A Leaf Spot of Florists' Geranium Incited by *Pseudomonas cichorii*

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ABSTRACT

Engelhard, A. W., Mellinger, H. C., Ploetz, R. C., and Miller, J. W. 1983. A leaf spot of florists' geranium incited by *Pseudomonas cichorii*. Plant Disease 67:541-544.

A new bacterial disease characterized by spots on the leaf, petiole, flower, and peduncle of the florists' geranium (*Pelargonium* × *hortorum*) was shown to be incited by *Pseudomonas cichorii*. An isolate from geranium was pathogenic to cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea*), chrysanthemum (*Chrysanthemum morifolium*), and a weed host, prickly poppy (*Argemone mexicana*). The causal organism produced a green fluorescent pigment on King's medium B and was arginine dihydrolase negative and oxidase positive, which identified it as *P. cichorii*. In greenhouse experiments, cupric hydroxide, cupric hydroxide with sulfur, and basic copper sulfate, when used singly or in combination with mancozeb, were moderately effective in controlling Pseudomonas leaf spot on *P.* × *hortorum* cultivars Heidi and Ringo plants.

Additional key words: *Pseudomonas erodii*

In 1975, a leaf spot disease of florists' geranium *Pelargonium* × *hortorum* Bailey) was found on the cultivars Sincerity and Yours Truly in Fort Myers, FL. The spots were dark brown, mostly about 5 mm in diameter, and unlike those previously reported for any disease on geranium. A bacterium was isolated that, upon inoculation into healthy geranium leaves, produced spots similar to those from natural infections. The pathogen was identified as *Pseudomonas cichorii* (Swing.) Stapp. The disease has been found numerous times on geraniums in greenhouses and outdoors since 1975.

Reports of bacterial diseases on geranium (*Pelargonium*) were made in the United States and France in 1890 (7,23) and in 1912 (9) but inoculation studies were not conducted. In 1914, Lewis (18) described a bacterial leaf spot of *Pelargonium* and *Erodium*. He named the causal agent *Pseudomonas erodii*. *P. erodii* is now considered a species incertae sedis (3). Some but not all symptoms and aspects described by Lewis coincide with those in our study. In 1923, Brown (2) described a bacterial leaf spot incited by *Bacterium* (*Xanthomonas*) *pelargonii*. Dodge and Swift (4) reported a stem rot caused by bacteria isolated from a leaf spot similar to that described by Brown but did not indicate the organism was *X. pelargonii*. Hellmers (10) and Munnecke (21) found *X. pelargonii* was the incitant of both the bacterial leaf spot and stem rot phases of this disease. Also, Koucheki (14,15) reported a pseudomonad on *P.* × *hortorum*, Baudin (1) associated a wilt of *P. capitatum* with *P. solanacearum*, and Passalacqua (22) associated a green fluorescent bacterium with a leaf spot of *Pelargonium*. Recently, Strider et al (25) showed that *P. solanacearum* was a pathogen of *Pelargonium*.

The objectives of this paper are to describe a disease of florists' geranium incited by *P. cichorii*, report results of inoculation studies with the pathogen on *Pelargonium* and other hosts, and discuss disease control. Preliminary reports describing this disease have been published (5,6).

Florida Agricultural Experiment Stations Journal Series No. 4170.

Accepted for publication 7 December 1982.

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SYMPTOMS

Leaves. Foliar symptoms on geranium plants vary with weather conditions. When plants are exposed to rainfall, water-soaked dark brown to black irregular-shaped necrotic areas 5–10 mm or larger develop (Fig. 1). The necrotic areas may enlarge along veins and coalesce to encompass large sections of a leaf. The entire leaf may become necrotic and curl when extensive infection occurs. Chlorosis develops in the tissues adjacent to lesions, usually after 2 days. When spots become numerous, general chlorosis of the leaf occurs. Severely affected leaves abscise.

Under less favorable moisture conditions for the development of the disease, ie, when plants are exposed to dews, overhead watering, or occasional wetting, sunken lesions 2–10 mm in diameter with tan centers that may be slightly raised develop on both leaf surfaces (Fig. 2). The tan centers may be surrounded by a dark margin and a chlorotic halo. The halo may be as wide as the diameter of the lesion or may be very small to nonexistent on a young leaf spot. Large lesions, apparently formed when several small lesions coalesce, may have several small centers that appear as "eyes" 1–2 mm in diameter.

