Local and Systemic Effects of NaCl on Root Composition, Rhizobacteria, and Fusarium Crown and Root Rot of Asparagus

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ABSTRACT

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The role of NaCl in suppression of Fusarium crown and root rot of asparagus was investigated in split root culture so the direct effects of NaCl on the root and rhizosphere could be separated from effects that were translocated to the nontreated root side. One side of the root system was exposed to 100 ml of 0, 0.5, or 1.0% NaCl, while the other side received deionized water. Both sides of the root system were inoculated with conidial suspensions of the pathogens *Fusarium oxysporum* and *F. proliferatum*. When plants were harvested and assayed, root lesions and CFUs of *F. oxysporum* or *F. proliferatum* per centimeter of root from both exposed and nonexposed roots decreased as the NaCl rate increased to 1.0%, but the reduction relative to the control was significantly greater on roots that were directly exposed to NaCl (51% reduction in root lesions) than on adjacent nonexposed roots (31% reduction in root lesions). On both sides of the root systems, disease suppression with NaCl

was associated with increases in the rhizosphere densities of fluorescent pseudomonads and Mn-reducing bacteria in the rhizosphere soil. In addition, as the NaCl rate increased, root tissues had marked reductions in malic acid and amino acids while concentrations of Cl and Mn increased in equal proportions on both sides of the root system. Chloride ions were absorbed in greater amounts than Na ions, and were more mobile in the plant than Na. Plants treated with 1% NaCl (171 meq of Cl⁻ per liter) had soil leachates 1 week later of 47 meq of Cl⁻ per liter from pots exposed to NaCl, but in the adjacent nonexposed pots, the amount of Cl in the leachates slowly increased over the course of the study to 20 meq/liter, presumably through the root exudation. These findings suggest that suppression of Fusarium crown and root rot of asparagus with NaCl may be due to multiple mechanisms. Maximum suppression occurs when NaCl is directly applied to roots, but suppression still occurs on distal non-treated roots resulting from systemic mechanisms. The latter mechanism may be associated with a root-mediated alteration in the rhizobacteria.

Additional keywords: *Asparagus officinalis*, mineral nutrition.

Chloride has long been applied to crops as a companion ion for ammonium, potassium, and calcium fertilizers, yet in the last few decades, these applications have been made more with the aim of supplying chloride than the cations (5,7,13,33). The effect of chloride on plant health and disease suppression has been recognized, but the mechanisms remain unclear (13,27). Chlorides affect nitrification, plant water potential, root exudation, and manganese and phosphate availability (11,13,34). Inasmuch as all these factors directly or indirectly influence root disease, chloride may act through many mechanisms that are not mutually exclusive from each other. For example, root exudation is influenced by water potential and osmoregulation (17). When a root cell absorbs Cl ions, concentration of organic anions is reduced (29) and the amount of organic carbon exuded during root exudation is reduced. Similarly, in acidic soils, chloride inhibits nitrification and keeps nitrogen available in ammoniacal forms longer (32). When Cl is added to acid soils, Mn oxides are reduced, Mn availability is increased (25), and disease severity is lessened (22). Applications of Cl are also associated with an increase in microbes that biologically increase Mn availability (6,22). Many of these microbes have disease-suppressive or plant growth-promoting properties (6,21). The contribution of each mechanism toward disease suppression is difficult to separate because many mechanisms are functionally dependent on each other. However,

the effect of NaCl can be separated into the broad category of direct effects on the rhizosphere system and indirect effects on the host.

The majority of examples where Cl fertilization enhanced crop yields have been in environments with considerable disease pressure (13). Asparagus (*Asparagus officinalis* L.), a salt-tolerant plant (14), is susceptible to Fusarium crown and root rot, caused by *Fusarium oxysporum* (Schlecht.) emend. Snyder & Hans and *F. proliferatum* (Matsush.) Nirenberg (synanamorph = *F. moniliforme* Sheld. emend. Snyder & Hans) (10). *F. oxysporum* is commonly found on roots of young plants, whereas *F. proliferatum* becomes the dominant colonist in older fields (8). Both species are isolated in roughly equal proportions from crown tissue (8). Over time, most asparagus plantings decline because of damage caused by Fusarium crown and root rot and other pest-related stresses and because of buildup of alleopathic toxins in the soils (15). Losses occur from fewer and smaller spears and crown death. When NaCl is applied to declining fields, disease severity is reduced and fern vigor and yield are increased (5,6,32). Sodium chloride was superior to other Cl salts such as KCl or CaCl₂ in reducing disease severity (5,31), however there was no added benefit of applying NaCl to asparagus fields that were healthy (31; W. H. Elmer, unpublished data). Applications of NaCl also have been effective in reducing damage from early decline of asparagus (9), a disease condition that results when asparagus is replanted into old asparagus soils (2).

