Leaf Spot of Southern Magnolia Caused by *Pseudomonas cichorii*

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ABSTRACT

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A new leaf spot of southern magnolia (*Magnolia grandiflora*) was found to be caused by *Pseudomonas cichorii*. Newly unfolded leaves had dark brown lesions covering as much as two-thirds of the leaf area. Symptoms were less severe on older foliage. Pathogenic bacterial isolates from *M. grandiflora* matched *P. cichorii* in morphology and physiological and biochemical test results. *M. grandiflora, M. macrophylla, M. soulangeana*, and *M. tripetala* developed foliar lesions after artificial inoculation. Sprays of tri-basic copper sulfate (2.4 g/L) or copper hydroxide (1.2 g/L) applied at weekly intervals during periods of active shoot growth suppressed disease. Disease severity was less with ground-level irrigation than with overhead irrigation.

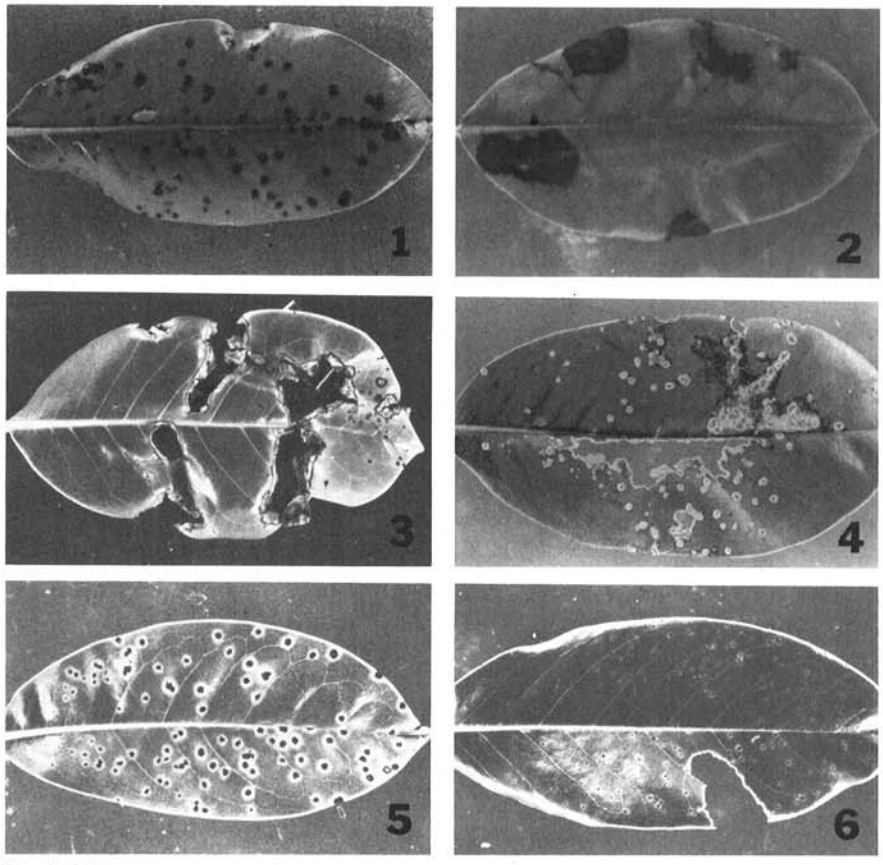
In the spring of 1981, an unidentified leaf spot on southern magnolia was observed in two locations in southern Alabama. Symptoms initially appeared as small, dark brown spots (1–2 mm in diameter) sometimes surrounded by faint yellow halos (Fig. 1). Spots developed individually or in small groups and coalesced into large, irregular lesions (Fig. 2). Tissue in the center of the lesion deteriorated into a fragile gray net and ultimately weathered away into irregular holes (Figs. 3 and 4). Leaf splitting along the midrib, foliar distortion, and leaf drop were associated with severe infection. Young, newly unfolded leaves were particularly susceptible, with large, dark brown lesions and holes often developing over as much as two-thirds of the leaf (Figs. 2–4). Expanding leaves showed black spots (2–10 mm in diameter) surrounded by thin yellow halos (1–2 mm wide) (Fig. 5). Mature current-season foliage typically showed small black specks (1 mm in diameter) surrounded by thin pale yellow halos (1 mm wide) (Fig. 6).

