Verticillium—Induced Scorch and Chlorosis in Ash[1]

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Abstract

Verticillium dahliae appears to be the causal agent of a common disorder of white and green ash that has increasingly affected nursery and landscape trees in the Upper Midwest. Affected trees are without wilting or vascular discoloration typical of Verticillium symptoms on other woody hosts, although upper branch and unilateral distribution patterns remain common and important clues. Symptoms include considerable light green to chlorotic foliage followed by irregular leaf scorch, defoliation and branch dieback. The fungus was best isolated from leaf petioles on potato dextrose agar containing 100 ppm iprodione + 200 ppm chloramphenicol. Repeated and intensive sampling was often necessary to detect the fungus. Symptom recurrence in following years was common but erratic and unpredictable. A discussion of environmental influences and control possibilities is included.

Index words: Verticillium wilt, disease, ash, scorch.

Species used in this study: Green ash (*Fraxinus pennsylvanica* Marshall); white ash (*F. americana* L.); verticillium wilt (*Verticillium dahliae* Kleb.).

Significance to the Nursery Industry

Verticillium disease is of much greater importance in ash trees in the Upper Midwest than was previously recognized, and can affect up to 50% or more of trees in severely infested soil. Classical symptoms involving wilt and internal discoloration are only infrequently encountered, but foliage chlorosis, scorch, and uneven necrosis, in addition to branch kill and disfiguration are common. Both landscape and nursery trees are susceptible, but symptoms vary greatly from year to year. Cool seasons favor disease expression. Nursery fields vary in disease potential. Proper field selection and management through careful rotation and weed control are suggested. Concerted efforts to isolate the fungus with novel techniques are encouraged where laboratory tests have failed to confirm suspect samples.

Introduction

Individual landscape and nursery trees of both green ash (*Fraxinus pennsylvanica* Marshall) and white ash (*F. americana* L.) have exhibited a mild to severe decline of undetermined causation in Wisconsin and the Upper Midwest for many years. In the last decade, however, the frequency and concern increased substantially. Commercial arborists were the first to call our attention to the problem, and in a random survey of 27 arborists in 1984, 22 of them reported an increased concern over what was referred to as 'severe scorch'. In the late summer of 1985 a nursery reported an unusual 'top chlorosis' problem that affected approximately 5% of some 11,000 seedlings and young trees of mixed white and green ash cultivars. The next year another nursery reported severe scorch and branch dieback involving nearly half of their more than 5,000 ash trees.

Ash in the Northeast were reportedly affected by several significant disorders, especially dieback (7) and yellows (12). However, the symptoms we encountered appeared to be different in many respects.

Symptoms were highly variable, both in their onset, severity and range of host responses. First symptoms were often subtle and easily overlooked by casual observation, with one or more branches along one side or in the upper part of a tree appearing off-color. The foliage assumed a light green to yellow color initially, followed by more dramatic, tan to brown blotches. Symptoms often evolved into an irregular necrotic pattern along the leaf margins, resulting in a condition referred to by nurserymen and arborists as a "scorch decline". In some cases the necrosis affected interveinal tissue more than the leaf margins. A faint chlorotic margin typically separated the green from the brown or tan necrotic tissue. Severe leaf crinkling sometimes occurred, and defoliation of leaflets and/or leaves on affected branches typically followed. Symptoms may have been influenced by varietal effects. For instance, 'Autumn Purple' white ash foliage generally assumed an olive-gray color initially, rather than yellow, and the symptoms often occurred more suddenly than in green ash observed.

Symptoms sometimes spread to additional branches during the season, but in other trees remained confined to one or a few small branches. Buds and cambium tissue of affected branches generally remained healthy in appearance during the current growing season. However, buds and bark shrivelled and became discolored on those branches that did not survive into the next season. No internal discoloration in the vascular elements appeared with any of these symptoms.

Initial symptoms occurred at any time during the summer after mid-June, though they more often showed up in July or early August. When symptoms appeared early in the season, very few lateral twigs developed on the affected branches, and the terminal growth was much more reduced than normal. Branches occasionally re-foliated during the same season, but the next spring severely affected branches were usually dead.