Frequently, infection occurs only on leaf margins. Necrotic areas 2–4 mm wide extend from a small part to the entire margin of a leaf, causing cupping and distortion as the leaf grows. Marginal infections may occur on leaves of any age but are common on young seedlings, especially those receiving frequent overhead water. Dry weather slows the development of symptoms and spread of the disease.

Flowers. Individual buds turn black and fail to open or the entire inflorescence turns dark brown to black. Necrosis usually extends down the peduncle for about 1 cm. Individual lesions occur on the peduncle but are not common.

Stems. No stem infections have been observed on naturally infected plants. Inoculated plants develop superficial tan to brown areas that do not enlarge. Cuttings soaked in bacterial suspensions of the pathogen root with no evidence of disease.

MATERIALS AND METHODS

Three to six detached leaves of each of the cultivars Sincerity (two experiments), Irene, Dawn, Improved Minnetonka, Pink Fiat, and Pink Camellia were washed in soapy water to remove spray residues. They were immersed in 0.54% sodium hypochlorite for 1 min, rinsed in sterile water, and aseptically wounded with 5–20 pinpricks per leaf. The pathogen was grown on two petri plates for 48 hr at 28 C under constant cool-white fluorescent light on the medium of King et al (KMB) (12). The petri plates were filled with sterile deionized water to

prepare a bacterial suspension. The resulting suspension was spread over the wounded leaves with a 3-mm-diameter inoculating loop or a pipette. The leaves were placed singly over moist vermiculite in petri plates and exposed to continuous cool-white fluorescent light at 28 C.

In another inoculation experiment, five leaves on each of five plants of the cultivars Fireflash and Ringo were rubbed on the adaxial surfaces with a sterile cotton swab dipped in a suspension of the pathogen prepared by flooding two 48-hr cultures grown on KMB with sterile saline solution (0.85% NaCl). Also, three leaves on each plant were pricked five times with a sterile dissecting needle. The adaxial and abaxial surfaces of the leaves were then sprayed at 15 psi with the bacterial suspension used in the previous experiment. A polyethylene bag was placed over each plant to maintain high humidity. All plants were maintained at 26 C. Leaves of control plants were treated similarly but were sprayed with sterile saline solution.

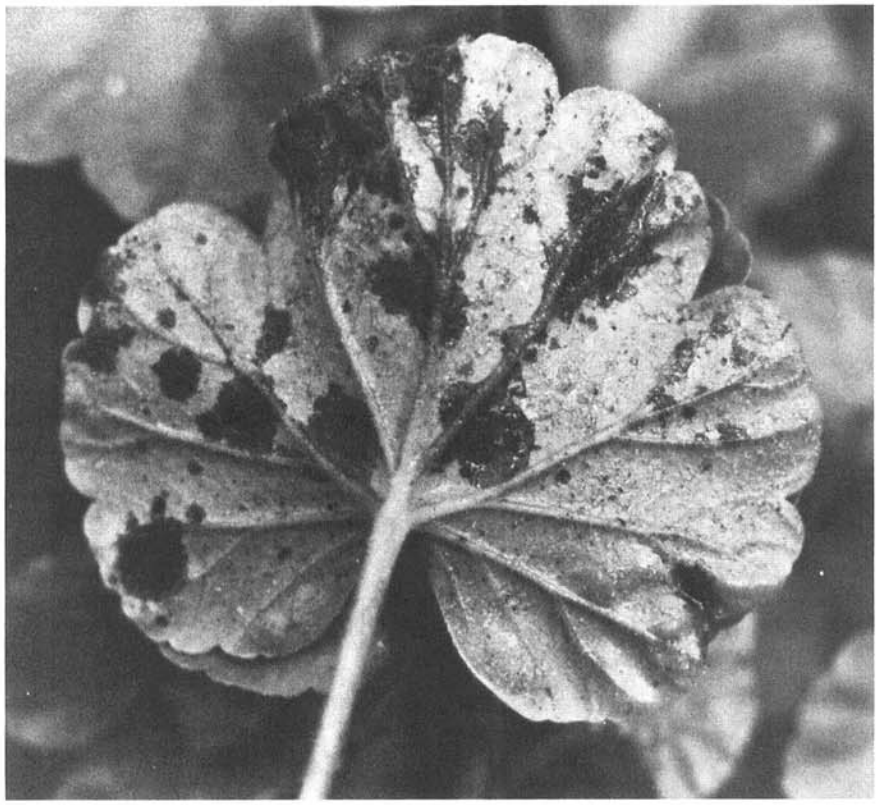

Fig. 1. Leaf symptoms on *Pelargonium* × *hortorum* 'Ringo' incited by *Pseudomonas cichorii* on outdoor-grown plants the morning after a rainy day. Note irregular-shaped lesions of varying sizes, enlargement along veins, and water-soaked appearance of lesions.

