A New Foliar Blight of *Impatiens* Caused by *Pseudomonas syringae*

D. A. COOKSEY, Associate Professor, Department of Plant Pathology, University of California, Riverside 92521, and S. T. KOIKE, Farm Advisor, University of California Cooperative Extension, 118 Wilgart Way, Salinas 93901

ABSTRACT

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Large water-soaked and necrotic lesions were observed on leaves of *Impatiens* cultivar New Guinea Hybrid in a commercial greenhouse in Santa Barbara County, California. An oxidase-negative, fluorescent bacterium was consistently isolated from diseased tissues and identified as *Pseudomonas syringae*. Rapid water-soaking and necrotic symptoms resulted after New Guinea Hybrid and *I. wallerana* were inoculated with this bacterium. Hypersensitivity developed within 24 hr after inoculation of tobacco, tomato, common bean, and mung bean. No reactions occurred after inoculation of *Begonia* × *semperflorens-cultorum*, *Dieffenbachia maculata*, or *Calendula officinalis*. The bacterium was resistant to 1.6 mM $CuSO_4$.

An outbreak of a foliar disease of *Impatiens* cv. New Guinea Hybrid was observed in a commercial greenhouse in Santa Barbara County, California, in 1989. Symptoms included large water-soaked and necrotic lesions that originated either at the leaf margins or in interveinal areas. Large populations of bacteria were consistently observed by microscopic examination of diseased tissues, and an oxidase-negative, fluorescent bacterium was consistently isolated on King's medium B agar. This report describes a copper-resistant strain of *Pseudomonas syringae* Van Hall that is the causal agent of this new disease.

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MATERIALS AND METHODS

Identification of causal agent. Tissue sections from lesions on original diseased plant material or inoculated leaves were examined with phase-contrast microscopy. The examination revealed bacterial streaming from diseased tissues into distilled water. To isolate the bacteria, a small amount of diseased tissue was crushed in a drop of sterile water, and then the resulting suspension was streaked onto King's medium B (12) or yeast-dextrose-calcium carbonate medium (17). The strains recovered were purified by streaking to single colonies on fresh media several times. Identification of bacteria to species was done by the tests listed in Table 1.

Plant inoculations. Tomato (*Lycopersicon esculentum* Mill. 'President'), tobacco (*Nicotiana tabacum* L. 'Burley'), common bean (*Phaseolus vulgaris* L.), and mung bean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) were grown from seed in pots (10-cm diameter) containing steam-treated U.C. soil mix (3). Tomato, bean, and mung bean were chosen as potential hosts that a strain of *P. syringae* pv. *syringae* with a broad host range might infect; tobacco was selected as a plant that frequently has a hypersensitive

response to infiltration of pathogenic species of *Pseudomonas* (17).

Impatiens cv. New Guinea Hybrid, common impatiens (*I. wallerana* Hook.), pot marigold (*Calendula officinalis* L. 'Mighty Yellow Marietta'), wax begonia (*Begonia* × *semperflorens-cultorum* Hort. 'Viva'), and *Dieffenbachia maculata* (Lodd.) G. Don 'Perfection' were purchased from local nurseries and repotted in U.C. mix in 10-cm pots. Marigold, begonia, and *Dieffenbachia* were chosen as potential hosts that are frequently found in nurseries adjacent to *Impatiens* plants.

Bacterial colonies were grown on King's medium B agar for 48 hr and then suspended in sterile distilled water. The suspension was diluted to about 5×10^7 cfu/ml, based on optical density readings with a Klett-Summerson colorimeter. The suspensions were either infiltrated into leaf tissues with a syringe (without a needle) or sprayed gently onto leaf surfaces until runoff with an aerosol-propelled spray unit. Both infiltration and spray inoculations were done twice, on two plants each time. Control plants were treated with water only. Plants were maintained on a greenhouse bench with natural light and received intermittent misting for 30 sec every 10 min during a 12-hr period each day.

