Anatomy of a Tolerant Chrysanthemum Cultivar Infected with *Fusarium oxysporum* f. sp. *chrysanthemi*

Barbara A. Stuehling and Paul E. Nelson

Former graduate research assistant and professor, Department of Plant Pathology, Fusarium Research Center, The Pennsylvania State University, University Park 16802.

Contribution 1205, Fusarium Research Center, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication 12 August 1980 as Journal Series Paper 6046.

Portion of a thesis by the senior author submitted in partial fulfillment of the requirements for the M.S. degree, The Pennsylvania State University.

Accepted for publication 20 February 1981.

ABSTRACT

Stuehling, B. A., and Nelson, P. E. 1981. Anatomy of a tolerant chrysanthemum cultivar infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. Phytopathology 71:1162-1168.

Plants of *Chrysanthemum morifolium* 'Mandalay' were inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*, and stem portions were cultured to determine the location of the fungus. Other stem portions were fixed, dehydrated, embedded, sectioned, and stained to examine changes in the anatomy of infected plants. Stems frequently were colonized, but symptoms were limited in severity and the fungus was restricted to the xylem. Breakdown of xylem parenchyma and vessel elements; occlusion of vessel elements by gums; accumulation of pectic substances; and hypertrophy of cells surrounding, and sometimes replacing, the xylem occurred in the primary xylem. Tyloses were present in a few cases. Responses in the secondary xylem included proliferation of hypertrophied parenchyma and formation of partially differentiated tracheary elements. Hyphae of the pathogen were sparse and limited primarily to xylem vessel elements. Occasionally, other xylem cells were colonized. The hyphae were continuous in infected stems. The limited host responses to infection did not appear to be the major reason for the tolerance of this cultivar.

The literature on Fusarium wilt of chrysanthemum and the pathogens involved has been reviewed thoroughly (8). The disease is caused by *F. oxysporum* f. sp. *tracheiphilum* race 1 (2). Cultivar reactions to both *formae speciales tracheiphilum* and *chrysanthemi* have been described (9–11). Although both pathogens incite similar symptoms, the reactions of individual cultivars to infection vary. Chlorosis, wilt, necrosis, vascular discoloration, twisting of leaves, stem necrosis, and stunting appear in various combinations. The occurrence of specific symptoms and their severity depend primarily on the interaction of the cultivar and temperature of the soil and air. The pattern of symptom development in cultivars also varies; symptoms appear first at the plant apex and move down in some cultivars, rather than starting at the base of the plant, as in most vascular wilts. Temperatures above 28 C favor disease development and play a major role in symptom development.

Several cultivars may remain symptomless although colonized by the pathogen (12). Cultivars such as Mandalay may show initial symptoms of the disease, but these symptoms disappear; the plant continues to grow and produce a flower crop, even though it is colonized by the fungus.

The effect of *F. oxysporum* f. sp. *chrysanthemi* on tolerant chrysanthemum cultivars has not been studied in detail, and the basis for tolerance is unknown. This study was initiated to determine the changes that take place in the anatomy of a tolerant cultivar, Mandalay, when infected by *F. oxysporum* f. sp. *chrysanthemi*; the extent of colonization by the pathogen; and the role of anatomic features of the cultivar Mandalay in determining its tolerance.

MATERIALS AND METHODS

Culture-indexed, rooted cuttings of *Chrysanthemum morifolium* (Ramat.) Hemsl. (California-Florida Plant Corp., Fremont, CA 94538) were planted in 12.5-cm clay pots in a 1:1:1 (v/v) mixture of peat, perlite, and Hagerstown silty loam. The soil mix and pots were heat-treated with aerated steam at 78.8 C for 35 min before use. Pots were placed into a bench containing steam-treated perlite. Heating cables were used to control the temperature of the soil mix. After a 1-wk acclimation for the potted cuttings, the soil temperature was increased gradually to about 27 C. The single-stemmed plants were grown in continuous light to prevent floral initiation and to maintain conditions of prior research on Fusarium wilt of chrysanthemum (8). Water was supplied through a Chapin system (23), which avoids splashing between adjacent pots and distributes water uniformly to each pot. A 20-20-20 fertilizer (Peters Fertilizer Products, Allentown, PA 18100) containing ammoniacal nitrogen was applied weekly.

F. oxysporum (Schlecht.) emend. Snyd. & Hans., f. sp. *chrysanthemi* Litt., Armst., & Armst. (3) isolate FRC-0-693 from the Fusarium Research Center collection of The Pennsylvania State University was used as the pathogen throughout this study. This isolate is known to be virulent on chrysanthemum (8). The fungus cultures were obtained initially from infected chrysanthemum plants growing in Florida. Cultures of FRC-0-693 originating from single spores were grown on potato-dextrose agar slants for about 2 wk. Tubes were sealed with cigarette paper and a $CuSO_4$ gel to prevent contamination by mites (21). Cultures were grown 43 cm below 40W fluorescent lights operating on a 12-hr photoperiod at 21–22 C (22).

The chrysanthemum cultivar Mandalay was found to be tolerant to Fusarium wilt in preliminary experiments. Twenty-four plants were arranged randomly on a greenhouse bench and surrounded by a barrier row of the same cultivar.

