Bacterial Leaf and Stem Rot of Geranium in Minnesota

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ABSTRACT

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A severe and widespread epidemic of leaf and stem rot of geranium (*Pelargonium* × *hortorum*) caused by *Xanthomonas campestris* pv. *pelargonii* occurred on both greenhouse- and field-grown plants in Minnesota in 1983. The pathogen survived up to 221 days in air-dried leaves placed on the soil surface of a greenhouse bench but was not detected after 11 days in diseased leaves that were buried in soil. When introduced onto leaves or into wounds of 17 common nonhost species of commonly propagated greenhouse ornamental plants, the bacterium could be detected in wounds of 11 and on leaves of three of the species 28 days later. Removal of significant amounts of foliage from host plants significantly increased disease severity in two of four cultivars. Plants subjected to long photoperiods did not appear to be more susceptible. All commercial geranium cultivars tested were susceptible.

Additional key words: host range, predisposition, survival

Commercially grown zonal and ivy geranium (*Pelargonium* × *hortorum* Bailey and *P. peltatum* L.) plants in varying stages of decline from several greenhouses were brought to our attention in the spring and summer of 1983. Leaves had water-soaked angular lesions as well as advanced necrotic lesions. Stems on wilting or dying plants frequently possessed irregularly shaped light brown lesions. An abundance of bacteria exuded from diseased material placed in water. Symptoms matched those described for bacterial blight (8), and we suspected the causal agent to be *Xanthomonas campestris* pv. *pelargonii*. Losses were severe in both greenhouse and outdoor plantings. Our objective was to isolate the causal agent, evaluate cultivar resistance, and establish length of pathogen survival in host tissues under greenhouse conditions and possible survival in nonhost floricultural plants. Also, because the disease was not observed during winter months, information on factors that might enhance host susceptibility and methods to detect presence of the pathogen in winter-grown stock plants were evaluated.

MATERIALS AND METHODS

Isolations were made from leaf spots or stems of wilted plants via dilution plating on nutrient agar or on Kado's medium 523 (2). Pigment formation was determined on nutrient agar, King's medium B, and yeast-dextrose carbonate (YDC) in accordance with methods outlined by Schaad (7). Suspecting that the bacterium was *X. campestris* pv. *pelargonii*, we proceeded to isolate and grow the bacterium on the semiselective medium reported by McGuire et al (4) to be a good substrate for *X. c.* pv. *pelargonii*.

Pathogenicity and survival. Four plants each of 10 commonly grown cultivars of geranium plants in the four- to eight-leaf stage were inoculated by spraying suspensions of the bacterium on undersurfaces of leaves or by placing 2 μl of suspension adjusted turbidometrically to contain 1×10^8 colony-forming units (cfu) per milliliter in stem wounds. Stem wounds were made with a sharp-pointed scalpel, inserted so that the cutting edge was at a 90° angle to the stem axis; the point extended about to the center of the stem. All experiments were made in a greenhouse held at 28 ± 5 C on plants obtained from a commercial grower. Plants originated from indexed stock plants certified to be free of *X. c.* pv. *pelargonii*. Young plants grown in peat moss in plastic pots 10 × 10 cm and fertilized once a week with 250 ppm of Peters (15:16:17 NPK) Geranium Special were used in all inoculation trials. To evaluate possible epiphytic survival on leaves of geranium and other floricultural plants normally grown with geranium, bacterial suspensions containing about 1×10^8 cfu/ml were gently sprayed on upper and lower surfaces of leaves until runoff (but without water-soaking) of 10 cultivars of zonal geranium and 17 other common greenhouse-grown floricultural plants. Zonal geranium cultivars included Cherry Blossom, Crimson Fire, Irene, Penny, Picardy, Pink Camelia, Saturn, Sincerity, Toreador, and Yours Truly. The nonhost crops were: begonia (fiberous) Charms and Lady Francis (*Begonia* × *semperflorens-cultorum* Hort.); begonia (tuberous) Nonstop Pink (*B.* × *tuberhybrida* Voss); chrysanthemum Jackpot (*Chrysanthemum* × *morifolium* Rarat); chrysanthemum Yellow Daisy (*C. frutescens* L.); coleus (*Coleus* × *hybridus* Voss); fuchsia Little Beauty (*Fuchsia* × *hybrida* Hort.); geraniums Happy Thoughts and Wilhelm Languth (*P.* × hortorum); ivy geraniums Beauty of Eastbourne, Cornell, Mexicana Ivy, Shone von Grenchen, and Sybil Holmes (*P. peltatum*); geranium Martha Washington (*Pelargonium domesticum* L.); Salmon impatiens (*Impatiens wallerana* Hook. f.); New Guinea impatiens (*I.* × *hybridus*); lantana Golden Plume (*Lantana camara* L.); spike (*Cordyline indivisa* Steud.); asparagus spring rye (*Asparagus densiflorus* (Kunth) Joessp), verbena (*Verbena* × *hybrida* Voss); and vinca vine (*Vinca major* L.). Stems of plants were inoculated by dipping a pointed scalpel in the suspension and then making three stab wounds 2–5 mm apart on a selected stem location; wounds extended about to the center of the stem. String tags were used to mark leaves sprayed, and a wick pen was used to mark locations of stem inoculations. Leaves were sampled after 9, 25, and 28 days. A 7-mm-diameter cork borer was used to cut 10 disks from five marked leaves; subsequently, each group of disks was placed in a flask that contained a diluent of 0.5% saline and 0.1% Tween 20. Flasks were then placed on a shaker for 20 min, and loops of suspension were streaked on semiselective medium. Stems were sampled after 9, 25, and 28 days. A cork borer was used to cut out a 16-mm section of stem containing the inoculation site; stem sections were then ground in a mortar with 1 ml Tween-saline and similarly streaked on the semiselective medium. All plates were incubated at 27 C and observed periodically for 7 days. Relative abundance of bacteria was estimated on a rating scale of 0–4 (0 = no growth, 4 = typical colonies were abundant and confluent). A sterile needle was dipped into one or more selected colonies that grew on agar surfaces and stabbed into leaf midveins and petioles of geranium cultivar Irene as a test for pathogenicity. Persistence or growth of the bacterium in stems was monitored via dilution plating of crushed stems on semiselective medium 1, 2, 3, 4, 7, 9, and 11 days after inoculation.