Determination of copper and streptomycin sensitivity. Bacteria were grown on a mannitol-glutamate agar medium (11) supplemented with yeast extract at 0.25 g/L (MGY medium) for 48 hr. Colonies were suspended to about 5×10^8 cfu/ml in sterile distilled water, and triplicate 10-μl samples were spotted onto MGY agar containing different levels of cupric sulfate (ranging from 0 to 3.2 mM). The minimum inhibitory concentration (MIC) of cupric sulfate was the concentration that inhibited confluent growth of the culture after 3 days at 28 C. Streptomycin sensitivity was determined by streaking cultures onto MGY medium supplemented with streptomycin at 5 μg/ml.

RESULTS

Identification of causal agent. Diseased plants from a commercial greenhouse had large water-soaked and brown to dark gray necrotic areas on leaves; some leaves were deformed. High populations of rod-shaped, motile bacteria were observed streaming into distilled water by phase-contrast microscopy of tissue sections from the lesions. Fluorescent, oxidase-negative bacteria were recovered in high numbers from diseased tissues. These bacteria were identified as *P. syringae* (Table 1). Two strains, designated PSI1 and PSI2, were selected for further analysis. Both strains were confirmed as the causal organism of the foliar blight of New Guinea Hybrid of *Impatiens* through Koch's postulates.

A gram-negative, oxidase-negative, yellow, mucoid bacterium with a single polar flagellum was isolated from a single sample but in numbers lower than those of *P. syringae* isolated from the same sample. The yellow bacterium was tentatively identified as a species of *Xanthomonas*, but it was not pathogenic to *Impatiens*.

Plant inoculations. Water-soaking occurred within 24 hr after leaves on New Guinea Hybrid and *I. wallerana* plants were infiltrated with PSI1 or PSI2. Water-soaking spread beyond the infiltrated area after 48 hr, and a gray to black necrosis developed within 72–96 hr. Leaves on spray-inoculated plants developed water-soaked leaf spots in interveinal areas and marginal water soaking 48 hr after inoculation. The lesions in the interveinal areas and margins of the leaves expanded over a period of several days to form large necrotic areas (Fig. 1). Disease symptoms on the inoculated plants were similar to those caused by natural infections on plants in the commercial greenhouse. Secondary infections occurred on new leaves several days after inoculation. Numerous motile, rod-shaped bacteria were observed streaming from sections of diseased tissues 24 hr after inoculation by infiltration; similar observations were made with lesions formed after spray

Table 1. Comparison of two presumptive strains of *Pseudomonas syringae* isolated from diseased *Impatiens* with *P. syringae* pv. *syringae*

Test	Reactions[a]	
	***P. syringae* PSI1 and PSI2**	***P. syringae* pv. *syringae*[b]**
Gram reaction[c]	−	−
Fluorescence on King's medium B	+	+
Oxidase reaction[c]	−	−
Polar flagella[d]	+	+
Levan production from sucrose[d]	+	+
Gelatin hydrolysis[d]	+	+
Arginine dihydrolase[d]	−	−
Nitrate reduction[d]	−	−
Ice nucleation activity[d]	+	+
Hypersensitive response on tobacco[d]	+	+

[a] + = Positive, − = negative.
[b] Results as reported by Krieg and Holt (13).
[c] Methods as described by Gerhardt (8).
[d] Methods as described by Schaad (17).

Fig. 1. Water-soaking and necrotic symptoms formed on leaves after spray inoculation with strain PSI1 of *Pseudomonas syringae*: **(A)** *Impatiens wallerana* and **(B and C)** *Impatiens* cv. New Guinea Hybrid.

