Etiology

Corky Root of Lettuce in California Caused by a Gram-Negative Bacterium

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ABSTRACT

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In greenhouse and field experiments, corky root of lettuce was shown to be caused by a gram-negative bacterium with one lateral flagellum. In the greenhouse, seedlings of iceberg lettuce inoculated with gram-negative bacteria isolated from corked lettuce roots developed symptoms similar to those observed in the field. Bacteria isolated from the roots of these seedlings caused corky root symptoms in subsequent pathogenicity tests. These bacteria were identical to those isolated originally from field samples. Corky root symptoms appeared initially on the taproot and later on secondary roots, as observed in root observation boxes. Small field plots were infested with bacterial suspensions at three concentrations. Even at the lowest concentration (about 10^8 bacteria per square meter), the plants became severely diseased, while plants in control plots remained healthy. Identical bacteria were reisolated from corked roots. Head dry weight and marketable yield were progressively decreased at increasing inoculum levels.

Corky root of lettuce has become increasingly important in the Salinas and Santa Maria valleys in California in recent years. The disease occurs on various soil types ranging from sandy loam to clay. It has not been observed in other areas of California so far. Early symptoms consist of banded yellow areas on the taproot, while internal tissues are still healthy. Later, the taproot and main laterals become very rough (corked), dark greenish-brown, and brittle. The taproot may be substantially shortened, and lateral roots may be numerous near the surface but sparse or nonexistent at deeper levels in the soil. Ultimately, internal yellowing or browning of the taproot may occur. More severe symptoms of corky root usually appear in the second lettuce crop in a growing season. Severely infected plants remain undersized and often are too small to be harvested. Similar symptoms have been reported for lettuce in New York (12,13), Wisconsin (1), Florida (11), Canada (4), and Italy (7). In New York, Wisconsin, and Italy the disease was attributed to toxic substances released from decomposing lettuce debris (1,2,6,12).

Three decades ago, Grogan and Zink (10) described a disease of lettuce with symptoms somewhat similar to those of corky root, but with more distinct internal discoloration. In greenhouse experiments, they demonstrated that those symptoms could be induced by free ammonia liberated from fertilizers such as ammoniated ammonium nitrate and chicken manure. In a field experiment at Salinas, nitrogenous fertilizers, especially ammoniated ammonium nitrate, increased root symptoms (specifically internal necrosis) compared with the controls (10).

These results were confirmed by Hoff and Newhall in New York (14), but not by Busch and Barron (4) or Amin and Sequeira (1).

More recently, Patterson et al (20) reported that corky root could be controlled with methyl bromide, chloropicrin, or metam-sodium, and that corky root could be induced in the greenhouse by incorporation of a small amount of infested soil into sterile planting mix (1:999, v/v). The corky root agent could be eliminated from soil by heating to 53 C for 10 min, passing through a 0.45-μm Millipore filter, or mixing soil with novobiocin (50 ppm). Waters and Grogan (22) reported the isolation and partial identification of a bacterium that induced severe symptoms in a lettuce cultivar susceptible to corky root, and only mild symptoms in cultivars tolerant to the disease. However, proof of pathogenicity according to Koch's postulates has not been published.

In this paper we demonstrate that a gram-negative bacterium isolated from corked roots of lettuce caused typical corky root symptoms when susceptible plants were inoculated. Furthermore, we describe the development of the disease as observed in root observation boxes, and the relationship between inoculum levels in the field and disease severity and yield loss.

MATERIALS AND METHODS

All experiments were performed with iceberg lettuce (*Lactuca sativa* L.) cultivar Salinas and a gram-negative bacterium, originally isolated from corked roots of iceberg lettuce from Salinas, CA, in 1984. The bacteria were routinely grown in the following medium: 5.0 g of casein hydrolysate, 2.5 g of glucose, 1.3 g of $K_2HPO_4 \cdot 3H_2O$, 0.5 g of KNO_3, 0.5 g of $MgSO_4 \cdot 7H_2O$, 60.0 mg of $Ca(NO_3)_2 \cdot 4H_2O$, and 11.0 g of Agar Noble per liter of distilled water. This medium (called S-medium) was developed by C. M. Waters after repeated failure to isolate any pathogens using ordinary media.

