Resistance in Seedlings of the Family Geraniaceae to Bacterial Blight Caused by *Xanthomonas campestris* pv. *pelargonii*

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ABSTRACT

Dunbar, K. B., and Stephens, C. T. 1992. Resistance in seedlings of the family Geraniaceae to bacterial blight caused by *Xanthomonas campestris* pv. *pelargonii*. Plant Dis. 76:693-695.

Leaves on 5-wk-old seedlings of *Geranium* and *Pelargonium* species grown in culture tubes containing 15 ml of Hoagland's solution solidified with 0.7% agar were swabbed with a 10^7 cfu/ml cell suspension of *Xanthomonas campestris* pv. *pelargonii*. Eight weeks after inoculation all seedlings from susceptible *P.* × *hortorum* cultivars and from *P. zonale, P. frutetorum, P. fulgidum, P. fruticosum, P. alchemilloides, P. inquinans, P. acraeum*, and *P. capitatum* were severely blighted. These susceptible species had an average of more than 50% tissue blighted 3 wk after inoculation, and 50–100% of the inoculated plants were dead within 8 wk. However, *P. cordifolium, G. nodosum, G. napalense, G. sylvaticum, G. richardsonii*, and *G. ibericum* seedlings had significantly less blighted tissue than *P.* × *hortorum*. These resistant species had an average of less than 50% tissue blighted 3 wk after inoculation, and 0–18% of the inoculated plants died after 8 wk. *P. cordifolium* and *G. ibericum* seedlings had the lowest levels of blighted tissue 3 wk after inoculation.

Bacterial blight of geranium, caused by *Xanthomonas campestris* pv. *pelargonii* (Brown) Dye, is the most serious disease of the garden geranium (*Pelargonium* × *hortorum* L. H. Bailey). Resistance to bacterial blight has not been reported for cutting- (12,13,24) or seed-propagated garden geranium (6,24). *X. c. pelargonii* infects both *Pelargonium* and *Geranium* species (22). Only a small number of the more than 300 species from these genera have been evaluated for bacterial blight resistance (6,13,22). Certain of these species may be sources of bacterial blight resistance that can be transferred to horticulturally important *Pelargonium* species. Certain previously identified sources of resistance cannot be transferred easily to *P.* × *hortorum*. For example, regal geranium (*P.* × *domesticum* L. H. Bailey) cultivars are resistant to bacterial blight but cannot be sexually crossed with *P.* × *hortorum* (7).

Protocols that evaluate disease resistance of shoots, roots, plantlets, or seedlings grown in culture tubes have been termed in vitro techniques, tests, assays, or screens (1,2,6,9–11,23). An in vitro assay could be useful in screening Geraniaceae species for bacterial blight resistance (6). Protocols for such assays have been developed for evaluating disease resistance of plants to pathogenic bacteria (1,6,8,20,21), fungi (2,9,14,16–19, 23,26), and nematodes (10,11). Hosts screened include seedlings of asparagus (2,23), alfalfa (9), and wheat (16,17); plantlets of aspen (14), potato (20,21), geranium (6), and peach (11); shoots of papaya (19), larch (18), and peach (8); and root explants of soybean (10). Such protocols have been suggested for rapidly screening large amounts of germ plasm in a small controlled environment (2,6,8, 19,23).

An in vitro assay has been developed for detection of bacterial blight resistance in *Pelargonium* plantlets (6). Five-wk-old plantlets of *P.* × *hortorum* grown in culture tubes and inoculated with *X. c. pelargonii* developed leaf spots, leaf blight, and wilt and died within 3 wk; similar symptoms appeared within 4 wk after inoculation of plants grown in the greenhouse (6). In the present investigation, this in vitro assay was used to screen seedlings of Geraniaceae species for resistance to bacterial blight.

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Accepted for publication 13 February 1992.

