). Disease severity increased steadily after inoculation, but there were noticeable increases every 7–10 days, which suggested activity of secondary inoculum. Disease also was more severe in the 100 meq L^{-1} salt treatments than in the 0 or 50 meq L^{-1} treatments at each inoculum level. Shoot symptoms of disease included chlorosis and senescence of lower leaves, followed by leaf wilt and necrosis of the lower stem. Leaf size decreased with increased salt concentration, but no other symptoms of salt toxicity (chlorosis of leaf margins) occurred except in association with the highest inoculum level × highest salt treatment combination in the second experiment.

Regression analysis showed a significant relationship between disease severity and salt accumulation in the 50 and 100 meq L^{-1} treatments ($P = 0.0008$ and 0.0001, respectively) (Fig. 1). Dummy variable regression showed that the slopes of the regression lines (Fig. 1) were significantly different (Table 1), and an F test indicated that the models should not be combined for a common slope (not shown). Thus, increasing sodium accumulation in shoots reflected an interaction between salt concentration and disease severity. In addition, parameter estimates (Table 1) showed that the intercept for the 100 meq L^{-1} treatment was significantly different from the 50 meq L^{-1} treatment. This difference demonstrates a significant salt effect in the absence of disease.

Short-term $^{22}Na^+$ uptake. Influx rate and accumulation of $^{22}Na^+$ in root tissues were significantly influenced by disease and time. Compared to healthy roots, the rate of sodium uptake by diseased

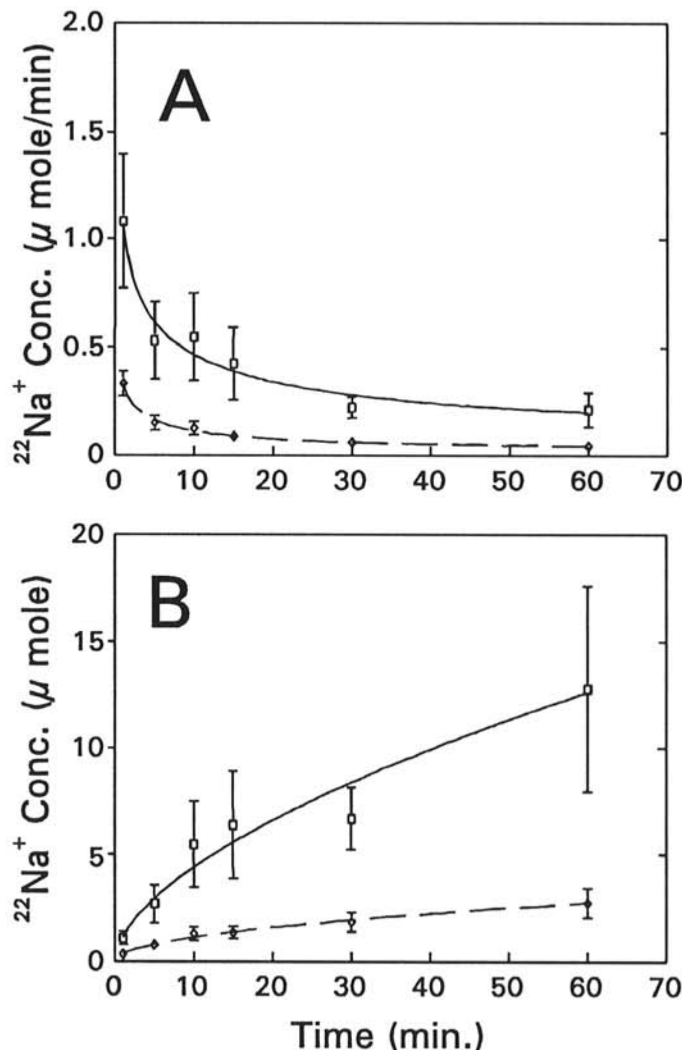

Fig. 2. Uptake of $^{22}Na^+$ by healthy and *Phytopthora cryptogea* infected safflower roots. **A,** Uptake rate (μmol min^{-1} g^{-1} root fresh wt). **B,** $^{22}Na^+$ concentration in roots over time (μmol g^{-1} root fresh wt). Each point represents the mean of 15 or 16 plants; ◇ represents healthy roots, and □ represents roots inoculated with *P. cryptogea.* Error bars show 95% confidence about the mean, and absence of error bars indicates symbol size is larger than the confidence interval. Lines were fitted to observed values by natural log transformations and regression of means. Regression parameters are presented in Table 3.

