Histopathology of A Susceptible Chrysanthemum Cultivar Infected with *Fusarium oxysporum* f. sp. *chrysanthemi*

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ABSTRACT

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Chrysanthemum morifolium 'Yellow Delaware' rooted cuttings grown in steam-treated peat-perlite-soil mix at 18–24 or 29–32 C were inoculated after 2 wk of growth with 100 ml of a conidial suspension of one of two isolates of *Fusarium oxysporum* f. sp. *chrysanthemi*. Terminal leaves of infected plants grown at soil temperatures of 29 to 32 C became chlorotic and twisted after 2 wk and eventually plants wilted and died. The lower temperatures delayed or prevented development of severe foliage symptoms and anatomical changes. Light microscopy of stained sections of stem tissue, together with results of fungus isolations showed the fungus to be systemic in the stem from the stem base to the upper limit of fungus colonization. Conidia were not observed in advance of mycelia in xylem vessels, indicating that culture-indexing should detect the fungus. Infection by either isolate resulted in plugging of xylem vessel elements with gum and pectinaceous materials, hypertrophy and hyperplasia of xylem parenchyma cells, abnormal activity of the vascular cambium derivatives, the formation of cavities within xylem tissue, and eventual colonization of phloem and cortex parenchyma cells, which resulted in their collapse. The collapse and necrosis of these cells resulted in a black streak appearing on the stems of severely wilted plants.

A Fusarium wilt disease of the florist's chrysanthemum, *Chrysanthemum morifolium* (Ramat.) Hemsl., has been reported in the United States and abroad for many years. Since there has been considerable confusion and uncertainty as to which *forma specialis* of *Fusarium oxysporum* causes this disease, a brief summation is given to show why we chose to work with f. sp. *chrysanthemi* of that species. In 1932, Tilford (27) described a wilt and stem rot of chrysanthemum caused by *Fusarium* sp. Coe (7) consistently isolated a species of *Fusarium* from wilted plants in Florida in 1957, but was not able to reproduce the disease. A wilt and stem rot of older chrysanthemum plants was described by Jackson and McFadden (15) in 1961 in Florida and the pathogen was reported to be *F. oxysporum* but attempts to reproduce the disease were rarely successful.

Toop (28) studied a wilt disease caused in chrysanthemum cultivar Encore by *F. oxysporum* and judging from a reference in USDA Agricultural Handbook 165 (31) he concluded that *F. oxysporum* f. sp. *callistephi* was the proper name for the chrysanthemum pathogen (3). Horst (14) obtained a subculture of the fungus from Toop and reported that he was unable to demonstrate pathogenicity of this fungus to three aster cultivars, one of which was known to be highly susceptible to *F. oxysporum* f. sp. *callistephi*. Armstrong and Armstrong (2,3) inoculated the chrysanthemum cultivar Encore with isolates of *F. oxysporum* f. sp. *callistephi* obtained from wilted aster plants and demonstrated that *F. oxysporum* f. sp. *callistephi* was not pathogenic to Encore. Armstrong and Armstrong (3) then inoculated Encore plants with isolates of 40 other recognized *formae speciales* and races of *F. oxysporum* and a subculture of the same fungus used by Toop. Toop's isolate and *F. oxysporum* f. sp. *tracheiphilum* race 1 were the only isolates pathogenic to Encore, indicating that the *forma specialis* with which Toop worked was in reality f. sp. *tracheiphilum* race 1. Toop's Fusarium isolate and Armstrong's isolate of *F. oxysporum* f. sp. *tracheiphilum* race 1 were both pathogenic to the cowpea cultivars that serve as differentials for race 1 of *F. oxysporum* f. sp. *tracheiphilum* (3).

In 1966, Littrell obtained an isolate of *F. oxysporum* from wilted plants of the chrysanthemum cultivar Yellow Delaware (18) and compared this isolate to a subculture of the fungus used by Toop. The isolate from Yellow Delaware was different in that it was pathogenic to Encore but not to the cowpea cultivar Climax, which is known to be susceptible to race 1 of *F. oxysporum* f. sp. *tracheiphilum*. Toop's isolate of *F. oxysporum* f. sp. *tracheiphilum* was pathogenic to the cowpea cultivar Climax and Encore chrysanthemum, but not to the chrysanthemum cultivar Yellow Delaware. In 1970, Armstrong and Armstrong obtained an isolate of *F. oxysporum* from wilted Yellow Delaware plants (4). Cross inoculations confirmed Littrell's results, and showed that the isolate of *F. oxysporum* from Yellow Delaware differed from f. sp. *tracheiphilum* race 1. They proposed that the isolate from wilted plants of chrysanthemum cultivar Yellow Delaware be named *F. oxysporum* (Schlecht.) emend. Snyd. & Hans., f. sp. *chrysanthemi* Litt., Armst., & Armst. (4). At present, these two *formae speciales* of *F. oxysporum* are the only ones reported to cause a wilt disease of chrysanthemum (11). Further work (5) has shown that susceptible cultivars inoculated with f. sp. *chrysanthemi* exhibit severe symptoms 10 days after inoculation and often become severely stunted and die. If the same cultivars are inoculated with f. sp. *tracheiphilum* race 1, symptoms do not appear until 4 wk after inoculation and are less severe and less extensive than those produced by f. sp. *chrysanthemi*. Only mild stunting occurs and the plants rarely die. Therefore, work reported in this paper deals only with f. sp. *chrysanthemi*.