Accordingly, the mechanisms of disease suppression in asparagus with NaCl are unclear. There is no evidence that NaCl applied at rates of 560 to 1,120 kg/ha is fungicidal (5), but this does not rule out a fungistatic effect. Amir et al. (1) suggested that NaCl might reduce the competitive saprophytic ability of the Fusarium

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wilt pathogen on date palm and enhance antagonistic microbes in the rhizosphere. Although salt-treated asparagus plants had greater numbers of fluorescent pseudomonads and Mn-reducing bacteria per gram of root soil than untreated plants, there was no detectable increase in strains that were antagonistic to *Fusarium* spp. on treated plants (6). However, a correlation was observed between disease suppression and the Mn content in roots treated with these microbes. It is not clear whether a direct exposure to NaCl increases these microbes or whether changes in root exudation were more important in their proliferation. Carbon substrates which are readily available in roots and root exudates consist of simple sugars, amino acids, and organic acids. These compounds are rapidly utilized by soil bacteria (26,39). Alteration in the level or composition of these substrates may affect rhizobacteria and the level of infection by root pathogens.

In the current experiments, a split root pot culture system was employed to separate the direct effects of NaCl on the soil system from those caused indirectly through changes mediated by the plant roots. The objectives were to separate the local from the systemic effects of NaCl on asparagus roots and to determine whether changes in heterotrophic bacteria, fluorescent pseudomonads, or Mn-transforming microbes were associated with roots in these studies.

MATERIALS AND METHODS

Split pot culture. Seeds of asparagus 'Mary Washington' (Johnny's Select Seeds, Albion, ME) were agitated for 1 h in 20% household bleach (1.05% NaHClO₂), rinsed three times with distilled H₂O, and soaked in distilled water at 30°C for 72 h. Seeds were germinated in trays with 36 cells (6.0 × 6.6 cm, 100 ml per cell) filled with potting mix (ProMix BX; Premier Brand Inc., New Rochelle, NY) and held for 8 weeks in the greenhouse. Seedlings received Hoagland's solution (19) (50 ml per cell) after 4 weeks and were irrigated as needed with deionized H₂O. The 36-cell trays were separated into individual cells. The bottom of each cell was carefully cut out with scissors. The root ball was teased apart and one half of the roots were placed in 10- × 10-cm plastic pots and the other half was placed into a similar adjacent pot. Thirty-six split pots were prepared and set on greenhouse benches. Pots were filled with a soil mixture that contained asparagus field soil mixed with potting mix at the rate of 1:2 (vol/vol). Soil came from a 9-year-old asparagus field with a history of *Fusarium* crown and root rot that was plowed under in June 1998. Soil dilutions onto Komada's selective agar (KSM) (23) revealed 13,900 CFU of *Fusarium* spp. per gram of soil. Field soil was passed through a 5-mm sieve and mixed in a cement mixer with the potting mix for 20 min. Hydrated lime was added to the mix at 3.1 g/liter soil mix to achieve a soil pH of 7.0 as determined on a 1:1 soil/water slurry (vol/vol) 1 week later. Soil mix (11% moisture) was stored in plastic-lined buckets in the greenhouse and used for all experiments.

Treatments and sampling. Three weeks later, one side of the pot of each plant was arbitrarily chosen to receive 100 ml of 0.5 or 1.0% NaCl (wt/vol) or deionized water without NaCl. The adjacent pot side was designated as the nonexposed root side and received 100 ml of deionized water at the same time. Seven days later, the soil in both pots was drenched with 50 ml of spore suspension of both *Fusarium* pathogens. Cultures of *F. oxysporum* (isolate FOA50) and *F. proliferatum* (isolate 95WT) were grown on potato carrot agar (4) for 7 days at 25°C, rinsed with distilled water to remove conidia, and adjusted to 10⁶ spores per ml. The NaCl treatments were re-applied 6 weeks after inoculation.

Leachates (75 ml per pot) were collected every 2 weeks beginning 4 weeks after transplanting for a total of five samplings. Pots were placed in plastic trays and deionized water was slowly added until approximately 75 ml was collected in the tray. Leachates were bulked according to block (three replicates per block) and

centrifuged for 5 min at 3,000 × g to remove soil particles. Samples were frozen at -20°C and thawed later for Cl analysis. Two-milliliter aliquots from each bulked group were analyzed at each sampling time for Cl (meq) with a chloridometer (Buchler Cotlove Inc., Fort Lee, NJ). Values were read from NaCl standard curves.

Soil and root assays. Twelve weeks after planting, the divided root systems were separated from each other and from the fern. Rhizosphere soil was shaken loose from the detached feeder roots and immediately diluted in sterile saline (8.5 g of NaCl per liter). Rhizosphere pH was measured in a 1:1 soil/water slurry by a pH meter. Tenfold dilutions were prepared from the rhizosphere soil and (0.1 ml) spread onto plates of 10% tryptic soy agar (TSA; Difco Laboratories, Detroit) (dilutions 10⁻⁴ to 10⁻⁵ g/ml), King's B agar (4) (dilutions 10⁻³ to 10⁻⁴ g/ml), Mn-dioxide agar (10% TSA, 5.0 g of Mn-dioxide, 20.0 g of sucrose, and 15.0 g of agar) (dilutions 10⁻² to 10⁻³ g/ml), and KSM (23) (dilutions 10⁻² g/ml). Soil moisture was determined independently. Plates were incubated in the dark at 25°C. There were three plates per dilution. Colonies of heterotrophic bacteria were counted daily on the TSA plates for 3 days and added together. Total counts between 30 and 300 colonies per plates were used for estimating bacterial densities. Fluorescent pseudomonads were counted under black light after 2 days and Mn-transformers were counted 1 week later. Mn-oxidizers produced a blackened deposit, whereas Mn-reducers produced a clear zone around the colony. Colonies of *F. oxysporum*-like and *F. proliferatum*-like fungi were counted after 7 days by observing the colonies under ×100 magnification. Microbial densities were expressed as CFU per gram of soil (dry weight equivalent).

