Populations of *Rhizomonas suberifaciens* on Roots of Host and Nonhost Plants

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ABSTRACT

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The influence of plant species and cultivar on populations of *Rhizomonas suberifaciens* (causal agent of corky root of lettuce) on roots was investigated in field and growth chamber experiments. Populations on the roots of resistant and susceptible cultivars (440-8 and Salinas, respectively) of iceberg lettuce (*Lactuca sativa* L.) grown in microplots were measured over time by an enzyme-linked immunosorbent assay (ELISA). Populations recovered from roots decreased over time and ranged from 4×10^3 to 4×10^5 cfu/g of fresh root. Populations recovered from Salinas roots were equal to or higher than those recovered from 440-8. In growth chamber experiments, populations of a rifampicin-resistant strain of *R. suberifaciens* on the roots of these cultivars were compared with those of prickly lettuce (*Lactuca serriola* L.) and a nonhost (barley, *Hordeum vulgare* L.). Root populations were determined by dilution plating on rifampicin-amended medium 6, 12, 18, and 24 days after inoculation. At all harvest dates, the susceptible lettuce cultivar Salinas contained higher populations than the other plant species and cultivars. At three harvest dates, barley had lower densities than the other plant species and cultivars. Prickly lettuce and the resistant lettuce cultivar 440-8 had similar populations at all but one harvest date. Populations of *R. suberifaciens* on roots of potted plants fluctuated over time and did not fit significantly to any simple model. The results support previous observations that 440-8 and prickly lettuce are resistant to *R. suberifaciens* because they supported lower populations of the bacterium. *R. suberifaciens* did not increase on barley roots but survived for at least 24 days.

Rhizomonas suberifaciens (25), causal agent of corky root of lettuce (*Lactuca sativa* L.), is a major pathogen of lettuce in coastal areas of California (17). The main symptoms are corky, brittle, greenish brown lesions on taproots and main laterals (23). *R. suberifaciens* is the sole member of the genus *Rhizomonas* and is most closely related to *Sphingomonas paucimobilis* (25), formerly *Pseudomonas paucimobilis* (11,27), a free-living, nitrogen-fixing bacterium from rice fields (2) and an opportunistic human pathogen (11). DNA-rRNA hybridization indicated close relationships of *R. suberifaciens* to the plant-infecting genera *Agrobacterium* and *Rhizobium* (12). The host range of *R. suberifaciens* is restricted to the Asteraceae, subfamily Liguliflorae, tribe Cichorieae (21). Although *Lactuca* spp. (4) and lettuce cultivars (4,9,19) exhibiting less corky root have been identified, no studies have determined the ability of the bacteria to grow on or in roots of resistant or susceptible hosts.

R. suberifaciens is oligotrophic (25) and grows only slowly on a medium developed for this organism (S medium) (23). For isolation of *R. suberifaciens* from lettuce roots, this medium is amended with 30 ppm of streptomycin (23). The amended medium is not very selective, however, and it is extremely difficult to isolate this pathogen from its host. Quantification of *R. suberifaciens* from infected roots or infested soil has not been successful so far (A. H. C. van Bruggen, *unpublished*).

Population dynamics of *R. suberifaciens* on different host plants may have ecological and epidemiological consequences. When resistant cultivars of iceberg lettuce become commercially available in California, extensive plantings are likely. This may increase selection pressure for strains with more virulence genes to lettuce than the currently predominant strains (26). However, if the bacterium grows and survives equally well on resistant and susceptible plants, adaptation to resistant plants may be unlikely because increased virulence might not increase reproductive fitness of the pathogen.

Information about relative populations of *R. suberifaciens* on and in the roots of different plant species is of interest because survival of the bacteria is poorly understood. Growth on nonhosts or weeds of the tribe Cichorieae could contribute to survival between lettuce crops (21). Previously, rye (*Secale cereale* L.) planted as cover crop suppressed corky root slightly in a subsequent lettuce crop compared with a fallow treatment (22). The bacterium has been isolated from symptomless roots of rye and broad bean (*Vicia faba* L.) (21,22). However, continuous cropping of susceptible cultivars of lettuce has become common over the past 20 yr (8). During that same period, increased disease severity and loss from corky root has been reported (17). Therefore, the bacterium may grow more profusely on susceptible hosts than on nonhost plants.