[1]Received for publication September 1, 1993; in revised form March 7, 1994. We appreciate the grant provided by the **Horticultural Research Institute, 1250 I Street, N.W., Suite 500, Washington DC 20005**, in partial support of this research , and we especially appreciate the support and hospitality accorded us by the two nurseries who played key roles in this research. Mr. Jim Hanson was gracious in providing equipment, as were other arborists and nurserymen who assisted. The manuscript was significantly improved by the technical assistance or Mr. Steve Vicen and Ms. Catherine Smejkal in its preparation and Drs. Robert Patton and Douglas Rouse for its review.

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Table 1. **Annual (current season) "scorch decline" symptoms expressed in 21 'Marshall Seedless' green ash on the UW-Madison campus, 1984–1988.**

Symptom expression	Year and number of trees				
	1984	1985	1986	1987	1988
Symptomless	13	13	8	13	19
Slight/moderate	6	3	8	4	0
Severe	2	5	3[z]	2	0
Dead	0	0	2	0	0

[z]Two trees removed after 1986 season.

Another pattern encountered less frequently was a condition, described as 'top chlorosis', which occurred in younger nursery stock in late summer. Affected trees showed progressive yellowing, bronzing and leaf blotching, which moved from the top downward. The associated bark assumed a bronzed or a tan appearance. Foliage of 'Autumn Purple' white ash turned purple in the upper portion of the tree, and the associated bark was generally darker, somewhat mottled and wrinkled. The tree tops were dead the following spring.

A number of known diseases and disorders are associated with one or more of these symptoms. The leaf scorching and marginal chlorosis symptoms attributed to diseases caused by *Xylella fastidiosa* Wells (6, 14), a recently recognized bacterial pathogen, are remarkably similar to certain phases of the 'scorch decline' syndrome we have observed. Verticillium wilt (*Verticillium dahliae* Kleb. and *V. albo-atrum* Reinke and Berth.) also produces several of these symptoms, especially the sudden collapse of foliage on certain branches. Both white and green ash are hosts of Verticillium (10), and we were initially suspicious of this pathogen. However, the extent of chlorosis and lighter green coloration, the generally slow symptom development along with the absence of wilting symptoms and internal vascular discoloration differed from the classical pattern in woody ornamentals. Furthermore, many efforts to isolate Verticillium were negative in several laboratories in the Upper Midwest, including our own.

Several local theories for the disorder also emerged, including the possibilities of winter injury, herbicide damage and potassium deficiencies.

The primary purpose of our research was to determine the cause of 'scorch decline'. The pattern of symptom development and probability of symptom recurrence in individual trees was additional information we wanted to determine, together with strategies for possible control measures. A preliminary report concerning etiology has been published (19).

Materials and Methods

This study was conducted from 1984 through 1988. Trees on the University of Wisconsin-Madison campus were used for landscape surveys and isolation material. Two commercial nurseries with large numbers of ash trees each including several cultivars of green and white ash, were major cooperators in making their trees available for research and observation purposes.

Surveys and Observations. Twenty-one 'Marshall' seedless green ash on the campus, planted the same year and measuring 20–30 cm (8–12 in dbh) were observed prior to onset of fall color for decline symptoms each year from 1984–1988. A severity ranking of 0 (none), 1 (slight), 2 (moderate), 3 (severe) or 4 (dead) was assigned. Selected trees showing symptoms were sampled for pathogen detection, periodically, as were other trees on the campus that showed symptoms in 1987.

Frequency and/or recurrence of symptoms on nursery stock were examined several times from 1985–1987, and two quantitative estimates of prevalence and severity were made. In 1985, 25 trees from 7.5–12.5 cm (3–5 in) dbh and approximately 6.5 m (20 ft) tall, were selected for uniformly severe evidence of scorch decline and marked for examination in 1986. Branch mortality was recorded the following spring, and recurrence of foliage symptoms were noted in July and August with the use of severity indices as indicated in Table 2. On July 7 and August 12, 1987, all trees in a three-year-old nursery block which had a high incidence of scorch decline the previous year, were evaluated for symptoms. 'Autumn Purple' white ash and 'Summit' green ash were examined on both dates, but 'Newport' green ash was examined only on the first date.