Fig. 2. Leaf symptoms incited by *Pseudomonas cichorii* on a commercially produced geranium pot plant. Note round leaf spots, necrotic areas along leaf margins, coalescing lesions, and leaf distortion.

A similar experiment was conducted with both geranium and *C. morifolium* plants, the latter a known host of *P. cichorii* (19). Eight vegetative plants of the chrysanthemum cultivar Iceberg and three flowering plants each of the geranium cultivars Ringo and Fireflash were used. Three leaves on each of these plants were pricked five times with sterile needles and four needle pricks each 2 cm apart in a linear row were made on the peduncles of the flowering geranium plants. The plants were then sprayed at 15 psi with a suspension of a geranium isolate. Controls were sprayed with sterile saline only. In addition, leaves and stems on four plants of the cultivar Ringo were pricked with a sterile needle dipped into a culture of *P. cichorii* isolated from the chrysanthemum cultivar Giant No. 4 Indianapolis Yellow. Each plant was covered with a polyethylene bag and placed in the greenhouse for 4 days at a daily mean low and high temperature of 21 and 33 C, respectively.

Plants known to be hosts of *P. cichorii* (three plants each of cultivars Heidi geranium, Burpeeana cauliflower, Rio Verde cabbage, and rooted cuttings of cultivar Manatee Iceberg chrysanthemum (19,24) were individually planted in 10-cm-diameter plastic pots filled with a sterilized (82 C for 30 min) soil mix amended with 2.5 cc 18-6-12 Osmocote controlled-release fertilizer per pot. Two weeks after potting, these plants were inoculated with an isolate of *P. cichorii* from geranium. The pathogen was grown in nutrient broth cultures on a wrist-action shaker for 30 hr. Bacterial cells were then concentrated with an International Equipment Centrifuge at 2,100 rpm for 15 min. Resultant pellets were resuspended in sterile saline at 8.5 g NaCl/L deionized H_2O to a concentration of about 5×10^6 cells per milliliter for inoculation treatments. Sterile disposable hypodermic syringes were used for injecting about 2 ml of the bacterial suspension into each of five leaves on each plant. Sterile saline was used for the control plants. Symptoms were rated 5 days after inoculation. A similar but separate test was done with prickly poppy, *Argemone mexicana* L., a weed host (H. A. Bingham, *unpublished*).

Disease control. Geranium seedlings, cultivar Heidi (one per 10-cm pot), were sprayed with Kocide 101 (cupric hydroxide, copper equivalent 50%), Kocide 404S (cupric hydroxide + sulfur, copper equivalent 17.5 and 15.5% sulfur), Manzate 200 (80% zinc ion + manganese ethylenebisdithiocarbamate), or tribasic copper sulfate (basic copper sulfate, copper equivalent 53%). All treatments

were replicated four times in a completely randomized block design. Plants were sprayed to runoff with a 10-L hand-held sprayer pressured with CO_2 to 55 psi. After the initial spray dried, all plants except the uninoculated controls were inoculated by spraying with an atomized suspension of *P. cichorii* from geranium containing 6.2×10^8 cells per milliliter. All plants were then placed in a polyethylene-covered chamber in which a humidifier was operating constantly. The plants were sprayed again with the chemicals 6 days later and were placed back in the chamber for 4 more days. Disease ratings were made using the Horsfall-Barratt method (11).

In all experiments, isolates were used that produced a green fluorescent pigment on KMB (17) and were arginine dihydrolase negative (26) and oxidase positive (16). Reisolations of the pathogen were made on KMB from selected plants in each test. Some reisolated fluorescent colonies were retested for pathogenicity.

RESULTS

After wounding of detached leaves and application of inoculum with a loop or cotton swab, water-soaked lesions 5–10 mm in diameter developed 24–48 hr later at the wounded sites and also at unwounded sites. Complete breakdown of the leaf tissue occurred after 72 hr. All cultivars tested were susceptible.

