Histopathology of Mimosa Infected with Fusarium oxysporum f. sp. perniciosum

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ABSTRACT

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Proliferation of the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *perniciosum,* in naturally-infected mimosa tree *(Albizia julibrissin)* tissues was studied during disease development. Once infection occurred in the roots, the pathogen invaded shoot tissues via the vessel elements of the latest growth ring. Microconidia were found in vessels lacking mycelium, which suggested that they move in the transpiration stream. Gum deposits appeared first in pit apertures of vessel elements, then increased and completely coated or occluded xylem vessels at the time of wilt symptoms. Abundant mycelial growth occurred in vessel elements of wilted trees, and hyphae invaded vasicentric and ray parenchyma cells via pit apertures. Using the ray parenchyma system, the pathogen invaded laterally into all parts of branches and twigs. Subsequent to defoliation, the pathogen emerged from the host through lenticels in the bark on the surface of which it produced sporodochia bearing masses of macroconidia.

Additional key words: Albizia julibrissin, vascular wilt.

In the first detailed description of Fusarium wilt of the mimosa tree or mimosa (*Albizia julibrissin* Durazz.) caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *perniciosum* (Hepting) Toole, Hepting reported vascular discoloration and the presence of the fungus in all xylem elements of trunk sapwood at the time of appearance of wilt (3). He speculated that the brown gummy substance within invaded xylem vessels originated from xylem parenchyma cells and then was exuded into vessels. Although conidia were observed in vessels, their occurrence was considered rare in a subsequent study (12). Since vascular discoloration was limited primarily to the outer growth ring of xylem vessels, infections were believed to have occurred in the spring. The decay of infected plant tissues was considered the primary means for return of the pathogen to soil. Although the wilt fungus sporulated on the bark of defoliated trees (10), spore production in nature was considered meager and unimportant in dissemination of the pathogen.

The study described herein was initiated to determine the pathway of fungus invasion into host tissues and to determine what symptoms were expressed by the host as tissue invasion progressed. A preliminary report on a portion of the pathological anatomy described has been given (6).

MATERIALS AND METHODS

Twelve trees ranging from 6 to 12 years of age and 12.7 and 25.4 cm in diameter at breast height were sampled periodically from July, 1970 to November, 1971 in both the mountain and piedmont regions of Virginia. Mimosa trees that exhibited wilt symptoms and trees in close proximity to infected trees were selected for further study. At the time of tissue collection, trees were carefully examined and disease symptoms were recorded. To determine the presence of the pathogen, tissues adjacent to those excised for histopathology were cultured on chloramphenicol-amended glucose-yeast extract agar (cGYEA) containing 5 g glucose, 1 g yeast extract, 200 mg chloramphenicol, 20 g agar and 1 liter of water. Tissues for histopathology were fixed either in formalin-acetic acid-alcohol (8) at field temperatures or acrolein solution (1:10, v/v) maintained near 0 C with ice in a styrofoam cooler.

Isolates of *F. oxysporum* were single spored and the cultural characteristics were studied on the potato-dextrose agar (PDA) described by Toussoun and Nelson (13). Pathogenicity was confirmed by using the standard root dip spore suspension method to inoculate seedlings in the greenhouse (11).

Fixed tissues were dehydrated in a tertiary butyl alcohol series (8), infiltrated with Tissuemat paraffin (MP 56-57 C), and imbedded in Paraplast-Plus (MP 56-57 C). Sections were cut using a stainless steel knife and a rotary microtome. A chrome alum-gelatin adhesive was used to

fix sections to slides by the method described by Pappas (4). A safranin-fast green staining procedure (8) was used in studies of host anatomy, whereas a procedure utilizing Pianese IIIB (9) was most useful for observation of pathological aspects of infected tissues.

RESULTS

Observations on symptomatology.—During the two seasons of observation, natural disease incidence first was detectable in late June and early July. By August, symptomatic trees had become more common along roadsides and in landscape plantings. Chlorosis and leaf epinasty were the first symptoms observed. Vascular discoloration occurred in the outermost growth ring and extended down the trunk into the roots where it was most intense.

In a few cases near Blacksburg, symptomatic trees developed bleeding trunk and branch lesions marked by white, jelly-like clumps of sap and a continuous flow of exudate down the trunk. The efflux from these lesions released a sweet ethanolic odor which seemed attractive to certain insects. Trees that developed symptoms and wilting in midsummer often produced adventitious sprouts that persisted until fall frosts.

