Association Between Mn-Reducing Root Bacteria and NaCl Applications in Suppression of Fusarium Crown and Root Rot of Asparagus

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Accepted for publication 2 September 1995.

ABSTRACT

Elmer, W. H. 1995. Association between Mn-reducing root bacteria and NaCl applications in suppression of Fusarium crown and root rot of asparagus. Phytopathology 85:1461-1467.

Ten field tests were conducted to determine if the suppression of Fusarium crown and root rot of asparagus by NaCl was associated with changes in the densities and characteristics of root-colonizing bacteria. A total of 1,114 strains of root-colonizing bacteria were isolated from NaCl-treated and nontreated feeder roots (80 to 200 per test) and rated for their ability to reduce Mn on a Mn-dioxide medium, for UV fluorescence, and for antagonism against *Fusarium oxysporum* and *F. proliferatum*. In addition, bacterial densities on roots, levels of Mn in roots, and marketable yield were measured. In seven out of 10 tests, NaCl-treated roots had a greater percentage of Mn-reducing bacteria than control roots. Overall, the combined data revealed a significant increase in the small fraction of Mn-reducing bacteria on NaCl-treated roots compared to control roots ($P = 0.006$). In five out of six tests, roots from NaCl-treated plots had an average of 19% more Mn per gram of root than roots from control plots ($P = 0.08$). The NaCl treatment did not affect the densities of bacteria on roots or the percentage of strains that were antagonistic to the *Fusarium* spp. The percentage of strains that fluoresced under UV light on King's B medium was significantly higher in half of the tests and, overall, was significant at $P = 0.097$. Fluorescent pseudomonads were more likely to be Mn-reducing strains than were nonfluorescing bacteria ($P = 0.019$). Twenty-eight out of 30 Mn-reducing strains were *Pseudomonas* spp. (64% fluorescent pseudomonads) and two were *Serratia* spp. Only seven out of 20 Mn-reducing strains suppressed disease on asparagus transplants in greenhouse tests. The disease-suppressive strains of *Serratia* spp. and *P. corrugata* were associated with higher levels of Mn in the roots of asparagus transplants grown in noninfested soils than in nontreated roots, but there were other Mn-reducing strains that did not affect disease or Mn levels. The interrelationships among NaCl treatments, suppression of Fusarium crown and root rot, and increases in Mn-reducing rhizobacteria tended to support the hypothesis that increased Mn availability contributes to disease resistance in asparagus.

Additional keywords: *Asparagus officinalis*, mineral nutrition, rock salt, soilborne disease.

Numerous reports show chloride salts are suppressive to disease (7,16,39,42,54), but the mechanism(s) of disease suppression is(are) not completely understood. The presence of chloride is known to inhibit soil nitrification (27), and plant water potential and root exudation also are affected (7,14,40). It has been proposed that chloride may alter host resistance (40). These studies are in accord with observations on grain crops demonstrating increased Mn availability following chloride applications (26,27,29), in that Mn also was implicated in host resistance (21,22,30).

Factors which affect Mn availability and resulting disease are often associated with changes in microbial populations in the rhizosphere (27,35,43,44). For example, ammonium nitrogen applied to wheat increases tissue levels of Mn (29), increases root colonization by *Pseudomonas* spp. (44), and suppresses take-all (29,34,44). Huber and McCay-Buis (29) reported that suppression of take-all of wheat with ammoniacal fertilizers combined with nitrification inhibitors decreases Mn-oxidizing rhizobacteria and increases Mn availability. Rogers (35) found that green manure amendment to soil increases the Mn-reducing microorganisms and suppresses potato scab. The effect of chloride on Mn availability and microbial changes in the rhizosphere have not been adequately studied.

Many early publications reported that rock salt (NaCl) liberally applied to asparagus fields improves yields and reduces weed competition (4,6,36,37,51,53). However, following the development of herbicides in the 1940s, there was little mention of NaCl usage in asparagus culture. After 1940, the number of reports of a destructive disease of asparagus called Fusarium crown and root rot, caused by *Fusarium oxysporum* Schlechtend.:Fr. and *F. proliferatum* (T. Matsushima) Nirenberg (synamorph *F. moniliforme* J. Sheldon), dramatically increased in North America (8,20, 23,31). The coincidence between the end of NaCl use and the increase in disease reports prompted experiments which showed that single applications of NaCl (560 kg/ha) suppresses Fusarium crown and root rot and increases marketable yields (15). Soil from NaCl-treated and nontreated field plots did not differ in *Fusarium* populations, but in greenhouse experiments fewer colonies of *F. oxysporum* and *F. proliferatum* grew from NaCl-treated roots than nontreated roots (12,15). In addition, ferns of NaCl-treated plants had higher levels of carbohydrates and nutrients, especially Fe and Mn, which were commonly increased 25 to 50% when compared to controls (13,15; W. H. Elmer, *unpublished data*).

