A Foliar Disease of *Fittonia verschaffeltii* Caused by a Pathovar of *Xanthomonas campestris*

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ABSTRACT

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A foliar disease of *Fittonia verschaffeltii* (nerve plant) has been observed in several central Florida nurseries. A pathovar of *Xanthomonas campestris* caused veinal necrosis on fully expanded leaves of all cultivars and marginal necrosis on some plants. Strains of this pathovar from *F. verschaffeltii* infected *Aphelandra squarrosa, Crossandra infundibuliformis, Stenandrium lindenii, Xantheranthemum igneum, Pachystachys lutea*, and *Ruellia brittoniana* (members of the Acanthaceae). The bacterium had a wide host range and caused symptoms similar to those caused by xanthomonads found on plants in eight other families.

Fittonia verschaffeltii (Lem.) Coem., the nerve plant, is commonly grown for dish gardens, terrariums, and hanging baskets. Recently, a foliar disease of all cultivars of *F. verschaffeltii* was observed in several central Florida nurseries. Symptoms included veinal necrosis on fully expanded leaves, marginal necrosis, and water-soaking. Lesions were dry and brittle when the foliage was allowed to dry. Small necrotic flecks were also observed. A bacterium was consistently isolated from diseased leaves.

The objectives of this research were to identify a causal agent, to investigate the host range of the causal agent, and to determine the distribution of this disease. A preliminary report has been published (3).

MATERIALS AND METHODS

Pathogen identification. The suspect pathogen was isolated by grinding approximately 0.5 cm^2 of symptomatic leaf tissue in approximately 2–3 ml of sterile deionized water (SDW) in a sintered glass tissue grinder. The resulting suspension was streaked onto Difco nutrient agar (NA) and King's medium B (KMB) (14) and incubated at approximately 27 C for 3–5 days. Single colonies of a yellow bacterium consistently isolated from symptomatic tissue were purified by serial transfers and stored in vials of SDW.

The bacterial strains were characterized by means of the following tests: Gram reaction (15); asparagine utilization (7); xanthomonadin production (9); casein hydrolysis (7); acid production from glucose, arabinose, and mannose (14); gelatin liquefaction (14); aesculin hydrolysis (14); growth at 36 and 41 C (14); growth on starch (SX) medium (14); production of fluorescent pigment on KMB (14); mucoid growth on yeast extract-dextrose-calcium carbonate (YDC) medium (14); oxygen requirement using Hugh-Leifson's medium (14); and pectolysis on crystal violet-pectate (CVP) medium (14). Hypersensitivity was tested on *Capsicum annuum* L. 'Early Calwonder' (pepper), *Lycopersicon lycopersicum* (L.) Karst. ex Fariv. 'Bonny Best' and 'Ace' (tomato), and *Nicotiana tabacum* L. 'Hick's' (tobacco). A strain of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye was compared with the suspect pathogen in all tests.

Pathogenicity trials. Five strains of the suspect pathogen from different hosts were used in pathogenicity trials: *F. v.* var. *argyroneura* (Coem.) Nichols (white nerve plant) = X1, *F. v.* var. *argyroneura* 'Minima' (miniature fittonia) = X2, *F. v.* var. *argyroneura* 'Minima Variegata' (variegated miniature fittonia) = X3, *Justicia* sp. L. = X4, and *F. v.* var. *verschaffeltii* (Lem.) Coem. (pink fittonia) = X6.

All strains used as inoculum were grown on NA for 2 days at 27 C. Bacteria were removed from agar surfaces by flooding with sterile 0.1 M $MgSO_4$ solution and then gently rubbing colonies on the agar surface with a sterile cotton swab. Resulting suspensions were adjusted to approximately 1×10^8 cfu/ml (50% transmittance at 600 nm with a spectrophotometer).

Pathogenicity of strains of the suspect pathogen was tested on *F. v.* var. *argyroneura* planted in 12.5-cm pots containing steam-treated (1.5 hr at 90 C) Canadian peat and pine bark (1:1, v/v) amended (after steaming) with 4.4 kg of Osmocote (19:6:12, Sierra Chemical Co., Milpitas, CA 95035), 4.0 kg of dolomitic lime, and 0.9 kg of Micromax (micronutrient source, Sierra Chemical Co.) per cubic meter. Plants were exposed to intermittent mist (5 sec/30 min from 0800 to 2000 hours daily) for 24 hr before inoculation. The plants were then inoculated to runoff with a pump-action hand sprayer, enclosed in polyethylene bags for 7 days, and maintained under intermittent mist throughout the experiment. Control plants in all experiments were treated with sterile $MgSO_4$ only. Plants were irrigated by hand two or three times weekly as needed. All tests were performed in a greenhouse with a temperature range of 18–36 C and a maximum light level of 570 $\mu E \cdot s^{-1} \cdot m^{-2}$. Plants were evaluated for symptoms 7 and 14 days after inoculation, and isolation from symptomatic tissue

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was performed as described above. There were three plants per treatment, and the test was performed three times.

