Verticillium Wilt of Maples: Symptoms Related to Movement of the Pathogen in Stems

W. A. Sinclair, K. L. Smith, and A. O. Larsen

Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
We thank Howard Lyon for help with the illustrations.
Accepted for publication 6 August 1980.

ABSTRACT

Sinclair, W. A., Smith, K. L., and Larsen, A. O. 1981. Verticillium wilt of maples: Symptoms related to movement of the pathogen in stems. 71:340-345.

Saplings of *Acer rubrum* in which the main stems were wound inoculated in September with *Verticillium dahliae* did not develop foliar necrosis or wilt. Infections were compartmentalized within xylem present at the time of inoculation. In the first 10 mo, *V. dahliae* grew only an average of 45 cm upward and 10 cm downward from the wound; no additional growth occurred in the next 14 mo. Xylem discoloration extended beyond the fungus. Greenhouse-grown, 2-yr-old seedlings of *A. platanoides* were inoculated by a split-root technique that provided infected, nonwounded stems on healthy roots. In these plants, *V. dahliae* moved from the xylem sheath of one growth period to that of the next. This movement occurred both by direct radial growth and by growth into bud traces and from there into new shoots and the new xylem sheath. In naturally and experimentally infected maples, acute symptoms (wilt and intercostal necrosis) occurred only if *V. dahliae* was present in xylem of the current growth period. The fungus extended into shoots and petioles of sprouts on main stems, but not into those on topmost or outermost twigs. The extent of branch dieback caused by *V. dahliae* between growing seasons was not related to previous symptoms. Wilt occurred in consecutive years in about half of the naturally infected *A. platanoides, A. rubrum,* and *A. saccharum* that were observed. Trees in remission or showing only chronic symptoms (scorch and suppressed growth) had infections confined to xylem at least 1 yr old. *V. dahliae* was isolated from xylem up to 3 yr old from trees in remission. These observations and results indicate that infections in stem xylem may enlarge during several seasons before death of the tree or dieback of major branches occurs, and that after each season an infection is subject to compartmentalization that can lead to remission of acute symptoms.

Verticillium dahliae Kleb. causes wilt, decline, dieback, or death in hundreds of diverse plant species. Among the many woody suscepts (18,23,34), maples (*Acer* spp.) are most often damaged in the northeastern USA. Infection has become increasingly common in nurseries and young landscape plantings during the last 20 yr because many shade tree nurseries have been established on land where infected herbaceous crops once grew. Some trees which become infected in nurseries show no symptoms until after transplanting. Thus, the pathogen is disseminated with planting stock to new sites.

Although the causal fungus shows some pathogenic specialization among both woody and herbaceous hosts (1,13,19,36), most isolates are virulent on the highly susceptible maples. In the sapwood of these trees the pathogen causes lesions consisting of dark-colored, elongate, necrotic regions often referred to as "vascular discoloration" or "streaking." These may be accompanied by acute external symptoms: wilting, intercostal necrosis and death of leaves, and death of branches or entire plants. Chronic symptoms may ensue. These include: stunted, chlorotic, and deformed foliage, leaf scorch, slow growth, abnormally heavy seed crops, and dieback (9,16,27,38).

Trees that survive after the first display of symptoms may show acute symptoms the next year, but such recurrence is unpredictable. Several years may pass before the infected tree again shows wilt. Inactivation of the pathogen during hot summer weather, as reported for apricot and olive in California (37,39), is unknown in the northeastern USA, and isolation records suggest that inactivation is probably not responsible for remission of acute symptoms in maples. Localization (5) and compartmentalization (32) of infection may be involved, but these concepts have neither been tested for Verticillium infections of perennial plants nor reconciled with recurrence of acute symptoms.

As the disease is not invariably fatal, measures to prolong the life of infected plants have been suggested (34,35) despite the scarcity of information about year-to-year behavior of the pathogen in woody suscepts.

Objectives of the work reported here were to characterize vertical and radial movement of *V. dahliae* from wounds in stems, to learn if the fungus can move from the xylem sheath of one growth period to that of the next in nonwounded stems, and to relate this information to recurrence of acute symptoms in infected trees.

0031-949X/81/03034006/$03.00/0

MATERIALS AND METHODS

Observations of naturally infected trees. Two groups of infected maples were observed: One group (26 stems) was a mixture of Norway, red and sugar maples (*Acer platanoides* L., *A. rubrum* L., and *A. saccharum* Marsh., respectively) 4–10 cm dbh, in a shade tree nursery, observed 1969–1972. The other group consisted of 28 Norway maples 20–30 cm dbh in a landscape planting, observed 1975–1976. Branches or stems were dissected to learn the association between internal and external symptoms and to allow isolation of *V. dahliae*. The fungus was isolated on potato-dextrose agar (PDA) or ethanol-aureomycin-streptomycin agar (EASA) (26).