Water uptake and translocation studies. The unilateral pattern of symptom expression, accompanied by leaf scorch, suggested possible blockage or physiological disruption of the vascular system, which would result in impaired water

Table 2. **Severity in 1986 of spring branch mortality following previous year (1985) "scorch decline" activity, and subsequent summer recurrence of symptom expression in 10 white and 14 green ash nursery trees.**

Severity index[z,y]	Number and percentage of trees affected					
	Spring branch kill[z]		Symptom recurrence in current season growth[y]			
	May 5		July 21		August 29	
(species):	white	green	white	green	white	green
0	3 (30%)	7 (50%)	6 (60%)	4 (29%)	2 (20%)	2 (14%)
1	—	—	1 (10%)	4 (29%)	5 (50%)	2 (14%)
2	1 (10%)	2 (14%)	1 (10%)	4 (29%)	0 (0%)	5 (36%)
3	4 (40%)	4 (29%)	0 (0%)	2 (14%)	0 (0%)	4 (29%)
4	2 (20%)	1 (7%)	0 (0%)	0 (0%)	1 (10%)	1 (7%)
5	—	—	2 (20%)	0 (0%)	2 (20%)	0 (0%)
Total	10	14	10	14	10	14

[z]Branch kill severity index: 0 = no dead branches; 2 = 10–25%; 3 = 25–50%; and 4 = >50% branch mortality.

[y]Current season severity index: 0 = symptomless; 1 = slight; 2 = moderate; 3 = severe; 4 = very severe; and 5 = tree killed.

Table 3. Symptom dynamics on July 7 and August 12, 1987 in a nursery block with a history of "scorch decline" and containing 100 'Autumn Purple' white, 56 'Newport' and 52 'Summit' green ash.

	Tree response (numbers and percentage							
	symptomless		carryover dead branches		carryover + new symptoms		new symptoms only	
Cultivar	July 17	Aug 12	July 17	Aug 12	July 17	Aug 12	July 17	Aug 12
'Autumn Purple'	71 (71%)	52 (52%)	12 (12%)	8[z] (8%)	0 (0%)	3 (3%)	17 (17%)	37 (37%)
'Summit'	24 (46%)	9 (17%)	19 (37%)	10 (19%)	2 (4%)	11 (21%)	7 (13%)	22 (43%)
'Newport'[y]	12 (21%)	— —	34 (61%)	— —	5 (9%)	— —	5 (9%)	— —

[z]One tree killed by unrelated canker.

[y]August 12 data not taken.

translocation. We used two approaches to test this hypothesis. The first was a syringe injection, modified after the technique used by Lee, et al (11) for the diagnosis of citrus blight. In July, 1986 31 10 cm (4 in) dbh nursery trees ranging from symptomless to severe scorch, including both green and white ash, were selected and indexed for severity. A small hole was drilled into the wood on either side of each tree, care being taken with the diseased trees to select a 'diseased' and 'symptomless' side of the tree as much as possible. A 30 ml disposable syringe fitted with a needle of the same diameter as the hole was then filled with 25 ml of water and the needle inserted into one of the two holes. Water was then injected by pressure on the syringe for one minute, the amount of uptake was recorded, and the process repeated with the second hole.

Two white ash, one symptomless and one showing recent, moderately severe symptoms, were injected on August 12, 1986 with a viable dye as a second way to demonstrate effect of the disease upon vascular function. A Hanson electric battery pump (Hanson and Associates, P. O. Box 7604, Madison, WI) capable of delivering 15 psi and equipped with a plastic harness containing Hanson tree injection heads was used to inject a .01% basic fuchsin solution into the trees 60 cm (2 ft) above the soil line. The symptomless tree, measuring 17 cm (7 in) dbh was injected first, using four injector heads. The diseased ash was larger, measuring 28 cm dbh (11 in), so seven injector heads were used. The trees were dissected immediately after injection and examined for the purple stain. Cross-sections of the roots and the stem at the soil line were examined for possible downward movement, while cuts at, and three representative points above, the injection site provided evidence of upward distribution.

