Genetics

The Genetics of Corky Root Resistance in Lettuce

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ABSTRACT

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Corky root is a serious disease of lettuce (*Lactuca sativa*) that causes deterioration in the root system of infected plants. A bacterium was confirmed to be a major etiological agent by fulfilling Koch's postulates. Five hundred and fifty-five accessions of *Lactuca* spp. were screened for the lack of corky root symptoms after being challenged by this bacterium. Seedlings were grown in the greenhouse and inoculated at the second or third true-leaf stage with a bacterial suspension. Plants were evaluated for symptom expression 30 days after inoculation. Highly resistant plants exhibited minimal root discoloration with no cracking of the taproots. Susceptible plants showed dark brown discoloration with cracking extending into the cortical regions. High levels of resistance were identified in 19 accessions of *L. sativa*, *L. serriola*, *L. saligna*, *L. dentata*, *L. virosa*, and *Lactuca* sp. Data from F_1, F_2, F_3, three-way crosses, and selfed backcross progenies demonstrated that this resistance was conferred by a recessive allele at a single locus (*cor*). Allelism tests indicated that the same locus determined resistance in most resistant accessions evaluated.

Additional keywords: disease screen, germ plasm, *Lactuca*.

Corky root is a serious disease of lettuce (*Lactuca sativa* L.) that has become of economic concern in major production areas of California (21). The disease, described in greater detail by van Bruggen (28), causes a slow progressive deterioration of the root system of infected plants (1–3,24). Fields continuously cropped to lettuce may become severely infected, resulting in substantial crop losses.

The etiology of corky root has been controversial. *Aphanomyces* sp. (20) *Pythium* sp. (6,10), *Fusarium* sp. (6), *Botrytis cinerea* (18), *Xanthamonas vitians* (4), and *Pseudomonas rhizoctonia* (26), as well as an unidentified organism (13,27), have been invoked as casual agents of symptoms similar to lettuce corky root. Abiotic factors have also been proposed. Marlatt (19) in Arizona described the root rot of unknown origin that increased in severity after heavy applications of manure. Grogan and Zink (9), studying a similar root rot, attributed the disease to toxic concentrations of ammonia from decomposition of organic or inorganic fertilizers. Hoff and Newhall (12) concurred with the findings of Grogan and Zink and introduced the descriptive name corky root. Busch and Barron (5), however, were unable to show involvement of toxic concentrations of ammonia in muck soils of Ontario; they proposed that some other toxic product was involved. This was supported by Amin and Sequiera (2,3) who partially purified phytotoxic substances from decomposing plant residues, suggesting their involvement in the development of corky root. Studies by Hartnett and Lorbeer (11) corroborated the work of Amin and Sequiera in greenhouses and controlled environment chambers.

Recently, Waters and Grogan (29) identified a slow-growing, hard-to-isolate bacterium as causing corky root. This bacterium was rod shaped (0.3–0.6 by 0.6–1.4 μm), aerobic and microaerophilic, oxidase (+), and catalase (−) and had a single lateral flagella, and optimal growth at 28–30 C. Colonies were circular, appearing umbonate and translucent. Old colonies in culture established raised edges and contorted wrinkles toward the center. The bacterium has been reported to produce a toxin (14) that is of low molecular weight (< 340 mw), heat stable, and soluble in polar solvents.

In the absence of genetically resistant cultivars, strategies to control the disease have been inadequate. Fumigation with methyl bromide or chloropicrin can be effective but has usually been uneconomical. Cultural practices that improve drainage and reduce soil compaction (i.e., cover cropping, raised beds, infrequent irrigation, and deep plowing) are thought to minimize the severity of corky root (21). Resistant cultivars seem to offer the only satisfactory method of long-term control.

Several cultivars with resistance to corky root have been developed. The genetic basis of the resistance in lettuce to corky root, however, was unknown. Three potential sources of resistance (PI175739, PI174229, and PI171669) were identified by Dickson (8) using corky root-prone soils under field and greenhouse conditions. Similarly, a greenhouse seedling screen was used to evaluate 172 accessions of *L. sativa* in a study by Sequiera (24,25), who released the resistant cultivars, Marquette, Montello, and Green Lakes. The resistance was thought to be controlled by several recessive genes.

The present study was undertaken to identify new sources of resistance and to determine its genetic basis. The newly isolated

bacterium was used to develop a reliable seedling screening procedure. The use of the bacterium was justified by fulfilling Koch's postulates. Resistant accessions were identified by screening several hundred lines representing both wild and cultivated *Lactuca* spp. The genetics of resistance was studied in F_2, F_3, three-way crosses, and selfed backcross progenies. Allelism tests were made to identify the number of loci conferring resistance.

