Histopathology of the Apical Leaves of a Susceptible Chrysanthemum Cultivar Infected with *Fusarium oxysporum* f. sp. *chrysanthemi*

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ABSTRACT

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The apical leaves of Yellow Delaware chrysanthemum plants inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi* were frequently chlorotic and asymmetric. The pathogen was not present in all leaves with these symptoms, nor was it absent from all symptomless leaves. Unequal tissue development on opposite sides of the midvein caused an asymmetric structure. Presence of the pathogen in the plant appeared to accelerate differentiation in leaf tissues. Vascular tissue throughout the entire lamina of infected plants was well differentiated, in contrast with the leaves of uninoculated plants.

Additional key words: Chrysanthemum morifolium.

Chrysanthemum morifolium (Ramat.) Hemsl. 'Yellow Delaware' infected with *Fusarium oxysporum* (Schlecht.) emend. Snyd. & Hans., f. sp. *chrysanthemi* Litt., Armst., & Armst. shows an initial symptom of asymmetry and chlorosis of the apical leaves beginning as early as 14 days after inoculation. Advanced symptoms of Fusarium wilt include chlorotic and/or wilted leaves, necrotic leaves, stunted plants, and necrotic streaks in the stem (2).

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This study was undertaken to determine the anatomy of the asymmetric leaves, the cause of this unique symptom, and the presence or absence of the fungus in leaves (with or without symptoms) of infected plants.

MATERIALS AND METHODS

Rooted cuttings of chrysanthemum cultivar Yellow Delaware (California-Florida Plant Corp., Fremont, CA 94538) were potted and placed on a greenhouse bench. Plants were soft-pinched 3 wk

after potting to promote branching and thus increase the number of stem apexes. The plants were grown in continuous light to prevent floral initiation. Water was supplied through a Chapin (9) system. A 20-20-20 fertilizer (Peters Fertilizer Products, Allentown, PA 18100) containing ammoniacal nitrogren was applied weekly.

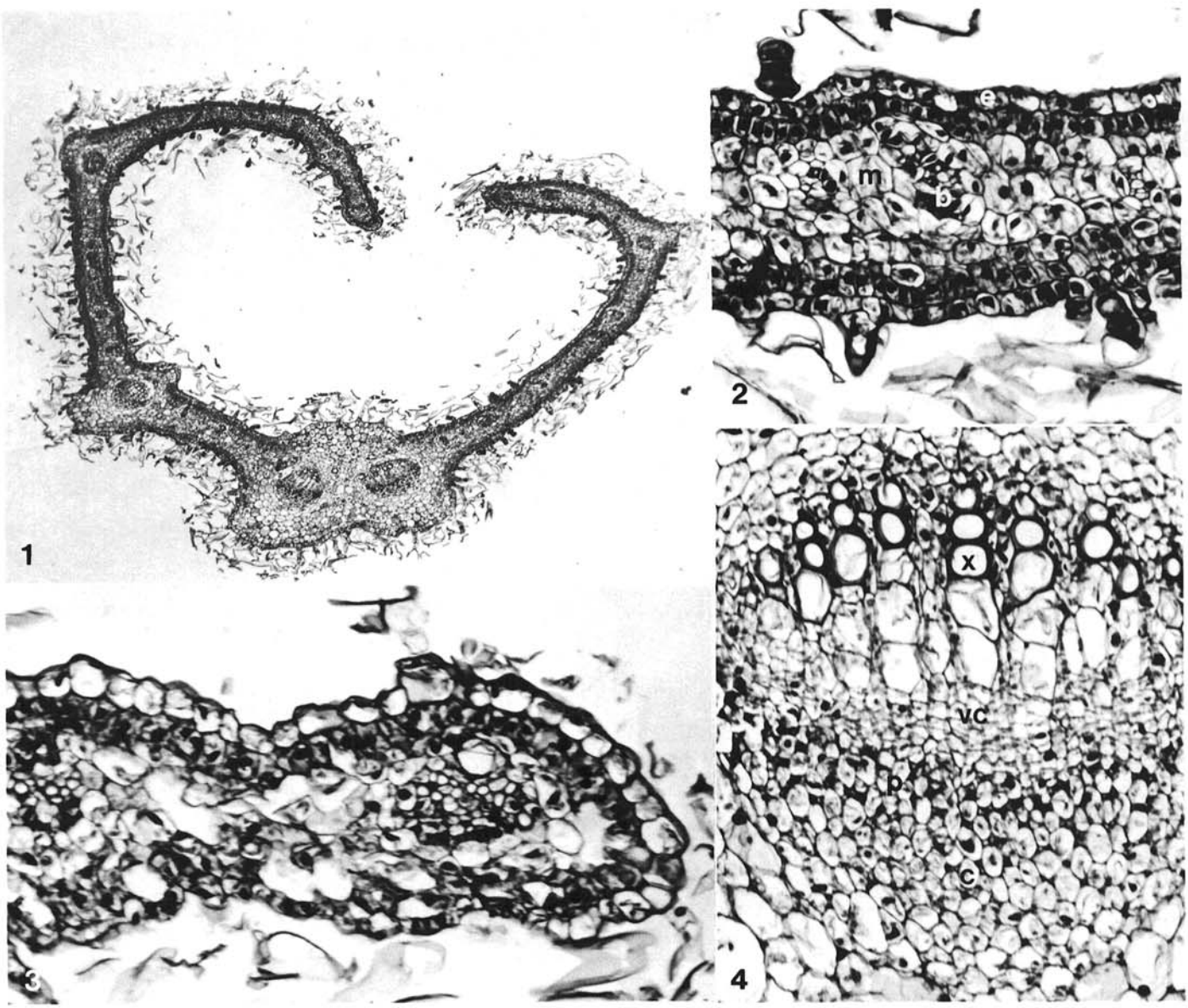
F. oxysporum f. sp. *chrysanthemi* isolate FRC-0-693 from the Fusarium Research Center collection, The Pennsylvania State University, was used as the pathogen in the first two experiments. Cultures originating from single spores were grown on potato-dextrose agar slants maintained 43 cm below 40W fluorescent lights operating on a 12-hr photoperiod at 21–22 C (6) for 2 wk. Sporodochial-type cultures were selected for preparing inoculum. Spores and bits of mycelium were suspended in tap water, and the suspension was adjusted to a concentration of approximately 60,000 spores per milliliter.