The goal of this research was to determine the population dynamics of corky root bacteria on highly susceptible and less susceptible lettuce cultivars, a resistant wild lettuce species, and a nonhost. A preliminary report was published (15).

MATERIALS AND METHODS

Populations of *R. suberifaciens* on susceptible and resistant lettuce cultivars in the field. Populations of *R. suberifaciens* from the roots of resistant and susceptible iceberg lettuce cultivars were compared in microplot experiments (23) at Davis, CA, with an enzyme-linked immunosorbent assay (ELISA). Approximately 10 wk before planting lettuce in the fall of 1989 and spring of 1990, the soil was treated with 500 kg/ha methyl bromide + chloropicrin (53:47%, v/v ratio, liquid mixture under pressure) to reduce populations of *R. suberifaciens* that might have remained from previous experiments. Seeds of iceberg lettuce Salinas (corky root susceptible) and an F5 pedigree breeding line of Salinas and Green Lake, 440-8 (corky root resistant), were planted in two rows on 1-m-wide beds in 10 plots (1×2 m) each. Five plots of each cultivar were sprinkled with 2 L each of a 4-day-old culture of *R. suberifaciens* CA1 (23) grown in S broth (23). The concentration of the bacterial suspension was 5×10^5 cfu/ml and was confirmed by dilution plating (1:9) on solid S medium. The other five plots of each cultivar received 2 L of water as a control. In the fall of 1989, plants from two additional noninfested microplots of one cultivar each, located 10 m from the other 20 plots, were used as clean controls for ELISA tests. In the spring, control plots remained disease free, and additional control plots at a distance were not needed.

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Each experiment was arranged in a completely randomized design with five replications of two cultivars in infested and noninfested plots. One month after sowing, the plants were thinned to 30 cm between plants. Standard fertilization, irrigation, and pest control practices were used (23). In the fall, one plant was dug from each plot with a shovel at 23, 36, 50, and 72 days after planting. One root system per plot, consisting of the taproot plus secondary and some feeder roots, was used for determination of populations of *R. suberifaciens* with ELISA. In the spring, 15, 8, 3, 1, and 1 root systems were collected from each plot 37, 46, 58, 73, and 90 days after planting, respectively. Total fresh weights of the root samples ranged from 5 to 10 g. The roots were 2–25 cm long depending on sampling date. The spring sampling method, which used more root tissue early in the growing season compared with the fall method, was an improvement on the fall method because the ELISA procedure was not sensitive enough to identify bacteria on small, single roots. Samplings occurred 5–7 days after irrigation in an attempt to sample at equal soil water potentials.

Determination of populations of *R. suberifaciens* with ELISA. An indirect, double monoclonal-polyclonal antibody sandwich ELISA technique (24) was used to estimate numbers of *R. suberifaciens* in and on the roots. Monoclonal antibody MAb-Rs1 was produced as described by Alvarez et al (1) and van Bruggen et al (24), and polyclonal antibody CA1-Ab1 as described by van Bruggen et al (20). One day before roots were sampled, wells of microtiter plates were coated with a preparation of monoclonal antibody/coating buffer (1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$, and 0.2 g of NaN_3 in 1 L of H_2O adjusted to pH 9.6 with HCl), 1:2,000, v/v, and incubated for 1 h at 37 C and then overnight at 4 C. The next morning, the plates were washed three times with phosphate-buffered saline (PBS: 8.0 g of NaCl, 0.2 g of KH_2PO_4, 1.15 g of Na_2HPO_4, 0.2 g of KCl, and 0.2 g of NaN_3 per liter, pH 7.4) plus 0.01% Tween 20. Roots were rinsed briefly to remove large clumps of adhering soil, weighed, and rated for disease severity using a Horsfall-Barratt scale with 12 levels (16). A thick suspension of crushed roots was made with mortar and pestle plus 0.2 ml of glass beads (0.20–0.30 mm Art. Nr 0.854 160/4 B Braun, Mesungen, Germany) and about half as much sample buffer (20 g of polyvinylpyrrolidone and 2 g of bovine serum albumin in 1 L of PBS plus 0.01% Tween 20) as the fresh weight of a root sample. Duplicate wells were filled with 200 μl of crushed roots in sample buffer and incubated for 1 h at 20 C and then overnight at 4 C. To obtain a standard curve relating absorbance to bacterial numbers, six serial dilutions (1:3) of a 4-day-old S broth culture of strain CA1 in sample buffer also were included in the microtiter plates. To determine bacterial concentration for the standard curves, a second dilution series (1:9) was prepared in sterile distilled water + 0.01% Tween 20 (because *R. suberifaciens* did not survive in sample buffer) and plated on S medium. The next morning, microtiter plates were washed three times, filled with polyclonal antibody/sample buffer, 1:500, v/v, and incubated for 2 h at 37 C. The plates then were washed three times, filled with antirabbit alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) in sample buffer at 1:1,000, v/v, and incubated at 37 C for 2 h. Next, the plates were washed three times and filled with substrate buffer (97 ml of diethanolamine and 0.2 g of NaN_3 in 903 ml of water, pH 9.8) + 1.0 mg/ml of *p*-nitrophenol phosphate. Absorbance at 405 nm was read after 75 min on a Maxline kinetic microplate reader (Molecular Devices, Menlo Park, CA).