MATERIALS AND METHODS

Inoculum. The isolate of the bacterium was collected in the Salinas Valley, CA, by Waters and Grogan (29). Aliquots of the culture were stored at -80 C in 15% glycerol. So far cultures have been stored at -80 C for 3 yr with no detectable loss in viability and pathogenicity. Cultures stored at 4 C in autoclaved water or on agar plates declined significantly in viability after 6 mo.

Inoculum was prepared by inoculating 500 ml of S-broth (2.5 g of casein hydrolysate, 1.25 g of glucose, 0.65 g of $K_2HPO_4 \cdot 3H_2O$, 0.25 g of KNO_3, 0.25 g of $MgSO_4 \cdot 7H_2O$, 30 mg of $Ca(NO_3)_2 \cdot 4H_2O$: C. M. Waters, Campbell Soup Co., Agric. Res., Davis, *personal communication*) from a scrape of the frozen culture. The inoculated broth was incubated at 29 C for 5–7 days in 1-L flasks on a rotary shaker at 100 rpm until the bacterial suspensions had approximately 1.5 A at 600 nm (log phase cultures, about 6×10^7 viable cells per milliliter).

Koch's postulates. Seeds were surface sterilized for 2 hr in 3% sodium hypochlorite and rinsed three times in sterile distilled water. To check on surface sterilization, samples of seeds were placed on bacterial nutrient medium; this resulted in no bacterial growth, demonstrating that the seeds were aseptic. Seeds (one or two per box) were placed in sealed plastic boxes (GA-7, Magenta Corp., Chicago) containing sterile vermiculite and Hoagland's solution (approximately 50 ml) and incubated at 27 C with 14 hr of light (300 $\mu E/m^2/s$) per day. After 3 wk, groups of 12 seedlings were inoculated with either water, S-broth alone, or a bacterial suspension in S-broth. Bacteria were reisolated from infected seedlings. A fresh group of seedlings were grown aseptically as before in Magenta boxes inoculated with water, S-broth alone, or a bacterial suspension prepared using the reisolated bacteria. The experiment was repeated twice.

Seedling screen. To identify sources of corky root resistance, plants were grown in moist vermiculite in plastic seedling trays (51 cells, each 3 cm diameter). Plants were thinned to one per cell providing eight or nine plants per accession. Seedlings were maintained in a greenhouse at 25–30 C during the day. Inoculations were made at the second to third true-leaf stage. Two milliliters of bacterial suspension (about 1.2×10^8 viable cells) was placed on the plant apex and allowed to flow down the hypocotyl into the vermiculite. Each screen included both susceptible and resistant checks. Salinas, Vanguard 75, Salad Bowl, Valmaine, and Winter Density were used as susceptible cultivars; Marquette, Montello, and Green Lakes were included as resistant cultivars.

Evaluation. Individual plants were evaluated 3–4 wk after inoculation. A rating of 0–9 was assigned to each plant based on the extent of taproot deterioration by using a disease scale (Fig. 1). Plants within each accession were averaged to provide a disease severity index (DSI). A rating of less than 4 was considered resistant. Ratings greater than 4 had pronounced fracturing (cracking) of the taproot, which provided entry for secondary microorganisms leading to extensive decay. Lines with a DSI value of 1.75 or below were considered highly resistant as the existing resistant eastern cultivars, Green Lakes, Montello, and Marquette, had DSI = 1.21 ± 0.18, 1.47 ± 0.36, and 1.60 ± 0.43, respectively. This study focused on high levels of resistance to identify lines with resistance which was at least as effective as those already available.

Genetic studies. The genetics of resistance was evaluated by screening 20 F_2 progenies (3,041 F_2 plants), 11 selfed three-way cross and backcross progenies (1,429 plants), and 19 F_3 families (820 plants). A limited number (six) of F_1 hybrids was also evaluated. The F_2 progenies resulted from crosses between resistant and susceptible lines, their reciprocals, and crosses

between two resistant lines to test for allelism. The commercial cultivars, Salinas, Calmar, Vanguard 75, and the breeding lines Early Flower (23) and FSL176 (Kihara and Michelmore, *unpublished*), were used as susceptible parents. Cultivar Green Lakes was used as one of the resistant parents in most allelism tests. In each tray containing segregating populations, both parents were included as checks.

