

Tolerance in Greenhouse Geraniums to *Pythium ultimum*

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ABSTRACT

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Four cultivars of geranium (*Pelargonium* × *hortorum*) were used to study their resistance to two strains of *Pythium ultimum*. In two separate experiments, the cultivars tested responded similarly to the treatments in terms of plant death, dry weight, plant volume, and symptom evolution. One strain was significantly more virulent than the other, but there was no significant interaction between the cultivars and the strains, indicating that the source of inoculum did not affect the respective response of the cultivars to the treatments. An average of 20% mortality was recorded after the inoculations. The growth of all inoculated surviving plants was severely delayed compared with the controls. However, two of the cultivars, after the initial stress of the inoculations, were able to resume a growth rate comparable to the controls and did not exhibit symptoms (except for growth delay) after 4 wk. The two other cultivars never recovered fully from the initial attack by *P. ultimum*. This relationship between the remission of symptoms and plant growth resumption of inoculated plants of some cultivars seems to indicate a defense reaction from the plant.

The production of *Pelargonium* × *hortorum* L. H. Bailey has constantly increased in Québec in the past few years (7) because of the growing popularity of seed-propagated geraniums. The importance of this industry has forced producers to pay more attention to pests that reduce quality and yield.

The pathogen *Pythium ultimum* Trow has been reported as the major cause of yield loss in geranium production (2,5). The impact of this pathogen has been compounded by the use of silver thiosulfate (STS), a product preventing early petal shattering, which causes premature death of plants infected with *P. ultimum* (3). In Québec, although STS application is not common, *P. ultimum* causes substantial losses in greenhouse geranium production. This disease can be partially controlled by chemical methods and cultural practices (4). However, elimination of the pathogen remains a difficult task because of its easy introduction and difficult detection in the cultures (8,9). Despite the ubiquity of this pathogen, there are no geranium cultivars reported to be immune to *P. ultimum*, although some cultivars have been reported to be more resistant in terms of plant death, growth, and flowering delay (1).

Symptoms caused by *P. ultimum* on geranium have recently been described in detail by Hausbeck et al (2). However,

in light of the existence of geranium cultivars showing variation in resistance to *P. ultimum*, it would be interesting to follow the evolution of the disease in an attempt to better understand what triggers this differential resistance and also to determine if the host response depends on the strains of *P. ultimum* used. The objective of this work was to study the progression of the disease on geranium plants by comparing the virulence of two strains of *P. ultimum* on four geranium cultivars commonly produced in Québec.

MATERIALS AND METHODS

Plant propagation. Geranium (*Pelargonium* × *hortorum*) seeds (F1 hybrid) of cultivars Orbit White, Hollywood Red, Ringo Salmon, and Red Elite (Jack Van Klaveren Ltd., St. Catharines, Ontario) were scarified for 2 min in sulfuric acid and distributed in single-cell trays filled with a 6:1:1 (v/v) peat moss/perlite/vermiculite mix, plus a starter fertilizer (Pro-Mix BX Special, Premier Enterprises, Rivière-du-Loup, Québec). After 30 days, seedlings were transferred to a similar substrate without fertilizer (Pro-Mix BX). Plants were fertilized with 100 ppm of a 15-30-15 solution of N-P-K the first week, 200 ppm the second week, and 300 ppm the following weeks. The experiments were conducted in the greenhouse where day/night temperatures were 22/19 C and supplemental lighting ($100 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR) was supplied by high-pressure sodium lamps for a photoperiod of 16 hr. The first experiment was run from 23 October to 18 December 1989 and the second from 29 October to 24 December 1990, for a total of 8 wk for each experiment.

Inoculation. Inoculum was prepared from two strains of *P. ultimum* (Barr 144 and Barr 447) (Biosystematics Research Institute, Ottawa, Ontario) that were virulent on geranium when tested in preliminary experiments. The strains were grown on Difco potato-dextrose agar (PDA). Inoculation was done following the method of Mellano et al (6). For each plant, four 10-mm-diameter disks taken from the margin of 5-day-old cultures of each strain were inserted 3 cm deep into the substrate 3 cm away from the stem of 30-day-old plants. Sterile PDA disks were used for control. The experimental design consisted of three treatments (control, Barr 144, and Barr 447) on four cultivars and was repeated twice. Each experimental unit consisted of 10 plants randomly distributed in the greenhouse.