Sporodochial-type cultures were selected for preparing inoculum. Spores and bits of mycelium were suspended in tap water and adjusted to a concentration of approximately 60,000 spores per milliliter, as measured with a hemacytometer. Roots were wounded by pushing a metal spatula down through the soil at five equally spaced sites around the stem, and 100 ml of the spore suspension was poured over the soil around each plant. Control plant roots also were wounded, and tap water was added. Wounding has been shown to decrease the latent period, although it is not required for infection (8).

Plants were observed for symptom development after

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/81/11116207/$03.00/0

inoculation. Once symptoms appeared, they were described and recorded every 3–4 days for the remainder of the experiment.

Sampling for histologic work and to check for the presence of the fungus began 2 wk after inoculation and continued weekly for 6 wk. The stems of one control and three inoculated plants were severed with a razor blade at the soil line and carefully stripped of leaves and branches. Each stem was traced on lined paper before being surface-sterilized in 10% Clorox (5.25% sodium hypochlorite) for 5 min. Sterile razor blades were used to cut stems into 5-mm pieces; alternate sections were cultured to check for the fungus, and the remaining sections were fixed. A thin slice was made from each even-numbered section and placed on carnation leaf agar (22). Odd-numbered sections were placed in numbered vials containing Rawlin's formalin-aceto-alcohol fixative no. 1 (19).

After the initial sampling period, it was determined that more plant material was needed for sectioning. Plants in the barrier row were sampled as described except that only the lower 12.5 cm of the stem was used to obtain samples for culturing and fixation. Sampling in this manner continued for another 6 wk. Cultures were examined after about 4 days, and the presence or absence of *F. oxysporum* in each stem section was recorded.

Fixed stem sections were dehydrated in a tertiary butyl alcohol series (14), infiltrated, and embedded in Paraplast (Sherwood Medical Industries, St. Louis, MO 63103). Paraffin infiltration was done in a vacuum oven at 252 mm Hg pressure to aid in infiltration of the desiccated pith cells. Before sectioning, a solution of 90 ml of 1% sodium lauryl sulfate (Dreft) and 10 ml of glycerol (1) was used for 16–24 hr to soften the tissue. Samples were sectioned on a rotary microtome at 10–12 μm, and ribbons were mounted on chemically cleaned slides with Haupt's adhesive (14). Sections were stained with Johansen's quadruple stain (14). Histochemical tests (13,20) for suberin (Sudan IV), cellulose (polarized light), wound gum (phloroglucinol, orcinol reactions), and pectic substances (iron absorption method) were used to detect these substances in host tissues.

All sections were examined under a Leitz Ortholux research microscope and photographed on Kodak Plus-X Pan film with a Leitz Aristophot camera with a 10.16 × 12.70 cm (4 × 5 in.) Graflex back.

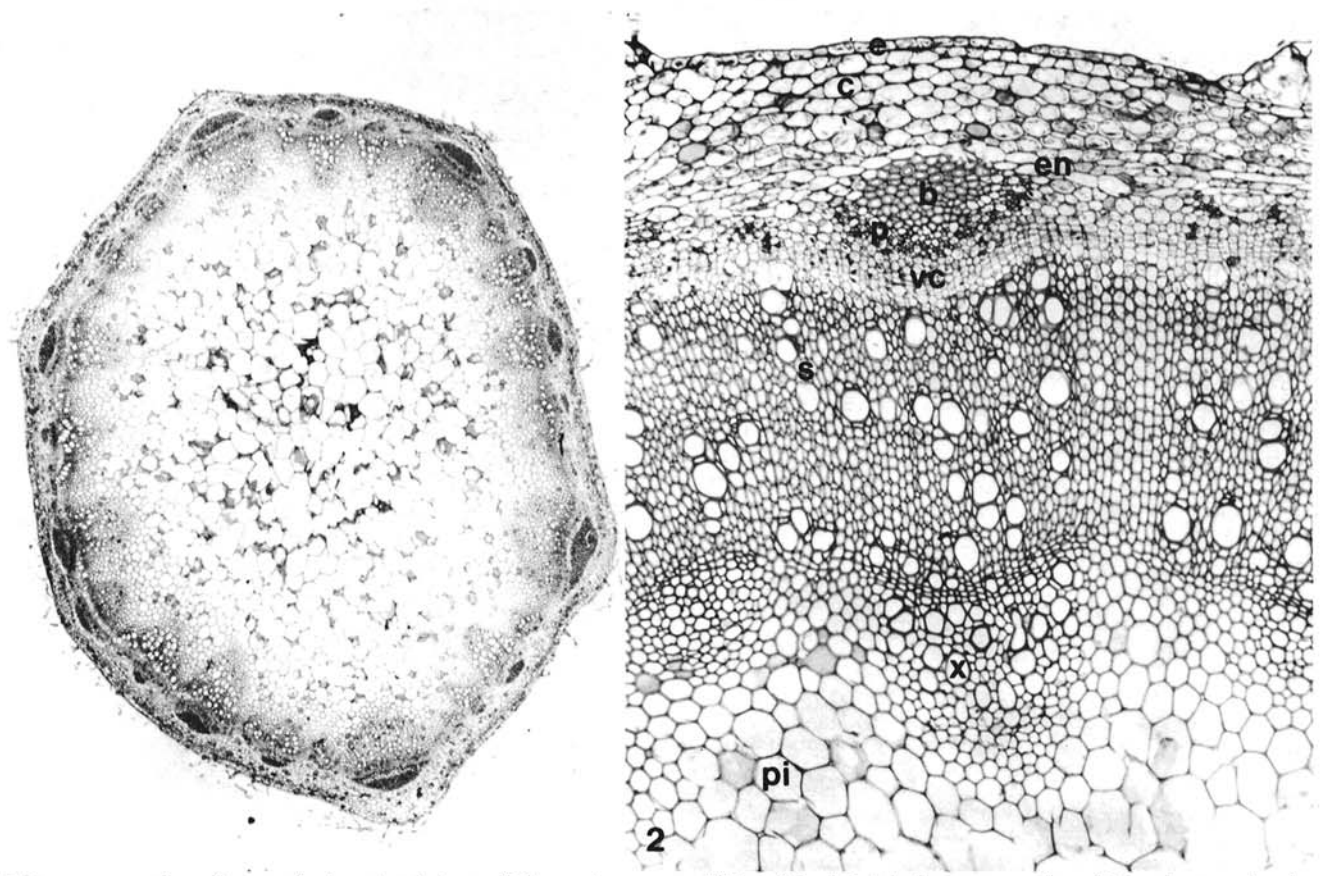
A second experiment using the cultivar Mandalay was set up to determine symptoms and supplement data from the first experiment. Thirty plants were grown as described above, and 20 were inoculated with *F. oxysporum* f. sp. *chrysanthemi* isolate FRC-0-693. Symptoms were recorded weekly, and sampling began 3 wk after inoculation. Two inoculated plants and one control were sampled as described earlier, except that stem sections were made at intervals of several centimeters.