Standard curves of absorbance vs. concentration of *R. suberifaciens* in S broth (cfu/200 μl) were prepared for each sampling date. Linear regression lines were obtained from three to five bacterial concentrations that showed significant absorbance ($R^2 = 0.93$–0.99). Absorbance values of root samples from noninfested plots were used as controls to correct for absorbance due to root tissue. Since absorbance values for healthy plants were not significantly different from those for sample buffer, populations of *R. suberifaciens* on healthy roots were considered negligible. Only average absorbances of root samples greater than two standard deviations above the mean of the roots from noninfested plots were considered significant for determination of bacterial numbers. Healthy roots from all noninfested resistant and susceptible cultivar plots were used at all harvests except in the fall at 23 days when only the five noninfested plots plus the isolated plots, both planted with Salinas, were used (because of an error in sampling), and in the fall at 72 days when only the isolated plots were used (because of a lack of suitable roots in noninfested plots within the experiment). Root populations were analyzed statistically as $\log_{10}$ cfu/g of fresh root and $\log_{10}$ cfu/root.

Populations of *R. suberifaciens* from roots of barley, prickly lettuce, and two cultivars of iceberg lettuce. Roots of 18-day-old plants of iceberg lettuce cultivars Salinas and 440-8, prickly lettuce, and barley cultivar Kombar were rinsed to remove adhering soil mix, dipped in a suspension of *R. suberifaciens* CA1R for 5 min, and transplanted into 10-cm-diameter pots that contained pasteurized Yolo sandy loam. Strain CA1R was a spontaneous mutant of *R. suberifaciens* CA1 that was resistant to 100 ppm of rifampicin (14). Both strains had the same growth rate in broth culture and were equally pathogenic to lettuce (14), indicating equal fitness (5). The bacterial suspension was made by sonication of several 6-day-old colonies in 5 ml of sterile distilled water plus 0.02 ml Tween 20 for 30 s with an ultrasonic cell disruptor (Heat Systems-Ultrasonics, Farmingdale, NY, model MS-50) at 80% capacity. Cell concentration was determined with a Spectronic 20 colorimeter at 650 nm, adjusted to approximately 1.0×10^6 cfu/ml by dilution, and confirmed by dilution plating (1:9) 0.1-ml aliquots onto S medium amended with 100 ppm of rifampicin, 100 ppm of cycloheximide, and 50 ppm of benomyl (SRCB medium).

The pots were placed in individual saucers in three growth chambers programmed for 14-h light periods at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$, with temperatures of 27 C during the light period and 18 C in the dark. Pots were watered daily with amounts proportional to the size of each plant. Water did not stand in the saucers for the entire day. On days 1, 7, 13, and 19, all pots were fertilized with Hoagland's nutrient solution (10).

Immediately after the root dip inoculation and on days 6, 12, 18, and 24, roots were assessed for populations of strain CA1R and disease severity. Immediately after inoculation, roots of nine plants per species in three replications were blotted dry on paper towels and weighed. For the posttransplanting samples, nine plants of each plant species or cultivar (three from each growth chamber) were removed from the pots, rinsed superficially (without scrubbing) under tap water to remove loosely adhering soil, blotted dry, and weighed. Roots from all samplings were ground into pulp in a mortar containing 0.2 cm^3 of glass beads and 0.75–2.0 ml of sterile distilled water + 0.01% Tween 20, depending on the amount of tissue. Root samples ranged from 0.05 to 10 g and included the taproot, secondary roots, plus most of the small feeder roots. The suspensions were plated as tenfold dilutions in 0.1-ml aliquots on SRCB medium and colonies were counted after 7–14 days at 28 C. Populations of bacteria were analyzed statistically as $\log_{10}$ cfu/g of fresh root and as $\log_{10}$ cfu/root.