Field experiment. In a natural thicket of red maple seedlings 4–7 yr old and 2–5 m tall, 113 stems were inoculated 17 September 1974 with a suspension of conidia plus microsclerotia introduced into drill wounds 50 cm above ground level. The wounds, 4.8 or 6.4 mm in diameter (according to the tree's stem size), were 2 cm deep and slanted 45 degrees downward from the surface-sterilized bark surface. The inoculum, a composite of six isolates of *V. dahliae* previously shown to be pathogenic to Norway maple seedlings, was prepared by mixing microsclerotial cultures grown on PDA with an equal volume of conidial suspension containing 10^7 cells per milliliter in a blender for 60 sec. The conidial suspension had been prepared by flooding and scraping 2-wk-old PDA cultures, then adjusting cell concentration on the basis of haemocytometer counts. Each small wound received 0.25 ml and each large wound 0.5 ml of inoculum. Similar wounds in 31 trees received only water. After inoculation, wounds were wrapped with Parafilm® (American Can Co., Greenwich, CT 06830).

On each of 10 dates during the next 24 mo, at least 10 inoculated and three control trees were randomly chosen, harvested, and dissected. For each tree, the length of the column of discolored wood upward and downward from the point of inoculation was recorded. Isolations of fungi were attempted on EASA from disks cut 5 mm from each side of the inoculation point and at 10-cm

vertical intervals to beyond the limits of the discolored column.

Greenhouse experiment. Dormant, bare-rooted, 2-yr-old Norway maple seedlings that had been grown in sterilized rooting medium in a greenhouse were assigned in groups of 20 to five treatments. The seedlings were then planted in soil-sand-vermiculite (1:1:1, v/v) mixture in 2.7-L pots and given alternating periods of growth in a greenhouse and rest at 0 C in a dimly lit storage room. While in the greenhouse the plants received supplemental light when necessary to maintain a daily photoperiod of at least 14 hr, and they occasionally received dilute soluble fertilizer through the watering system. Treatments applied in the greenhouse experiments were:

Treatment 1—Stem inoculation. Two weeks after planting, each stem was surface-disinfested with 70% ethanol at 10 cm above soil line, wounded as in the field experiment, and inoculated with 0.5 ml of triturated PDA culture of isolate VS1 of *V. dahliae*. The inoculum contained 1.25×10^4 microsclerotia plus 8×10^7 spores per milliliter. Isolate VS1 was one of those used in the field experiment.

Treatment 2—Root inoculation. Two weeks after planting, roots on two sides of each plant were wounded by forcing a knife into the rooting medium. Ten milliliters of inoculum as in treatment 1 were discharged into a columnar zone in each region of wounded roots by means of a Cornwall syringe with a cannula 10 cm × 1.5 mm inside diameter. The cannula was first inserted fully into the rooting medium, then slowly withdrawn as inoculum was discharged.

Treatment 3—Isolated-root inoculation. Before potting, one first-order lateral root was separated from the root mass of each plant. From a point just below the attachment of this root the plant axis was cut upward with a coping saw to 4 cm above the root collar (8–10 cm above the isolated root). The main root mass was then planted in a 2.7-L pot and the isolated root was planted in the same medium in a 500-ml container (satellite container) taped to the side of the pot (Fig. 1). Freshly cut surfaces were covered with a grafting compound to retard drying, and the trees were allowed to begin growth. Sixty trees were thus prepared during 12 wk. Two weeks after the last tree was potted, 20 were selected randomly for treatment 3, and the isolated root of each tree received 10 ml of inoculum as in treatment 2. The satellite container of each isolated root was then covered with tape to prevent splash-transfer of *V. dahliae* inoculum to the pot during watering. Inoculations in treatments 1, 2 and 3 were done 21–22 September 1977. Isolated roots were severed and their containers were separated from the pots 6 wk after inoculation. This was 2 wk after the first foliar symptoms were noted.

Treatment 4—Isolated root inoculation after one cycle of growth and rest. Forty trees prepared as for treatment 3 were given one cycle of growth and rest to provide time for compartmentalization (32) of wounds made during preparation. On 13 March 1978 after 12 wk of growth and 15 wk of rest they were inoculated as in treatment 3 with a suspension of isolate VS1 containing 1.5×10^4 microsclerotia plus 2.89×10^7 spores per milliliter. These trees and all that remained in other treatments were given an additional 20 wk of growth and 19 wk of rest followed by 23 wk of growth, and were dissected in mid-May, 1979.

Treatment 5—Uninoculated control. Trees were potted as in treatment 1 and received no further treatment until dissection.

Although the growth periods did not coincide with natural growing seasons, they did occur within different calendar years. Thus, we designate age classes of tissue by the years in which they formed. When seedlings showed acute foliar symptoms for the second time (or after 20 mo for asymptomatic seedlings) they were dissected and examined for discolored xylem which could indicate possible routes of radial movement of the pathogen. Where these were found, bits of discolored tissue from surface-disinfested (0.5% NaOCl for 2 min) plant parts were incubated on PDA, and the presence or absence of *V. dahliae* was recorded after 2 wk. Discolored stem and (or) root xylem representing tissue present at the time of inoculation also was incubated in most cases to confirm infection by *V. dahliae*. If no discolored xylem was found, a nondiscolored sample was incubated.