roots was significantly ($P < 0.01$) higher at all times (Fig. 2A). This resulted in significantly ($P < 0.01$) higher $^{22}Na^+$ accumulations in diseased roots over the term of the experiment (Fig. 2B). Although there was a highly significant ($P < 0.01$) inoculation × time interaction that influenced uptake rate in roots (Table 2), the interaction was less significant ($P = 0.058$) for total accumulation (Table 2).

The concentration of $^{22}Na^+$ in foliar tissues increased steadily in all plants over the first 30 min (Fig. 3). After 60 min, however, the foliar tissues of diseased plants had significantly higher concentrations than healthy plants (Fig. 3). Analysis of variance showed that shoot accumulation was significantly influenced by time and a time × inoculation interaction (Table 2). Regression coefficients relating to all rate and accumulation data (Figs. 2,3) are presented in Table 3.

$^{22}Na^+$ efflux. At the end of the efflux experiments, 25–77% of the roots of inoculated plants were infected. Because this range of infection severity could influence interpretation of the efflux data, plants were grouped into three infection severity classes: healthy (noninoculated plants), mild (29–39% root infection), and moderate (42–77% root infection). Some inoculated plants were discarded for having either insufficient or excessive root infection. There were eight, three, and seven plants assigned to the three infection severity classes, respectively.

Compared to healthy roots, diseased roots lost considerably more $^{22}Na^+$ to an iso-osmotic external solution (Fig. 4). On a percentage basis, healthy roots lost 7–10% of accumulated $^{22}Na^+$ over a 10-h period (Fig. 4A), whereas moderately diseased roots lost up to 55% (Fig. 4C). Plants with mild root infection lost approximately 30% of accumulated $^{22}Na^+$ (Fig. 4B). Lines were fitted to the observations by regression (Table 4).

The relatively greater cumulative loss of $^{22}Na^+$ from diseased roots (Fig. 4B,C) was associated with a higher efflux rate. In all plants, efflux of $^{22}Na^+$ was highest during the first minute in unlabeled solution and then declined quickly to a low constant rate. The efflux rate from healthy roots was initially 2 μM $^{22}Na^+$ min^{-1} g^{-1} root fresh wt but dropped quickly to approximately 0.01 μM $^{22}Na^+$ min^{-1} g^{-1} root fresh wt. The efflux rate from infected roots, however, was 2.5–20 times that of healthy roots, depending on disease severity (data not shown).

TABLE 2. Effects of experimental replication, disease, and time on the influx rate and accumulation of $^{23}Na^+$ in roots of safflower cv. Gila

Source	df	MS	Denominator df	Denominator MS	F	$P > F$
Influx rate in roots						
Rep[a]	2	0.0567	0.4	0.0221	2.56	0.5762
Inoc	1	6.4557	2.0	0.0158	408.00	0.0024
Rep × Inoc	2	0.0158	10.2	0.0685	0.23	0.7984
Time	5	1.5069	10.1	0.0747	20.18	0.0001
Rep × Time	10	0.0746	10.0	0.0682	1.09	0.4452
Inoc × Time	5	0.3983	10.1	0.0683	5.83	0.0088
Rep × Inoc × Time	10	0.0682	156.0	0.1100	0.62	0.7949
Accumulation in roots						
Rep	2	10.8442	1.1	14.7152	0.74	0.6313
Inoc	1	916.2960	2.0	10.6628	85.93	0.0114
Rep × Inoc	2	10.6616	10.1	25.3979	0.42	0.6682
Time	5	187.0731	10.0	29.5122	6.34	0.0066
Rep × Time	10	29.5309	10.0	25.4500	1.16	0.4093
Inoc × Time	5	79.8307	10.0	25.4377	3.14	0.0582
Rep × Inoc × Time	10	25.4500	156.0	17.6522	1.44	0.1668
Accumulation in shoots						
Rep	2	0.116	0.8	0.271	0.43	0.7518
Inoc	1	4.635	2.0	0.370	12.52	0.0714
Rep × Inoc	2	0.370	10.5	0.438	0.85	0.4568
Time	5	38.319	10.1	0.332	115.39	0.0001
Rep × Time	10	0.330	10.0	0.430	0.77	0.6579
Inoc × Time	5	5.016	10.1	0.431	11.62	0.0006
Rep × Inoc × Time	10	0.430	153.0	1.626	0.264	0.9878

