Epidemiology and Control of Bacterial Leaf Spot and Stem Rot of Pelargonium hortorum

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ABSTRACT

Xanthomonas pelargonii, the causal agent of bacterial leaf spot and stem rot of *Pelargonium hortorum,* was disseminated by splashing water. Leaf infection and spotting followed inoculation of *Pelargonium* plants with water droplets containing a suspension of the pathogen. The pathogen spread systemically from leaf spots and caused stem rot and wilting of other leaves. Wound-inoculation of *Pelargonium* roots resulted in infection with delayed symptoms. Cuttings removed from these plants were also infected, but were symptomless. Weekly streptomycin or fixed copper sprays delayed symptom expression, but did not prevent disease development.

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The geranium, *Pelargonium hortorum* Bailey, is a major floricultural crop in the U.S. As geranium production became more intensified following World War II, bacterial leaf spot and stem rot caused by *Xanthomonas pelargonii* (Brown) Starr and Burkholder emerged as a serious production problem (1, 4). Cuttings removed from infected stock plants have been demonstrated to be symptomless carriers of *X. pelargonii* (2). The basic control procedure is to exclude the pathogen during growth of the crop. No method is currently available for control of disease outbreaks that occur through inadvertent use of infested stock.

The objectives of this study were: (i) to study the means by which *X. pelargonii* is disseminated under greenhouse conditions; (ii) to apply this information toward modifications in cultural practices; and (iii) to evaluate chemicals which may be useful in controlling the disease.

MATERIALS AND METHODS.—The isolate of *X. pelargonii* used was obtained from diseased plants in an Ohio greenhouse. Initial identification of the pathogen (Ohio-2) was by comparison with a known *X. pelargonii* isolate (XP-42) provided by R. S. Dickey, Cornell University. Ohio-2 was comparable to XP-42 in terms of acid and gas production on 12 carbohydrate sources (2).

Pathogenicity of isolates was determined by a cutting inoculation technique (3, 6). Entire plants were used to demonstrate the range of symptoms. The pathogen was commonly reisolated, purified by streaking, and reinoculated into plants. Ohio-2 was repeatedly found to be more pathogenic than XP-42.

Inoculum was prepared by seeding potato-dextrose broth with *X. pelargonii* from potato-dextrose agar (PDA) slants. Potato-dextrose broth was adjusted to a starting pH of 6.8 by the addition of approximately 3 ml/liter 1.0 M K_2HPO_4 and 3 ml/liter 1.0 M KH_2PO_4. The broth cultures were incubated for 48 h at room temp on a shaker (100 cycles/min). Bacterial cells were harvested by centrifugation at 10,000 *g* for 10 min. The cell pellet was resuspended in 0.001 M phosphate buffer at pH 6.8. The amount of buffer added was equal to the volume of the medium from which the cells were harvested.

Pelargonium hortorum 'Sincerity', 'Irene', and 'Orange Glow' were used in this study. The disease severity was evaluated using a 0 to 5 scale, as follows: 0 = healthy; 1 = one leaf with leaf spots or wilt, no stem lesions; 2 = up to one-half of the plant wilted, leaf-spotted or defoliated, no stem lesions; 3 = same as 2 but with visible stem lesion; 4 =

TABLE 1. Rate of recovery of *Xanthomonas pelargonii* from plant parts 3 wk after leaf inoculation of *Pelargonium hortorum* with droplets of a bacterial suspension[a]

Plant	Leaf spot development[b]	Isolations of Pathogen[c] A	B	C	D
1	4/11	0/3	2/29	0/15	2/15
2	6/28	1/5	8/37	0/7	0/7
3	3/14	3/3	6/6	4/5	4/5
4	3/23	2/3	7/16	2/4	1/4
5	1/26	1/3	15/18	1/1	2/2
6	6/15	0/7	0/10	-	-
7	1/26	0/8	0/15	-	-

[a]Droplets (0.5 ml) of 0.001 M phosphate buffer containing approximately 5×10^6 bacterial cells were carefully placed on *P. hortorum* leaves to avoid wounding. Petioles of inoculated leaves were ringed with lanolin and the plants were watered with Chapin irrigation system.

[b]Fractions indicate the number of inoculated areas which contained leaf spots versus the total number of inoculations.

[c]Locations of isolation attempts: A = petioles of inoculated leaves; B = stem pieces; C = petioles of noninoculated leaves; D = blades of noninoculated leaves.

[d]Fractions indicate successful isolations versus total attempted.

plant totally wilted, advanced stem rot or multiple stem lesions; 5 = dead.

