Bacterial Leaf Spot of Gloxinia

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ABSTRACT

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A nonfluorescent, aerobic, gram-negative bacterium was isolated from necrotic spots in leaves of gloxinia (*Sinningia speciosa*) seedlings. The organism induced a hypersensitive reaction in pepper and tobacco. Upon artificial inoculation, lesions that turned brown (similar to those in the field) were reproduced. On the basis of biochemical and physiological tests, the bacterium was identified as a *Pseudomonas* sp.

A leaf spot of gloxinia characterized by an interveinal brownish necrosis has been observed for several years on seedlings of gloxinia (*Sinningia speciosa* (Lodd.) Hiern.) in Florida. Spotted plants are unmarketable. In the summer of 1982, a bacterium was isolated consistently. Upon artificial inoculation, it induced symptoms similar to those on naturally infected plants. This is apparently the first report of a bacterial disease of gloxinia.

MATERIALS AND METHODS

Isolation and identification of the pathogen. Leaf sections from affected gloxinia plants were cut at the interface of healthy and diseased tissue. The tissue was triturated in sterile, distilled water and loopfuls of the suspension were streaked on petri plates of nutrient yeast-dextrose agar (NYDA) (5). Plates were incubated at 28 C for 48 hr.

The following tests were used to characterize the unknown and known strains of *Pseudomonas avenae, P. caryophylli, P. marginata, P. cepacia,* and *P. corrugata*: Gram stain (11); accumulation of poly-β-hydroxybutyrate (11); production of phenylalanine deaminase (13), fluorescent pigments (6), levan (9), and cytochrome oxidase (5,8); ability to hydrolyze starch and liquefy gelatin (11); tolerance to NaCl (5); protease activity; inhibition by 0.1% triphenyltetrazolium chloride (TTC) (11); arginine dihydrolase activity (15); hypersensitive reaction of tobacco and pepper (7); nitrate reduction (10); lipolysis of Tween 80 (12); oxygen requirement (2,4); flagella stain (3); morphological characteristics (2); and growth at 41 and 37 C on slants of NYDA.

Nutritional tests were conducted as described by Misaghi and Grogan (10), except Noble agar was used in place of oxoid agar. All compounds were filter-sterilized (0.22-μm pore size) 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 petri plates of each medium. Plates were incubated at 25 C for 7 days and evaluated for extensive growth. Slight growth was considered negative.

Pathogenicity tests. Seedlings of gloxinia Improved Red Velvet, about 3–4 wk after transplanting into 2-in. square pots, were inoculated with a bacterial suspension (10^8 colony-forming units per milliliter). The suspension or distilled water (control) was applied with a cotton swab to leaves previously dusted with Carborundum. Inoculated plants were placed under alternating mist (10 sec every 20 min) from 0700 to 1900 hours daily for the duration of the experiment or placed in polyethylene bags for 48 hr, then held on the greenhouse bench. The experiment was replicated four times, each replicate consisting of one plant. The experiment was repeated three times.

RESULTS AND DISCUSSION

The disease occurred during the summer when temperature and humidity were high and appeared to be a problem only in seedling flats watered from overhead. Once the seedlings were transplanted to individual pots, the disease ceased to develop. The spots on naturally infected plants were observed to be interveinal, ranging from small (2–3-mm) brownish areas to larger areas that encompassed much of the individual leaf panels. The spots did not appear to spread once the seedlings were transplanted to pots. Stems were unaffected.

The bacterium induced necrotic blotches on artificially inoculated leaves (Fig. 1). These lesions, similar to naturally occurring ones, were distinctly water-soaked and dark brown. Two of the strains induced extensive necrosis, whereas only slight necrosis resulted from a third strain. Control plants inoculated with deionized water showed only slight browning as a result of rubbing injury.

A whitish, nonfluorescent bacterium was isolated on NYDA. The bacterium was a gram-negative rod 0.8 × 1.5–3.1 μm. It was a strict aerobe and had a single polar flagellum. In physiological and biochemical tests (Tables 1 and 2), strains of the pathogen were unlike *P. avenae, P. corrugata, P. caryophylli, P. cepacia,* and *P. marginata.*

All gloxinia strains utilized asparagine, saccharate, gluconate, and serine. The gloxinia strains *P. alcaligenes* Monias (L.) and *P. lemoignei* Delafield,

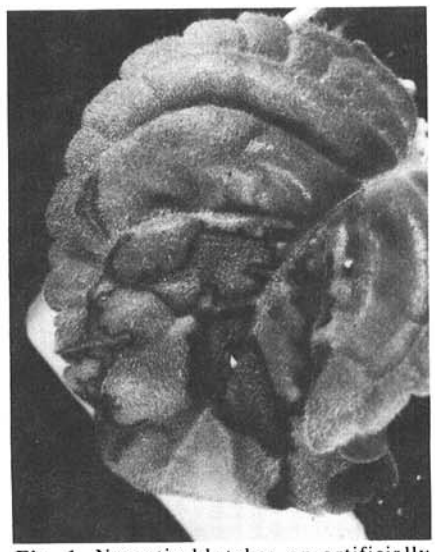

Fig. 1. Necrotic blotches on artificially inoculated gloxinia leaf induced by a *Pseudomonas* sp.

