Verticillium Wilt of Yellow Poplar

A. L. Morehart, F. M. Donohue III, and G. L. Melchior

Associate professor of Plant Pathology, and graduate research assistants, respectively; Plant Science Department, University of Delaware, Newark 19711. Present address of the second author: American Cyanamid Company, Agricultural Division, P.O. Box 400, Princeton, NJ 08540.

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ABSTRACT

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The pathogenicity of *Verticillium dahliae* and *V. albo-atrum* to yellow poplar was demonstrated. Five *V. dahliae* and two *V. albo-atrum* isolates from four host species were tested for pathogenicity to six hosts. The order of relative susceptibility of these hosts, from most to least, was eggplant, maple, tomato, yellow poplar, potato, and dahlia. Cultures from root and soil samples collected in a survey from 96 yellow poplar sites located in woodlots throughout Delaware indicated an incidence of *Verticillium* spp. of 14.6%. *V. albo-atrum* was isolated from the roots of asymptomatic mature yellow poplar trees and diseased yellow poplar seedlings; whereas, *V. dahliae* was isolated only from rhizospheres. Eleven isolates obtained from the survey all induced disease when inoculated into yellow poplar or Bonnie Best tomato seedlings.

Additional key words: vascular disease, soil fungi, *Liriodendron tulipifera*.

Verticillium wilt of yellow poplar (*Liriodendron tulipifera* L.) caused by *Verticillium dahliae* Klebahn was first reported in 1929 on a specimen tree in France (8). Engelhard and Carter (11), in 1956, reported that *Verticillium albo-atrum* Reinke & Berthold was found to be the primary pathogen of a wilted yellow poplar tree. In the same year, Waterman (35) isolated *V. albo-atrum* (microsclerotial form; ie, *V. dahliae*) from a yellow poplar tree growing in an urban area of Connecticut. Inoculation of yellow poplar trees in a nursery with this isolate produced wilt symptoms and positive reisolations in 40 of 42 attempts. In 1960, Kessler and True (20) isolated *V. dahliae* from a wilted yellow poplar tree growing in a forested area of West Virginia. Himelick (16) summarized the known occurrences of *V. albo-atrum* attacking trees and shrubs and reported that 18 naturally infected yellow poplar trees had been observed in Illinois. Eight of 20 artificially inoculated trees became infected. Recently, Smith and Neely (32) reported successful inoculation of yellow poplar trees with eight different isolates of *V. dahliae*. In Delaware, *Verticillium* wilt of yellow poplar was observed on seedlings and occasionally on mature trees in both urban and forest settings (7).

This study was undertaken to assess the occurrence of *Verticillium* species in the rhizosphere of yellow poplar trees in Delaware and to better understand the relationship, if any, between isolates of *V. dahliae* and *V. albo-atrum* which attack commonly grown herbaceous plants and their relative pathogenicity to yellow poplar.

MATERIALS AND METHODS

Verticillium isolates. Six isolates of *Verticillium* spp. were obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852. The isolates and host sources were: *V. albo-atrum* ATCC 13542 from *Acer platanoides* L. (Norway maple); *V. dahliae* ATCC 11405 from *Mentha piperita* L. (peppermint); *V. dahliae* ATCC 18697 from *Solanum tuberosum* L. (potato); *V. albo-atrum* ATCC 13391 from *Solanum tuberosum* L. (potato); *V. dahliae* ATCC 16535 from *Lycopersicon esculentum* Mill. (tomato); *V. albo-atrum* ATCC 26001 from *Lycopersicon esculentum* Mill. (tomato). Eleven isolates were cultured from infected yellow poplar seedlings, root tissue from asymptomatic mature yellow poplar trees or the rhizospheres of mature yellow poplar trees growing in Delaware. A total of 96 yellow poplars, 32 in each of three counties, were designated for rhizosphere sampling. Each group of 32 trees contained four tree-diameter-at-breast-height (dbh) classes, namely: 2.5–7.6 cm, 10.2–15.2 cm, 17.8–22.9 cm, and 25.4–30.5 cm. Soil samples were collected from the crown periphery of each tree for a period of 2 yr. Samples were taken to a depth of 15.2 cm below the soil surface with a sterile 1.9-cm diameter soil sampling tube. Subsamples collected at random from each quadrant around the tree were combined, placed in a new polyethylene bag and taken directly to the laboratory. The soil in each sample was mixed and a 30-g subsample was diluted 1:50 (v/v) with sterile tap water. The soil suspension was stirred for 2–3 min and 0.3-ml aliquots were pipetted into 9-cm-diameter petri plates containing 15 ml of 0.5% alcohol agar (26) or water agar supplemented with 100 μg/ml of streptomycin. The soil suspension was distributed evenly over the agar surface with a sterile glass rod. Cultures were incubated 5 days in the dark at 22–24 C. Subcultures of fungal colonies were made on potato dextrose agar (PDA), Czapek-Dox agar (CDA), and Talboys' prune extract medium (33). Root tissue samples were cultured on natural media by the methods of Hansen and Snyder (14) and those of McKeen and Thorpe (24).

