Host Range of *Rhizomonas suberifaciens*, the Causal Agent of Corky Root of Lettuce

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ABSTRACT

van Bruggen, A. H. C., Brown, P. R., and Jochimsen, K. N. 1990. Host range of *Rhizomonas suberifaciens*, the causal agent of corky root of lettuce. Plant Dis. 74:581-584.

Greenhouse experiments were conducted to investigate if *Rhizomonas suberifaciens*, the causal agent of corky root (CR) of lettuce, was pathogenic on various winter cover crops, vegetable crops, and weed species in eight plant families. *R. suberifaciens* induced CR symptoms on endive, common sowthistle, and prickly lettuce of the Compositae but not on safflower and sunflower. The pathogen was reisolated from each of the susceptible hosts, and the reisolated strains were similar to the original strain in affinity to polyclonal antibodies and pathogenicity on lettuce. Inoculation of species of the Chenopodiaceae, Cruciferae, Cucurbitaceae, Gramineae, Leguminosae, Solanaceae, and Umbelliferae with *R. suberifaciens* did not result in visible symptoms, except for a hybrid carrot cultivar that reacted with root proliferation and orange-brown discoloration of feeder roots. However, the pathogen was not reisolated from these plants. An open-pollinated carrot cultivar did not develop symptoms. *R. suberifaciens* was isolated from the root surface of inoculated broad bean, safflower, and soybean plants without CR symptoms. Strains of *R. suberifaciens* were also isolated from field-grown tomato, melon, sowthistle, and prickly sowthistle with CR symptoms. These strains were pathogenic on lettuce and sowthistle but not on tomato and melon. So far, only members of the Compositae closely related to lettuce have shown susceptibility to *R. suberifaciens*.

Corky root (CR) of lettuce has been reported in California (8,13), Florida (6), New York (7), Ontario (3), Wisconsin (1), and Italy (4). The causal agent was identified as a gram-negative bacterium in California (13). Recently, similar strains were isolated from muck soils of Florida, Wisconsin, and New York (11). These strains were pathogenic on the lettuce cultivar Salinas and caused symptoms typical of CR in the greenhouse. Strains of the bacterium that caused CR had a very characteristic fatty acid profile with 2-hydroxy fatty acids rather than 3-hydroxy fatty acids (11). A similar fatty acid profile had only been described for *Pseudomonas paucimobilis* (which is not a true *Pseudomonas* and should be reclassified [14]). Results of physiological and biochemial tests with strains that induced CR on lettuce did not correspond to those of any existing genus. Thus, a new genus and species, *Rhizomonas suberifaciens*, were proposed for strains of this bacterium (14).

Because the causal agent of CR was identified only recently, very little is known about the pathogen and its life cycle. Typical CR symptoms were observed on wild lettuce (*Lactuca serriola* L.), common sowthistle (*Sonchus oleraceus* L.), and prickly sowthistle (*S. asper* (L.) Hill) in commercial lettuce fields in California and on endive (*Cichorium endivia* L.) in a commercial endive field in Florida (van Bruggen, *unpublished*). D'Ercole (4) observed CR symptoms on endive in Italy but believed that the disease might be caused by toxins liberated from decomposing lettuce debris. Brown and Michelmore (2) screened over 500 accessions of *Lactuca sativa, L. saligna, L. serriola*, and other *Lactuca* species for resistance to strain CA1 of *R. suberifaciens*. There was a wide range of susceptibility levels, but only two accessions were immune. These belonged to *L. dentata* and an unidentified *Lactuca* species that could not be crossed with *L. sativa* (2). So far, all species that are demonstrated hosts of *R. suberifaciens* are in the genus *Lactuca*. *R. suberifaciens* has not been isolated from other genera in the family Compositae (such as *Sonchus* and *Cichorium*), and strains of this bacterium from lettuce have not been tested for pathogenicity on these or other genera.

Another corky root disease has been described for tomato and melon (5). The symptoms can be similar to those of CR of lettuce, but the causal agent is a fungus, *Pyrenochaeta lycopersici* (5). The pathogen has been isolated from both tomato (5) and melon in California (D. Gubler, *personal communication*).