Fusarium wilt of chrysanthemum, which is caused by *F. oxysporum* f. sp. *chrysanthemi*, can cause significant losses in chrysanthemum crops; these losses may occur year after year due to carry-over of the organism in infected propagation stock, the persistence of the organism in soil, and the difficulty in controlling it once it becomes established in a soil (10–12). Diagnosis is frequently complicated by the variability of symptoms on various cultivars and because the symptoms may resemble those of nutrient

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deficiencies, Pythium root rot, or excess water (10,12).

Because there is confusion in the literature about the cause of this disease, its economic importance, the unusual symptoms, and the threat posed by infected symptomless plants, further work on it seemed warranted.

The purpose of the research reported here was to study the comparative anatomy of inoculated and uninoculated plants of the susceptible cultivar Yellow Delaware, and to study the effect of soil temperature on colonization of host tissue by the fungus.

MATERIALS AND METHODS

Rooted cuttings, 7–10 cm tall, from culture-indexed plants of chrysanthemum cultivar Yellow Delaware (Yoder Brothers, Inc., Barberton, OH 44203) were potted singly in 12.5-cm-diameter clay pots containing steam-treated (aerated steam at 78.8 C for 35 min) 1:1:1 (v/v) peat-perlite-soil mix. The soil used was Hagerstown silty loam.

Several isolates of *F. oxysporum* f. sp. *chrysanthemi,* obtained from diseased chrysanthemum plants from Florida were maintained as lyophilized cultures at the Fusarium Research Center of The Pennsylvania State University. Based on the results of a pathogenicity test employing eight of these Fusarium isolates, two of the most virulent, designated 0-693 and 0-734, were selected. These isolates have been deposited in the Fusarium Research Center collection as FRC-0-693 and FRC-0-734. Inoculum was prepared by growing single-spore cultures on potato-dextrose agar (PDA) slants for 14 days under fluorescent lights (on a daily 12-hr off/on cycle) at 21–22 C (30). After 14 days, spores were harvested in sterile water blanks and a hemacytometer was used to adjust the spore concentration to approximately 60,000 spores per milliliter. The spore suspensions contained microconidia, macroconidia, and some mycelial fragments.

Eighty potted plants were placed on a greenhouse bench equipped with heating cables to maintain soil temperatures of 29–32 C (12). Pots were packed in moist perlite to maintain uniform soil temperature. An additional 80 potted plants were placed on a greenhouse bench without heating cables or perlite and maintained at ambient soil temperatures of 18–24 C. Soil temperatures were monitored and recorded by using copper-constantan thermocouples. Continuous lighting prevented flowering. A Chapin watering apparatus was used to water all plants uniformly and to avoid splashing from pot to pot (32). On both benches, soil pH remained at 5.5 and the plants were fertillzed once a week with a 20-20-20 ammoniacal-nitrogen fertilizer.

Plants were inoculated 14 days after potting (12–15 cm tall) by pouring 100 ml of the spore suspension over the soil surface immediately after root wounding. Roots were wounded by plunging a spatula into the soil at five equally spaced sites around the stem. Inoculation with root wounding, while not necessary for symptom development, greatly increases the rate of symptom development (29).

Of the 80 plants on each bench, 35 were inoculated with isolate 0-693, 35 with isolate 0-734, and 10 were used as controls. Roots of control plants were wounded as described and 100 ml of sterile water was poured over the soil surface. Treatments were randomly arranged on each bench.

Two days after inoculation, and thereafter at twice-weekly intervals, all plants are rated for symptom expression. The Engelhard and Woltz system (10) for rating symptom expression was used: 1 = apical leaf chlorosis; 2 = symptoms for rating 1, plus curvature of leaf and/or stem; 3 = symptoms for rating 2 plus stunting; 4 = symptoms for rating 3, plus wilting; and 5 = dead plant.

Randomly selected plants from both benches were sampled on the same twice-weekly schedule as mentioned above until symptoms appeared. Sampling continued until a representative number of plants at the various stages of symptom development was obtained. Sampling was discontinued 7 wk after inoculation although rating of the plants for symptom expression continued for 6 mo after inoculation.

Sampling consisted of severing stems at the soil line and cutting off all leaves. Stems were surface sterilized in a 10% Clorox (5.25% sodium hypochlorite) solution for 5 min, placed on a sheet of paper lined at 5-mm intervals, and cut into 5-mm sections by using sterilized razor blades; each section was assigned a number from the base of the stem to the top. Even-numbered stem sections were placed in petri dishes of carnation leaf agar (CLA) (30) to determine host tissue colonization. Selected isolates from the top, middle, and bottom regions of infected stems were lyophilized and used later to inoculate chrysanthemum Yellow Delaware plants to check pathogenicity to verify their identity as *F. oxysporum* f. sp. *chrysanthemi.* Odd-numbered stem sections were placed in Rawlin's formalin-acetic acid-alcohol fixative # 1 (24), dehydrated in a tertiary butyl alcohol series (17), and embedded in Paraplast (Sherwood Medical, St. Louis, MO 63103). Embedded material was softened in 1% sodium lauryl sulfate (Dreft detergent) and glycerol (9:1, v/v) (1) for ~18 hr and sectioned at 10 μm. Longitudinal and transverse sections were mounted with Haupt's adhesive and stained with Johansen's Quadruple stain (17).

