Airborne Spore Dispersal and Recolonization of Steamed Soil by Fusarium oxysporum in Tomato Greenhouses

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ABSTRACT

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A crown and root rot of greenhouse tomatoes caused by *Fusarium oxysporum* has become a problem in Ohio and Ontario, Canada, during the past few years. Repeated attempts to control the disease by steam-disinfestation of soil have failed. In naturally infested commercial greenhouses, microconidia of pathogenic isolates of *F. oxysporum* were trapped from air. The sources of these airborne microconidia were presumed to be infected tomato stems, straw mulch, and partially decomposed tomato vines in outside dump piles; large numbers of microconidia were recovered from all of these substrates. Flats of autoclaved soil were atomized with suspensions of microconidia to determine their recolonization rate in steamed soil. The number of propagules per gram of soil increased by 10^3 after 3 days and by 10^4 after 1 wk. After 2 wk, populations stabilized at about 1,000/g of soil, regardless of initial inoculum concentrations. This investigation suggests that the failure of soil steaming to control Fusarium crown and root rot was due to recontamination of freshly steamed soil by airborne microconidia of the pathogen.

Additional key words: Fusarium crown and root rot, epidemiology.

A new and extremely destructive crown and root rot of greenhouse tomatoes caused by *Fusarium oxysporum* Schlect. was first reported in Japan in 1974 (29). In the same year, it was observed in greenhouses in Ohio and in Ontario, Canada (4, 7, 12). The disease has subsequently appeared throughout most of the greenhouse tomato industry in these areas, frequently causing serious losses (7, 11). A similar disease has been reported in winter field tomato production areas in California and Florida (5, 15, 22). At present, no satisfactory control is available (7, 11).

Most commercial tomato greenhouses in Ohio are equipped with steam tiles 30-35 cm below the soil surface. Yearly disinfestation by steaming tarped soil beds at 80-85 C for 4-6 hr is standard procedure to eliminate weed seeds, soil insects, nematodes, and soil-borne pathogens. This procedure, however, has been completely ineffective in controlling Fusarium crown and root rot and in some cases severe losses have resulted in subsequent tomato crops (7). Although pathogenic *Fusarium* spp. traditionally have been considered soil-borne and not commonly dispersed in air (9), they have been reported in a few aerial trapping studies (17, 18, 26). Considering the well-known problem of reinfestation of soil after steaming (3, 14, 25), we decided to determine if the *F. oxysporum* responsible for crown and root rot of tomatoes is airborne in naturally infested greenhouses and, if so, to (i) determine which propagules are airborne and where they originate, and (ii) evaluate the importance of these propagules in reestablishing the pathogen in steam-treated soils. A preliminary report of this work has been published (19).

MATERIALS AND METHODS

Spore trapping. — Petri plates containing a Fusarium-selective agar medium developed by Komada (13) were placed randomly in four naturally infested commercial greenhouses at various heights from 0 to 1.5 m above the soil at a density of 500-700 plates per hectare. Plates were exposed for 2 hr, then returned to the laboratory to incubate for 5 days. Unless indicated otherwise, cultures throughout this study were incubated at laboratory ambient temperature (about 25 C). After incubation, 5- to 10-mm-diameter colonies, visually identified as *F. oxysporum,* were subcultured on potato-dextrose agar (PDA) slants for pathogenicity tests.

Pathogenicity tests. — Initial screening of nearly 1,000 isolates of *F. oxysporum* recovered from trap plates throughout this study was accomplished using a modification of a technique described by Sanchez et al. (20). A known pathogenic isolate of the fungus (F-1) isolated from a greenhouse tomato stem naturally infected with *F. oxysporum* was included as a control throughout these studies. Test isolates were grown on PDA slants for 7-10 days. Spores were then washed from slants with sterile distilled water and filtered through cheesecloth. The resulting suspensions (2×10^6 spores/ml) were almost exclusively microconidia with only a few macroconidia present. Thirty tomato seeds (cultivar Ohio MR-13) were soaked in sterile distilled water for 20-30 min and then in

spore suspensions for 1-2 min. The seeds were germinated on 2% water agar (10/plate) in petri plates incubated in the dark at 25 C for 3 days. Following germination, plates were moved to 20 C in constant light for 7-10 days to promote seedling growth and disease development. Suspected pathogens were selected for further testing according to the degree of stunting and necrosis which they produced on inoculated seedlings. Often, a distinct browning reaction could be seen in hypocotyl areas.

