Crown and Leaf Rot of Statice Incited by a Bacterium Resembling *Pseudomonas caryophylli*

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ABSTRACT

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A crown rot of statice (*Limonium sinuatum*) plants was observed in Manatee County, FL. A nonfluorescent, aerobic, gram-negative bacterium was isolated. Upon artificial inoculation, symptoms similar to those in the field were reproduced. On the basis of biochemical and physiological tests, the bacterium was identified as *Pseudomonas caryophylli*

A rot affecting the crown and leaves of statice (*Limonium* spp.) plants has been observed for several years in field plantings in Florida, with losses estimated as high as 10% in a planting. Some of the symptoms resemble those of Colletotrichum crown rot (4,5). In autumn 1982, a bacterium was isolated that, when inoculated into statice plants, induced symptoms similar to those found on naturally infected plants in the field. We identified the causal agent as *Pseudomonas caryophylli* on the basis of biochemical and physiological characteristics of the pathogen. This is believed to be the first report of a bacterial disease of statice.

MATERIALS AND METHODS

Isolation and identification of the pathogen. Crowns from wilted statice plants were cut longitudinally, revealing a dark discolored pith. A sample at the interface of healthy and diseased tissue was triturated in sterile, distilled water. Symptomatic leaf tissue showing red veins was treated similarly. A loopful of the suspension was streaked on plates of nutrient yeast-dextrose agar (NYDA) (9) and the plates were kept at 28 C for 48 hr.

The following tests were used to characterize the bacterial isolates: Gram stain (15), production of cytochrome oxidase (9,12), accumulation of poly-β-hydroxybutyrate (15), production of phenylalanine deaminase (18), fluorescent pigmentation (10), ability to hydrolyze starch and liquefy gelatin (15), tolerance to NaCl (9), protease activity (16), levan production (13), inhibition by 0.1% triphenyltetrazolium chloride (TTC) (9), growth at 41 and 37 C on slants of NYDA, arginine dihydrolase activity (19), hypersensitive reaction of tobacco and pepper (11), nitrate reduction (14), lipolysis of Tween 80 (17), oxygen requirement (7), flagella stain (6), and morphological characteristics (3).

Nutritional tests were conducted as described by Misaghi and Grogan (14), except Noble agar was used in place of oxoid agar. All compounds were filter-sterilized and added to make a final concentration of 0.2% (w/v). A suspension of bacterial cells grown on NYDA for 48 hr was adjusted to 10^8 cells per milliliter. A loopful of each strain was streaked on duplicate plates of each medium. Plates were incubated at 25 C for 7 days and evaluated for extensive growth. Slight growth was rated negative.

Cultures. Nonfluorescent bacteria for comparison with the statice strains were obtained as follows: *P. marginata* strains F1 and W3 and *P. cepacia* strains ATCC 17759 and ATCC 25416 from M. Sasser, Department of Plant Science, University of Delaware, Newark; *P. corrugata* strain PSU 388 from F. L. Lukezic, Department of Plant Pathology, Pennsylvania State University, University Park; *P. avenae* strains PA78-5, PA117, and VS-1 from R. D. Gitaitis, Department of Plant Pathology, Coastal Plain Experiment Station, University of Georgia, Tifton; and *P. caryophylli* strains 1251 and 1255 from R. S. Dickey, Department of Plant Pathology, Cornell University, Ithaca, NY.

Pathogenicity tests. Four-week-old plants of *Limonium sinuatum* (L.) Mill. 'Lavender Queen' were transplanted in 10-cm pots and grown in a soilless medium. About 2–3 wk after transplanting, the plants were inoculated by inserting in the crown an insect needle laden with bacteria. The plants were then placed in a greenhouse at 25–32 C and subjected to alternating mist (10 sec every 20 min) from 0700 to 1900 hours daily. In a second experiment, plants were inoculated by infiltrating the crown with bacterial suspension at 10^8 colony-forming units

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per milliliter. The plants were enclosed in transparent polyethylene bags for 7 days at 28 C, then unbagged and replaced on the greenhouse bench. The experiment was replicated four times, with each replicate consisting of one plant per 10-cm pot.