Four days after wounding and swab- or sprayer-inoculating of attached leaves of the cultivars Fireflash and Ringo, many dark brown necrotic spots 3–5 mm in diameter developed on both wounded and unwounded leaves. Where large lesions up to 15 mm in diameter developed and sometimes coalesced, adjacent tissues became increasingly chlorotic. No chlorosis was associated with the smaller lesions. In the most severe reaction, large sections of a leaf became necrotic, collapsed, and died. Newly developing leaves, less than 2.5 cm in diameter, had numerous dark brown necrotic lesions along the margins. No reaction was evident on control plants.

The cultivars of *P.* × *hortorum* that were tested and shown to be susceptible were the cutting types Dawn, Improved Minnetonka, Irene, Sincerity, Pink Fiat, and Pink Camellia and the seedling types Fireflash, Heidi, Ice Queen, Ringo, and Pink Lady. Ringo Salmon, Ringo Scarlet, Showgirl, Red Express, Rosita, Mustang, and Bright Eyes were observed to be susceptible under commercial production conditions.

In reciprocal inoculations on geranium and chrysanthemum with an isolate of *P. cichorii* from each host, typical symptoms developed on both hosts.

After injection-inoculation of geranium and four previously described hosts of *P. cichorii* with an isolate from geranium, positive disease reactions were produced on all including cauliflower, cabbage, chrysanthemum, and prickly poppy.

Table 1. Chemical control of Pseudomonas leaf spot on *Pelargonium* × *hortorum* 'Heidi' geranium plants[w]

Treatment (rate a.i./L)	Disease rating after 10 days
Water control (inoculated)	3.3 c[x,y]
Water control (uninoculated)	1.8 a
Kocide 101 77W 1.2 g	2.0 ab
Kocide 101 77W 0.6 g	2.3 ab
Kocide 404S 0.4 ml (Cu) + 0.4 ml (S)[z]	1.8 a
Kocide 404S 0.2 ml (Cu) + 0.2 ml (S)	2.0 ab
Kocide 101 77W + Manzate 200 80W 1.2 g + 1.8 g	2.0 ab
Tribasic copper sulfate 1.2 g	2.8 ab
Tribasic copper sulfate 0.6 g	2.3 bc
Tribasic copper sulfate + Manzate 200 80W 1.2 + 1.8 g	2.0 ab

[w] Plants were sprayed on day 1 and day 6, inoculated on day 1 after spraying, and rated on day 10.
[x] Disease rating = mean of four replicates evaluated with the Horsfall-Barratt rating system: 1 = 0% disease, 2 = 0–3% disease, and 12 = 100% disease.
[y] Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.
[z] Cu as copper equivalent and S as elemental sulfur.

Disease severity was reduced with sprays containing copper with the exception of the lower rate of tribasic copper sulfate (Table 1). Presence of sulfur or mancozeb in the sprays did not significantly alter the level of disease. No phytotoxicity was associated with any of the materials.

DISCUSSION

P. cichorii is becoming a more important phytopathogen in subtropical regions. Among the ornamental and vegetable crops susceptible are cabbage (24; H. A. Bingham, *unpublished*), cauliflower (24), celery (27), lettuce (8), escarole, green beans and watermelon (24), *C. morifolium* (19), Gerbera daisy (20), and several foliage plants (13). Smith (24) lists 16 vegetables susceptible to *P. cichorii* and most are grown in Florida. Prickly poppy, a native weed, is known to be a host and reservoir of the pathogen (H. A. Bingham, *unpublished*).

There was little host specificity among the isolates of *P. cichorii* evaluated, supporting the data of Bingham (*unpublished*) who used 67 isolates and six hosts.

Copper-containing fungicides were shown to provide some control of bacterial leaf spot of geranium. McFadden (19) demonstrated that weekly sprays of basic copper sulfate at 4 lb/100 gal provided satisfactory control of bacterial leaf spot on chrysanthemum. Such high rates of copper, however, are phytotoxic to geranium plants (*unpublished*). Thus, chemical control of the disease on geranium with copper-containing sprays does not appear promising. The broad host range, the nonspecificity of the isolates, the potential phytotoxicity of copper sprays for disease control, and the importance of water in disease spread and development would indicate a high potential for the development of epidemics on geraniums in Florida and other locations with warm, wet, or rainy climates.