Pathogenicity tests were performed in the greenhouse with isolates that produced a positive reaction in the petri-plate test. Spore suspensions were prepared as described previously, and 6- to 8-cm-tall tomato seedlings (cultivar Ohio MR-13) grown in steam-treated soil were uprooted, root-dipped in these suspensions, and then replanted in steam-treated soil. Ten seedlings were planted per plastic tray (18 × 38 × 12 cm), with two trays per isolate. Plants were grown in a greenhouse at 17-22 C with a 12-hr, 100-200 hlux photo period. After 10 wk, plants were uprooted and sectioned to observe the basal, cortical browning reaction characteristic of Fusarium crown and root rot. Disease severity was rated using an index of 0 (no observable symptoms) to 3 (severe cortical browning).

Temperature studies.—Isolate F-1 was used to evaluate effects of temperature on growth of the pathogen and on disease development. Colonies were grown on PDA plates for 7-10 days. Mycelial-agar disks (5-mm in diameter) were cut from a colony with a cork borer and each was placed in the center of a fresh PDA plate. Plates were incubated in the dark at various temperatures from 8-35 C. Radial growth was measured daily for 1 wk on five replicate plates at each temperature. The experiment was done twice.

Four-wk-old Ohio MR-13 tomato seedlings were inoculated and planted as previously described. Two trays of 10 plants each were grown in controlled environment chambers set for a 12-hr photoperiod at constant temperatures of 20, 25, and 30 C. After 6 wk, plants were uprooted and examined as described previously. The experiment was done twice.

Identification of aerial propagules. — Petri plates containing a thin layer (about 2 mm thick) of Komada's agar were exposed in a naturally infested greenhouse for 1-2 hr. Plates were returned to the laboratory, incubated for 18-24 hr and then observed under a dissecting microscope at × 30. Microscopic colonies of *Fusarium* were selected by the characteristic right-angle branching pattern of young mycelia (Fig. 1-A). Sections of thin agar containing these colonies were aseptically removed, transferred to glass slides and observed under a compound microscope. Colonies arising from a single visible spore were photographed and then aseptically transferred to Komada's medium to continue growth. Conclusive identification of these isolates was accomplished by using the pathogenicity tests described previously.

Recolonization studies. — To test the ability of microconidia of *F. oxysporum* to recolonize freshly steamed soil, wooden flats (38 × 60 × 8 cm) were filled with 10 liters of moist soil collected from a commercial tomato greenhouse. Each flat was covered with aluminum foil and autoclaved for 5 hr. Ten ml of conidial suspension, prepared as previously described and adjusted to 10^2, 10^3, and 10^4 spores/ml were atomized on the surface of autoclaved soil after cooling to ambient temperature. Infested flats were incubated in a greenhouse at 17-22 C. Samples were taken daily from each flat by removing about 10 cc of soil with a sterile scoop from each of eight to ten scattered locations throughout the flat. Samples were thoroughly mixed and a 10-g subsample was removed, added to 90 ml of cold 0.1% water agar, and agitated on a vortex mixer for about 1 min. Two additional 10-fold serial dilutions were made as above and 1 ml of each dilution was pipetted onto a petri plate containing Komada's medium. Plates were air-dried in a sterile-air hood for about 30 min. and incubated for 5 days. At that time, Fusarium colonies, 3-5 mm in diameter, could be easily counted. The experiment was repeated several times.

The recolonization potential of chlamydospores formed in autoclaved soil in the studies just described also was tested. Chlamydospore concentrations in soil were determined by dilution plating. One g or 0.1 g, of colonized soil (about 5,000 chlamydospores/g) then were distributed evenly over the surface of freshly autoclaved soil in flats prepared as previously described. These flats were incubated and assayed as above in parallel studies with flats infested with microconidia.