Isolation procedures. Attempts to isolate *Verticillium*, or other possible fungal pathogens, were made primarily with modifications of potato dextrose agar (PDA), supplemented with nutrient dextrose agar (NDA), ethyl alcohol and streptomycin agar (ES), following standard laboratory techniques. Wood chips from stem tissue, and less frequently root tissue, were selected for most of the early isolation efforts. Woody material was prepared by flaming after first rinsing in 90% ethanol. The bark was then aseptically removed and wood chips from 0.5–1.0 cm in size were excised and placed on the agar in petri dishes. Leaf tissues were surface sterilized sequentially in 70–95% ethyl alcohol (ETOH) (60 sec), 20% Chlorox (120 sec) and ETOH again (30 sec) before rinsing in sterile water and plating sections on the appropriate agar media. Plates were then allowed to incubate on the laboratory bench for 7 days at 22–25°C (71–75°F). In 1987 a semi-selective media was developed to control *Alternaria* and bacterial growth by incorporating 100 ppm iprodione active ingredient (a.i.) (Chipco 26019) and 200 ppm chloramphenicol into PDA (icPDA). Isolation attempts from sumac (*Rhus* sp.), redbud (*Cercis canadensis* L.) and velvetleaf (*Abutilon theophrasti* Medic.) showing suspect symptoms were made in 1987 and from linden (*Tilia cordata* Mill.) in 1986.

Efforts to isolate the bacterium *Xylella fastidiosa* were made in July, 1986. Four to eight stem sections were taken from each of 6 suspect green ash trees on the UW-Madison campus. Five were plated on a semi-solid media, and one in a broth substrate, by the use of techniques similar to those described by Davis, et al (4).

Inoculation studies. Two cultures of *V. dahliae*, one isolated from nursery ash and another from linden in 1986, were used to determine whether the chlorotic and scorch symptoms could be caused by this pathogen. Thirty one-year-old 'Summit' green ash seedlings, planted in 20 cm (8 in) containers and maintained in outdoor cold frames, were inoculated on June 12, 1987 with an injection of spores from the ash isolate; another 30 were inoculated on July 17 by a mycelial implantation technique in which a small amount of mycelium into a wound beneath bark that was opened with a sterile scalpel. The resulting bark flap was replaced

Table 4. Uptake of water by syringe injection into two sides of 12 symptomless green and white ash, 3–5 in dbh, versus 19 trees with varying unilateral "scorch decline" symptoms in late July, 1986.

		Average uptake (ml/min) per tree side[z]		Total uptake/ tree
Severity index	No. trees	symptomless	with symptoms	
symptomless	12	25	—	50
slight	3	25	8	33
moderate	4	25	7	32
mod. severe	7	18	4	22
severe	4	6	0	6
> 50% symptoms	1	0	0	0

[z]maximum of 25 ml water/side in 60 seconds.