MATERIALS AND METHODS

Seeds from *Pelargonium* and *Geranium* species and from *Brassica oleracea* var. *capitata* L. were surface-disinfested by submerging first in 95% ethanol for 1 min, then in 20% (v/v) household bleach (5.25% sodium hypochlorite) solution containing 0.5 ml of Tween 20 per liter for 30 min. Seeds were rinsed three times in sterile distilled water. The seeds were germinated on moist filter paper in petri dishes sealed with Parafilm M (American National Can, Greenwich, CT) (6), transferred to Hoagland's solution (3) solidified with agar (HSS) (5), and cultured (6). Inoculum was prepared from liquid shake cultures grown for 48 hr in a modified (6) complete Lederberg (15) medium using strains X-1 and X-7 of *X. c. pelargonii* and a strain of *X. c. campestris* (Pammel) Dowson (6). Strains X-1 and X-7 were selected because they produced more leaf blight on plants of *P.* × *hortorum* at 2 wk after inoculation than other strains tested (6).

Seedlings were inoculated 30–35 days after the germinated seeds had been placed onto HSS medium in culture tubes. Upper surfaces of individual leaves were gently rubbed with a sterile cotton swab that had been moistened with inoculum. The tubes were sealed with Parafilm M for 2 days and incubated at 24 C with 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light, and a 16-hr photoperiod provided by cool-white fluorescent lamps. The Parafilm M was removed after 2 days, and the plants were incubated as before. Three weeks later the plants were rated for disease on a scale of 1–6: 1 = no symptoms; 2 = <20% tissue blighted; 3 = 20–50% tissue blighted; 4 = 51–75% tissue blighted; 5 = >75% tissue blighted; 6 = plant death. Percentage of surviving seedlings was recorded 8 wk after inoculation.

Seedlings of the geranium cultivar White Orbit (Ball Seed Co., West Chicago, IL) were inoculated as described above with a cell suspension of *X. c. pelargonii* at 0, 10^3, 10^5, 10^7, or 10^9 cfu/ml to determine the effect of inoculum concentration on blight severity of seedlings grown in tubes. There were six single-plant replicates (one plant per culture tube) per treatment, arranged in a completely random design, and the experiment was repeated once.

The geranium cultivar White Orbit and cabbage seedlings were inoculated with 10^7 cfu/ml of strain X-1 of *X. c. pelargonii* and *X. c. campestris* to test the reaction of seedlings grown in tubes to another pathovar of *X. campestris* (not pathogenic to geranium). Control plants were swabbed with sterile tap water. There were eight single-plant replicates per treatment arranged in a randomized factorial design with three inocula (two bacterial plus water) and two plant species. The test was repeated once.

The susceptibility of different species of Geraniaceae to *X. c. pelargonii* was determined with seedlings from *P.* × *hortorum, P. capitatum* (L.) L.'Hér. ex Ait., *P. zonale* (L.) L.'Hér. ex Ait., *P. frutetorum* R. A. Dyer, *P. fulgidum* (L.) L.'Hér. ex Ait., *P. fruticosum* (Cav.)

Willd., *P. alchemilloides* (L.) L.'Hér. ex Ait., *P. inquinans* (L.) L.'Hér. ex Ait., *P. reniforme* Curtis, *P. cordifolium* (Cav.) Curtis, *P. acraeum* R. A. Dyer, *G. nodosum* L., *G. napalense* Sweet, *G. sylvaticum* L., *G. richardsonii* Fisch. & Trautv., *G. viscosissimum* Fisch. & C. A. Mey., and *G. ibericum* Cav. These plants were inoculated with 10^7 cfu/ml of strain X-1 or X-7 of *X. c. pelargonii.* The experiment had a completely random design with six single-plant replicates per treatment and was repeated at least once for each species.

Analysis of variance was performed on disease-rating data for all experiments. Regression analysis was performed on the disease-rating data from the inoculum concentration experiment. The means from the Geraniaceae experiment were separated using LSD test ($P = 0.05$).