Host range tests were performed in a greenhouse to investigate the importance of weed species, winter cover crops, and various vegetable crops as alternative hosts for *R. suberifaciens*. Isolations were made from field-grown tomato, melon, sowthistle, and prickly sowthistle with corky root symptoms to determine if *R. suberifaciens* was associated with corky root on these plants.

Accepted for publication 26 January 1990 (submitted for electronic processing).

MATERIALS AND METHODS

Preparation of inoculum. All cultures of *R. suberifaciens* were routinely grown in S-medium [5.0 g enzymatic casein hydrolysate (Sigma Chemical Co., St. Louis, MO), 2.5 g glucose, 1.3 g $K_2HPO_4 \cdot 3H_2O$, 0.5 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 60 mg $Ca(NO_3)_2 \cdot 4H_2O$, and 11.0 g Agar Noble per liter, pH 7.2]. Inoculum for the host range tests was prepared from 4-day-old cultures of *R. suberifaciens* in S-medium broth. The cultures were centrifuged for 20 min at 9,150 *g*, and the pellet was resuspended in distilled water as described previously (11). Colony-forming units were estimated with a spectrophotometer (Spec-20, Bausch & Lomb Optical Co., Rochester, NY) based on a standard curve with 12 concentrations ranging from 1×10^8 to 5×10^9 cfu/ml. The concentrations (cfu/ml) for the standard curve were determined from eight 10-fold dilutions of a 4-day-old culture of strain CA1 that were plated on S-medium.

Pathogenicity tests with strain CA1 from lettuce. Two series of greenhouse experiments were conducted to test various crop and weed species for susceptibility to strain CA1 isolated from lettuce in California. Table 1 lists the families, genera, and species tested.

In the first tests, five seeds of 12 cover or rotation crops were planted in 4-L plastic pots with noninfested soil or soil infested with strain CA1. The lettuce cultivar Salinas was planted similarly as a control. The soil, a 2:1 (v/v) mixture of Chualar loam (13) and river sand, was autoclaved for 2 hr at 120 C and allowed to air out for 15–20 days. Then, 4-L samples of the soil mixture were mixed with 50 or 100 ml of distilled water (controls) or a suspension of a 4-day-old culture of strain CA1 (3×10^7 cfu/ml). Each treatment comprised five single-pot replications arranged in randomized complete blocks on greenhouse benches. The plants were grown under either six 60W fluorescent tubes or two 400W multivapor lamps, with a day length of 14 hr. The light intensity at plant level was 400–1,000 $\mu E \cdot m^{-2} \cdot s^{-1}$ during daylight and 100–200 $\mu E \cdot m^{-2} \cdot s^{-1}$

after sunset. Two weeks after planting, the plants were thinned to three plants per pot. Two months after planting, the roots were rated for severity of CR on a 0–9 scale (2). The percentage of the surface of the taproot that is yellow or corky is the main criterion at the lower end of this scale, i.e., 0–6, and depth of fissures on the taproot and shoot symptoms (yellowing, wilting, and stunting) are the main features at the upper end, i.e., 7–9 (2). Isolations were made from one to five roots of each plant species with symptoms (CR or other symptoms) as described previously (11,13). Bacterial colonies that looked like strain CA1 were tested for affinity to polyclonal antibodies produced against this strain and for pathogenicity on lettuce seedlings (11,13). The experiment was conducted three times—in the fall, spring, and winter during 1987–1989; average maximum/minimum temperatures were 28/21, 29/22, and 24/19 C, respectively.