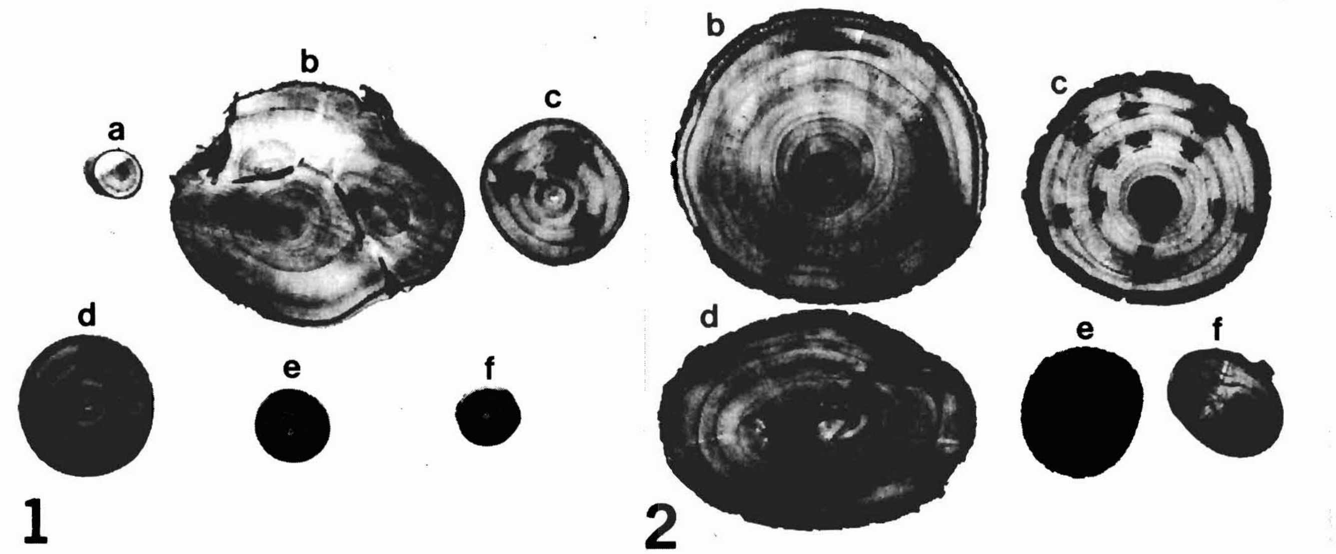

Fig. 1. **1–1: Stem (and root) cross sections of a symptomless white ash tree injected in August, 1986, with an aqueous solution of basic fuchsin (viable dye), demonstrating uninterrupted uptake and distribution in the vascular system: a = root 6 inches below soil line; b = stem at soil line; c = stem at injection site; d–f sequential stem sections above injection site, distance not measured. 1–2: Similar cross sections of a "scorch decline" tree showing limited dye coloration and illustrating restricted vascular uptake and distribution.**

and covered with masking tape to avoid desiccation. The same procedure was repeated on a second set of trees with the linden isolate while a third set of trees were used as 'wound only' controls. The spore suspensions containing 1×10^6 spores/ml were prepared from inoculum grown for 10–14 days on PDA in room light and then injected into the stem in three places with a 27 gauge hypodermic needle and 1 ml syringe. Approximately 0.01 ml was allowed to be drawn into the transpiration stream at each site.

Trees were randomly placed in an open cold frame, watered every other day, and observed for symptoms. After symptoms developed, approximately one third of the trees were destructively sampled for *Verticillium*. Detached bark, wood chips, petioles and petiolules were surface sterilized for 60 sec in 95% ethyl alcohol, 120 sec in 20% Chlorox, another 30 sec in alcohol, then rinsed in sterile distilled H_2O before plating on either PDA or icPDA. The remaining trees were observed for the remainder of the season, at which time their roots (and containers) were trenched into soil to allow for survival and branch-kill observations the following spring.

On May 28, 1988 an additional 10 green ash saplings were inoculated with the spore suspension technique described above. This time a *V. dahliae* isolate obtained from the other nursery in 1987 was used. On June 14, five additional seedlings were similarly inoculated with an isolate from velvetleaf. Wounded, non-inoculated controls were included. Re-isolation of the fungus followed symptom expression.

Results and Discussion

Surveys and observations. Whereas new symptoms appeared in the landscape trees during four of the five years under surveillance, (Table 1), their recurrence in any individual tree was not predictable. For instance, one tree showed mild symptoms and another severe symptoms the first year, but there was no recurrence in any succeeding years. However, another two showed symptoms from 1984–1987. One tree showed moderate symptoms in 1984, 1986 and 1987, but none in 1985. Of the 21 trees, eight never showed symptoms. Two trees were removed in 1986 because of increasing severity over the three year period. Only a few twigs remained alive when they were removed.

Branches killed by the disease were common in the landscape trees the following season after symptom expression, and dead branches were found in the nursery as well in 1986 (Table 2). Seven of 10 (70%) green and 7 of 14 (50%) white ash showed at least some branch mortality. By season's end current season symptoms had recurred in 80% of the white and 86% of the green ash observed. A similar pattern of branch mortality and recurrent symptoms was evident in nursery trees in 1987 (Table 3), but in this block we did not have data on the previous year's disease incidence. These results illustrate how severe the disease can become in a nursery situation.

Water uptake and translocation. Water and viable-dye injections demonstrated clearly that vascular conductivity was severely disrupted (Table 4 and Fig. 1). Syringe injection of water was quick and uniform in symptomless trees (Table 4), and was similarly efficient on the symptomless side of trees showing slight to moderate symptoms. But uptake was sluggish or absent on the side showing scorch, and was even diminished on both sides of those trees with greater symptoms.

