

Tolerance of Half-Sib Families of Red Maple to *Verticillium* Wilt

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The authors thank W. O. Masters and D. E. Wuertz for technical assistance.

Accepted for publication 12 December 1972.

ABSTRACT

Seedlings representing six half-sib families of red maple were inoculated with *Verticillium dahliae*. After inoculation, differences among families in foliar symptoms and in the suppression of height growth were highly significant. Illinois and Arkansas sources showed the least foliar symptom development and growth reduction, and Pennsylvania and Minnesota sources the most. Twenty-five percent of the Illinois seedlings showed

less than 10% foliar symptoms after two inoculations. All of the Pennsylvania and Minnesota seedlings showed more than 10% foliar symptoms after two inoculations. Isolation of the pathogen from seedlings showing less than 5% foliar symptoms indicated tolerance rather than resistance to the fungus.

Phytopathology 63:673-676

Verticillium wilt caused by *Verticillium dahliae* Kleb. is a common disease of maple species. The fungus is present in the soil as short-lived mycelia and conidia or as more persistent microsclerotia. On red maple (*Acer rubrum* L.), first symptoms include wilting and dying of the leaves of one or several branches. The widespread and destructive nature of this disease on red maple is well-known (4, 9). Unfortunately, no cultural or chemical treatments are reliable in preventing or diminishing disease symptoms.

One possible control method heretofore untried is the development and release of resistant or tolerant cultivars. Natural resistance to *Verticillium* has been demonstrated in strawberries (2, 5), tobacco (7), tomato (8), cotton (13), and olive (15), but not within any of the important maple species. This study was designed to determine the possible presence of resistance or tolerance to *Verticillium dahliae* Kleb. within and among six half-sib families of red maple.

MATERIALS AND METHODS.—Open-pollinated seed was collected from one native red maple tree in each of the following locations: Zaleski, Ohio; Carbondale, Illinois; New Brunswick, New Jersey; Ely, Minnesota; Glenwood, Arkansas; and University

Park, Pennsylvania. In August 1971, seed which had been stratified in moist sand for 3 months at 34°F was planted in 7-in plastic pots filled with a 2:2:1 mixture of peat:perlite:soil. After germination, 24 seedlings from each source were placed in a greenhouse under a 16-hr photoperiod, and fertilized biweekly with modified Hoagland's solution (6). Seedlings from all sources were placed in a completely randomized design on greenhouse benches.

Preliminary studies demonstrated significant differences in virulence to red maple seedlings among isolates of the fungus cultured from different hosts. Therefore, a mixture of isolates from five different origins was used to make a microsclerotial and conidial suspension for inoculation. Isolates were grown in plastic petri dishes on potato-dextrose agar for about two months before blending the 100 X 10 mm fungus-agar discs in tap water. Three discs of each isolate were blended in 500 ml of water. The macerated contents from each of the five isolates were then combined into a single microsclerotial-conidial suspension. On January 4, 1972, a 1-cm long knife wound was made in the stem of each seedling at a point about one-third the seedling height up from

the base of the stem. Three drops of the inoculum were added to the wound on each of 12 seedlings of each half-sib family. No spore suspension was added to the other 12 seedlings, which served as controls within each family. In a pilot study, this method had eliminated transplant stress and had proved more effective in introducing the fungus into the host than by dipping roots in spore suspension of *V. dahliae*.

Height measurements and estimates of the percentage of the total leaf area expressing foliar symptoms (including defoliation) were made weekly. From seedlings that showed more than 5% foliar symptom development, reisolation of the fungus was attempted from a 5-cm long segment of the main stem taken 5 cm above the root collar. Isolation of the fungus was attempted from petioles from seedlings that showed little or no foliar symptoms. This procedure was followed to avoid sacrificing potentially resistant clones.

On March 23, 1972, all inoculated seedlings that had shown 5% or less foliar symptoms were reinoculated in the manner described above.

TABLE 1. Average percentage of foliar symptoms of red maple seedlings inoculated 1/3/72 with *Verticillium dahliae*

Source of half-sib family	2/8/72	2/15/72	2/22/72	2/29/72	3/7/72
	%	%	%	%	%
Illinois	6 a ^a	11 a	14 a	26 a	30 a
Arkansas	10 a	16 a	18 ab	28 a	32 a
Ohio	16 ab	25 ab	39 bc	48 ab	61 b
New Jersey	31 bc	37 bc	42 cd	50 b	58 b
Minnesota	35 c	44 bc	49 cd	56 b	62 b
Pennsylvania	36 c	51 c	58 d	65 b	73 b

^aValues followed by the same letter in the same column are not significantly different at the .05 probability level by Duncan's New Multiple Range Test. Averages were based on 12 seedlings per family.