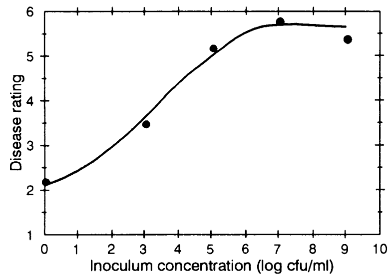

Fig. 1. Effect of concentration of *Xanthomonas campestris* pv. *pelargonii* on disease severity of in vitro-grown seedlings of geranium cultivar White Orbit rated 3 wk after leaf inoculation. 1 = No symptoms; 2 = <20% blighted tissue; 3 = 20–50% blighted tissue; 4 = 51–75% blighted tissue; 5 = >75% blighted tissue; 6 = plant death. $Y = 2.1 + 0.1X + 0.2X^2 - 0.02X^3$, R^2 value = 0.66 (F test for Y-intercept: $P < 0.0001$; F test for cubic regression: $P < 0.04$).

All statistical analyses were performed using SAS software (SAS Institute, Cary, NC).

RESULTS

Seedlings of susceptible Geraniaceae species developed circular water-soaked spots, leaf blight, or leaf wilt while petiole remained erect, then developed dry rot or died. Water-soaked spots were observed within the first week after inoculation of susceptible seedlings with 10^7 cfu/ml of *X. c. pelargonii.* Leaves with lesions often became completely blighted within 1–2 wk, and plant death occurred within 3–4 wk. After 8 wk in tubes, uninoculated control plants had five to seven leaves and appeared healthy; however, one or two of the lower leaves on these plants were often dead or senescent.

The percentage of blighted tissue on plants of *P.* × *hortorum* at 3 wk after inoculation was correlated with inoculum level (Fig. 1). An inoculum level of 10^3 cfu/ml of *X. c. pelargonii* was too low to ensure consistent leaf blight and plant death. Three weeks after inoculation, 36% of the plants inoculated with 10^3 cfu/ml of *X. c. pelargonii* were dead or had more than 75% blighted tissue, but 64% of these plants had less than 50% blighted tissue. In contrast, with 10^5, 10^7, and 10^9 cfu/ml, 81–100% of the seedlings were dead or had more than 75% tissue blighted. An inoculum level of 10^7 cfu/ml was selected for use in subsequent experiments.

Though the environment in the tubes during the 3-wk incubation period was conducive to disease development, seedlings of *P.* × *hortorum* inoculated with *X. c. campestris* or sterile water had no leaf spots, wilt, dry rot, or death. However, one or two of the lowest leaves on most of these plants were dead or senescent. These old dead leaves looked like blighted tissue; as a result, *P.* × *hortorum* plants inoculated with *X. c. campestris* or water were rated as having some blighted tissue (mean disease rating [MDR] = 2.0 and 2.3). In contrast, over 50% of the plants inoculated with *X. c. pelargonii* (MDR = 4.0) were dead. All cabbage seedlings were dead 3 wk after inoculation with *X. c. campestris* (MDR = 6.0). Cabbage seedlings had no symptoms 3 wk after inoculation with water (MDR = 1.0), but 55% of the cabbage seedlings inoculated with *X. c. pelargonii* (MDR = 2.9) had developed irregular water-soaked spots, and 20% were dead. The effect of inoculum and inoculum-host interaction on disease was highly significant (F test, $P < 0.001$), but host species was not significant.

The species of Geraniaceae tested had a highly significant effect on the percentage of blighted tissue per plant (F test, $P < 0.0001$) (Table 1). Seedlings of *P. zonale, P. frutetorum, P. fulgidum, P. fruticosum, P. alchemilloides, P. inquinans, P. acraeum, P. capitatum,* and *P.* × *hortorum* developed water-soaked spots, leaf blight, wilt, and plant death. These susceptible species had an average of more than 50% tissue blighted 3 wk after inoculation and 50–100% of inoculated plants dead within 8 wk. Resistant species had an average of less than 50% tissue blighted 3 wk after inoculation, and 0–18% of inoculated plants were dead after 8 wk. These resistant species had small, circular, necrotic leaf spots. The spots observed on *G. nodosum, G. napalense, G. sylvaticum,* and *G. richardsonii* were brown, whereas the spots observed on *P. cordifolium,* and *G. ibericum* were reddish brown.