Asparagus is favored by slightly acidic to neutral soils, in which Mn and other trace elements are less available than soils with low pH (25). Rhizobacteria that increase Mn availability would be beneficial in these soils, and they may also play a direct role in disease suppression. Traits that are common to disease-suppressing rhizobacteria have included antagonism toward pathogens (18,41,52) and UV fluorescence as associated with siderophore production (32,38,52). However, in view of past studies, the ability to increase Mn availability deserves attention as another potential characteristic of beneficial microbes.

The objectives of this work were to determine if the disease-suppressive applications of NaCl could affect Mn levels in roots,

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marketable yields, and densities of root-colonizing bacteria in fields of asparagus declining from Fusarium crown and root rot, and to determine if these bacterial populations differed in characteristics such as the ability to reduce Mn in vitro, antagonism against *Fusarium* spp., and/or UV fluorescence. A third objective was to determine if Mn-reducing bacteria could influence disease and Mn levels in the roots.

MATERIALS AND METHODS

Field history and treatments. Ten separate tests were conducted in the spring and summer of 1991 to 1994 in six different 4- to 10-year-old experimental field plots and in one commercial asparagus field in Madison, CT (Table 1). Experimental plots were initially established with 1-year-old asparagus ('Syn 4-56') crowns (Nourse Farms, Whately, MA) or 1-year-old transplants ('Mary Washington') grown from seed in the greenhouse. Crowns (or transplants) were placed 0.3-m apart into trenched rows spaced 1.5-m apart. Plots in tests 1, 2, 8, 9, and 10 contained between three to five crowns per plot, and plots in tests 6 and 7 contained 13 to 15 crowns per plot. In the commercial field (tests 3 to 5), eight plots (3-m long) were selected from three rows that were approximately 30-m long.

In each test, there were four replicate plots treated with NaCl and four plots were left untreated to serve as controls. In all test plots, insects were controlled with timely applications of insecticides. Weeds were removed by hand in test plots 1, 2, 8, 9, and 10. In other plots, glyphosate (Roundup 4WSL) was applied before the spears emerged in the spring, and applications of metribuzin (Sencor 75DF) and napropamide (Devrinol 50DF) were applied at label rates after spears were harvested. Soils in each test plot were limed in the fall as required to maintain a soil pH of 6.0 to 7.0, which was determined at the time treatments were applied.

Sodium chloride (common rock salt) was broadcast (560 kg/ha) over the crowns and on the soil out to 0.75 m from the crown on four of the eight plots in each field on the specified dates mentioned (Table 1). All plots were fertilized at the same time with 560 kg/ha of 10-10-10, N-P_2O-K_2O fertilizer. Spears were harvested from treated plots three times a week for 2 to 5 weeks (depending on the age of the planting), trimmed to 22.5 cm, weighed, and compared to control plots to assess the disease-suppressing effect of NaCl. No yield was available from the commercial field (tests 3 to 5).

Sampling and enumeration of root-colonizing bacteria. In all tests except test 8, soil cores (22.5 × 3-cm diameter) were removed 4 to 5 weeks after the NaCl was applied. Test 8 was sampled a year after the last application of NaCl was made. Five soil cores were taken with a soil auger from each replicate plot in the rows, approximately 12 to 15 cm from the crown, and bulked. Since feeder roots are the site of *Fusarium* infection (45), they were separated from storage roots with forceps and placed into beakers. Roots were briefly immersed in 1% sodium hexametaphosphate, rinsed in tap water, surface-treated in 0.2% Na hypochlorite for 1 min, and rinsed three times in sterile distilled water. This procedure removed adhering soil and surface inhabitants. Excess water was removed from the roots by pressing them between sterile, absorbent paper towels. Roots were weighed and, depending on the amount, between 100 to 200 mg was placed in sterile 125-ml Erlenmeyer flasks with enough sterile saline (0.9% wt/vol) to yield 4.0 mg of roots/ml of saline. Roots were agitated for 45 min on a wrist-action shaker, whereupon the saline rinsates were serially diluted with sterile saline to yield 4×10^{-5}, 4×10^{-6}, and 4×10^{-7} g of root/ml. Aliquots (0.2 ml) of diluted saline rinsate were spread onto nutrient agar (Difco Laboratories, Detroit) in 10-cm diameter petri plates. Plates were prepared in triplicate and incubated in the dark for 2 to 3 days at 25°C. Plates that contained between 30 and 300 colonies were counted and used to estimate densities of root-colonizing bacteria per gram of root. The counts from the three plates from each replicate plot were averaged, expressed as log colonies of bacteria per gram of root, and used in statistical analyses. When possible, the roots were saved, bulked according to treatment, and frozen for analyses described below. Isolation for *Fusarium* spp. was not done because of the difficulty in differentiating pathogens from morphologically identical nonpathogens in field soil.