Roots of 5-wk-old plants were wounded about 2.5 cm from the stem 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. Control plants were treated similarly except water was substituted for inoculum.

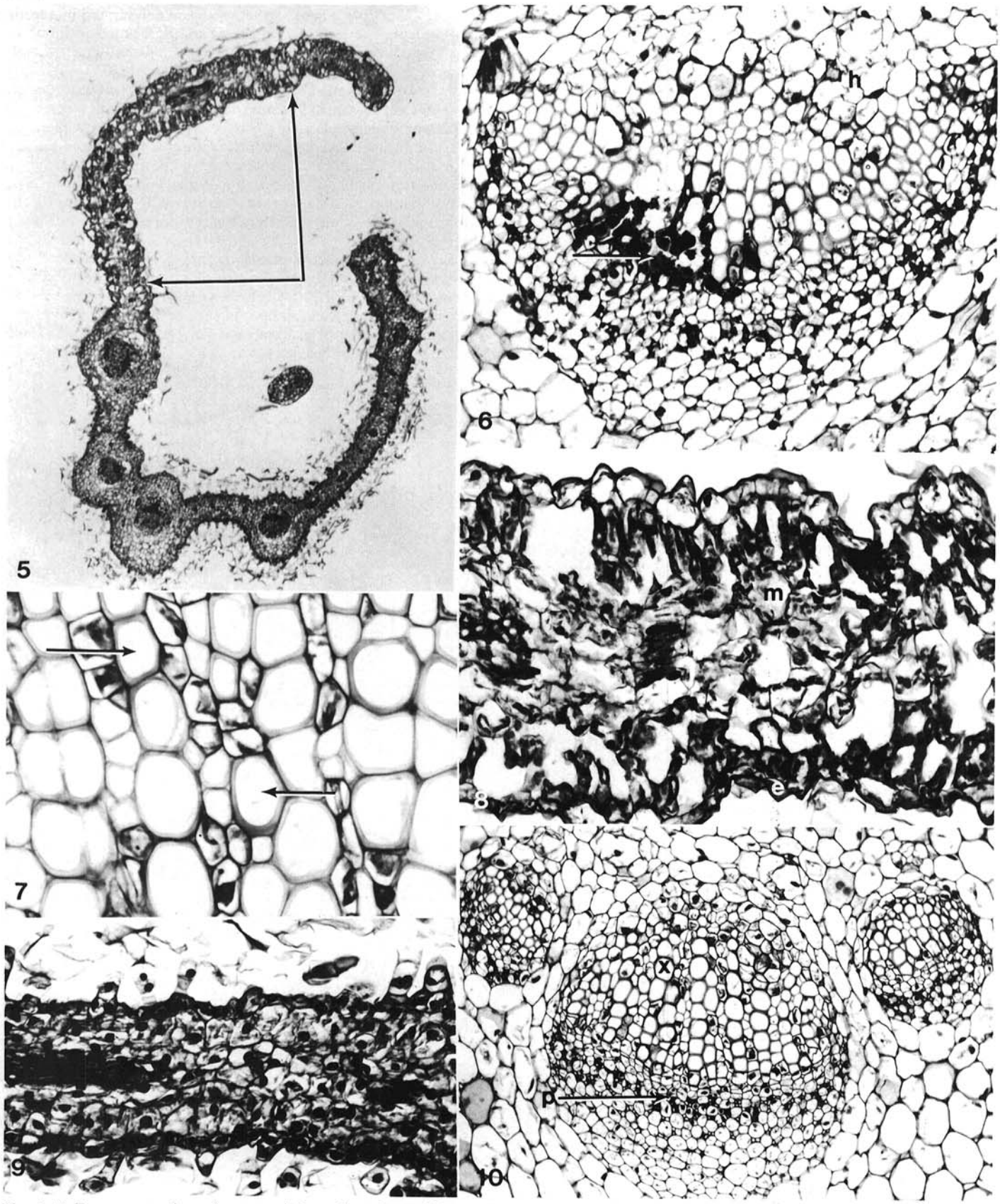
Leaf samples for culturing and sectioning were collected from three separate experiments over a 14-mo period. In the first experiment, 15 asymmetric leaves were removed carefully from the stem of inoculated plants with a razor blade 3 wk after inoculation. Leaves to be sectioned were placed in formalin-aceto-alcohol fixative No. 1 (4). Fixed specimens were dehydrated in a tertiary butyl alcohol series (3), infiltrated, and embedded in Paraplast (Sherwood Medical Industries, St. Louis, MO 63103). Leaf tissue was softened overnight in a solution of 90 ml of 1% sodium lauryl sulfate (Dreft) solution and 10 ml of glycerol (1) before sectioning. Sections were cut at 10 μm on a rotary microtome and stained with Johansen's quadruple stain (3) and Safranin and Fast Green (5).