RESULTS

Koch's postulates. Koch's postulates were fulfilled using the aseptic lettuce seedlings, confirming that the bacterium could play an important etiological role in corky root of lettuce. Seedlings
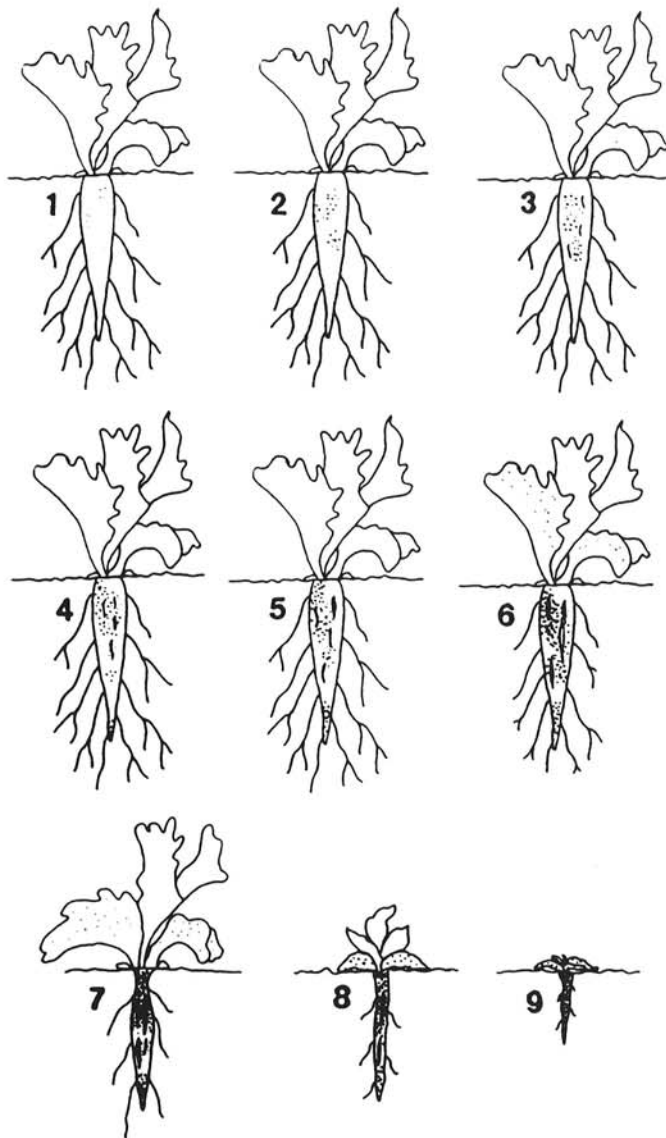

Fig. 1. Corky root disease severity scale. 0) No symptoms, completely white roots; 1) small areas of yellow discoloration ($<5\%$) on the taproot; 2) yellowing to light brown areas of discoloration over 5–10% of the taproot; 3) yellowing to light brown over 5–10% of the taproot with small (1–2 mm) superficial cracks; 4) light brown to dark brown discoloration over approximately 20% of taproot and longitudinal cracks (2–3 mm) penetrate epidermis; 5) brown to dark brown over 25–30% of taproot and pronounced cracks (3–5 mm long); 6) dark brown discoloration over 30–40% of taproot with cracks (3–8 mm long) penetrating to cortical region, some noticeable reduction of secondary root growth; 7) dark brown over 40–60% of taproot, longitudinal cracks penetrate well into cortex, chlorosis of lower leaves under water stress, some girdling of taproot; 8) dark brown over 60–80% of taproot, girdling at soil level, large longitudinal cracks and severely reduced root system, seedlings wilt under water stress; 9) seedlings dead, taproot dark brown with minimal secondary root growth, and leaves necrotic.

treated with a pure suspension of the isolated bacterium developed corky root symptoms within 4 wk. Seedlings inoculated with water or S-broth alone never developed symptoms. All seedlings inoculated with the bacterium developed symptoms. The bacterium could only be reisolated from symptomatic plants inoculated with the bacterium. Fresh aseptically grown seedlings inoculated with a pure suspension of the reisolated bacterium again exhibited corky root symptoms. These data supported the development of a seedling screen using the newly isolated coryneform bacterium.

Germ plasm screen. Five hundred and fifty-five accessions of *Lactuca* spp. were screened for resistance to corky root. Symptoms ranged from a white taproot with a few bands of yellow lesions (rated 1) that usually did not interfere with root development to a severely necrotic taproot with extensive fractures (rated 9). Accessions displaying a DSI of less than 1.75 were retested to confirm their resistance and eliminate any escapes. Only the highly resistant lines with DSI = 1.75 or below were studied in detail as the existing resistant cultivars, Green Lakes, Montello and Marquette, had DSI = 1.21 ± 0.18, 1.47 ± 0.36 and 1.60 ± 0.43. High levels of resistance were identified in 16 accessions in addition to the three resistant cultivars, Green Lakes, Montello, and Marquette (Table 1). Two accessions, PI234204 and PI274376, were not sexually compatible with *L. sativa*. One accession, PI289064, was a heterogeneous population with an average DSI of 2.25; two resistant plants were selfed (289064-1 and 289064-2) and when reevaluated showed high levels of resistance (DSI = 0.97 ± 0.23 and 1.08 ± 0.17, respectively). Lower levels of resistance (DSI = 1.75 to 3.99) were identified in a further 40 accessions (Table 2). These lines were not studied further.