Pathogenicity tests. Lettuce seedlings were grown in 5-cm-diameter pots with vermiculite for 10 days, and then inoculated by pouring 5 ml of bacterial suspension (7×10^8 cells per milliliter) around the stembase. Bacterial concentrations were determined with a Petroff Hausser and Helber counting chamber (Hausser Scientific, Blue Bell, PA). To prepare a bacterial suspension in water, the bacteria were cultured in S-broth for 4 days and centrifuged at 7,500 rpm for 20 min. The pellet was resuspended, centrifuged, washed, centrifuged, and resuspended in the same amount of sterile water as the original broth. Control plants were treated similarly, but with sterile water only. During the experiment, the plants were watered alternately with 30–50 ml of water, 30–50 ml of 0.005 M $Ca(NO_3)_2$ + 0.005 M KNO_3, 30–50 ml of water, and 30–50 ml of 1/2 Hoagland's solution. The pots were placed at least 50 cm apart to avoid contamination. There were 20 plants per treatment, randomized in two blocks. Five plants were harvested on four consecutive dates. The plants were rated for corky root on a 0–10 scale, with 10% increments of yellow or necrotic areas on the taproot and hypocotyl (0 = no symptoms, 10 = necrosis on 91–100% of the taproot and hypocotyl). Mean percentages of diseased taproot area were calculated using the midpoints of the infection classes. Three plants were used to determine dry weights and two were used for isolations. Each root system was washed in running distilled water and placed in 20 ml of sterile distilled water in large test tubes. The tubes were sonicated for 10 min in an ultrasonic cleaner (B-12, Branson Cleaning Equipment Co., Shelton, CT) and vortexed for 3 min. Root extracts were filtered through a 0.650-μm Millipore filter (Micronsep, Honeoye Falls, NY) and 0.04 ml of the filtrate and of diluted filtrate (10^{-2}) were plated on a selective medium. The roots were then soaked in 0.5% sodium hypochlorite for 1 min, rinsed in 10 ml of sterile water, and comminuted in a mortar. Undiluted and diluted (10^{-2}) tissue extracts (0.04 ml) were plated on a selective medium. The selective medium was the S-medium plus 30 mg of streptomycin sulfate per liter. All isolates producing colonies similar to the original isolate were tested for pathogenicity on 2-wk-old lettuce seedlings by pouring a 5-ml bacterial suspension (about 10 bacteria per milliliter) onto the stem bases. The data were analyzed with the analysis of variance procedure of SAS (SAS Inst., Inc., Cary, NC).

Root observation boxes. Ten root observation boxes were constructed from plastic waste baskets (31 × 36 × 56 cm, No. 2850, Rubbermaid Inc., Wooster, OH). Observation windows were 21.5 × 41.5 cm, and were covered by 0.5-cm thick Plexiglas. The boxes were filled with Chualar loam (about 45% clay, 35% silt, 15% sand, and 5% gravel) from the USDA experiment station at Salinas. The area from which the soil was collected had been fallow for many years and presumably was not infested with corky root bacteria. The soil was packed to a bulk density of 1.43–1.45 kg/L, and the initial soil moisture was adjusted to 17%. In the first experiment, half of the boxes were infested with corky root bacteria by pouring 50 ml of a bacterial suspension (10^7 cells/ml) around the stem bases of 18-day-old lettuce seedlings of cultivar Salinas. At the end of the experiment, infested and control soils were kept separate: The same soil was used in a second experiment after adding additional bacterial suspension to the infested soil (125-ml suspension with 3×10^7 cells/ml to about 500 kg of soil). In both experiments, the boxes were arranged according to a randomized complete block design with five replications. The plants were watered when the soil water potential dropped to −300 mbar as indicated by tensiometers. The plants were fertilized with calcium nitrate (0.1 g of N) and complete fertilizer at a rate of 1.4 g of N, 0.7 g of P, and 0.7 g of K per box equivalent to about 225 kg of N and 73 kg of P and K per hectare. The roots growing along the Plexiglas wall were traced on acetate sheets twice a week during the first experiment, and once a week during the second experiment. From the acetate sheets total root length was assessed with a map measure (type 40, Silva Compass, Binghamton, NY).

Field experiment. Microplots (1 × 2 m^2) were constructed at U.C. Davis, by sinking fiberglass walls to a depth of 45–50 cm into the soil. The plots were fumigated with methyl bromide plus chloropicrin (3:1 by weight) at a rate of 113 g per plot, 3 mo before infestation with corky root bacteria. Fertilizer (16-20-0) was incorporated in all plots at a rate of 77 g per plot. Two additional side-dressings of 55 g of ammonium nitrate were applied per plot, 41 and 66 days after planting. This amounted to a total of 224 kg of N and 70 kg of P per hectare, a rate commonly used in the Salinas Valley. Lettuce cultivar Salinas was planted in two rows per bed (50 cm wide) and thinned to one plant per 30 cm 25 days later. There were four treatments: three inoculum levels of the corky root bacterium and a control. Ten liters of 5-day-old broth cultures containing 3×10^8, 3×10^7, 3×10^6, or 0 cells per liter was sprinkled on the beds immediately after planting. The experiment had a randomized complete block design with five replications.

The plots were sprinkled with 10 L of water every other day until emergence. Subsequently, 85 L of water was applied in the furrows, at about weekly intervals.

At harvest, 95 days after planting, the roots were scored for corkyness by using a 0–5 scoring scale (Fig. 1). The scoring scale for the field differed from that for the greenhouse, because in the field, all roots became diseased, whereas in the greenhouse only the taproots were affected. The corky root data were analyzed in chi-square tests using MINITAB (Statistics Department, The Pennsylvania State University, University Park, PA).

Fresh weights were determined for all heads, and heads >900 g were considered marketable. Dry weights of heads and roots were obtained for six plants per plot. The heads were dried in a drying shed at about 40 C for 2 wk, and the roots were dried in an oven at 80 C for 2 days. The weight data were analyzed using the General Linear Models Procedure of SAS (SAS Inst., Inc., Cary, NC).

