Fusarium oxysporum f. sp. perniciosum on Fusarium-Wilted Mimosa Trees

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ABSTRACT

The presence of *Fusarium oxysporum* f. sp. *perniciosum* was determined in 1,200 lenticels of 20 Fusarium-wilted mimosa trees (*Albizia julibrissin*) that became symptomatic either 1-2 years before, or during the observation period. *F. oxysporum* was isolated and identified in culture from 38, 83, and 79% of lenticellar tissues from trees that exhibited symptoms at the time of sampling, and 1 or 2 years previously, respectively. About 90, 85, and 96% of these cultures, respectively, produced typical foliar symptoms of Fusarium wilt on root-dip-inoculated greenhouse seedlings, thereby confirming the identity of the fungus as *F. oxysporum* f. sp. *perniciosum*. Two of 240 lenticels from asymptomatic trees yielded the pathogen. Many lenticels in which sporodochia of a *Fusarium* sp. were observed were located on the underside of branches. The high incidence of the pathogen in lenticels of these diseased trees constitutes a new report. These findings suggest that lenticellar sporodochia may function as major sources of inoculum from which epiphytotics may occur, especially after periods of warm, humid weather that stimulates sporulation. Accordingly, we emphasize the importance in disease control of prompt destruction of symptomatic trees.

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Recent observations of mimosa trees (*Albizia julibrissin* Durazz.) succumbing to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *perniciosum* (Hepting) Toole, included the abundant presence of salmon- or orange-colored sporodochia of *Fusarium* produced predominantly in lenticels on the trunk and lateral branches. Although Toole (6) and Snyder et al. (4) reported the occurrence of the pathogen on the bark of infected trees, they emphasized the presence of saprophytic fusaria, or those non-pathogenic to the mimosa tree. No detailed investigation has been reported on the incidence of the pathogen on the bark of the diseased host. Because of the importance of potential inoculum sources on the bark in the epiphytology of this disease, we investigated the frequency of occurrence and identity of *F. oxysporum* produced in these lenticellar sporodochia. A preliminary report has been given (5).

MATERIALS AND METHODS.—The 24 trees observed in this study were approximately 16 years old and constituted an ornamental landscape planting in an irregular fashion on a gentle, west-facing slope at the University arboretum near Blacksburg, Virginia. They were variously spaced, some solitary, while others were in loosely-arranged groups, possibly within root-grafting distance of each other. Fusarium wilt was first diagnosed by the senior author on four trees during the summer of 1970, followed by seven and nine trees in 1971 and 1972, respectively, all of which were used in this study. Four asymptomatic, apparently healthy, specimens on the same site were included as controls.

In September, 1972, 12 bark samples (1×4 cm^2) per tree were removed at random with a wood chisel on the multi-stemmed trunk and lateral branches yielding lenticels visibly exhibiting sporodochia of *Fusarium* sp. They were placed in moist chambers at room temperature for 3 days to induce sporulation, and stored at 4 C until isolations were made.

Biopsy tissues from 5 separate lenticels per bark sample were removed by a sterile probe and placed either on glucose-yeast extract agar (GYEA) (5 g D-glucose, 1 g yeast extract, and 20 g agar per liter), or on a modified Nash and Snyder's PCNB medium (2). Both media were supplemented with microelements (0.2 mg Fe^{+++}, 0.2 mg Zn^{++} and 0.1 mg Mn^{++} per liter) and 200 μg/ml chloramphenicol; the PCNB agar did not contain streptomycin. Plates were incubated under continuous cool fluorescent white light at 28 C for about 9 days when macroscopic and microscopic observations were made.

To confirm the form species identity of *F. oxysporum* isolates as determined morphologically, 8- to 10-week-old greenhouse-grown mimosa seedlings were inoculated by a root dip-spore suspension technique (4). Foliar and vascular symptoms were indexed 6-8 weeks after inoculation.

RESULTS AND DISCUSSION.—Fusarium wilt was first noted in the University arboretum in July, 1970. During the next two years, the disease progressed in a generally radiating fashion from the initial infection center, killing more trees each successive year. This suggested the possible production and liberation of inoculum from the enlarging infection center. Upon close examination of the infected trees during late summer, we observed numerous prominent salmon- or orange-colored sporodochia of *Fusarium*, produced primarily within lenticels (Fig. 1), but also along some linear bark fissures; macroconidia only were found in these fruiting bodies. T. Matuo (*personal communication*, 1973) also indicated a similar phenomenon in Fusarium branch blight of mulberry trees (*Morus alba* L.) caused by *F. solani* App. & Wr. emend Snyd. f. sp. *pisi* (Jones) Snyd. & Hans. that was not mentioned in a recent report (1).

Phipps (3) found that wood beneath the sporodochia was discolored and many vessels therein were plugged or contained gums, while vessels in wood beneath normal-appearing lenticels on the same branches of pathologic trees were not occluded and appeared normal. Although

he could not differentiate the pathogen in cortical tissues of the stem by staining procedures, the swelling of lenticels preceding lenticellar sporodochia formation suggested the presence of fungal growth and production of mycelium. Phipps (3) also found that lenticels on defoliated branches exhibited the disruption of closing layers and filling tissues that was coincident with sporodochium development; at that time, hyphae were visible in parenchyma cells in the region of the cambium and among filling cells in lenticels. Phipps' (3) and our findings suggest that, after initial root and later trunk and branch invasion, the pathogen emerges at the lenticels where it fruits.