Depending on the test, between 45 and 100 bacterial colonies were sampled from predetermined quadrants on the plates and increased in culture test tubes containing 3 ml of sterile Luria-Bertani (LB) broth (Difco Laboratories). Equal numbers of colonies were sampled from plates that contained the NaCl-treated roots and control roots. Once a turbid suspension developed in the broth (24 to 72 h), a loopful of each strain was transferred to the three media described below.

Microbial assays. Assays were conducted to detect Mn reduction, UV fluorescence, and antagonism toward the *Fusarium* pathogens. The ability to reduce Mn was detected on a Mn-dioxide agar (5 g of Mn-dioxide, 30 g of sucrose, 1 g of Difco yeast extract, 15 g of agar) (35). Each strain was streaked down the center of two 10 × 60-mm diameter petri plates and incubated in the dark for 4 weeks at 25°C. Organisms capable of reducing the insoluble black Mn-dioxides were detected by a clearing around the colonies. Each strain was rated for its Mn-reducing ability on a scale of 1 to 4, in which 1 = no clearing (non-Mn reducer), 2 =

TABLE 1. History of asparagus fields treated with NaCl and sampled for root-colonizing bacteria

Test no.	Location	Cultivar[a]	Date planted	Date(s) NaCl applied[b]	Date(s) roots sampled	No. of strains recovered[c]	Soil pH[d]
1	Hamden, CT	B	1989	April 1991	May 1991	199	6.4
2	Hamden, CT	B	1989	April 1992	June 1992	114	6.9
3	Madison, CT	B	1989	April 1991	May 1991	98	6.3
4	Madison, CT	B	1989	April 1992	May 1992	83	6.3
5	Madison, CT	B	1989	June 1992	July 1992	99	...
6	Windsor, CT	A	1983	April 1987–91	June 1991	120	6.3–6.9
7	Windsor, CT	B	1990	June 1992	July 1992	120	6.2
8	Hamden, CT	A	1986	May 1987–91	August 1992	128	6.2–6.9
9	Hamden, CT	B	1989	May 1994	June 1994	45	7.1
10	Hamden, CT	B	1989	April 1994	June 1994	108	7.2

[a] A = asparagus cultivar Mary Washington, B = asparagus cultivar Syn 4-56.
[b] NaCl was broadcast over the rows at 560 kg/ha.
[c] Approximately equal numbers of bacteria from salt-treated and control roots were recovered from each field.
[d] Soil pH was determined at the time when NaCl treatments were applied.

Fig. 1. Mn-reduction rating scale for asparagus root-colonizing bacteria grown on Mn-dioxide agar at 25°C for 4 weeks: 1 = no clearing (non-Mn reducer); 2 = slight clearing (slight Mn reducer); 3 = moderate clearing (moderate Mn reducer); and 4 = complete or almost complete clearing (strong Mn reducer).

slight clearing (slight Mn reducer), 3 = moderate clearing (moderate Mn reducer), and 4 = complete or almost complete clearing (strong Mn reducer) (Fig. 1). Representative strains that received a Mn-reducing rating of 3 or 4 were again subcultured in LB broth (Difco Laboratories) and stored in 15% glycerol at –40°C.

UV fluorescence was detected by viewing 2-day-old colonies of each strain on Kings' B medium (10) under a short wave UV light and scored as fluorescent or nonfluorescent. In vitro antagonism to the two *Fusarium* spp. was detected by pairing the two *Fusarium* spp. on either side of a fresh bacterial lawn streaked in the center of a 10 × 60-mm diameter petri plate containing potato-dextrose agar (10). Duplicate plates were sealed with parafilm, incubated in the dark at 25°C for 7 days, and then rated for zones of inhibition around the bacterial strain on a scale of 1 to 4, in which 1 = no antagonism, 2 = slight antagonism, 3 = moderate antagonism, and 4 = strong antagonism.

Identification of Mn-reducing bacteria. Single cells of 30 Mn-reducing bacteria were selected from freshly streaked nutrient agar plates and increased in the dark on Tryptic soy agar (TSA) (Difco Laboratories) for 24 h at 25°C. Bacteria were identified to genera based on their utilization of carbon substrates as provided by Biolog Identification System (version 3.0) (Biolog Co., Hayworth, CA). Representative strains were further identified using fatty acid analysis (Analytical Services Inc., Essex Junction, VT).