Survival of the pathogen was evaluated by use of heavily infected air-dried leaves (16.5 g) and petioles (3.0 g) chopped in a

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Table 1. Persistence of *Xanthomonas campestris* pv. *pelargonii* on leaf surfaces and in stems of host and nonhost plants

Host	Time after application of bacteria (days)[a]										Bacteria per stem ($\times 10^5$)	
	9				25				28			
	Assay[b]		Symptoms[b]		Assay		Symptoms		Assay			
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Day 2	Day 9
Begonia (fiberous), Lady Francis	0	0	N	N	0	0	N	N	0	3	0	0
Begonia (tuberous), Nonstop Pink	1	0	N	N	0	2	N	N	0	2	–	–
Begonia (variegated), Charms	0	0	N	N	0	0	N	N	0	1	0	0
Chrysanthemum, Jackpot	2	2	N	N	0	4	N	N	0	3	36	5
Chrysanthemum, Yellow Daisy	2	2	N	N	0	2	N	N	0	2	3	1
Coleus	0	2	N	N	0	0	N	N	0	2	6	2
Fuschia, Little Beauty	2	2	N	N	1	0	N	N	1	3	4	0
Martha Washington (*P. domesticum*)	–	–	–	–	3	3	N	N	–	–	–	–
Geranium, Beauty of Eastbourne (ivy)	3	3	N	SW	1	4	SW	SW	3	4	–	–
Geranium, Cornell (ivy)	3	3	N	SW	3	4	SW	SW	2	4	–	–
Geranium, Mexican Ivy (ivy)	–	–	–	–	–	–	SW	SW	2	4	–	–
Geranium, Shone von Grenchen (ivy)	–	–	–	–	–	–	SW	SW	3	4	–	–
Geranium, Sybil Holmes (ivy)	3	3	N	SW	1	4	SW	SW	3	4	–	–
Geranium, Happy Thoughts (zonal)	3	3	N	SW	2	–	N	D	–	–	–	–
Geranium, Irene (zonal)	3	3	N	SW	1	4	D	D	1	4	9	4,300
Geranium, Wilhelm Languth (zonal)	3	3	N	SW	1	4	D	D	3	3	–	–
Impatiens, Salmon	2	2	N	N	0	1	N	N	1	2	2	4
Impatiens, New Guinea	2	2	N	N	0	3	N	N	0	2	3	7
Lantana, Golden Plume	2	2	N	N	0	1	N	N	0	2	–	–
Spike	2	–	N	N	0	0	N	N	0	0	–	–
Spring rye	–	2	–	N	0	1	N	N	0	0	–	–
Verbena	2	2	N	N	0	1	N	N	1	3	–	–
Vinca vine	–	2	–	N	0	0	N	N	0	0	–	–