Isolations were made from one plant per plot. The roots were washed under running tap water, soaked in 0.5% sodium hypochlorite for 1 min, and washed in sterile distilled water. Pieces of tissue on the border between healthy and diseased areas were comminuted in a mortar with 10 ml of sterile water. Fifty microliters of tissue extract and diluted extract (10^{-2}) was plated on the selective medium described above.

Despite careful irrigation and physical separation of the plots, some of the control plants became infected. Root aphids (*Pemphigus bursarius* (L.)) were observed in both infested and

control plots and were checked for the presence of corky root bacteria. Soil samples with root aphids were collected from three infested and three control plots. Ten aphids per sample were transferred to a mortar with about 1 ml of alcohol. The alcohol was flamed off, and the aphids were comminuted in 10 ml of sterile distilled water. Fifty microliters of the undiluted and diluted (10^{-2}) extract were placed onto plates of the selective medium. All isolates with colonies similar to the original isolate were checked for pathogenicity as described under pathogenicity tests.

Preliminary identification. Corky root bacteria were smeared onto glass slides, stained according to Hucker's modification of the Gram-stain procedure (21), and then examined under a light microscope at 1,000×. In addition, a loop of corky root bacteria was added to a drop of KOH solution (3%) and checked for stringiness (8). Lipopolysaccharides were extracted with a phenol-water mixture according to Westphal and Jann (23). Lyophilized lipopolysaccharide was tested for the presence of 2-keto-3-deoxyoctanoic acid (KDO) by the method of Osborn (19). *Clavibacter michiganense* subsp. *michiganense* or *Rhodococcus fascians* and *Pseudomonas fluorescens* were used as gram-positive and -negative controls.

Bacteria were checked with a transmission electron microscope for the presence of a flagellum. Corky root bacteria were grown on S-broth, and centrifuged in an Eppendorf microcentrifuge for 5 min. The pellet was resuspended in sterile distilled water. A collodion-coated copper grid was floated in the bacterial suspension mixed with 0.4% potassium phosphotungstate (pH 7.0) (9). The grid was air dried and viewed with a Zeiss Model 109 transmission electron microscope operating at 80 kV.

To visualize the cell wall, small pieces (2 mm^3) of S-agar with a colony of corky root bacteria were fixed in 4% *para*-formaldehyde in 0.1 M phosphate buffer (pH 7.0) for 18 hr, and dehydrated in a graded ethanol series. The pieces were embedded in Epon (18) and sectioned with a glass knife. The sections were double stained with uranyl acetate and lead citrate and viewed with the same electron microscope operating at 50 kV.

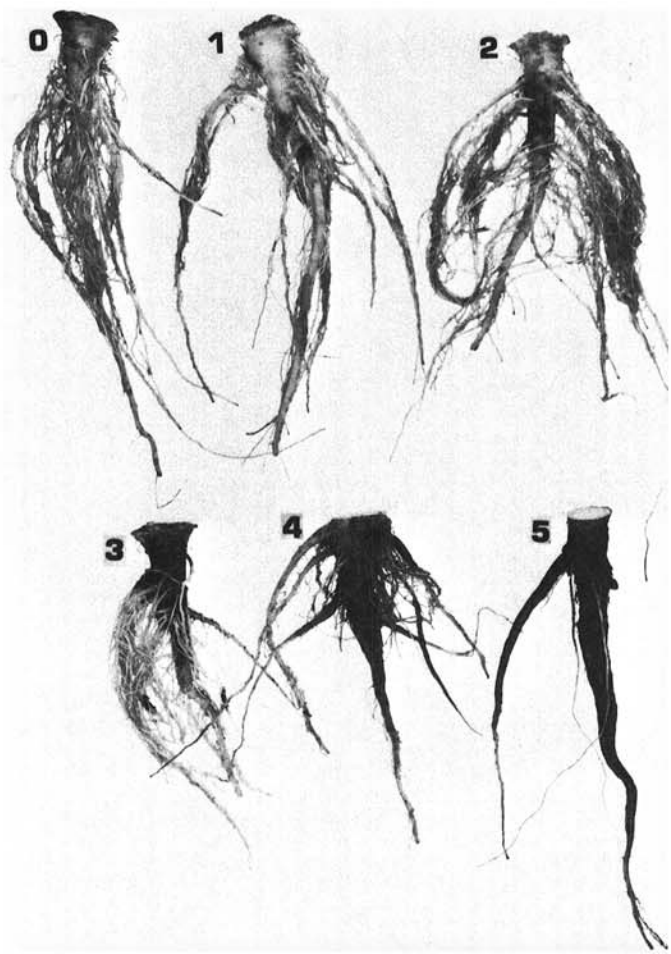

Fig. 1. Scoring scale (0–5) for corky root of lettuce in a field experiment. At score 5 the roots were completely corked and brittle.

