Sources of Species of Fusarium in Northern Hardwood Forests

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ABSTRACT

Several species of *Fusarium*, capable of causing Fusarium canker of sugar maple, were found in the soil and air in forest stands and in bark of healthy maple trees. Air samples were collected in Pennsylvania sugar maple stands under various weather conditions between April and November. Soil and bark samples were collected in the same stands. Two isolates each of *Fusarium solani* and *F. roseum*, and three of *F. tricinctum*, were collected from 25,000 ft^3 of air sampled. One hundred-eleven isolates of *Fusarium* were obtained from forest soil and maple bark. Sixty, 26, 15, and one of the isolates recovered were *F. solani, F. roseum, F. tricinctum,* and *F. rigidiusculum*, respectively. Thirty-two of the *F. solani* isolates and the one isolate of *F. roseum* from soil caused cankers on one or more of eight inoculations. Sixteen, 11, 12, and one of the bark isolates of *F. solani, F. roseum, F. tricinictum,* and *F. rigidiusculum*, respectively, caused cankers. In addition, all three isolates of *F. tricinctum* and one isolate of *F. roseum* recovered from air caused cankers. All pathogenic species of *Fusarium* were obtained from bark at 5.5 m or less aboveground. There was no apparent association between isolate pathogenicity and the tree face from which it was isolated. More pathogenic isolates were recovered from stands with moderately and severely cankered trees than from stands with trees only slightly affected.

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Fusarium solani (Mart.) Appel & Hans. causes an annual canker of sugar maple (*Acer saccharum* Marsh.) (14), poplar (1), cottonwood (2), musizi (3), yellow poplar (4), aspen (6), tupelo (17), and oak (19). Fusarium canker of sugar maple, which is found throughout the natural range of sugar maple in Pennsylvania, develops during the dormant season of the host. It occurs most frequently on the lower portion of the stem (14), and severe cankering has no apparent effect on diameter growth.

Little is known about the sources of inoculum and the methods of dissemination of the pathogen. The results of Toole (18) and Schreiber (13) suggest that the pathogen might be present in the soil. Snyder & Toussoun (15) suggest that *F. solani, F. oxysporum* (Schl.) Snyd. & Hans., and *F. roseum* (Lk.) Snyd. & Hans. probably are found in soil, water, and air during some phase of their life cycles.

Fusarium solani is rarely found fruiting on trees in nature, even around cankers.

Lukezic & Kaiser (5) demonstrated the capability of *F. roseum* 'Gibbosum' to become airborne at low wind speeds. It is generally thought that species of *Fusarium* are not wind-borne because their conidia are produced primarily in wet sporodochia.

The study reported herein had the following objectives: (i) to determine whether the soil in sugar maple stands and the bark of healthy (uncankered) sugar maple trees were sources of inoculum; and (ii) to determine whether wind is an important method of dissemination of *Fusarium* inoculum.

MATERIALS AND METHODS.–Soil and bark samples were collected in two sugar maple stands each in Huntingdon, Clearfield, and Potter Counties, Pa. Based on the number of visible cankers in a sample plot in each stand, the stands were classified as light (0-3 cankers/tree), moderate (4-10 cankers/tree), or severe (11+ cankers/tree).

Soil samples were collected during June and July of 1966. Ten samples were removed from the A_1 horizon (top layer of mineral soil beneath duff) in each stand. One gram of each sample was finely ground in a mortar, mixed with 35 ml of sterile glass-distilled water, and thoroughly blended in an Omni-Mixer for 2 min at 10,000 rpm. The suspension was then diluted by 10^{-2} to insure adequate separation of colonies that developed subsequently in culture, and 10 ml were transferred to agar plates at the rate of 1 ml/plate. A modified Martin's medium (22) containing 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g Bacto peptone, 10 g dextrose, and 20 g agar/liter of sterile distilled water was used. Rose bengal was added to the medium at a concentration of 1:30,000 (w/w) to restrict the size of contaminant fungus colonies. The medium, after sterilization at 15 psi for 20 min, was cooled to 48 C, and streptomycin sulfate was added to it. Both Wensley & McKeen (22) and Parmeter & Hood (12) have used modified Martin's medium successfully to isolate and enumerate colonies of species of *Fusarium*.