The roots in each divided pot were washed free of soil and approximately half of the roots (2.0 to 4.0 g) were rewashed in deionized water, labeled, frozen, and freeze-dried. Disease severity and colonization by *Fusarium* spp. was determined on the remaining half. Roots were surface-disinfested for 4 min in 4% household bleach (0.21% NaHClO₂), rinsed in deionized water, and blotted dry on paper towels. Feeder roots approximately 1.0 to 4.0 cm long were placed on KSM (23). The petri dishes containing the roots were placed over a 1.0-cm grid, and the total root length was estimated using the line-intersect method (37). The fraction of intersects where a reddish lesion occurred was counted and used to estimate disease severity [% root lesions = (number of intersects with root lesions/total intersects) × 100]. Plates were incubated at room temperature for 5 days, and the number of *Fusarium* colonies that grew from the roots was counted and used to estimate root infection (*Fusarium* colonies per centimeter of feeder root). Approximately 1.5 to 2.0 m of feeder roots was sampled from each pot.

Root tissue assays. Frozen roots were freeze-dried, ground in a Wiley mill, and passed through a 1.0-mm sieve. Dried root tissue from the replicate pots in each block was bulked (four plants per block, three blocks per experiment) and stored at -20°C until analyzed for amino acids, organic acids, carbohydrates, and elemental composition.

Amino acid analysis. Two 500-mg samples of freeze-dried root tissue were removed from each bulked sample and added to test tubes with 5 ml of water, placed under vacuum for 10 min, vortexed, and filtered (0.45 µm). Two aliquots (0.1 ml) were removed from each test tube and were each assayed for amino groups using a ninhydrin assay. Standard curves were prepared using glycine. The four values from each bulked sample were averaged and expressed as milligrams of amino groups per gram of root tissue.

Carbohydrate analysis. Total nonstructural carbohydrates were determined on each bulked sample by assaying two 50-mg samples and digesting in α amylase (Sigma Chemical, St. Louis) according to the procedures described by Streeter and Jeffers (36). Reducing sugars were determined colorimetrically and quantified with standard glucose concentrations (36). The values from each bulked sample were averaged and expressed as milligrams of glu-

cose per gram of tissue. Soluble carbohydrates were determined on reducing sugar extracted from filtered (0.45 µm) extracts of dried root tissue (100 mg per 2 ml of H₂O) from each bulked sample. Two determinations were performed on each digested sample. The values from each bulked sample were averaged and expressed as milligrams of glucose per gram of tissue.

Malic acid analysis. Two 100-mg samples of freeze-dried root tissue from each bulked sample were added to test tubes with 3.5 ml of water, placed under vacuum for 10 min, vortexed, and brought up to 10 ml with distilled water. A filtered (0.45 µm) aliquot of the sample (0.2 ml) was assayed using test kits for malic acid (Boehringer Mannheim Corp., Indianapolis) according to the manufacturer's instructions. L-Malic acid was determined spectrophotometrically by measuring the NADH produced following the oxidation of L-malic acid with NAD in the presence of malate dehydrogenase. Standard curves were prepared with authentic malic acid. Values were expressed as milligrams of malic acid per gram of tissue.

Elemental analysis. Total N was determined by digesting 0.25 g of root tissue from each bulked sample in H₂SO₄ followed by colorimetric analysis with the Nessler reaction. Phosphorus, K, Na, Ca, Mg, S, Fe, Mn, Zn, Cu, and B were analyzed by microwaving 0.5 g of root tissue in HCl and HNO₃ in a microwave (CEM MDS 81D; CEM Co., Matthews, NC) and using inductively-coupled-plasma-emission (ICP) spectroscopy on an ICP-OES spectrophotometer (Thermal Jarrel ARL 3520; Fison Instruments, Dearborn, MI). Free chlorides were detected by titration of a filtered 1% (wt/vol) suspension using a chlorido-

meter. Mineral concentrations were expressed as micromoles per gram of tissue.

Statistical procedures. The experiment was set up as a balanced randomized block design with four blocks and three replicate plants per block ($n = 12$). The experiment was repeated the following year. A test for interactions between treatment and experiment was not significant for the variables examined so data from both data sets were combined ($n = 24$). Transformations of the data were made when the data were not normally distributed. Microbial densities were all transformed to the Log (base 10). Percent root lesions were transformed to the arcsine of the square root. The values in the tables represent nontransformed data. Stepwise regressions determined the best fit model for NaCl rate (X) on the measured variable (Y) as detected on the NaCl-exposed and nonexposed root systems. Terms were rejected if P was >0.15 . Comparison between treated and untreated root systems at each rate of NaCl was done according to Bonferroni's test at $P < 0.05$. Correlation coefficients were generated using Bonferroni's correction. All statistical operations were performed using Systat Statistical Software version 10 (SPSS Inc., Chicago).