RESULTS

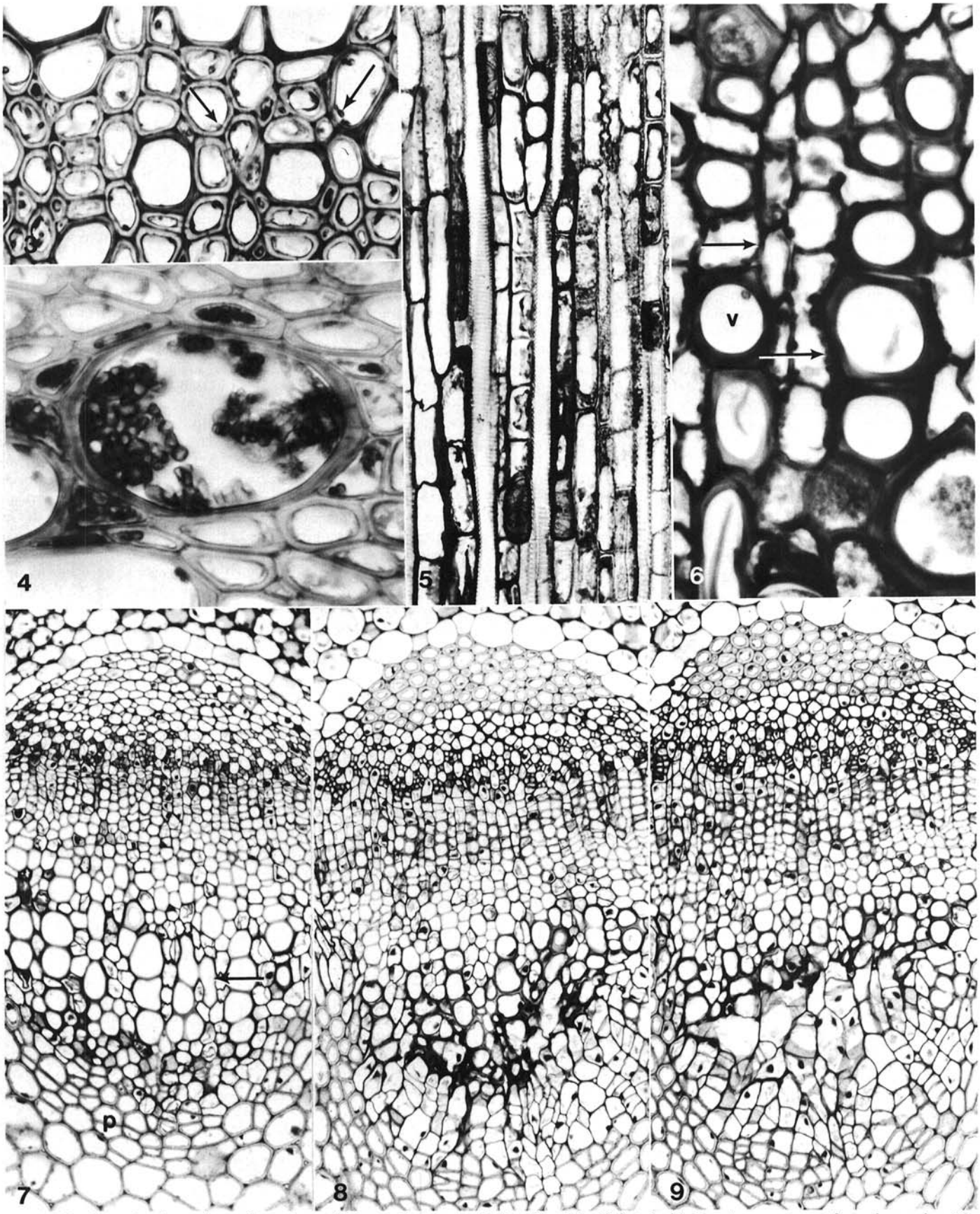
Symptomology. Symptoms occurred on the cultivar Mandalay about 3 wk after root inoculation. Some plants showed asymmetric terminal leaves and general chlorosis. When the experiment was repeated, some plants showed twisting and chlorosis of apical leaves, some plants were chlorotic and stunted, and some had combinations of these symptoms. Less than one-third of the inoculated plants showed visible symptoms. Symptoms ceased to develop about 5 wk after inoculation.