Disease symptoms and dead plants were recorded daily for 8 wk after inoculation. At the end of that period, plants that survived were oven-dried at 65 C for 48 hr. Aerial and root portions were then weighed separately. To confirm the presence of *P. ultimum*, plants from each treatment and each cultivar were randomly selected at the end of each experiment. Two 1-cm-long root segments were sampled, rinsed, and disinfested with 70% ethanol for a few seconds and with 1% sodium hypochlorite for 1 min. Roots were then rinsed and placed on 0.8% water agar to allow fungal growth. Identification was based on fungal morphological and microscopical features according to Van Der Plaats-Niterink's key (10).

Plant growth and symptom evolution. To measure the cumulative effects of *P. ultimum* on growth, plant volume was calculated weekly, using the formula for a volume of a cylinder (2). The width was averaged from two perpendicular measures, and the height from the base of the plant to the top of the canopy was recorded. During the same 8-wk period, a reaction rating was attributed weekly to each plant to quantify disease symptoms. An arbitrary scale was used where 0 = absence of symptoms, 1 = slight chlorosis of a few leaves, 2 = chlorosis of several leaves, 3 = leaf chlorosis and stem necrosis, and 4 = plant death.

The effects of the treatments were analyzed by ANOVA and compared by contrasts or mean separation tests. The software SuperANOVA (Abacus Con-

cepts, Berkeley, CA) was used for the statistical analysis.

RESULTS

Inoculation. In both experiments, symptom development after inoculation of *P. ultimum* was as previously described (3). All cases of mortality were recorded within the first 3 wk. Beyond that period, symptoms typical of infection by *Pythium* slowly receded on several surviving plants, and at the end of the experiments, plants showed mostly symptoms of delayed growth. For the two experiments, an average of 20% mortality was recorded. This percentage of mortality varied among the cultivars, with Orbit White and Ringo Salmon each losing 30% of their plants and Hollywood Red and Red Elite losing 15 and 5%, respectively. *P. ultimum* Barr 144 accounted for 66% of the deaths, compared with 34% for Barr 447. In all root segments sampled, *P. ultimum* was reisolated from roots of inoculated plants but not of control plants.

When comparing dry weights after 8 wk, there was no significant interaction between cultivars and treatments in either experiment. Data from the four cultivars were pooled for further analyses. Inoculated plants were significantly lighter than controls ($P = 0.0001$), whereas *P. ultimum* Barr 144 accounted for more weight loss than Barr 447 ($P = 0.005$) (Fig. 1). At the end of the experiments, dry weights of aerial parts of inoculated plants were significantly reduced compared with the controls by 45, 46, 36, and 30% for Orbit White, Ringo Salmon, Hollywood Red, and Red Elite, respectively, whereas root dry weights were significantly reduced by 52, 52, 50, and 35%, respectively.

Plant growth and symptom evolution. In the first experiment, volume of inoculated plants became significantly lower than the controls at week 2 for all cultivars (Fig. 2A). These differences increased progressively in the following weeks and were more important in the case of Orbit White and Ringo Salmon. Inoculated plants of Red Elite and Hollywood Red, although still smaller in volume, appeared to resume a growth

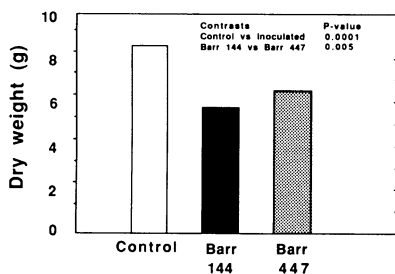


Fig. 1. Average dry weights of geranium plants 8 wk after being transplanted into an unfested soilless root medium (control) and a soilless root medium infested with *Pythium ultimum* Barr 144 or Barr 447.

rate comparable to the controls. The same trends were observed in the second experiment (Fig. 2B), with the exception that volumes of inoculated plants were not significantly different from the controls until week 4 for Hollywood Red and were still not statistically different after week 4 for Red Elite. After week 4 for both experiments combined, inoculated plants of Orbit White and Ringo Salmon reached only 31 and 31.5% of the volume of the controls, respectively, which was significantly less than values of 47 and 56% for Hollywood Red and Red Elite, respectively.