Even though rifampicin was used to suppress contaminant bacteria, many soilborne bacteria grew on the plates and could have been confused with strain CA1R. To test that colony counts included CA1R only, a total of 240 putative CA1R colonies were examined using plates from the first and last harvests, the four plant species/cultivars, and three replications. Eight contaminant colonies that did not resemble *R. suberifaciens* also were tested. All 240 putative CA1R colonies were positive for *R. suberifaciens* in the indirect monoclonal/polyclonal double bed above. None of the contaminant colonies was positive.

To compare populations calculated from dilution plates with those calculated from absorbance values in ELISA, root samples with different corky root severities also were subjected to the ELISA procedure used for the field samples. No significant absorbances were observed, however, probably because the root samples were too small to allow detection of *R. suberifaciens*.

The experiment was performed twice and arranged in a randomized complete block design with three growth chambers as blocks and four different plant species/cultivars as treatments. Because

overall trends were similar, only one experiment is presented.

Statistical analysis. Statistical computations were made using Statistical Analysis Systems (release 6.03, SAS Institute Inc., Cary, NC). Horsfall-Barratt disease severity scores were converted to percentage of the taproot area showing corkiness (16) before statistical analysis. The SAS general linear models procedure was used to perform regression, analysis of variance, and standard deviation calculations on bacterial populations from field and growth chamber experiments. Linear, quadratic, and cubic polynomial models were tested for bacterial populations over time from the growth chamber experiment. Linear contrasts and contrast estimates for the growth chamber experiments were obtained with the ESTIMATE statement.

RESULTS

Disease severities and populations of *R. suberifaciens* on field grown plants. Corky root disease severity increased over time in all plots infested with *R. suberifaciens* during both spring and fall (Fig. 1). Disease severity was greater on Salinas than on 440-8 and greater in the fall than in the spring. Plants from control plots remained almost completely disease free (less than 0.1% incidence) in both seasons.