Host range tests. Host range of two strains of the suspect pathogen was determined for members of nine plant families. Three plants from each family were inoculated per treatment, and each host range test was performed three times. Inoculation procedures were those previously described. Plants were evaluated for symptoms weekly for 4 wk. Pathogenicity was determined on the following members of the Acanthaceae: *F. v.* var. *argyroneura, Aphelandra squarrosa* Nees (zebra plant), *Crossandra infundibuliformis* (L.) Nees (firecracker flower), *Pachystachys lutea* Nees (golden shrimp plant), *Ruellia brittoniana* E. Leonard, *Xantheranthemum igneum* (Linden) Lindau (bronze vein plant), and *Stenandrium lindenii* N. E. Br. (golden vein plant). Other cultivars of *F. verschaffeltii* were not evaluated for susceptibility to this pathogen because a source of disease-free stock was not available. Members of eight other plant families were inoculated: *Brassaia actinophylla* Endl. (schefflera) in the Araliaceae, *Dieffenbachia maculata* (Lodd.) G. Don 'Camille' and *Syngonium podophyllum* Schott. 'White Butterfly' in the Araceae, *Begonia* × *semperflorens-cultorum* Hort. (wax begonia) in the Begoniaceae, *Pelargonium* × *hortorum* L. H. Bailey (bedding geranium) in the Geraniaceae, *Codiaeum variegatum* (L.) Blume 'Gold Star' (croton) in the Euphorbiaceae, *Ficus benjamina* L. (weeping fig) in the Moraceae, *Strelitzia reginae* Ait. (bird of paradise) in the Musaceae, and *Pilea spruceana* Wedd. (friendship pilea) in the Urticaceae.

Strains of several named and unnamed pathovars of *X. campestris* were inoculated onto a susceptible host plus *F. v.* var. *argyroneura* to determine the pathogenicity of each strain on both plant species. The following isolates were used: *X. c.* pv. *hederae* (Arnaud) Dye (2) from *B. actinophylla, X. c.* pv. *dieffenbachiae* (McCulloch & Pirone) Dye (11) from *Philodendron* sp. Schott., *X. c.* pv. *syngonii* pv. nov. Dickey & Zumoff (6) from *S. podophyllum, X. c.* pv. *begoniae* (Takimoto) Dye (1) from *Begonia* sp., *X. c.* pv. *poinsettiicola* (Patel, Bhatt, & Kulkarni) Dye (13) from *C. variegatum, X. c.* pv. *pelargonii* (Brown) Dye (4) from *P.* × *hortorum*, and unnamed pathovars from *F. benjamina* (G. W. Simone, *unpublished*), *P. spruceana* (12), and *S. reginae* (5).

Nursery survey. A survey of *F. verschaffeltii* in central Florida was done to determine disease distribution. Symptomatic leaves were collected and isolations were made as previously described. Plants surveyed included *F. v.* var. *argyroneura, F. v.* var. *pearcei* Nichols (red fittonia), *F. v.* var. *verschaffeltii, F. v.* var. *argyroneura* 'Minima,' *F. v.* var. *argyroneura* 'Minima Variegata,' and *F. v.* var. *argyroneura* 'Black Empress' (black fittonia). Pathogenicity of the isolated strains was verified on *F. v.* var. *argyroneura* as previously described.

