Comparisons of Three Bacterial Leaf Spots of *Hibiscus rosa-sinensis*

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ABSTRACT

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Three bacterial leaf spot diseases were observed on *Hibiscus rosa-sinensis* during the 1983–1984 season in central and southern Florida. *Pseudomonas cichorii*, *P. syringae*, and *Xanthomonas campestris* pv. *malvacearum* (*X. c. malvacearum*) were isolated in pairs and singly from symptomatic tissue. Lesions caused by *P. cichorii* that developed within 3 days of inoculation were up to 1 cm wide, irregularly shaped, and had a distinctive black border with a separate purple margin. *P. cichorii* was most severe on mature leaves, especially the oldest. Lesions caused by *P. syringae* developed 5–14 days after inoculation and were usually 1 mm wide, angular, and usually without a halo. Most lesions formed on immature leaves or those recently expanded and commonly caused distortion. Lesions caused by *X. c. malvacearum* formed 7–14 days after inoculation and resembled those caused by *P. syringae*. These lesions formed on mature leaves only and were frequently surrounded by a chlorotic halo. Leaves infected by *X. c. malvacearum* commonly abscised.

Several bacterial leaf spot diseases, including those caused by *Pseudomonas cichorii* (Swing.) Stapp, *P. syringae* van Hall, and *Xanthomonas campestris* pv. *malvacearum* (E. F. Sm.) Dows. (*X. c. malvacearum*) have been reported on *Hibiscus rosa-sinensis* L. in Florida (1). Over a 12-mo period, each of these organisms was isolated from lesions on numerous cultivars of hibiscus, sometimes singly and sometimes in pairs. Symptoms generally were confined to a few foliar lesions that were angular and surrounded by a chlorotic halo in some instances, with the central necrotic tissue tan to black. The following research was conducted to establish pathogenicity and identity of bacterial pathogens of hibiscus and compare symptom development.

MATERIALS AND METHODS

Cuttings of hibiscus cultivar Brilliant Red were obtained from commercial producers and rooted under intermittent mist in the following steam-treated potting medium: Canadian peat (50%), cypress shavings (25%), and pine bark (25%). The medium was amended with 4.4 kg of Osmocote 19-6-12 slow-release fertilizer (Sierra Chemical Co., Milpitas, CA), 4.2 kg dolomite, and 0.9 kg of Micromax (micronutrient source also from Sierra) per cubic meter. Plants were grown in 10- or 15-cm-diameter plastic pots in a glasshouse at 18–33 C and a maximum light level of 200 μmol s^{-1} m^{-2}.

Strains of suspected pathogens were obtained by grinding individual lesions in a scintered glass tissue grinder and streaking onto Difco nutrient agar (NA) plates. After incubation for 2 days at 32 C, colonies of suspected pathogens were isolated onto fresh NA plates and purified by three subsequent single-colony transfers. Colonies to be used as inoculum grew on NA plates at 32 C for 2 days before use. Inoculum concentration was adjusted to 1×10^8 colony-forming units (cfu) per milliliter with sterilized, deionized water and a spectrophotometer (50% transmittance at 600 nm). Plants were placed on a glasshouse bench and received intermittent mist (5 sec/30 min from 0800 to 2000 hours daily) for 24 hr before inoculation and during incubation. Plants were inoculated with a bacterial suspension applied to runoff with a pump-action hand sprayer and covered with a polyethylene bag. After 48 hr, plants were removed from plastic bags and arranged on the bench in a randomized complete block design with a single pot as the experimental unit. Natural light levels ranged from 150 to 250 μmol s^{-1} m^{-2} and temperatures ranged from 15 to 32 C. The tests reported were conducted between 1 January 1984 and 1 April 1985.

Pathogenicity and symptomatology. Bacteria suspected to be pathogens were of three types initially designated *Pseudomonas* I, *Pseudomonas* II, and *Xanthomonas*. Pathogenicity tests were performed as described, with four strains of *Pseudomonas* I, six strains of *Pseudomonas* II, and six strains of *Xanthomonas*. The first pathogenicity test employed plants wounded with insect pins (10 wounds to each of three leaves per plant). All other tests used nonwounded plants only. Noninoculated control plants sprayed with water only were included in each trial. Three to five plants were used for each treatment, and each strain was tested either two or three times. Reisolation of suspected pathogens was performed as described for original isolations.

Development of symptoms caused by pathogens was investigated using three strains of each pathogen type that had caused symptoms in previous tests. Plants were observed for number, shape, size, and color of lesions every 2 days (starting 2 days after inoculation) until no differences were noted (usually after 21 days). This test was performed three times using three plants per treatment each time.