Strong emphasis for disease control must be based primarily on obtaining plants free of the disease and following procedures that exclude the pathogen from the growing area. Maintaining strict sanitary procedures in the production area is essential. The importance of water in disease development with *P. cichorii* on chrysanthemum was emphasized by McFadden (19), so maintaining the plants under cover, as compared with outdoors where the plants are exposed to rain and dew, is also important to disease control.

LITERATURE CITED

1. Baudin, P. 1935. Les maladies des plantes a parfum topicales. Rev. Mycol. 20:73-112.
2. Brown, N. A. 1923. Bacterial leafspot of geranium in the eastern United States. J. Agric. Res. 23:361-372.
3. Buchanan, R. E., and Gribbons, N. E., eds. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams & Wilkins Co., Baltimore. 1,268 pp.
4. Dodge, B. O., and Swift, M. E. 1932. Black stem rots and leaf spot of pelargonium. J. N.Y. Bot. Gard. 33:97-103.
5. Engelhard, A. W. 1980. Bacterial leaf spot—a new geranium disease. Florists' Rev. 166(4311):53-55.
6. Engelhard, A. W., Mellinger, H. C., Ploetz, R. C., and Miller, J. W. 1982. A new leaf spot disease of geranium incited by *Pseudomonas cichorii*. (Abstr.) Phytopathology 72:977.
7. Galloway, B. T. 1890. Diseases of geraniums. J. Mycol. 6:114-115.
8. Grogan, R. G., Misaghi, I. J., Kimble, K. A., Greathead, A. S., Ririe, D., and Bardin, R. 1977. Varnish spot, destructive disease of lettuce in California caused by *Pseudomonas cichorii*. Phytopathology 67:957-960.
9. Heald, F. D., and Wolf, F. A. 1912. A plant disease survey in the vicinity of San Antonio, Texas. U.S. Dep. Agric. Bur. Plant Ind. Bull. 226:86.
10. Hellmers, E. 1952. Bacterial leaf spot of *Pelargonium* (*Xanthomonas pelargonii* (Brown) Starr and Burkholder) in Denmark. Trans. Dan Acad. Tech. Sci. 4:1-40.
11. Horsfall, J. G., and Barratt, R. W. 1945. An improved grading system for measuring plant diseases. (Abstr.) Phytopathology 35:655.
12. King, E. D., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pycocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
13. Knauss, J. F. 1973. Common diseases of tropical foliage plants: II. Bacterial diseases. Florists' Rev. 153(3958):27-28, 73-80.
14. Koucheki, H. K. 1973. Observations in the etiology of disease of *Pelargonium hortorum*. Plant Dis. Rep. 57:284-288.
15. Koucheki, H. K. 1973. The role of seed in transmission of a disease of *Pelargonium hortorum*. Plant Dis. Rep. 57:909-911.
16. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 178:703.

17. Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bact. 29:470-489.
18. Lewis, I. M. 1914. A bacterial disease of *Erodium* and *Pelargonium*. Phytopathology 4:221-233.
19. M Fadden, L. A. 1961. A bacterial leaf spot of florists' chrysanthemums, *Chrysanthemum morifolium*. Plant Dis. Rep. 45:16-19.
20. Miller, J. W., and Knauss, J. F. 1973. Bacterial blight of *Gerbera jamesonii* incited by *Pseudomonas cichorii*. Plant Dis. Rep. 57:504-505.
21. Munnecke, Donald E. 1954. Bacterial stem rot and leaf spot of *Pelargonium*. Phytopathology 44:627-632.
22. Passalacqua, T. 1933. La variegatura patologia del *Pelargonium* ed altre picnite. R. As. Bot. Palermo Lav. 4:201-240.
23. Prillieux, E., and Delacroix, G. 1890. La gangrene de la tige de la Pomme de terre, Maladie bacillaire. C. R. Acad. Sci. 111:208-210.
24. Smith, M. A., and Ramsey, G. B. 1946. Bacterial zonate spot of cabbage. Phytopathology 46:210-213.
25. Strider, D. L., Jones, R. K., and Haygood, R. A. 1981. Southern bacterial wilt of geranium caused by *Pseudomonas solanacearum*. Plant Dis. 65:52-53.
26. Thornley, M. J. 1960. The differentiation of *Pseudomonas* from the gram negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23:37-52.
27. Wedgeworth, H. W. 1931. Bacterial spot and early blight of celery. Fla. Agr. Exp. Stn. Annu. Rep. 156. 184 pp.