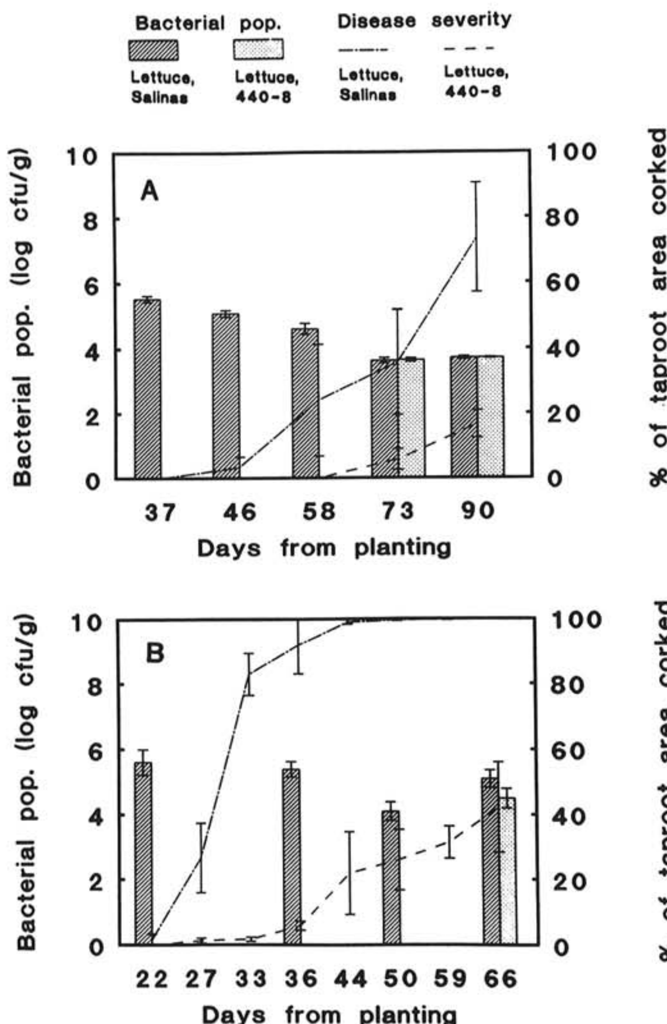

Fig. 1. Populations of *Rhizomonas suberifaciens* (bars) and disease severities (lines) on roots of two cultivars of iceberg lettuce, Salinas and 440-8, grown in microplots in **A**, spring 1990, and **B**, fall 1989. Populations were determined by an indirect, double monoclonal-polyclonal antibody sandwich enzyme-linked immunosorbent assay (ELISA). Disease severities were expressed as percentage of the taproot showing corkiness. Populations and disease severities for each sampling date and cultivar are the means of five microplots with 1–15 roots per microplot.

The presence of *R. suberifaciens* in or on the roots from infested plots was detected with the ELISA procedure. Significant absorbance values (two standard deviations above those of the control roots) were observed for Salinas at each sampling date in both fall and spring field seasons (Fig. 1). However, absorbance values for 440-8 were significant only at the final and the final two sampling dates for fall and spring seasons, respectively. Absorbance values for roots from control plots were very similar and only slightly higher than those for the sample buffer.

Populations of *R. suberifaciens* calculated from absorbance values fluctuated little during both growing seasons. Populations expressed as cfu/g of fresh root declined slightly over time (Fig. 1). This decline was significant in the spring only ($y = -0.04x + 6.77$, $P = 0.014$, $R^2 = 0.90$). Densities expressed as cfu/root remained stable (data not shown).

The lowest level of detection was approximately 4×10^3 cfu/g of fresh root. Populations recovered from roots ranged from 4×10^3 to 3×10^5 cfu/g of fresh root for Salinas and from less than 4×10^3 to 5×10^3 cfu/g of fresh root for 440-8. In the fall, populations varied from 10^4 to 4×10^5 for Salinas and from less than 4×10^3 to 2×10^5 cfu/g of fresh root for 440-8. Populations recovered from Salinas were always higher than those recovered from 440-8 except for the final two sample dates in the spring season.

Although populations of *R. suberifaciens* were similar or the same on both cultivars at the final harvest dates in fall and spring, respectively, disease severity was much higher on Salinas than on 440-8 in both seasons (100 vs. 42% in fall and 78 vs. 18% in spring for Salinas and 440-8, respectively).

Disease severities and populations of *R. suberifaciens* on host and nonhost plants in growth chambers. Disease severity of corky root increased over time, with symptoms first visible 12 days after inoculation (Fig. 2). Corkiness was most severe on Salinas, increasing to approximately 60% of the taproot surface area by 24 days. Disease severity was much lower on both 440-8 and prickly lettuce with 2–6% of the root surface area showing corkiness by day 24. No disease symptoms were seen on barley.

R. suberifaciens was recovered from the roots of all of the different plants tested at all sampling dates. Although dipped in the same concentration of inoculum, significantly more bacteria were recovered from barley than from the other plants (1.6×10^6 vs. 1.3×10^5 cfu/g of fresh root) within minutes after inoculation. Following inoculation, the bacterial densities varied with each sampling date (Fig. 3), but no simple model provided sufficient fit to describe *R. suberifaciens* population dynamics over time in both experiments. Consequently, each harvest date was

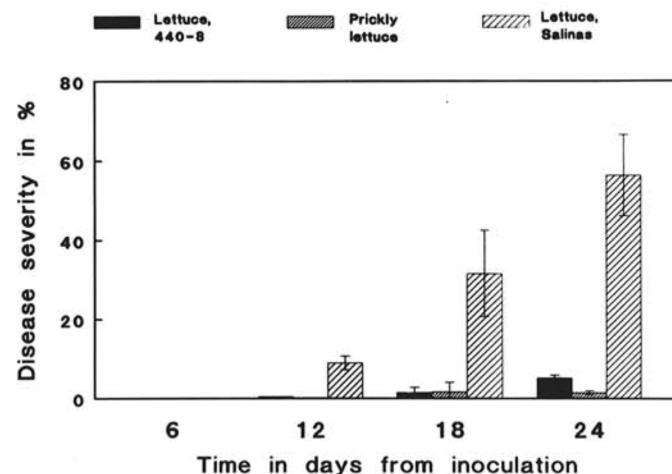

Fig. 2. Disease severity over time for roots of prickly lettuce and two cultivars of iceberg lettuce, Salinas and 440-8, dipped in a suspension of *Rhizomonas suberifaciens* CA1R. Disease severity was calculated as percentage of the taproot surface area showing corkiness and is the mean of three replicates (containing three roots each) for each plant at each sampling date.

analyzed separately with analysis of variance and two sets of orthogonal contrasts (Table 1).

