Disease Symptomatology and Variation in Susceptibility of Seed-Propagated Hybrid Geranium Varieties to *Xanthomonas campestris* pv. *pelargonii*

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ABSTRACT

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Ten of the 63 seed-propagated F_1 hybrid geranium varieties were found to possess some resistance to *Xanthomonas campestris* pv. *pelargonii* based on either a foliar disease rating index or plant survival. In two of the more resistant varieties, rapid leaf abscission and absence of a systemic population of the bacteria in the vascular system was observed. The optimal plant age for screening plants for resistance was 80–95 days after seeding. At this plant age, applications of chlormequat chloride to reduce the growth of plants had no effect on disease incidence or severity. Supplemental high-pressure sodium illumination also had no effect. The development of bacterial wilt on diploid seed-propagated geranium was similar to that on tetraploid cutting geranium except that in the former, lesions often coalesced into large, blighted, necrotic areas, a symptom not normally observed on the latter. The moderately resistant cultivars identified in this study appear to be useful for breeding programs.

Bacterial wilt, also known as bacterial stem rot and leaf spot, of tetraploid cutting geraniums (*Pelargonium* × *hortorum* Bailey) is caused by *Xanthomonas campestris* pv. *pelargonii* (Brown) Dye (1). This disease has been recognized since 1923 as producing serious losses during all stages of plant production. A diverse range of symptoms has been observed in geraniums (3,4,7,8,10). Localized symptoms include water-soaked lesions 2–3 mm in diameter on the lower leaf surface, often surrounded by a yellow halo. The lesions dry out and become necrotic. Eventually the entire leaf wilts and dies.

In systemically infected plants, V-shaped lesions develop in leaves as water-soaked areas bounded by veins. Ultimately, the lesions become necrotic with chlorotic edges, and the leaves may wilt and die while still attached to an apparently healthy petiole.

Bacterial wilt has been traditionally associated with tetraploid cutting and ivy geraniums ($2n=36$) because the bacterium is easily spread during asexual propagation of the plant. Because diploid geraniums ($2n=18$) are grown from seed, bacterial wilt problems were not anticipated. However, during the past 4 yr the disease was observed in seed-propagated geraniums submitted to the Plant Disease Diagnostic Clinic of Michigan State University (S. K. Perry, *personal communication*). With the growing popularity of seed-propagated geraniums, concerns have risen regarding *X. c.* pv. *pelargonii* and its possible effect on the seed-propagated geranium industry. Resistance to *X. c.* pv. *pelargonii* may be an important disease control strategy because current chemical controls are ineffective (3). Essentially nothing is known of genetic differences in resistance in seed-propagated geranium varieties. The objectives of the research reported here were to 1) determine whether resistance is present in available seed-propagated geranium varieties and 2) observe the disease development of bacterial wilt on seed-propagated geraniums caused by *X. c.* pv. *pelargonii*.

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MATERIALS AND METHODS

Sixty-three F_1 hybrid geranium varieties derived from inbred parents were obtained from the Ball Seed Co., West Chicago, IL, and the Department of Horticulture, Michigan State University, East Lansing. Seeds were sown onto a moist, soilless medium for plant production (Sunshine Mix, Blend 1, Fisons-Western Corp., Vancouver, BC, Canada) in 18 × 13 × 6 cm trays, 20 seeds per tray. These trays were incubated at 24 C with misting as needed to prevent the medium from drying. At approximately 35 days, the 2- to 5-cm seedlings were transplanted into 9.6-cm-diameter clay pots containing the same potting medium.

The plants were grown in a 20-hr light period with illumination by high pressure sodium (HPS) lights (58.56 $\mu mol \cdot s^{-1} \cdot m^{-2}$) at 24 C (day) and 21 C (night). Plants were hand-irrigated daily, and fertilizer (200 ppm liquid aqueous solution of 20% N, 20% P_2O_5, 20% K_2O) was injected at each watering through the irrigation line; 200 ppm of phosphoric acid was added to the water to maintain pH at 6. The plants were 80 days old (45 days after transplanting) when inoculated.