RESULTS

Naturally infected trees. Wilt and foliar necrosis invariably were associated with necrosis of the youngest age class of xylem somewhere in the affected branch or stem. The wood first appeared water-soaked, then assumed the greenish-brown to black color typical of infection in maples (16,27,38). If all foliage on a branch suddenly wilted (usually in September–October), a large region of necrotic xylem could be found extending from the trunk into the base of that branch. Virtually all the sapwood there was discolored. Similarly, if a tree died, the xylem of an entire cross section of the trunk had been killed. Discolored xylem did not regularly extend into small branches and twigs; exceptions were those that originated low on the trunk. In cross sections with darkly discolored xylem in peripheral rings, the centrally located wood often was discolored shades of brown distinctly lighter than that of the peripheral infected wood. *V. dahliae* could be isolated only from the darkly discolored xylem, and we saw nothing to indicate previous infection of the central tissues by that fungus. *V. dahliae* was, however, readily isolated from darkly discolored wood ensheathed by one to three annual layers of healthy sapwood in branches that showed no external symptoms. Stunting, leaf scorch, and heavy fruit crops were associated with xylem lesions which involved most of the transverse area of the stem or branch but which were overlaid by thin sheaths of healthy wood. Elongate cankers, as previously noted (16,38), sometimes occurred on main stems where xylem lesions reached the cambium. *Cytospora* sp. and *Nectria cinnabarina* Tode sporulated in the cankers.

External symptoms appearing in the autumn were not good predictors of subsequent damage. For example, 26 nursery trees showed wilt in 1970 or 1971, but only 12 of these showed wilt or intercostal necrosis in 1972. In the landscape planting 28 infected trees were observed through two seasons. Seven that had no external symptoms in 1975 sustained an average of 31% branch dieback during the 1975–1976 dormant season. In 20 other trees that showed chronic or acute symptoms in 1975, average branch mortality during dormancy was 14–15%. Thus, dieback was a major symptom not necessarily preceded by other external symptoms. Wilt occurred in consecutive years in 13 trees, while five trees were externally asymptomatic in both years. Four of 15 trees which showed wilt in 1975 had no foliar symptoms the next year.

Field experiment. No foliar symptoms developed in the wound-

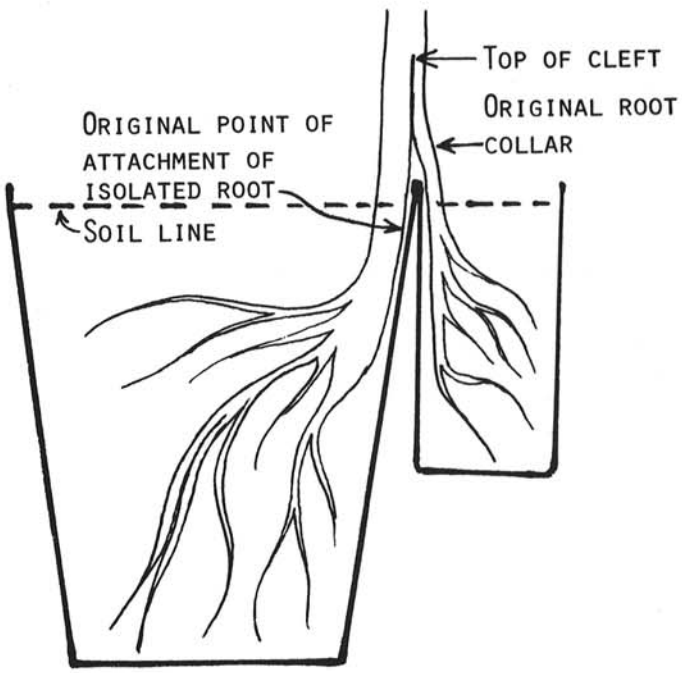

Fig. 1. Scheme for obtaining stem infections by *Verticillium dahliae* in maples on healthy roots. The isolated root in a satellite container is inoculated and, after appearance of foliar symptoms, severed at the soil line.

inoculated red maples. Lesions much larger than those associated with control wounds developed in the xylem of all inoculated stems (Fig. 2), but these infections were restricted to xylem present at the time of inoculation. Discoloration was neither observed in, nor could *V. dahliae* be isolated from, xylem formed in 1975 or 1976. Lesions in 1974 xylem had extended by July 1975 to an average of 60 cm upward and 15 cm downward from the inoculated wounds, and did not enlarge thereafter (Fig. 2). Discoloration usually extended several centimeters beyond the farthest point from which *V. dahliae* was recovered. One year after inoculation this fungus was recovered on the average only 43 cm upward and 10 cm downward from inoculated wounds. The extent of discolored xylem, but not its color or pattern, distinguished the control stems from those inoculated with *V. dahliae*. Discolored xylem in controls extended less than 10 cm up or down after 1 yr but, unlike that caused by *V. dahliae*, continued to extend during the 2nd year (Fig. 2). Diameter of the wounds (6.4 or 4.8 mm) had no apparent influence on the amount of discolored xylem. Some drill wounds of both sizes had not closed after 2 yr.