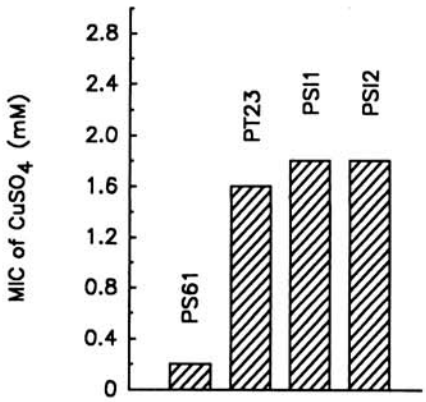

Fig. 2. Minimum inhibitory concentration (MIC) of cupric sulfate for strains of *Pseudomonas syringae isolated from Impatiens* compared with MICs for strain PS61 (previously characterized as copper-sensitive) and copper-resistant strain PT23.

inoculation. Fluorescent, oxidase-negative bacteria were consistently isolated from the diseased tissues. No symptoms were observed on control plants.

Inoculation of tomato, tobacco, common bean, and mung bean with infiltration of the two strains of *P. syringae* resulted in an apparent hypersensitive response within 24 hr. A dry, brown necrosis occurred after each infiltration; this necrosis did not spread beyond the initial infiltrated area during the 2-wk incubation period. No symptoms developed on these plants after spray inoculation with PSI1 or PSI2. Similarly, no symptoms occurred when PSI1 was infiltrated into or spray-inoculated on *D. maculata, Begonia* × *semperflorens-cultorum,* or *C. officinalis.*

The *Xanthomonas* species isolated from one *Impatiens* sample did not cause any reaction in leaves of *I. wallerana* following spray or infiltration inoculations. We have not yet conducted pathogenicity tests of this *Xanthomonas* strain on other plant species.

Determination of copper sensitivity. The MIC of cupric sulfate for strains PSI1 and PSI2 of *P. syringae* was nine times that of strain PS61 of *P. syringae* pv. *syringae* , previously characterized as copper-sensitive (4), and similar to that of the copper-resistant strain PT23 (4) of *P. syringae* pv. *tomato* (Fig. 2). In two separate experiments, confluent growth of PSI1 and PSI2 was inhibited in each of the triplicate spots on MGY agar containing 1.8 mM cupric sulfate; growth was confluent at the next lower level tested (1.6 mM).

The recovered *Xanthomonas* species was not tested on the full range of copper concentrations, but it grew confluently on MGY containing 1.0 mM cupric sulfate.

P. syringae strain PSI1 did not grow when streaked onto media containing streptomycin at 5 μg/ml. PSI2 was not tested for streptomycin sensitivity.

DISCUSSION

A bacterial blight of *Impatiens,* characterized by a rapid water-soaking and expansion of necrosis leading to some leaf deformation, appears to be caused by a pathovar of *P. syringae.* This bacterium has not been previously reported as a pathogen of *Impatiens* and, to our knowledge, the disease has not been reported on *Impatiens.* Since an extensive host range study was not conducted, we do not know if the strains from *Impatiens* represent a new pathovar of *P. syringae.* However, the failure of these strains to cause lesions in tomato, bean, mung bean, and the ornamental plants tested suggests that the pathogen has a limited host range.

The strains of *P. syringae* isolated in our study were resistant to copper in vitro. We routinely screen new isolates of bacterial pathogens for copper resistance to further our understanding of the distribution and potential spread of copper resistance in bacterial pathogen populations. Copper resistance has been reported only recently in other *P. syringae* pathovars (2,4,9,19) and in *X. campestris* pv. *vesicatoria* (1,15). In each case, the pathogens were exposed to periodic selection pressure from copper bactericides. Although the *Impatiens* grower in our study had not used copper sprays to control this bacterial disease, copper compounds are used for some other nursery crops. It is possible that the bacterium was exposed to copper on another host or that the strain acquired a copper resistance plasmid from another bacterium that was exposed to copper sprays as a pathogen or epiphyte on another plant. For example, plasmid-determined copper resistance is now common in strains of *P. syringae* pv. *tomato* from California (7). Tomato transplants on which copper sprays are used can be found close to *Impatiens* and other bedding plants in retail nurseries. In two nurseries, tomato transplants with visible bacterial speck lesions were found near *Impatiens* plants (Cooksey, *unpublished*), suggesting that opportunities for genetic exchange between the pathogens of tomato and *Impatiens* could occur. The copper-resistant *Xanthomonas* species that we isolated from *Impatiens* is another possible donor of copper resistance genes. The relationship of copper resistance genes in the strains of *P. syringae* from *Impatiens* to copper resistance genes in other copper-resistant plant pathogens (5,14,16,18,19) is under investigation.