RESULTS

Pathogenicity tests. Ten days after inoculation, the cotyledons and first leaves of plants inoculated with bacteria developed a chlorotic mottling. Eleven and 15 days after inoculation, inoculated plants had yellowish-brown lesions on the taproot. The taproots showed severe corking 18 and 25 days after inoculation (Table 1). The lateral roots did not become infected, possibly because they were mainly located along the edges of the pots. The control plants remained healthy. The dry weights of the roots and shoots were significantly reduced ($P < 0.01$) by corky root at all harvest dates (Table 1).

Despite 30 ppm of streptomycin in the selective medium, various bacterial colonies could grow on this medium, so that it was quite difficult to distinguish the slow-growing corky root bacteria (colonies become visible after about 10 days of incubation at 27 C). Corky root bacteria form initially translucent but later opaque, umbonate, compact colonies, which ultimately become wrinkled and have raised edges on S-medium (Fig. 2). Against fluorescent light, the colonies appear yellowish. Corky root bacteria were reisolated from 75% of the root washings or tissue extract of plants with corky root symptoms (56% of the root washings and 44% of the tissue extract). More positive isolations were made from plants with yellow root lesions than from those with corked roots. No corky root bacteria were isolated from healthy plants.

Root observation boxes. In the first experiment, the first necrotic lesions were observed on the stem bases 5 days after inoculation. The stem bases became constricted, and the lower leaves of inoculated plants turned yellow. The taproots and stem bases of inoculated plants were so severely corked that no adventitious roots were formed. The roots of control plants remained healthy (except for one plant that became slightly infected). In the second experiment, when the soil was uniformly infested with corky root bacteria, the first symptoms were yellow areas on the taproots about 10 cm below the soil line at 17 days after planting. Two weeks later, most taproots in infested soil were dying, and many adventitious roots were formed in the upper soil layers. At harvest, 68 days after planting, the upper 10 cm of the taproots was severely corked and brittle.

The visible root length was reduced by inoculation with corky root bacteria, but the difference was only significant 35 days after inoculation in the first experiment (Fig. 3). In the second experiment, many adventitious roots were formed in the top 10 cm when the taproot had died, about 20 days after planting, resulting in an increase in total root length of infected plants. The differences in root length were not significant on the various observation dates.

The dry weights of shoots and roots were significantly reduced by corky root in both experiments (Table 2).

Field experiment. Plants in infested plots became severely

TABLE 1. Effect of inoculation with corky root bacteria suspended in water on corky root severity, and dry weights of shoots and roots of lettuce seedlings grown in vermiculite

Treatment	Days after inoculation	Taproot affected (%)	Dry weight shoot (mg)	Dry weight roots (mg)
Control	11	0 (0)[a]	150 (24)	35 (2)
	15	0 (0)	357 (23)	85 (9)
	18	0 (0)	549 (34)	144 (12)
	25	0 (0)	1,111 (117)	354 (37)
Inoculated	11	27 (15)	149 (10)	26 (3)
	15	49 (13)	287 (14)	50 (6)
	18	65 (19)	494 (57)	76 (23)
	25	71 (13)	746 (271)	137 (33)

[a] Standard deviation.

affected by corky root (Fig. 4), while most of those in control plots remained healthy (Table 3). There were no significant block effects. The differences in corky root severity between inoculum levels were significant (Table 3). In the field experiment, there was a significant increase in the dry weight of the taproots by corky root infection ($P < 0.01$) (Fig. 5), as opposed to a decrease in dry weight in the greenhouse experiments. In cross section, the central core of severely corked roots was greyish-brown and seemed more dense. The diameter of severely corked taproots appeared to be wider than that of healthy taproots. On moderately corked roots, the corked ridges seemed to be hypertrophic, whereas the areas between the ridges seemed to reflect normal growth. The dry weight of the heads was progressively decreased at increasing inoculum levels (slope $= -1.55 \pm 0.47$ $P < 0.01$) (Fig. 6). Within each plot, the variability in fresh weight of the heads was increased by corky root ($P < 0.01$), and the number of marketable heads was progressively reduced as inoculum levels increased (slope $= -1.02 \pm 0.09$ $P < 0.01$) (Fig. 7). Again there were no significant block effects.

Corky root bacteria were reisolated from 88% of the tissue extracts from plants with corky root symptoms, and not from plants without symptoms. Corky root bacteria were also isolated