In the second series of experiments, various vegetable crops and weeds grown in 5-cm-wide plastic pots containing vermiculite were tested for susceptibility to strain CA1. Five milliliters of 10^7–10^8 cfu/ml or distilled water were poured at the base of the stem of 2-wk-old seedlings. There were five or six single plant replications. The experiment was conducted three times, and all plant species were tested at least twice. Inoculated and control plants were placed in separate insect-proof cages at least 50 cm apart to avoid possible dispersal of the pathogen by fungus gnats (midges, belonging to the family Chironomidae) or splashing water. The cages were placed under multivapor lamps (see above) that were switched on for 14 hr per day. The light intensity at plant level was 400–1,000 $\mu E \cdot m^{-2} \cdot s^{-1}$ during daylight and 140-200 $\mu E \cdot m^{-2} \cdot s^{-1}$ after sunset. The maximum/minimum temperatures for the fall, winter, and spring of 1987–1989 were 28/21, 27/22, and 29/23 C, respectively. The plants were fertilized as described previously (11). One month after inoculation, the plants were uprooted and rated for severity of CR as described above. Isolations were made from two or three inoculated and control plants. Presumptive colonies of the pathogen were tested for reaction with polyclonal antibodies and pathogenicity on lettuce seedlings.

Chi-square tests were performed on frequency distributions of plants in disease severity classes for those species that were susceptible to the pathogen. The data of similar experiments were combined.

Isolations from naturally infected roots. Roots with symptoms typical of corky root previously associated with *P. lycopersici* were collected from four fresh market tomato plants (cultivar not known) and six melon (Persian, cantaloupe, and crenshaw) plants in Monterey County and Stanislaus County, California, respectively, between July and September of 1987. In addition, roots with CR symptoms similar to those on lettuce were collected from a sowthistle plant and a prickly sowthistle plant in lettuce fields in Watsonville and Salinas, California, in September 1988 and May 1989, respectively. Root samples (5 g) were rinsed under running tap water, sonicated in 20 ml of sterile distilled water, and comminuted in a sterile mortar with 10 ml of sterile distilled water as previously described for lettuce roots (11,13). The cell suspensions obtained from the root surface by sonication and from comminuted roots were filtered through a 0.65-μm sterile filter, and 0.04 ml of filtered suspension (undiluted and 10-fold diluted) was spread onto plates of S-medium amended with streptomycin sulfate (11,13). The plates were incubated at 28 C for 10 days. Slow-growing colonies similar to those of strain CA1 were tested for affinity to polyclonal antibodies as described above. Strains that reacted positively were tested for pathogenicity on Salinas lettuce plants and on the plant species from which the strains were isolated (tomato cv. Earlypak 7 or cantaloupe cv. PMR 45 and sowthistle obtained from a field in Yolo County, California). Five milliliters of approximately 10^8 cfu/ml were dispensed at the stem base of two to four seedlings of each plant species. Noninoculated plants and plants inoculated with strain CA1 served as negative and positive controls. One month after inoculation, the roots were inspected for symptoms and scored on the 0–9 scale. Reisolations of *R. suberifaciens* were made as described above.

Table 1. Plant species included in pathogenicity tests with *Rhizomonas suberifaciens*, the causal agent of corky root of lettuce

Family Genus and species	Common name	Cultivar	Source[a]
Chenopodiaceae			
Beta vulgaris L.	Red beet	Ruby Queen	NK
	Swiss chard	Fordhook Giant	NK
Spinacia oleracea L.	Spinach	Bloomsdale Longstanding	NK
Compositae			
Lactuca sativa L.	Lettuce	Salinas	HM
L. serriola L.	Prickly lettuce	···	Field
Sonchus oleraceus L.	Common sowthistle	···	Field
S. asper (L.) Hill	Prickly sowthistle	···	Field
Cichorium endivia L.	Endive	Green Curled	NK
Carthamus tinctorius L.	Safflower	Nebraska 10	AGR
Helianthus annuus L.	Sunflower	Giant Greystripe	NK
Cruciferae			
Brassica oleracea L.	Broccoli	Green Goliath	BP
	Cabbage	Red Acre	NK
	Cauliflower	Snowbally Y Improved	NK
B. napus L.	Rapeseed	BO482	JS
Cucurbitaceae			
Cucumis melo L.	Melon	Topmark	HM
		PMR 45	HM
Gramineae			
Triticum durum Desf.	Wheat	UC '87	AGR
Sorghum sudanense L.	Sudan grass	Piper	Unknown
Secale cereale L.	Rye	Merced	CCIA
Lolium perenne L.	Ryegrass	···	Field
Avena sativa L.	Oats	Sierra	CCIA
Hordeum vulgare L.	Barley	Kombar	NK
Leguminosae			
Vicia faba L.	Broad bean	Bell Bean	SM
Glycine max (L.) Merr.	Soybean	Pershing	USDA
		Williams	USDA
		Douglas	USDA
Lupinus luteus L.	Lupine	Texas Bluebonnet	NK
Cicer arietinum L.	Chickpea	Suratato	CCIA
Solanaceae			
Lycopersicon esculentum Mill.	Tomato	Earlypak 7	PS
Umbelliferae			
Daucus carota L.	Carrot	Chantenay Red Cored	NK
		Sierra Hybrid	NK