Inheritance of resistance. Crosses between resistant and susceptible accessions produced F_1 hybrids that were allowed to self. The few F_1 individuals that were screened were all susceptible and died without setting seed. The majority of F_2 progenies segregated in the ratio of one resistant to three susceptible suggesting that a single recessive gene conferred resistance (Table

3). To confirm the monogenic inheritance, F_1 individuals were crossed or backcrossed to susceptible lines and their progeny were selfed. Unfortunately, many of these hybrid individuals succumbed to a disease of unknown etiology in the greenhouse before setting seed. The 1,429 progeny plants of 11 selfed three-way and BC_1 hybrids analyzed either segregated in a 1:3 ratio, resistant to susceptible, or all the plants were susceptible (Table 4). There was an approximately 1:1 ratio of segregating to nonsegregating families supporting the presence of a single recessive gene for resistance.

F_3 family analyses from the cross Green Lakes $\times$ Salinas exhibited no segregation (all plants resistant) or segregated one resistant to three susceptible (Table 5). No homozygous susceptible F_3 families were observed; this was probably due to homozygous susceptible F_2 plants failing to set seed before succumbing to corky root. All F_3 families from susceptible F_2 plants segregated 1 resistant to 3 susceptible. Several of the families from resistant F_2 plants, however, also segregated one resistant to three susceptible, indicating that some of the F_2 plants may have been misclassified or that other loci or modifier genes are involved. The majority of segregation data from F_3 families support the existence of a single recessive gene for resistance; however, further studies are needed to clarify the basis for the observed segregation in progeny from resistant F_2 plants.

The cross between Early Flower and the resistant cultivar, Marquette, deviated from the 1:3 ratio in two evaluations of the

TABLE 1. Taxonomic distribution of lines highly resistant to corky root among species of *Lactuca*

Species	Lines screened[a] (no.)	Lines resistant (no.)	Designation of resistant lines	DSI ± s.d.[b]	Origin
L. sativa	507	5	Montello	1.47 ± 0.36	U.S.A.
			Green Lakes	1.21 ± 0.18	U.S.A.
			Marquette	1.60 ± 0.43	U.S.A.
			PI491010	1.57 ± 0.55	Turkey
			PI491011	1.75 ± 0.52	Turkey
L. augustana	3	0			
L. serriola	20	5	PI255665	0.50 ± 1.17	Afghanistan
			PI289064-1	0.97 ± 0.23	Hungary
			PI289064-2	1.08 ± 0.17	Hungary
			PI491249	0.25 ± 0.46	Greece
			PI491250	0.25 ± 0.46	Greece
			PI491251	0.37 ± 0.37	Greece
L. squarrosa	1	0			
L. saligna	12	6	PI261653	0.55 ± 0.52	Portugal
			PI491206	1.22 ± 0.44	Greece
			PI491204	0.62 ± 0.52	Greece
			PI490999	1.42 ± 0.53	Turkey
			UC83UK2	0.62 ± 0.52	U.K.
			UC83US1	1.42 ± 0.78	U.S.A.
L. dregeana	1	0			
L. livida	1	0			
L. dentata	1	1	PI234204[c]	0.44 ± 0.53	Japan
L. virosa	3	1	UC83UK1[c]	1.00 ± 0.00[d]	U.K.
L. perennis	3	0			
Lactuca spp.	1	1	PI274376[c]	0.63 ± 0.43	Afghanistan

[a] Complete list of lines screened is available on request.
[b] DSI ± s.d. = Disease Severity Index and standard deviation (see text). Only those lines which were at least as resistant as Marquette are included.
[c] Not sexually compatible with *L. sativa*.
[d] Only screened once and all 10 individuals expressed high degree of resistance.

TABLE 2. Taxonomic distribution of accessions with partial resistance to corky root among species of *Lactuca*