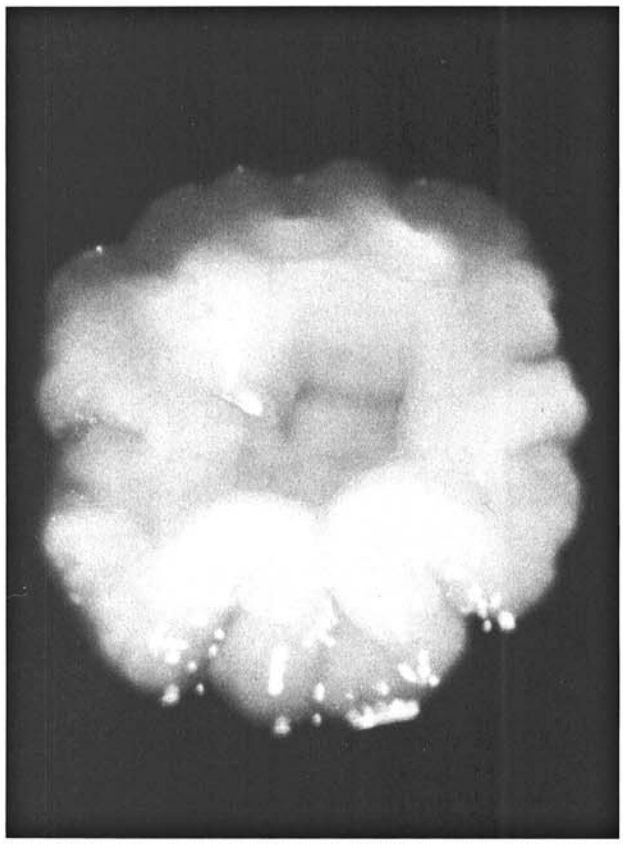

Fig. 2. Three-week-old colony of gram-negative bacteria, causal agent of lettuce corky root on S-medium.

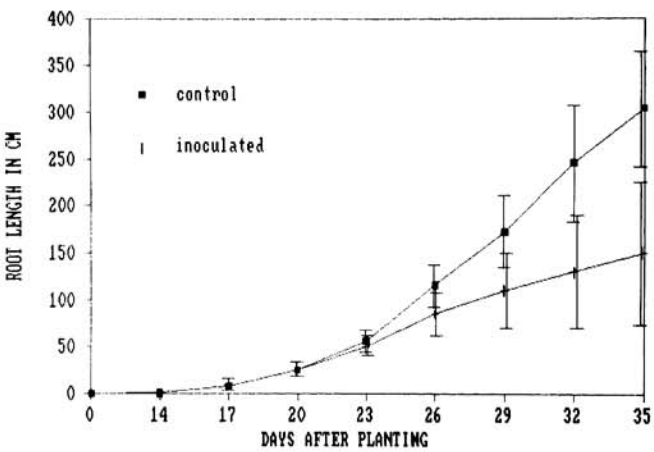

Fig. 3. Effect of inoculation with corky root bacteria on increase in root length against a Plexiglas wall over time. Vertical bars denote standard deviations (N = 5).

TABLE 2. Effect of inoculation with corky root bacteria on dry weight (g) of shoots and roots of lettuce grown in root observation boxes

Expt.	Plant part	Control	Inoculated
1[a]	Shoot	9.99 (3.73)[b]	1.04 (0.62)**[c]
	Root	0.33 (0.17)	0.02 (0.02)*
2[d]	Shoot	17.91 (4.23)	9.63 (1.22)**
	Root	1.37 (0.15)	0.98 (0.29)*

[a] 62 days after planting.
[b] Standard deviation.
[c] **, Significant at $\alpha = 0.01$; *, significant at $\alpha = 0.05$.
[d] 68 days after planting.

Fig. 4. Typical corky root symptoms on lettuce roots as observed in microplots infested with corky root bacteria.

TABLE 3. Severity of corky root disease of lettuce in microplots infested with a 10-L suspension of corky root bacteria or water[a]

Bacteria per ml	Number of lettuce plants with a corky root score of:					
	0	1	2	3	4	5
0	62	6	0	2	0	0
3×10^6	0	0	2	3	57	8
3×10^7	0	0	0	0	55	15
3×10^8	0	0	0	0	41	29

[a] X^2 (total) = 290.28, df = 9, $\times < 0.01$.
X^2 (control vs. inoculated) = 263.59, df = 3, $\times < 0.01$.
X^2 (3×10^6 vs. 3×10^7) = 7.17, df = 2, $\alpha = 0.05$.
X^2 (3×10^7 vs. 3×10^8) = 6.50, df = 1, $\alpha < 0.05$.

from root aphids from one infested plot, and two control plots (which apparently had become contaminated accidentally).

Preliminary identification. Under the light microscope, the bacteria appeared variable in size, ranging from very small rods to slender filamentous threads. The filamentous threads were sometimes clearly branched, but aerial mycelium was not formed. The rods often occurred in strings from two to four or more cells.

The KOH test indicated that corky root bacteria were gram-positive. However, with Hucker's Gram stain, the bacteria stained as gram-negative. Lipopolysaccharides were extracted from corky root bacteria and *Pseudomonas fluorescens*, but not from *Rhodococcus fascians*. The KDO test on partially purified lyophilized lipopolysaccharides was positive for the first two species and negative for the last one.

Transmission electron micrographs showed a cell wall similar to that of *Pseudomonas fluorescens* (Fig. 8). The bacterium had a single, lateral flagellum (Fig. 9). Based on electron micrographs, the rod-shaped bacteria measured 0.6–1.4 by 0.3-0.6 μm.