Histochemical tests (16,25) were carried out on selected tissue sections to detect the following chemical constituents: pectin (iron absorption method), wound gum (orcinol, phloroglucinol reactions), suberin (Sudan IV), and cellulose (polarized light).

Stained sections were examined with a Leitz Ortholux research microscope and photographed on Kodak Plus-X Pan film in a Leitz Aristophot camera fitted with a 10.16 × 12.70-cm Graflex back and Kodak Wratten gelatin filters.

RESULTS

Plants grown at soil temperatures of 29 to 32 C. As there was no apparent difference in virulence between the two isolates of *Fusarium* used, the following summarizes the symptoms as they occurred on plants inoculated with either isolate. Initial symptoms of chlorosis and twisting of the apical leaves (Figs. 1 and 2) occurred 14–40 days after inoculation as reported in the literature (13). Some plants remained symptomless 6 mo after inoculation. Many plants with chlorotic and twisted apical leaves were also stunted. Wilted leaves were first observed (Fig. 3) on a few plants as early as 21 days after inoculation and on most plants after 7 wk. The rate of symptom development varied among individual plants; 7–40 days elapsed between an initial rating of 1 or 2 and a rating of 4.

Chlorotic and/or wilted leaves often developed on only one side of the stem from the stem apex to some point down the stem. If axillary shoots were developing on plants with these symptoms, shoots on the side of the plant free from symptoms were usually longer than those on the affected side which appeared stunted and usually developed characteristic disease symptoms.

Following severe wilting of several leaves, a black necrotic streak appeared on the stem, usually on the upper half of the plant. The streak not only extended vertically in both directions, but eventually girdled the stem. Eventually the entire shoot became black and necrotic. Several plants showed this symptom 3 mo after inoculation. Pale-orange sporodochia often appeared on necrotic shoots. Spores collected from these sporodochia were grown in culture and identified as *F. oxysporum.*

Plants grown at soil temperatures of 18 to 24 C. In contrast to the plants grown at soil temperatures of 29–32 C, symptom development among plants grown at 18–24 C was delayed and extended over a longer time period. Initial symptoms (ie, chlorosis and twisting of apical leaves) were observed on a few plants 4–6 wk after inoculation. No plants had wilted leaves or were stunted 7 wk after inoculation. After 3 mo, a few wilted leaves occurred on some plants, but symptom development never approached the severity found at higher soil temperatures. Indeed after 6 mo, most plants were still symptomless.

Pathogen isolations. Petri dishes of CLA with the 5-mm stem sections placed on the agar surface, one to a dish, were incubated under standard environmental conditions (30). Within 2 days, white mycelium was observed growing directly from the vascular area of stem sections from plants with severe symptoms. Mycelia of *Fusarium* outgrew bacterial and fungal contaminants and were

identified without difficulty.

At soil temperatures of 29–32 C initial symptoms of twisting of apical leaves and chlorosis appeared 2–3 wk after inoculation on plants 25–30 cm tall. *Fusarium oxysporum* often could not be isolated from the 4–6 cm of stem tissue immediately below chlorotic shoots showing twisted leaves. Several of the plants colonized as described above were symptomless. Isolations from successive stem pieces demonstrated that the fungus in these stems was in a continuous column from the soil line to the upper limit of fungus growth with no uncolonized stem pieces in between.

Isolates of *F. oxysporum*, obtained from the top, middle, and bottom regions of infected plants and lyophized, were used 8 mo later to inoculate Yellow Delaware plants. Of the 88 isolates from 46 different plants, 77 (87%) were pathogenic to Yellow Delaware, indicating that the *Fusarium* isolated from diseased stem tissue was *F. oxysporum* f. sp. *chrysanthemi*.

Histology of uninoculated plants. The anatomy of uninoculated plants of the chrysanthemum cultivar Yellow Delaware agrees with the description for the primary tissues of the chrysanthemum cultivar, Giant # 4 Indianapolis White (22). In Yellow Delaware, as the plant matures, an interfascicular vascular cambium arises from the interfascicular parenchyma cells and begins to form secondary tissue similar to that found in woody dicots. Phloem tissue differentiates first, followed by secondary xylem. Eventually a continuous cylinder of secondary tissues form (Fig. 4), consisting mainly of secondary xylem composed of pitted vessels, tracheids, nucleated libriform fibers with simple pits, and sparse amounts of paratracheal xylem parenchyma.

Collateral leaf traces and associated leaf gaps occurred regularly in stem tissue of Yellow Delaware. Some were seen at the point where they initially diverged from the vascular cylinder and others at the bases of leaf petioles. In addition, small, amphicribral cortical bundles (Fig. 5) were often observed throughout the stem and may be leaf traces (9).