Species	Lines resistant (no.)	Designation of resistant lines	DSI[a] ± s.d.	Origin
L. sativa	36	PI278065	2.18 ± 1.22	Turkey
		PI491006	2.33 ± 3.67	Turkey
		PI175736	2.38 ± 1.17	Turkey
		PI278066	2.60 ± 1.52	Turkey
		PI278086	2.89 ± 1.83	Turkey
		PI278110	3.00 ± 1.19	Turkey
		PI250428	3.00 ± 1.82	Czechoslovakia
		PI278071	3.14 ± 1.21	Turkey
		PI261654	3.20 ± 0.91	Netherlands
		PI206964	3.20 ± 0.96	Turkey
		RHT57	3.25 ± 1.83	[Breeding line]
		PI164940	3.38 ± 2.38	Turkey
		PI273205	3.40 ± 0.52	England
		PI278090	3.40 ± 0.89	Turkey
		PI273596	3.42 ± 1.38	Germany
		PI179297	3.44 ± 0.88	Turkey
		PI206966	3.44 ± 1.29	Turkey
		PI278094	3.50 ± 1.77	Turkey
		Dandie	3.55 ± 0.53	[Cultivar]
		PI273616	3.55 ± 1.25	Netherlands
		PI172916	3.63 ± 2.05	Turkey
		PI339262	3.64 ± 1.80	Turkey
		PI204706	3.67 ± 1.22	Turkey
		PI278084	3.71 ± 1.70	Turkey
		PI274358	3.78 ± 1.34	Poland
		PI381932	3.80 ± 1.40	France
		PI181883	3.83 ± 0.98	Syria
		PI212015	3.86 ± 0.90	Iran
		PI289061	3.86 ± 1.73	Hungary
		PI381933	3.86 ± 1.77	France
		Kinemontepas	3.87 ± 1.12	[Cultivar]
		Blondine	3.87 ± 1.76	[Cultivar]
		PI179295	3.88 ± 0.99	Turkey
		PI289059	3.88 ± 2.32	Hungary
		PI257288	3.90 ± 1.80	Spain
		PI372907	3.94 ± 2.93	Netherlands
L. serriola	3	LSE/57/15	3.08 ± 0.28	England
		PI274372	3.08 ± 1.62	USSR
		PI253467	3.27 ± 1.52	Yugoslavia
L. saligna	1	PI491208	3.67 ± 0.50	Greece

[a] DSI = Disease Severity Index (see text). Those lines that had a DSI of 1.75 to 3.99 were considered to have partial resistance.

same F_2 population (Table 3). Although Marquette is a highly inbred cultivar, its resistance was variable, with some individuals showing susceptible responses (DSI 2 to 5). Therefore, resistance in Marquette may be readily influenced by environmental factors. This correlated with the partial resistance that has been observed in Marquette in the field.

Significant deviations from simple inheritance ratios were obtained in the interspecific crosses between UC83US1 (*L. saligna*) and FSL176 or Vanguard 75 (both *L. sativa*) and between PI490999 (*L. saligna*) and Salinas (*L. sativa*). Bias in the small F_2 populations from these interspecific crosses was not unexpected (16). The crosses were only successful when *L. saligna* was used as the maternal parent. The F_1 hybrids have poor seed set (less than one seed per capitulum rather than approximately 18 in normal capituli) and abnormal meioses (O. Ochoa, U. C. Davis, *personal communication*). Of the seeds that did set, some had aborted embryos or endosperm and failed to germinate. The seedlings that emerged often had abnormal leaf and stem morphologies. Some of the interspecific crosses involving *L. saligna* had insufficient F_2 seed for analysis.

Allelism tests. F_2 progenies from crosses between many of the resistant accessions and the cultivar Green Lakes showed no segregation for resistance (Table 6). Resistance was, therefore, determined at the same locus in Green Lakes as in the other 10 resistant lines tested. As only one locus was involved, it was unnecessary to analyze many of the possible combinations of resistant lines. These allelism tests demonstrated that a single locus confers resistance in many of the lines identified.

Segregation was observed in crosses involving *L. saligna*. In one screen, 31 out of 137 individuals exhibited a susceptible response from the cross PI261653 (*L. saligna*) × Green Lakes (Table 6); however, the F_2 were stunted by a pesticide applied before inoculation. This stress may have induced susceptibility to corky root. A subsequent screening of the same F_2 population showed no segregation. In another screen, 37 out of 143 individuals from

TABLE 3. Segregation of resistance to corky root in F_2 progenies from crosses between resistant and susceptible accessions of *Lactuca* spp.

Cross	Number of F_2 seedlings R:S[a]	Chi^2 (1:3)
L. sativa × *L. sativa*		
Green Lakes(R)[a] × Salinas(S)	35:111	0.037
Montello(R) × Salinas(S)	30:83	0.07
Marquette(R) × Salinas(S)	35:119	0.173
Calmar(S) × Montello(R)	102:264	1.46
Salinas(S) × PI491010(R)	42:145	0.55
Early Flower(S) × Marquette(R)	17:99	6.080**
Early Flower(S) × Marquette(R)	21:98	3.43
L. sativa × *L. serriola*		
Salinas(S) × PI491251(R)	56:166	0.000
Salinas(S) × PI289064-1(R)	35:117	0.210
Salinas(S) × PI289064-2(R)	32:122	1.250
Early Flower(S) × PI491251(R)	28:98	0.295
Early Flower(S) × PI491251(R)	37:73	3.93*
Early Flower(S) × PI289064-1(R)	33:92	0.067
Early Flower(S) × PI289064-2(R)	50:126	0.916
L. serriola × *L. Sativa*		
PI289064-1(R) × Salinas(S)	36:87	1.03
PI289064-2(R) × Salinas(S)	28:93	0.134
PI255665(R) × Salinas(S)	46:128	0.122
PI2898064-2(R) × Early Flower(S)	46:121	0.449
L. saligna × *L. sativa*		
PI261653(R) × Salinas(S)	17:60	0.21
US83UC1(R) × FSL176(S)	18:14	15.04**
US83UC1(R) × Vanguard 75(S)	2:29	4.75*
PI490999(R) × Salinas(S)	2:27	4.15*

[a] R = resistant; S = susceptible. *Significant at 5% level. ** Significant at 1% level.