Several chemicals were evaluated for use in controlling the disease: 17% streptomycin (Agri-Strep, Merck & Co., Rahway, N.J.) was used alone and in combination with adjuvants L-324, Multifilm X-77 and Regulaid (Colloidal Products, Petaluma, Calif.); 83% cupric hydroxide (Kocide 101, Kennecott Copper Corp., New York, N.Y.); HPMTS (2-hydroxypropyl methane thiosulfonate, as 80% E.C., 25 WP, or 50 WP, Buckman Laboratories, Inc., Memphis, Tenn.) was used alone and in combination with WSCP adjuvant (Buckman Laboratories); Bunema (potassium *N*-hydroxymethyl-*N*-methyl dithiocarbamate, 40% E.C., Buckman Laboratories); and MBR 10995 (Experimental 25 WP, Commercial Chemicals Div., 3M Company, St. Paul, Minn.).

TABLE 2. Inhibition of *Xanthomonas pelargonii* by various chemicals in filter paper disks on potato-dextrose agar

Material	Inhibition Zone (mm)[a] Concn of material (active ingredient, μg/ml)[b] 50	100	500
Streptomycin	4	5	-[c]
Streptomycin + 60 μg/ml (4 oz/100 gal) L-324	5	6	-
HPMTS	8	11	16
HPMTS + 2% WSCP	9	12	15
HPMTS (50 WP)	7	9	13
HPMTS (25 WP)	4	5	9
Cupric hydroxide	0[d]	-	-
Bunema	-	0	2
MBR 10995	3	6	13

[a]Inhibition zones were measured 48 h after placing the disks on seeded agar plates and were defined as the distance in mm from edge of paper disk to edge of bacterial growth. Each zone value is the mean of 20 replicates.

[b]Filter paper disks were soaked in various concns of chemicals listed and placed on potato-dextrose agar plates that had previously been seeded with 48-h shake culture suspension of *X. pelargonii*.

[c]Dashes indicate that materials were not tested at these concns.

[d]Inhibited *X.* pelargonii. at 50 μg/ml in liquid culture.

For in vitro evaluation, filter paper disks were soaked in dilutions of these chemicals, dried, and plated on PDA plates seeded with *X. pelargonii*. After 48 h, inhibition zones were measured between the paper disk and the edge of the bacterial "lawn" at the largest distance.

To test in vivo effects of these chemicals, flats of 30 rooted geranium cuttings or groups of 25 potted plants were sprayed weekly for varying lengths of time. Two plants in the center of each group of plants were inoculated by rubbing the leaves with cotton swabs that had been dipped in a bacterial suspension (about 10^8 cells/ml). Overhead watering provided dissemination of *X. pelargonii* to the noninoculated plants. Disease ratings and phytotoxicity were noted weekly.

None of the isolates lost pathogenicity or ability to cause leaf spot during 36 mo of storage at 8 C with monthly transfers. Isolates Ohio-2 and XP-42 survived 10-mo storage on PDA slants at 8 C without transfer and retained their ability to cause both leaf spot and stem rot symptoms.

RESULTS.—To demonstrate the effect of overhead watering on disease spread, blocks of Sincerity and Irene plants in 10.2-cm (4-inch) diam pots were arranged pot-to-pot on benches in nine rows of 12 pots. Five diseased plants were spaced in the blocks, and they were overhead watered for 50 days. At the same time, other blocks of plants were watered by Chapin-type irrigation tubes. After 44 days, every overhead-watered Sincerity plant was diseased, with an average severity rating of 3. After 50 days, all the Irene plants were diseased, also averaging 3 on the severity rating. The plants watered by Chapin tubes remained symptomless.

To determine whether spread of the pathogen could occur during cutting propagation, six dozen cuttings were rooted under mist in groups of 12 in 51.6 cm^3 (8-in^3) compressed lignin propagating blocks (BR-8 blocks, Famco Inc., Medina, Ohio). One cutting in each BR-8 block group was inoculated. All of the originally inoculated cuttings rotted without rooting after 21 days. Cuttings located closest to the inoculum source were most frequently diseased. At 1, 2, 3, 4, and 5 inches from the inoculated cuttings, 44, 42, 16, and 8%, respectively, of cuttings had leaf spots.

To determine whether the pathogen could cause the complete disease syndrome from leaf inoculation, 0.05-ml droplets containing approximately 5×10^6 bacterial cells in 0.001 M phosphate buffer were carefully placed on leaf adaxial surfaces. The plants were Chapin-irrigated in 10.2-cm (4-inch) diam pots. Aluminum foil was placed over the Chapin tubes and the soil at the base of the plants to prevent water from splashing onto the upper parts of the plants. The petioles of inoculated leaves were ringed with lanolin to prevent bacteria from running down the petiole to the stem. Leaf spots occurred in 17% of the cases where the droplets had been placed on plants (Table

1). After 3 wk, isolations were made from all areas of the inoculated plants. The bacteria moved in varying degrees from leaf spots in all but two of the test plants (Table 1).