Histology of inoculated plants. Although symptoms appeared later on plants grown at soil temperatures of 18–24 C than on plants grown at 29–32 C, there were no observable differences in anatomical changes among plants with comparable symptom ratings or infected by different isolates. The following description of the anatomy of infected plants therefore applies to all observed combinations of Fusarium isolate and soil temperature.

Host colonization. Fungal hyphae and conidia, never observed with uninoculated plants, were first observed in a few primary and secondary xylem vessel elements of externally symptomless but

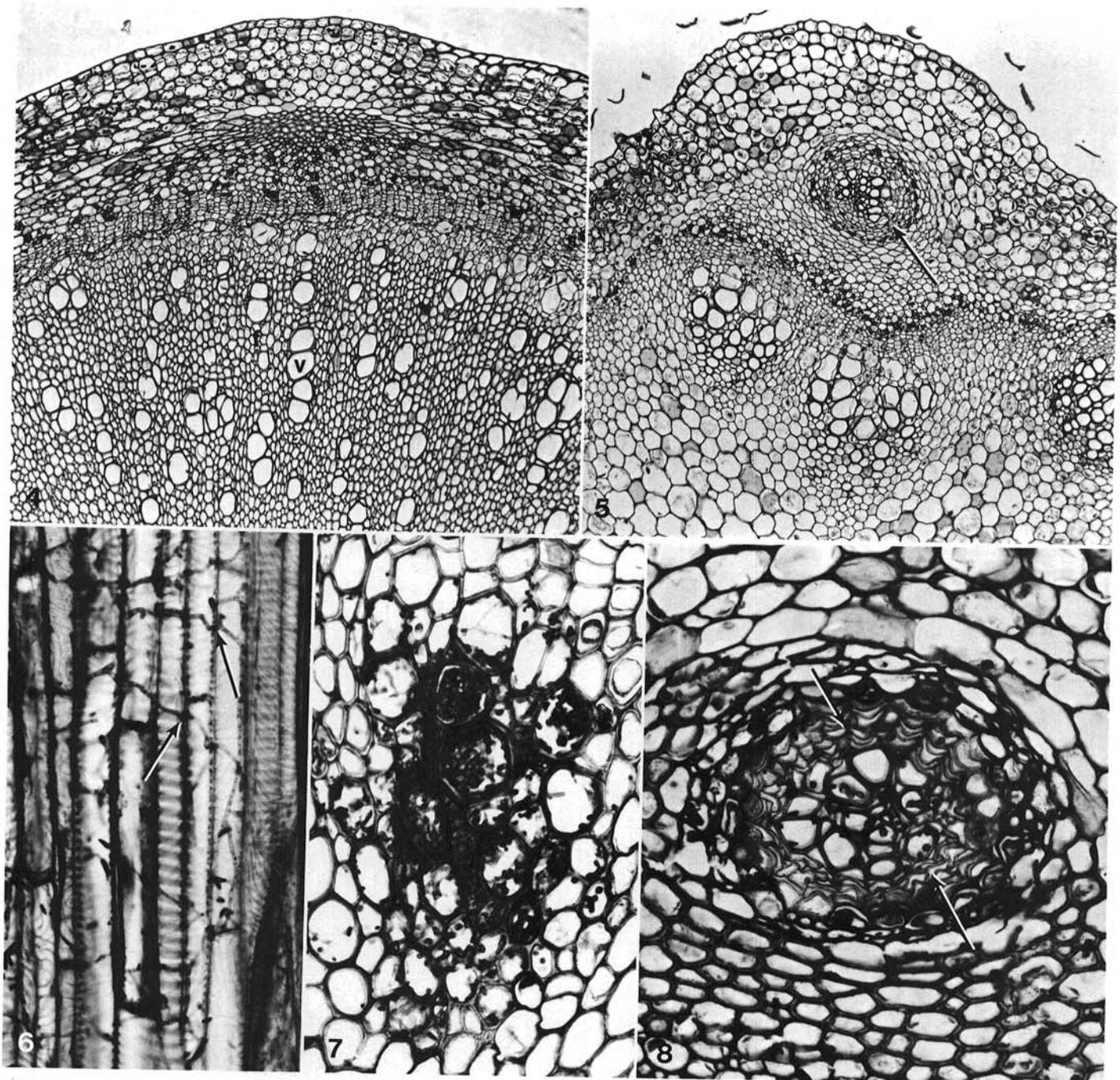
Figs. 1–3. A comparison of *Chrysanthemum morifolium* 'Yellow Delaware' plants infected with *Fusarium oxysporum* f. sp. *chrysanthemi* with uninfected plants. **1,** Terminal portions of shoots from infected (right) and uninfected (left) showing chlorosis and twisting of apical leaves on the infected shoot. **2,** Second and fourth leaves from the left are from infected shoots and show unilaterial chlorosis and twisting. The other leaves are from uninfected shoots. **3,** Infected plant showing wilted, necrotic leaves on the upper part of the plant.

inoculated plants. Hyphae were always observed in stem tissues to whatever stem height isolation results indicated the fungus to be present. Hyphae were observed between the secondary wall thickenings of contiguous vessel elements, indicating passage from one xylem vessel element to another (Fig. 6).