Species identification. All isolates used in this study were identified according to the following characters: formation of resting mycelium (*V. albo-atrum*) or microsclerotia (*V. dahliae*) on Talboys' prune extract agar, growth response on PDA and CDA at various temperatures, conidial size, and the presence or absence of dark pigmentation in the conidiophore base cell of cultures grown on natural media (31)

Pathogenicity studies. The ATCC isolates were tested for pathogenicity on four herbaceous host plants, and on saplings of maple and yellow poplar. Eggplant (*Solanum melongena* L. 'Black beauty'); tomato (*Lycopersicon esculentum* Mill. 'Bonnie Best'); potato (*Solanum tuberosum* L. 'Katahdin'), and dahlia (*Dahlia pinnata* Cav.) were grown from seeds in flats of pasteurized peatlite mixture. After several true leaves had formed, plants were removed from flats and inoculated by the root-dip method (2). Propagule suspensions of *Verticillium* isolates were prepared from 14-day-old-cultures grown on Czapek-Dox broth. The contents of each flask, approximately 100 ml, were blended at top speed for 1 min in

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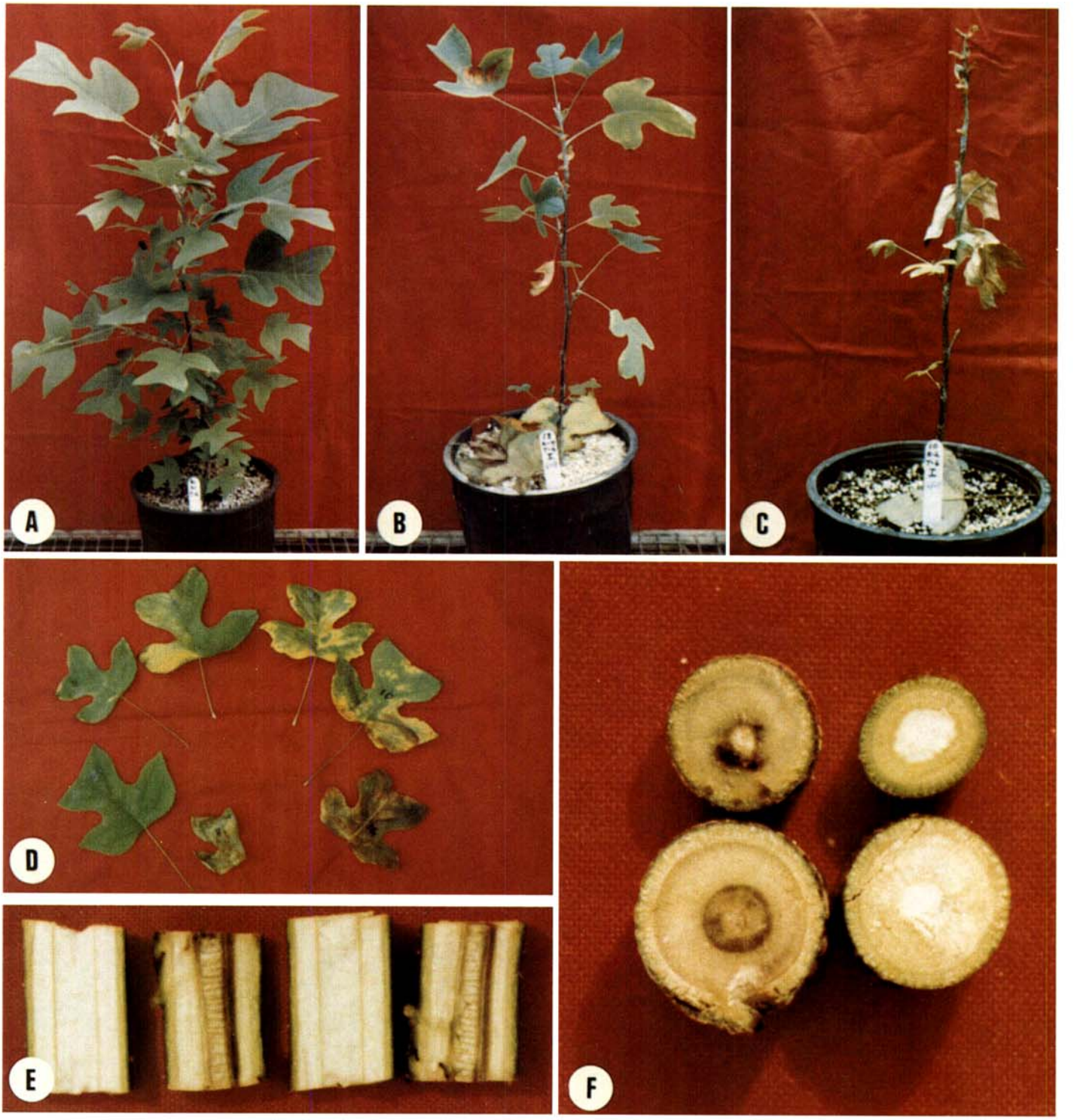

