Leaf Spot and Blight of *Asplenium nidus* Caused by *Pseudomonas gladioli*

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ABSTRACT

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A leaf spot and blight of *Asplenium nidus* (bird's nest fern) caused extensive losses in many Florida nurseries. A nonfluorescent pseudomonad was isolated consistently from affected ferns. Comparisons of this organism with *Pseudomonas asplenii*, a fluorescent pseudomonad causing similar symptoms on the same host, indicated differences in the host ranges of the two organisms but not in virulence on bird's nest fern. *P. asplenii* was highly virulent on the original host only. It caused slight symptoms on five of 10 other ferns tested. The nonfluorescent pseudomonad was highly virulent on four of the 11 ferns tested and avirulent on only two varieties of ferns. On the basis of biochemical and nutritional tests, the nonfluorescent pseudomonad was identified as *P. gladioli*, a pathogen of gladiolus. *P. gladioli* was pathogenic on bird's nest fern and the fern pathogen was pathogenic on gladiolus.

Additional key words: *Adiantum, Davallia,* foliage plants, *Pelleae, Platycerium, Pteris*

Bacterial blight of *Asplenium nidus* L. (bird's nest fern) was described in 1946 (1) and the causal organism was named *Phytomonas asplenii* Ark & Tomp. (*Pseudomonas asplenii* (Ark & Tomp.) Savulescu). Until recently, this was the only reported bacterial disease of this fern or any other small fern grown for the foliage market in Florida.

During the winter of 1982–1983, a serious leaf spot and blight of bird's nest fern caused severe losses to the crop in many central Florida nurseries. Symptoms included small (1-mm-diameter) tan to reddish lesions surrounded by a dark brown or purplish margin (Fig. 1). These lesions expanded rapidly along leaf veins, and under moist conditions, resulted in leaf and plant death. Control measures attempted by growers were unsuccessful in eliminating the disease, even under dry conditions. Similarities between these symptoms and those reported by Ark and Tompkins (1) seemed to indicate that this disease was caused by *P. asplenii*. The purpose of this research was to identify the causal agent of this disease and compare it with *P. asplenii* on bird's nest fern and 10 other small ferns produced for the foliage market.

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MATERIALS AND METHODS

Symptomatic tissue from bird's nest ferns was surface-disinfested in 0.52% NaOCl for 3 min, rinsed in sterilized deionized water (SDW), and crushed in a scintered glass tissue grinder. The resulting suspension was streaked onto Difco nutrient agar (NA). Plates were incubated at about 26 C for 2 days and individual colonies of the suspect pathogen were transferred to additional NA plates three successive times.

Inocula were produced from cultures on NA at 26 C for 2 days. A suspension was made in SDW and adjusted to about 1×10^8 colony-forming units (cfu) per milliliter with a spectrophotometer (50% transmission at 600 nm). Inoculations were performed by spraying test plants with the suspension to runoff and enclosing plants in a polyethylene bag for 3 days. Plants were removed from the bags and placed randomly on a greenhouse bench and watered from overhead twice daily. Greenhouse temperatures ranged from 20 to 32 C and maximum light level was 200 μE m^{-2} sec^{-1}. Plants were incubated for at least 2 wk before the number of lesions per plant was recorded and reisolation of the suspect pathogen was attempted. All plants were obtained from commercial growers and potted in either 5- or 10-cm-diameter plastic pots.

Pathogenicity of the suspect pathogen on *Asplenium nidus*. Eight cultures of the suspect pathogen were tested for pathogenicity on bird's nest fern plants. Each treatment, including water-inoculated controls, consisted of three plants. This test was performed three times.

Comparison of the host ranges of the

suspect pathogen and *P. asplenii*. *P. asplenii* strains ATCC 10855 and ATCC 23835 were obtained from the American Type Culture Collection. The methods described were used to produce inocula of *P. asplenii* as well as the suspect pathogen. Treatments consisted of two *P. asplenii* cultures, two cultures of the suspect pathogen, and a water-inoculated control. Three of each of the following ferns were used in each treatment: *Adiantum* L. (maidenhair fern), bird's nest fern, *Cyrtomium falcatum* (L. f.) k. Presl. (holly fern), *Davallia fejeensis* Hook (rabbit's foot fern), *Nephrolepis exaltata* L. Schott (Compacta Boston fern and Fluffy Ruffle Boston fern), *Pelleae rotundifolia* (G. Forst) Hook (button fern), *Platycerium bifurcatum* (Cav.) C. Chr. (common staghorn fern), *Pteris cretica* L. 'Albo-lineata' (variegated table fern), *P. cretica* 'Wilsonii' (crested table fern), and *P. ensiformis* Burm. f. 'Victoriae' (silver table fern). Plants were inoculated as described and reisolation of bacteria was attempted using both NA and King's medium B (KMB) (6). This test was performed three times.