Bacterial numbers recovered from barley were significantly lower than those from any of the other plants for the four post-inoculation harvests (Fig. 3). The highest populations were recovered from the susceptible lettuce cultivar Salinas. Populations recovered from the resistant cultivar 440-8 and prickly lettuce were intermediate to those of barley and Salinas.

Orthogonal contrasts indicated that populations of *R. suberifaciens* recovered from barley were significantly lower than those from other plants at all harvest dates (Table 1). The numbers of bacteria recovered from Salinas were significantly higher than those from prickly lettuce and 440-8 at days 12, 18, and 24. Throughout the experiment, bacterial populations from 440-8 and prickly lettuce did not differ significantly except on day 18 when the population on 440-8 was higher than that on prickly lettuce ($P = 0.01$).

In the second set of orthogonal contrasts, populations of *R. suberifaciens* recovered from Salinas were higher than those from all other plants at all harvest dates (Table 1). Bacterial densities from barley were significantly lower than those from prickly lettuce and 440-8 at days 6, 12, and 18.

DISCUSSION

The ELISA method was successfully used to quantify populations of *R. suberifaciens* on field-grown roots, although the minimum level of detection was relatively high (4×10^3 cfu/g of fresh root) and populations on the resistant lettuce cultivar 440-8 could not be measured early in the season. The maximum population on Salinas was less than 10^6 cfu/g of fresh root and tended to decline over time despite the increase in disease severity. Competition from other, perhaps faster growing, organisms may have been responsible (13).

The detection level of the ELISA method was too high to allow quantification of *R. suberifaciens* on smaller, potted roots. Dilution plating of root suspensions on rifampicin-amended S medium, on the other hand, resulted in significant differences in populations of *R. suberifaciens* on the species and cultivars used in growth chamber experiments. Larger populations of the bacteria and higher disease severities were observed in and on roots of Salinas compared with those of 440-8 or prickly lettuce, confirming previous reports that 440-8 and prickly lettuce were resistant to *R. suberifaciens* based on severity scores only (4,9,19,21). Similar correlations between resistance and bacterial populations occurred with *Clavibacter michiganense* ssp. *sepedonicum* on potato (7).

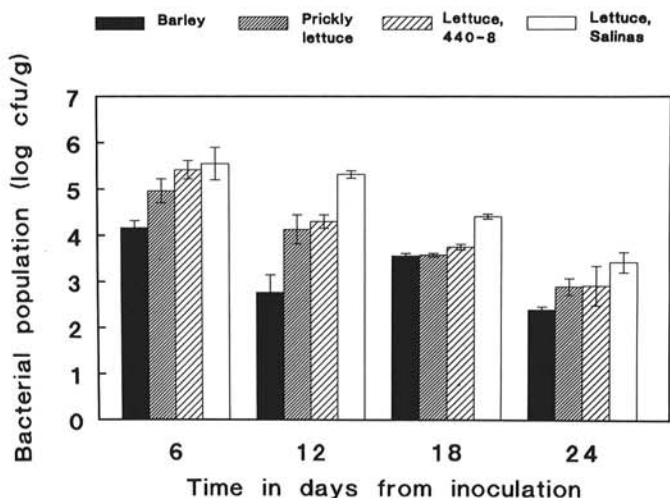

Fig. 3. Populations of *Rhizomonas suberifaciens* CA1R on roots of barley, prickly lettuce, and iceberg lettuce cultivars Salinas and 440-8 grown in 10-cm-diameter pots in a growth chamber. Populations for each sampling date are the means of three replicates per plant species or cultivar, determined by dilution plating of root macerates on S medium containing rifampicin.