Identification of pathogens. The following biochemical tests were performed to characterize the suspect pathogens in vitro. Tests for the *Pseudomonas* spp. were: arginine dihydrolase (20), oxidase (16), oxygen requirement (10), fluorescein production (14), Gram reaction (19), casein hydrolysis (19), gelatin hydrolysis (19), levan production (19), growth at 36 or 41 C (19) β-glucosidase production (9), and asparagine utilization (7). Tests for the *Xanthomonas* sp. were performed according to Dye (7): Gram reaction, oxygen requirement, growth at 36 or 41 C, asparagine utilization, aesculin hydrolysis, mucoid growth on glucose-yeast-chalk agar, gelatin hydrolysis, casein hydrolysis, and urease production. In addition, production of xanthomonadin (11) for *Xanthomonas* sp., and hypersensitive reactions to all pathogens were tested on *Capsicum annuum* L. 'Early Calwonder'(pepper), *Lycopersicon esculentum* L. (Mill.) Karst. ex Fariv. 'Bonny Best' (tomato), and *Nicotiana tabacum* L. 'Hick's' (tobacco) (15). Appropriate positive and negative controls were used for each test performed.

Host ranges of strains of *X. c. malvacearum* were compared with those of *X. campestris* isolates from hibiscus using Brilliant Red hibiscus, *Gossypium hirsutum* L. 'Acala 44' (cotton), and *Hibiscus esculentus* L. 'Crimson Spineless' (okra). Three strains of *X. c. malvacearum* were obtained from D. Gabriel, University of Florida at Gainesville. All plants were used after at least two mature leaves had formed. Five plants of each species were inoculated as described with either water or *X. campestris* from cotton (three

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strains) or hibiscus (three strains). Percentage of adaxial foliar surface infected was determined after 5 days. Reisolation of pathogens was performed as described.

Temperature effects on disease development. The effects of various constant temperatures on symptom development were evaluated using Plant Growth Chamber E 30 B (Percival Manufacturing Co., Boone, IA). A single growth chamber was used for each of the following temperatures: 15, 18, 21, 24, 27, 30, and 33 C. Plants were placed in growth chambers after inoculation with a single strain of one of the three suspected pathogens by the method described earlier and maintained in polyethylene bags for 3 days (*Pseudomonas* I) or 7 days (*Pseudomonas* II and *Xanthomonas*). The number of lesions per plant was recorded after 3 days (*Pseudomonas* I) or between 10 and 21 days (*Pseudomonas* II and *Xanthomonas*). Each test was performed with five plants per temperature and was repeated twice.

RESULTS

Pathogenicity and symptomatology. Each strain of the suspected pathogens caused lesions on hibiscus. Frequently a single lesion contained two of the three suspect pathogens. Lesions caused by the three pathogens differed in appearance and time of development. Reisolation of the pathogens was successful.

Symptoms caused by each of the three pathogens were distinctive. Plants inoculated with *Pseudomonas* I developed lesions on the oldest leaves primarily although lesions also formed on fully expanded mature leaves. Lesions formed within 3 days of inoculation and were 2–10 mm in diameter. They were generally surrounded by a double border of black (adjacent to necrotic tissue) and purple (adjacent to outer margin) (Fig. 1). The central necrotic portion of lesions was tan to whitish, and the overall shape of the lesions was slightly angular or rounded. Generally, no more than 10 lesions formed on a single leaf inoculated with *Pseudomonas* I. In the first trial, plants were artificially wounded with insect pins. Those inoculated with *Pseudomonas* I developed lesions at 80–90% of the wound sites with abscission of oldest leaves common. Lesions also formed in nonwounded tissue of mature leaves.

Lesions that formed on plants inoculated with *Pseudomonas* II did not appear until 5–14 days after inoculation. These lesions formed on the most recently expanded leaves and on all immature leaves. Lesions on immature leaves were pinpoint to 0.5 mm in diameter, black to brown, and caused distortion and puckering. Lesions on more completely expanded leaves were angular and up to 3 mm in diameter (Fig. 2). The centers of these lesions were dark brown to black. As many as 100 lesions could be counted on single leaves of plants inoculated with *Pseudomonas* II. No lesions formed at wound sites.

Symptoms caused by *Xanthomonas* developed between 7 and 14 days after inoculation, primarily on the oldest leaves. They were dark brown to black, angular, and pinpoint to 2 mm in diameter (Fig. 3). As many as 300 lesions formed on single leaves, with abscission of leaves common. In contrast to those caused by *Pseudomonas* II, lesions caused by *Xanthomonas* frequently were surrounded by a wide yellow halo that could be many times wider than the necrotic portion. Sometimes a few lesions caused the entire leaf to turn chlorotic and abscise. No lesions formed at wound sites (Fig. 3).