[a]NK = Northrup King Seed Company; HM = Harris Moran Seed Company; Field = Yolo County, California; AGR = Agronomy, University of California, Davis; BP = Burpee Seed Company; JS = Johnny's Seed Company; CCIA = California Crop Improvement Association; SM = Sacramento Milling; USDA = USDA Northern Soybean Germplasm Collection, Urbana, Illinois; PS = Petoseed Company.

RESULTS

Pathogenicity tests with strain CA1 from lettuce. In the first series of experiments with autoclaved soil mix

infested with strain CA1, severe symptoms were observed on all inoculated lettuce plants. From 40 to 60% of the surface of most taproots was dark brown with deep longitudinal cracks (score 6 or 7). Some taproots were girdled and the plants were wilted (score 8). *R. suberifaciens* was reisolated from 80% of the plants tested for the presence of the bacterium. Of the noninoculated lettuce plants, 60% became contaminated and had mostly small superficial yellow lesions on the taproot (score 1 or 2) or sometimes larger yellow lesions (up to 15% of the taproot) and small superficial cracks (score 3). The pathogen was not isolated from nonsymptomatic plants. None of the other crops (barley, broad bean, broccoli, lupine, oat, rapeseed, rye, ryegrass, safflower, soybean, Sudan grass, and wheat) had symptoms typical for CR. Inoculated broad bean and soybean plants had some dark brown to black lesions on the taproot and lateral roots, but the noninoculated plants had similar symptoms. The pathogen was isolated once from a broad bean root and once from a root of the soybean cultivar Williams, both with atypical lesions. In one experiment, a single safflower plant in infested soil had minor black streaks on the taproot, and the bacterium was recovered from that plant.

In the second series of experiments in which seedlings grown in vermiculite were inoculated with strain CA1, symptoms typical of CR were observed on endive, lettuce, prickly lettuce, and sowthistle. Lettuce and endive were severely and moderately diseased and had scores ranging from 5 to 9 and from 1 to 6, respectively. Prickly lettuce and sowthistle developed symptoms similar to those on resistant lettuce cultivars (2), namely, yellow lesions and superficial cracks on the taproot. The scores for prickly lettuce and sowthistle ranged from 1 to 3 and from 0 to 4, respectively. In chi-square tests, lettuce was most susceptible, followed by endive, then sowthistle, and then prickly lettuce. None of the other plant species (beet, broad bean, cabbage, carrot, cauliflower, chickpea, melon, safflower, soybean, spinach, Sudan grass, sunflower, Swiss chard, and tomato) developed any symptoms, except for a hybrid carrot cultivar. The latter developed a slight brown discoloration of the feeder roots in the first and third experiments and an orange-brown discoloration and proliferation of the feeder roots in the second experiment. Noninoculated plants remained healthy in the first two experiments. In the third experiment, about one-half of the noninoculated lettuce and sowthistle plants had some yellow lesions on the taproot (score 1). In the first experiment, the pathogen was not recovered from diseased roots, probably because the tissue extracts were not filtered and the pathogen was overgrown by saprophytes. In the second experiment, the pathogen was reisolated from filtered root extracts of both lettuce plants and one endive plant of the two symptomatic plants tested but not from that of a sowthistle plant with CR symptoms. In the third experiment, *R. suberifaciens* was recovered from all three lettuce plants, from two of the three endive plants, and from one of the three prickly lettuce and sowthistle plants from which isolations were made. The bacterium was not isolated from any of the plants without symptoms or from the hybrid carrot with the atypical symptoms on the feeder roots.