Isolations indicated replacement of *V. dahliae* by other microorganisms in discolored wood during the 2nd yr. For example, in 10 trees which yielded *V. dahliae* at least 30 cm above the point of inoculation in September 1976, the fungus was present in eight stems at 30 cm, six stems at 20 cm, and five stems at 10 cm. Other microorganisms isolated included species of *Trichoderma, Fusarium,* and several unidentified fungi and bacteria.

Greenhouse experiment. Wilt and foliar necrosis were associated with presence of *V. dahliae* in shoots and often in the bases of petioles of 2-yr-old Norway maples. Most trees inoculated in 1977, regardless of inoculation method, showed recurrent wilt and died or were dissected in 1978. Ten infected trees did not show recurrent wilt. When these were dissected at the end of the experiment (20 mo), columns of discolored xylem from which *V. dahliae* was isolated extended 60–135 (avg 90) cm above the inoculation sites.

Most stem-inoculated trees died back to the point of inoculation, but 17 of 20 began growth from the base after the first rest period (Table 1). Wilt recurred in 12 trees, and discoloration caused by *V. dahliae* was found in 1978 xylem of all 17 trees. In five trees, the discoloration of 1978 xylem occurred only in new shoots, not in the sheath overlying infected 1977 xylem. This indicated direct passage of *V. dahliae* from 1977 xylem into 1978 shoots. *V. dahliae* was isolated from xylem traces of 1977–1978 buds that failed to open on three of these trees (Table 1).

Of 20 trees inoculated in the main root system, 15 began growth after the first rest period. Only three showed recurrent wilt, but all 15 had discolored 1978 xylem (Table 1).

The isolated-root inoculations (treatments 3 and 4) were designed to allow *V. dahliae* to enter stem xylem produced after the time of wounding and, thus, outside of any wound-associated xylem compartment. The isolated roots with their satellite containers were removed after severing these roots at the soil line, 5–6 cm below the top of the cleft which separated the plant axis from the isolated root (Fig. 2). This was done as trees began to show symptoms, or (for asymptomatic trees) 6 wk after inoculation in treatment 3, or 4 mo after inoculation in treatment 4. We hoped that this wounding of root tissue away from the main axis would not stimulate wound-associated compartmentalization in the stem. After removal of isolated roots, the stems were served by healthy root systems.

When isolated roots were inoculated soon after preparation (treatment 3), stems of 15 trees became infected, and 10 showed recurrent wilt (Table 1). In only three trees did a xylem lesion extend below the cleft toward the main root system, and the

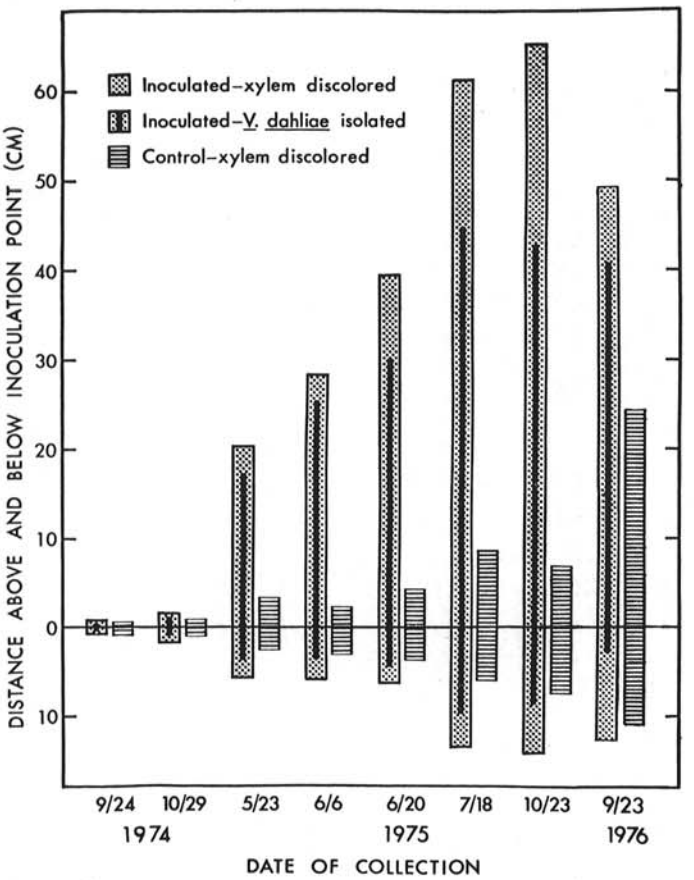

Fig. 2. Extent of discoloration and movement of *Verticillium dahliae* from points of inoculation in stems of red maple wound-inoculated 17 September 1974 and destructively sampled at intervals thereafter. A group of 10 inoculated trees and three uninoculated controls in which similar wounds received only water were dissected on each date. Beginning 6 June 1975, discoloration associated with *V. dahliae* extended significantly ($P = 0.05$) farther upward than did that from control wounds. Differences in lengths of discolored columns between successive sampling dates were significant only for the interval 20 June to 18 July 1975.