TABLE 2. Average percentage of foliar symptoms after second inoculation of red maple seedlings with *Verticillium dahliae*

Source of half-sib family	No. of trees ^a reinoculated on 3/23/72	Average symptom development (%) on 6/7/72	% of original 12 trees showing < 10% symptom's after two inoculations
Illinois	3	5	25
Arkansas	2	7	17
Pennsylvania	2	35	0
Ohio	1	40	0
Minnesota	1	50	0
New Jersey	0	--	--

^aAll of these seedlings showed less than 5% foliar symptoms as a result of the first inoculation.

RESULTS.—Symptom development.—Wilting, yellowing, and interveinal browning of leaves were the first symptoms to develop. Families differed significantly in the average percentage of foliar symptoms; these differences were apparent as early as one month after inoculation (Table 1). Seedlings from the New Jersey, Pennsylvania, and Minnesota sources displayed the greatest degree of foliar symptoms. The Illinois and Arkansas families displayed the least amount of foliar symptoms, and were slowest in developing the first symptoms of the disease.

By March 22, 1972, eight of the 12 inoculated seedlings from Arkansas and seven of the 12 Illinois seedlings showed less than 50% foliar symptoms. In contrast, all but two each of the inoculated Pennsylvania and Minnesota seedlings showed more than 50% symptoms.

Results from the second inoculation again indicated the relatively high tolerance of the Illinois and Arkansas families to *V. dahliae* (Table 2). None of the Illinois and Arkansas seedlings displayed more than 10% symptom development on June 7, 1972. However, all of the seedlings representing the Pennsylvania, Ohio, and Minnesota families developed at least 25% foliar symptoms after the second inoculation.

In early June, seedlings that had been subjected to a second inoculation were outplanted to a field site. In mid-July, all but the Minnesota seedling were surviving. The Illinois and Arkansas trees appeared most vigorous. Several of the less vigorous seedlings had set healthy buds and stopped growing.

Growth.—Growth rate was decreased in trees from all sources as a result of inoculation; but there were significant differences among sources in the extent of the growth depression (Fig. 1B). Differences in growth rate among families were also expressed by the noninoculated seedlings (Fig. 1A).

The Arkansas and Illinois families, which displayed the lowest average percentage of foliar symptoms (Table 1), also showed the least amount of growth retardation after inoculation (Fig. 1B). Conversely, the susceptible families of Minnesota and Pennsylvania grew the least. This agrees with the data of Talboys et al. (12), who found that inoculation of strawberries with *Verticillium* species reduced growth more in susceptible than in resistant varieties. The difference between the Arkansas and New Jersey families in growth response to inoculation is especially evident. Growth of the noninoculated seedlings of these two sources is almost identical (Fig. 1A). However, growth patterns after inoculation were markedly different. The New Jersey seedlings displayed rapid growth during the first 2 weeks, but this pattern was soon followed by almost complete cessation of growth from the 3rd to the 9th week after inoculation. In contrast, Arkansas seedlings were still growing rapidly 9 weeks after inoculation (Fig. 1B).

Isolation and discoloration.—Within 3 months after the first inoculation, *V. dahliae* was recovered

from 71% of those trees showing at least 5% symptom development. Eighteen percent of the attempted isolations were negative. Of those trees showing less than 5% symptoms, most attempts to isolate the fungus from petioles were successful.

There were no significant differences among families in the presence or absence of vascular discoloration. Discoloration was present in the form of grayish-green streaks or bands in the sapwood. The percentage of trees showing discoloration within each family ranged from 90% in the New Jersey and Arkansas sources to 100% in the Illinois, Ohio, and Minnesota sources. Discoloration was present in several trees showing no foliar symptoms. Conversely, a few trees did not show discoloration despite their high degree of foliar symptom expression and the presence of the fungus.

DISCUSSION.—This is the first report of tolerance to *V. dahliae* in *A. rubrum*, or within any other maple species. Highly significant differences in tolerance among the half-sib families used in this study indicate a promising degree of additive genetic variance in the control of Verticillium wilt. Theoretical models show that differences among half-sib families account for one-fourth of the additive genetic variance in the parental population, the other three-fourths being variation within the progenies. Significant additive variance for *Verticillium* resistance has also been found in cotton (13) and hops (14). With the use of half-sib families, where the male parents are unknown, it is difficult to estimate the degree of dominant gene action; presence of this mode of action will not be known until full-sib progeny can be evaluated.