Pathogenicity of the suspect pathogen to gladiolus. The pathogenicity of four cultures of the suspect pathogen and three cultures of *P. gladioli* Severini was tested on both bird's nest fern and *Gladiolus* × *hortulanus* L. H. Bailey. Three plants of each were each inoculated with a culture of the suspect bacterium, *P. gladioli*, or SDW using the methods described, except the plants were wounded before inoculation with a sterilized dissecting needle. Gladioli were wounded both on the leaves (five wounds) and the corms (one wound). Bird's nest ferns were wounded five times on each of three leaves per plant. This test was repeated once without wounding.

Characterization of the suspect pathogen. The following tests were employed to characterize the suspect pathogen: arginine dihydrolase (12); oxidase reaction (8); oxygen requirement using Hugh-Leifson's medium (4); fluorescin production using KMB (6); Gram reaction (10); poly-β-hydroxybutyrate storage (10); potato and onion rot; hydrogen sulfide production (2); casein hydrolysis (11); gelatin hydrolysis (2); starch hydrolysis (10); levan production on NA amended with 5% sucrose (10); growth at 41 C in nutrient broth (10); nitrate reduction (9); and hypersensitive reaction on *Capsicum annuum* L. (Early Calwonder pepper), *Lycopersicon lycopersicum* (L.) Karst. ex Fariv. (Bonny Best tomato), and *Nicotiana tabacum* L. (Hick's tobacco) (7). Each test was performed four times using duplicate tubes or plates and six of the eight isolates of the suspect pathogen. Nutritional studies were performed twice as described by Misaghi and Grogan (9), except Noble agar was used in place of Oxoid agar. All carbon sources were filter-sterilized and added to give a final concentration of 0.2% (w/v). A suspension of bacterial cells grown on nutrient yeast dextrose agar (5) was adjusted to 1×10^8 cfu/ml and a loopful was streaked on duplicate plates of each medium. Plates were incubated for 7 days at 25 C and evaluated for growth; slight growth was considered negative. The following bacteria were included as controls: *P. caryophylli* (Burk.) Starr & Burk., *P. cepacia* Burk., and *P. gladioli*. All three were used in nutritional trials, but only *P. gladioli* was used in the biochemical trials.

Fig. 1. Naturally occurring Pseudomonas leaf spot of *Asplenium nidus* (bird's nest fern) caused by *P. gladioli*. Symptoms caused by *P. asplenii* are identical. (Courtesy V. Jane Windsor)

Table 1. Susceptibility of some small ferns to *Pseudomonas asplenii* and the bird's nest fern bacterium

Fern species tested	*P. asplenii*	Bird's nest fern cultures
Adiantum sp.	Slight[a]	Slight
Asplenium nidus	High	High
Cyrtomium falcatum	Slight	Slight
Davallia fejeensis	None	Moderate
Nephrolepis exaltata 'Fluffy Ruffle'	None	None
Nephrolepsis exaltata 'Compacta'	None	None
Pelleae rotundifolia	None	Slight
Platycerium bifurcatum	None	High
Pteris cretica Variegated table	Slight	Moderate
Pteris cretica Crested table	Slight	High
Pteris ensiformis	Slight	High

[a] Degree of susceptibility to the bacterium: none = no symptoms developed in any plants, slight = 1–5 lesions developed on at least 10% of the plants tested, moderate = 5–30 lesions developed on at least 50% of the surface of 50% of the plants, and severe = all plants affected in all tests showing at least 50% of their surface damaged.

RESULTS

Pathogenicity of the suspect pathogen to *Asplenium nidus*. A gram-negative, rod-shaped bacterium was isolated consistently from infected bird's nest fern. Pathogenicity trials on the host were positive for all eight cultures and the causal organism was readily reisolated from these plants. Symptoms were indistinguishable from those noted on the naturally infected bird's nest fern. Water-inoculated control plants remained free of symptoms during the trials and no pathogenic organisms were isolated from them.

Comparison of host ranges of the suspect pathogen and *P. asplenii*. Each of the four cultures tested was pathogenic to bird's nest fern. Symptoms caused by *P. asplenii* were identical to those caused by the suspect pathogen on commonly susceptible plants and the same as those originally described and photographed by Ark and Tompkins (1). Both pathogens were successfully reisolated from lesions. The suspect pathogen had a wider host range than *P. asplenii* and caused more severe symptoms on inoculated plants (Table 1). After inoculation with the suspect pathogen, silver table fern developed reddish lesions 1–3 mm wide scattered across the fronds, whereas staghorn fern had black sunken lesions 3–15 mm wide (Fig. 2). Both Boston fern cultivars were highly resistant to the suspect pathogen in these tests and developed no lesions. Symptoms on the remaining ferns were lesser in degree and were pinpoint to 2 mm wide and generally tan. The suspect pathogen was reisolated from each of the ferns with symptoms but not from any control plants or ferns without symptoms.

P. asplenii caused symptoms only on rabbit's foot fern, silver, variegated, and crested table ferns, and maidenhair fern (Table 1). Symptoms on these plants consisted of 1-mm necrotic areas on frond edges and tips and generally failed to continue enlarging, even under high moisture conditions. *P. asplenii* was

Fig. 2. Pseudomonas leaf spot of *Platycerium bifurcatum* (staghorn fern) resulting from artificial inoculation with *Pseudomonas gladioli*.