Table 1. Bacterial blight in seedlings of Geraniaceae species grown in vitro[x]

Species	MDR[y]	% Surviving[z]
Pelargonium cordifolium	1.6 a	100
Geranium ibericum	2.1 a	100
G. napalense	2.6 a	100
G. richardsonii	2.3 a	83
G. nodosum	2.7 a	83
G. sylvaticum	2.7 a	83
P. reniforme	2.7 a	69
G. viscosissimum	3.8 b	50
P. fulgidum	4.2 bc	42
P. capitatum	4.3 bc	43
P. alchemilloides	4.3 bc	0
P. × *hortorum* cv. Red Orbit	4.7 bcd	34
cv. White Orbit	4.7 bcd	44
P. frutetorum	4.7 bcd	33
P. acraeum	4.8 bcd	17
P. zonale	5.3 cd	33
P. fruticosum	5.2 cd	17
P. inquinans	5.4 d	4

[x]Plants were rated for severity of blight 3 wk after inoculation of 5-wk-old seedlings with 10^7 cfu/ml of *Xanthomonas campestris* pv. *pelargonii.* Seedlings were grown on solidified Hoagland's solution in a test tube.

[y]Mean disease rating. 1 = No symptoms; 2 = <20% blighted tissue; 3 = 20–50% blighted tissue; 4 = 51–75% blighted tissue; 5 = >75% blighted tissue; 6 = plant death. Means with the same letter are not significantly different by LSD test ($P = 0.05$).

[z]The percentage of surviving seedlings was recorded 8 wk after inoculation.

DISCUSSION

Resistance to bacterial blight has been observed in *P.* × *domesticum, P. acerifolium* Cav., *P. tomentosum* Jacq., *P. scarboroviae* Sweet, *P. scabrum* (L.) L.'Hér ex Ait., *P. betulinum* (L.) L.'Hér. ex Ait., *P. grandiflorum* Willd., *P. multicaule* Jacq., and *P. hispidum* (L. f.) Willd. (6,13). The tests reported here suggest that *P. cordifolium* should be included on the list of bacterial blight–resistant *Pelargonium* species. Resistance was found in *G. sylvaticum* and *G. yedoense* Franch. & Sav. (22). We confirmed that *G. sylvaticum* possessed resistance. Additionally, we found resistance in *G. nodosum, G. napalense, G. richardsonii,* and *G. ibericum.*

The seedlings in the in vitro assay developed symptoms similar to tissue culture plantlets (6). Seedlings and plantlets of *P.* × hortorum were highly susceptible to *X. c.* pv. *pelargonii,* the compatible pathogen, but resistant to *X. c. campestris,* the incompatible bacterium (6). Greenhouse-grown plants of *P. zonale, P. inquinans, P. capitatum, P.*

fulgidum (13), and *P.* × *hortorum* (6,24) were found to be susceptible to *X. c. pelargonii*. These species were also susceptible in our tests with the seedling assay. In contrast, *P. cordifolium* has been observed to be resistant both as seedlings and as mature plants in the greenhouse (4).

Knauss and Tammen (13) suggested that bacterial blight resistance could be most easily introduced into popular *P.* × *hortorum* cultivars if it could be found in *P.* × *hortorum* (13). However, useful resistance has not been observed in it (12,13,24,25). The major genetic contributors to the hybrid species *P.* × *hortorum* are most likely *P. zonale, P. inquinans, P. scandens* J. F. Ehrh., and *P. frutetorum* (7). *P. zonale, P. inquinans*, and *P. scandens* have been observed to be susceptible to bacterial blight (14), and we found here that *P. frutetorum* is susceptible. We also confirm the susceptibility of *P. zonale* and *P. inquinans*. Therefore, none of the parents of *P.* × *hortorum* have resistance to *X. c. pelargonii.*

Seed-propagated geraniums have become a major part of the geranium market (5). Resistance to bacterial blight that is expressed in the seedling would be desirable. Previous bacterial blight–screening procedures have used mature plants (13,24,25), but the procedure in this paper can identify resistance expressed in seedlings.

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