RESULTS

The amount of chloride in the leachates of nontreated pots was negligible (<3.0 meq of Cl per liter, <80 ppm of Cl) over the course of the study in both repetitions. Data from the first study are presented in Figure 1. As expected, pots treated with the highest rate of NaCl (1%) had high levels of chloride in the leachate, between 30 and 60 meq of Cl per liter. Chloride levels in the leachates were always highest in the samples that were taken following the applications of NaCl. Leachates from the non-exposed pots of treated plants had Cl levels similar to controls until 10 weeks when they slowly increased to 20 meq of Cl per liter.

The roots of control plants had reddish elliptical root lesions typical of those caused by both *Fusarium* spp. on approximately 35% of the feeder roots (Table 1). As NaCl rates increased to 0.5%, the percentage of lesions on roots exposed to NaCl dropped to 21% and dropped to 17% on roots exposed to 1.0% NaCl. However, the reduction was not as great on the root half that was not exposed to NaCl which dropped from 36 to 27% at the 0.5% rate and to only 25% at the 1.0% rate of NaCl. The best-fit relationships using stepwise regression for the percent lesions (Y [arcsine transformed]) versus NaCl rate (X) yielded linear equations for roots exposed to NaCl ($Y = 0.71 - 0.11X$; $r^2 = 0.49$; $P < 0.001$) and for the nonexposed root side ($Y = 0.70 - 0.07X$; $r^2 = 0.31$; $P = 0.024$). At NaCl rates of 0.5 and 1.0%, the percent root lesions were reduced more on roots directly exposed to NaCl than on nonexposed roots ($P = 0.040$ and 0.031 , respectively). Similarly, the best-fit equation for the number of colonies of *Fusarium* spp. per centimeter of root (Y) versus NaCl rate (X) was

Fig. 1. Chloride (milliequivalent/liter) in leachate from soils from a split pot experiment with asparagus treated with NaCl and sampled over time. Treated pot root side exposed to NaCl (▲); treated pots but roots not exposed to NaCl (●); control pots, exposed root side (Δ); and control pots, roots not exposed (○). Arrows indicate the time of NaCl application.

TABLE 1. Effect of application of NaCl on root lesions and colonies of *Fusarium* spp. growing from asparagus roots in split root pots

NaCl rate of application (%) ^v	% Root lesions ^w			<i>Fusarium</i> colonies per cm of root ^x		
	Exposed to NaCl	Not exposed to NaCl	<i>P</i> value	Exposed to NaCl	Not exposed to NaCl	<i>P</i> value
0	34.79 ^y	36.63	NS	0.67	0.76	NS
0.5	20.12	27.40	0.040	0.52	0.59	NS
1.0	16.93	25.24	0.031	0.48	0.61	0.023
Best fit regression models (<i>P</i>) ^z						
Linear fit	<0.001	0.024	—	0.012	0.031	—

^v Total amount applied in two applications of 50 ml of H₂O.
^w Percent root lesions = number of line/root intersects where a root had a lesion/total intersects) × 100].
^x Root colonies determined on Komada's selective medium (22) and root length determined using the line-intersect method (36).
^y Values represent the means of 24 plants (12 replicates per experiment). NS = not significant at $P = 0.05$.
^z Equations were determined using stepwise regression for linear and quadratic terms. Percent root lesions [Y (arcsine transformed)] exposed to NaCl rate (X): $Y = 0.71 - 0.11X$, $r^2 = 0.49$; percent root lesions [Y (arcsine transformed)] not exposed to NaCl rate (X): $Y = 0.70 - 0.07X$, $r^2 = 0.31$; colonies per cm of root (Y) exposed to NaCl rate (X): $Y = 0.74 - 0.09X$, $r^2 = 0.29$; colonies per cm of root (Y) not exposed to NaCl rate (X): $Y = 0.81 - 0.08X$, $r^2 = 0.25$.

linear for roots exposed to NaCl ($Y = 0.74 - 0.09X$; $r^2 = 0.29$; $P < 0.012$) and for the nonexposed root side ($Y = 0.81 - 0.08X$; $r^2 = 0.25$; $P = 0.031$). Both sides of the root systems had similar levels of colonization except for the higher rate where NaCl-exposed roots had fewer colonies per centimeter of root than on the adjacent nonexposed roots ($P = 0.023$).

Log densities of fluorescent pseudomonads per gram of soil (Y) increased linearly with increased NaCl in rhizosphere of roots that were exposed to NaCl ($Y = 5.7 + 0.09X$; $r^2 = 0.33$; $P = 0.007$) and around roots that were not exposed to NaCl ($Y = 5.7 + 0.10X$; $r^2 = 0.25$; $P = 0.016$) (Table 2). Numbers approximately doubled at the highest rate of NaCl (1.0%). Similarly, log densities of Mn-reducing bacteria (Y) increased linearly as NaCl rate (X) increased, but only in rhizosphere soil of nonexposed roots ($Y = 3.5 + 0.3X$; $r^2 = 0.31$; $P < 0.001$). Densities were more than three-fold greater at the highest NaCl rate. The densities of fluorescent pseudomonads or Mn-reducing microbes did not differ between NaCl-exposed and nonexposed soils, suggesting that exposure to the NaCl treatment did not directly affect their increase. Total heterotrophic bacteria in the rhizospheres of NaCl-exposed and nonexposed soils were not affected by treatment with NaCl (range = log 7.79 – log 7.92 CFU/g of soil; mean = log 7.84 CFU/g of soil) (data not shown). Mn-oxidizing microbes were also not affected by NaCl treatment (range = log 3.89 – log 4.10 CFU/g of soil; mean = log 4.00 CFU/g of soil) (data not shown). NaCl did not affect rhizosphere soil pH (range 6.84 to 7.02, mean 6.9) or the combined densities of *F. oxysporum* and *F. proliferatum* (range = log 3.84 – log 3.97 CFU/g of soil; mean = log 3.91 CFU/g of soil) (data not shown).