Plates were examined after 5 to 14 days' incubation at 21 C. All isolates of *Fusarium* that appeared different morphologically were subcultured and stored. Single spore cultures of each isolate were established and maintained on potato-dextrose agar. Subsequently, a conidial suspension was prepared and

5 ml were poured into a tube containing a 2:1:1 mixture of soil, peat, and perlite. The tubes were capped and stored at 10 C. Isolates of *Fusarium* maintain their pathogenicity longer when stored in this manner, since culture variability is reduced (20).

We made pathogenicity tests by inoculating trees in November 1966. Cankers that developed from the inoculations were collected in July 1967. We prepared inoculum by growing isolates on sterilized rye grain mixed with an equal volume of sterile distilled water. The inoculum was incubated for 2 weeks.

The bark and cambium of five healthy sugar maple trees of the intermediate crown class were sampled between July and September 1967, in the same stands from which the soil samples had been collected. Samples (3-inch^2) were removed from the north, east, south, and west faces at 1-m intervals along the stem to the first main branch bearing live foliage (average of 4.6 m); an average of 30 samples was removed from each of the five trees. Trees in the intermediate crown class were sampled because the most severely cankered trees were in this class. In the laboratory, each sample was mixed thoroughly with 50 ml of sterile distilled water for 2 min at 10,000 rpm in the Omni-Mixer. A 10-ml sample was drawn from each resulting suspension and was added to 10 plates of modified Martin's medium at the rate of 1 ml/plate.

Air samples were collected with midget impingers. It was assumed that microconidia or ascospores would most likely be disseminated by wind and, owing to their relatively small size, would be collected relatively efficiently with midget impingers. Air was drawn through the samplers at the rate of 4 ft^3/min or (0.12 m^3) with a vacuum pump calibrated with a wet-test flow meter (Precision Scientific, Chicago, Ill.). A manifold of Tygon tubing designed to accommodate 12 impingers was attached to the vacuum pump. The impingers were emplaced at 0.3 m aboveground and were arranged in a circle with a 7.6-m radius. A gasoline-driven 2,500-w generator was used as the power source. The impingers were placed ca. 75 m from the generator to eliminate any influence of the generator on air movement.

Prior to sampling, each impinger was washed and sterilized with a solution of potassium dichromate and sulfuric acid, rinsed several times with sterile glass-distilled water, and partially filled with 10 ml of sterile distilled water. The intake and outlet orifices were sealed with wax tape until they were used.

To test the ability of the impingers to trap large spores such as the macroconidia of *F. solani*, a conidial suspension of *F. solani* was atomized into the atmosphere several feet from impingers in the laboratory and in the field. In both instances, spores were collected and their viability was determined by growing them on culture media.

After sampling was completed, water in the impingers was plated on the modified Martin's medium at the rate of 1 ml/plate. Using twelve impingers, 1,000 ft^3 (or 30 m^3) of air were collected on each sampling day. Sampling was done 1 day each week in one of the three stands previously sampled for bark and soil. One stand in each of Huntingdon, Clearfield, and Potter Counties was sampled under a variety of weather conditions from April through October 1967.

Inoculations with isolates collected from bark and air in 1967 were made in November of that year. Wounds that extended to the xylem were made in the trees by means of an 11/64-inch bit that had been sterilized in alcohol and was driven by a cordless electric drill. Each isolate was introduced twice into each of four trees, twice on each cardinal face. Two wounds into which sterile grain was introduced also were made on each tree and these served as controls. Moist sterile cotton was tied over each wound. Trees in the intermediate crown class generally were used for the inoculations.

Cankers that developed from the inoculations were harvested in July 1968, and isolations were made. The bark was removed and the canker surface was sterilized with 72% ethanol and flamed. Chips were removed from the margins of the cankers and placed on modified Martin's medium. Species of *Fusarium* recovered from a canker were identified and compared with the isolate used for the inoculation. All species' identifications were confirmed by Paul E. Nelson, Department of Plant Pathology, The Pennsylvania State University, University Park. The term "species" refers to those species of *Fusarium* recognized by Snyder & Toussoun (15).