Isolations from naturally infected roots. Two strains of *R. suberifaciens* were isolated from the surface of tomato roots with CR obtained from the Salinas Valley. These strains reacted positively with the polyclonal antibodies, caused symptoms on lettuce (score 3–9), and were reisolated from infected lettuce plants. However, none of the strains from tomato caused characteristic corky root symptoms when inoculated onto tomato seedlings. One strain from diseased melon (crenshaw) roots induced moderately severe (score 2–5) CR symptoms on lettuce but no symptoms on melon (cantaloupe cv. PMR 45). Another strain from melon (crenshaw) caused only slight symptoms on lettuce (score 0–2) and a brown discoloration in distinct bands on a secondary root of melon (cantaloupe cv. PMR 45). However, *R. suberifaciens* was not reisolated from this plant.

Two strains isolated from sowthistle roots with CR symptoms were positive in polyclonal antibody tests. One was pathogenic on lettuce (score 1–6), and the other was not. One strain from prickly sowthistle with CR was serologically related to strain CA1, caused severe corky root on both lettuce (score 7 on each of four plants) and sowthistle (score 6 on each of four plants), and was reisolated from both plant species.

DISCUSSION

Of the plant species tested to date, only members of the Compositae family closely related to lettuce were susceptible to CR. These belong to the subfamily Liguliflorae and the tribe Cichorieae. Two members of the Compositae outside this tribe, safflower and sunflower, did not develop symptoms typical of CR. Moreover, the disease has not been observed in commercial fields of safflower or sunflower in the Sacramento Valley, California (van Bruggen, *unpublished*). Two members of the Cichorieae, cultivated lettuce and endive, developed more severe symptoms than the wild species, prickly lettuce, sowthistle, and prickly sowthistle. However, the initial symptoms, yellow bands on the taproot and main lateral roots followed by corky ridges, were the same for all plants.

The atypical symptoms that developed on an inoculated hybrid carrot were reminiscent of those associated with ammonia injury (9) and might have been induced in response to ammonia produced by *R. suberifaciens* from nitrate (10,14).

Strains of *R. suberifaciens* isolated from sowthistle, prickly sowthistle, melon, and tomato induced CR on lettuce and sowthistle. Neither the strains from melon or tomato nor strain CA1 from lettuce induced CR symptoms on melon or tomato. However, the melon and tomato cultivars used for pathogenicity tests were not the same as the cultivars from which the melon and tomato strains of *R. suberifaciens* were obtained. Thus, we can only conclude that tomato cv. Earlypak 7 and cantaloupe cv. PMR 45 were not susceptible to strain CA1 from lettuce and two additional strains obtained from field-grown tomato and melon. In a separate experiment, conducted in cooperation with Nina Shiskoff, tomato cv. VF6203 inoculated with *P. lycopersici* developed symptoms typical of CR, while the same cultivar inoculated with strains of *R. suberifaciens* isolated from the surface of corky tomato roots remained healthy (van Bruggen, *unpublished*). Thus, at present, *P. lycopersici* is the only known pathogen that causes CR on tomato and melon.

R. suberifaciens was isolated from the surface of roots of broad bean, safflower, and soybean inoculated with strain CA1; from field-grown melon and tomato; and from field-grown broad bean and rye in earlier studies (van Bruggen, *unpublished*). Moreover, CR was observed on lettuce grown in soil recently brought into production after pasture or forest (7,12). Thus, *R. suberifaciens* may be a common rhizosphere organism, and crop rotation may not be as effective a control measure as was hoped (12). Research is currently under way to determine the relative growth rates of *R. suberifaciens* on various susceptible and nonsusceptible plants of both cultivated and wild species to help clarify the potential effects of crop rotation on corky root of lettuce.

ACKNOWLEDGMENTS

We thank Nina Shiskoff and Amy Amann for supplying tomato and melon roots, respectively, with corky root symptoms. This research was partially funded by the California Iceberg Lettuce Research Program and the University of California Statewide IPM Project.

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