Identification of pathogens. Table 1 summarizes the results of biochemical tests used to identify the pathogens. *Pseudomonas* I was identified as *P. cichorii* on the basis of oxidase and arginine dihydrolase reactions (Table 1). *Pseudomonas* II was identified as a pathovar of *P. syringae* and was distinguished from *P. syringae* pv. *syringae* by nutritional studies and the lack of syringomycin production (12). *Xanthomonas* was identified as *X. campestris* on the basis of the tests of Dye (7). Cultures of *X. campestris* from hibiscus and cotton caused lesions on each of the hosts tested. The most virulent of the strains from either cotton or hibiscus were compared for percentage of the plant infected. Inoculation with the strain from hibiscus resulted in an average of 33, 71, and 41% of adaxial foliar surface infected for hibiscus, cotton, and okra, respectively. Inoculation with the strain from cotton resulted in an average of 20, 76, and 38% plant infection for hibiscus, cotton, and okra, respectively. These results indicate that the strains of *X. campestris* from hibiscus can be identified as *X. c. malvacearum.*

Temperature effects on disease development. The three pathogens had different optimum temperatures for disease development. Greatest numbers of lesions developed at 27 C and lesions

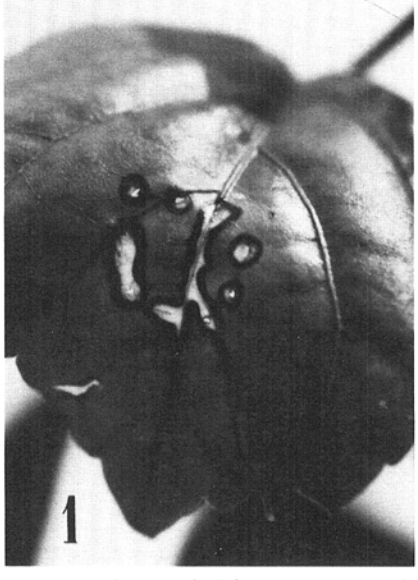

Fig. 1. Leaf spot of *Hibiscus rosa-sinensis* caused by *Pseudomonas cichorii*. Characteristic tan lesions with purple and black margins formed after artificial inoculation of mechanically wounded tissue.

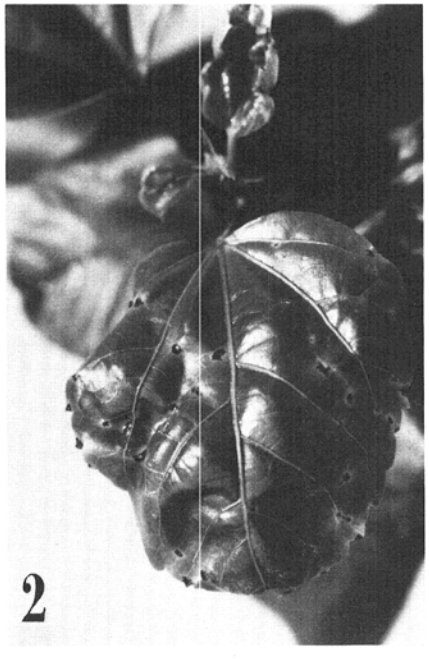

Fig. 2. Pinpoint lesions and puckering of immature leaves after artificial inoculation of *Hibiscus rosa-sinensis* with *Pseudomonas syringae*.

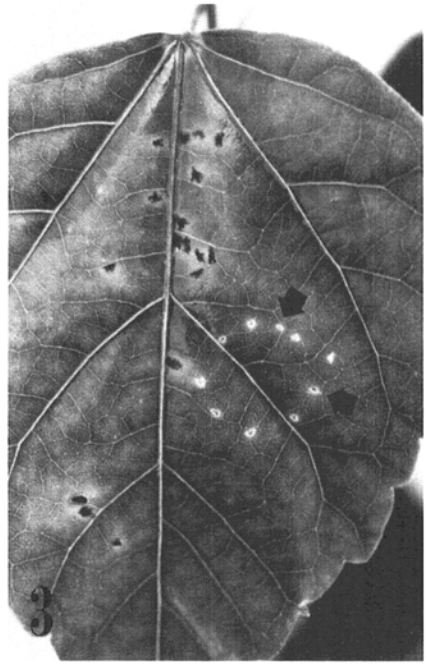

Fig. 3. Angular lesions on an older leaf of *Hibiscus rosa-sinensis* after artificial inoculation with *Xanthomonas campestris* pv. *malvacearum*. Lesions are surrounded by a chlorotic halo and do not occur in mechanically wounded tissue (arrows).

developed at 30 C or higher on plants inoculated with *P. cichorii* (Table 2). Plants inoculated with *P. syringae* developed the greatest number of lesions at 15–18 C; few lesions developed on plants maintained at 21 C or higher. *X. c. malvacearum*, however, caused high numbers of lesions on plants maintained between 24 and 33 C. Although the temperature range for each pathogen overlapped that of another, their optima differed.