RESULTS.—A majority of 111 isolates of *Fusarium* recovered from the soil, bark, and air sampled was isolated from bark and soil (Table 1). *F. solani* was recovered most frequently, followed by *F. roseum* and *F. tricinctum*. One isolate of *F. rigidiusculum* (Brick) Snyd. & Hans. was recovered from bark in a moderately cankered stand. *Fusarium oxysporum* and *F. moniliforme* (Scheld.) Snyd. & Hans. also were recovered.

Pathogenicity tests showed that 67% of the 111 isolates caused cankers. Thirty-nine, 31, and 4 of the bark, soil, and air isolates, respectively, caused cankers. Inoculations with *F. oxysporum* and *F. moniliforme* did not result in cankers (Table 2).

All isolates of *F. tricinctum* and the single isolate of *F. rigidiusculum* were pathogenic. *Fusarium solani* induced the largest cankers, which ranged in size from

TABLE 1. Frequency of isolation of species of *Fusarium* from hardwood forests

Species	Bark No./1,000 samples	Soil No./60 samples	Air No./288 samples	Total
F. solani	20	38	2	60
F. roseum	23	1	2	26
F. tricinctum	12	0	3	15
F. rigidiusculum	1	0	0	1
F. oxysporum	3	3	0	6
F. moniliforme	2	1	0	3

TABLE 2. Number of isolates of species of *Fusarium* pathogenic to sugar maple

Species	Bark	Soil	Air
F. solani	16/20[a]	31/38	0/2
F. roseum	11/23	1/1	1/2
F. tricinctum	12/12	0/0	3/3
F. rigidiusculum	1/1	0/0	0/0
F. oxysporum	0/3	0/3	0/0
F. moniliforme	0/2	0/1	0/0

[a] Number of pathogenic isolates per total number of isolates.

TABLE 3. Occurrence of pathogenic isolates of species *Fusarium* in tree bark in relation to tree face from which they were isolated

Species	North	East	South	West
F. solani	2/16[a]	7/16	2/16	5/16
F. roseum	7/11	2/11	1/11	1/11
F. tricinctum	3/12	3/12	3/12	3/12
F. rigidiusculum	0/1	0/1	1/1	0/1
All species	12/40	12/40	7/40	9/40

[a] Number of pathogenic isolates (for each face) per total number of pathogenic isolates of a given species isolated from bark.

2.7 to 5.5 cm wide × 4.5 to 24.5 cm long. Cankers also developed commonly around some of the nails used to hold identification tags on inoculated trees, and usually *F. solani* or *F. tricinctum* could be isolated from them.

Fusarium solani was found more frequently in healthy bark on the east and west faces, but *F. roseum* was recovered most often from the north face. Pathogenic isolates of *F. tricinctum* were recovered in almost equal numbers regardless of the face sampled. There apparently was no association between isolate pathogenicity and the tree face from which it was isolated, although fewer pathogenic isolates were recovered from the south face (Table 3).

All pathogenic species of *Fusarium* originally isolated from bark were recovered at 5.5 m or less aboveground (Table 4). The average sampling height was 7.6 m. Sixty-five percent of the isolates were recovered below 1.8 m and, except for *F. tricinctum*, the pathogenic species were recovered most frequently at 1.8 m or below.

The condition of the stands sampled was not directly related to the frequency of isolation of pathogenic isolates, but a higher number of pathogenic isolates was recovered from moderately and severely cankered stands than from lightly affected stands (Table 5).

Thirty-four, 25, 21, and 20% of the cankers that resulted from the inoculations were located on the north, south, west, and east faces, respectively.

DISCUSSION.—*Fusarium solani* has been considered as the organism responsible for Fusarium canker of sugar maple. The results of our studies indicate that *F. tricinctum, F. roseum*, and *F. rigidiusculum* are capable of causing similar cankers on sugar maple.

Fusarium solani is probably the most important species involved because it is the species most commonly isolated from natural cankers and is associated with the highest frequency with both bark and soil. *Fusarium tricinctum* and *F. roseum* were occasionally recovered from natural cankers, but not so frequently as was *F. solani*. A higher number of pathogenic isolates was recovered from bark; comparisons among sources were precluded, however, because of differences in the procedures used to sample them. The frequency of isolation of *F. solani* from natural cankers is relatively low, but its pathogenicity and its abundance at potential infection courts have been demonstrated.