Table 2. Comparison of bird's nest fern bacterium cultures and *Pseudomonas gladioli* strains with certain biochemical tests

Test	Bird's nest fern cultures[a]	*P. gladioli* cultures
Oxidase (+)	2[b] (very weak)	3
Arginine dihydrolase	0	0
Levan production	0	0
H_2S production	0	1
Oxygen requirement (obligate aerobe)	6	3
Polyhydroxybutyrate storage	6	3
Nitrate reduction	0	0
Growth at 41 C	0	0
Potato rot	0	0
Onion rot	6	3
Hydrolysis of:		
Casein	6	3
Gelatin	6	3
Starch	0	0
Hypersensitive reaction on:		
Pepper	6	3
Tobacco	6	3
Tomato	6	3
Pathogenic to:		
Bird's nest fern	6	3
Gladiolus	6	3

[a] Six cultures of the fern pathogen were tested and three cultures of *P. gladioli* (one culture obtained from R. E. Stall, University of Florida).
[b] Number of cultures positive for each characteristic.

Table 3. Comparison of bird's nest fern cultures with nonfluorescent pseudomonads for utilization of certain compounds as a sole carbon source for growth

Compound tested	*Pseudomonas* species[a]			
	caryophylli (2)	*cepacia* (1)	*gladioli* (4)	Bird's nest fern (6)
Adonitol	0	1	4	6
β-Alanine	NT[b]	1	4	6
D-Arabinose	2	1	2	0
L-Arginine	2	1	4	6
L-Asparagine	0	1	2	6
Betaine	2	1	4	6
Cellobiose	2	1	4	6
Citraconate	0	1	4	6
Erythritol	0	1	4	0
Fructose	NT	1	0	6
Geraniol	NT	0	0	0
D-Gluconate	NT	1	4	6(?)[c]
Glucose	2	1	4	6
DL-Lactate	2	1	4	2(?)
Levulinic acid	0	1	1(?)	6(?)
Mannitol	NT	1	4	6
Mesotartrate	0	0	0	0
L-Rhamnose	2	0	0	0
D-Ribose	2	1	4	6
Saccharate	0	1(?)	4	6
D-Sorbitol	NT	1	4	6
Sucrose	2	1	1	0
D-Tartrate	0	0	0	0
Trehalose	2	1	4	6
Tryptamine	0	1(?)	0	0
L-Xylose	2	1	4	6

[a] Numbers in parentheses indicate number of cultures of each species used.
[b] Not tested.
[c] Questionable response.

successfully reisolated from these ferns but not from control plants or ferns inoculated with the suspect pathogen.

Pathogenicity of the suspect pathogen to gladiolus. Cultures of *P. gladioli* caused numerous lesions on the leaves of inoculated bird's nest ferns. Symptoms were identifical to those caused by cultures of the suspect pathogen, and both organisms were readily reisolated from their respective treatments. Similarly, the suspect bacterium caused both leaf spots and corm lesions on inoculated gladiolus like those caused by the *P. gladioli* cultures on this host. Both bacteria were reisolated from their respective treatments.

Characterization of the suspect pathogen. The suspect pathogen was gram-negative, rod-shaped, and nonfluorescent on KMB, which distinguished it from *P. asplenii*. Several nonfluorescent pseudomonads were tested as comparisons and the results for *P. gladioli* as well as those of the nonfluorescent fern pathogen are presented in Tables 2 and 3. Most characteristics of the fern pathogen were identical to those of *P. gladioli* strains (Table 2). All isolates were capable of rotting onions but not potatoes. A nonfluorescent, yellow-brown pigment formed on KMB and sometimes on NA, a reported characteristic of *P. gladioli* (3). Carbon utilization tests further established the similarity between isolates of *P. gladioli* and the unknown fern pathogen with a 98%S (Table 3) (percentage similarity, Misaghi and Grogan [9]). These data indicate that the fern isolates are *P. gladioli*.

DISCUSSION

Bacterial blight may become very serious in the fern industry because the host range of *P. gladioli* appears quite broad and includes some other flowering crops, such as *Chrysanthemum morifolium* Ramat. (florist's chrysanthemum), *Schefflera arboricola* Hayata ex Kanehira (dwarf schefflera), and *Gerbera jamesonii* Bolus ex Hook f. (Transvaal daisy) (A. R. Chase, *unpublished*). Control of this bacterial disease must be based on keeping the foliage dry and using bacteria-free plants because pesticides have been unsuccessful in eliminating this disease once it has started.

There are very few similarities between *P. gladioli* and *P. asplenii*, although both organisms cause similar symptoms on the bird's nest fern. Production of fluorescin by *P. asplenii* allows a rapid differentiation between these two pathogens of ferns. *P. gladioli* also has a broader host range than *P. asplenii* on foliage plants, which further distinguishes the two pathogens. We believe this is the first report of *P. gladioli* causing a serious disease of a foliage plant.

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