Sources of air borne spores.—Plant materials commonly found in or around tomato greenhouses were tested as potential sources of microspores. Numerous samples of straw mulch, tomato stems, tomato leaves and fruits on the ground, and partially decomposed tomato vines from outside dump piles were collected. Samples were cut into 2- to 3-cm-long sections and incubated in petri dish moist chambers for 4-6 days. Each sample then was aseptically transferred to a test tube containing 5 ml of sterile distilled water, agitated on a vortex mixer, and 1 ml of the suspension was plated on Komada's medium. Isolates of *F. oxysporum* collected in this manner were subjected to the tomato seedling assay and greenhouse tests to verify pathogenicity.

RESULTS

Initial attempts to detect airborne propagules of *F. oxysporum* in a naturally infested, commerical tomato greenhouse were made in late October, when the tomato crop was mature and when numerous diseased plants were evident. Plates exposed for 2 hr contained about 40-50 *Fusarium* sp. colonies per plate after 5 days of incubation. Of 92 isolates of *F. oxysporum* recovered from air in the initial study, three were pathogenic in both petri dish and greenhouse tests. Considering the inefficiency of our trapping technique, this frequency (about 3%) indicates that the total number of airborne propagules of the crown and root rot pathogen present in the greenhouse must have been considerable. The pathogen also was easily isolated from naturally-infested soil, with propagule counts ranging from 100-3,000/g.

After initial air and soil sampling, all plants were removed from the greenhouse, the soil was rotovated, tarped, and steam-treated at 80-85 C for 4-6 hr. In an effort to eliminate airborne propagules of the pathogen, all interior surfaces of the greenhouse were sprayed with a 5-10% solution of formaldehyde with an air-blast orchard

sprayer (27, 28). Two wk after disinfestation, the pathogen could not be detected in either air or soil by random sampling. However, after 7 wk, the pathogen was detected again in the air, although the total number of *F. oxysporum* colonies averaged less than five per trap plate. Trapping at monthly intervals throughout the development of this crop showed a gradual increase in the number of airborne propagules of *F. oxysporum*. Maximum numbers, however, were only about 10% of those present at the end of the previous crop when the formaldehyde treatment had not been used. In trapping studies in three other naturally infested commercial greenhouses, pathogenic isolates of *F. oxysporum* also were recovered from air and soil. Of 320 isolates of *F. oxysporum* recovered from air throughout this study and subjected to both petri dish and greenhouse pathogenicity tests, 13 caused the crown and root rot disease.

Isolate F-1 of the pathogen grew maximally on PDA at 24-26 C, with rapidly declining growth above 31 C (Fig 2-A). In pathogenicity tests, symptoms were severe at 20 and 25 C, but only light to moderate at 30 C (Fig. 2-A). The observation that this disease is favored by relatively cool soil temperatures also has been made in Japan (29).

Microscopic examination of 18- to 24-hr-old colonies originating from propagules trapped from air showed that they developed from microconidia averaging 4 × 9 μm (Fig. 1-B, C, D). In a few cases, germinating *Fusarium* macroconidia were found on trap plates, but isolates originating from these propagules were never *F. oxysporum*.

In recolonization studies, propagule concentrations in autoclaved soil artifically infested with microconidia increased on the average by 10^3 after 3 days and 10^4 after 1 wk (Fig. 2-B). Two wk after infestation, propagule concentrations had stabilized at about 1,000/g soil, regardless of initial inoculum concentration. Microscopic examination revealed that these propagules were exclusively chlamydospores averaging 5-7 μm in diameter. Chlamydospores produced in this manner and reintroduced onto freshly autoclaved soil remained at the same concentrations 3 wk after introduction, while propagule