Effects of Mn-reducing bacteria on Fusarium crown and root rot of asparagus. Twenty strains of Mn-reducing bacteria were tested for their ability to suppress disease on asparagus transplants in the greenhouse. Bacterial colonies were grown on TSA for 24 h at 25°C in the dark. Methods for growing the transplants ('Mary Washington'), producing the inoculum, and preparing the soil are published elsewhere (12,15). Roots of the transplants were placed in 100 ml of a 1% carboxymethyl cellulose (CMC) suspension containing 10^8 bacteria cells/ml for 30 min. Control plants were exposed to distilled water containing 1% CMC. Five transplants were each placed into 1-liter plastic pots filled with soil, that were infested with *F. oxysporum* and *F. proliferatum*, and held in the greenhouse for 10 weeks. Each pot received 100 ml of Hoagland's nutrient solution (24) weekly. Fresh weights of the entire plant were recorded, and root lengths were measured as described by Tennant (48). The experiment was repeated once.

Five out of the 20 Mn-reducing strains were selected from above and tested further for their ability to affect disease and to increase root concentrations of Mn. Two of the strains (SC105 and P6296) had been previously rated as disease-suppressive, and three strains (P6270, P385, and P2185) did not affect disease. Experiments were conducted as above, but an equal number of transplants were treated with the bacteria and placed in pots filled with noninfested soil. After 8 weeks, plants were examined and rated for disease as described above. Feeder roots were separated from storage roots, freeze-dried, and assayed for Mn levels. There were five replicates and the experiment was repeated once.

Elemental analyses. Dried feeder roots were ground with a mortar and pestle and digested by placing 0.5 g of tissue with 5 ml of HCl and 10 ml of HNO_3 in a CEM Microwave Digestion System 81D microwave (CEM Corp., Matthews, NC) according to the instructions from the manufacturer. Samples were brought up to a volume of 50 ml with deionized water. Levels of Mn were determined by inductively coupled plasma spectroscopy (28) on an Applied Research Laboratory 3520 inductively coupled plasma optical emission spectrophotometry (ICP-OES) spectrophotometer (Fisons Instruments, Dearborne, MI) and expressed as μmol/g of dry weight.

Statistical procedures. A Student's *t* test with unequal variances was used to detect statistical differences between the total

TABLE 2. Effect of NaCl on yield of asparagus

Test[b] no.	Spear weight[a] (metric tons/ha)		P value[c]
	Salt-treated	Control	
1	6.00	4.98	0.05
2	7.55	6.48	0.05
6	2.08	1.66	0.05
7	1.87	1.30	0.04
8	4.00	3.15	0.09
9	7.81	6.30	0.05
10	8.20	8.23	ns
Mean	5.35	4.58	0.01

[a] Spear weight represents the total marketable trimmed (22.5 cm) yield.
[b] Test refers to different field experiments consisting of four replicate plots either treated or not treated with NaCl (560 kg/ha); see text for details. No yields were taken in tests 3 to 5.
[c] *P* value determined from Student's *t* test. ns = $P > 0.10$.

TABLE 3. Effect of NaCl on bacterial densities and concentrations of Mn in asparagus roots in fields affected by Fusarium crown and root rot

Test[a] no.	Colonies/g of root (× 10^4)[b]		P value	Mn levels (μmol/g of root)	
	NaCl	Control		NaCl	Control
1	24.0	4.3	0.05	...[c]	...
2	912.5	875.0	ns	...	...
3	17.1	1.9	0.01	...	...
4	61.3	82.6	ns	2.5	2.3
5	185.0	150.0	ns	5.3	4.5
6	99.0	73.5	ns	...	...
7	300.0	118.1	ns	2.0	1.8
8	102.0	113.0	ns	5.8	4.3
9	580.0	990.0	ns	1.7	1.8
10	468.0	76.4	0.02	2.0	1.5
Mean	274.9	248.5	ns	3.2	2.7 ($P = 0.08$)[d]

[a] Test refers to different field experiments consisting of four replicate plots either treated or not treated with NaCl (560 kg/ha).
[b] Bacterial densities were log-transformed and compared using Student's *t* tests at $P = 0.05$. The overall totals were compared using Student's paired *t* test.
[c] Mn analyses were not available for these tests.
[d] Means separated using the Student's paired *t* test.