Fig. 1. Symptoms of Verticillium wilt in yellow poplar in Delaware. **A,** healthy tree at time of inoculation; **B,** typical foliar symptoms a few weeks following inoculation; **C,** diseased tree, showing advanced symptoms; **D,** a progression of typical foliar symptoms; and (E–F) characteristic vascular discoloration in **E,** longitudinal section and **F,** cross section.

a Waring Blendor, collected by centrifugation at 2,000 *g*, washed twice with sterile distilled water and resuspended in 100 ml of sterile distilled water. Dilution plate assay indicated a concentration of 4×10^6 viable propagules per milliliter. Microscopic study indicated that the viable propagules were conidia (65–80%), hyphae, or clumped hyphae (20–35%). The root systems of treated plants, except potato, were immersed for 10 min in the inoculum suspension and then planted in 12.7-cm-diameter plastic pots containing peatlite. Potato plants were inoculated in the stem by hypodermic injection of 0.1 ml of the inoculum suspension. Control plants were treated exactly as inoculated plants except that sterile distilled water replaced the inoculum. Six replicate host plants were used for each *Verticillium* isolate. All plants received a similar moisture and fertility regimen during the study period. Disease ratings were made weekly for 8 wk after inoculation. McKinney's (25) infection index (infection index = (sum of numerical ratings × 100) ÷ (total no. of inoculated plants × 3) was used to express numerically the extent of disease development. Infection indexes were correlated with disease ratings as follows: no disease = 0.00; very slight = 0.75; slight = 1.00; moderate = 2.00; and, severe = 3.00. After the final disease ratings were recorded (usually 8 wk), all test plants were cut into 10-cm sections with pruning shears which were dipped in 70% ethanol between cuttings. Each section was labeled and placed in plastic bags. Samples not processed in one day were stored at 0 C. The 10-cm sections were halved and one portion was sectioned radially to expose any vascular discoloration. The other portion was surface sterilized 10 min with a 1:9 (v/v) solution of commercial Clorox (5.25% sodium hypochlorite) then given a 2-min treatment in 70% ethanol. The tissue was then cut into 5-mm sections with a sterile scalpel. Sections were cultured as outlined for root tissue samples.

Three-year-old yellow poplar saplings and 2-yr-old maple saplings were planted on 1.83-m centers in separate 6 × 6 Latin square design in field plots. Inoculum of the various ATCC isolates was prepared as previously described. All inoculations were made by the wound-inoculation method of Caroselli (4). Inoculum consisted of propagules from 14-day-old shake cultures prepared as previously detailed. Each of the inoculated yellow poplar trees received 5 ml of inoculum and each of the inoculated maple trees received 1 ml of inoculum. Control trees were treated identically except the inoculum was replaced with sterile distilled water, or the trees were wounded with no additional treatment. Seasonal growth

TABLE 1. Characterization of *Verticillium albo-atrum* and *V. dahliae* isolates by means of conidial size and conidiophore base-cell pigmentation taken from infected host tissues; and, mycelial resting form, and growth temperature response on potato-dextrose agar