An additional 12 symptomatic leaves were surface sterilized for 1 min in 10% Clorox (5.25% sodium hypochlorite), and portions of the petiole were placed on carnation leaf agar (CLA) in petri dishes (6). The petri dishes were maintained under lights for approximately 3 days, then observed for the presence of *F. oxysporum*.

Leaves from infected plants in the second experiment were sampled 3 wk after inoculation to determine the location of *F. oxysporum* in the leaf. Six leaves with symptoms were removed carefully from infected plants and surface-sterilized. Leaves were cut into transverse strips 2 cm wide, and each leaf piece was placed on CLA in petri dishes.



Figs. 1–4. Transverse sections of leaves from uninoculated plants of chrysanthemum cultivar Yellow Delaware. **1,** Section of an entire leaf showing the uniform appearance of the lamina. Note the abundant trichomes on the leaf surface (×40). **2,** Portion of a leaf section showing the epidermis (e), mesophyll (m), and vascular bundle (b) (×505). **3,** Portion of a section of the leaf margin showing initial differentiation of the mesophyll (×245). **4,** Portion of a section of a leaf midvein showing the bundle cap (c), phloem (p), vascular cambium (vc), and xylem (x) (×240).



Figs. 5–10. Transverse sections of asymmetric leaves from plants of chrysanthemum cultivar Yellow Delaware inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*. **5,** Leaf section in which differentiation has occurred in a portion of the lamina (arrows) (×40). **6,** Section of the leaf midvein in which cell breakdown has occurred (arrow). Note the hypertrophied cells (h) and the accumulation of dark-staining material (×230). **7,** Close-up of a portion of the leaf vascular bundle in Fig. 10, showing well-differentiated xylem vessel elements (arrows) (×681). **8,** Portion of a leaf section showing differentiated mesophyll cells (m) and collapsed abaxial epidermal cells (e) (×235). **9,** Portion of a leaf section from the same leaf as the section in Fig. 8, showing a compact, undifferentiated mesophyll (×185), **10,** Section through a leaf midvein showing well-differentiated xylem (x) and phloem (p) (×155).

In the third experiment, chrysanthemum plants were inoculated with isolates of *F. oxysporum* f. sp. *chrysanthemi* recovered from several different chrysanthemum cultivars in earlier experiments. Plant apexes containing three to seven leaves were removed 14, 18, or 25 days after inoculation. Leaves were surface sterilized, and petiole sections were cultured as before. Sections containing *F. oxysporum* were noted, as well as the presence or absence of visible leaf symptoms.

RESULTS

Leaf morphology. The apical leaves exhibited symptoms 2–3 wk after inoculation, becoming chlorotic and/or asymmetric as described by Emberger and Nelson (2). Curling and puckering of the leaf were common; increased growth on one side of the lamina appeared to cause the uneven development of the leaf.