TABLE 4. Effect of NaCl on recovery of Mn-reducing bacteria, fluorescent pseudomonads, and bacteria antagonistic to *Fusarium* spp. from asparagus roots affected by Fusarium crown and root rot

Test[a] no.	% Mn reducers[b]			% Fluorescent pseudomonads[c]			% Exhibiting antagonism[d]		
	NaCl	Check	P	NaCl	Check	P	NaCl	Check	P
1	20	23	ns	50	49	ns	20	19	ns[e]
2	35	11	0.03	66	83	0.05	3	26	0.01
3	11	5	0.02	36	28	ns	7	5	ns
4	2	0	ns	16	0	0.01	0	0	...
5	33	2	0.01	2	12	0.05	0	0	...
6	14	12	ns	92	59	0.03	0	0	...
7	18	7	0.05	0	10	0.01	0	0	...
8	18	6	0.05	40	14	0.01	...	-	...
9	15	0	0.05	48	16	0.01	15	40	0.03
10	18	3	0.05	65	58	ns	1	2	ns
Mean	18	7	0.006	42	33	0.09	5.1	10.2	ns

[a] Test refers to different field experiments consisting of four replicate plots either treated or not treated with NaCl (560 kg/ha).
[b] Mn reducers determined on a medium that contained insoluble Mn-dioxide.
[c] Fluorescence was detected by viewing 2-day-old culture on King's B medium under UV light. The Kruskal-Wallis Rank Test was used to examine whether NaCl applications affected the densities of root-colonizing fluorescent pseudomonads. The total means were similarly compared.
[d] Antagonism against *F. oxysporum* and *F. proliferatum* was determined in vitro on agar plates and rated as 1 = no antagonism, 2 = slight antagonism, 3 = moderate antagonism, and 4 = intense antagonism. Those strains that received a rating of 3 or 4 were scored as exhibiting antagonism. Within each test, antagonism was compared using the Kruskal-Wallis Rank Test. The total means were similarly compared.
[e] ns = $P > 0.10$.

log number of root-colonizing bacteria from NaCl-treated roots and nontreated roots in each test (47). When the ratings of bacterial characteristics were plotted by frequency, they did not fit a normal distribution; therefore, the nonparametric Kruskal-Wallis Rank Test ($P = 0.05$) (9) was used. Although it was technically invalid to consider the different tests as true repetitions or blocks of the same experiment, since the fields differed in age, cultivars, and the time that treatments and samples were made, comparisons using the 10 tests were cautiously made to reveal overall patterns. Greenhouse tests were analyzed using Tukey's test following arcsine transformation, when necessary, to establish homogeneity of variance (47). All analyses were conducted with Systat Statistical Software (SYSTAT Inc., Evanston, IL).

RESULTS

Effect of NaCl on disease and yield of asparagus. Sodium chloride applications improved the vigor and increased the average marketable yield in five out of the seven tests in which yields were taken (Table 2). The wide range of yields was caused by the difference in cultivars, disease pressure, and the number of times the spears were cut. The ferns in plots that were treated with sodium chloride were usually more dense, their roots usually whiter, and had less discoloration than those in control plots roots (data not shown).

Effect of NaCl on Mn levels and densities of root-colonizing bacteria. Asparagus roots were available in only six tests, and in each test the samples had to be bulked to obtained enough tissue for Mn analysis. Therefore, the intratest comparisons could not be done. However, a paired *t* test (47), using each test as a pair of treated and nontreated values, was used. In five out of six tests, NaCl-treated roots had an average of 19% more Mn than controls ($P = 0.081$). The number of root-colonizing bacteria was greater from NaCl-treated roots than from controls in only three out of the 10 tests, and there was no overall trend in the number of root-colonizing bacteria (Table 3).

Effect of NaCl on the phenotypes of root-colonizing bacteria. Bacteria that cleared the insoluble Mn-dioxide agar were labeled as Mn-reducing bacteria, and in some test plots up to 35% of the strains had this trait (Fig. 1). Those strains that received a rating of 4 (strong Mn reducer) usually began to clear the black agar in less than 7 days. This reaction was presumed to be catalyzed by a diffusible enzyme because the reduction occurred rapidly and at some distance from the bacterial lawn. The Mn-reduction that was rated as 2 (slight Mn reducer) took approximately 3 to 4 weeks to occur, was in close proximity to the bacterial lawn, and was probably a result of microbial acid production.

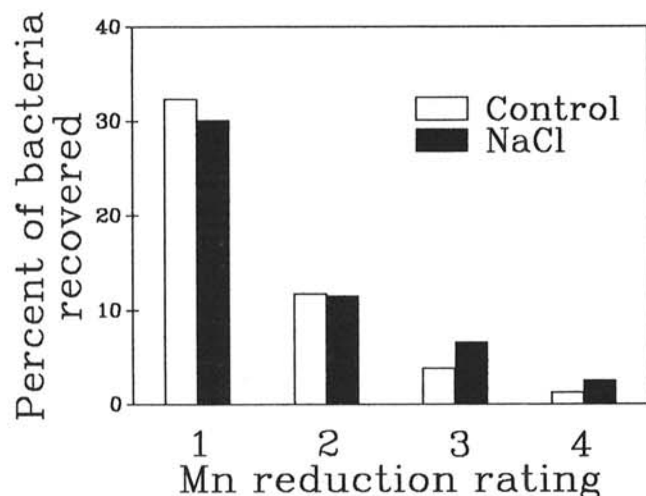

Fig. 2. Percentage of strains (out of 1,114) recovered from untreated (open bars) and NaCl-treated (filled bars) asparagus roots. The Mn-reduction ratings are 1 = no clearing (non-Mn reducer), 2 = slight clearing (slight Mn reducer), 3 = moderate clearing (moderate Mn reducer), and 4 = complete or almost complete clearing (strong Mn reducer). Treatment differences were significantly different as determined by Kruskal-Wallis Rank Test ($P = 0.006$).