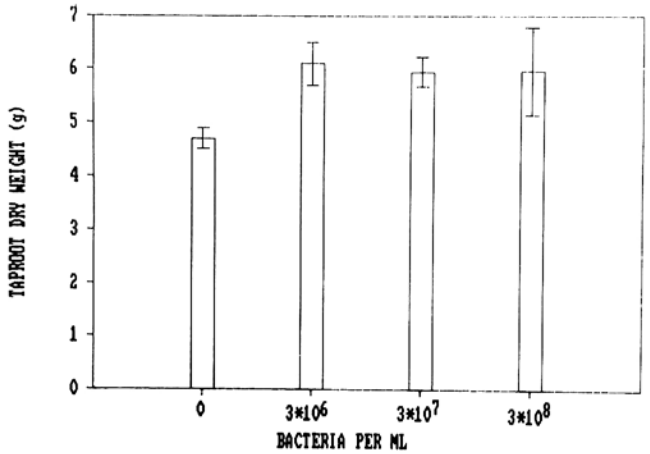

Fig. 5. Effect of infestation of microplots with various concentrations of corky root bacteria (0, 3×10^6, 3×10^7, and 3×10^8 bacteria per milliliter in 10 L per plot) on dry weight (g) of lettuce taproots at harvest time. Vertical bars denote standard deviations (N = 5).

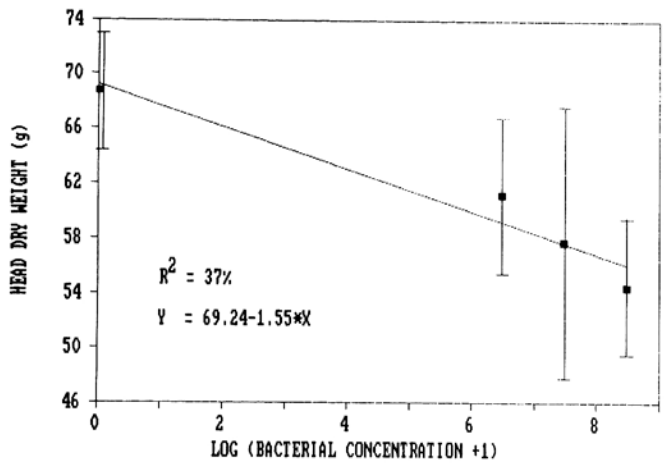

Fig. 6. Relation between head dry weight (g) of lettuce and the logarithm (base 10) of the concentration of corky root bacteria added to microplots. Vertical bars denote standard deviations (N = 5).

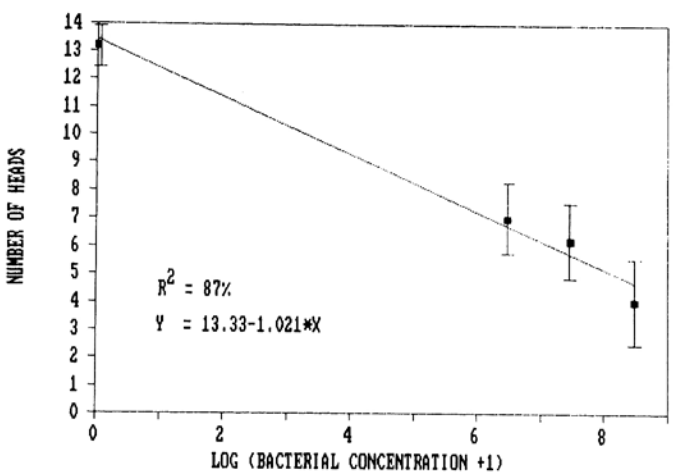

Fig. 7. Relation between number of marketable heads of lettuce and the logarithm (base 10) of the concentration of corky root bacteria added to microplots. Vertical bars denote standard deviations (N = 5).

DISCUSSION

In this paper we demonstrated that, in California, corky root of lettuce is caused by a gram-negative bacterium. Previously, the same isolate of this bacterium was thought to be gram-positive (22). The corky bacterium has one lateral flagellum and a cell wall similar to that of gram-negative bacteria (16). Further characterization of this bacterium is in progress.

The corky root bacterium initially affects the taproot, especially where lateral roots emerge, as observed in root observation boxes in the greenhouse. Ultimately, the whole root system may become affected, resulting in stunted plants with small heads. Under field conditions we demonstrated a significant yield reduction at a relatively low initial inoculum level (10^8 bacteria per square meter of surface area).

In microplots, the dry weights of corked taproots (without laterals) were significantly higher than those of healthy taproots. Visually, the corked taproots seemed denser. This is in contrast to the results obtained in the greenhouse, where the dry weights of corked taproots were reduced compared with healthy controls. This difference could perhaps be attributed to higher inoculum and