Isolations. Attempts to isolate *F. oxysporum* f. sp. *chrysanthemi* from stems of plants began 2 wk after inoculation. The pathogen was recovered from 58% of the assayed plants, irrespective of symptom development. In the initial 6-wk sampling period, the pathogen was present in 5–86% of the stem sections from all plants sampled.

Elapsed time after inoculation did not seem to affect the amount of stem tissue colonized. Most plants sampled 8–14 wk after inoculation contained *F. oxysporum* throughout the 12-cm stem portion sampled, but occasionally the fungus was restricted to the lower 3–7 cm. Colonization of stems by the fungus was continuous



Figs. 1–2. Transverse sections of stems of uninoculated plants of *Chrysanthemum morifolium* 'Mandalay'. **1,** Transverse section of the entire stem, showing the arrangement of vascular bundles and the other stem tissues (×16). **2,** Portion of a transverse stem section showing the epidermis (e), cortex (c), endodermal layer (en), fiber bundle cap (b), phloem (p), vascular cambium (vc), secondary xylem (s), primary xylem (x), and pith (pi) (×64).



Figs. 3–9. Portions of sections of stems from plants of *Chrysanthemum morifolium* 'Mandalay' infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. **3,** Part of a transverse section showing hyphae (arrows) in the tracheids and xylem vessel elements (×440). **4,** Part of a transverse section showing hyphae partially occluding a xylem vessel element (×768). **5,** Part of a longitudinal section showing darkly stained chromophilic xylem parenchyma cells (×205). **6,** Part of a transverse section showing the initial stages of disintegration of the walls of parenchyma cells (arrows). Note the hypha in the adjacent xylem vessel element (v) (×768). **7–9,** Transverse sections of stem vascular bundles, showing several stages in the disintegration of the primary xylem and hypertrophy of xylem parenchyma cells. **7,** Hyperplasia of the preimedullary fibers (p) has occurred. Note the crushed xylem vessel elements (arrow) (×170). **8,** Disintegration of xylem parenchyma cells and vessel elements has occurred, and the area is surrounded by an accumulation of pectic material. Hypertrophy of parenchyma cells around the xylem has occurred (×165). **9,** Hypertrophied parenchyma cells now occupy much of the area that was primary xylem tissue (×165).

from the base to the uppermost section sampled except in one case.

Histology of uninoculated plants. Transverse and longitudinal stem sections of control plants were examined to determine the anatomy of uninoculated Mandalay stems (Figs. 1 and 2). The stem anatomy was similar to the descriptions for the cultivars Giant No. 4 Indianapolis White (17) and Yellow Delaware (8).

Histology of inoculated plants. Sparse mycelium of *F. oxysporum* was present in limited areas of the transverse stem sections. Hyphae were present generally in older xylem vessel elements, often one to a few strands per cell (Fig. 3). Some cells were partially occluded (Figs. 4 and 5). In a few plants, hyphae were present throughout the tracheids (Fig. 3), parenchyma, and vessel elements of the xylem. Conidia were abundant in some colonized cells, sometimes filling the entire length of a vessel element.

Limited anatomic changes, frequently confined to a small area of the transverse sections, occurred in response to the pathogen and were present in nearly all sections examined. Tissues showing no response were very commonly found next to or surrounding areas containing the pathogen and showing anatomic changes. The presence of *F. oxysporum* in the immediate area was not correlated with anatomic changes, nor did such changes always develop in colonized tissues.

Anatomic changes occurred in the vascular cambium, xylem, and outer regions of the pith. Other tissues were unaffected, except a concentric vascular bundle in which the phloem was disintegrated.

The primary xylem was the first tissue to show pathological change. Xylem parenchyma cells became chromophilic (Fig. 5) and eventually disintegrated (Figs. 5 and 6). The walls of xylem vessel elements were thinner than those in the controls, and sometimes the cells appeared crushed (Fig. 7). Eventually, the vessel elements disintegrated in severely affected stem areas (Figs. 8 and 9). Reduction of cellulose content and cell disintegration were readily visible as reduced birefringence of cell walls (Fig. 10). Cell disintegration usually occurred in small areas but seldom led to cavity formation. Dark-staining materials (Figs. 8 and 11) that accumulated next to areas of disintegration appeared to be products of cell disintegration. Histochemical tests for pectinaceous materials were positive (Fig. 14) in most cases. Occasionally, this material gave a positive test for wound gum.

Infected stem areas contained a few vessel elements and parenchyma cells plugged by dark-staining compounds (Fig. 13). These vascular plugs usually gave a positive reaction with phloroglucinol, indicating the presence of wound gum; however, some gave a positive reaction for pectic materials. In one section, hyphae were observed growing through a plugged xylem vessel element.