TABLE 4. Segregation of resistance to corky root in selfed progenies from backcrosses between resistant and susceptible accessions of *Lactuca* spp.

Pedigree	Number of progenies R : S[a]	Chi^2 (1:3)
[Salinas(S)[a] × 289064-1(R)] × Salinas	43:113	0.42
Early Flower(S) × [289064-2(R) × 255665-1(R)]	29:121	2.26
Salinas(S) × [Early Flower(S) × 289064-2(R)]	0:144	
Salinas(S) × [255665-1(R) × Green Lakes (R)]	12:48	0.56
[UC83US1(R) × Valmaine(S)] × Vanguard 75(S)	4:343	
[Salinas(S) × 289064-1(R)] × Salinas(S)	18:69	0.64
Salinas(S) × [289064-2 × Early Flower(S)]	0:74	
Early Flower(S) × [289064-2(R) × 255665-1(R)]	16:58	0.29
[Early Flower(S) × 289064-1(R)] × Salinas(S)	0:91	
Salinas(S) × [289064-2(R) × Early Flower(S)]	0:81	
Salinas(S) × [255665-1(R) × Green Lakes(R)]	45:120	0.34

[a] R = resistant; S = susceptible.

TABLE 5. Segregation of resistance to corky root in F_3 families from cross between Green Lakes and Salinas

F_3 Family	Rating of F_2 plant	F_3 Segregation R:S[a]	X^2 (1:3)	DSI of resistant progeny
1	1	41:0		1.00 ± 0.00
2	1	141:0		1.02 ± 0.14
3	1	10:27	0.019	1.00 ± 0.00
4	1	37:0		1.00 ± 0.00
5	1	9:19	0.43	1.00 ± 0.00
6	1	9:20	0.28	1.00 ± 0.00
7	2	9:32	0.07	1.22 ± 0.44
8	2	32:0		1.00 ± 0.00
9	1	38:0		1.00 ± 0.00
10	1	40:0		1.00 ± 0.00
11	7	11:31	0.98	1.45 ± 0.52
12	7	7:34	0.03	1.28 ± 0.49
13	7	10:30	0.13	1.10 ± 0.31
14	7	11:27	0.04	1.00 ± 0.00
15	7	7:24	0.15	1.00 ± 0.00
16	7	12:29	1.14	1.00 ± 0.00
17	7	14:28	0.03	1.21 ± 0.47
18	7	9:31	0.01	1.00 ± 0.00
19	7	11:32	0.01	1.00 ± 0.00

[a] R = resistant, S = susceptible.

TABLE 6. Segregation of susceptibility and resistance in progenies from crosses between resistant accessions of *Lactuca* spp.

Cross	Number of F_2 seedlings R:S[a]	DSI ± s.d. of resistant progeny
L. sativa × *L. sativa*		
Montello × Green Lakes	161:0	1.26 ± 0.45
PI491010 × Green Lakes	160:0	1.03 ± 0.17
L. serriola × *L. sativa*		
PI289064-1 × Green Lakes	170:0	1.13 ± 0.34
PI289064-2 × Green Lakes	179:0	1.16 ± 0.36
PI255665 × Green Lakes	163:0	1.02 ± 0.13
PI491251 × Green Lakes	162:9	1.56 ± 0.75
L. serriola × *L. serriola*		
PI289064-2 × PI255665	151:8	1.38 ± 0.68
PI255665 × PI289064-2	159:0	1.09 ± 0.36
PI255665 × PI289064-1	156:0	1.03 ± 0.19
L. saligna × *L. sativa*		
PI261653 × Green Lakes	95:31[b]	1.20 ± 0.47
PI261653 × Green Lakes	67:0	
PI490999 × Montello	15:5[c]	1.00 ± 0.00
PI491204 × Green Lakes	106:37[c]	1.19 ± 0.33
L. saligna × *L. serriola*		
PI261653 × PI289064-1	26:0	
L. saligna × *L. saligna*		
PI261653 × US83UC1	204:0	1.08 ± 0.37
PI261653 × US83UC1	100:127[c]	1.25 ± 0.41

[a] R = resistant; S = susceptible.
[b] Biased by application of pesticide (see text).
[c] See text for explanation.

the cross PI491204 (*L. saligna*) × Green Lakes, five out of 20 individuals from the cross PI490999 × Montello, and 127 out of 227 individuals from the cross PI261653 (*L. saligna*) × UC83US1 (*L. saligna*) showed a susceptible response. Occasional susceptible progeny were also observed in two other crosses (Table 6); in both these cases the DSI of the resistant progeny was slightly higher. In all populations there was a large excess of resistant progeny and, therefore, the segregation ratios deviated significantly from the ratio of one resistant to 15 susceptible expected if two unlinked loci were involved in resistance. It was, therefore, tentatively concluded that resistance was determined by the same locus in both *L. sativa* and *L. saligna*. The susceptible progeny may be the consequence of the resistant allele in at least one of the parental lines being influenced by the environment or due to modifier genes.