Amino acids and malic acid concentrations (milligram per gram of root) in the roots decreased linearly as NaCl increased

regardless of whether they were directly exposed to NaCl treatment or not (Table 3). Compared with controls, amino acids were 17 and 22% less in roots exposed to the 1% rate and nonexposed roots, respectively. There were no differences in concentrations of amine products between exposed and in the adjacent nonexposed roots treated at the same level of NaCl. Amino acids (Y) decreased linearly as NaCl rate (X) increased for roots exposed to NaCl ($Y = 3.18 - 0.12X$; $r^2 = 0.46$; $P = 0.010$) and for roots on the nonexposed side ($Y = 2.54 - 0.23X$; $r^2 = 0.43$; $P < 0.001$). At the highest NaCl rate (1.0%), root concentrations of malic acid were reduced 88 and 93% in NaCl-exposed and nonexposed roots, respectively. The relationship between malic acid levels (Y) and NaCl rate (X) also was linear for roots exposed to NaCl ($Y = 11.66 - 3.74X$; $r^2 = 0.62$; $P = 0.007$) and for the roots that were not exposed to NaCl ($Y = 11.57 - 3.99X$; $r^2 = 0.67$; $P = 0.012$). Root concentrations of malic acid did not differ regardless of whether or not they were exposed to NaCl. Soluble root carbohydrates were decreased 33 and 15%, but not significantly on NaCl-exposed and nonexposed roots as NaCl increased to 1% rate (data not shown). Similarly, total nonstructural root carbohydrates decreased 37 and 9%, but not significantly on NaCl-exposed and nonexposed roots as NaCl increased (data not shown).

Roots that were exposed or not exposed to NaCl both accumulated Na and Cl ions as the NaCl rate increased (Table 4). A linear relationship best described Cl (Y) concentration (micromoles per gram of root tissue) data in roots that were exposed to the NaCl rates (X) ($Y = -1.07 + 67.72X$; $r^2 = 0.94$; $P < 0.001$) and on roots not exposed to NaCl rate (X) ($Y = -1.29 + 60.93X$; $r^2 = 0.94$; $P < 0.001$). Uptake of Na (Y) into the roots also followed a linear pattern for roots that were exposed to NaCl ($Y = -9.86 + 36.26X$; $r^2 = 0.91$; $P < 0.001$). A quadratic relation described the uptake of

TABLE 2. Effect of application of NaCl on recovery of fluorescent pseudomonads and Mn-reducing microbes in rhizosphere soil sampled from asparagus roots in split root pots (log CFU/g of soil)

NaCl rate of application (%) ^v	Fluorescent pseudomonads ^w			Mn-reducing microbes ^x		
	Exposed to NaCl	Not exposed to NaCl	<i>P</i> value	Exposed to NaCl	Not exposed to NaCl	<i>P</i> value
0	6.19 ^y	6.13	NS	3.43	3.09	NS
0.5	6.29	6.27	NS	3.45	3.38	NS
1.0	6.42	6.34	NS	3.64	3.67	NS
Best fit regression model (<i>P</i>) ^z						
Linear fit	0.007	0.016	–	NS	0.001	–

^v Total amount applied in two applications of 50 ml of H₂O.

^w Determined by serial dilution onto King's B agar and viewed under UV light.

^x Determined on Mn-dioxide agar and view as clearing in agar.

^y Values represent the means of 24 samples from 24 different plants (12 replicates per experiment). NS = not significant at $P = 0.05$.

^z Equations were determined using stepwise regression for linear and quadratic terms. Log fluorescent pseudomonads (Y) exposed to NaCl rate (X): $Y = 5.7 + 0.09X$, $r^2 = 0.33$; Log fluorescent pseudomonads (Y) on nonexposed roots to NaCl rate (X): $Y = 5.7 + 0.10X$, $r^2 = 0.25$; Mn-reducing microbes (Y) on roots that were not exposed to NaCl rate (X): $Y = 3.1 + 0.11X$, $r^2 = 0.31$.

TABLE 3. Effect of application of NaCl on the composition of amine compounds and malic acids (milligram per gram dry root) in asparagus roots grown in split root pots

NaCl rate of application (%) ^v	Total amine compounds ^w			Total L-malic acid ^x		
	Exposed to NaCl	Not exposed to NaCl	<i>P</i> value	Exposed to NaCl	Not exposed to NaCl	<i>P</i> value
0	2.25 ^y	2.33	NS	8.49	8.58	NS
0.5	2.03	1.94	NS	3.03	1.62	NS
1.0	1.86	1.81	NS	1.00	0.60	NS
Best fit regression model (<i>P</i>) ^z						
Linear fit	0.010	<0.001	–	0.007	0.012	–

^v Total amount applied in two applications of 50 ml of H₂O.