Isolate	*Verticillium* species	Host or source	Conidial dimensions[b]		Conidiophore base-cell pigmented	Type of resting mycelium	Optimal growth range (C)	Growth at 30 C
			Average (μm)	SD (± μm)				
4	*V. albo-atrum*	Potato	5.0 × 2.6	0.8 × 0.3	+	DM[c]	20–27	−
6	*V. albo-atrum*	Tomato	5.1 × 2.5	0.5 × 0.3	+	DM	20–25	−
8	*V. albo-atrum*	Y poplar[c]	5.3 × 2.3	0.8 × 0.2	+	DM	20–27	−
9	*V. albo-atrum*	Y poplar	4.5 × 2.8	0.5 × 0.2	+	DM	23–27	−
12	*V. albo-atrum*	Y poplar	5.5 × 2.6	0.7 × 0.2	+	DM	23–27	−
13	*V. albo-atrum*	Y poplar	5.5 × 3.0	0.9 × 0.6	+	DM	20–27	−
15	*V. albo-atrum*	Y poplar	5.0 × 2.8	0.5 × 0.5	+	DM	20–27	−
16	*V. albo-atrum*	Y poplar	4.8 × 2.5	0.6 × 0.3	+	DM	20–27	−
1	*V. dahliae*[a]	Maple	4.4 × 2.1	0.7 × 0.4	−	MS	23–29	+
2	*V. dahliae*	Mint	4.5 × 2.0	0.6 × 0.3	−	MS	23–27	+
3	*V. dahliae*	Potato	4.6 × 1.8	0.6 × 0.4	−	MS	25–29	+
5	*V. dahliae*	Tomato	4.8 × 1.7	0.9 × 0.4	−	MS	23–27	+
7	*V. dahliae*	Soil	4.6 × 1.9	0.5 × 0.2	−	MS	20–29	+
10	*V. dahliae*	Soil	4.4 × 1.9	0.6 × 0.4	−	MS	20–27	+
11	*V. dahliae*	Soil	4.5 × 1.9	0.7 × 0.3	−	MS	20–27	+
14	*V. dahliae*	Soil	4.2 × 2.0	0.6 × 0.5	−	MS	20–29	+
17	*V. dahliae*	Soil	4.3 × 1.9	0.8 × 0.4	−	MS	20–27	+

[a] ATCC isolate 13642 catalogued as *V. albo-atrum* and later found to be *V. dahliae*.
[b] Average of 100 conidia for each isolate.
[c] Abbreviations used: MS = microsclerotia; DM = dark mycelia; and Y poplar = yellow poplar.

TABLE 2. Relative pathogenicity of *Verticillium* spp. isolates, obtained from yellow poplar or yellow poplar rhizospheres, to yellow poplar seedlings and Bonnie Best tomato plants

Isolate	Species	Source	Infection index[a]		Reisolation[b] (%)	
			Tomato	Yellow poplar	Tomato	Yellow poplar
8	*V. albo-atrum*	seedling	3.00 B[c]	2.61 B	100	80
16	*V. albo-atrum*	seedling	3.27 B	2.68 B	100	100
9	*V. albo-atrum*	root	5.07 A	4.84 A	100	100
12	*V. albo-atrum*	root	4.27 A	2.18 B	67	22
13	*V. albo-atrum*	root	2.67 B	1.39 BC	78	45
15	*V. albo-atrum*	root	4.73 A	3.04 B	100	87
7	*V. dahliae*	rhizosphere	1.20 C	0.38 C	50	33
10	*V. dahliae*	rhizosphere	2.67 B	1.10 BC	78	56
11	*V. dahliae*	rhizosphere	5.27 A	0.73 C	100	50
14	*V. dahliae*	rhizosphere	1.87 C	0.46 C	50	50
17	*V. dahliae*	rhizosphere	1.73 B	0.66 C	68	50
	Controls[d]		0.00 C	0.00 D	0	0

[a] Values represent the average of 12 plants of each host species per isolate as assessed by McKinney's index. See text for an explanation of McKinney's index.
[b] Positive identification of the pathogen on pieces of infected host tissue incubated in a moist chamber or on media composed of host tissue.
[c] Numbers followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[d] Controls, in each case, consisted of plants which were uninoculated and unwounded; wounded and inoculum replaced by sterile distilled water; and wounded alone. No infections were recorded for any control plants.

was recorded for each tree by measuring tree height from the soil line to the apex. Diameter was measured with a caliper, 15 cm above the soil for yellow poplar and 5 cm above the soil line for maple.