Correlation between greenhouse screen and field conditions. Seedling resistance was previously correlated to field resistance (8,24) based on an unknown etiology and screening for resistance using corky root-prone soils. To confirm that resistance identified in the greenhouse using the bacterium was correlated with resistance in the field, resistant accessions identified in the greenhouse were evaluated for resistance in the field. The accessions were grown in corky root-prone soils in Salinas and Santa Maria in collaboration with Dr. E. J. Ryder and D. Miltz. In another evaluation, breeding lines selected for resistance in the field by E. J. Ryder (*unpublished data*) were subjected to screening in the greenhouse. In both evaluations, resistance identified in the greenhouse using the bacterium correlated precisely with resistance identified in the field.

DISCUSSION

The bacterium isolated by Waters and Grogan (29) can play a major role in the etiology of corky root in lettuce. In the greenhouse, seedlings inoculated with the bacterium routinely developed the same characteristic corky root symptoms as exhibited by diseased plants in the field; the bacterium could be readily reisolated from inoculated seedlings. The resistance identified in the greenhouse using the bacterium correlated precisely with resistance identified in the field. The fulfillment of Koch's postulates confirmed the potential of the bacterium as an etiological agent of corky root.

Several outstanding questions remain concerning the role of the bacterium in disease development. The presented data confirmed the observations of Waters and Grogan (29) and van Bruggen (28) that the bacterium is capable of causing corky root symptoms, although the data do not, however, preclude the involvement of other organisms or factors in the field. Circumstantial observations suggest other organisms or factors can also be involved. Phytotoxic substances have been isolated from soil containing decomposing lettuce residues (1,3). Early studies of corky root attributed increases in disease severity to excessive amounts of nitrogenous fertilizers (e.g., urea), large amounts of manure, organic soils, or soils continuously cropped to lettuce (2,3,5,12,18–20); the influence of nitrogen levels on corky root development should be reevaluated. In each of these situations, there is a high level of nitrogen-containing nutrients available. Thorough epidemiological studies are needed to understand better the relationship between this bacterium and corky root disease. The coryneform bacterium is potentially part of a complex set of interacting factors in the etiology and epidemiology of corky root in lettuce.

Resistance to corky root was simply inherited, being determined by a single recessive allele at a single locus in the highly resistant lines tested. We have designated this gene *cor*. Not all of the highly resistant lines identified could be evaluated in allelism tests due to sexual incompatibility with *L. sativa*, sterility problems in F_1 hybrids with *L. saligna*, or loss of F_1 individuals to a disease of unknown etiology. Segregation and allelism data consistently indicated that recessive alleles at a single locus conferred corky root resistance in all lines; however, independent loci for resistance in untested lines cannot be excluded. Also, the genetic basis for partial resistance (DSI = 1.75 to 3.99; Table 2) was not studied and

is unknown. Reactions of Marquette were less stable than that of Green Lakes, varying within and between screens. Marquette has the same allele for corky root resistance as Green Lakes (24,25), suggesting that the former cultivar has modifying genes that are sensitive to environmental influences. Resistance may have evolved several times as resistance was identified in accessions of geographically and taxonomically diverse origins. There may therefore be several alleles for resistance. Different alleles may determine different levels of resistance and may account for the susceptible progeny in allelism tests involving *L. saligna* accessions. Alleles from *L. saligna* and Green Lakes could be independently backcrossed into a susceptible crisphead cultivar to study this possibility.

The severity of corky root may be influenced by several physiological factors. In this study there was a larger difference between susceptible and resistant accessions at higher temperatures (approximately 25–30 C). Sensitivity to changes in the environment (22) is a common characteristic of resistance to bacterial diseases. In tomatoes, resistance to *Pseudomonas solanacearum* can be significantly affected by temperature (15), light intensity, and photoperiod (15,17).

The results of this study are of significant value to the plant breeder. The ability to screen seedlings in the greenhouse for corky root provides rapid and reliable identification of resistant genotypes. Development of resistant varieties through a backcross program provides precise control over introgression of the recessive gene. Such a breeding program can be carried out in any environment and only small numbers of progenies are required; this will enable rapid release of resistant cultivars. As resistance is recessive, a generation of selfing is required before evaluation. Homozygosity, however, can be fixed in a single generation.