Hypertrophy and hyperplasia of the xylem parenchyma were other host responses to infection by *F. oxysporum* f. sp. *chrysanthemi*. Usually, these responses were visible first in the preimedullary fiber region (Fig. 7). Large thin-walled cells occurred around the xylem (Figs. 8 and 11). The hypertrophied cells frequently filled the space that occurred when the original primary xylem cells disintegrated (Fig. 9). The irregularly shaped cells often were arranged in files, radiating from the xylem toward the pith. In one section, hypertrophied cells surrounded an area of colonized cells that failed to stain typically, causing a faded appearance (Fig. 11). Hyphae were present in the xylem vessel elements, and pectic materials had accumulated on the walls of the surrounding cells (Fig. 14).

In some areas, cell disintegration, tyloses (Fig. 12), and an accumulation of gum and/or pectin occurred in vessel elements of the primary and secondary xylem. Tyloses were found in only a few plants; they were small and did not fill the lumen of the vessel element.

Different host responses occurred in the secondary xylem. Although cell breakdown and vascular plugging were present, they occurred less frequently than in the primary xylem. Alteration of the cell orientation and structure, apparently due to incomplete differentiation of the cambial derivatives, typified the changes in the secondary xylem. This xylem was composed of small, rectangular, thick-walled cells (Fig. 15) appearing like stunted vessel elements and hypertrophied parenchyma cells. Composition of the tissue varied, sometimes being predominantly hypertrophied parenchyma (Fig. 17) with scattered, partially differentiated tracheary elements. Other sections primarily contained incompletely differentiated tracheary elements arranged in orderly files (Fig. 19) with occasional strands of parenchyma cells. Sometimes both alterations of cell structure were observed in the same transverse stem section. The type of host response in the secondary xylem was not related to plant age, location in the stem, or severity of symptoms. The vascular cambium often was not present in areas of parenchyma proliferation (Fig. 20) and was distorted (Fig. 18) or indistinct in other regions.

Incomplete differentiation of cambial derivatives of the secondary xylem became evident in most plants 4 wk after inoculation, most frequently in the upper portions of the plant. Some transverse stem sections showed this host response to infection throughout the vascular cylinder; in others, it was limited to a few vascular bundles. Sometimes, typical xylem cells were produced, again resulting in the formation of a band of incompletely differentiated tissue.

Concentric amphicribral bundles in the cortex frequently showed more cell disintegration than did the normal vascular tissue. In these bundles, only the xylem appeared to be affected. Patterns of changes in the anatomy of the plant stem were inconsistent in many cases. Some of the most severe changes occurred in plants sampled within 6 wk of inoculation. The area of the transverse stem section showing changes varied considerably. In many stem sections, changes appeared in one third to one half of the cross-sectional area. Anatomic changes tended to be more severe above the stem base, particularly those changes characterized by incomplete differentiation of the secondary xylem. Hyphae were more numerous in the lower areas of the stem. Changes in anatomy of the infected stem were visible above the area colonized by the fungus. Hypertrophy, cell disintegration, and partially differentiated secondary xylem were present in small areas in some uncolonized stem sections (Fig. 16).

Results of the histochemical tests showed wound gum in many vascular plugs and in some areas of disintegrating xylem parenchyma. The phloroglucinol test showed small quantities of lignin in some cells of the incompletely differentiated secondary xylem. The histochemical test for pectic materials showed a stronger reaction in much of the hypertrophied xylem parenchyma tissue than in the control tissues. Strong positive reactions for pectin also occurred in the chromophilic and disintegrating xylem parenchyma, in occasional vascular plugs, and in material accumulated (Fig. 14) around the areas of cell disintegration.