Table 1. Physiological and biochemical reactions of five strains of *Xanthomonas campestris* isolated from members of the Acanthaceae and one strain of *X. c.* pv. *vesicatoria* from tomato

Test	Acanthaceae xanthomonads[a]	*X. c.* pv. *vesicatoria*
Gram reaction	−	−
Hypersensitive reaction[b]	+	+
Aerobic	+	+
Mucoid growth on YDC (yeast extract-dextrose-calcium carbonate medium)	+	+
Asparagine utilization	−	−
Xanthomonadin production	+	+
Acid production: arabinose	+	+
glucose	+	+
mannose	+	+
Growth on SX (starch medium)	+	+
Fluorescent pigment on King's medium B	−	−
Pectolysis on CVP (crystal violet-pectate medium)	+	+
Aesculin hydrolysis	+	+
Casein hydrolysis	+	+
Urease production	−	−
Gelatin liquefaction	+	+
Growth at: 36 C	+	+
41 C	−	−

[a] X1 from *Fittonia verschaffeltii* var. *argyroneura* (white nerve plant), X2 from *F. v.* var. *argyroneura* 'Minima' (miniature fittonia), X3 from *F. v.* var. *argyroneura* 'Minima Variegata' (variegated miniature fittonia), X4 from *Justicia* sp., and X6 from *F. v.* var. *verschaffeltii* (pink fittonia).
[b] On pepper, tomato, and tobacco.

Table 2. Responses of plants in the Acanthaceae to inoculation with strains of a pathovar of *Xanthomonas campestris* isolated from members of the Acanthaceae

Host plant tested	Pathogenicity of *X. campestris* strains[a]				
	X1	X2	X3	X4	X6
Fittonia verschaffeltii var. *argyroneura*	9[b]	9	9	3	9
Aphelandra squarrosa	9	9	9	5	9
Crossandra infundibuliformis	9	9	9	1	9
Pachystachys lutea	ND	ND	7 HR	ND	9 HR
Ruellia brittoniana	ND	ND	6 HR	ND	6 HR
Stenandrium lindenii	ND	ND	9	ND	9
Xantheranthemum igneum	ND	ND	9	ND	9

[a] X1 from *Fittonia verschaffeltii* var. *argyroneura* (white nerve plant), X2 from *F. v.* var. *argyroneura* 'Minima' (miniature fittonia), X3 from *F. v.* var. *argyroneura* 'Minima Variegata' (variegated miniature fittonia), X4 from *Justicia* sp., and X6 from *F. v.* var. *verschaffeltii* (pink fittonia). ND = pathogenicity not determined, HR = hypersensitivelike reaction.
[b] Number of diseased plants out of nine inoculated (three plants per treatment, test performed three times). Symptoms included veinal and marginal necrosis and small, angular, necrotic lesions.

RESULTS

Pathogen identification. An aerobic, gram-negative bacterium that formed yellow colonies was consistently isolated from *F. verschaffeltii* with foliar blight. All strains of this bacterium produced a

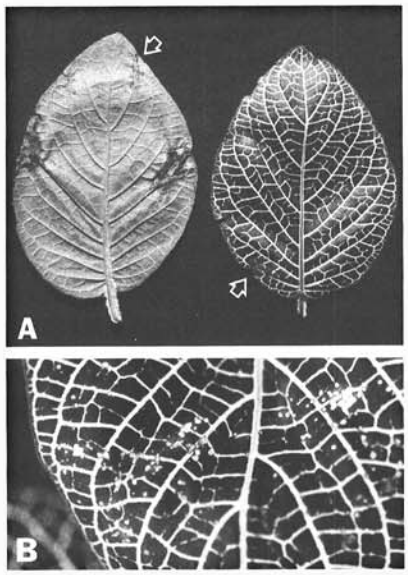

Fig. 1. (A) Veinal necrosis (arrows) on *Fittonia verschaffeltii* var. *argyroneura* 14 days after inoculation with a strain of *Xanthomonas campestris* isolated from this host. **(B)** Small, tan, circular lesions on *F. v.* var. *argyroneura* that are possibly due to a hypersensitivelike reaction.

hypersensitive reaction on pepper, tomato, and tobacco. The five strains tested were physiologically and biochemically similar to *X. campestris* (Table 1) (14).

Pathogenicity trials. Typical symptoms of veinal necrosis with some marginal necrosis developed on fully expanded leaves of inoculated plants approximately 7 days after inoculation. Small, tan, circular lesions also occurred; these appeared to be a hypersensitivelike reaction (Fig. 1). The pathogen was easily isolated from inoculated, symptomatic tissue. All five strains were pathogenic on *F. v.* var. *argyroneura* (Table 2). The strain from *Justicia* sp. was apparently less virulent than strains from *F. verschaffeltii*, since it produced disease in only three of nine plants inoculated.

Host range tests. The strains from *Fittonia* were pathogenic on the following members of the Acanthaceae: *A. squarrosa, C. infundibuliformis, S. lindenii*, and *X. igneum* (Table 2). Disease symptoms were most severe on *A. squarrosa* in experimental inoculations (Fig. 2). *C. infundibuliformis, S. lindenii*, and *X. igneum* produced fewer lesions than other inoculated plants. A hypersensitivelike reaction was produced on leaves of *P. lutea* and *R. brittoniana*.