^w Determined spectrophotometrically following reaction with ninhydrin.

^x L-Malic acid was determined spectrophotometrically by measuring the NADH produced following the oxidation of L-malic acid with NAD in the presence of malate dehydrogenase.

^y Values represent the means of eight samples from 24 different plants (three bulked plants per sample). NS = not significant at $P = 0.05$.

^z Equations were determined using stepwise regression. Amino acids (Y) in roots exposed to NaCl rate (X): $Y = 3.18 - 0.12X$, $r^2 = 0.46$; amino acids (Y) in roots not exposed to NaCl rate $Y = 2.54 - 0.23X$, $r^2 = 0.43$; malic acid (Y) on roots exposed to NaCl rate (X): $Y = 11.66 - 3.74X$, $r^2 = 0.62$; malic acid (Y) on roots that were not exposed roots to NaCl rate (X): $Y = 11.57 - 3.99X$, $r^2 = 0.67$.

Na (micromoles per gram of root tissue) in roots that were not exposed to NaCl (X) ($Y = 39.47 - 20.44X + 7.89X^2$; $r^2 = 0.82$; $P < 0.001$), indicating that there was reduced translocation of Na from the exposed root side to the adjacent roots at the 1% rate. On the other hand, Cl was easily translocated into nonexposed roots and concentrations of Cl did not differ between the roots exposed to NaCl and those that were not. Concentrations of Mn (micromoles per gram of root tissue) also increased with NaCl rate in both exposed roots and their adjacent nonexposed roots. Manganese concentrations were 15 and 35% higher than controls in roots exposed and not exposed to 1% NaCl, respectively. Mn (Y) in roots treated with NaCl increased linearly for roots exposed to NaCl (X) ($Y = 0.41 + 0.11X$; $r^2 = 0.60$; $P = 0.047$) and for roots that were not exposed to NaCl ($Y = 0.29 + 0.12X$; $r^2 = 0.38$; $P = 0.037$).

The two disease variables, percentage of root lesions and number of *Fusarium* colonies per cm of root, were correlated with each other ($r = 0.64$; $P < 0.001$) and with the malic acid concentrations in the roots ($r = 0.44$, $P < 0.01$ and $r = 0.51$, $P < 0.001$, respectively) (Table 5). The percentage of root lesions and the number of *Fusarium* colonies were negatively correlated with the log fluorescent pseudomonads in the rhizosphere ($r = -0.62$, $P < 0.001$ and $r = -0.46$, $P < 0.01$, respectively) and with the amount of Na ($r = -0.67$, $P < 0.001$ and $r = -0.54$, $P < 0.01$, respectively) and Cl per gram of root tissue ($r = -0.64$, $P < 0.001$ and $r = -0.52$, $P < 0.001$, respectively). Although malic acid concentrations were associated more with estimates of root colonization ($r = 0.51$; $P < 0.001$) and the percent root lesions ($r = 0.44$; $P < 0.01$), the log densities of fluorescent pseudomonads and Mn-reducers in rhizosphere were associated more with changes in the amount of amino acids in the root tissue ($r = -0.56$, $P < 0.01$ and $r = -0.41$, $P < 0.01$, respectively). There was a strong inverse relationship between the concentration of Cl and malic acid in the roots ($r = -0.71$; $P < 0.001$). Concentrations of Mn were only marginally correlated with Na concentrations ($r = 0.39$; $P < 0.05$), Cl concentrations ($r = 0.32$; $P < 0.05$), and with the densities of Mn-reducing microbes ($r = 0.43$; $P < 0.05$).

DISCUSSION

The exact mechanisms of how Cl amendments suppress *Fusarium* crown and root rot of asparagus disease are still unclear, but two areas where it could affect disease are those that have direct effects against the pathogen and those that have effects on the resistance of the plant (13). The current study tried to separate these responses using split root culture. Although significant reductions in disease were observed on both sides of the root system, there was more suppression when the NaCl was applied directly to the roots and suggest that multiple mechanisms may be operating in disease suppression.

Although NaCl was not toxic to *Fusarium* spp., at the rates used in this study as evidenced by its inability to reduce densities of *F. oxysporum* and *F. proliferatum* in rhizosphere soils, disease suppression was greater in the pots that were directly exposed to NaCl. Sodium chloride may have caused a fungistatic effect in these soils and prevented the onset of pathogenesis. Assays of the leachates for Cl showed that the soil-water concentration in treated pots remained between 30.0 to 60.0 meq of Cl^- during the study. Amir et al. (1) also found that *F. oxysporum* Schlechtend.:Fr. f. sp. *albedinis* Belaaf was unaffected by certain rates of NaCl, whereas wilt disease of date palm was suppressed when NaCl was applied. They suggested NaCl could reduce competitive saprophytic ability of the pathogen while increasing antagonistic microbes (1). Antagonism was not examined in the current study, but a previous field study on asparagus examined microbes associated with NaCl-treated and untreated roots and found no difference in antagonism to *Fusarium* in vitro (6). These studies did not rule out antagonism in situ.