Inoculation. Inoculum was prepared from a culture of *X. c.* pv. *pelargonii* originally isolated from geraniums grown in greenhouses in Detroit, MI. This strain, AP-10, was found to be as aggressive on various seed-propagated and cutting geranium varieties as other strains of *X. c.* pv. *pelargonii* collected from around the country (12). Bacteria from cultures stored in 0.85% NaCl (w/v) at 4 C were streaked onto Lederberg's complete medium (LCM) in petri plates (9). Lederberg's complete agar medium consists of 10 g of casamino acids, 5 g of yeast extract, 3 g of K_2HPO_4, 1 g of KH_2PO_4, and 15 g of agar suspended in 1,000 ml of sterile distilled water. A single colony was aseptically transferred to a 125-ml flask containing 50 ml of complete broth (LCM less the agar) (9). The flask was shaken on a rotary shaker at 180 rpm for 2 days. The bacterial culture was diluted with sterile distilled water to about 1×10^6 cfu per milliliter as determined by standard dilution-plating and turbidimetric assays.

Before inoculation, flower buds were removed to prevent the development of Botrytis blossom blight, which is common to geraniums held in mist chambers. The plants were sprayed to runoff with the bacterial suspension and were placed in a mist chamber at 24 C. Control plants were similarly treated with complete

broth diluted with sterile distilled water only. The plants were misted to keep leaf surfaces moist. After 3 days, the plants were removed from the mist chamber and placed on a greenhouse bench under HPS lighting at 29 C day and 21 C night temperatures and watered as described previously. Foliar disease was rated after 21 days based on a scale of 1–4, where 1 = no visible disease, 2 ≤ 25% of leaf area diseased, 3 = 25–50% of leaf area diseased, and 4 ≥ 50% of leaf area diseased. After 28 days, the number of dead plants was recorded and survivors were checked for the presence of the bacteria in the vascular tissue, using a modification of the methods developed by Yount and Rhoads (13). A 2.0-cm stem section was removed from each plant approximately 8–10 cm above the soil line, washed in a 0.25% sodium hypochlorite solution for 10 min, and rinsed in sterile saline solution. A 0.25-cm section was trimmed from each end and the center portion was crushed in 5 ml of sterile saline solution. The resulting suspension was streaked onto LCM plates with a sterile loop and the plates were incubated at room temperature. Yellow, mucoid colonies appearing in 3 to 4 days were presumptive for *X. c.* pv. *pelargonii*.

Treatments. Preliminary tests with two seed-propagated geranium varieties, Pinwheel Salmon and Salmon Express, were conducted to determine effects on disease development of plant age, chlormequat chloride (CCC), or lighting. For plant age, plants were inoculated in 10-day increments beginning 10 days after transplanting and continuing up to full flower development (approximately 70 days). CCC, a commercial growth retardant, was sprayed on 35-day-old plants to leaf wetness at a rate of 1,500 ppm. Tap water was applied to control plants. Plants were inoculated at 0, 10, 20, 30, and 40 days following the CCC treatment as described above. To test the effect of HPS lighting, 80-day-old plants were inoculated and exposed for 20 hr per day to HPS illumination (58.56 $\mu mol \cdot s^{-1} \cdot m^{-2}$) or held in a 12-hr photoperiod (normal day length) without supplemental HPS light.

In the studies of the effect of CCC and plant age on host susceptibility, treatments were completely randomized on a single greenhouse bench. There were 10 single-plant replications of each treatment. In the test of supplemental illumination with HPS, equal numbers of plants were placed in adjacent greenhouses that differed only in light source. Both of these tests were conducted five times. The plant age studies were subject to an analysis of variance including single degrees of freedom for trend effects. The CCC treatments were subjected to an analysis of variance and a correlation was made between CCC and non-CCC treated plants. Lighting effects were compared using an analysis of variance.