[a] Leaf surfaces: suspension of 1×10^8 bacteria per milliliter fogged on leaves until wet. Stems: 2×10^5 bacteria placed on stem wound.
[b] 0 = Pathogen not present via plate assay on selective medium, 1 = one or two colonies, 2 = 3–100 colonies, 3 = more than 100 colonies, 4 = confluent colony growth from plate streak dilutions, and – = not done (84/84 and 64/64 individual colonies isolated from leaf surfaces and isolated from stem punctures, respectively, were pathogenic). Symptoms: N = none, SW = systemic wilt, and D = dead plant.

blender (one experiment) or used whole (two experiments) and placed in nylon bags 2×5 cm^2. Each bag contained 0.75 g of the mixture. Bags were then placed under greenhouse benches or on sand surface of benches, buried 3–5 cm deep in bench sand, or suspended in air 1 m above benches. Periodically thereafter for 3 wk, bags were removed and contents ground in 7.5 ml of water, and a portion of the ground sample was injected into leaves, petioles, and stems of the susceptible geranium cultivar Irene.

Predisposition of host to infection. To evaluate the effects of day length and temperature on development of disease, susceptible geranium cultivars Toreado and Irene were inoculated at 20 or 27–30 C under day lengths of 6, 10, 16, 20, or 24 hr. Two leaves from young plants (four- to 10-leaf stage) were inoculated by severing midveins at center of the leaf and depositing 2 μl of a suspension containing 1×10^8 cfu/ml into the wound. Disease severity was evaluated over a period of 4–20 days on a scale of 0–3, where 0 = no disease, 1 = water-soaked tissues surrounding inoculation site or necrosis on half or less of leaf, 2 = wilted leaf or more than half leaf necrotic, and 3 = wilted or dead plant.

In a second experiment, plants were stressed by removing all stems with flowers and all but one of the remaining branch stems of cultivars Irene, Picardy, Toreado, and Saturn immediately before inoculating. Plants were about 2 mo old, were flowering, and had two to four branching stems each. All plants were grown in growth chambers with a 16-hr day at temperatures of 18–22 C at night and 27–29 C during the day. Wounds at the centers of midveins of six leaves per plant were inoculated as described. Disease severity was evaluated 20 days later according to percentage of wilt: 1 = none, 2 = 1–25, 3 = 26–50, 4 = 51–75, 5 = 76–100, and 6 = dead plant.

RESULTS

Pathogenicity. A relatively slow-growing pale yellow bacterium was consistently isolated from leaf spots or stems of wilted plants. Regardless of inoculation method, symptoms of disease (water-soaking, wilt, or death) appeared within 3–5 days. The pathogen initially caused angular water-soaked leaf spots on spray-inoculated leaves, but infection quickly became systemic as leaves wilted, petioles shriveled, stems became necrotic, and plants died in rapid succession. Forty plants of 10 commonly grown cultivars of geranium all became systemically diseased or died within 26 days of inoculation. In four experiments, the bacterium persisted on leaves of geranium for at least 1 mo but tended to decline on nonhosts (Table 1). The bacterium also persisted in stem wounds of nonhosts.

Four cultures of the pathogen originally isolated from plants obtained from different growers were gram-negative rods, mucoid, cream-pale yellow on nutrient agar, and deeper yellow on YDC, did not hydrolyze starch, grew profusely and were lipolytic on a medium semiselective for *Xanthomonas*, and were motile via a single polar flagellum.

Survival. Populations of the bacterium remained viable in infected plant material suspended in air, placed on the soil surface under greenhouse benches, placed on the soil surfaces of greenhouse benches, and buried in sand in greenhouse benches (Table 2).

Predisposition to infection. Day length was not a significant factor in disease development, but long days had an important effect on health of both inoculated and uninoculated plants. Inoculated plants at 27–30 C showed water-soaking within 6 days and varying degrees of systemic wilt within 20 days, whereas those at 20 C showed water-soaking within 6 days and necrosis of the inoculated leaf plus occasional petiole collapse and systemic wilt within 20 days. Severe chlorosis developed in both geranium cultivars Irene and Toreado within 10 days of exposure to 20-hr days or continuous light; this effect was more severe at the higher temperature.