DISCUSSION

P. cichorii (6,8,13), *P. syringae* (3,18), and *X. campestris* pathovars (5,17) are common pathogens of ornamental plants. The fact that these organisms each cause leaf spot of hibiscus in Florida is not surprising since they are present on many other crops in this state. Two of these diseases have not been adequately described before this report, but the pathogens have been isolated from hibiscus for many years (1,2,4). The recent increase in use of many hibiscus cultivars perhaps explains the apparent rise in prevalence of their bacterial diseases.

Each bacterial pathogen of hibiscus caused distinct symptoms and displayed a distinct response to temperature. *P. syringae* was isolated more frequently in Florida during the winter months and *X. campestris* and *P. cichorii* were isolated more frequently during the summer months (B. C. Raju, *personal communication*). These observations correspond to the temperature ranges identified for each pathogen in preliminary trials. Because the pathogens appear to be equally damaging and no effective control of any of them can be achieved using commercially available bactericides, differences between the diseases are commercially unimportant at present. These diseases are most common during hibiscus production and do not continue to develop under most landscape conditions. Limiting water applications, timing applications for periods when rapid drying occurs, and adequate plant spacing to allow air circulation should minimize severity of bacterial leaf diseases of hibiscus.

Table 1. Biochemical and biological characterizations of bacterial pathogens of *Hibiscus rosa-sinensis*

Test	Number of isolates positive[a]		
	***Pseudomonas* I (four isolates)**	***Pseudomonas* II (six isolates)**	***Xanthomonas* (six isolates)**
Pathogen of *Hibiscus*	4	6	6
Hypersensitive reaction			
Tobacco	1	5	3
Tomato	2	6	6
Pepper	4	3	6
Oxygen requirement (aerobe)	4	6	6
Gram reaction (negative)	4	6	6
Fluorescein production	4	6	NA[b]
Arginine dihydrolase	0	0	NA
Oxidase reaction (positive)	4	0	NA
Levan production	0	6	NA
β-Glucosidase production	4	6	NA
Asparagine utilization	4	6	0
Casein hydrolysis	0	0	6
Gelatin hydrolysis	0	0	6
Mucoid growth	NA	NA	6
Aesculin hydrolysis	NA	NA	6
Urease production	NA	NA	6
Xanthomonadin production	NA	NA	6
Growth on SX medium	NA	NA	1

[a] *Pseudomonas* I, *Pseudomonas* II, and *Xanthomonas* were identified as *P. cichorii*, *P. syringae*, and *X. campestris* pv. *malvacearum*, respectively. Three standard strains of each taxon were compared with, and did not differ from, the respective hibiscus pathogens in these tests.
[b] NA = not applicable.

Table 2. Effects of various constant temperatures on number of lesions caused by *Pseudomonas cichorii*, *P. syringae*, or *Xanthomonas campestris* pv. *malvacearum* on *Hibiscus rosa-sinensis*

Temperature (C)	Disease severity ratings for each of three tests[a]								
	P. cichorii			*P. syringae*			*X. campestris* pv. *malvacearum*		
	1	2	3	1	2[b]	3	1	2	3
15	1.3	1.0	1.6	2.5	1.3	1.7	1.0	1.0	1.0
18	1.0	1.2	1.6	2.7	1.3	2.5	1.6	1.0	1.0
21	1.8	2.0	2.4	1.0	2.0	1.4	2.0	1.0	1.0
24	2.2	1.0	2.6	1.0	1.3	1.6	3.5	1.0	1.4
27	2.8	1.7	3.2	1.0	2.0	1.2	4.3	1.3	1.0
30	NT[c]	1.0	1.0	1.3	1.0	1.0	NT	3.3	1.6
33	NT	1.1	1.0	1.0	1.0	NT	NT	3.0	1.4

[a] Disease severity was rated on the following scale: 1 = no lesions, 2 = 1–15 lesions, 3 = 16–30 lesions, 4 = 31–50 lesions, and 5 = more than 50 lesions per plant (some leaf abscission).
[b] The *F*-test for variation associated with temperature was significant at $P = 0.01$ for all tests except *P. syringae* test 2.
[c] NT = not tested.

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