[a] Rep = experimental replication; inoc = inoculation with *Phytophthora cryptogea*.

DISCUSSION

These experiments clearly show that *P. cryptogea* infection of safflower roots altered normal Na^+ levels in plant tissues. Sig-

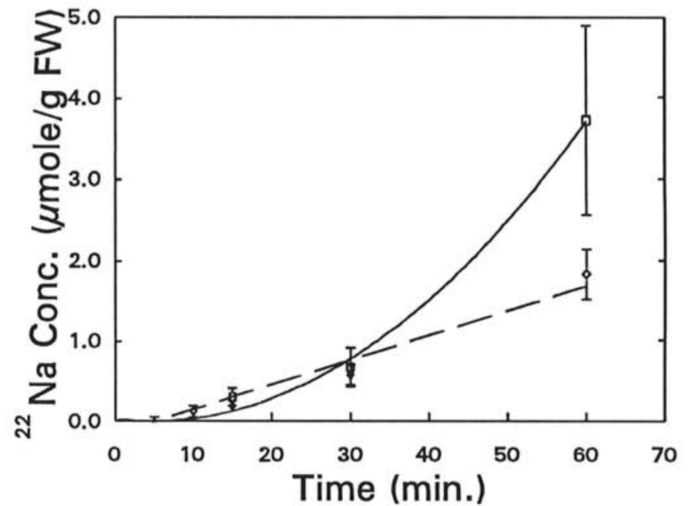

Fig. 3. Accumulation of $^{22}Na^+$ in the shoots of healthy and *Phytophthora cryptogea* infected safflower plants over time (μmol ^{22}Na g^{-1} root fresh wt). Each point represents the mean of 15 or 16 plants; ◇ represents healthy plants, and □ represents plants infected by *P. cryptogea.* Error bars show 95% confidence about the mean, and absence of error bars indicates symbol size is larger than confidence interval. Regression parameters are presented in Table 3.

TABLE 3. Regression coefficients and related statistics for the acumulation of $^{22}Na^+$ in shoots and roots and the rate of $^{22}Na^+$ uptake in roots

Variable	df	Healthy plants: Parameter estimate	Healthy plants: Standard error	P	Diseased plants: Parameter estimate	Diseased plants: Standard error	P
Rate of accumulation in roots							
Intercept	1	0.3310	0.0102	0.0001	1.0691	0.0773	0.0008
ln Time[a]	1	−0.1273	0.0102	0.0011	−0.3266	0.0770	0.0240
(ln Time)^2	1	0.0141	0.0024	0.0099	0.0279	0.0181	0.1910
Net accumulation in roots							
Intercept	1	−1.0675	0.0630	0.0001	0.1264	0.1502	0.4475
ln Time	1	0.5028	0.0234	0.0001	0.5874	0.0557	0.0005
Net accumulation in shoots							
Intercept	1	−0.3543	0.2974	0.2994	0.1053	0.0191	0.0118
Time	1	0.0638	0.0105	0.0037	0.0119	0.0019	0.0086
Time^2	1	...	...	...	0.0003	0.00003	0.0017

[a] Diseased plants were modeled with the ln(time)^2 for comparison to the healthy treatment.

nificant increases in uptake and efflux occurred when root rot severity was ≥40% and ≥30%, respectively. These levels of root infection are well below those that can induce wilt in safflower (8).