Those bacteria that received a Mn-reduction rating of 3 or 4 were found in greater percentages on NaCl-treated roots than on control roots in seven out of the ten tests (Table 4). However, because those bacteria with a Mn-reduction rating of 1 (non-Mn reducers) were the most common and those with a rating of 4 (strong Mn reducers) were rare, a skewed distribution resulted. The effect of NaCl was statistically compared by the Kruskal-Wallis Rank Test. In intratest comparisons, seven out of 10 tests had significantly more Mn-reducing bacteria on NaCl-treated roots than on nontreated roots. When the 10 tests were combined and compared, significantly more Mn-reducing bacteria colonized on NaCl-treated roots than roots from control plots ($P = 0.006$) (Fig. 2).

NaCl applications were associated with significant differences in the proportion of fluorescent pseudomonads in seven out of the 10 tests (Table 4). However, in two of the seven fields that were affected, NaCl-treated roots had a smaller proportion of fluorescent pseudomonads than control roots; whereas, in the other five fields, the NaCl treatment was associated with significantly larger increases in the proportion of fluorescent pseudomonads than in controls. The proportion of fluorescent pseudomonads varied considerably between fields, and there was no consistent pattern between changes in fluorescent pseudomonad densities and yield increases. Since the data were binary, meaning the values were scored as fluorescent or nonfluorescent, the rank data of each test were combined and compared to results from the controls by the Kruskal-Wallis Rank Test. The overall effect of NaCl on the percentage of root-colonizing fluorescent pseudomonads was significant at $P = 0.097$. Fluorescent pseudomonads were statistically more likely to be Mn-reducing bacteria than were the nonfluorescent bacteria (Kruskal-Wallis Rank Test, $P = 0.019$) (data not shown).

TABLE 5. The effect of Mn-reducing bacteria on Fusarium crown and root rot of asparagus in greenhouse tests

Strain	Percent diseased roots[a]		Identification
	Experiment I	Experiment II	
SC105	7.4*[b]	6.2*	*Serratia* spp.[c]
P6100	7.2*	20.0	*Pseudomonas* spp. (fluorescent)[d]
P785	7.4*	...	*Pseudomonas putida* biotype A[c]
SC67	7.4*	11.7*	*Pseudomonas corrugata*[d]
P6296	8.7*	13.4*	*Pseudomonas corrugata*[d]
P6297	...	13.8*	*Pseudomonas marginalis*[c]
SC33	8.7*	5.4*	*Pseudomonas* spp. (fluorescent)[d]
P386	8.9*	13.7*	*Pseudomonas* spp. (fluorescent)[d]
P6276	9.7*	15.1	*Pseudomonas* spp. (fluorescent)[d]
P452	...	7.4*	*Pseudomonas* spp. (fluorescent)[d]
P2211	10.7*	10.8*	*Pseudomonas* spp. (fluorescent)[d]
P6270	13.0	15.0	*Pseudomonas fluorescens* biotype A[d]
P373	13.8*	...	*Pseudomonas* spp. (fluorescent)[d]
P6193	14.0	36.0	*Pseudomonas* spp. (fluorescent)[d]
P385	14.1	14.4	*Pseudomonas fluorescens* biotype A[c]
P6287	14.9	56.0	*Pseudomonas* spp. (fluorescent)[d]
P381	16.4	9.2*	*Pseudomonas corrugata*[d]
P2198	17.0	29.0	*Pseudomonas* spp. (fluorescent)[d]
P2185	17.0	14.0	*Serratia* spp.[c]
P352	18.7	9.8*	*Pseudomonas corrugata*[d]
Control	38.0	37.0	

[a] Disease was assessed by determining the percentage of root length that was diseased using the line intersect method.
[b] Values represent the means of five plants, values that are followed by an asterisk are significantly different from their respective controls according to Tukey's test ($P = 0.05$). Values were arcsine-transformed prior to analysis.
[c] Identification based on fatty acid analysis (Analytical Services Inc.).
[d] Identification based on carbon utilization on Biolog GN plates (Biolog Co.).