RESULTS AND DISCUSSION

In naturally infected plants, chlorotic and necrotic leaves are intermingled with green leaves in the rosette. Decay of fibrous roots occurs before crown rot, but the crown and plant eventually die. On mature plants, chlorotic and necrotic leaves are concentrated on one side of the rosette. At the bases of individual leaves, a black decay occurs that gradually advances up the petiole. Affected leaves may have unilateral chlorosis and necrosis or the entire leaf may become necrotic. The midvein and lateral veins may have a pronounced red color in localized portions of either green or chlorotic areas of leaves. Cross sections of the crown showed a black, decayed area corresponding closely to the insertions of diseased leaves. The black decay spreads across the crown. Black streaks occur in the white crown tissue as the decay advances. A red color may be diffused through the crown tissue. Affected crowns may give off a putrid odor during advanced stages of the disease. Some of the symptoms are similar to those of Colletotrichum crown rot (4,5).

A yellow-brownish nonfluorescent bacterium that becomes dark brown on NYDA was isolated from the crown at the interface of healthy and discolored tissues. In artificially inoculated plants, the bacterium was isolated readily from the red veins but not from the crown. The bacterium was a gram-negative rod, 0.8 × 1.5–3.1 μm. It was a strict aerobe with one or more polar flagella. Results of biochemical and physiological tests are presented in Tables 1 and 2.

All symptoms described were produced by the two strains by both inoculation methods. Early symptoms on young plants included water-soaking at the leaf bases. As disease progressed, chlorosis and red coloration of the veins were prominent (Fig. 1A). In many instances, entire inoculated plants placed in

Table 1. Comparison of statice strains and other nonfluorescent pseudomonads in physiological and biochemical tests

Test	Number of strains positive in test[a]					
	Statice strains	*Pseudomonas avenae*	*P. caryophylli*	*P. marginata*	*P. cepacia*	*P. corrugata*
Hypersensitive reaction of tobacco and pepper	2	3	2	2	1	2
Oxidase	2	3	2	2	2	2
Starch	0	0	0	0	0	0
Phenylalanine deaminase	0	0	0	0	0	0
Gelatin liquefaction	0	0	0	2	1	2
Lipase	2	3	0	2	2	0
Proteases	2	3	0	2	2	2
Levan	2	0	2	0	0	0
Nitrate reduction	0	0	0	0	0	2
Agrinine dihydrolase	0	0	0	0	0	2
Accumulation of poly-β-hydroxybutyrate	2	2	2	2	2	2
Growth at 41 or 37 C	41	41	41	37	41	37

[a] Two strains of each species were tested, except three for *P. avenae*.

Table 2. Comparison of the statice strains and other nonfluorescent pseudomonads in nutritional tests

Compound	Number of strains that utilized nutrient[a]					
	Statice strains	*Pseudomonas avenae*	*P. caryophylli*	*P. marginata*	*P. cepacia*	*P. corrugata*
Betaine	2	0	2	2	2	2
Citroconate	0	0	0	2	2	0
Asparagine	2	3	2	2	2	2
Sucrose	2	0	2	2	2	2
Mesaconic acid	0	0	0	2	0	0
Levulinate	0	0	0	1	2	0
D-Ribose	2	3	2	2	2	2
Sorbitol	2	2	2	2	2	1
Arginine	2	0	2	2	2	2
Adonitol	0	0	1	2	2	0
Valine	0	0	0	2	2	0
Saccharate	0	2	2	1	2	2
D(−)-Tartrate	0	2	0	2	0	0
Glucose	2	3	2	2	2	2
Cellobiose	2	0	2	2	2	1
Meso-Tartrate	0	0	0	2	2	1
Mannitol	2	2	2	2	2	2
Trehalose	2	0	2	2	2	2
Arabinose	2	3	2	2	2	2
Tryptamine	0	0	0	0	2	0
DL-Threonine	0	0	0	2	2	0
Gluconic Acid	2	3	2	2	2	2
L-Threonine	0	3	2	2	2	1
L-Rhamnose	2	0	2	0	0	1
Serine	0	3	0	2	2	0
Arabitol	2	0	1	2	2	0

[a] Two strains per species were tested, except three for *P. avenae*.