Chloride salts cause other changes in the soil. Inhibition of nitrification and solubilizing of Mn oxides occurred in acid soils following Cl treatment (25,32). However, these effects diminished as pH increased to 6.6 (25) and therefore, were probably not a factor in the current study where rhizosphere soil pHs were between 6.8 to 7.0. In addition, if there was a chemically mediated increase in Mn availability by NaCl on the exposed side of the roots, one would expect higher Mn levels than on the nonexposed roots because Mn is not highly mobile in plants. The comparable increase in Mn in exposed and nonexposed roots was probably mediated by the Mn-reducing microbes which were equally stimulated on both root systems. If there were other direct effects of NaCl applications on microbial populations, these assays did not detect them.

The highly mobile Cl ions that were transported from roots exposed to NaCl to nonexposed roots also were strongly associated with the reductions in malic acid and the amino acid groups in the tissue. These reductions were expected as the uptake of Cl^- ions suppresses the production of organic acids and amino acids (29). It is reasonable to assume that roots low in malic acid and amino acids produced exudates that were low in these substrates. Krafczyk et al. (24) reported that compared with KNO_3 , the addition of KCl to maize plants reduced levels of malic, citric, oxalic, and fumaric acids along with glutamic acid, aspartic acid, glycine, and serine levels in the root tissue and in root exudates. Their work with potassium forms on maize found that KCl significantly reduced soluble carbohydrates when compared with K_2SO_4 (24). The current study observed only insignificant reductions. If these changes in root exudates were occurring in asparagus, they may be responsible for the stimulation of fluorescent pseudomonads and those microbes that could reduce Mn. Bacteria in both of these groups have disease-suppressing abilities (6). In

TABLE 4. Effect of application of NaCl on concentrations of Na, Cl, and Mn (micromoles per gram) in roots of asparagus grown in split root pots

NaCl rate of application (%) ^x	Na			Cl			Mn		
	Exposed to NaCl	Not exposed to NaCl	P value	Exposed to NaCl	Not exposed to NaCl	P value	Exposed to NaCl	Not exposed to NaCl	P value
0	30.17 ^y	26.91	NS	19.26	19.43	NS	0.28	0.26	NS
0.5	62.04	26.70	0.001	95.43	90.62	NS	0.32	0.27	NS
1.0	99.21	49.17	0.001	153.30	141.32	NS	0.33	0.34	NS
Best fit regression model (P) ^z									
Linear fit	0.001	NS	—	0.001	0.001	—	0.047	0.037	—
Quadratic fit	NS	<0.001	—	NS	NS	—	NS	NS	—

^x Total amount applied in two applications of 50 ml of H_2O .

^y Values represent the means of six samples from 24 different plants (four root systems per sample). NS = not significant at $P = 0.05$.

^z Equations were determined using stepwise regression. Na (Y) in roots exposed to NaCl rate (X): $Y = -9.86 + 36.26X$, $r^2 = 0.91$; Na (Y) in root not exposed to NaCl rate (X): $Y = 39.47 - 20.44X + 7.89X^2$, $r^2 = 0.82$; Cl (Y) on roots exposed to NaCl rate (X): $Y = -1.07 + 67.72X$, $r^2 = 0.94$; Cl (Y) on roots that were not exposed to NaCl rate (X): $Y = -1.29 + 60.93X$, $r^2 = 0.94$; Mn (Y) on roots exposed to NaCl rate (X): $Y = 0.31 + 0.01X$, $r^2 = 0.60$; Mn (Y) on roots that were not exposed to NaCl rate (X): $Y = 0.29 + 0.12X$, $r^2 = 0.38$.

addition, other specific plant growth-promoting microbes may have been increased in the rhizosphere. Many of these microbes can induce systemic resistance (38). He et al. (18) demonstrated that nonpathogenic strains of *F. oxysporum* could activate defense mechanism in asparagus roots when subsequently infected with pathogenic *Fusarium* spp. Because field soil containing natural populations of *F. oxysporum* was used in these experiments, the role of nonpathogenic *F. oxysporum* cannot be ruled. This hypothesis is currently being studied.

Altered root exudation may be one mechanism of how chloride affects disease. Because microbes thrive on the carbon-rich compounds, their numbers naturally increase in the rhizosphere (26,39). Gilbert et al. (16) suggested that root health may be associated with a reduction in this rhizosphere effect and that suppression of root exudation may make a root less susceptible to infection. The current study supports this hypothesis so far as the evidence indicates a presumed reduction in organic and amino acid exudates. In contrast to the hypothesis, the total numbers of heterotrophic bacteria were not affected and fluorescent pseudomonads and the Mn-reducing microbes increased as root severity declined. However, fluorescent pseudomonads and the Mn-reducing microbes represent only a small fraction of the total bacterial densities. He et al. (18) reported preinoculation of asparagus roots with nonpathogenic *F. oxysporum* followed by inoculation with pathogenic *F. oxysporum* produced root exudates that inhibited the pathogens. Because these studies were not designed to capture and analyze root exudates, it is not known if substrates being released could adversely affect the microbial communities directly. However, when the leachates were concentrated 10 times and placed in wells in agar containing chlamydo-spores of the pathogenic *F. oxysporum* (strain FOA50), there was no effect on germination or germ tube growth (W. H. Elmer, unpublished data).