Although root infection clearly altered plant-Na^+ relations, the underlying mechanisms are not known yet. Infection might cause physical or physiological damage to root membrane systems, thus reducing selective permeability. For example, the greater efflux of $^{22}Na^+$ from diseased roots than from healthy roots suggests a breakdown of Na^+ compartments within root cells. Also, the increased accumulation of Na^+ in shoot tissues of diseased plants in long-term and short-term exposures indicates that normal root functions of Na^+ exclusion and compartmentation are impaired by Phytophthora root rot. Diseased roots allowed more Na^+ to move upward into foliar tissues than healthy roots. Although no histological studies were conducted, *Phytophthora* infection can cause extensive damage to root organelle membranes (27), even in cells distant from the point of invasion.

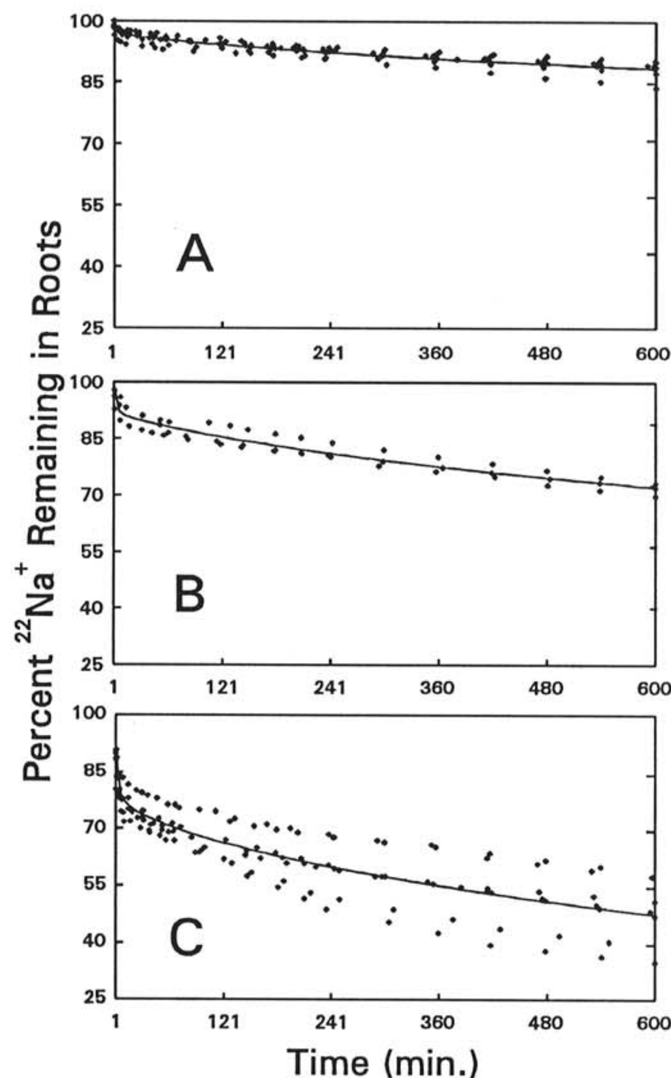

Fig. 4. Efflux of $^{22}Na^+$ from healthy and *Phytophthora cryptogea* infected safflower roots over time. Symbols represent individual observations from experimental units. **A,** Observations from eight noninoculated controls; **B,** observations from three plants with 29–39% root infection; and **C,** observations from seven plants with 42–77% root infection. Lines were fitted to the observations by regression. Regression parameters are presented in Table 4.

Phytophthora-induced changes in membrane permeability could explain the increased rate of Na^+ uptake and higher concentrations in diseased plants in the long- and short-term experiments, but this is not the only possible explanation for the results. A major impact of Phytophthora root rots is increased resistance to water transport (9). This causes plant water stress and, if severe, leads to wilt. In hydroponic culture, however, safflower can withstand substantial root infection (≥70%) before wilt symptoms develop (T. R. Weicht and J. D. MacDonald, *unpublished*). The ability of safflower to tolerate such severe infection is due to increased activity by the remaining healthy roots. Safflower can compensate for decreased hydraulic conductivity in diseased roots by increasing water flux through healthy portions of the root system (17). This is in contrast to *Eucalyptus* spp., for which water flux is very sensitive to even small amounts of root infection (7).

