Variation in Response of Norway Maple Cultivars to *Verticillium dahliae*

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ABSTRACT

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Significant variation in foliar symptoms and crown dieback was observed over 16 mo among 13 cultivars of grafted Norway maples after stem inoculation with *Verticillium dahliae*. Cultivars Jade Glen and Parkway showed the fewest symptoms and Crimson King and Greenlace the most, with wide variation among the other cultivars. In a second study, six of the same 13 cultivars were propagated on their own roots and root-inoculated with the fungus. Crimson King and Greenlace again showed high susceptibility, with high mortality (as much as 80%) and/or incidence of foliar symptoms. The cultivars expressing the greatest tolerance (fewest symptoms and lowest mortality) to root inoculation were also the most tolerant of stem inoculation.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a widely distributed disease of maples (8–11). The fungus invades the root system from the soil, where it is present as mycelia and conidia and as persistent microsclerotia. The disease in maples is characterized by vascular discoloration, wilting of leaves, shorter twig growth, and dieback of branches (10,15).

Zimm (15) was first to show that Norway maple (*Acer platanoides* L.) expressed more foliar symptoms and dieback in response to the fungus than did sugar (*A. saccharum* Marsh.) and red (*A. rubrum* L.) maples. Norway maple was the most susceptible of 10 species tested in one study (11). Verticillium wilt of Norway maple has been found in many production nurseries (4). A commercial nursery in Oregon once lost 85% of transplanted Norway maples to this disease (1). Verticillium wilt also contributes to the decline of Norway maple in landscapes and along streets (7,10).

Tolerance to *Verticillium* has been found in woody species such as red maple (13) and American elm (*Ulmus americana* L.) (6). Except for some preliminary studies (4,14), however, little research has been done to identify or exploit variation in tolerance to this pathogen within Norway maple. The present studies were designed to detect the possible tolerance of certain cultivars of Norway maple to *V. dahliae*. Grafted and own-rooted trees of commercially available cultivars were examined in two separate studies. Throughout this paper we will use "tolerance" to mean the relative ability of certain trees to respond to the presence of a fungus with neither pronounced foliar symptoms nor mortality. We use tolerance rather than "resistance" because resistance is so often used to mean fungal exclusion or lack of colonization (2), an extreme that we did not observe.

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MATERIALS AND METHODS

Grafted plants. Dormant 1.2–1.8 m tall grafted 2-yr-old whips (saplings) of 13 Norway maple cultivars, on seedling understocks of unknown origin, were planted in 50-L plastic pots in a peat:perlite:nonsterile soil (2:2:1) mix in April 1982 and placed outdoors at Delaware, Ohio. Trees were arranged in a randomized block design with 15 blocks. One tree of each cultivar in each block was designated for inoculation with *Verticillium*, and another tree of each cultivar was placed in each of five of the 15 blocks and designated for control, for a total of 15 inoculated and five control trees representing each cultivar. The cultivars used were Greenlace, Superform, Columnare Compacta, Schwedleri, Cleveland, Emerald Queen, Parkway, Jade Glen, Crimson King, Summershade, Silver Variegated, Globosum, and Royal Red. Trees were watered by drip irrigation as needed and allowed to grow for 1 yr before inoculation treatments were made.

Inoculum was prepared by growing five isolates of *V. dahliae* in Czapek-Dox broth on a rotary shaker (125 rpm) for 4 days at 24 C. The isolates used were recovered from infected Japanese maple (*A. palmatum* Thunb.), Norway maple, potato (*Solanum tuberosum* L.), sugar maple, and viburnum (*Viburnum dilatatum* Thunb.). Aliquots of each isolate were combined into a single conidial suspension with a final concentration of 6×10^6 conidia per milliliter. Similar mixtures of these isolates had been used to infect maples in previous studies. On 3 May 1983, 30 ml of conidial suspension or sterile distilled water (for controls) was poured into an inverted collar on the stem of each tree about 1 m above the root collar, 0.9 m above the graft union. The diameter range of the stem where wounds were made was 30–45 mm. These collars were formed of aluminum foil and sealed with warm paraffin. Puncture wounds 6 mm deep $\times$ 10 mm wide were then made with a chisel on four sides of the trunk below the level of liquid in the collar. Wounds were made directly into the stems in order to determine if scions from different cultivars differ intrinsically in their response to invasion by *Verticillium*.

The percentage of the total leaf surface area with disease symptoms was estimated by three observers on 9 June, 23 June, and 14 July 1983. The percentage of the crown with a combination of branch dieback, wilt, and foliar necrosis was estimated on 5 June and 7 September 1984. A consensus symptom score from the three observers was used for data analysis, for all dates.

All trees were sampled to determine the presence or absence of the fungus on 6 June 1983, approximately 5 wk after inoculation. The proximal and distal sections of petioles were taken from two leaves on side branches 15 cm above the inoculation point on each tree. Sections about 1.5 cm long were swirled in 20% hydrogen peroxide (H_2O_2) for 45 sec, rinsed for 5 sec in sterile water, and placed on Czapek-Dox agar in petri plates. Care was taken to insert a portion of the section into the agar with the remaining tissue protruding above the agar surface. Plates were sealed with paraffin film, incubated at 22 C, and examined by microscope periodically over 4 wk to determine the presence of *V. dahliae*.