The increase in Mn-reducing microbes following treatment with NaCl also was observed on asparagus in field studies (6). It is still not clear what signal promoted their increase. Although many Mn-reducers were fluorescent pseudomonads, the correlation between these two phenotypes was weak (6). In fact, Ghiorse (15) stated that the ability to reduce Mn has never been a reliable taxonomic trait. The Mn-reducing trait exists in many aerobic facultative soil bacteria, and it may be inducible in some. Huber and McCay-Buis (21) reported that the biocontrol agents *Bacillus subtilis* strain A-13 and *Pseudomonas fluorescens* 2-79 reduced Mn and suppressed disease when applied under an ammoniacal-N regime, but

they oxidized Mn and increased disease when nitrates were applied. Plants also can increase Mn availability in slightly acid soils by releasing organic acids to acidify the rhizosphere and increased Mn availability (28). In neutral soils, it is not clear how a reduction in malic and amino acids might increase Mn-reducing microbes. However, Ghiorse (15) suggested that in neutral soils adding glucose and organic acids would increase microbial oxidation of Mn through increased pH following the metabolic utilization of these substrates. The findings reported here found no significant effect on Mn-oxidizing microbes. However, if the trait is inducible in microbes, the application of NaCl may reduce the carbon flow in the root exudates, suppress Mn oxidation, and stimulate the microflora to be Mn-reducing.

One obvious role that Mn could have in increasing host resistance is in the deposition of ligneous defense barriers (3,22). Synthesis of these products requires many reactions in the shikimic acid pathway which contain enzymes activated by Mn (3). Lignin barriers are implicated with reduced *Fusarium* infection (12,18,35). Although Mn deficiency is rarely observed in asparagus, inadequate levels may occur in neutral soils (20) and may lead to increased root susceptibility. Since microbes account for most Mn transformations in neutral soil (15), asparagus may use chloride in its osmoregulation to alter its exudation products to promote an Mn-reducing microflora.

Asparagus responds more favorably to NaCl than other sources of chloride (5,31), but Na applied in the absence of Cl provides little benefit (30). Uptake of Na was suppressed relative to Cl concentration, but still reached levels typical of macroelements. Sodium also was less mobile in the root system than Cl. The preference for NaCl over other chloride forms may result from the ability of asparagus to restrict and compartmentalize Na uptake. Other cations like K, Ca, and NH₄ that are physiologically active may cause toxicity when applied in the rates required for Cl disease suppression. Because asparagus retains most of its nutrients from year to year in its vast root system, addition of excessive amounts of any cation other than Na may create an imbalance. Schneider (33,34) argued that a specific ratio between cations and Cl was associated with *Fusarium* disease suppression on celery.

Until new management practices or resistant cultivars are available, growers must avoid stress or relocate their plantings to new land where *Fusarium* densities in the soil are low. Sodium chloride, in the form of rock salt, has been one strategy to invigorate old fields that are declining from *Fusarium* crown and

TABLE 5. Pairwise correlation coefficients (*r*) for variables describing *Fusarium* crown and root rot, microbial densities in the rhizosphere soil, and root composition of asparagus plants grown in split pot culture and treated with different rates of NaCl^a

Variables	Root lesions ^v	CFU/cm of root ^w	Fluorescent pseudomonads ^x	Mn reducers ^x	Amino acids ^y	Malic acid ^y	Na ^z	Cl ^z
Disease								
Root lesions	—	—	—	—	—	—	—	—
CFU/cm of root	0.62***	—	—	—	—	—	—	—
Rhizosphere soil								
Fluorescent pseudomonads	0.62***	0.46**	—	—	—	—	—	—
Mn reducers	0.30*	0.26	0.27	—	—	—	—	—
Root composition								
Amino acids	0.11	0.36*	0.56**	0.41*	—	—	—	—
Malic acid	0.44**	0.51***	0.32	0.32	0.45**	—	—	—
Na	0.67***	0.54***	0.43*	0.21	0.25	0.46**	—	—
Cl	0.64***	0.52***	0.36*	0.34*	0.33*	0.71***	0.74***	—
Mn	0.26	0.21	0.16	0.43	0.18	0.22	0.38*	0.32*

^a Pearson's correlation coefficients for pairwise tests that include 36 values/variable; values followed by *, **, or *** are significant at *P* = 0.05, 0.01, or 0.001, respectively, and corrected using Bonferroni's correction factor.

^v Percent root lesions = number of line/root intersects where a root had a lesions/total intersects) × 100].

^w Colony forming units of *Fusarium oxysporum* or *F. proliferatum* per centimeter of root determined on Komada's selective medium (23); root length was determined by the line-intersect method (37).

^x Values expressed as Log CFU per gram of rhizosphere soil.

^y Values expressed as milligrams per gram of dry root weight.

^z Values expressed as micromoles per gram of root.

root rot. Although NaCl may suppress disease by multiple mechanisms, the systemic activity of chloride may allow growers to band-apply NaCl instead of a broadcast application and minimize sodicity on heavier clay soils.

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