Symptom evolution, based on host reaction rating, revealed essentially two distinct patterns of response among the cultivars tested (Fig. 3). In both experiments, average symptom rating reached its maximum value usually after week

1 for inoculated plants of all cultivars. Beyond this period, inoculated Red Elite and Hollywood Red plants became progressively less symptomatic until their symptom rating reached that of the controls at week 4 in seven out of eight cases. In contrast, inoculated plants of Orbit White and Ringo Salmon never recovered fully from the initial stress caused by the infection; the host reaction rating, after reaching its peak after week 1, did not recede over the 4-wk period to reach that of the controls. Trends observed in the first (Fig. 3A) and the second experiment (Fig. 3B) were consistent for all cultivars and both strains tested.

DISCUSSION

P. ultimum is responsible for important losses in geranium production (5).

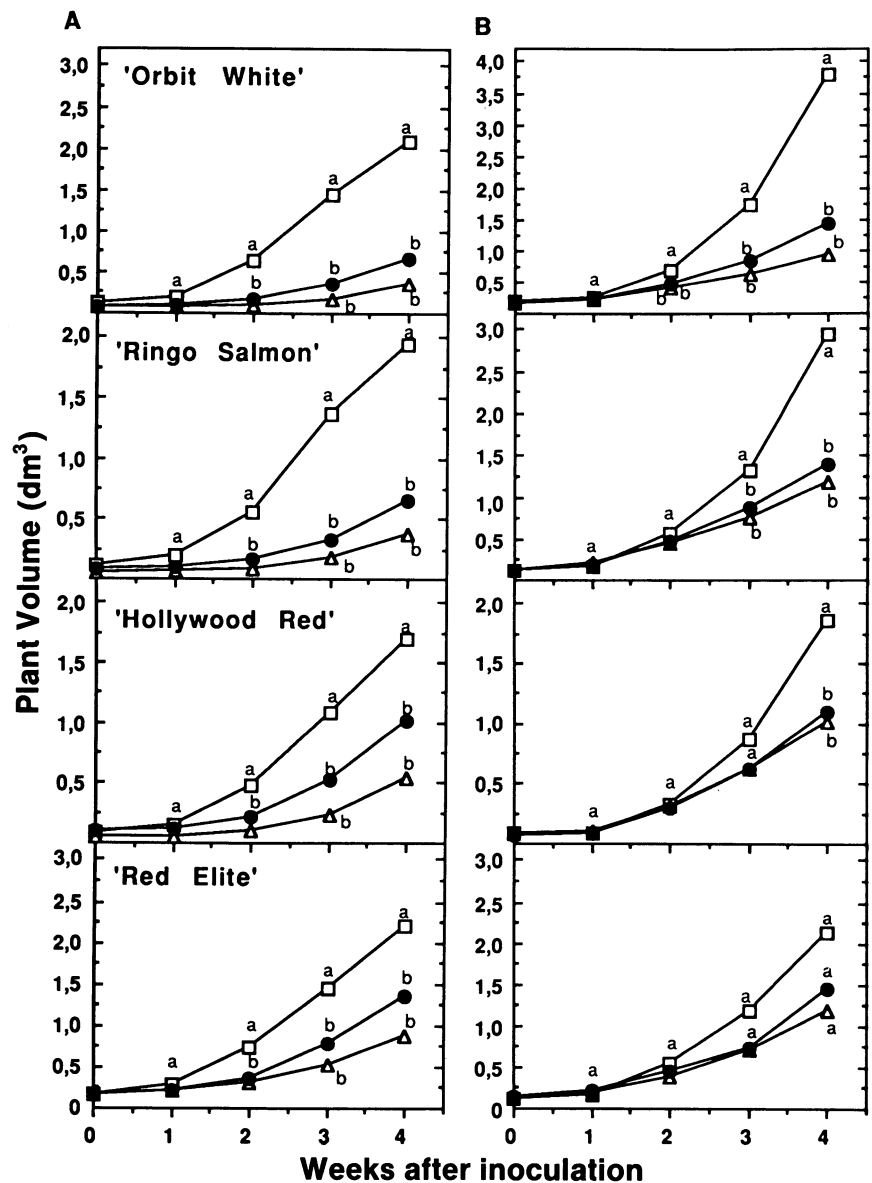


Fig. 2. Average plant volume of four cultivars of geranium transplanted at 30 days into an unfested soilless root medium (control) (\square) and a soilless root medium infested with *Pythium ultimum* Barr 144 (Δ) or Barr 447 (\bullet). (A) Experiment run from 23 October to 18 December 1989; (B) experiment run from 29 October to 24 December 1990. Values with the same letter are not statistically different according to LSD ($P = 0.05$).

Although there are no geranium cultivars reported to be immune to the pathogen, Hausbeck et al (1) reported that some cultivars may exhibit different levels of resistance in terms of plant death, plant stunting, and flowering delay. Results from this study corroborate these findings because they clearly demonstrate that the cultivars tested responded differently to inoculations with *P. ultimum*. These differences were consistently measured in plant death, dry weight, plant volume, or symptom rating among cultivars in the two separate experiments. These observations strongly suggest that the

resistance of geranium cultivars to *P. ultimum* is under genetic control because environmental conditions were the same during both experiments.