In September 1984, trees were cut down. The central leader of each tree, from the point of inoculation to the top, was cut into six sections of equal length. Four wood chips from each section were

aseptically plated onto Czapek-Dox agar amended with 100 ppm of streptomycin and novobiocin, then incubated at 18 C for up to 4 wk to detect *V. dahliae*.

Data for crown symptoms were transformed to arcsine, then subjected to analyses of variance, followed by Duncan's multiple range tests for mean comparisons. Data for presence or absence of fungus were analyzed by 2×2 contingency tests.

Own-rooted plants. Dormant rooted cuttings, 25–35 cm tall, of Parkway, Columnare Compacta, Jade Glen, Crimson King, Greenlace, and Globosum were planted into 7-L containers in a mixture of peat, perlite, pine bark, and soil (4:4:1:1) in the spring of 1985. These six cultivars were chosen because they represented extremes of high and low susceptibility in the 1983–1984 study. They were then placed outdoors at Washington, D.C., in a split-plot randomized block design with 20 blocks (Crimson King had 10 blocks only) and assigned to one of the following treatments: 1) root inoculation in 1986, 1987, and 1988; 2) root inoculation in 1987 and 1988; 3) water control for treatment 1; and 4) water control for treatment 2. In this split-plot design, cultivars were used for major plots and the four treatments were used for minor plots. Each cultivar or treatment was represented by one plant in each block. Therefore, except for Crimson King, which had 10 replications, each treatment for each cultivar had 20 replications.

Inoculum of *V. dahliae* was prepared by growing isolates from Norway maple and eggplant (*S. melongena* L.) in Czapek-Dox broth on a gyratory shaker at 500 rpm for 7 days (isolates used in the preceding grafted study were not available for this study). Aliquots were combined to make a mixed conidial suspension. Before inoculation, a wood chisel, 2.5 cm wide, was inserted 7 cm deep into the soil of each container in two places. In 1986, 200 ml of inoculum (2×10^6 conidia per milliliter) was added to soil in treatment 1 on 2 April and 1 May. On 8 May 1987 and 13 April 1988, 200 ml of inoculum (4×10^6 conidia per milliliter) was added to the pots of treatments 1 and 2. Control trees received 200 ml of distilled water on each treatment date. Fungal recovery was attempted from two petioles in the top tenth and bottom tenth of each plant in May, June, and August 1988. Petiole sections 1.5 cm long were disinfested for 10 min in 1.05% sodium hypochlorite, then rinsed three times in sterile water. Sections were incubated on Czapek-Dox agar and examined for up to 4 wk to determine presence or absence of the fungus. Remaining live trees were sacrificed in May 1989 and sampled for length of aboveground vascular stem discoloration and presence of the fungus. Stem sections 1.5–2.0 cm were taken at 1 cm and 25 cm above the root collar, disinfested, and plated as before.

Survival was recorded in August 1987, April 1988, and August 1988. The percentage of the leaf surface area showing foliar symptoms was recorded on each tree 5 August 1986, 11 June 1987, and 30 June 1987.

RESULTS

Grafted plants. Analyses of variance indicated highly significant differences among cultivars for foliar and crown symptoms recorded in 1983 and 1984. One cultivar, Greenlace, showed as high as 73% and another, Jade Glen, showed no more than 2% crown symptoms throughout the experiment (Table 1). The cultivars most severely affected were Greenlace and Crimson King, followed closely by Globosum, Royal Red, and Cleveland. Those consistently least damaged were Jade Glen, Parkway, and Columnare Compacta (Table 1).

Successful attempts to recover the fungus from petioles in June 1983 varied significantly among cultivars, with a range from 25% in Jade Glen to 92% in Superform (Table 1). Variation among cultivars in fungal recovery from wood chips in September 1984 was less than from petioles in June 1983 but was significant (Table 1). There was a lack of consistent association between severity of symptoms and frequency of pathogen recovery (Table 1). The fungus was recovered from one control tree in 1983.

Own-rooted plants. The survival of *Verticillium*-inoculated Crimson King and Greenlace was significantly reduced compared with that of uninoculated trees in 1988 (Table 2). Crimson King also had

Table 1. Response of grafted Norway maple cultivars to stem inoculation with *Verticillium dahliae*

Cultivar	Average % foliar symptoms 14 July 1983	Average % crown symptoms 7 Sept. 1984	Percentage of trees from which *V. dahliae* was recovered June 1983	Sept. 1984
Greenlace	35 ab[y]	73 a	67 abc	...[z]
Crimson King	41 a	37 b	77 ab	67 ab
Globosum	14 cde	35 b	62 abc	50 ab
Royal Red	25 bc	28 bc	75 abc	71 a
Cleveland	17 cd	27 bcd	84 ab	44 ab
Summershade	7 def	18 cde	73 abc	47 ab
Emerald Queen	5 ef	14 cde	87 ab	69 a
Silver Variegated	3 f	13 def	87 ab	31 b
Schwedleri	7 def	10 ef	86 ab	69 a
Superform	7 def	7 ef	92 a	73 a
Parkway	1 f	7 ef	36 c	50 ab
Columnare Compacta	2 f	6 ef	86 ab	29 b
Jade Glen	2 f	1 f	25 c	71 a

[y]Values within a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test or to contingency tests.
[z]Insufficient trees available to sample adequately.