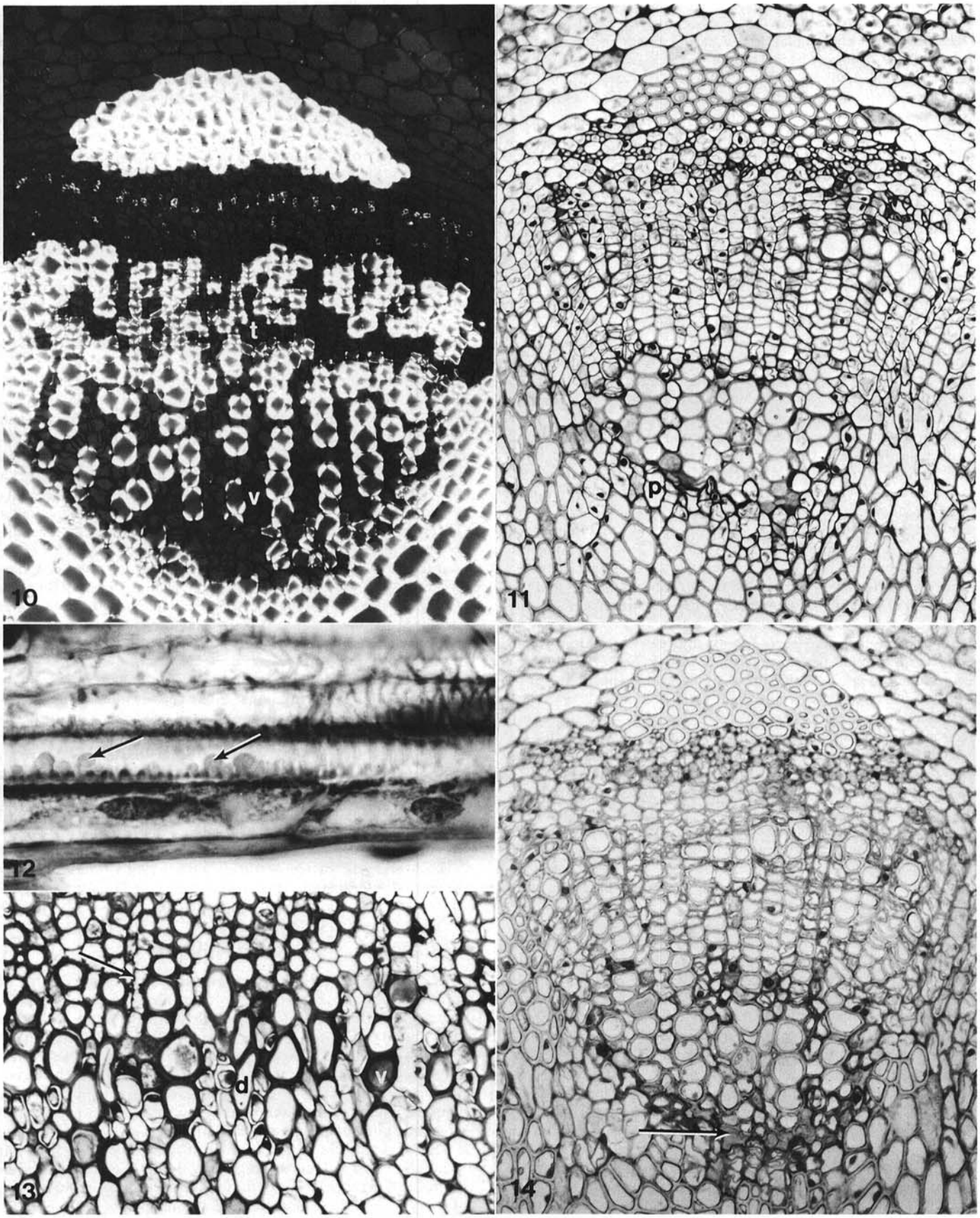
A few sections reacted positively with Sudan IV, indicating the presence of suberin in the area surrounding the faded central cells. Some vascular plugs also gave a positive reaction with Sudan IV.

DISCUSSION

Responses of the chrysanthemum cultivar Mandalay to colonization by *F. oxysporum* f. sp. *chrysanthemi* resemble in most respects those of the more susceptible cultivar Yellow Delaware. Inoculation techniques used on Yellow Delaware (8) were suitable for use with other cultivars. Unpublished data have suggested that wounding chrysanthemum roots is unnecessary; however, wounding hastened infection and colonization and also approximates the root wounding that occurs when rooted cuttings are planted in a commercial operation.

Visible symptoms of Fusarium wilt were much less severe in Mandalay than in Yellow Delaware. Symptoms generally were limited to a slight chlorosis of the foliage and chlorosis and twisting of some apical leaves. Occasionally, stunted plants were observed. In contrast, more susceptible cultivars frequently showed wilt and necrosis of leaves, necrosis of cortical tissues in the stem, and severe stunting.

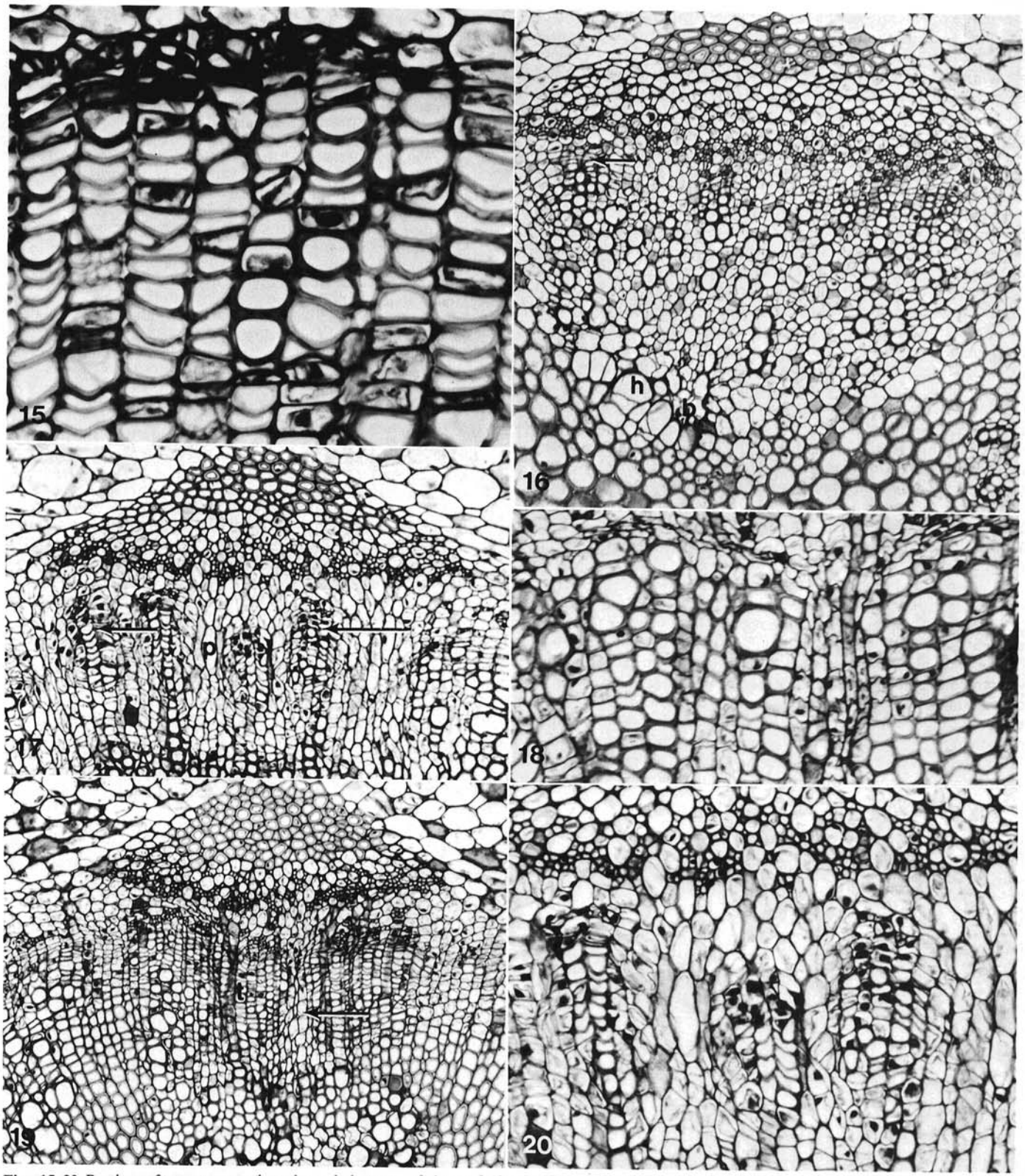
Mandalay stem tissues frequently were colonized for a considerable distance, though mycelium was sparse within any given transverse section. Individual cells usually contained only a few hyphae. Mycelium infrequently plugged xylem vessel elements, as was reported to occur in Yellow Delaware (8). Tracheids and