We did not determine the type of morphological unit that gave rise to the isolates recovered from the different sources. Warcup (21) reported that most fungus colonies developing on soil dilution plates arise from spores. Nash et al. (8) state that *F. solani* exists in field soils as chlamydospores. Sometimes *F. solani* and *F. tricinctum* have been found in cultivated, but not in noncultivated, soils (9). Some investigators have been unable to recover certain species of *Fusarium* from forest soils (11, 16), whereas others have had no difficulty (10). We did not isolate *F. tricinctum* from forest soils, but several

TABLE 4. Occurrence of pathogenic isolates of *Fusarium* in tree bark in relation to height above ground

Species	Height above ground (m)						
	0	0.9	1.8	2.7	3.7	4.6	5.5
F. solani	5/16[a]	3/16	2/16	0/16	2/16	3/16	1/16
F. roseum	1/11	6/11	4/11	0/11	0/11	0/11	0/11
F. tricinctum	0/12	1/12	2/12	1/12	2/12	5/12	1/12
F. rigidiusculum	0/1	0/1	1/1	0/1	0/1	0/1	0/1
All species	6/40	10/40	9/40	1/40	4/40	8/40	2/40

[a] Number of pathogenic isolates (for each height) per total number of pathogenic isolates of a given species isolated from bark.

TABLE 5. Number of pathogenic isolates of *Fusarium* in relation to amount of disease in stands sampled

Species	Canker incidence[a]		
	Light	Moderate	Severe
F. solani	1/16[b]	11/16	4/16
F. roseum	0/11	10/11	1/11
F. tricinctum	0/12	9/12	3/12
F. rigidiusculum	0/1	1/1	0/1
All species	1/40	31/40	8/40

[a] Amount of disease in a stand was established on the basis of a sample plot which was classified as light, moderate, or severe if the trees had, respectively, 0-3, 4-10, or 11+ cankers/tree.

[b] Number of pathogenic isolates (for stands of different disease levels) per total number of pathogenic isolates.

other *Fusarium* species were isolated from this source without any difficulty.

F. rigidiusculum, which was isolated only once during this study, was obtained from bark. This species is rare in the northern hemisphere.

Our findings suggest that both forest soil and healthy sugar maple bark are important sources of inoculum of species of *Fusarium* capable of causing cankers in sugar maples. Bark is probably the most important source since the inoculum is present at potential infection courts. The pathogen may be carried to the bark by splashing rain or possibly incorporated in soil that is either splashed or wind-borne to the tree stems. Emerging seedlings might contact inoculum in the soil and the pathogen subsequently develops saprophytically in the bark. We do not know if air and bark were sampled with comparable efficiency. Therefore, the importance of wind as a transport medium for Fusarium spores is difficult to evaluate. Though fewer isolates were recovered from air than from bark and soil, it is possible that critical spore release periods were missed.

Skelly & Wood (14) reported that canker north and south faces than on the east and west faces of sugar maples. In our study, pathogenic isolates of *F. solani* were recovered most frequently from the east and west faces. *Fusarium roseum* was isolated most frequently from the north face and *F. tricinctum* was isolated with almost equal frequency from all faces.

Skelly & Wood (14) found more cankers on the frequency varied inversely with height above ground. We found that the frequency with which pathogenic isolates were recovered also varied inversely with height above ground. These data suggest that the pathogen occurs in the "healthy bark" of sugar maples and that cankers may develop when wounds in the bark extend to the vicinity of the cambium. Such wounds might result from wind, a sudden drop in temperature, and/or insect attack.

A decline in virulence of isolates has been associated with cultures maintained and stored on nutrient media, whereas storage of isolates as chlamydospores in soil apparently preserves such isolate characteristics as virulence. In preliminary studies, we observed that *F. solani* on potato-dextrose agar changed from a pionnotal and sporodochial morphology to a mycelial type of growth. This has been observed and discussed by others (8). Concurrently, a marked reduction in virulence occurred. Maloy (7) noted comparable changes for *F. solani* f. sp. *phaseoli*. The virulence of stored isolates recovered from induced cankers cannot be compared with stock isolates for purposes of identification if changes in the morphology of stored isolates have occurred.

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