Those bacteria that had antifungal activity against *F. oxysporum* also inhibited the hyphal growth of *F. proliferatum*. However, treatment with NaCl did not increase the percentage of bacteria that exhibited antagonism against the pathogenic *Fusarium* spp. in vitro in any of the 10 tests (Table 4). In fact, antagonism was rarely observed and, in two tests, it was significantly more common among bacteria associated with roots in nontreated control plots than among bacteria from NaCl-treated roots. In addition, strains that exhibited antagonism were significantly less likely to be Mn-reducing bacteria than those that exhibited no antifungal activity ($P < 0.001$) (data not shown).

Identification of Mn-reducing strains. Of 30 strains, 28 belonged to the genus *Pseudomonas* and 18 (64%) were fluorescent pseudomonads. These included closely related species of *P. fluorescens* biotype A, *P. marginalis*, and *P. putida* biotype A. All nonfluorescent pseudomonads were identified as *P. corrugata*. The two other strains were identified as *Serratia* spp.

Effects of Mn-reducing bacteria on Fusarium crown and root rot of asparagus. Twenty strains of Mn-reducing bacteria were tested for their ability to suppress Fusarium crown and root rot in the greenhouse (Table 5). Eleven or 12 strains suppressed disease. However, only seven strains consistently suppressed the disease in both tests.

Five Mn-reducing strains were tested again for their ability to influence disease and affect the mineral composition of asparagus roots. Two of these were previously shown to be disease-suppressive, while the others did not affect disease. The two disease-suppressive strains, *Serratia* spp. (SC105) and *P. corrugata* (P6296), were again effective in suppressing disease with larger plant weights and root lengths than controls, but only SC105 *(Serratia* spp.) significantly increased Mn levels in the roots of plants grown in noninfested soils (Table 6). Strain P2185 *(Serratia* spp.) did not suppress disease and, in both repetitions of this trial, this strain was associated with more disease than control plants. Although healthy plants treated with P2185 showed no visual damage, the levels of Mn in the roots was 36% less than in control roots.

DISCUSSION

Increases in asparagus yield were strongly correlated with increased vigor of the fern canopy (11) and with less Fusarium crown and root rot (15). In five out of seven tests in which yields were recorded, NaCl applications resulted in higher yields than control plots. However, yield increases were not consistently associated with increases in the number of root-colonizing bacteria, proportion of fluorescent pseudomonads, or bacteria that exhibited antagonism. However, in seven out of 10 tests, NaCl applications caused detectable shifts in the small proportion of rhizobacteria that could reduce Mn in vitro (Table 4) and, when all 10 tests were combined, the NaCl effect was highly significant ($P = 0.006$). Moreover, in five out of six comparisons, the NaCl treatment increased Mn levels in the roots by 19% when compared to controls ($P = 0.08$).

A number of possible mechanisms, not mutually exclusive, have been proposed to explain how increasing Mn availability can influence disease (21,22,30). Increased concentrations of Mn at the root surface may exert a fungistatic effect on *Fusarium* spp. High concentrations of Mn are toxic to *Streptomyces scabies* and suppress potato scab (33). This might explain past greenhouse studies (15; W. H. Elmer, *unpublished data*) in which NaCl applications suppressed disease, reduced root colonization, and increased Mn tissue levels, but did not affect the total densities of the *Fusarium* spp. in bulk soil. Secondly, Mn could indirectly affect host susceptibility through its effect on root exudation. Mn-activated enzymes affect nitrogen metabolism and photosynthesis (5). It is possible that changes in root exudation could deprive the pathogen of needed substrates to overcome fungistasis. On the other hand, altered exudation could favor a disease-suppressive microflora. Although fluorescent pseudomonads are frequently associated with healthy vigorous roots and have been implicated in disease suppression (32,38,52), the current study found this bacterial group to be associated with NaCl-treated roots in only half of the tests ($P = 0.097$). In addition, there was no evidence that NaCl treatment favored a microflora that was antagonistic to the *Fusarium* spp. However, it is possible that changes in root exudation resulted in higher populations of the Mn-reducing rhizobacteria, but it is not clear if this microbial trait had any direct or indirect role in the suppression of Fusarium crown and root rot. A third mechanism suggests that increased Mn availability could directly affect host defense by activating enzymatic systems which produce ligneous defense barriers (5,22,30). Inasmuch as lignin deposition in asparagus roots has been implicated as a barrier to infection by *Fusarium* spp. (17,45), it would be reasonable to assume that roots that manufacture and deposit lignin faster would be more resistant to infection than roots less efficient in lignin deposition.