In plants with chlorotic and twisted apical leaves, hyphae and conidia occurred with greater frequency in xylem vessel elements within the stem. Some xylem vessel elements and adjacent secondary xylem fibers were occluded with hyphae and conidia (Fig. 7), but much of the secondary xylem and many vascular bundles remained free of the fungus. Infected vascular bundles commonly occurred only on one side of the stem. In the upper stem, prior to leaf wilting, some leaf traces and concentric cortical vascular bundles, believed to be leaf traces, contained hyphae (Fig. 8).

Many more vascular bundles and most of the secondary xylem was infected in stems of plants with wilted leaves. Also, hyphae were observed in the pith at this time (Fig. 9). Colonization of phloem parenchyma and cortical cells occurred in plants with black stem necrosis. Colonized cortical cells collapsed (Fig. 10), although endodermoid cells containing hyphae within the cortex seemed more resistant to collapse (Fig. 10).

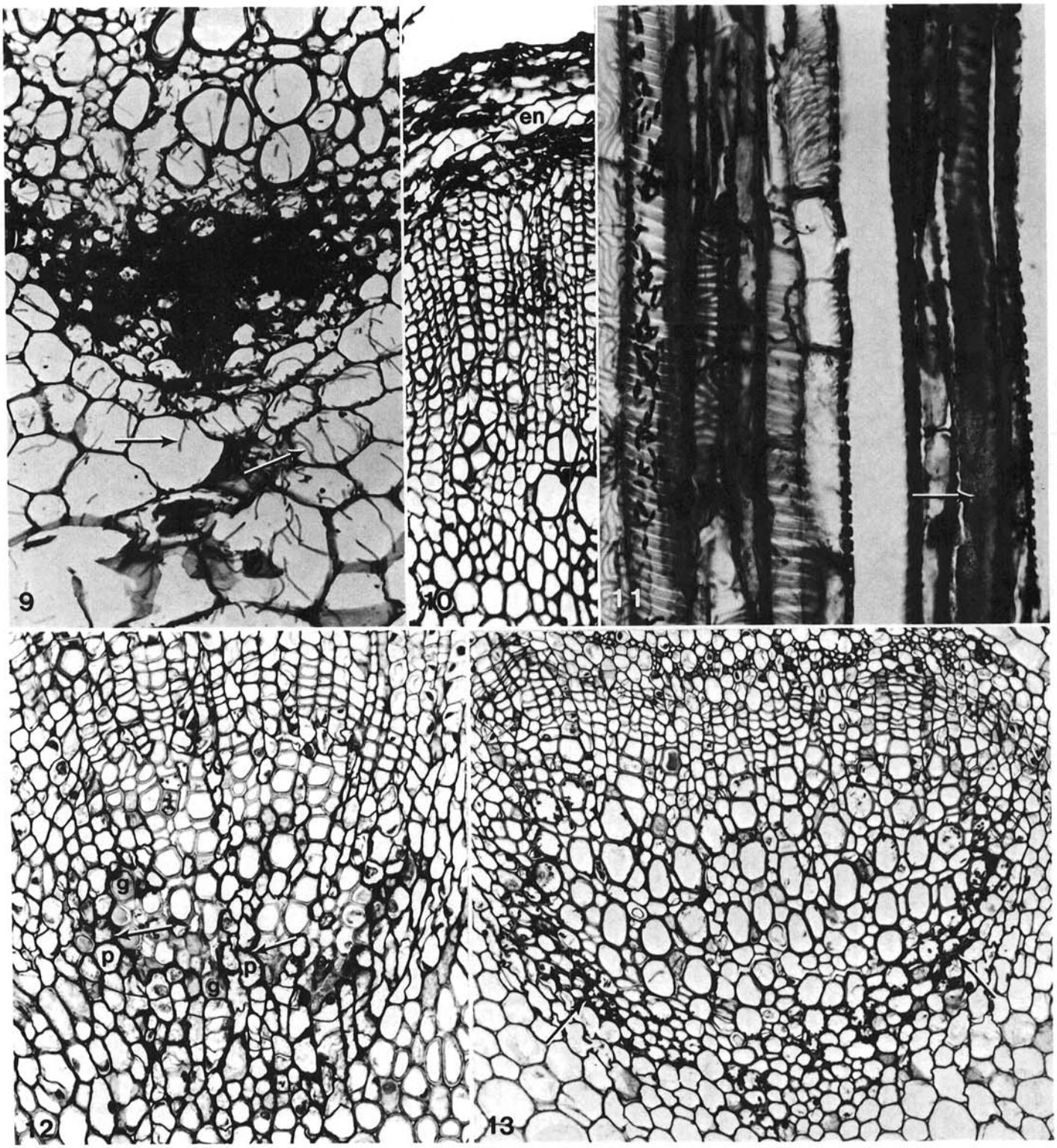
Host responses. The major anatomical host responses observed in chrysanthemum plans infected by *F. oxysporum* f. sp.