TABLE 1. Infection, recurrence of infection and symptoms, and radial movement of *Verticillium dahliae* via bud traces in artificially inoculated Norway maple seedlings[a]

Treatment: (inoculum applied to:)	Stems (infected/ total)	Infected stems (no. that survived to 2nd growth period)	Stems with: recurrent xylem infections	Stems with: recurrent external symptoms	Stems with: *V. dahliae* in bud traces
1. Wound in mainstem	20/20	17	17	12	3
2. Main root system	20/20	15	15	3	0
3. Isolated root, in same growth period as preparation[b]	15/20	12	11	10	4
4. Isolated root, after a rest period[b]	13/39	12	8	0	3
5. Control	0/20	···	0	0	0

[a] Two-year-old bare rooted seedlings were potted, inoculated in various ways, and allowed to complete one and one-half or two and one-half cycles of growth in a greenhouse and rest at 0 C before dissection and isolation of *V. dahliae*. Trees that showed recurrent wilt at the time of the second symptom display were dissected.

[b] Trees were inoculated via a root which was segregated from the main root mass by splitting the plant axis from a point just below the root to 4 cm above root collar, and planting it in a satellite container attached to the main pot. Inoculation was performed soon after preparation of the satellite (treatment 3) or after a rest period of 15 wk (treatment 4).

maximum distance of this discolored xylem was 3 cm, all in 1977 xylem. Thus the movement of *V. dahliae* from 1977 to 1978 xylem had occurred either in the stem or in the isolated root above the cut. Dissection and isolation indicated that in four trees the fungus had crossed from 1977 to 1978 xylem at bud traces. Discoloration in the 1977 xylem sheath of the main stem was contiguous with discolored xylem in 1978 shoots, but 1978 xylem in the main stem was not discolored. In six trees, xylem lesions involved both 1977 and 1978 tissue in the main stem, but a site of radial crossover could not be determined.

When isolated roots were inoculated in 1978 after a cycle of growth and rest (treatment 4), only 13 of 39 trees became infected in the main axis (Table 1). Ten of these showed wilt; none showed recurrent wilt. Three trees that had infections extending below the soil line toward the main root system were removed from the experiment. Of 10 trees with infections confined above the soil line, three had discoloration extending from 1978 xylem into 1979 shoots and petioles, and two of these trees yielded *V. dahliae* from unopened 1978–1979 buds in which the xylem traces were discolored (Fig. 3).

Uninoculated controls showed no external symptoms, and neither discolored xylem nor *V. dahliae* was found in any of 10 that were dissected.

DISCUSSION

Verticillium dahliae is ubiquitous and is becoming increasingly destructive in landscapes populated by susceptible woody plants. Schemes for managing woody ornamentals exposed to or infected by *V. dahliae* will require more information than has heretofore been available about invasion of the perennial woody plant body and year-to-year movement of the pathogen within it. The Verticillium wilt syndrome in maples can not be reconciled with concepts of movement of insect-vectored xylem pathogens in trees (4,8,17). Previous studies of the disease in woody hosts (2,7,9,10,12,13,22,36,39) provide a partial basis for managing infected plants. Our observations broaden this basis and provide insight into the year-to-year development of *V. dahliae* in maples.

Rate and extent of movement within the host. *V. dahliae* moved slowly in wound-inoculated red maple stems, only an average of 43 cm upward in 10 mo. This was equivalent to about 2.36 mm/day during the 6 mo warm enough to allow significant fungal growth. In potted Norway maples held 20 mo, including 371 days in the greenhouse, *V. dahliae* caused lesions that extended only an average of 90 cm above the soil line in plants which were more than twice that height. The fungus thus moved somewhat less than 2.42 mm/day while the trees were in the greenhouse. Mycelium of *V. dahliae* grows 2.5–4.0 mm/day at 15–28 C on common media (9). Thus, all known movement of the fungus in these maples occurred at rates that could have been achieved solely by mycelial growth. Caroselli (9,10) and Santamour (29) reported similar results, but Caroselli (9) also observed columns of discolored xylem that indicated movement at rates averaging up to 50 mm/day in some inoculated trees. Rapid passive movement of *V. dahliae* occurs in root-inoculated hops (30) and cotton (15). Also, in dissecting the red maples, we occasionally found evidence of passive movement for short distances: thin, discontinuous columns of discolored xylem from which we isolated *V. dahliae*. Banfield (3) noted discontinuous discolored streaks in elm stems inoculated with *Verticillium* sp. Determinants of the incidence and extent of rapid passive distribution in maples are unknown.

Discolored, presumably necrotic xylem extended beyond the pathogen above and below points of inoculation in stems (Fig. 2). Smith and Neely (36) also noted this. In addition, *V. dahliae* moved much more rapidly than did the columns of wound-associated discoloration in control stems. Perhaps the fungus, by inactivating xylem parenchyma at a distance from advancing mycelium, was able to temporarily evade the compartmentalizing process which normally restricts microorganisms that enter stem wounds (32). Cessation of vertical movement of *V. dahliae* after 1 yr in stem-inoculated maples may, however, have been due to compartmentalization.