Another observation of interest is that *P. ultimum* Barr 144 was consistently more virulent than Barr 447 regardless of the variables measured or the cultivars tested. On one hand, this demonstrates that *P. ultimum* strains have different virulence levels, but on the other hand, the lack of interaction between the cultivars and the treatments indicates that, within the limits of our experiments, the source of inoculum did not play a

major role in assessing the tolerance of geranium cultivars against *P. ultimum*.

The fact that some cultivars were better able to recover from an infection by *P. ultimum* than others, as measured by a time-course study of plant volume and symptom rating, has never been reported in the literature. The relationship observed between the remission of symptoms and plant growth of inoculated plants seems to indicate a defense reaction from the plant. In fact, the resumption of relatively normal growth by Red Elite and Hollywood Red plants was associated with symptom remission, whereas the weaker growth of Orbit White and Ringo Salmon plants corresponded to the persistence of severe symptoms. The mechanisms allowing a geranium plant to recover from such an infection are unknown at this time. Whalen (11) has suggested the possibility that geraniums produce fungistatic metabolites that reduce the activity of *P. ultimum*. However, her work was done in relation to the use of STS and did not establish whether those metabolites were induced (phytoalexins) or constitutive.

As reported in this study and others (2,5), the impact of *P. ultimum* on geranium was characterized by plant death (20% in our experiments) or severe growth reduction. This latter symptom, although less spectacular, accounts probably for more important economic losses in geranium production because it allows *P. ultimum* to go undetected until most of the damage is done. This explains in part why *P. ultimum* is one of the most serious problems in geranium and other crops where the mode of action is similar (9). No geranium cultivars are known to be immune to the fungus, but results presented here suggest that some cultivars have the genetic constitution to recover from the initial shock of infection and suffer less yield loss. A better understanding of the defense mechanisms inducing these reactions would undoubtedly prove useful in producing geraniums or other crops more resistant to *P. ultimum*.