Figs. 4–8. Sections through the stems of uninfected *Chrysantheumum morifolium* 'Yellow Delaware' plants and plants infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. **4,** Portion of a transverse section of an uninfected stem showing secondary growth. Note the thickwalled fibers (f) and vessels (v) of the secondary xylem (×72). **5,** Portion of a transverse section of an uninfected stem showing a cortical amphicribral vascular bundle (arrow) (×79). **6,** Portion of a longitudinal section of an infected stem showing hyphae (arrows) passing through the annular and reticulate secondary wall thickenings (×365). **7,** Portion of a transverse section of an infected stem showing several xylem vessel elements partly or entirely occluded with hyphae (×350). **8,** Section of a cortical amphicribral vascular bundle showing hyphae in the xylem vessel elements and gum deposition on adjacent cell walls and distortion and collapse of these cells (arrows) (×320).

chrysanthemi were: plugging of xylem vessel elements with pectinaceous materials and wound gum; hypertrophy and hyperplasia of xylem parenchyma in infected xylem tissue and of pith cells bordering infected vascular bundles; disintegration of xylem parenchyma cells and formation of cavities within the primary xylem tissue of some infected vascular bundles; and dysfunction of the derivatives of the vascular cambium. It is important to note that these host responses do not occur independently but are interrelated and occur in intimate association.

Deposition of gum and pectinaceous materials. A few xylem vessel elements in symptomless, inoculated plants were occluded with pectic substances or with wound gum. Vessel elements



Figs. 9–13. Portions of sections of stems of *Chrysanthemum morifolium* 'Yellow Delaware' plants infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. **9,** Transverse section showing hyphae (arrows) in the pith cells adjacent to a heavily colonized vascular bundle (×210). **10,** Transverse section from a stem with the black streak symptom showing collapsed cortical and phloem cells. Endodermoid cells (en) are not collapsed but contain hyphae (arrow) (×170). **11,** Portion of a longitudinal section of an infected stem showing conidia and hyphae in xylem vessel elements and occlusion of a vessel element with gum (arrow) (×365). **12,** Section of an infected vascular bundle showing xylem vessel elements occluded with gum (g), and adjacent hypertrophied parenchyma cells (p) with pectinaceous materials deposited on the cell walls (arrows) (×195). **13,** Section showing an infected vascular bundle with hyphae in the vessel elements and gum deposition (arrows) at the border between the bundle cells and the pith cells (×150).

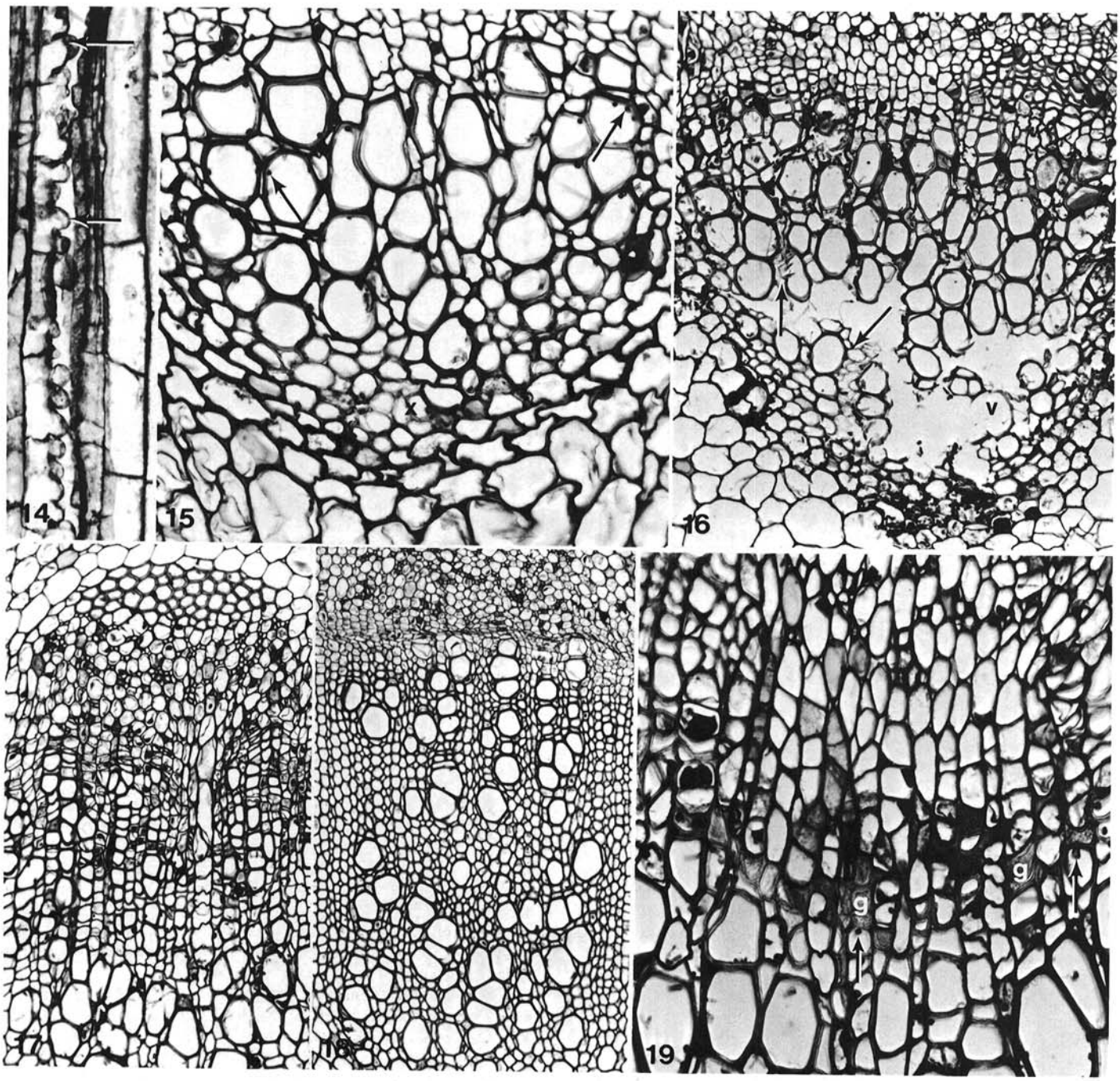
occluded with wound gum (Fig. 11) occurred more frequently in vascular bundles and secondary xylem as symptom severity increased. Gum deposition was almost always associated with the presence of hyphae in nearby cells.