This study was designed to identify the pathogen of the leaf spot on southern magnolia, to determine its pathogenicity to some other *Magnolia* spp., and to identify some cultural and chemical treatments effective in disease control.

MATERIALS AND METHODS

Tissue from the margins of large, spreading lesions on unfolding leaves; black, circular spots on expanding leaves; 1-mm-diameter spots on mature leaves; and healthy leaves was immersed in 0.5% sodium hypochlorite for 1 min, dipped in sterile water, and allowed to air-dry. Small sections were cut from these tissue pieces and placed on nutrient agar and potato-dextrose agar in petri plates. Alternatively, surface-sterilized tissue was triturated in two or three drops of sterile water and incubated for 1 hr. A loopful of this suspension was streaked onto nutrient agar. Culture plates were incubated at 24 C for 48 hr. Isolations were made from diseased leaves collected at two locations in Alabama.

Pathogenicity. Pathogenicity was tested in two experiments using three cell suspensions containing 10^6 colony-forming units (cfu) per milliliter. Each suspension was prepared from 48-hr-old nutrient agar cultures of bacteria isolated from an individual magnolia lesion. Suspensions were swabbed onto the upper and lower surfaces of the youngest four to six leaves on container-grown magnolias (9.0–15.5 dm tall) potted in a pine bark medium. Controls were inoculated with sterile water. After inoculation, plants were covered with plastic bags for 24 hr. Each treatment included four single-plant replicates arranged in a completely randomized block design with 3.1 dm between plants. Overhead irrigation (equivalent to 12 mm



Figs. 1–6. Symptoms on *Magnolia grandiflora* naturally infected by *Pseudomonas cichorii*. **(1)** Initial appearance of small, dark brown spots on young foliage. **(2)** Large, irregular lesions developing on newly unfolded leaves. **(3 and 4)** Gradual deterioration of necrotic tissue. **(5)** Discrete black spots with yellow halos on expanding leaves. **(6)** Small, black specks with thin yellow halos on mature, current-season foliage.

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of rainfall) was applied for 1 hr each day. Six weeks after inoculation, symptoms were recorded and isolations were made from large, spreading lesions, from spots 2–10 mm in diameter, from specks 1 mm in diameter, and from apparently healthy tissue. Isolates were check for gram reaction (9), fluorescence on King's medium B (9), lipid hydrolysis using Tween 80 (10), and oxidase production (9).

In a test for effects of wounding on pathogenicity, both wounded (dusted with Carborundum on upper and lower leaf surfaces) and unwounded southern magnolia plants were swabbed with a cell suspension of one isolate (A). Control plants were treated with Carborundum plus water or water alone. Plants were maintained in a shadehouse.

In a second test, pathogenicity of two isolates (B and C) was evaluated on unwounded southern magnolias in 3.8-L containers exposed to full sun. Inoculated plants and controls were kept together, but a group of untreated magnolias was maintained at a 31-dm distance.

Susceptibilities of *M. grandiflora* L. (southern magnolia), *M. soulangeana* Soul. (saucer magnolia), *M. tripetala* L., and *M. macrophylla* Michx. to the bacterium were tested using the procedures described, except plants were maintained in a shadehouse and control plants were kept 31 dm from inoculated plants.

Identification. Four isolates from diseased southern magnolias were identified by the following tests: gram reaction (9,11), number and arrangement of flagella (4), fluorescin production on King's medium B (9), lipid hydrolysis using Tween 80 (10), growth at 41 C (8), oxidase production (9), arginine dihydrolase production (8), and hypersensitivity reaction on *Capsicum annuum* L., *Lycopersicon lycopersicum* (L.) Karst. ex Fario, and *Nicotiana tabacum* L. (1,5).

Four isolates of *Pseudomonas cichorii* (Swingle) Stapp obtained from A. R. Chase, University of Florida, IFAS, Agricultural Research Center, Apopka, were included in the tests. All isolates were grown in Difco nutrient agar for 18–48 hr before testing, and all tests were performed in duplicate.