Although many micronutrients become less available to plants as soil pH rises from 6.0 to 7.0, Mn availability is most affected (25). In fact, large changes in Mn availability in soils with pH between 6.0 to 7.0 are almost exclusively mediated by microbial activity (1,2,19). Nevertheless, the study of Mn-transforming bacteria in plant disease has been limited to only a few reports (29,35,49,50). Timonim and colleagues (49,50) first showed that elevated densities of Mn-oxidizing bacteria in the rhizosphere of oats causes the grey speck disease, but found that tolerant cultivars and disease-suppressive soil treatments would not support this microflora. Rogers (35) demonstrated that organic residue amendments increase Mn-reducing microorganisms and suppress potato scab. However, he detected no appreciable increases of Mn^{+2} in bulk soil extracts and concluded that Mn availability was not increased enough to be toxic to *Streptomyces scabies*. An alternate explanation may have been that Mn availability was increased in the rhizosphere and soil associated with the tubers in which densities of Mn-reducing microbes may be higher and infection would occur, but tissue analyses were not done to test this assumption. Huber and McCay-Buis (29) reported that take-all disease is less severe when wheat seeds are treated with Mn-reducing bacteria, while disease is markedly greater when Mn-oxidizing strains are applied to seeds. It is probable that Mn-transforming microbes will be implicated in other disease systems.

In the present study, strains of Mn-reducing bacteria were less likely to exhibit antagonism than non-Mn-reducing strains. Al-

TABLE 6. The effect of Mn-reducing bacteria on asparagus growth, Mn availability, and Fusarium crown and root rot of asparagus

Species	Strain	Fresh weight[a] (g)	Root length[b] (m)	Mn concentration[c] (μmol of Mn/g of root)
Disease-suppressive				
Pseudomonas corrugata	P6296	2.58*[d]	1.82*	6.5
Serratia spp.	SC105	3.18*	2.40*	7.0*
Not disease-suppressive				
P. fluorescens	P6270	1.52	1.43	5.9
P. marginalis	P385	1.58	1.42	5.4
Serratia spp.	P2185	1.02	1.03	3.2
Control		1.36	1.12	5.0

[a] Includes ferns and roots.
[b] Root length determined using modified line intersect method.
[c] Determined using inductively coupled plasma spectrophotometry following acid digest of freeze-dried feeder roots.
[d] Values represent the means of five plants, values that are followed by an asterisk are significantly different from their respective control according to Tukey's test ($P = 0.05$).

though greenhouse tests provided evidence that some of the Mn-reducing bacteria could suppress disease and increase Mn uptake, the effect was not associated with all the Mn-reducing bacteria. One strain of *Serratia* spp. (SC105) consistently suppressed disease and increased Mn levels in the roots of plants grown in noninfested soils, while another strain of *Serratia* spp. (P2185) provided no benefit and tended to lower the Mn concentrations relative to the control. The reason for these differences is unexplained. Strains of *S. liquefaciens* were shown to suppress Fusarium wilt on carnation and to produce inhibitory chitinases (46), but no inhibition was detected in the current study.

It was of interest to find that fluorescent pseudomonads were more probable to be strong reducers of Mn-dioxide than nonfluorescent bacteria. This observation raised the question of whether siderophores, which are produced by fluorescent pseudomonads, have a role in Mn transformations (32). Furthermore, it may be significant that many of the Mn-reducing bacteria identified in this study belong to species which have been implicated in disease suppression (52). The frequent categorization of root bacteria as plant-growth promoting rhizobacteria or deleterious rhizosphere microorganisms (38) could also relate to the ability of these microbes to increase or decrease Mn availability, respectively.

A number of factors could affect the ability of microbes to reduce Mn. Since the clearing of the insoluble Mn-dioxide in vitro was probably mediated enzymatically (3), the conditions governing this reaction would be dependent on nutrition, pH, and temperature. In addition, the enzyme(s) may not be constitutively produced by all strains. Ghiorse (19) reported that strains of *Bacillus* spp. oxidize or reduce Mn, depending on the stage of its life cycle. Huber and McCay-Buis (29) reported that strains of *B. subtilis* and *P. aureofaciens* oxidize or reduce Mn, depending on the redox potential of the soil, which is affected by fertilization and cropping practices. Moreover, the composition of the root exudates could also affect the ability of bacteria to transform Mn. Alexander (1) and Ghiorse (19) reported that the presence of glucose would favor microbial reduction of Mn, while, in neutral soils, the presence of organic acids, such as citrate or malate, stimulate Mn oxidation. Since applying chloride inhibits malate synthesis in asparagus roots (13) and other crops (40), one may question whether NaCl applications could reduce malate levels excreted by roots and, thus, stimulate a Mn-reducing microflora.

In summary the Mn-reducing rhizobacteria were increased by the disease-suppressive NaCl treatments, but their collective role in the disease remains vague. While the Mn-reducing trait was not consistently associated with disease-suppressing ability, these findings suggest that the ability to reduce Mn may be a property of a disease-suppressive microbial community.

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