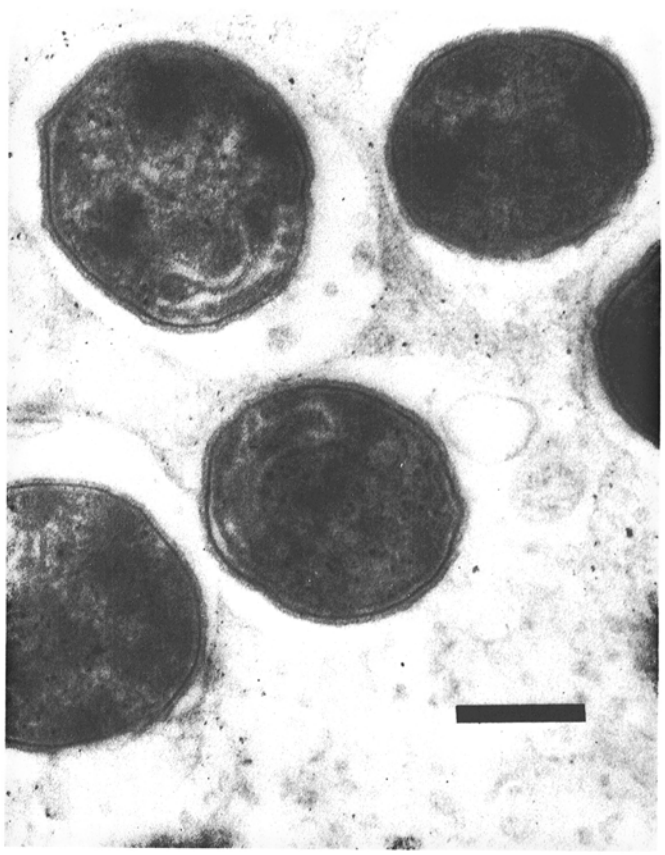

Fig. 8. Transmission electron micrograph of corky root bacteria embedded in epon. Scale line = 0.2 micron.

soil moisture levels in the greenhouse, resulting in more severe symptoms earlier in the development of the plants.

In root observation boxes, one of the control plants became diseased, and it seems likely that the unautoclaved field soil used in this experiment carried a low level of the bacteria. This soil originated from a Salinas area that had been fallow for many years. Hartnett and Lorbeer (13) reported the occurrence of lettuce corky root in soil that had been virgin before the lettuce crop.

The pathogenicity tests were repeated several times before we succeeded in keeping the controls healthy. Apparently, corky root bacteria spread easily from plant to plant by splashing water. Amin and Sequeira (2) also reported that untreated plants developed initial symptoms of root rot, but ascribed this to crowding of the roots in small pots. However, we were able to keep crowded roots healthy, if the pots were spaced at least 50 cm apart and watering were carried out cautiously to prevent accidental contamination.

Some of the control plants in the microplots became infected. Because corky root bacteria were isolated from root aphids, perhaps these insects transmitted the disease. It is unlikely that the bacteria originated from the soil because corky root has not been observed in lettuce-growing districts of the Central Valley of California, and because the soil was fumigated before the experiment.

So far, the corky root bacterium has not been found in other areas with corky root. However, for those areas, a bacterial causal agent cannot be excluded. Experiments in which corky root was attributed to toxins liberated by decomposing lettuce debris were performed in the field (1) or in greenhouses using field soil (2,6,7,12,13). In one experiment, perfusates from an autoclaved soil-plant residue mixture reduced germination of lettuce seed, but the authors suggested that microbial contamination could not be excluded (2). A toxin extracted from decomposing lettuce debris caused necrosis of roots of very young seedlings (2). Typical symptoms including corked roots were obtained only using the crude extract. This crude extract could have contained bacteria. Autoclaved crude extract also caused corky root symptoms, indicating that the toxic substance was heat stable (2). This toxic substance might have been produced by a bacterium similar to the one isolated from corked lettuce roots in Salinas: A heat stable toxin has been isolated from a culture filtrate of the Salinas isolate (15).

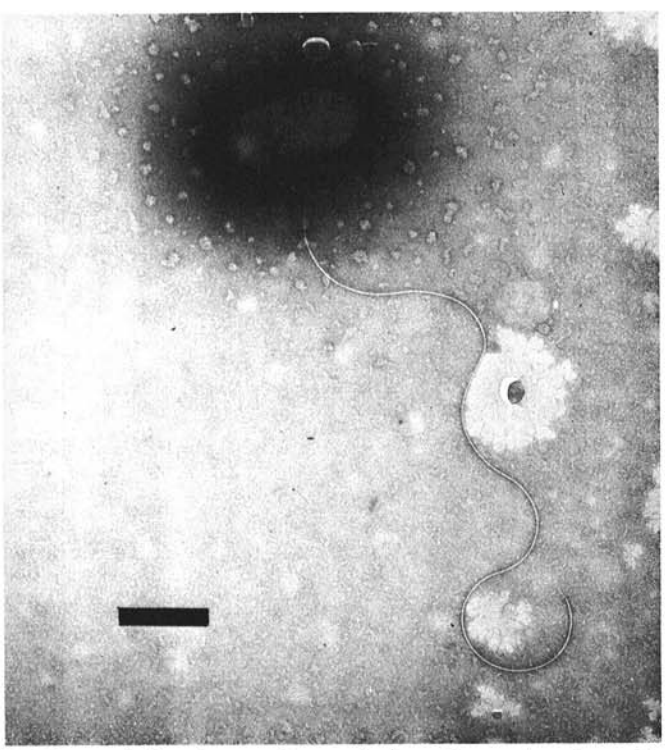

Fig. 9. Transmission electron micrograph of a corky root bacterium with lateral flagellum. Scale line = 0.5 micron.