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Table 1. Comparison of the gloxinia strains and selected nonfluorescent pseudomonads in physiological and biochemical tests

Tests	Gloxinia strains (3)[a]	*Pseudomonas alcaligenes*[b] (1)	*P. lemoignei*[b] (1)	*P. avenae* (3)	*P. caryophylli* (2)	*P. marginata* (2)	*P. cepacia* (2)	*P. corrugata* (2)
Hypersensitivity of tobacco, pepper	3[c]	NR	NR	3	2	2	1	2
Oxidase	3	1	1	3	2	2	2	2
Starch	0	0	0	0	0	0	0	0
Phenylalanine deaminase	0	NR	NR	0	0	0	0	0
Gelatin liquefaction	0	?	0	0	0	2	1	2
Lipase	3	NR	NR	3	0	2	2	0
Proteases	3	NR	NR	3	0	2	2	2
Levan	0	0	NR	0	2	0	0	0
Nitrate reduction	0	0	0	0	0	0	0	2
Arginine dihydrolase	3	1	0	0	0	0	0	2
Accumulation of poly-β-hydroxybutyrate	3	0	1	2	2	2	2	2
Growth at 41 or 37 C	37	41	41	41	41	37	41	37

[a] Number of strains.
[b] Results for *P. alcaligenes* and *P. lemoignei* are from Stanier et al (14).
[c] Number of strains positive for a particular test. NR = not reported, ? = questionable results. *P. avenae* provided by R. D. Gitaitis, *P. caryophylli* provided by R. S. Dickey, *P. marginata* and *P. cepacia* provided by M. K. Sasser, and one strain of *P. corrugata* provided by F. L. Lukezic.

Table 2. Comparison of the gloxinia strains and selected nonfluorescent pseudomonads in nutritional tests[a]

Test	Gloxinia strains (3)[a]	*Pseudomonas alcaligenes*[b] (1)	*P. lemoignei*[b] (1)	*P. avenae* (3)	*P. caryophylli* (2)	*P. marginata* (2)	*P. cepacia* (2)	*P. corrugata* (2)
Betaine	0[c]	0	0	0	2	2	2	2
Citroconate	0	0	0	0	0	2	2	0
Asparagine	3	NR	NR	3	2	2	2	2
Sucrose	0	0	0	0	2	2	2	2
Mesaconid acid	0	0	0	0	0	2	0	0
Levulinate	0	0	0	0	0	1	2	0
D-Ribose	0	0	0	3	2	2	2	2
Sorbitol	0	0	0	2	2	2	2	1
Arginine	0	1	0	0	2	2	2	2
Adonitol	0	0	0	0	1	2	2	0
Valine	1	0	0	0	0	2	2	0
Saccharate	3	0	0	2	2	1	2	2
D(−)-tartrate	2	0	0	2	0	2	0	0
Glucose	0	0	0	3	2	2	2	2
Cellobiose	0	0	0	0	2	2	2	1
Mesotartrate	0	NR	NR	0	0	2	2	1
Mannitol	0	0	0	2	2	2	2	2
Trehalose	0	0	0	0	2	2	2	2
Arabinose	0	0	0	3	2	2	2	2
Tryptamine	0	0	0	0	0	0	2	0
DL-Threonine	0	NR	NR	0	0	2	2	0
Gluconate	3	0	0	3	2	2	2	2
L-Threonine	0	0	0	3	2	2	2	1
L-Rhamnose	0	0	0	0	2	0	0	1
Serine	3	0	0	3	0	2	2	0
Arabitol	0	NR	NR	0	1	2	2	0

[a] Number of strains.
[b] Results for *P. alcaligenes* and *P. lemoignei* are from Stanier et al (14).
[c] Number of strains positive for a particular test. NR = not reported.

Doudoroff, Palleroni, Lusty, & Contopoulou have a very limited nutritional utilization. Although the gloxinia strains do not utilize arginine as the sole carbon source, they do synthesize arginine dihydrolase. This may not be unique to this bacterium but it is certainly uncommon. Because of its ability to produce arginine dihydrolase, this bacterium does not fit in with *P. lemoignei* of section III of *Pseudomonas* (1). Because the bacteria did not utilize betaine and arginine and did not require growth factors (characteristics of either section II or III of *Pseudomonas*), the strains were placed in section I of *Pseudomonas* in Bergey's Manual (1). The bacterium did not reduce nitrate. Unlike *P. alcaligenes*, the gloxinia strains utilized serine but not arginine. Although several of the characteristics of the bacterium are not in line with *P. alcaligenes* (1), the bacterium is most closely related to that organism.

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