The development of genetically resistant cultivars offers an effective long-term control strategy for corky root. Pathogen variation has not been reported, but it has not been thoroughly investigated. Bacterial pathogens tend to have less variability than fungal pathogens and their spread is usually slow. If the resistance mechanism is passive, there would be a smaller tendency for the pathogen to actively overcome resistance than as is the case in gene-for-gene interactions (7). Further studies are needed on the mechanism(s) of resistance, potential bacterial variation, and the epidemiology of corky root in lettuce.

LITERATURE CITED

1. Amin, K. S. 1965. Nature of corky root rot disease of lettuce. Ph.D. thesis. University of Wisconsin, Madison. 107 pp.
2. Amin, K. S., and Sequiera, L. 1966. Role of certain factors in the etiology of corky root rot of lettuce. Phytopathology 56:1047-1053.
3. Amin, K. S., and Sequiera, L. 1966. Phytotoxic substances from decomposing lettuce residues in relation to the etiology of corky root rot of lettuce. Phytopathology 56:1054-1061.
4. Brown, N. A. 1918. Some bacterial diseases of lettuce. J. Agric. Res. 13:367-388.
5. Busch, L. V., and Barron, G. L. 1963. Root rot of head lettuce in Ontario. Can. J. Plant Sci. 43:1054-1061.
6. Coons, G. H. 1922. Diseases of field and vegetable crops in the U.S. for 1921. Plant Dis. Rep. Sup. 22:409.
7. Crute, I. R. 1985. The genetic bases of relationship between microbial parasites and their hosts. Pages 80-142 in: Mechanisms of Resistance to Plant Diseases. R. S. S. Fraser, ed. Martinus Nijhoff/Dr. W. Junk Publ.
8. Dickson, M. H. 1963. Resistance to corky root rot in head lettuce. Am. Soc. Hortic. Sci. 82:388-390.
9. Grogan, R. G., and Zink, F. W. 1956. Fertilizer injury and its relationship to several previously described diseases of lettuce. Phytopathology 16:416-422.
10. Haenseler, C. M. cited in A. J. Mix 1945 Diseases of lettuce and other vegetables observed in Northern New Jersey. Plant Dis. Rep. 29:556-557.
11. 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.
12. 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.
13. Jagger, I. C. 1940. Brown blight of lettuce. Phytopathology 30:53-64.

14. Kao, J., Mitten, D. H., and Milich, C. A. 1984. Identification of a host-specific toxin produced by the corky root rot bacterial pathogen of lettuce. Phytopathology 76:844.

15. Krausz, J. P., and Thurston, H. D. 1975. Breakdown of resistance to *Pseudomonas solanacearum* in tomato. Phytopathology 65:1272-1274.

16. Lindqvist, K. 1960. Cytogenetic studies in the serriola group of *Lactuca*. Hereditas 46:75-151.

17. Lozano, J. C., and Sequiera, L. 1970. Differentiation of races of *Pseudomonas solanacearum* by a leaf infiltration technique. Phytopathology 60:833-838.

18. MacNeill, B. H. 1953. A Botrytis root rot condition in lettuce. Plant Dis. Rep. 37:618-619.

19. Marlatt, R. B. 1955. Brown stele of lettuce. Plant Dis. Rep. 39:827-828.

20. Mix, A. J. 1945. Aphanomyces root rot of lettuce, pepper and eggplant seedlings in Northern New Jersey. Plant Dis. Rep. 29:649-650.

21. Patterson, C. L., Grogan, R. G., and Campbell, R. N. 1986. Economically important diseases of lettuce. Plant Dis. 70:982-987.

22. Russell, G. E. 1981. Bacterial diseases. Pages 169-198 in: Plant Breeding for Pest and Disease Resistance. Butterworth and Co. Woburn, MA.

23. Ryder, E. J. 1985. Use of early flowering genes to reduce generation time in backcrossing, with specific application to lettuce breeding. J. Am. Soc. Hortic. Sci. 110:570-573.

24. Sequiera, L. 1970. Resistance to corky root rot in lettuce. Plant Dis. Rep. 54:754-758.

25. Sequiera, L. 1978. Two root rot resistant varieties of head lettuce. Wis. Agric. Exp. Stn. Rep. 2 pp.

26. Thomas, R. C. 1922. A bacterial rosette disease of lettuce. Ohio Agric. Exp. Stn. Bull. 359:197-214.

27. Valleau, W. D. 1921. Disease of field and vegetable crops in the United States in 1921. Plant Dis. Rep. Sup. 16:282.

28. van Bruggen, A. H. C., Grogan, R. G., Bogdanoff, C. P., and Waters, C. M. 1988. Corky root of lettuce in California caused by a Gram-negative bacterium. Phytopathology 78:1139-1145.

29. Waters, C. M., and Grogan, R. G. 1984. Identification of the bacterium causing lettuce corky root. (Abstr.) Phytopathology 74:857.