The sensitivity of the *Impatiens* pathogen to streptomycin suggests that streptomycin treatments might be effective in control of this disease. However, the development of streptomycin resistance, including a recent report of plasmid-determined resistance (6), has been reported in several pathovars of *P. syringae* (10). These reports emphasize the need to develop alternative bactericides and other control methods.

LITERATURE CITED

1. Adaskaveg, J. E., and Hine, R. B. 1985. Copper tolerance and zinc sensitivity of Mexican strains of *Xanthomonas campestris* pv. *vesicatoria,* causal agent of bacterial spot of pepper. Plant Dis. 69:993-996.
2. Anderson, G. L., and Lindow, S. E. 1986. Occurrence and control of copper tolerant strains of *Pseudomonas syringae* on almond and citrus in California. (Abstr.) Phytopathology 76:1118.
3. Baker, K. F., ed. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23. 332 pp.
4. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato:* Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.
5. Bender, C. L., and Cooksey, D. A. 1987. Molecular cloning of copper resistance genes from *Pseudomonas syringae* pv. *tomato.* J. Bacteriol. 169:470-474.
6. Burr, T. J., Norelli, J. L., Katz, B., Wilcox, W. F., and Hoying, S. A. 1988. Streptomycin resistance of *Pseudomonas syringae* pv. *papulans* in apple orchards and its association with a conjugative plasmid. Phytopathology 78:410-413.
7. Cooksey, D. A. 1987. Characterization of a copper resistance plasmid conserved in copper-resistant strains of *Pseudomonas syringae* pv. *tomato.* Appl. Environ. Microbiol. 53:454-456.
8. Gerhardt, P., ed. 1981. Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, DC. 524 pp.
9. Gitaitis, R. D., McInnes, T. B., and Jones, J. B. 1985. A survey of Georgia and Florida for the presence of copper tolerant phytopathogenic bacteria. (Abstr.) Phytopathology 75:1288.
10. Jones, A. L. 1982. Chemical control of phytopathogenic prokaryotes. Pages 399-414 in: Phytopathogenic Prokaryotes. Vol. 2. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.
11. Keane, P. J., Kerr, A., and New, P. B. 1970. Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. Aust. J. Biol. Sci. 23:585-595.
12. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
13. Krieg, N. R., and Holt, J. G., eds. 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1. Williams & Wilkins, Baltimore. 964 pp.
14. Malvick, D., and Bender, C. 1988. Host range studies and partial characterization of a copper-resistance plasmid in *Xanthomonas campestris* pv. *vesicatoria.* (Abstr.) Phytopathology 78:1587.
15. Marco, G. M., and Stall, R. E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. Plant Dis. 67:779-781.
16. Mellano, M. A., and Cooksey, D. A. 1988. Nucleotide sequence and organization of copper resistance genes from *Pseudomonas syringae* pv. *tomato.* J. Bacteriol. 170:2878-2883.
17. Schaad, N. W., ed. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. American Phytopathological Society, St. Paul, MN. 164 pp.
18. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria.* Phytopathology 76:240-243.
19. Sundin, G. W., Jones, A. L., and Fulbright, D. W. 1989. Copper resistance in *Pseudomonas syringae* pv. *syringae* from cherry orchards and its associated transfer in vitro and in planta with a plasmid. Phytopathology 79:861-865.