Isolates of *V. albo-atrum* and *V. dahliae* obtained from yellow poplar seedlings, roots, mature trees, or rhizospheres were tested for pathogenicity to tomato (Bonnie Best) and yellow poplar seedlings. The procedures used were identical to those previously outlined.

RESULTS

***Verticillium* spp. isolates.** Cultural studies of 768 soil and root tissue samples, obtained over a 2-yr period from 96 yellow poplar sites located throughout Delaware, indicated that *Verticillium* spp. were present at 14 of 96 sites (14.6%). Four isolates of *V. albo-atrum* were cultured from the root tissues of asymptomatic 12, 18, and 28-yr-old yellow poplar trees; two isolates were obtained from obviously diseased yellow poplar seedlings. A second pathogen *Cylindrocladium scoparium* Morg. also was isolated from one of these seedlings. Five isolates of *V. dahliae* were cultured from the rhizospheres of healthy 15- to 30-yr-old yellow poplars. Repeated culturing of root, trunk, and branch tissues obtained from each of these sites failed to yield *V. dahliae*.

Identification of isolates. Seventeen *Verticillium* isolates were identified on the basis of mycelial resting type on PDA and Talboys' medium, growth response on PDA at 30 C, the presence or absence of dark pigmentation in the conidiophore base cell, and conidial size (Table 1). The most consistent features which distinguished *V. albo-atrum* from *V. dahliae* were the presence of dark pigmentation in the conidiophore base cell and the absence of growth at 30 C. Only six of nine *V. dahliae* isolates consistently formed microsclerotia on Talboys' medium. Conidial size was highly variable for both species but *V. albo-atrum* conidia were generally larger than those of *V. dahliae*. These data agree with those of previous taxonomic studies (5,18,19,30,31).

Pathogenicity studies. *V. albo-atrum* and *V. dahliae* isolates obtained from soil and herbaceous or woody host plants all were pathogenic to yellow poplar and each of the other test host plants. *V. albo-atrum* isolates 4, 6, and 9, and *V. dahliae* isolate 1 were the most pathogenic to yellow poplar (Tables 2 and 3). Symptoms of *Verticillium* infection in yellow poplar usually were first seen 2–3 wk after inoculation. Initially, the lower leaves became chlorotic with obvious sectoring. Severely affected leaves became necrotic and abscised. All inoculated yellow poplar trees had some degree of dark brown to black vascular discoloration (Fig. 1). Reisolation attempts were positive for all inoculated plants except for yellow poplar saplings inoculated with isolates 1 and 2 (Table 4).

In cross-inoculation tests, isolates 1, 4, 5, and 6 were, on the basis of average infection index, equally pathogenic to the six kinds of host plants that were tested; isolate 2, *V. dahliae* from mint, was the least pathogenic isolate tested (Table 3). Eggplant and maple were the most susceptible hosts, yellow poplar and tomato were intermediate, and potato and dahlia were the least susceptible (Table 3). The relative pathogenicity of isolate 4 to potato, eggplant, and tomato was similar to that reported by Robinson et al (28) in the original pathogenicity study of this isolate. Comparison of *V. albo-atrum* isolates 3 and 5 from potato and tomato, respectively, and *V. dahliae* isolates 4 and 6 from potato and tomato, respectively, showed that *V. albo-atrum* isolates generally were more pathogenic.

Another measure of the pathogenicity of *V. albo-atrum* and *V. dahliae* isolates to yellow poplar and maple was the influence of infection on seasonal growth (Table 4). Most of the maple treatments and half of the yellow poplar treatments were taller than the controls. Significant differences in diameter were found with yellow poplar.

TABLE 3. Relative pathogenicity of *Verticillium* spp. to yellow poplar and maple saplings in field plots and selected herbaceous test hosts under greenhouse conditions