Movement of *V. dahliae* downward was only a small fraction of its movement upward, amounting to less than 10 mm/mo in both field and greenhouse trees. The fungus apparently has negligible ability to move proximally in woody stems and, thus, is localized when inoculated in distal tissues (9,11,12). In addition, wound-associated compartmentalization, as we and also Santamour (29) observed in the field, may help to contain the fungus. Thus, infection through pruning wounds, although demonstrable (12), is of no practical consequence.

Symptoms related to tissue invasion by *V. dahliae*. In trees displaying wilt or intercostal necrosis, *V. dahliae* invariably was found in xylem of the current season, extending even into petioles of 3-yr-old trees in the greenhouse. But presence of the fungus in current xylem was not always accompanied by wilt. Apparently a lesion that causes wilt may either extend into shoots or cause dysfunction of virtually an entire transverse section of xylem somewhere below the shoots. These observations are compatible with descriptions of Verticillium wilt in herbaceous plants (14,24,28). Lesions in wood 1 yr or more of age caused either chronic or no external symptoms.

Zimm (40) first noted that *V. dahliae* can reach petioles of maples. If common, petiolar infections could be important in the dispersal of *V. dahliae* in nurseries and landscapes. Infection of leaves of herbaceous suscepts is well known. This aspect of maple wilt needs more study.

Routes of radial movement. There are four possible routes by which *V. dahliae* may pass from one sheath of xylem to the next: (i) direct growth; (ii) growth into xylem traces of buds, thence successively into new shoots and the subjacent xylem sheath on twigs and branches; (iii) radial growth in infected roots, then growth or passive movement upward into the current year's stem xylem; (iv) reinfection from soil, then upward movement as in (iii). Our observations suggest that vertical movement of *V. dahliae* in maples is often inadequate to allow ramification through a large plant body within one season if invasion begins at overwintering sites in roots or soil. Major dieback or death of large trees must be the result of infection during more than one season. Among our potted trees and those observed in landscape plantings and nurseries were many that, although infected, showed no wilt. The

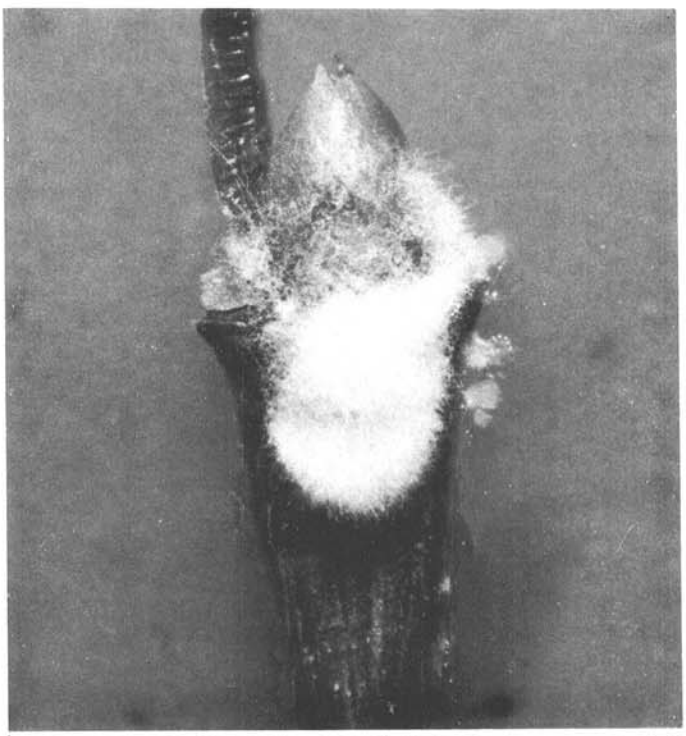

Fig. 3. *Verticillium dahliae* associated with a leaf scar and green bud of Norway maple 6 days after placing a surface-disinfested chip from a stem node on an agar medium.

development, and especially the recurrence, of acute symptoms apparently depended heavily upon radial movement of the pathogen in stems. In potted trees, contiguous infected sheaths of xylem in stems supported by healthy roots indicated direct radial growth of *V. dahliae* from one age class of sapwood to the next. Another route indicated by patterns of discolored xylem and the results of isolations was from 1-yr-old stem xylem, via bud traces to new shoots, and thence into nearby current season's stem xylem. In rose canes, buds also become infected (11).

Inconsistency of radial movement between age classes of xylem was indicated both by the percentages of naturally infected trees in which wilt did not recur annually (54 and 40% in the groups we observed), and by dissection of naturally infected branches. In 20 branches infected 1–5 yr, cross sections revealed 11 cases of discontinuous infection in which discoloration had skipped at least one xylem ring.

Infection by *V. dahliae* caused inhibition of meristematic activity in the greenhouse experiment and was associated with very slow growth of surviving symptomatic branches in naturally infected trees. Presumably the chance of radial movement of *V. dahliae* from one age-class of xylem to the next is enhanced by infection of an abnormally thin sheath of xylem.