Table 2. Survival of and symptoms on own-rooted Norway maple cultivars after root inoculation with *Verticillium dahliae*

Cultivar	Treatment[y]	Percentage of live individual trees with foliar symptoms affecting 5% or more of the total leaf surface area on 30 June 1987	Survival (% of control) on 8 Aug. 1988
Columnare Compacta	1	10	100
	2	20	89
	1 + 2	15	95
Crimson King	1	75*[z]	40*
	2	70*	20*
	1 + 2	72*	30*
Greenlace	1	5	65*
	2	15	63*
	1 + 2	10	64*
Globosum	1	5	89
	2	5	83
	1 + 2	5	86
Jade Glen	1	0	95
	2	0	95
	1 + 2	0	95
Parkway	1	5	95
	2	5	95
	1 + 2	5	95

[y]Trees in treatment 1 were root-inoculated in April 1986, 1987, and 1988; trees in treatment 2 were root-inoculated in April 1987 and 1988.
[z]Asterisk indicates significant difference from control, $P = 0.05$.

a high percentage of trees with foliar symptoms of 5% or greater; by 30 June 1987, more than two-thirds of the Crimson King trees were in this category (Table 2). Plants of Columnare Compacta, Globosum, Jade Glen, and Parkway showed no significant mortality or increase in foliar symptoms in response to *V. dahliae* (Table 2). The fungus was recovered in 1988 from six inoculated plants: one Crimson King, two Globosum, and three Greenlace. In 1989, the fungus was recovered from 41 of the remaining 166 live inoculated plants and from two of the 203 surviving control plants. Percent recovery varied among cultivars. The percent fungal recovery and the number of inoculated plants sacrificed, respectively, were 100% of 3 Crimson King plants, 35% of 37 Jade Glen, 32% of 25 Greenlace, 28% of 29 Globosum, 16% of 37 Parkway, and 9% of 35 Columnare Compacta. Average length of vascular discoloration in 1989 was 30 ± 2 cm for inoculated plants and 3 ± 0.4 cm for control plants.

DISCUSSION

These studies demonstrate important clonal variation within Norway maple in response to inoculation with. *V. dahliae.* Moreover, the response from stem (scion) inoculation was consistent with that from root inoculation. Crimson King and Greenlace showed high susceptibility and Jade Glen and Parkway showed low susceptibility, regardless of whether the fungus was introduced through the stem or through the roots. This suggests that tolerance factors may be present and functioning throughout the plant, rather than in the roots alone.

A possibility exists for the development of *Verticillium*-tolerant rootstocks for Norway maple through selection and breeding. Such rootstocks have been developed for olive (3), and the variability shown in our studies and in another study (14) offers hope that Verticillium wilt in susceptible Norway maple scions could be prevented by using tolerant rootstocks. Further research with reciprocal grafts of tolerant and susceptible clones of Norway maple is needed, however. Grafting of hop clones using various susceptible-resistant combinations of stocks and scions showed that the tolerant roots can prevent disease in susceptible scions (5). Such research with Norway maple could determine if disease development is impeded when susceptible scions are grafted onto tolerant understocks or if tolerant scions display this characteristic when grafted onto susceptible understocks.

A better alternative to grafting or budding may be the propagation of tolerant cultivars on their own roots. This technique initially may prove to be more expensive for the nursery industry than grafting or budding but would, in the long term, be advantageous, because the entire plant would be tolerant. Our study of own-rooted plants showed the feasibility of such an approach.

Inactivation of the *Verticillium* fungus has been shown to occur soon after inoculation of apricot trees (12). In contrast, the fungus was viable in most of our grafted Norway cultivars 16 mo after inoculation. Success of fungal recovery 5 wk after inoculation was greater in the more susceptible clones than in the more tolerant ones. Symptom development has been shown to be related to movement of the fungus (9). The fungus may initially have grown more slowly and therefore been harder to recover in the more tolerant clones used in our study. No clear association between tolerance and fungal recovery was apparent 16 mo after inoculation, however. We also successfully recovered the fungus from tolerant and susceptible own-rooted cultivars, even 1 yr after the final inoculation. Further studies are needed to ascertain the relationship (if any) between growth and movement of the fungus and the relative susceptibility of Norway maple clones to *V. dahliae.* Also needed are studies designed to determine the importance of variation found in these studies to disease development under natural or "field" conditions.

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