In the comparison of varietal susceptibility to *X. c.* pv. *pelargonii*, plants of 63 seed-propagated varieties were spray-inoculated with strain AP-10 of *X. c.* pv. *pelargonii* and were rated as described above. To maintain uniform plant size and to prevent growth to stages that are susceptible to Botrytis leaf spot, 33 of the varieties had been treated previously with CCC. Later, 30 of the 63 varieties were retested, except that plant mortality and presence of the bacteria in the vascular system were evaluated at 30 rather than 49 days after inoculation. According to environmental information collected with a computerized greenhouse environment control system, natural irradiance levels decreased rapidly during the first experiment (initiated 15 December), perhaps due to extended periods of cloudy weather. In contrast, plants in the second experiment (initiated 25 March) were exposed to rapidly rising natural irradiance levels. There was a minimum of 10 single-plant replications of each treatment. The foliar disease rating and plant survival data were subjected to an analysis of variance and the means were separated by an LSD test. Because the ranking of the cultivars for disease severity was similar between the tests, only data from one experiment are presented.

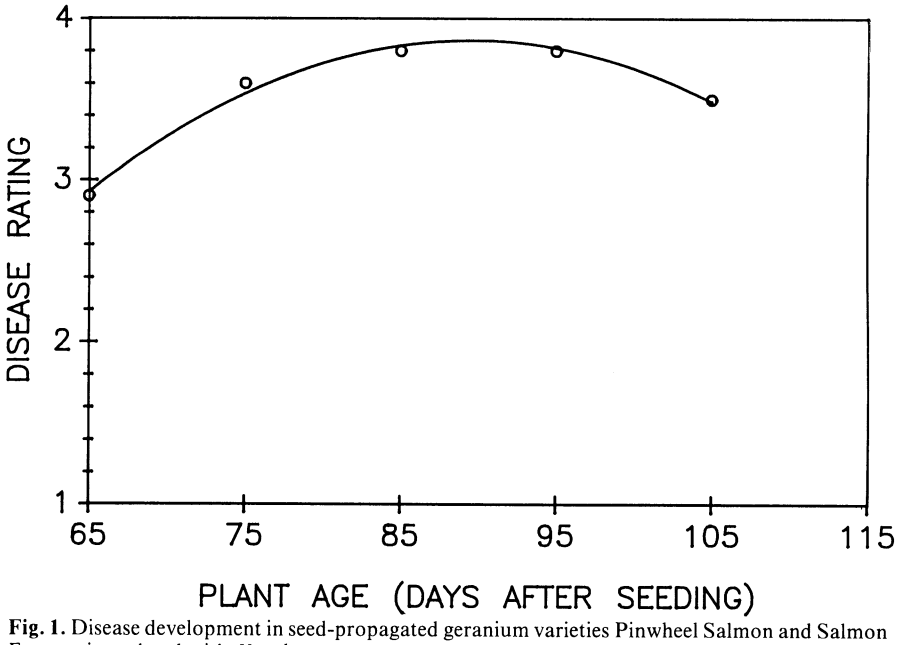

Fig. 1. Disease development in seed-propagated geranium varieties Pinwheel Salmon and Salmon Express inoculated with *Xanthomonas campestris* pv. *pelargonii* at different plant ages.

RESULTS AND DISCUSSION

Susceptibility to disease increased as plant age increased, such that the optimum time for inoculation was between 80 and 95 days after planting (45–60 days after transplanting) (Fig. 1). There was size variation among plants inoculated at 65 days as some plants took longer to recover from transplant shock. However, all plants appeared to be growing vigorously. The rapid increase in leaf area on the smaller plants may have affected disease severity recorded at 65 days, whereas by 75 days all plants were of uniform size and growth rate. Because plants inoculated soon after transplanting were too small to rate accurately, they were not included in Figure 1. In contrast, plants inoculated at 105 days (70 days after transplanting) were discarded due to infection of flower parts and leaf margins by *Botrytis* sp.

Chlorotic leaf margins, typical of CCC injury, were observed up to 10 days following application of CCC. Along with this phytotoxicity, plants inoculated up to 20 days following application of CCC were more susceptible to *X. c.* pv. *pelargonii* than were control plants (Fig. 2). However, by 30 or 40 days, plants in the two treatments were equally susceptible. The initial predisposition may have been associated with the marginal leaf chlorosis or some general plant stress and is in contrast to a recent report where CCC-treated hibiscus plants were more resistant to three bacterial leaf spots than control plants (2).

Supplemental HPS illumination had no effect on disease ratings. However, compared with plants grown under natural lighting, the plants grown under HPS matured faster and had larger, more uniform leaves, which facilitated disease ratings.