Numerous bright-orange sporodochia developed in lenticels of defoliated, diseased trees. As these bodies appeared, masses of macroconidia were produced, particularly during periods of high humidity. In numerous semipermanent mounts, only macroconidia (23-60 × 3.0-4.5 μm) with morphology closely comparable to that described for *F. oxysporum* (13) were observed.

In cross-sections of branches with lenticellar sporodochia, there was a consistent association of vascular discoloration with the lenticellar sporodochia, whereas wood beneath normal-appearing lenticels usually was not discolored.

Isolations, cultural characteristics, and proof of pathogenicity.—The pathogen, *F. oxysporum* f. sp. *perniciosum,* was easily obtained from diseased tissues and recognized on cGYEA by production of a deep-purple pigment and spores. On PDA, colonies developing from single spore transfers varied in color from rose or pale pink to pale salmon and exhibited either a raised mycelial or a pionnotal (slimy surface) growth habit. Occasionally, orange masses of macroconidia in sporodochialike clusters were produced in culture. Both

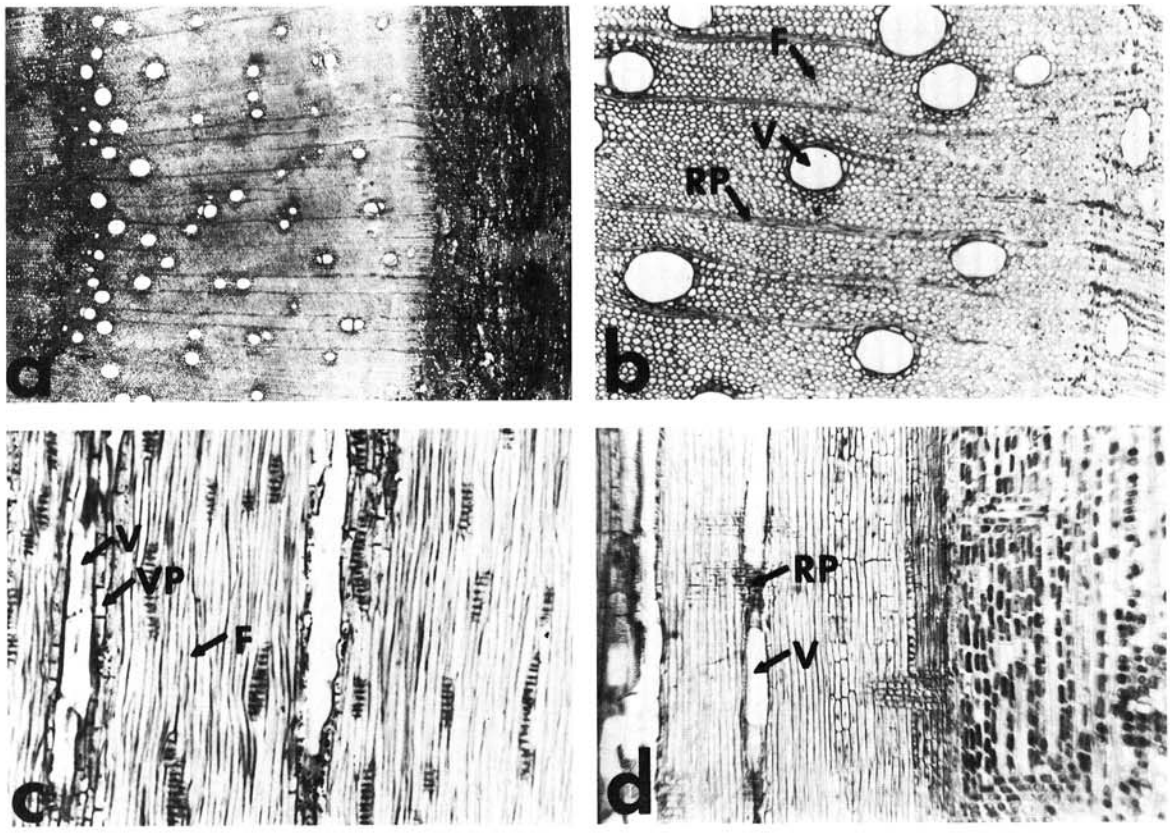

Fig. 1-(a-d). Anatomy of healthy, noninfected mimosa-tree *(Albizia julibrissin)* wood of the current growth ring. **a)** Cross-section of newly formed wood on 15 June 1971 (×25); **b)** Cross-section of wood illustrating typical arrangement of vessels, ray parenchyma, and fibers (×126); **c)** Longitudinal section of wood illustrating vasicentric parenchyma cells (×84); and **d)** Longitudinal section illustrating association of ray parenchyma and vessels and the extension of rays into the cambium and phloem tissues (×84). V = vessel, RP = ray parenchyma, VP = vasicentric parenchyma, and F = fibers.

the cultural characteristics of isolates and the morphology of conidia were typical of the descriptions of *F. oxysporum* (13) and *F. oxysporum* f. sp. *perniciosum* (10). Monosporic cultures of isolates made from internal tissues of roots, trunk, branches, and from conidia in lenticellar sporodochia were pathogenic.