Because we did not observe wilt symptoms on plants with 30–40% root infection, we assumed that water flux increased through the remaining healthy roots. The resulting mass flow of ions could have overwhelmed the ability of those roots to regulate Na^+ uptake and transfer. There is some evidence that increased transpiration can decrease normal K^+/Na^+ selectivity in favor of Na^+, possibly because of increased apoplastic transport around lateral roots and in meristematic areas in which endodermis differentiation is incomplete (20,21). Although changes in salt relations may be linked to changes in water transport, we believe the efflux experiment clearly shows that a major impact of disease is on root membrane function.

A portion of the increased $^{22}Na^+$ uptake in diseased roots reported here also could reflect fungal uptake, but we have assumed it to be insignificant. *P. cryptogea* can utilize Na^+ in osmotic adjustment, but it accumulates <1 mg of Na^+ g^{-1} dry wt mycelium at 100 meq L^{-1} of Na^+ (28). In the root tissues harvested here, fungal biomass represented only a small fraction of the harvested tissue.

Previous research has shown that *Phytophthora* spp. can survive and cause root infections under saline conditions (2,3,19,25). Our experiments show that light to moderate levels of Phytophthora root rot can significantly alter root-Na^+ interactions in safflower. Infection increased the rate of Na^+ uptake, decreased compartment efficiency, and allowed greater amounts of Na^+ to transfer to foliar tissues. Although plants may differ in sensitivity to *Phytophthora* infection or salinity, these results clearly show that root disease disrupts the function of salt regulation mechanisms, and this could be an important consideration in efforts to breed plants with improved salt tolerance.

TABLE 4. Parameter estimates, standard error, and probabilities of statistical models for root efflux experiments

		Healthy plants (0% root rot)[a]		29–39% Root rot[b]		42–77% Root rot[c]	
Variable	df	Parameter estimate	P	Parameter estimate	P	Parameter estimate	P
Intercept	1	98.01 ± 0.37	0.0001	99.90 ± 2.32	0.0001	90.29 ± 2.98	0.0001
LOG_{10} t[d]	1	−4.26 ± 1.09	0.0001	−16.01 ± 5.80	0.0080	−21.63 ± 7.84	0.0016
$(LOG_{10}\ t)^2$	1	3.31 ± 0.92	0.0004	10.88 ± 4.01	0.0091	12.61 ± 5.80	0.0307
$(LOG_{10}\ t)^3$	1	−1.13 ± 0.21	0.0001	−3.13 ± 0.81	0.0003	−3.73 ± 1.19	0.0113

[a] R-square = 0.86.
[b] R-square = 0.93.
[c] R-square = 0.80.
[d] Time in minutes.