When a symptomless tree was injected with an aqueous solution of basic fuchsin, the foliage throughout the tree developed a reddish tinge within a short time. Within the vascular system of the wood the dye could be found in the root 60 cm (24 in) below the injection site (lowest point examined) and upward throughout the tree (Fig. 1). By contrast, with the diseased tree only the foliage on branches free of symptoms showed a reddish color, and the internal vascular staining was similarly restricted, indicating disturbed translocation, although we did not examine for histological evidence of vascular plugging. Basic fuchsin was an effective dye for this purpose, but neither the foliage nor wood of the trees responded to acid fuchsin.

Isolation results. Although *V. dahliae* was a primary suspect of scorch decline for several years prior to the onset of this study, routine laboratory isolation efforts of our laboratory as well as those in surrounding states were negative. Our first success occurred following isolation attempts from several nursery trees from one nursery in August of 1986. Isolations from stems of 7 and roots from one of 10 suspect trees were positive, but neither stems nor roots from similar trees of the second nursery yielded the fungus.

Alternaria was a common inhabitant of the wood chips sampled, and grew from within the wood even following surface disinfection. When iprodione was added to the media in 1987 to inhibit this fungus, detection of Verticillium was made easier, and the frequency of isolation was increased. In one isolation comparison made in late summer of 1987 and involving 20 nursery trees with current season symptoms, we succeeded in isolating Verticillium from 12 trees on icPDA but only from 5 trees on antibiotic PDA. We also improved recovery when we began sampling leaf petioles and increasing number of wood chips sampled, e.g., as many as 20–30/tree.

Petiole sampling was especially helpful, not only with trees in this study, but in routine diagnostic laboratory analyses that have followed. Those petioles that were only loosely attached, e.g., whose abscission layer has formed, were especially likely to yield the fungus. Of the 12 successful isolations on icPDA reported earlier, the fungus was isolated only from petiole tissue of 7, and only from wood chips of 3 trees. It was recovered from both wood and petiole tissue of 2 trees, but it was found with much greater frequency from the latter. No method was consistently successful. Repeated and intensive sampling was sometimes necessary for confirmation.

Attempts to isolate *X. fastidiosa*, the xylem-limited bacterial pathogen of plants that has been reported increasingly as an incitant of scorch in urban shade trees of the Eastern United States, were not successful. It is a difficult pathogen to isolate. We are not aware of any reports of its isolation from the Upper Midwest on any crops. Consequently, when Verticillium isolation became increasingly common in our studies, we elected to concentrate our efforts on that pathogen. Further efforts to isolate *X. fastidiosa* were abandoned.

Inoculation studies. First symptoms with the ash isolate of *V. dahliae* appeared 21 days following inoculation as marginal chlorosis and scorching on the foliage of a few trees. Chlorosis became very apparent, especially in the upper leaves, followed by considerable uneven, brown marginal necrosis. Petioles were easily detached, although most leaves remained attached unless disturbed. While none of the trees died, growth was much reduced and trees were obviously stunted by season's end. Of 30 trees inoculated by spore suspension, 21 eventually showed symptoms along with another 17 of 30 green ash wound-inoculated with mycelia (Table 5). None of the trees inoculated with the linden isolate nor any of the control trees developed symptoms.

V. dahliae was recovered from all 15 of the trees showing symptoms that were sacrificed for this purpose (Table 6). It was also recovered from 2 of 6 symptomless trees that had been inoculated with the ash isolate, but the fungus was not found in any of 22 trees inoculated with the linden tree isolate nor the 10 non-inoculated control trees that were examined.

Branch mortality was observed the following spring in 15 of the 35 remaining trees inoculated with the ash isolate (Table 5). Unfortunately, some of the non-inoculated trees could not be located the following spring, but those that remained were symptomless as were all remaining trees inoculated with the linden isolate.

Fig. 2. Symptoms of light green to yellow foliage, irregular marginal necrosis and curling typical of "scorch decline" in green ash saplings following 1988 inoculations with *Verticillium dahliae* isolates obtained the previous year from ash (a and b) and velvetleaf (c).