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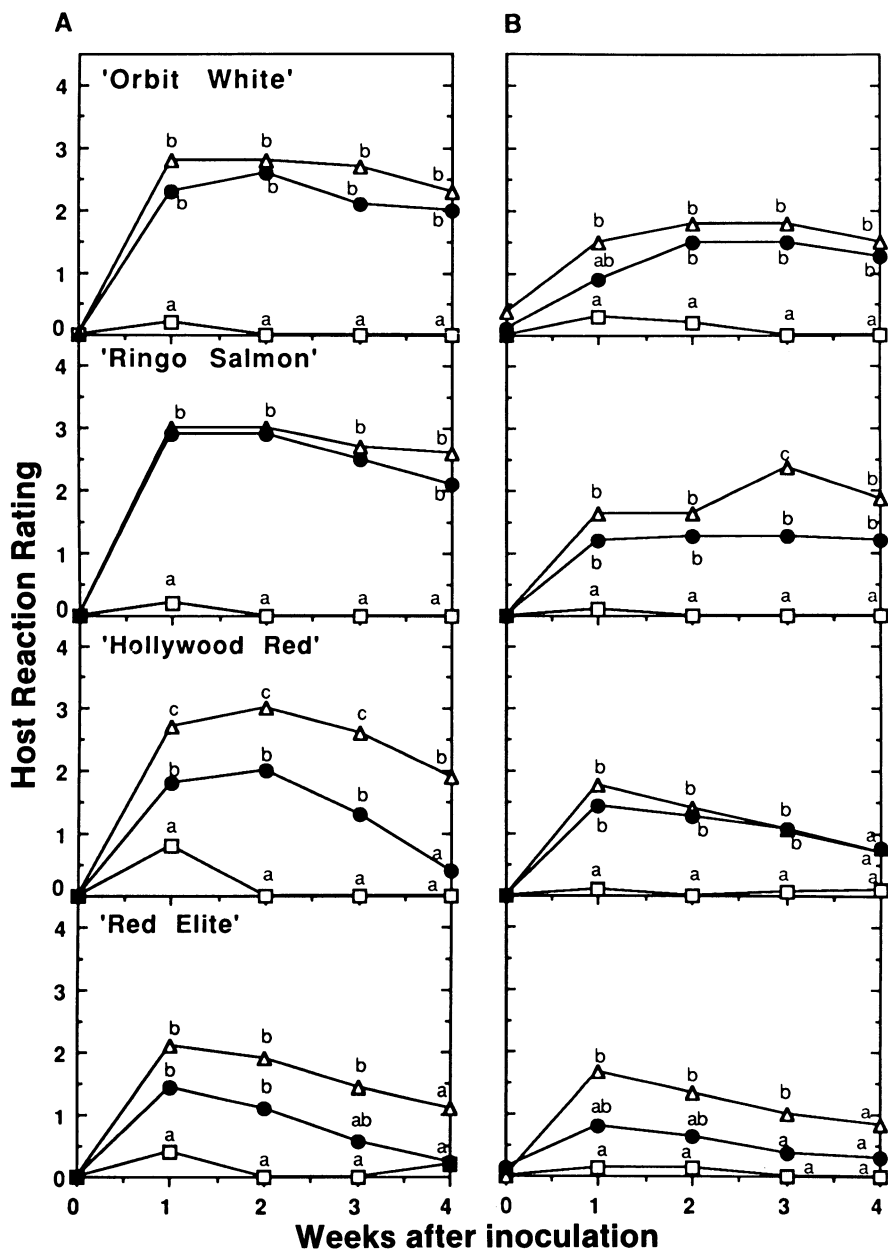


Fig. 3. Weekly evolution of symptoms on plants of four cultivars of geranium transplanted at 30 days into an uninfested soilless root medium (control) (□) and a soilless root medium infested with *Pythium ultimum* Barr 144 (△) or Barr 447 (●). (A) Experiment run from 23 October to 18 December 1989; (B) experiment run from 29 October to 24 December 1990. Symptom evolution was evaluated according to an arbitrary scale where 0 = absence of symptoms 1 = slight chlorosis of a few leaves, 2 = chlorosis of several leaves, 3 = leaf chlorosis and stem necrosis, and 4 = plant death. Values with the same letter are not statistically different according to LSD ($P = 0.05$).

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