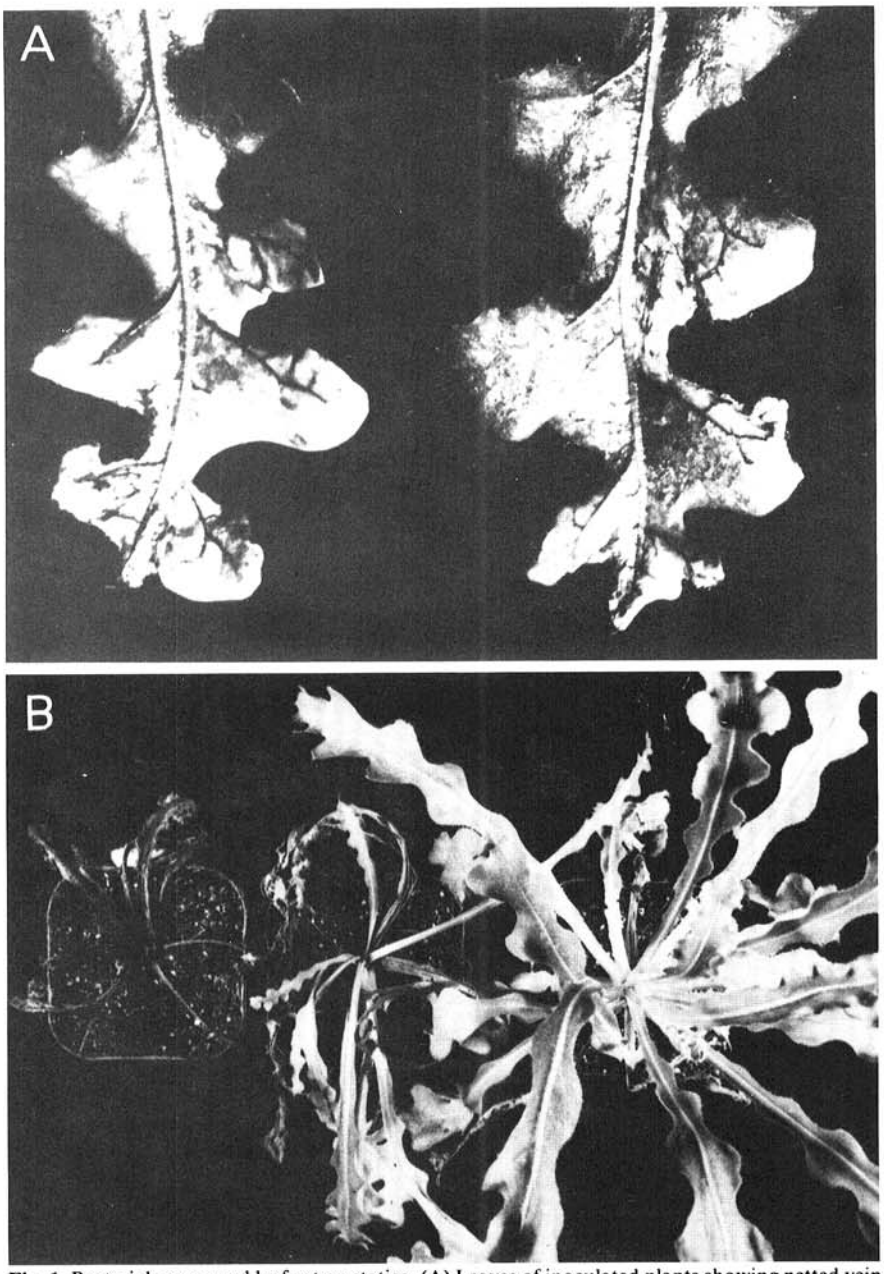

Fig. 1. Bacterial crown and leaf rot on statice. **(A)** Leaves of inoculated plants showing netted vein appearance. **(B)** Uninoculated control on left and two stages of inoculated plants in the middle and right.

intermittent mist died and easily broke off at the ground line (Fig. 1B).

On the basis of biochemical and physiological tests, the statice bacterium is *P. caryophylli*, a wilt pathogen (8) first identified in 1942 (2). The statice strains, like the other nonfluorescent pseudomonads, had a wide carbon utilization range. The percentage of similarity (%S) was determined using Misaghi and Grogan's technique (14). The %S of the statice strains with *P. caryophylli* was 90%; with *P. corrugata* it was 75%, with *P. avenae* and *P. marginata* it was 72%, and with *P. cepacia* it was 69%.

Previous literature (1) listed *P. caryophylli* as producing arginine dihydrolase, but the ATCC strains of *P. caryophylli* were negative in this test. In the past, this may have created some confusion as to the correct placement of bacterial strains that were not identified as *P. caryophylli*, because the ability to produce this enzyme is a characteristic for separation of several species of nonfluorescent pseudomonads.

Results of pathogenicity tests corroborate that the bacterium induces an extensive rot on the crown and leaf. The disease appears to be most prevalent in autumn, when plants are grown under a high moisture regime (as a result of the high precipitation) and high temperature.

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