The effects of trimming were associated with a distinct cultivar difference in susceptibility and increased disease attributable to trimming (Table 3).

DISCUSSION

The bacterium was able to survive on leaves or in wounds on stems of several ornamental species that might be grown near geraniums commercially. These plants could become a source of inoculum for new crops of geranium grown in the vicinity. Survival of the organism in debris from diseased plants in the greenhouse is consistent with other reports (6,8). Sanitation is therefore an

Table 2. Survival of *Xanthomonas campestris* pv. *pelargonii* in infected plant debris stored in four places in the greenhouse

Age of sample when assayed[a] (days)	Origin of sample: Air			Soil surface under bench			Soil surface on bench			Buried (3–5 cm in bench sand)		
	1[b]	2	3	1	2	3	1	2	3	1	2	3
0	+[c]	+	+	...	...	...	...	...	...	...	...	...
7	+	−	−	+	−	−	+	−	−	+	+	+
10	−	+	−	−	+	−	−	+	−	−	+	−
11	−	−	−	−	−	−	−	−	−	−	−	+
14	+	+	−	+	+	−	+	+	−	0	0	0
28	+	+	−	+	+	−	+	+	−	0	0	−
35	−	−	−	−	−	−	−	+	−	−	−	−
56	+	−	+	+	−	+	0	−	0	−	−	−
70	−	0	−	−	+	−	−	+	−	−	−	−
84	+	−	+	+	−	+	+	−	0	−	−	−
112	−	−	+	−	−	0	−	−	0	−	−	−
140	−	−	+	−	−	0	−	−	0	−	−	−
154	−	−	0	−	−	0	−	−	0	−	−	−
182	−	−	0	−	−	0	−	−	0	−	−	−
203	−	−	0	−	−	0	−	−	0	−	−	−
221	−	0	−	−	+	0	−	−	−	−	−	−

[a] Air-dried, infected plant material (0.75 g) ground with 7.5 ml water; needle dipped in slurry and used to stab leaves, petioles, and stems of susceptible geranium cultivar Irene.
[b] Trial.
[c] Bioassay (+) indicates survival, − indicates no assay, and 0 indicates no survival.

Table 3. Effect of foliage removal on disease development of four geranium cultivars inoculated with *Xanthomonas campestris* pv. *pelargonii*

Geranium cultivar	Treatment	Disease index[a] (mean)	Standard error	*F* value
Irene	Trim[b]	4.75	0.5439	0.13 NS
	No Trim	4.50	0.4282	
Picardy	Trim	4.58	0.2000	15.97**
	No Trim	3.00	0.3416	
Toreado	Trim	4.75	0.4425	12.50**
	No Trim	2.60	0.4000	
Saturn	Trim	6.00	0	...
	No Trim	6.00	0	...

[a] Disease index: 1 = no wilt, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%, and 6 = dead plant after 20 days.
[b] Plants with two to four branch stems and one to four flowering stalks were trimmed to one branch and no flowering stalks; six leaf midveins were severed on each plant, and 2×10^5 bacteria (2 µl of a suspension containing about 1×10^8 cfu/ml) was applied to wounds.

important consideration for geranium culture in previously infested greenhouses. Although, in our experiments, the pathogen disappeared within 11 days in buried leaves, survival for at least 3 mo in moist soil has been reported (6).

Our attempt to enhance sensitivity of plants by placing stress on the host plant (manipulation of the environment by varying light and temperature regimes) or by removing foliage from fast-growing plants in the early flowering stage was partially successful. A means of early detection of infected plants before the cutting and planting operation in early spring would be useful. In two of four cultivars tested, it appeared probable that removing foliage on mature plants could increase severity of the disease. This procedure could, however, predispose plants to other diseases or weaken planting stock. Others have reported increased susceptibility at high (27 C) night temperatures (3). In tests reported here, disease was more severe at 27–30 C than at 20 C but was not affected by day length varying from 6 to 24 hr.

Bacterial leaf spot and stem rot of geranium observed in Minnesota in 1983 appeared to be similar to that described by others (1,5,8). Our conclusion that the causal agent is probably *X. c.* pv. *pelargonii* was based on symptomatology, host range, presence of a non-water-soluble pigment in culture, mucoid growth, presence of a single polar flagellum, and growth on a medium semiselective for *Xanthomonas*. The origin of the pathogen was unknown. The disease was not common in 1984, 1985, and 1986.

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