Culturing. Apical leaves of infected plants were not consistently colonized by *F. oxysporum* f. sp. *chrysanthemi.* Although many chlorotic, asymmetric leaves did contain the pathogen, some did not, and some symptomless leaves were also colonized. The position of the leaf on the stem did not appear to influence colonization. Leaves containing the fungus were often located next to those free of the pathogen.

Histology of healthy leaves. Transverse sections of the blade of young apical leaves from healthy, uninoculated plants were observed (Fig. 1). Within the lamina, mesophyll cells next to the epidermis were differentiated as short, rectangular cells, giving the appearance of a multiple epidermis (Fig. 2). Chloroplasts were abundant in these cells, and nuclei were often conspicuous. Small vascular bundles appeared regularly throughout the lamina on either side of the large central midvein. The tissues of the vascular bundle included a bundle cap, phloem, xylem, and vascular cambium (Fig. 4). The xylem of these young leaves was not fully differentiated; only a few vessel elements were well formed. Large, thin-walled cambial derivatives comprised most of the xylem tissue. Oil ducts were often located next to the vascular bundles. Further blade expansion resulted from differentiation of the mesophyll tissue, beginning at the leaf margins (Fig. 3). Cells of the mesophyll became less compactly arranged as large intercellular spaces appeared throughout the tissue.

Histology of asymmetric leaves. Histology of chlorotic and asymmetric leaves produced on infected plants was variable. Some sections were uniform in appearance throughout the leaf section, while others varied considerably from one side of the midvein to the other. The presence of *F. oxysporum* f. sp. *chrysanthemi* within the plant appeared to accelerate differentiation in leaf tissues (Fig. 5).

Epidermal cells of asymmetric leaves were not uniform in size and shape and formed an irregular surface layer. Many cells of the abaxial leaf surface were crushed (Fig. 8). Nuclei were less visible in most epidermal cells than in those of the control leaves. In several cases, an epidermis intermediate in structure between healthy and asymmetric leaf tissue was present on one side of the lamina (Fig. 9). Trichomes were infrequent on the differentiated portions of the leaf.

The structure of the mesophyll varied considerably from the compact tissue of leaves from uninoculated plants. Large cavities formed throughout the tissue as partial differentiation of the cells into palisade and spongy parenchyma occurred (Fig. 8). The leaf blade increased in thickness in some areas to twice the thickness of healthy tissues. The isodiametric cells in both the healthy leaf tissue and young tissue of asymmetric leaves were replaced by irregularly shaped cells in distorted leaves. Distinct chloroplasts and nuclei were much less frequent in these tissues. Differences existed within leaf blades, and the lamina on one side of the midvein often appeared similar to healthy tissue while the other side exhibited the symptoms described. Chlorotic and distorted leaves contained well-differentiated xylem with fully matured vessel elements (Fig. 10). The large, thin-walled cambial derivatives seen in the leaves from uninoculated plants were absent (Fig. 7). This change in the vascular tissue was apparent in all samples from inoculated plants. Hyphae of *F. oxysporum* f. sp. *chrysanthemi* were visible in the xylem vessel elements of some leaf sections. Breakdown of xylem parenchyma and vessel elements frequently occurred in colonized areas, resulting in the accumulation of large amounts of cell by-products around adjacent cells (Fig. 6). Occlusion of vessel elements by unidentified materials and hypertrophy of xylem parenchyma cells occurred in heavily infected tissues. Hypertrophied cells replaced most xylem vessel elements and parenchyma in one leaf, and the remaining vessel elements were small and infrequent.

DISCUSSION

Infection of Yellow Delaware chrysanthemum plants by *F. oxysporum* f. sp. *chrysanthemi* accelerated the differentiation of leaf tissues. Advanced differentiation of vascular tissue was observed in all vascular bundles of asymmetric leaves. It was not necessary for the pathogen to be present in the leaf tissue for asymmetry to occur. In contrast to changes within the vascular tissues, mesophyll structure varied within the asymmetric leaf. Differentiation of the tissue was often more rapid in the portion of the blade on one side of the midvein, while the other side remained similar in structure to leaves from uninoculated plants.

The reason for this unequal response is unknown. The occurrence of symptoms in leaves apparently free of the pathogen suggests that the fungus produces a translocatable substance that causes the structural alterations or stimulates the host to produce some compound. Studies on the action of hormones within plants have shown that auxins stimulate differentiation of vascular tissue and formation of cambial layers (7). The accelerated differentiation of the vascular tissue of asymmetric chrysanthemum leaves may be the result of increased auxin levels. Mesophyll growth has been shown to be affected by adenine and similar substances, independently of vascular tissues (8).

Cell destruction caused by *F. oxysporum* f. sp. *chrysanthemi* in infected leaves resembles that in stem tissues (2). The reaction of more resistant chrysanthemum cultivars is not known, although infected plants of some of these cultivars show limited asymmetry and chlorosis of leaves.

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