LITERATURE CITED

1. Backlund, V. L., and Hoppes, R. R. 1984. Status of soil salinity in California. Calif. Agric. 38(10):8-9.
2. Blaker, N. S., and MacDonald, J. D. 1986. The role of salinity in the development of Phytophthora root rot of citrus. Phytopathology 76:970-975.
3. Bouchibi, N., van Bruggen, A. H. C., and MacDonald, J. D. 1990. Effect of ion concentration and sodium:calcium ratio of a nutrient solution on Phytophthora root rot of tomato and zoospore motility and viability of *Phytophthora parasitica*. Phytopathology 80:1323-1329.
4. Box, G. E. P., Hunter, W. G., and Hunter, J. S. 1978. Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building. John Wiley & Sons, New York.
5. Cahill, D., and Weste, G. 1983. Changes in respiration of seedling roots inoculated with *Phytophthora cinnamomi*. Phytopathol. Z. 106:51-62.
6. Cahill, D., Weste, G., and Grant, B. 1985. Leakage from seedling roots inoculated with *Phytophthora cinnamomi*. Phytopathol. Z. 114:348-364.
7. Dawson, P., and Weste, G. 1984. Impact of root infection by *Phytophthora cinnamomi* on the water relations of two *Eucalyptus* species that differ in susceptibility. Phytopathology 74:486-490.
8. Duniway, J. M. 1975. Water relations in safflower during wilting induced by Phytophthora root rot. Phytopathology 65:886-891.
9. Duniway, J. M. 1977. Changes in resistance to water transport in safflower during the development of Phytophthora root rot. Phytopathology 67:331-337.
10. Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley & Sons, New York. 412 pp.
11. Epstein, E., Norlyn, J. D., Rush, R. W., Kingsbury, R. W., Kelley, D. B., Cunningham, G. A., and Wrona, A. F. 1980. Saline culture of crops, a genetic approach. Science 210:399-404.
12. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347. 32 pp.
13. Ingraham, K., Luginbuhl, R., Schlotzhauer, S., and Watts, H. 1988. Pages 549-637 in: SAS/STAT User's Guide Release 6.03. SAS Institute Inc., Cary, NC.
14. Labanauskas, C. K., Stolzy, L. H., and Zentmyer, G. A. 1976. Effect of root infection by *Phytophthora cinnamomi* on nutrient uptake and translocation by avocado seedlings. Soil Sci. 122:292-296.
15. Läuchli, A. 1984. Mechanisms of nutrient fluxes at membranes of the root surface and their regulation in the whole plant. Pages 1-23 in: Roots, Nutrient and Water Influx, and Plant Growth. Soil Sci. Soc. Am., Crop Sci. Soc. Am., Am. Soc. Agron., Madison, WI.
16. Lessani, H., and Marschner, H. 1978. Relation between salt tolerance and long-distance transport of sodium and chloride in various crop species. Aust. J. Plant Physiol. 5:27-37.
17. Liddell, C., and Duniway, J. 1987. Changes in water uptake and root distribution occurring during the development of Phytophthora root rot in safflower. (Abstr.) Phytopathology 77:1745.
18. MacDonald, J. D. 1982. Effect of salinity stress on the development of Phytophthora root rot of chrysanthemum. Phytopathology 72:214-219.
19. MacDonald, J. D. 1984. Salinity effects on the susceptibility of chrysanthemum roots to *Phytophthora cryptogea*. Phytopathology 74:621-624.
20. Pitman, M. G. 1977. Ion transport into the xylem. Ann. Rev. Plant Physiol. 28:71-88.
21. Pitman, M. G. 1982. Transport across plant roots. Quart. Rev. BioPhys. 15:481-554.
22. Rawlings, J. O. 1988. Applied Regression Analysis: A Research Tool. Wadsworth and Brooks/Cole Advanced Books and Software, Pacific Grove, CA.
23. Shannon, M. C. 1984. Breeding, selection, and the genetics of salt tolerance. Pages 231-254 in: Salinity Tolerance in Plants. Strategies for Improvement. R. C. Staples and G. H. Toenniessen, eds. John Wiley & Sons, New York.
24. Swiecki, T. J., and MacDonald J. D. 1988. Histology of chrysanthemum roots exposed to salinity stress and *Phytophthora cryptogea*. Can. J. Bot. 66:280-288.
25. Swiecki, T. J., and MacDonald J. D. 1991. Soil salinity enhances Phytophthora root rot of tomato but hinders asexual reproduction by *Phytophthora parasitica*. J. Am. Soc. Hortic. Sci. 116:471-477.
26. Tennant, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63:995-1001.
27. Tippet, J. T., O'Brien, T. P., and Holland, A. A. 1977. Ultrastructure changes in eucalyptus roots caused by *Phytophthora cinnamomi*. Physiol. Plant Pathol. 11:279-285.
28. Woods, D. 1984. The water relations of mycelial growth by *Phytophthora cryptogea* and *Fusarium moniliforme*. Ph.D. thesis. University of California, Davis.