Disease control. Tests were designed in 1981 and 1982 to evaluate some chemical and cultural treatments for prevention of disease. Severely infected southern magnolias grown in a pine bark medium in 11.4-L containers were used in both experiments. Plants were exposed to full sun, and normal nursery cultural practices were followed with the exception of pesticide applications and irrigation. Spray treatments were applied weekly to the foliage until runoff, using a hand-pump compressed-air sprayer. An adjuvant, Nu-film 17 (1.3 ml/L), was included with each bactericide treatment.

In 1981, three bactericides were evaluated at two rates each per liter of water: copper hydroxide (Kocide 101, copper equivalent 50%, 1.2 and 2.4 g), tri-basic copper sulfate (basic copper sulfate, copper equivalent 53%, 2.4 and 4.8 g), and copper resinate (Citcop 4E, copper salts of fatty and rosin acids, 38 g of copper per liter, 2.5 and 5.0 ml). Exhalt 800, a sticker-extender composed of polymerized pinene, saturated napthenes, and paraffins, was also tested at 5 and 10 ml/L to determine if a layer of inert material would provide protection. Six single-plant replicates per treatment were arranged in a completely randomized design. For each weekly spray treatment, replicates were derandomized and grouped. Irrigation (equivalent to 12 mm of rainfall) was applied each day with overhead impulse sprinklers. Disease incidence and severity were evaluated after 7 wk of treatment. Results were evaluated using analysis of variance with orthogonal comparisons.

In 1982, four chemicals were evaluated in a factorial experiment comparing overhead irrigation with ground-level irrigation. The chemicals and rates tested (per liter of water) were: copper hydroxide (2.4 g), tri-basic copper sulfate (2.4 g), streptomycin sulfate (Agri-Strep 17, 1.2 g), and Bordeaux mixture (hydrated lime-copper sulfate mixture, 9.6 g). Within each irrigation plot, four single-plant replicates per treatment were arranged in a randomized block design with 3.1-dm spacing between containers. Disease incidence and severity were evaluated after 15 wk of treatment. Main effects and interactions were analyzed using analysis of variance.

Table 1. Physiological and biochemical characteristics of *Pseudomonas cichorii* and isolates from *Magnolia grandiflora*

Characterization test	No. of isolates positive of four tested	
	P. cichorii	From *M. grandiflora*
Oxidase (+)	4	4
Arginine dihydrolase production	0	0
Lipid hydrolysis	4	4
Growth at 41 C	0	0
Hypersensitivity on		
Tobacco	3	4
Pepper	3	4
Tomato	3	4

RESULTS AND DISCUSSION

Pathogenicity. Disease incidence and severity were similar on wounded and unwounded southern magnolia leaves after inoculation with magnolia isolate A. About 66% of the wounded leaves became infected (about 30% of the leaf area affected), whereas unwounded leaves showed 83% disease incidence (about 40% of the area diseased). Symptoms were similar to those observed on naturally infected plants. Control plants did not develop symptoms. The bacterium was reisolated from large lesions and from spots 2–10 mm in diameter but not from spots 1 mm in diameter or control plants.

Unwounded southern magnolias grown in full sun and inoculated with magnolia isolate B or C consistently developed lesions similar to those on naturally infected plants. All plants including controls developed severe symptoms involving 60% of the leaf area. Uninoculated magnolias kept 31 dm from the test magnolias did not show symptoms. The pathogen was reisolated from large lesions and from spots 2–10 mm in diameter but not from spots 1 mm in diameter or healthy tissues. We believe the control plants became infected as a result of water splash from the closely spaced inoculated plants under overhead irrigation.

Isolates A, B, and C have been deposited with the American Type Culture Collection, Rockville, MD.