Symptom development on seed-propagated geraniums was similar to that observed on cutting geraniums. Small, round, necrotic lesions initially appeared 7–10 days after inoculation. The lesions enlarged to about 3 mm in diameter and were surrounded by a chlorotic halo. In some cases, the lesions coalesced to form large necrotic areas on the leaf surface, a symptom that is not normally described

on cutting or ivy geraniums. Another type of leaf symptom observed was V-shaped necrotic lesions, which apparently accompanied vascular infection. These symptoms were usually accompanied by a wilt of the leaves with petioles remaining attached and rigid. Vascular discoloration was noted in some plants. As the infection progressed, the entire plant wilted and a rotting of the stem tissue occurred.

Disease susceptibility varied among the 63 varieties; the foliar disease ratings ranged from 2.4 to 4.0. (LSD at $P = 0.05$ was 0.6 and 0.7 for CCC and non-CCC treated varieties, respectively). All plants became diseased and some plants of each variety died. The survival rate among varieties ranged from 0 to 80% and was highly inversely correlated with the foliar disease ratings ($R^2 = -0.94$, $P = 0.01$). Almost all of the survivors were visually diseased within 30 days after inoculation. Initial lesions were observed within 7 days of inoculation for all varieties regardless of the final foliar rating. Localized lesions on leaves were approximately 2–3 mm in diameter among all varieties. Based on the two methods used for assessing susceptibility, varieties were divided into four groupings where <2 was considered a resistant reaction, 2–2.9 a moderately resistant reaction, 3–3.7 a susceptible reaction, and >3.7 a highly susceptible reaction. None of the varieties tested here were immune or resistant. However, 10 varieties, including Carefree Crimson, Exp. Rose PAC, Marathon, PAC Andretta, Cherry Diamond, Delta Queen, Orbit Red, Ringo Dolly, Smash Hit Red, and Orbit White (16% of all varieties tested), appeared to possess some resistance. The average foliar disease index for this group was 2.9 and the average percent plant survival was 76%. Carefree Crimson and Exp. Rose PAC were the most resistant varieties with foliar disease ratings of 2.4 and 2.5 and 80 and 78% plant survival, respectively. In nearly half of the plants of these varieties, 25% or less of the leaf surfaces were covered with localized lesions. Very few V-shaped lesions were observed.

On six varieties, Carefree Crimson, Marathon, Cherry Diamond, Ringo Dolly, Orbit White, and Delta Queen, many diseased leaves rapidly wilted, became necrotic, and abscised (14–20 days following inoculation). The leaf abscission may be a defense mechanism because it helped prevent systemic movement of the bacteria into the plant. No bacteria could be detected in the vascular system of surviving plants in either Cherry Diamond or Ringo Dolly, two of the varieties in which leaf abcission occurred.

Fifty-seven percent of all varieties tested were classified as susceptible. These included Cameo Exp. Scarlet PAC, Gala Sunbird, Ringo Salmon, Exp. F-1 Zoned Red 6X1027, Smash Hit Salmon, Ringo Rouge, Exp. F-1 White, Merlin, Orbit Appleblossom, Ice Queen, PAC Quix Improved, Red Express, Smash Hit Rose Pink, Exp. F-1 Zoned Red 6X891, Red Elite, Cherry Glow, Snowden, Gala Flamingo, PAC Sitta Improved, Ringo Rose, Rosita Improved, Showgirl, Smash Hit White, Pinwheel Salmon, Cherrie Improved, Ringo Scarlet, Salmon Express, Hollywood Star, Exp. Salmon PAC, Hollywood Red, Hollywood Salmon, Jackpot, Orbit Pink, and Piccaso. The average foliar disease index of this group was 3.4 and the average percent plant survival was 45%. Leaf spotting and blighting covered 50% of the leaf surfaces of half of the plant replicates within this group. Leaf blighting was more extensive because greater numbers of localized lesions covered leaf surfaces, causing larger areas of the leaf to yellow and dry out. Although the majority of the lesions were localized, more V-shaped lesions developed on the leaves of these varieties.