Occurrence of a lesion within a xylem sheath overlaid by a healthy sheath of normal thickness can be explained by vertical movement of the pathogen in the inner portion of a sheath already formed, but not close enough to the cambium to inhibit growth. Whether the fungus continues such vertical movement after one season is unknown. The lack of vertical progress after 1 yr in our red maples suggests not, although the compartmentalized infections in those trees may not have been typical. At the end of the greenhouse experiment, in three trees which did not have recurrent symptoms, *V. dahliae* was isolated 60–110 cm above the soil line in 1- or 2-yr-old xylem overlaid by sheaths of healthy xylem.

Localization and compartmentalization of infection. Evasion of localizing (5) and compartmentalizing (32) processes allows xylem pathogens to become widespread within host plants. Evasion leads to recurrent external symptoms. Localization often leads to remission. This is documented for Dutch elm disease (21) and also seems to occur in Verticillium wilt of maples.

The concept of compartmentalization (31–33) encompasses that of localization (5,6) and in addition identifies a significant role of the vascular cambium in producing a physico-chemical barrier (25) which surrounds all xylem present at the time of wounding. This prevents centrifugal enlargement of wound-associated infections. Whether such a barrier develops around xylem infections other than those originating at stem wounds is unknown.

Our experiments gave contrasting results regarding compartmentalization. In red maples in the field, no infections extended into xylem formed after the season of wounding and inoculation. Placing inoculum in wounds after seasonal growth of the trees had ceased (September) but when compartmentalizing processes were presumably strong (20) may have allowed unusually effective containment of *V. dahliae*. Santamour (29), however, noted similar compartmentalization in red and silver maples which he wound-inoculated with *V. dahliae* in June.

In Norway maples in the greenhouse, most infections extended into xylem formed after the first season regardless of the site and method of inoculation. The potted trees were much smaller than those in which compartmentalization has been documented, and the level of stress imposed by wounding plus infection may have precluded compartmentalization. Cambial activity was greatly suppressed or apparently absent in some parts of infected stems.

A hypothesis compatible with all observations is that xylem lesions caused by *V. dahliae* in stems *are* often compartmentalized, especially during the time between suscept growth periods, and that further invasion of the plant body depends upon the fungus either reaching apical meristematic regions (buds = holes in compartment walls) or approaching the vascular cambium so closely as to disrupt formation of the barrier zone. This disruption can lead to recurrent infection of current xylem, to canker formation, or both. In addition, the pathogen has the capability of annual ascent from roots into current stem xylem.

The common recommendation for care of infected trees—pruning and fertilization (34,35)—may now be understood in terms of the tree's response to infection, branch death, and wounding. When an infected branch dies or is removed, wound-associated compartmentalization supplements the tree's ability to localize xylem infections. These processes often confine the pathogen to xylem already present and thus prevent annual recurrence of acute symptoms. Fertilization, if done with care to a balance of major nutrients, benefits the infected tree by enhancing localization as shown by Dwinell (13), and by stimulating growth which lengthens the routes the pathogen must traverse from the xylem of one season to that of the next.