Histology of healthy tissues.—Since no anatomical studies of *A. julibrissin* were extant in the United States, a brief study was made to become familiar with the anatomy and staining characteristics of healthy tissues. Cross-sections of samples from branches revealed the presence of mostly solitary vessels with occasional clusters of two or three (Fig. 1-a, b). The arrangement of vessels and their size distribution was typical of that described for diffuse-porous wood, although a few variants were found with patterns of vessel development resembling ring-porous wood. The vessel elements had simple perforations throughout as small bordered pits that averaged 4 μm in diameter. Vessels were surrounded by a sheath of vasicentric parenchyma cells that frequently came in contact with either uniseriate or biseriate ray parenchyma (Fig. 1). Starch grains were abundant in parenchyma cells adjacent to vessel elements in the current year's growth ring. Libriform fibers, occasionally septate, were a major component of the woody tissues. No tracheids were observed.

Histopathology of tissues prior to and at the time of initial wilt symptoms.—Initial wilt symptoms commonly

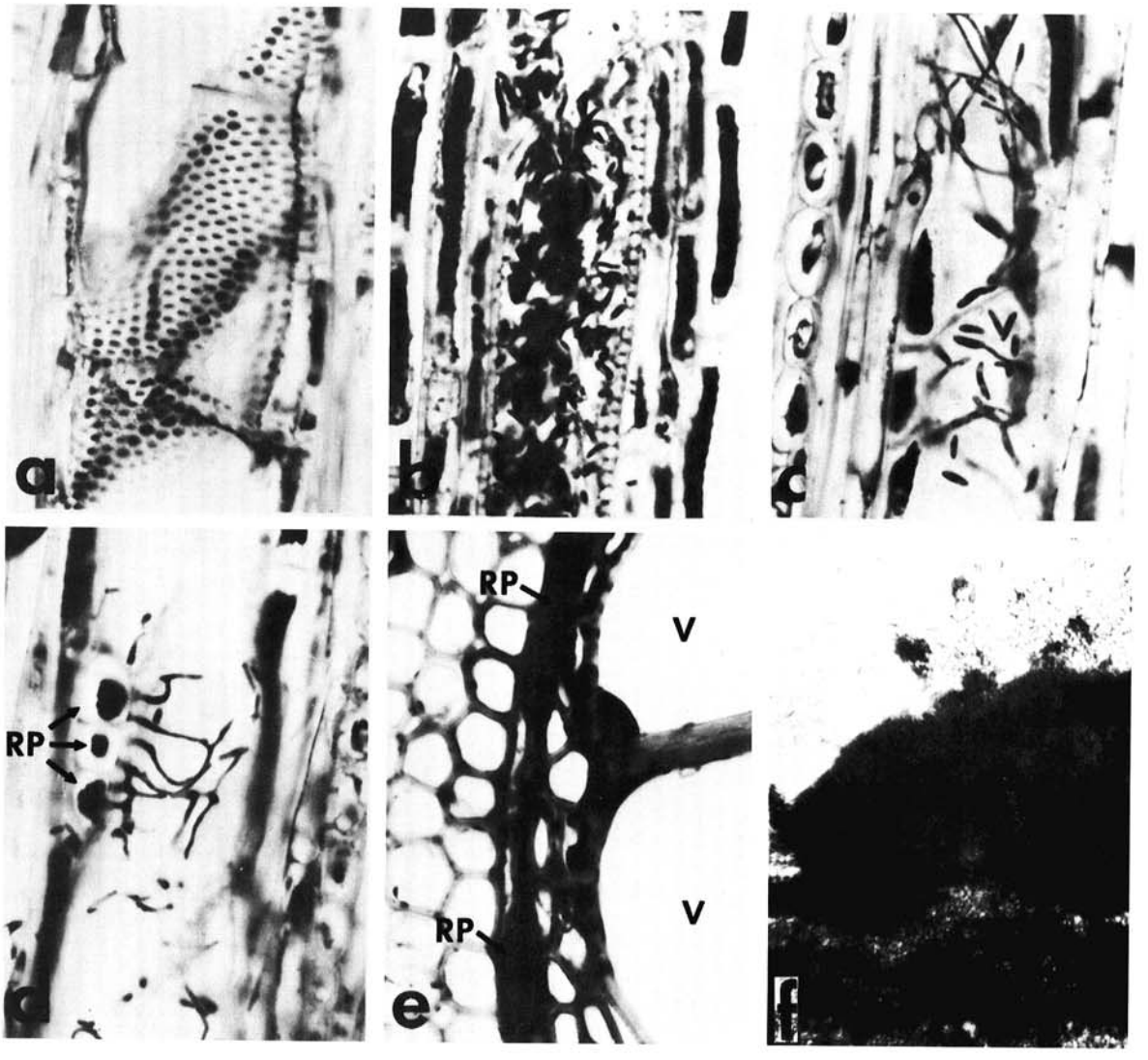

Fig. 2-(a-f). Anatomy of mimosa *(Albizia julibrissin)* tissues infected with the wilt pathogen, *Fusarium oxysporum* f. sp. *perniciosum*. **a)** Accumulation of gum deposits in pit apertures of a vessel element prior to foliar wilt symptoms (× 400); **b)** Accumulation of conidia below the endwall of a vessel element in wood just prior to wilt symptoms (× 400); **c)** Conidia production by mycelium in vessel elements at the time of initial wilt symptoms (× 400); **d)** Invasion of vasicentric parenchyma cells by mycelium in a vessel element (× 400); **e)** Gum-like substances in vessel elements (× 800); and **f)** Cross-section of a lenticellar sporodochium (with macroconidia) formed after wilt and defoliation (× 25). V = vessel, and RP = ray parenchyma.

were observed in only one or a few branches. This permitted sampling of tissues from both symptomatic and asymptomatic branches on the same tree. In several specimens from asymptomatic branches, gum deposits were detected in pit apertures of the vessel elements (Fig. 2-a). Microconidia, in the absence of mycelium, were found in the vessels of a few specimens from asymptomatic branches; the spores occurred in aggregates within vessel elements at points below endwalls (Fig. 2-b). Neither gums nor conidia were found in tissues from healthy trees.