Various bacteria were isolated from rotted or corked roots in the past but these bacteria were either nonpathogenic (1,10,17) or caused symptoms different from corky root (3,5). The isolation media used were common media, such as nutrient agar, on which the gram-negative corky root bacterium cannot grow (*unpublished*).

Symptoms similar to those of corky root were induced by ammonia or nitrite liberated from nitrogenous fertilizers or chicken manure when directly applied onto lettuce roots (10) or added to autoclaved muck soil (14). Corking of the roots usually was accompanied first by a pink and then by a brown discoloration of the stele. Infectious corky root, however, is only in extreme cases accompanied by internal necrosis of the root. Under field conditions (at Salinas), corky root severity was increased when nitrogenous fertilizers were applied, especially ammonia-liberating fertilizers (10). However, we can not exclude the possibility of enhanced susceptibility to a biotic agent under those conditions. In a field experiment at Davis, we observed that infection by corky root bacteria was increased by a side-dressing with ammonium nitrate (*unpublished*).

It would be interesting to determine whether lettuce reacts in a similar way to various nitrogen containing compounds such as ammonia, nitrite, secondary amines (2), and possibly a toxin produced by the corky root bacterium.

LITERATURE CITED

1. Amin, K. S., and Sequeira, L. 1966. Role of certain factors in the etiology of corky root rot of lettuce. Phytopathology 56:1047-1053.
2. Amin, K. S., and Sequeira, L. 1966. Phytotoxic substances from decomposing lettuce residues in relation to the etiology of corky root rot of lettuce. Phytopathology 56:1054-1061.
3. Brown, N. A. 1918. Some bacterial diseases of lettuce. J. Agric. Res. 13:367-388.
4. Busch, L. V., and Barron, G. L. 1963. Root rot of head lettuce in Ontario. Can. J. Plant Sci. 43:166-173.
5. Busch, L. V., and Carpenter, J. A. 1964. The presence of a water soluble toxin in lettuce root rot soil. (Abstr.) Can. Phytopathol. Soc. Proc. 31:23.
6. D'Ercole, N. 1981. La suberosi radicale della lattuga. Colture Protette 10:35-38.
7. D'Ercole, N. 1981. La suberosi radicale della lattuga. Terra e Vita 41:40-41.
8. Fahy, P. C., and Persley, G. J. 1983. Plant Bacterial Diseases, A Diagnostic Guide. Academic Press, Sydney. 393 pp.
9. Gabriel, B. L. 1982. Biological Electron Microscopy. Van Nostrand Reinhold, New York. 264 pp.
10. Grogan, R. G., and Zink, F. W. 1956. Fertilizer injury and its relationship to several previously described diseases of lettuce. Phytopathology 46:416-422.
11. Guzman, V. L. 1982. Yield and quality response of crisphead lettuce cultivars to seeding dates and farms in south Florida organic soils. Proc. Fla. State Hortic. Soc. 94:182-185.
12. Hartnett, J. P., and Lorbeer, J. W. 1968. Association of phytotoxins with lettuce root rot. (Abstr.) Phytopathology 58:1053.
13. Hartnett, J. P., and Lorbeer, J. W. 1971. The production of a noninfectious lettuce root rot under controlled environmental and soil conditions. Phytopathology 61:1153-1158.
14. Hoff, J. K., and Newhall, A. G. 1960. Corky root rot of iceberg lettuce on the mucklands of New York. Plant Dis. Rep. 44:333-339.
15. Kao, J., Mitten, D. H., and Milich, C. A. 1986. Identification of a host-specific toxin produced by the corky root rot bacterial pathogen of lettuce. (Abstr.) Phytopathology 76:844.
16. Lickfeld, K. G. 1976. Pages 127-176 in: Methods in Microbiology. Vol. 9. J. R. Norris and D. W. Ribbons, eds. Academic Press, Inc., New York.
17. Marlatt, R.B. 1955. Brown stele of lettuce. Plant Dis. Rep. 39:827-828.
18. Mollenhauer, H.H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39:111-114.
19. Osborn, M. J. 1963. Studies on the Gram-negative cell wall. I. Evidence for the role of 2-keto-3-deoxycotonate in the lipopolysaccharide of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. 50:499-506.
20. Patterson, C. L., Waters, C. M., and Grogan, R. G. 1981. Factors affecting corky root of lettuce in California. (Abstr.) Phytopathology 72:957.
21. Society of American Bacteriologists. 1957. Manual of Microbiological

Methods. McGraw-Hill Book Co., New York. 315 pp.
22. Waters, C. M., and Grogan, R. G. 1984. Identification of the bacterium causing lettuce corky root. (Abstr.) Phytopathology 74:857.
23. Westphal, O., and Jann, K. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Pages 83-91 in: Methods in Carbohydrate Chemistry. Vol. 5. R. L. Whistler, J. N. BeMiller, and M. L. Wolfram, eds. Academic Press, New York.