The pathogen was found to have a wide host range and caused symptoms similar to those caused by other xanthomonads on the following plants: *C. variegatum, B. × semperflorens-cultorum, P. × hortorum, P. spruceana*, and *S. reginae* (Table 3). A hypersensitivelike reaction was produced on *F. benjamina, D. maculata, S. podophyllum*, and *B. actinophylla*. *X. c.* pv. *poinsettiicola* and unnamed pathovars of *X. campestris* from *P. spruceana* and *S. reginae* produced similar disease symptoms on *F. v.* var. *argyroneura* as the isolates from that plant.

Nursery survey. Fourteen nurseries were surveyed in central Florida for the veinal necrosis disease of *Fittonia* spp. Some plants with symptoms characteristic of this disease were found in all nurseries. Many nurseries had only a few plants with symptoms, while a few nurseries had the disease in epidemic proportions. A pathogenic strain of the bacterium was recovered from 10 of 14 nurseries.

DISCUSSION

Veinal necrosis of *F. verschaffeltii* caused by a pathovar of *X. campestris* produces three different types of symptoms: veinal necrosis and marginal necrosis (the most common) and small, tan, circular lesions that appear to be a hypersensitivelike reaction. Lesions of all types occurred on the same leaf naturally and experimentally. The reaction types may be due to differences in resistance of sites on the leaf, as was suggested for crown rust of oats (10). Testing the hypothesis that the hypersensitivelike reaction is actually a susceptible reaction could involve an examination of the pathogen population dynamics (8).

The pathogen infected several members of the Acanthaceae, with symptoms being most severe on *A. squarrosa* in experimental inoculations. This disease is apparently not severe on these plants in nurseries, since the majority of *Fittonia* growers do not produce *Aphelandra*. On the basis of number of lesions produced, *C. infundibuliformis, S. lindenii*, and *X. igneum* appeared to be moderately resistant to the pathogen. The pathogen also infected plants in eight other plant families. Symptoms on these plants ranged from disease reactions similar to those produced by xanthomonads on that plant to what appeared to be hypersensitivelike reactions.

Such a wide host range is not common for xanthomonads as a whole but may be unique to those infecting ornamental plants. The unnamed pathovars of *X. campestris* from *Pilea* spp. and *Pellionia* spp. (12) and *S. reginae* (5) have host ranges including plants in other families. These pathovars and *X. c.* pv. *poinsettiicola* infected *F. v.* var. *argyroneura*, producing symptoms similar to those produced by the xanthomonad isolated from *Fittonia* cultivars. On the basis of the host range studies conducted, *X. c.* pv. *poinsettiicola* and the unnamed pathovars of *X. campestris* isolated from *P. spruceana* and *S. reginae* appear to be closely related to the pathovar isolated from *F. verschaffeltii*. Because pathovar nomenclature for *X. campestris* is based on the host range of the organism, it is not possible to name this pathovar at this time.

A pathogenic isolate of the bacterium was recovered from 71% of the nurseries surveyed. Many of the growers believed this problem was due to cold damage, fertilizer burn, phytotoxicity, or insect damage. The ability to recognize this problem as a biotic disease is the first step in choosing appropriate control measures. Control should begin with sanitation and proper horticultural practices to avoid spread of the pathogen among plants and plant species. Many of the plants found to be susceptible to this pathogen are grown side by side under ideal conditions for pathogen spread and disease development. In general, nurseries that kept foliar surface moisture as low as possible had lower levels of the disease than those in which the foliage stayed wet. Chemicals such as copper and strepto-

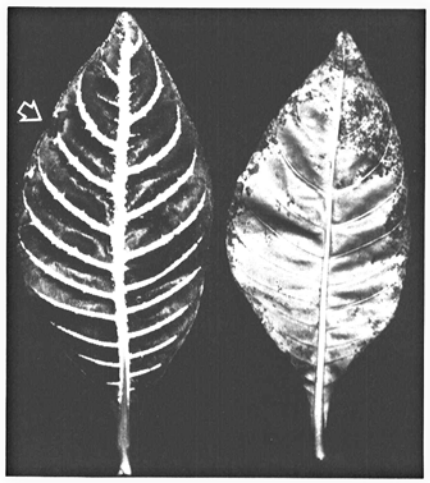

Fig. 2. Marginal, veinal, and interveinal necrosis on *Aphelandra squarrosa* 14 days after inoculation with a strain of *Xanthomonas campestris* isolated from *Fittonia verschaffeltii* var. *argyroneura*.