The third group, representing 27% of the varieties tested, appeared to be highly susceptible. These included Orbit Cherry Improved, Encounter Red, Exp. Scarlet PAC, Capri Deep Red, Exp. Hollywood White, Gala Amaretto, Rosita "80," Tiffany Red, Heidi, Mustang, Orbit Scarlet, Sprinter Scarlet, Sprinter White Type, Cherie "80," Exp. White PAC, Sprinter White, Gala Redhead, and Sprinter Salmon. The average foliar disease index was 3.9 and the percent plant survival was 4.5%. The few plants that survived to the end of the test were severely diseased. In this group, lesions covered between 50 and 100% of the leaf surfaces. The remainder of the leaf became first chlorotic and then necrotic. As the necrosis developed, the leaf collapsed but remained attached to a firm petiole. There was at least one V-shaped lesion on each plant, suggesting that rapid invasion into the vascular tissue was occurring. This may explain the extremely low plant survival rate.

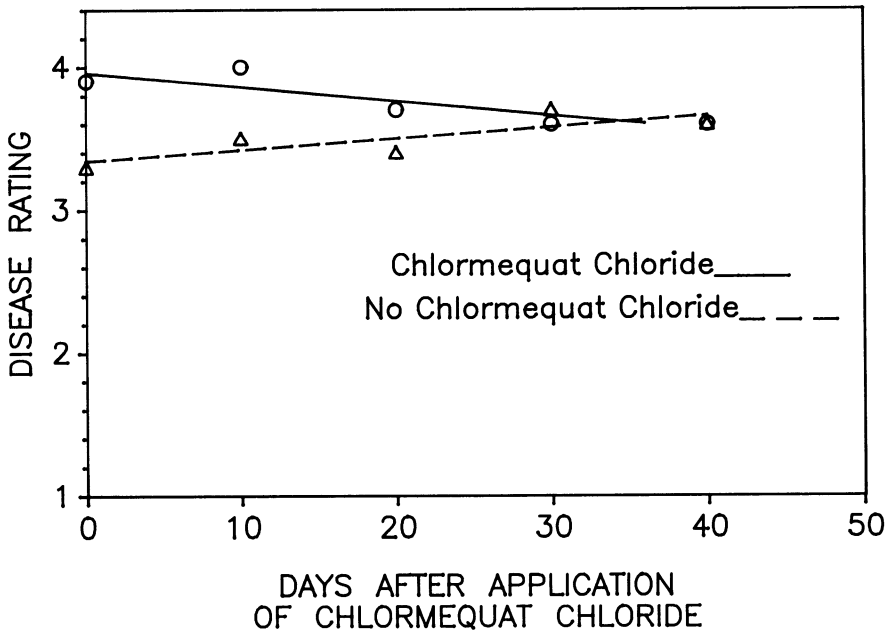

Fig. 2. Disease development in seed-propagated geranium varieties Pinwheel Salmon and Salmon Express inoculated with *Xanthomonas campestris* pv. *pelargonii* at various times following application of chlormequat chloride.

All single-plant replications of eight of the highly susceptible varieties (Exp. Hollywood White, Gala Ameretto, Mustang, Sprinter White Type, Cherie "80," Exp. White PAC, Gala Redhead, and Sprinter Salmon) died within 49 days after inoculation.

There was slightly less disease overall in the second test of 30 of the 63 cultivars, but the cultivars fell into the same disease susceptibility groupings.

This is the first report in which diploid geraniums used exclusively for seed-propagated varieties were screened for susceptibility to *X. c.* pv. *pelargonii*. With tetraploid cutting geraniums, no difference in resistance of varieties was observed in two reports. Recently, however, distinct varietal differences in susceptibility were found (6,8,11). Germ plasm resistant to *X. c.* pv. *pelargonii* would be useful to the seed-propagated geranium industry. Moderate resistance, rather than high resistance or immunity, was observed in this study. However, the resistance expressed in the 10 most resistant varieties was not sufficiently high to warrent recommending these varieties to growers. Even in this group, lesions were present on most surviving plants and significant numbers of plants were lost to the disease. However, it may be possible to amplify this resistance through tissue culture technology. Several of these moderately resistant varieties have been regenerated in vitro through tissue culture procedures (5).

Somaclones derived from these varieties are currently being screened in vitro with *X. c.* pv. *pelargonii*.

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