Table 5. Symptom expression in 1987 and 1988 of sapling 'Summit' green ash with *Verticillium dahliae*, inoculated in June 1987.

Inoculum source	Inoculation technique	Tree symptom responses[z] September[y] 1987	May[x] 1988
ash	puncture (spores)	21/30 (70%)[x]	13/18 (72%)
ash	wound (mycelium)	17/30 (70%)	2/17 (12%)
linden	puncture (spores)	0/30 (0%)	0/18 (0%)
linden	wound (mycelium)	0/30 (0%)	0/17 (0%)
control	puncture (water only)	0/30 (0%)	0/2 (0%)
control	wound (agar only)	0/30 (0%)	0/10 (0%)

[z]Number and percentage of symptomatic trees/total number trees evaluated.
[y]Symptoms included yellowing, wilting and collapse of foliage.
[x]Symptoms were dead branches.

The 1988 inoculations and subsequent isolations confirmed that *V. dahliae* obtained from ash showing chlorosis and 'scorch decline' symptoms were capable of producing symptoms typical of the disorder that we have been researching. The velvetleaf isolate that we used in the second inoculation trial also caused scorch symptoms (Fig. 2).

Through repeated fungal isolation, inoculations into ash with subsequent chlorosis and scorch symptom expression, followed by re-isolation, i.e., Koch's Postulates were satisfied, we conclude that *V. dahliae* is the incitant of 'scorch decline'. The water and dye injection studies, while themselves not conclusive, add further evidence of a vascular disease such as Verticillium.

A number of characteristics of the pathogen and its host relationship may account for earlier failures of its detection and recognition as the incitant of this disease. Variations in symptoms not considered as typical of 'Verticillium wilt' in woody hosts was one reason. Distinct vascular discoloration in woody hosts, a symptom widely accepted as suggestive of this disease (18), was absent throughout our studies. Nor did we encounter sudden wilting symptoms, although typical one-sided effects on the tree were common. Similarly, the gradual development of symptoms in many instances, the extent of chlorosis and the predominance of scorch symptoms were not typical of our previous experiences with Verticillium on other woody hosts. These factors encouraged consideration of other possible causes, especially when isolation attempts failed.

Himelick (10) may have been the first to succeed in isolating Verticillium from ash. He included ash in his list of hosts after he had done some preliminary work on the pathogenicity of *V. albo-atrum*. He noted only occasional faint tan streaks in ash. In personal correspondence (December, 1985), Himelick indicated the difficulty he had encountered with dependable isolation, and suggested the need to take many samples as one possible solution for its detection. Our results support the later, but provide an improved method of isolation and an expanded view of symptom expression.

Table 6. Results of attempts to re-isolate *Verticillium dahliae* from 'Summit' green ash from 28–40 days following inoculation.

Inoculum source	Inoculation technique	Number of trees sampled with symptoms	without symptoms	Isolations obtained
ash	puncture (spores)	7		7
ash	puncture (spores)		3	2
ash	wound (mycelium)	8		8
ash	wound (mycelium		3	0
linden	puncture (spores)		13	0
linden	wound (mycelium)		9	0
none	puncture (water only)		5	0
none	wound (agar only)		5	0

The foliage symptoms in several herbaceous plants infected with Verticillium more closely approximate those we have encountered with ash than do those of woody hosts. In sunflowers, tiny chlorotic spots appear on the leaves, which later become necrotic, resembling senescence (2). In potatoes the fungus is a major component in a complex that causes 'early dying', in which symptoms are difficult to distinguish from normal senescence. The fungus may initially involve only reduced growth, and uneven chlorosis of lower leaves (15). Marginal scorch occurs in Verticillium wilt-infected chrysanthemum, where investigations suggested that restricted water flow in the vascular elements of the foliage causes wilt to occur first near leaf margins (8). These experiences suggest that we need to broaden our concept of symptom expression from Verticillium infection in woody hosts as well. Perhaps the term 'wilt' should be given a lower priority in our concept of this disease.

That the fungus is difficult to detect can be accounted for substantially by two major factors. First, although Verticillium is recognized to be a vascular-invading pathogen (3), the colonization of vessels is often discontinuous, presumably because of its distribution by spores that move passively through the system and become established irregularly along the way. In chrysanthemum, conidia accumulate in petioles and basal portions of the midrib and lateral veins of leaves (1).