To further test the hypothesis that the pathogen travels from leaves to stems through petioles, 16 plants were inoculated on only one leaf. After 33 days, isolations were attempted. The pathogen was found in stem sections of 4 out of 12 plants which exhibited leaf spots at the inoculation site. Four of the 16 plants neither developed leaf spots nor yielded the pathogen from stem sections.

To determine whether infected cuttings could be removed and propagated from inoculated but symptomless stock plants, Sincerity plants in 15.2-cm (6-inch) diam pots were inoculated by dipping the roots into bacterial suspensions. Twenty days after inoculations, the plants showed no symptoms and cuttings were removed. Bacterial isolations were attempted from the bottom 2-3 mm of each cutting. The rest of the cutting was rooted under mist and potted after 16 days. Cultures from 24 of 46 cuttings contained the pathogen 19 days after potting and 34 of the 46 resulting plants exhibited symptoms of disease. Thirteen of these diseased plants were derived from cuttings that did not provide the pathogen in culturing. Nineteen died from bacterial stem rot during the 78 days, while nine did not develop symptoms. Of these nine, four were from cuttings in which the pathogen was detected by culturing.

HPMTS, Bunema, streptomycin, and MBR 10995 inhibited *X. pelargonii* in vitro (Table 2). However, no control was noted at subphytotoxic levels in the in vivo trials. Streptomycin and cupric hydroxide appeared to provide some control of the disease with only mild or occasional phytotoxicity (Table 3).

An experiment to more fully evaluate the usefulness of 17% streptomycin and 83% cupric hydroxide, was carried out over a longer period than previously used. Whereas the disease was under control in the sprayed blocks at 61 days, by 76 days little effective control was evident.

DISCUSSION.—*Xanthomonas pelargonii* has been reported to lose pathogenicity quickly in artificial culture (1, 2). Ohio-2 and XP-42 survived and were pathogenic after 10 mo at 8 C on PDA slants.

Splashing water easily disseminated the pathogen during either propagation in a mist area or routine overhead watering operations. Bacteria placed on leaves without intentional wounding caused lesions. Our studies demonstrated systemic movement of the pathogen from leaf spots. Furthermore, this systemic invasion led to the complete wilt and rot syndrome. Spread through petioles was proposed by Munnecke (2), but Kivilaan and Scheffer (1) were unable to demonstrate it.

As originally reported by Munnecke (2), we were also able to procure infected cuttings from apparently healthy stock plants. Plants resulting from these cuttings occasionally remained apparently healthy for weeks. As suggested by Wainwright and Nelson (5), detection of the pathogen by culturing one slice from the bottom of a cutting did not always reliably indicate the infestation of the cutting.

Several chemicals inhibited *X. pelargonii* in vitro but either failed to protect plants when applied weekly as a foliar spray or showed excessive phytotoxicity. Cupric hydroxide (83%) at 250 μg/ml and 17% streptomycin at 100 μg/ml were the two most effective treatments made. Both materials reduced disease severity as compared with the control, but caused some phytotoxicity. Both acted only to suppress, but not control, the disease; thus they cannot be recommended for use.

TABLE 3. Summary of the effects of 17% streptomycin and 83% cupric hydroxide as five weekly foliar sprays on mean disease ratings of *Pelargonium hortorum* plants[a]

Material	Rate (μg/ml)	Phyto-toxicity[b]	Disease Ratings[c]	
			Treated	Con-trol
Streptomycin	500	+++	1	1
Streptomycin	500 + L-324[d]	+++	0	1
Streptomycin	500 + X-77	+++	0	1
Streptomycin	100 + X-77	++	0	1
Streptomycin	100 + L-324	++	1	3
Streptomycin	100	+	1	4
Streptomycin	100 + Regulaid	++	2	4
Cupric hydroxide	600	−	1	1
Cupric hydroxide	360	−	1	1
Cupric hydroxide	240	+	2	4
Cupric hydroxide	120	−	0	3
Cupric hydroxide	60	−	2	3

[a]The table summarizes five experiments.

[b]− = no phytotoxicity; + = slight phytotoxicity; ++ = moderate phytotoxicity; and +++ = severe phytotoxicity.

[c]Disease ratings: 0 = healthy; 1 = one leaf with leaf spots or wilt, no stem lesion; 2 = up to 1/2 of plant wilted, leaf-spotted or defoliated, no stem lesion; 3 = same as 2 but with visible stem lesion; 4 = plant totally wilted, advanced stem rot or multiple stem lesions; 5 = dead.

[d]All adjuvants used at 60 μg/ml.

The lack of a truly effective chemical, the extreme ease with which the pathogen is disseminated, and the considerable delay between infection and symptom expression in plants, emphasize the need to exclude the pathogen for effective disease control. The use of culture-indexed stock plants grown with good sanitation practices remains the only method to avoid losses to this disease.

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