In plants with chlorotic and twisted apical leaves some vascular bundles showed several xylem vessel elements and xylem parenchyma cells occluded with gum (Fig. 12) which also seemed to be deposited on the walls of these cells. Adjacent xylem parenchyma cells free of gum often showed hypertrophy with deposition of pectic substances on the walls bordering the cells with gum (Fig. 12). Due to the deposition of gum and pectinaceous materials as well as the hypertrophy of the xylem parenchyma cells, the xylem tissue of these bundles appeared distorted and disorganized.

Other vascular bundles showed fewer vessel elements occluded with gum but if hyphae were present in cells near the pith, gum was frequently observed on or in the walls of xylem parenchyma cells between the bundle cells containing hyphae and the pith cells or fiber cells surrounding the bundle (Fig. 13).

Cells with gum associated with their walls frequently were distorted or partially collapsed (Fig. 8) as if under pressure. The cell walls were weakly birefringent upon examination with polarized light, indicating a decrease in cellulose content.

Xylem parenchyma cells showing hypertrophy protruded between the helical or annular secondary wall thickenings of some



Figs. 14–19. Portions of sections of stems of *Chrysanthemum morifolium* 'Yellow Delaware' plants infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. **14,** Longitudinal section showing the walls of the xylem parenchyma cells protruding between the annular secondary wall thickenings of the xylem vessel elements (arrows) (× 225). **15,** Section showing hyphae in some xylem vessel elements (arrows) and the deteriorating cell walls of some xylem parenchyma cells (x) (× 300). **16,** Transverse section of an infected vascular bundle showing disintegration of xylem parenchyma cells (arrows) and xylem vessel elements (v) resulting in gaps in the tissue (× 145). **17,** Transverse section showing dysfunction of vascular cambial derivatives. Note the files of thin-walled parenchyma cells and undifferentiated xylem vessel elements. Compare with Fig. 18 (× 150). **18,** Same transverse section as in Fig. 17 but from an area without the fungus. The thin-walled parenchyma cells are absent from the secondary xylem. Note the amount of secondary xylem compared to that in Fig. 17 (× 94). **19,** Transverse section showing cambial cells and their undifferentiated derivatives occluded with gum (g) and hyphae near these cells (arrows) (× 340).

xylem vessel elements plugged with gum to such an extent that the lumens of the vessel elements were often completely occluded (Fig. 14).

In plants with wilted leaves or other severe external symptoms, gum deposition was more abundant. In addition, it was strongly associated with other host anatomical responses and will be discussed where appropriate.

Hypertrophy and hyperplasia. In plants with wilted leaves, hypertrophy and hyperplasia of pith cells occurred adjacent to some infected vascular bundles in the upper portion of the stem. This resulted in the crushing of xylem cells containing gum deposits or having deteriorating walls. Hypertrophy and hyperplasia also occurred in combination with other host anatomical responses.

Disintegration of xylem tissue. In stems of inoculated symptomless plants some xylem parenchyma cells did not stain with normal intensity (Fig. 15) and the faded appearance may indicate cell wall deterioration. Small gaps resulting from disintegration of xylem tissue occurred in plants with chlorotic and twisted apical leaves. In plants with wilted leaves, a greater proportion of infected bundles had large cavities formed as a result of the disintegration of large amounts of xylem tissue. The cavities increased in size and serial sections (Fig. 16) showed that the xylem parenchyma cells disintegrated first, leaving isolated xylem vessel elements that also eventually disappeared. The cells surrounding the cavities often had gum or pectic substances deposited on the walls or in the cell lumens. Disintegrating xylem parenchyma cells gave a negative to weakly positive reaction for pectin indicating a loss of pectic materials from the walls and middle lamellae of these cells. Such areas were most often noted in bundles in the upper portions of stems.

Stem sections of several plants, some with chlorotic and twisted apical leaves and others with severe symptoms, often had some bundles with a few partially disintegrated cells and gum deposits occurring above the highest point in the stem from which the fungus could be isolated. Cross sections above these bundles revealed that the tissues gradually became indistinguishable from tissues of uninoculated plants.