Tissues from branches that exhibited wilt symptoms commonly contained conidia and mycelium of the pathogen in the vessel elements. Conidia were observed attached to conidiophores and in a free state (Fig. 2-c). Mature free-floating conidia stained deep red or purple, whereas those attached to conidiophores stained light pink. Only microconidia (6.0 - 12.5 × 2.0 - 3.5 μm) were observed and no evidence of budding conidia was found.

Mycelial growth was restricted mostly to vascular tissues and the neighboring vasicentric parenchyma cells. Where invasion of neighboring parenchyma cells was noted, the contents of these cells stained heavily (Fig. 2-d). Hyphae penetrated cells via pit apertures and no direct penetration was observed. Vascular discoloration occurred primarily, although not exclusively, in the outer growth ring. Cross- and longitudinal sections of tissues exhibited gum-like deposits on the walls of vessel elements and in several instances, vessels were occluded completely by these substances. These deposits were continuous with an identically staining material within vasicentric parenchyma cells, suggesting their leakage from parenchyma cells through pit apertures and into xylem elements (Fig. 2-e). Infected roots from a few selected trees had a similar deposition of substances in xylem parenchyma and vessel elements.

Histopathology of tissues from defoliated branches.—Mycelia and conidia were most abundant in tissues from defoliated branches. At this time, almost no starch grains were found in xylem tissue. Hyphal invasion often could be traced from vessel elements into vasicentric parenchyma cells and then into ray parenchyma. By invasion into the ray parenchyma system, the pathogen reached the cambium and phloem tissues. Frequently, appressorium-like swellings were noted in hyphae at points before constriction and passage through pit apertures. None of the histological stains permitted clear differentiation of hyphae in phloem tissues. Stems and branches with swollen lenticels contained masses of mycelium beneath the closing and filling tissues of lenticels. The growth of mycelium in lenticels then resulted in formation of sporodochia, previously alluded to by us as "lenticellar sporodochia" (11). Masses of macroconidia were produced by sporodochia particularly during wet periods (Fig. 2-f).