Figs. 10–14. Portions of sections through the stems of plants of *Chrysanthemum morifolium* 'Mandalay' infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. **10,** Part of a transverse section photographed with polarized light, showing a decrease in birefringence of thin-walled xylem vessel elements (v) and partially differentiated tracheary elements (t) (×152). **11,** Part of a transverse section showing weakly stained primary xylem surrounded by hypertrophied parenchyma cells (p). Note the hyphae in xylem vessel elements and the cells with accumulation of material on the wall around the weakly stained primary xylem (×205). **12,** Part of a longitudinal section showing tyloses (arrows) in a xylem vessel element (×688). **13,** Part of a transverse section showing plugged xylem vessel elements (v). Note the disintegrating parenchyma cells (arrow) and distorted xylem vessel elements (d) (×240). **14,** Part of a transverse section showing the results of the iron absorption test for pectic substances. Note the positive reaction of the material around the primary xylem, indicating the presence of pectic substances (arrow). This section was taken from the same 5-mm stem sample shown in Fig. 11 (×225).

parenchyma were colonized in a few sections. Hyphae were not seen growing through pit pairs into adjacent xylem vessel elements, although this may have occurred and may have accounted for the lateral spread of the pathogen. Vertical distribution of the fungus was continuous in nearly all cases, as in Yellow Delaware (8).

Culture indexing (16) remains a valid technique for detecting *F. oxysporum* f. sp. *chrysanthemi* in Mandalay. *F. oxysporum* was found by culturing to be distributed throughout the stems of symptomless Mandalay plants. Infected cuttings could be shipped around the country, spreading the pathogen very efficiently. This



Figs. 15–20. Portions of transverse sections through the stems of plants of *Chrysanthemum morifolium* 'Mandalay' infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. **15,** Atypical secondary xylem composed of partially differentiated tracheary elements (×608). **16,** An uncolonized portion of a stem, showing hypertrophy (h) and partial disintegration (b) of cells and partially differentiated tracheary elements (arrow) (×101). **17,** Incomplete differentiation of secondary xylem consisting of parenchyma cells showing hypertrophy (p) and files of distorted tracheary elements (arrows) (×110). **18,** Distorted cells of the vascular cambium have produced incompletely differentiated xylem cells (×270). **19,** Secondary xylem composed of partially differentiated tracheary elements (t) and strands of parenchyma cells (arrow) (×101). **20,** Close-up of a portion of the section in Fig. 17. Note the absence of the vascular cambium (×220).

appears to be an immediate problem in the chrysanthemum industry.

Vascular plugging occurred in infected chrysanthemums. Most plugs were composed of wound gum, although a few were primarily pectinaceous materials (8). Most vascular wilts have been associated with pectinaceous vascular plugs (6,18). Vascular plugging by gums and gels has been described (4) as a mechanism of resistance resulting in localization of the pathogen. Because chrysanthemum appears to be colonized by hyphae rather than conidia, the effectiveness of this mechanism may be reduced. Hyphae were seen within plugged vessels. Tyloses, like vascular plugs, are thought to play an important role in resistance by excluding the pathogen from uncolonized portions of the plant (4,5) but are not an important factor in the tolerance of Mandalay to *F. oxysproum* f. sp. *chrysanthemi* because of their scarcity and small diameter.

Hypertrophy and hyperplasia of pith and parenchyma cells have been reported in diseased plants of the susceptible chrysanthemum cultivar Yellow Delaware (8). Extensive hypertrophy was observed in Mandalay; thin-walled cells replaced areas of destroyed xylem cells. In Yellow Delaware, the disintegrated xylem was not replaced by other cells, and thus large cavities formed. Perhaps this response accounted for the greater severity of external symptoms in Yellow Delaware. Hypertrophy and hyperplasia have been attributed to increased levels of auxins in diseased plants (4,7). Indoleacetic acid is known to be produced by some *formae speciales* of *F. oxysporum* (15) and by wilt-diseased plants (7) and could play a role in chrysanthemum wilt.