Isolate	*Verticillium* species	Host[b]	Infection index[a]					
			Yellow poplar	Maple	Eggplant	Tomato	Potato	Dahlia
1	*V. dahliae*	maple	5.56 A[c]	4.84 AB	4.86 A	3.39 A	2.38 A	1.22 A
2	*V. dahliae*	mint	0.38 B	4.52 AB	0.20 B	1.12 B	0.33 B	1.29 A
3	*V. dahliae*	potato	0.36 B	2.47 B	5.46 A	2.68 B	2.47 A	0.94 A
4	*V. albo-atrum*	potato	5.44 A	3.94 AB	5.11 A	3.77 A	3.14 A	0.90 A
5	*V. dahliae*	tomato	0.78 B	6.73 A	5.44 A	5.44 A	0.58 B	1.00 A
6	*V. albo-atrum*	tomato	5.66 A	3.52 AB	4.42 A	5.24 A	2.72 A	1.32 A
7	Controls[d]		0.00 B	0.00 B	0.00 B	0.00 B	0.00 B	0.00 B

[a] Values represent the average of 36 plants for each herbaceous host and six for each woody host per isolate as assessed by McKinney's index. See text for an explanation of McKinney's index.
[b] Host from which the original isolate was obtained.
[c] Numbers followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[d] Controls, in each case, consisted of plants which were uninoculated and unwounded; wounded and inoculum replaced by sterile distilled water; and, wounded alone. No infections were recorded for any control plants.

TABLE 4. Seasonal growth of a 2-yr-old maple and of 3-yr-old yellow poplar saplings wound-inoculated in the spring with 4×10^6 and 2×10^7 viable *Verticillium* spp. propagules, respectively

Isolate	*Verticillium* species	Mean growth (cm)				Reisolation[c] (%)	
		Maple		Yellow poplar			
		diam	height	diam	height	Maple	Yellow poplar
1	*V. dahliae*	0.5[a]	46.3 AB[b]	0.08 B	1.6	33	0
2	*V. dahliae*	0.5	46.9 AB	0.30 AB	19.6	17	0
3	*V. dahliae*	0.5	49.6 AB	0.50 A	27.8	33	17
5	*V. dahliae*	0.3	16.3 B	0.20 AB	26.1	67	17
4	*V. albo-atrum*	0.5	56.0 A	0.30 AB	25.0	50	33
6	*V. albo-atrum*	0.6	56.6 A	0.10 AB	6.3	33	83
7	Controls	0.3	45.1 AB	0.30 AB	22.1	0	0
	LSD (0.05)	NS	34.1	0.36	NS		

[a] Numbers represent an average of six trees.
[b] Numbers followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[c] Number of trees yielding *Verticillium* spp. divided by total number of trees inoculated per isolate.

DISCUSSION

The results show that yellow poplar, like many other plant species, is susceptible to both *V. albo-atrum* and *V. dahliae* (10,31). Isolates of *V. albo-atrum* were more pathogenic to yellow poplar than were *V. dahliae* isolates. This agrees with other studies which found *V. albo-atrum* to be more pathogenic on numerous plant species than *V. dahliae* (19).

Yellow poplar was less susceptible than maple to either *V. albo-atrum* or *V. dahliae* as measured by disease index. This may account for the frequent reports of *Verticillium* wilt in maple, and the sparsity of such reports on yellow poplar. Isolation of *V. albo-atrum* from the roots of mature asymptomatic yellow poplars is an indication of resistance. Many host plants become systemically invaded by *V. dahliae* but remain asymptomatic (12,17,21). These trees, however, appear to have confined pathogen colonization to the extra-xylary tissues preventing primary xylem infection as described by Talboys (34). This defense mechanism may be a function of tree age in yellow poplar, since we did not observe such a reaction in artificially inoculated seedlings or saplings. Environmental influences, such as moisture stress and temperature, which are known to affect both resistance and pathogenicity (1–3,9,23,27), also may have a role in the absence of disease expression.

Verticillium spp. commonly occur in cultivated soils (6), but little is known of their distribution in forest soils. Lane and Witcher (22) found *Verticillium* spp. and *Acrostalagmus* spp., a widely used *Verticillium* synonym, in the rhizospheres of loblolly pine (*Pinus taeda*) and yellow poplar. The presence of *V. dahliae* in the rhizospheres of yellow poplars in forest settings indicates potential for disease development, since these isolates were pathogenic when inoculated into yellow poplar seedlings. It is probable that naturally occurring fungistatic inhibitors in most soils (29) or the absence of root exudates which provide the nutritional stimulus for microsclerotia germination (13), individually or collectively, inhibit microsclerotial germination.

An increase in Verticillium wilt incidence of yellow poplar seems likely because seedlings are susceptible to both *V. albo-atrum* and *V. dahliae*, inoculum is present in soils and tissues, and many yellow poplars are propagated in nurseries for forest planting (15).

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