Temperature is a second major factor, since it is a major contributor to Verticillium viability and symptom expression. In regions of high summer temperature, infected cotton plants reportedly recovered, at least temporarily, through a condition referred to as 'temperature-mediated recovery', and the fungus could not be isolated successfully. When cooler weather returned, symptoms also recurred, presumably following fungal re-invasion from the soil, and isolation could again be made (3). While we made no attempt to relate isolation successes with temperature, we did note higher success rates in late August and early September than in mid-July.

The complete absence of field symptoms in both landscape and nursery trees in 1988 is noteworthy (Table 1). The isolates we used grew optimally at 20°C (68°F), but showed only scant growth at 28°C (82°F) (highest temperature tested). June-August temperatures established record high levels that year. High temperatures for those three months that summer averaged 85.3°F, compared to 80.1, 78.4, 79.7, and 82.0 for the years 1984–1987.

Considerable confusion exists in the older literature relative to species identification of *V. albo-atrum* versus *V.*

dahliae. Himelick's pathogen would now be recognized as *V. dahliae*. (personal communications, 1985). The latter produces abundant microsclerotia, along with other less significant mycological differences. It also has a wider host range and greater adaptation to slightly warmer conditions than the former, thus making it more difficult to control by rotation (3), and probably through development of host resistance. In spite of the fact that one nursery field had been in alfalfa for the previous several years, we isolated *V. dahliae* from suspect linden, sumac, redbud, and velvetleaf. Isolates from all but the linden appeared identical to the ash isolate, and the velvetleaf isolate proved to be pathogenic to ash. The linden isolate was quite different phenotypically, and was not pathogenic to ash in our trials, possibly due to host-race specificity. Isolates from sumac and redbud were not tested.

Diseased velvetleaf plants were widespread, and may have been the major reservoir of the pathogen, since weeds are widely recognized as a means of Verticillium survival between years of production of susceptible crops (20). Velvetleaf is recognized as a host (17). Sickinger, et al (17) found the host range from velvetleaf isolates to be very limited, thus leading others to suggest the possibility of Verticillium's use as a possible biological control agent for the weed. This would appear ill advised, however, not only because of our experiences, but because Verticillium is a persistent soilborne pathogen, and it is recognized that its host specificity is quite unstable (5). In fact, in unpublished work, Green demonstrated that isolates obtained from tomato, potato, and velvetleaf and originally not pathogenic to soybeans became pathogenic to soybeans (*Glycina max* cv Miami) after five serial transfers to that host (personal communications).

Control or remission of symptoms by fungicide treatments is doubtful. We found injections of neither Arbotect fungicide (65 ml/2.5 cm dbh) nor Tetracycline antibiotic (2.5g/2.5cm dbh) into diseased trees to be of no benefit in trees showing advanced foliage symptoms (unpublished data). Uptake was limited to symptomless parts of the trees, just as we had encountered with water and dye injections. We did not inject trees showing incipient symptoms. Although unlikely to reduce further disease potential, pruning of dead branches the following year after symptom expression in landscape trees is suggested, since tree appearance would be improved, and the majority of diseased trees we have observed have remained aesthetically useful in subsequent years.

Rotation and weed control are suggested for long-term benefit of this soil-borne pathogen, although these practices will have limited value in reducing inoculum density to controllable levels in the short term (16). It may also be possible to use measurements of fungal inoculum density in the soil, or 'inoculum thresholds', as indicators of potential Verticillium problems in selecting future fields for growing susceptible nursery crops. Verticillium inoculum thresholds have been developed for potatoes (13). In limited trials that utilized the potato crop inoculum threshold index and compared inoculum density in soil samples from nursery fields exhibiting high versus low Verticillium histories in ash trees, results correlated well with what might be expected (unpublished data). More precise sampling procedures and establishment of risk thresholds applicable to the nursery industry could be very helpful in managing for Verticillium disease suppression.

In summary, Verticillium wilt is now recognized as a major disease of ash trees in the Midwest and deserves careful attention in nursery crop production and landscape tree management.

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