Considerable variation in response to inoculation existed within each family. For example, many of the Arkansas and Illinois seedlings developed more than 50% symptoms within 2 months after the first inoculation. Yet several other seedlings from the same sources showed less than 10% symptoms even after two inoculations. This wide range of response in a uniform environment is consistent with a quantitative rather than a qualitative mode of gene action. A polygenic scheme of resistance has also been suggested in cotton (13) and tobacco (7). However, Schaible et al. (11) described a dominant monogenic resistance to *V. dahliae* in tomatoes.

A breeding and selection program beginning with the use of half-sib families should concentrate on between-family as well as within-family differences in *Verticillium* resistance of red maple. A combined approach using clonal as well as family selection would be warranted.

The isolation of *Verticillium dahliae* several months after the first inoculation from those trees appearing healthy indicates "tolerance" rather than "resistance" (10). Survival of *Verticillium* species in healthy hosts has also been shown in other plants. Wright (16) found "surprisingly" large amounts of pathogen in resistant tobacco plants. The ability to tolerate the presence of the fungus has also been shown by Bringham et al. (2) and Talboys et al. (12) in strawberry, and by Busch & Schooley (3) in

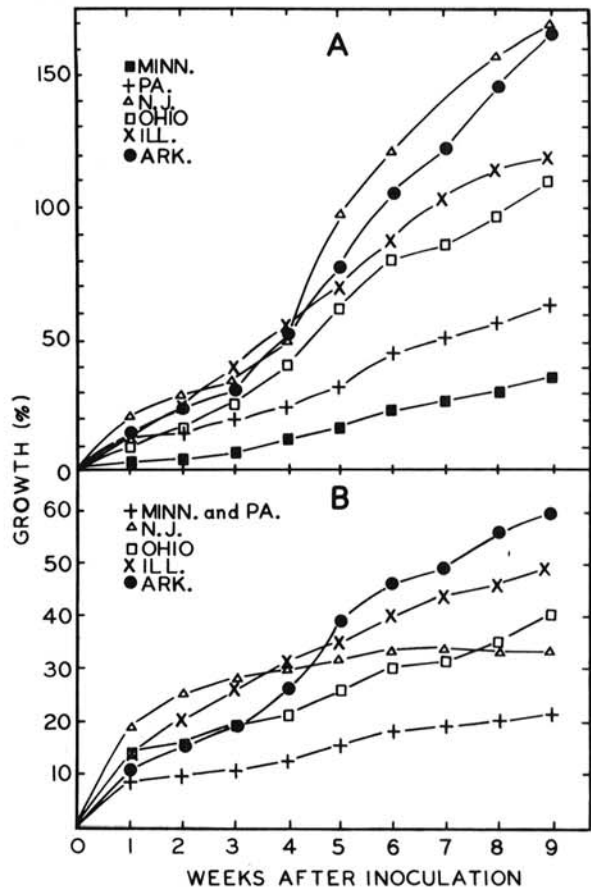


Fig. 1-A,B. Growth of red maple seedlings as a percentage of their height at the time of inoculation with *Verticillium dahliae*: A) Noninoculated seedlings. B) Inoculated seedlings.

chrysanthemum. Pegg & Dixon (8) found that in some tomato cultivars, susceptibility was directly proportional to the amount of mycelium present in the vessels, whereas in others a physiological tolerance mechanism independent of the degree of colonization appeared to operate.

There was some relationship between inherent growth rate and tolerance. For example, noninoculated seedlings of the Pennsylvania and Minnesota sources were slower in growth than those from other sources (Fig. 1A). The former sources also showed the greatest degree of symptom development. Conversely, the more resistant Arkansas and Illinois sources grew rapidly (Fig. 1A). However, the fastest-growing family, from New Jersey, was intermediate in susceptibility. Therefore, one cannot conclude that rapid growth and good vigor are unequivocally correlated with resistance to *V. dahliae*. However, such a close relationship between growth rate and tolerance has been shown for strawberries (5), and therefore may offer a preliminary selection index.

The outlook appears favorable for development of

Verticillium-tolerant clones of red maple. Additive variance is high, and several seedlings in the most tolerant families showed less than 10% foliar symptoms after two inoculations. Even those seedlings that were slightly more susceptible may have adequate "field tolerance" to be included in a breeding program. Varieties of tobacco have been used in this manner (7), and Berry & Thomas (1) presented evidence that intermediate resistance in mints is adequate for commercial production.

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