The incomplete differentiation of the secondary xylem was the most obvious anatomic response to infection in Mandalay. Cambial derivatives failed to mature into tracheids, fiber tracheids, and vessel elements; rather, they remained as small, rectangular or distorted thick-walled cells. This host response was noted also in Yellow Delaware (8). Partially differentiated tracheary elements sometimes were present within a proliferation of hypertrophied parenchyma in Mandalay. Vascular cambial cells were absent from these areas and did not appear to be involved in parenchyma production. A cambium usually was present outside the tracheary elements, although the cells often appeared distorted.

The tolerance of Mandalay to *F. oxysporum* f. sp. *chrysanthemi* is characterized by fewer changes in the anatomy and reduction or absence of visible symptoms compared with Yellow Delaware, even though colonization occurred. Although limitation of fungus growth in the primary xylem and parenchyma proliferation around infected xylem vessel elements appear to play a role in the limited symptom expression of this cultivar, anatomic barriers may not be the major tolerance mechanism.

LITERATURE CITED

1. Alcorn, S. M., and Ark, P. A. 1953. Softening paraffin-embedded plant tissues. Stain Technol. 28:55-56.
2. Armstrong, G. M., and Armstrong, J. K. 1965. Wilt of chrysanthemum caused by race 1 of the cowpea *Fusarium*. Plant Dis. Rep. 49:673-676.
3. Armstrong, G. M., Armstrong, J. K., and Littrell, R. H. 1970. Wilt of chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi*, forma specialis nov. Phytopathology 60:496-498.
4. Beckman, C. H. 1964. Host responses to vascular infection. Annu. Rev. Phytopathol. 2:231-252.
5. Beckman, C. H., Halmos, S., and Mace, M. E. 1962. The interaction of host, pathogen, and soil temperature in relation to susceptibility to Fusarium wilt of bananas. Phytopathology 52:134-140.
6. Beckman, C. H., and Zaroogian, G. E. 1967. Origin and composition of vascular gel in infected banana roots. Phytopathology 57:11-13.
7. Dimond, A. E. 1970. Biophysics and biochemistry of the vascular wilt syndrome. Annu. Rev. Phytopathol. 8:301-322.
8. Emberger, G., and Nelson, P. E. 1981. Histopathology of a susceptible chrysanthemum cultivar infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. Phytopathology 71:1043-1050.
9. Engelhard, A. W., and Woltz, S. S. 1971. Fusarium wilt of chrysanthemum: Symptomatology and cultivar reactions. Proc. Fla. State Hortic. Soc. 84:351-354.
10. Engelhard, A. W., and Woltz, S. S. 1972. Fusarium wilt of chrysanthemum. Bradenton Agric. Res. and Educ. Cent. (AREC) Mimeo. Rep. GC-1972-1. 4 pp.
11. Engelhard, A. W., and Woltz, S. S. 1972. The Fusarium wilt disease of chrysanthemum. Fla. Flower Grow. 9:1-5.
12. Engelhard, A. W., and Woltz, S. S. 1973. Pathogenesis and dissemination of the Fusarium wilt pathogens of chrysanthemum. (Abstr.) Phytopathology 63:441.
13. Jensen, W. A. 1962. Botanical Histochemistry. W. A. Freeman and Co., San Francisco. 408 pp.
14. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., New York. 523 pp.
15. Mace, M. E. 1965. Isolation and identification of 3-indoleacetic acid from *Fusarium oxysporum* f. *cubense*. Phytopathology 55:240-241.
16. Nelson, P. E., Tammen, J., and Baker, R. 1960. Control of vascular wilt diseases of carnation by culture-indexing. Phytopathology 50:356-360.
17. Pennypacker, B. W., and Nelson, P. E. 1972. Histopathology of carnation infected with *Fusarium oxysporum* f. sp. *dianthi*. Phytopathology 62:1318-1326.
18. Pierson, C. F., Gothoskar, S. S. Walker, J. C., and Stahmann, M. A. 1955. Histological studies on the role of pectic enzymes in the development of Fusarium wilt symptoms in tomato. Phytopathology 45:524-527.
19. Rawlins, T. E. 1933. Phytopathological and Botanical Research Methods. John Wiley & Sons, New York. 156 pp.
20. Rawlins, T. E., and Takahashi, W. N. 1952. Technics of Plant Histochemistry and Virology. National Press, Millbrae, CA. 125 pp.
21. Snyder, W. C., and Hansen, H. N. 1946. Control of culture mites by cigarette paper barriers. Mycologia 38:455-462.
22. Toussoun, T. A., and Nelson, P. E. 1976. *Fusarium*; A pictorial guide to the identification of *Fusarium* species. 2nd ed. Pa. State Univ. Press, University Park. 43 pp.
23. White, J. W. 1971. Irrigation. Pages 94–104 in: J. W. Mastalerz, ed. Geraniums: A Manual on the Culture, Diseases, Insects, Economics, Taxonomy, and Breeding of Geraniums. Pa. Flower Growers Assoc., Chalfont. 350 pp.