Although lettuce cultivars resistant to corky root have been grown for many years in areas outside California (9,19), new virulent strains of *R. suberifaciens* have not yet been observed. Resistance to *R. suberifaciens* is based on a single gene (4) and could potentially be overcome by strains of the pathogen with new virulent genes (26). Examples of both breakdown and persistence of single resistant genes have been published (6), however, and the likelihood of a new virulent race of *R. suberifaciens* is impossible to predict.

Another implication of growing resistant lettuce plants that support low populations of *R. suberifaciens* may be a decrease in the inoculum in soil responsible for infection of a following crop. Whether the reduction in inoculum would be biologically significant is not clear, since bacterial populations in and on roots of 440-8 and prickly lettuce were similar to those in and on roots of Salinas at the end of the season. However, this was mainly due to relatively low bacterial densities on Salinas late in the season, probably as a result of competition by saprophytes with *R. suberifaciens* on the severely rotted roots.

Populations of *R. suberifaciens* per gram of fresh root were higher on barley than on the other plants immediately after inoculation, although the roots were dipped in the same suspension of *R. suberifaciens*. The difference may be caused by root morphology. Barley has a fibrous root system while the other plants have taproots. Barley probably has a higher surface area per gram of root, presumably allowing adsorption of more bacteria. Despite a greater initial population of bacteria on barley, later populations were lower in barley than on other plants. Had the results been corrected for differences in root morphology, the differences between bacterial populations on the various plants might have been even larger. Future studies comparing bacterial densities on plants with different root systems might be improved by considering root surface area.

Despite the differences in root morphology, the results from the growth chamber experiments indicated that *R. suberifaciens* was able to survive on barley roots for at least 24 days. *R. suberifaciens* was introduced into pasteurized soil in this experiment, however, and survival in natural field soil might be suppressed by competitors. Nevertheless, roots of barley or other nonhosts may provide a place for *R. suberifaciens* to persist in the absence of hosts, similar to nonhost plants that harbor *Agrobacterium tumefaciens* (3). If true, crop rotations may be of limited value. Use of cover crops had little effect on corky root severity and yield loss of a subsequent lettuce crop (22), perhaps because the bacteria persisted on cover crop roots. However, corky root disease was equally severe following fallow soil, putting the significance of nonhost roots for bacterial survival in doubt (22). Research on *A. tumefaciens* suggested that survival was better in nonhost rhizospheres than in fallow soil (3).

TABLE 1. Contrast estimates for two orthogonal contrast sets comparing population sizes in log cfu/g of *Rhizomonas suberifaciens* recovered from roots of prickly lettuce, barley, and lettuce cultivars Salinas and 440-8 grown in 10-cm pots in a growth chamber and sampled 6, 12, 18, and 24 days after inoculation

	Plant species and cultivar contrast estimates				
	Orthogonal set 1			Orthogonal set 2	
Day[a]	Barley vs. others[b]	Salinas vs. prickly lettuce + 440-8	Prickly lettuce vs. 440-8	Salinas vs. others	Barley vs. prickly lettuce + 440-8
6	1.14***[c]	−0.37	0.47	−0.70**	1.02***
12	1.82***	−1.10***	0.18	−1.58***	1.45***
18	0.35***	−0.75***	0.18**	−0.78***	0.11*
24	0.68*	−0.53*	0.02	−0.70**	0.51

[a]Time in days from inoculation.
[b]Calculated from the mean of three replicates per plant species or cultivar for each sampling date.
[c]Significance levels: * = 0.05, ** = 0.01, *** = 0.001.

Soil populations were not measured in our experiments, and the role of roots as a major niche of *R. suberifaciens* is uncertain. Research with other bacterial root diseases, however, indicated that densities associated with roots or the rhizosphere were much higher than those in soil, and decaying roots were the major source of pathogenic bacteria (18). The degree to which our results apply to nonsterilized soils and natural resident populations was not determined.

Although the intention of this study was to model population dynamics of *R. suberifaciens* in and on roots, the results indicate that considerably more sampling dates, more stringent environmental control, and more effective measurement procedures would be required to reach this goal. Rapid growth and death rates of bacteria and the difficulty in maintaining environmental variables constant (e.g., soil moisture) may have affected the population densities observed. Population estimates taken at intervals approaching the doubling time of the bacterium (currently not known) would be more likely to produce bacterial population curves.

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