DISCUSSION

Based on the current observations of symptomatology and histopathology of Fusarium wilt of mimosa, the pathway of fungal invasion during pathogenesis now may be described. Previous reports (3, 12) have provided evidence that the pathogen resides in the soil where it infects roots of the host. All attempts to reproduce the disease by inoculation of above-ground parts of trees have been unsuccessful, whereas root inoculations consistently resulted in disease (3, 7). Once infection has occurred in roots, the production and release of microconidia into the sapstream provides a means for rapid colonization of above-ground parts. The presence of conidia in vessel elements prior to wilt symptoms and the absence of mycelium provided evidence of the role of spores in host colonization. The movement of spores of vascular wilt fungi has been documented in other fungal wilt diseases of trees, and probably is a major factor accounting for the rapid rate of disease development characteristic of mimosa wilt.

At the time of systemic invasion, the presence of gum-like substances in pit apertures and on the walls of vessel elements was first observed. Sections of roots and branches indicated that these substances originated from vasicentric parenchyma cells as proposed by Hepting (3). The continued release and possible translocation of these substances upward to areas of lower concentrations may account for their appearance early in the disease syndrome. According to Anderson et al. (1), the crude gums of at least three *Albizia* spp. are soluble in cold water up to concentrations of 5 percent. The accumulation of gums in vessel elements and discoloration in vasicentric parenchyma cells resulted in the characteristic vascular discoloration.

The symptoms of Fusarium wilt of mimosa differ from those of the other vascular wilt diseases in that tyloses were not observed in the vessel elements of the diseased trees. Chattaway (2) reported that tyloses are not formed in wood with pit aperture diameters less than 10 μm, and that the secretion of gums instead of tylose formation is the characteristic response to injury.

Once systemic invasion had occurred, wilt symptoms developed rapidly and the pathogen invaded vasicentric and ray parenchyma cells. Using the ray parenchyma system of the host, the pathogen proliferated laterally from vessel elements into cells of the cambium and phloem. In the final stage of pathogenesis, the pathogen emerged from the host through lenticels in the bark where sporodochia developed and produced masses of macroconidia. The occurrence of sporodochia on diseased trees and the period in which viable inoculum may be produced has been the subject of a recent study (11).

LITERATURE CITED

1. ANDERSON, D. M. W., G. M. CREE, J. J. MARSHALL, and S. RAHMA. 1966. Appendix on botanical nomenclature in the genus Albizia (Leguminosae). Carbohyd. Res. 2:63-69.
2. CHATTAWAY, M. 1949. The development of tylosis and secretion of gum in heartwood formation. Austr. J. Sci. Res. (Series B) Biol. Sci. 2:227-240.
3. HEPTING, G. H. 1939. A vascular wilt of the mimosa tree (Albizzia julibrissin). U. S. Dep. Agric. Circ. 535. 11 p.
4. PAPPAS, P. W. 1971. The use of a chrome alum-gelatin (subbing) solution as general adhesive for paraffin sections. Stain Technol. 46:121-124.
5. PHIPPS, P. M. 1972. Histopathology, pathology, and chemotherapy of Fusarium wilt of mimosa (Albizia julibrissin). M. S. Thesis. Va. Polytech. Inst. and State University, Blacksburg. 139 p.
6. PHIPPS, P. M., and R. J. STIPES. 1971. Some

histopathological observations on Fusarium wilt of "mimosa." Va. J. Sci. 22:107 (Abstr.).
7. PHIPPS, P. M., and R. J. STIPES. 1973. Artificial reproduction of Fusarium wilt of the mimosa tree under greenhouse and field conditions. Phytopathology 63:804. (Abstr.).
8. SASS, J. E. 1958. Botanical microtechnique. 3rd ed. Iowa State Univ. Press, Ames. 228 p.
9. SIMMONS, S. A., and R. A. SHOEMAKER. 1952. Differential staining of fungus and host cells using a modification of Pianese III B. Stain Technol. 27:121.
10. SNYDER, W. C., E. R. TOOLE, and G. H. HEPTING. 1949. Fusaria associated with mimosa wilt, sumac wilt, and pine pitch canker. J. Agric. Res. 78:365-382.
11. STIPES, R. J., and P. M. PHIPPS. 1975. Fusarium oxysporum f. sp. perniciosum on Fusarium-wilted mimosa trees. Phytopathology 65:188-190.
12. TOOLE, E. R. 1941. Fusarium wilt of the mimosa tree (Albizzia julibrissin). Phytopathology 31:599-616.
13. TOUSSOUN, T. A., and P. E. NELSON. 1968. A pictorial guide to the identification of Fusarium species according to the taxonomic system of Snyder and Hansen. The Pennsylvania State University Press, University Park. 51 p.