Dysfunction of cambium derivatives. Anatomical changes involving the vascular cambium were first observed in plants with chlorotic and twisted apical leaves. Many small undifferentiated cells were produced from the cambium of infected vascular bundles or secondary xylem with the resultant formation of files of small, square vessel elements formed amid small, thin-walled parenchymalike cells (Fig. 17). By comparison in the secondary xylem of uninoculated plants, vessel elements and fibers immediately adjacent to the cambial cells are almost fully differentiated (Fig. 4). Vessel elements have large diameters in comparison to fibers and are circular to oval in cross section. Fibers, as well as vessel elements, are thick-walled. In infected tissue the undifferentiated parenchymalike cells often proliferated until they began to separate clusters of the small, undifferentiated vessel elements from each other. This type of cambial activity resulted in a decrease in secondary xylem, which was apparent if the amount of secondary xylem above infected bundles was compared with that above uninfected bundles within the same stem cross section (Fig. 18). Whenever hyphae occurred near the undifferentiated cambial derivatives, gum was present on the walls and in the lumens of these cells and apparently prevented colonization of host tissues exterior to the gummed cells (Fig. 19).

In plants with wilted leaves the dysfunction of the vascular cambium derivatives was more severe and occurred farther down the stem. Often, there was little, if any, secondary growth and the undifferentiated cells frequently were plugged with gum. If several adjacent bundles were infected, these plugged cells often occurred in a continuous layer forming a barrier which prevented movement of the fungus to the exterior of the stem. In addition, the dissection of the undifferentiated xylem vessel elements by the strands of undifferentiated parenchyma cells was much more apparent. This effect was apparently due to hyperplasia and hypertrophy of the undifferentiated xylem parenchyma cells. In time, cambial cells were no longer recognizable in these parenchyma strands and the cells of these strands or rays were continuous with those of the phloem parenchyma.

In plants with black stem necrosis, there was increased deposition of gum and pectinaceous materials on or in the walls and lumens of the undifferentiated xylem vessel elements, especially in the cells bordering the dissecting strands of parenchyma cells. Despite this activity, hyphae colonized these parenchyma cells as well as the phloem parenchyma and cortex. The resultant collapse of the colonized cortical cells (Fig. 10) together with the deposition of dark materials in these cells resulted in the black streak symptoms on the stem. Fungal colonization of the cortex, with resultant cellular collapse, eventually occurred all around the stem.

DISCUSSION

To place the anatomical observations reported in this study in perspective, comparisons will be made with anatomical work done with other hosts infected with *F. oxysporum*.

In tomato (26) conidia are transported in the transpiration stream ahead of the hyphae. As a result, areas of stem tissue colonized by the fungus exist separated by areas with no colonization. This pattern of fungal colonization did not occur in carnation (21) nor was it observed in this study with chrysanthemum. Instead, hyphae could always be observed all the way up to but not beyond the point in the stem where isolation indicated the fungus was present. This continuity of fungal growth in stems of chrysanthemum suggests that culture-indexing would be a usable control technique, as it is in carnations, (20), because symptomless infected cuttings could always be detected by isolation from sections of stem tissue. Our results, however, apply to plants with single stems. Further tests will be required to extend these conclusions to commercial-type plants in which the growth of numerous flowering branches is induced (33).

Many of the anatomical symptoms observed with Fusarium wilt of chrysanthemum were similar to those described for other Fusarium wilt diseases. For example, vessel element plugs of pectinaceous materials occur in tomato (8,23) and plugs of wound gum as well as pectinaceous materials occur in carnation (21) and sweet potato (19). Also, hyperplasia and hypertrophy of the xylem parenchyma adjacent to infected cells occurs in tomato (6) and carnation (21).

Although dysfunction of the cambial derivatives occurs in tomato plants infected by *F. oxysporum* f. sp. *lycopersici* (6), it is not as severe as that observed in chrysanthemum in this study. Results of the dysfunction of cambial initials in chrysanthemum were two-fold. First, formation of secondary xylem was severely limited in areas of the stem where the fungus was most prevalent. Second, the formation of strands of hypertrophied and hyperplastic xylem parenchyma cells seemed to provide the points where hyphae began colonizing phloem and cortical parenchyma cells, leading to the collapse of these cells and to the black streak symptom and eventual necrosis of the entire shoot. Wedges of hypertrophied xylem parenchyma were also noted in carnation (21) but were not continuous with phloem tissue as in chrysanthemum.

The disintegration of xylem tissue observed in this study did not occur in tomato (6) but it did occur in carnation (21). Pectolytic enzymes may be involved in this disintegration as well as in several instances of the deterioration of xylem parenchyma cells found above the point in the stem where it was no longer possible to isolate the fungus.

Several chrysanthemum plants had chlorotic and twisted apical leaves but the fungus could not be isolated from stem sections just below these leaves or detected by microscopy. This suggests the involvement of a toxin(s), growth regulator(s), or possibly, a nutrient imbalance or deficiency in the appearance of these initial symptoms.

Wilted leaves first occurred near the top of the stem and progressed downward. The plugging of xylem vessel elements by conidia, hyphae, wound gum, and pectinaceous materials as well as the disintegration of xylem tissue and the dysfunction of the vascular cambium derivatives, all were most severe initially in the upper half of the stem. This may account for the initial symptoms of

chlorotic, curved apical leaves and the progression of wilted leaves from the top to the bottom of the plant, symptoms that are unusual for Fusarium wilt diseases.

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