Table 2. Chemical control of Pseudomonas leaf spot on *Magnolia grandiflora* in 1981

Treatment	Formulation (a.i./L)	Leaves infected (%)	Foliar disease rating[a]
Check	...	97	2.7
Copper hydroxide	1.2 g	66	1.9
	2.4 g	61	1.8
Tri-basic copper sulfate	2.4 g	56	1.7
	4.8 g	47	1.6
Copper resinate	2.5 ml	74	2.1
	5.0 ml	84	2.5
Exhalt 800	5.0 ml	85	2.4
	10.0 ml	97	2.7

Analysis of variance with orthogonal comparisons

Comparison	Probability of greater value of *F*	
Check vs. treatments	0.0001	0.0001
Copper compounds vs. Exhalt 800	0.0001	0.0001
Copper resinate vs. other copper compounds	0.0001	0.0001
Copper hydroxide vs. tri-basic copper sulfate	0.0081	0.0530
Copper hydroxide rate	0.4622	0.1656
Tri-basic copper sulfate rate	0.1539	0.3628

[a] Foliar disease rating: 1 = no lesions, 2 = less than 10% total leaf area damaged, and 3 = more than 10% total leaf area damaged.

With each of the four *Magnolia* spp. tested, about 45% of the inoculated leaves became spotted with typical black spots 1–10 mm in diameter. Six weeks after inoculation, most spots were dried and many irregular holes were present. Lesions did not develop on control plants, which were placed 31 dm from bacteria-inoculated plants to prevent spread of bacteria by water splash. The bacterium was reisolated from spots 2–10 mm in diameter on *M. macrophylla* and *M. grandiflora* but not from similar lesions on *M. soulangeana* and *M. tripetala*. Also, the bacterium was not reisolated from spots 1 mm in diameter or from control plants. Failure to isolate the bacterium from lesions on *M. soulangeana* and *M. tripetala* may have been related to the dried condition of the diseased plant material.

Identification. Cream-colored colonies of bacteria repeatedly grew onto nutrient agar from southern magnolia leaf tissue showing large lesions and black spots 2–10 mm in diameter. The isolates were fluorescent, gram-negative, rod-shaped bacteria with a single polar-flagellum. Results of biochemical and physiological tests are given in Table 1. Because test results with *P. cichorii* and isolates from southern magnolia were in close agreement, we concluded that the southern magnolia isolates were *P. cichorii*. Researchers in Florida recently identified *P. cichorii* as a foliar pathogen of florists' geranium (*Pelargonium* × *hortorum*) and dwarf schefflera (*Schefflera arboricola*) (1,3).

Disease control. In 1981, disease incidence (percentage of leaves infected) and disease severity (foliar disease rating) were suppressed by all treatments except Exhalt 800 (Table 2). Copper hydroxide and tri-basic copper sulfate were most effective, with tri-basic copper sulfate providing greatest control. Application rate was not significant ($P \leq 0.05$) with either compound.

Analysis of 1982 test results showed no differences among chemical treatments or between chemical treatments and control plants, and no interaction between irrigation and chemical treatment. Disease severity on hand-watered plants, however, was significantly lower ($P \leq 0.01$) than that on plants watered by overhead irrigation.

Although copper hydroxide and tri-basic copper sulfate provided the best disease control of the materials tested in 1981, these chemicals did not perform well in 1982 under more humid, rainy conditions. In 1982, disease incidence and severity on magnolias subjected to ground-level irrigation were less than on those under sprinkler irrigation. These results are in agreement with a report of *P. cichorii* on chrysanthemum where water was an important factor in disease development (6). Even when chemical and cultural control methods were combined, as in 1982, complete disease control was not achieved. As with some other bacterial diseases in greenhouse situations (3), control of this disease in nurseries will require strict sanitation practices in addition to chemical and/or cultural control treatments.

This is the first report of a bacterial leaf spot disease on southern magnolia. *P. syringae* and an unidentified *Pseudomonas* sp. were previously reported to cause leaf spot on *M. soulangeana* and a hybrid (*M. campbellii* × *M. soulangeana*, respectively) (2,7). During 1981–1984, the disease caused by *P. cichorii* was confirmed in two areas of Alabama. Because this leaf spot appeared to be a problem only on new succulent growth, spread of the bacterium was restricted to a few weeks during the growing season when new foliage was produced. Thus far, leaf spot caused by *P. cichorii* on southern magnolia does not appear to be a major problem in Alabama.

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