Table 3. Responses of selected ornamental plants to pathovars of *Xanthomonas campestris* on respective hosts and on *Fittonia verschaffeltii* var. *argyroneura*

Pathovar[a]	Host plant tested	Pathogenicity[b]	Pathogenicity of pathovar on *Fittonia*	Pathogenicity of *Fittonia* strain on each host
X. c. pv. *begoniae*	*Begonia × semperflorens-cultorum*	9*	0	9*
X. c. pv. *dieffenbachiae*	*Dieffenbachia maculata*	ND	ND	9 HR
X. c. pv. *hederae*	*Brassaia actinophylla*	7*	0	9 HR
X. c. pv. *pelargonii*	*Pelargonium × hortorum*	9*	0	9* HR
X. c. pv. *poinsettiicola*	*Codiaeum variegatum*	6*	9*	9*
X. c. pv. *syngonii*	*Syngonium podophyllum*	9*	0	9 HR
Unknown	*Ficus benjamina*	ND	ND	9 HR
Unknown	*Pilea spruceana*	9*	9* HR	9*
Unknown	*Strelitzia reginae*	9*	9* HR	9*
Unknown	*F. v.* var. *argyroneura*	9* HR	...	...

[a]Each pathovar was obtained from the host plant indicated except for *X. c.* pv. *dieffenbachiae*, which was obtained from *Philodendron* sp.
[b]Number of diseased plants out of nine inoculated (three plants per treatment, test performed three times). * = Typical disease reaction, ND = pathogenicity not determined, HR = hypersensitivelike reaction.

mycin used for control of other bacterial diseases may provide some control.

LITERATURE CITED

1. Ark, P. A., and Tompkins, C. M. 1939. Bacteriosis of tuberous begonia. Phytopathology 29:633-637.
2. Arnaud, G. 1920. Une maladie bacterienne du lierra (*Hedera helix* L.). C. R. Hebd. Seances Acad. Sci. 171:121-122.
3. Blake, J. H. 1987. A new disease of *Fittonia verschaffeltii* caused by a pathovar of *Xanthomonas campestris*. (Abstr.) Phytopathology 77:1732.
4. Brown, N. A. 1923. Bacterial leafspot of geranium in the eastern United States. J. Agric. Res. 23:361-372.
5. Chase, A. R., and Jones, J. B. 1987. Leaf spot and blight of *Strelitzia reginae* (bird-of-paradise) caused by *Xanthomonas campestris*. Plant Dis. 71:845-847.
6. Dickey, R. S., and Zumoff, C. H. 1987. Bacterial leaf blight of *Syngonium* caused by a pathovar of *Xanthomonas campestris*. Phytopathology 77:1257-1262.
7. Dye, D. W. 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N.Z. J. Sci. 5:393-416.
8. Hibberd, A. M., Stall, R. E., and Bassett, M. J. 1987. Different phenotypes associated with incompatible races and resistance genes in bacterial spot disease of pepper. Plant Dis. 71:1075-1078.
9. Irey, M. S. 1980. The taxonomic value of the yellow pigment of the genus *Xanthomonas*. M.S. thesis. University of Florida, Gainesville. 39 pp.
10. Luke, H. H., Barnett, R. D., and Pfahler, P. L. 1984. Postpenetration development of *Puccinia coronata avenae* in slow- and fast-rusting cultivars of *Avena byzantina*. Phytopathology 74:899-903.
11. McCulloch, L., and Pirone, P. P. 1939. Bacterial leaf spot of *Dieffenbachia*. Phytopathology 29:956-962.
12. Miller, J. W., and Chase, A. R. 1986. New disease of *Pellionia* and *Pilea* spp. caused by *Xanthomonas campestris*. Plant Dis. 70:346-348.
13. Patel, M. K., Bhatt, V. V., and Kulkarni, Y. S. 1951. Three new bacterial diseases of plants from Bombay. Indian Phytopathol. 4:142-151.
14. Schaad, N. W., ed. 1980. Laboratory Guide for Identification of Plant Pathogenic Bacteria. American Phytopathological Society, St. Paul, MN. 72 pp.
15. Suslow, T. V., Schroth, M. N., and Isaka, M. 1982. Application of a rapid method for Gram-differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology 72:917-918.