LITERATURE CITED

1. Adams, D. R., and Tattar, T. A. 1976. Effect of host source of inoculum on pathogenicity of *Verticillium albo-atrum* isolates in sugar maple. (Abstr.) Proc. Am. Phytopathol. Soc. 2:93-94.
2. Ashworth, L. J., Jr., and Zimmerman, G. 1976. Verticillium wilt of the pistachio nut tree: occurrence in California and control by soil fumigation. Phytopathology 66:1449-1451.
3. Banfield, W. M. 1941. Distribution by the sap stream of spores of three fungi that induce vascular wilt diseases of elm. J. Agric. Res. 62:637-681.
4. Banfield, W. M. 1968. Dutch elm disease recurrence and recovery in American elm. Phytopathol. Z. 62:21-60.
5. Beckman, C. H. 1966. Cell irritability and localization of vascular infections in plants. Phytopathology 56:821-824.
6. Beckman, C. H., Mueller, W. C., and Mace, M. E. 1974. The stabilization of artificial and natural cell wall membrances by phenolic infusion and its relation to wilt disease resistance. Phytopathology 64:1214-1220.
7. Born, G. L. 1974. Root infection of woody hosts with *Verticillium albo-atrum*. Ill. Nat. Hist. Surv. Bull. 31:205-249.
8. Campana, R. J. 1978. Inoculation and fungal invasion of the tree. Pages 17–20 in: W. A. Sinclair and R. J. Campana, eds. Dutch elm disease: Perspectives after 60 years. Search Agric. (Cornell Univ. Agric. Exp. Stn., Ithaca, NY) 8(5). 52 pp.
9. Caroselli, N. E. 1957. Verticillium wilt of maples. Rhode Island Agric. Exp. Stn. Bull. 335. 84 pp.
10. Caroselli, N. E. 1959. The relation of sapwood moisture content to the incidence of maple wilt caused by *Verticillium albo-atrum*. Phytopathology 49:496-498.
11. Dimock, A. W. 1951. Bud transmission of *Verticillium* in roses. Phytopathology 41:781-784.
12. Dochinger, L. S. 1956. New concepts of the Verticillium wilt disease of maple. (Abstr.) Phytopathology 46:467.
13. Dwinell, L. D. 1967. Selected interactions of *Verticillium dahliae, Pratylenchus penetrans,* major nutrient elements, *Acer saccharum* and *Ulmus americana*. Ph.D. thesis, Cornell University, Ithaca, NY. 262 pp.
14. Garber, R. H., and Houston, B. R. 1966. Penetration and development of *Verticillium albo-atrum* in the cotton plant. Phytopathology 56:1121-1126.
15. Garber, R. H., and Houston, B. R. 1967. Nature of Verticillium wilt resistance in cotton. Phytopathology 57:885-888.
16. Gravatt, G. F. 1926. Maple wilt. U. S. Dep. Agric. Circ. 382. 13 pp.
17. Henry, B. W., and Riker, A. J. 1947. Wound infection of oak trees with *Chalara quercina* and its distribution within the host. Phytopathology 37:735-743.
18. Himelick, E. B. 1969. Tree and shrub hosts of *Verticillium albo-atrum*. Ill. Nat. Hist. Surv. Biol. Notes 66. 8 pp.
19. Hoitink, H. A. J., Snydor, T. D., and Wilson, C. L. 1979. Resistance of maple cultivars and species to Verticillium wilt—a preliminary report. Ohio Agric. Res. Devel. Cent. Res. Circ. 246:46-47.
20. Kile, G. A. 1976. The effect of season of pruning and of time since pruning upon changes in apple sapwood and its susceptibility to invasion by *Trametes versicolor*. Phytopathol. Z. 87:231-240.
21. MacHardy, W. E. 1978. Mechanisms of resistance. Pages 25–26 in: W. A. Sinclair and R. J. Campana, eds. Dutch elm disease: Perspectives after 60 years. Search Agric. (Cornell Univ. Agric. Exp. Stn., Ithaca, NY) 8(5). 52 pp.
22. Malia, M. E., and Tattar, T. A. 1978. Electrical resistance, physical characteristics, and cation concentrations in xylem of sugar maple infected with *Verticillium dahliae*. Can. J. For. Res. 8:322-327.
23. McCain, A. H., Wilhelm, S., and Raabe, R. D. 1970. Plants resistant or susceptible to Verticillium wilt. Calif. Agric. Ext. Serv. Circ. AXT-40. 5 pp.
24. Misaghi, I. J., DeVay, J. E., and Duniway, J. M. 1978. Relationship between occlusion of xylem elements and disease symptoms in leaves of

cotton plants infected with *Verticillium dahliae*. Can. J. Bot. 56:339-342.
25. Mulhern, J., Shortle, W., and Shigo, A. 1979. Barrier zones in red maple: an optical and scanning microscope examination. Forest Sci. 25:311-316.
26. Nadakavukaren, M. J., and Horner, C. E. 1959. An alcohol agar medium selective for determining Verticillium microsclerotia in soil. Phytopathology 49:527-528.
27. Rankin, W. H. 1914. Thrombotic disease of maple. (Abstr.) Phytopathology 4:395-396.
28. Robb, J., Busch, L., and Lu, B. C. 1975. Ultrastructure of wilt syndrome caused by *Verticillium dahliae*. I. In chrysanthemum leaves. Can. J. Bot. 53:901-913.
29. Santamour, F. S., Jr. 1979. Inheritance of wound compartmentalization in soft maples. J. Arboric. 5:220-225.
30. Sewell, G. W. F., and Wilson, J. F. 1964. Occurrence and dispersal of Verticillium conidia in xylem sap of the hop (*Humulus lupus* L.). Nature 204:901.
31. Shigo, A. L., and Hillis, W. E. 1973. Heartwood, discolored wood, and microorganisms in living trees. Annu. Rev. Phytopathol. 11:197-222.
32. Shigo, A. L., and Marx, H. G. 1977. Compartmentalization of decay in trees. U. S. Dep. Agric. Inform. Bull. 405. 73 pp.
33. Shortle, W. C. 1979. Compartmentalization of decay in red maple and hybrid poplar trees. Phytopathology 69:410-413.
34. Sinclair, W. A., and Johnson, W. T. 1975. Verticillium wilt. Cornell Tree Pest Leafl. A-3 (revised). 7 pp.
35. Smith, L. D. 1979. Verticillium wilt of landscape trees. J. Arboric. 5:193-197.
36. Smith, L. D., and Neely, D. 1979. Relative susceptibility of tree species to *Verticillium dahliae*. Plant Dis. Rep. 63:328-332.
37. Taylor, J. B. 1963. The inactivation of *Verticillium albo-atrum* in apricot trees. (Abstr.) Phytopathology 53:1143.
38. Van der Meer, G. H. H. 1926. Verticillium wilt of maple and elm seedlings in Holland. Phytopathology 16:611-614 + pl.
39. Wilhelm, S., and Taylor, J. B. 1965. Control of Verticillium wilt of olive through natural recovery and resistance. Phytopathology 55:310-316.
40. Zimm, L. A. 1918. A wilt disease of maples. (Abstr.) Phytopathology 8:80-81.