Although lenticellar sporodochia were visible on bark specimens when samples were collected, incubation in moist chambers greatly stimulated sporulation; many lenticels exhibited slimy masses of spores after 3 days. In most cases, pure cultures of *F. oxysporum* from diseased trees resulted from lenticellar explants on GYEA and PCNB media. Lenticels from healthy trees yielded predominantly dark-spored moniliaceous members of the Fungi Imperfecti. The agar color reaction by *F. oxysporum* on the PCNB medium was yellowish, while on GYEA or PDA, it was pink to purple.

Table 1 includes data on isolation frequency of *F. oxysporum* from lenticellar tissues. The pathogen was obtained in significantly higher levels from symptomatic than from asymptomatic trees in which only 2 of 240 lenticels (0.8%) yielded *F. oxysporum* f. sp. *perniciosum*. In addition, the pathogen was isolated more frequently from trees which exhibited initial disease symptoms 1-2 years prior to the observation period than from those becoming symptomatic during the observation period. This indicated that a time period was required to permit the pathogen to egress radially at the lenticels where it produced sporodochia. Phipps (3) did not observe lenticellar sporodochia until after trees had been defoliated. Although the raw data of individual replicates are not given in Table 1, considerably more variation in recovery of *F. oxysporum* occurred among replicates of trees which exhibited initial symptoms during 1972, than among those which did so during 1970 and 1971; we believe that trees yielding the higher number of *F. oxysporum* isolations from their lenticels had become

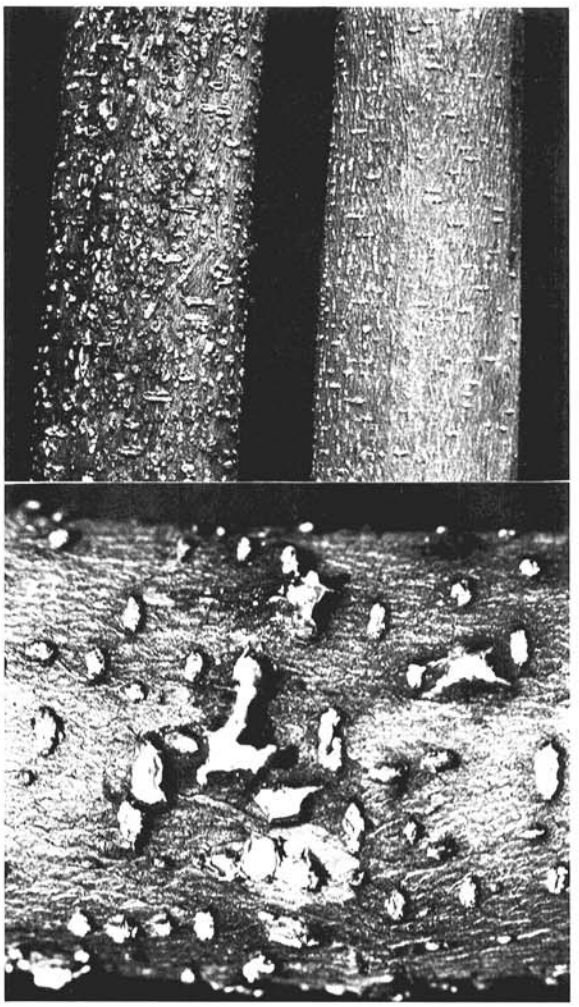

Fig. 1. Lenticellar sporodochia on bark of mimosa-tree infected with *Fusarium oxysporum* f. sp. *perniciosum*. Upper left: section of branch from diseased tree (ca. ×0.5). Upper right: comparable branch from healthy tree. Lower photo: enlarged (×3) view of erumpent sporodochia.

TABLE 1. Recovery of *Fusarium oxysporum* isolates from lenticels of Fusarium-wilted and healthy mimosa trees and their pathogenicity to mimosa-tree seedlings[a]

Tree Class	Trees sampled (no.)	Lenticels yielding *F. oxysporum*[b] (%)	Lenticellar *F. oxysporum* cultures producing Fusarium wilt symptoms (%)	
			Foliar Symptoms	Vascular discoloration
Healthy	4	0.8 A	100	100
Initial disease, 1972	9	38.0 B	90	87
Initial disease, 1971	7	83.0 C	85	86
Initial disease, 1970	4	79.0 C	96	91

[a] 12 bark samples per tree and 5 lenticellar isolations per sample were studied, totaling 1,440 lenticellar observations.
[b] Numbers not followed by the same letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.

infected during the previous summer, while those yielding lesser numbers had become infected at later periods. Bark on the trunk and larger branches of trees exhibiting initial disease in 1970 was cracking and falling off the trees about 2 years after initial symptoms.

Although no attempt was made to quantify the amount of surface area of diseased trees yielding lenticellar sporodochia, the potential number of spores is very great. Since the bark of these trees yields great numbers of *F. oxysporum* f. sp. *perniciosum* fruiting bodies, we suggest that these sporodochia constitute a major source of inoculum by which epiphytotics may occur, at least under this specific landscape situation. Accordingly, we emphasize the possibility of these phenomena occurring in other geographic locations, and therefore the importance in disease control of the prompt destruction of symptomatic trees, preferably by burning.

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