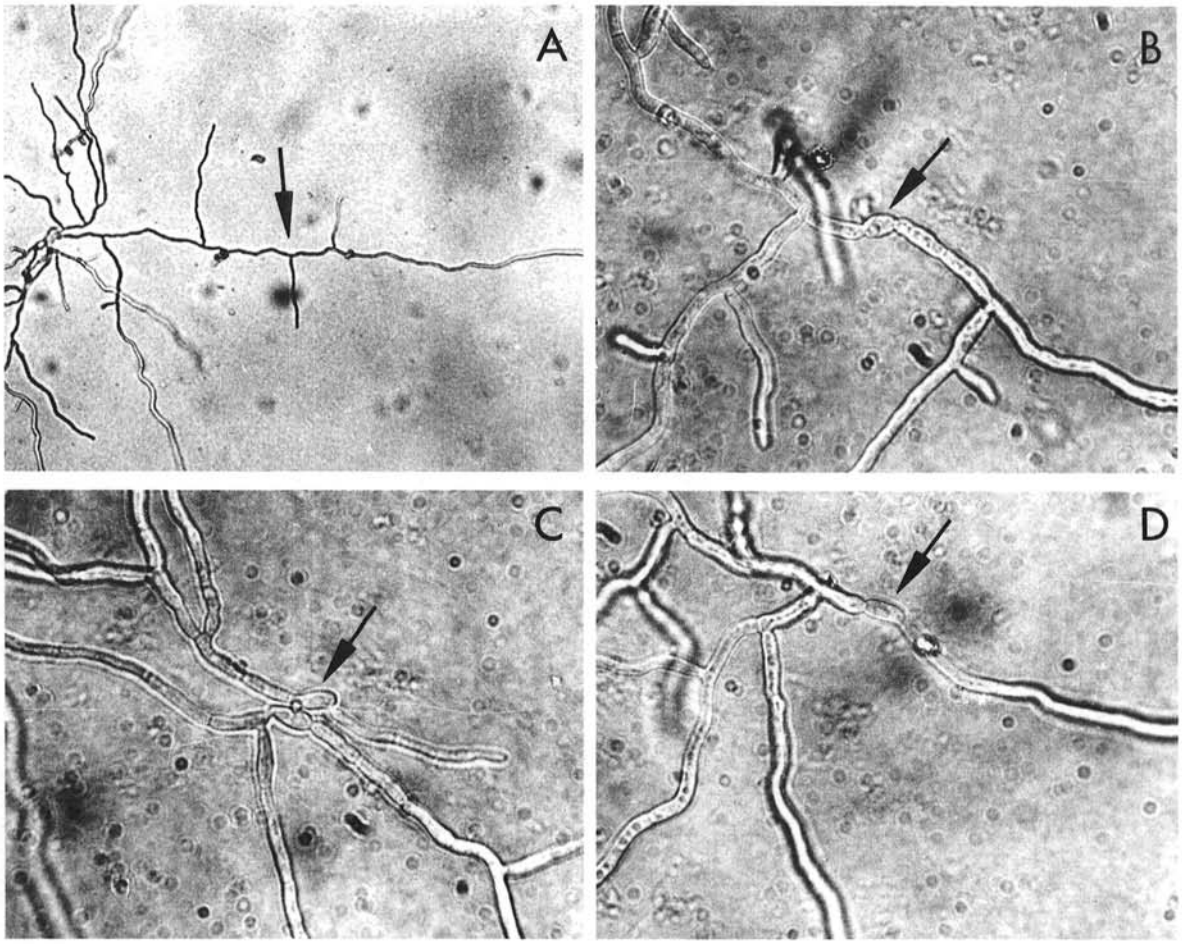

Fig. 1-(A to D). **A)** Hyphae of *Fusarium oxysporum* on Komada's agar medium (13). Right-angle branching pattern of young hyphae 18-24 hr after microconidia were trapped from air in an infested greenhouse (× 125). **B, C, D)** Germinating microconidia (arrows) 18-24 hr after being trapped as above (× 625).

concentrations in parallel flats amended with microconidia increased rapidly as previously shown.

Collections of plant material and mulch from naturally infested commerical greenhouses showed that pathogenic isolates of *F. oxysporum* were recovered easily from microconidia formed on basal stems of infected tomato plants and on straw mulch, and partially decomposed tomato vines in outside dump piles.

DISCUSSION

The failure of steam-treatment of soil to control Fusarium crown and root rot of greenhouse tomatoes was due primarily to recontamination of freshly steamed soil by airborne microconidia of the pathogen. Although these propagules are present in relatively low numbers in terms of total airspora, especially after disinfestation with formaldehyde, they can produce localized areas of high inoculum concentrations where they settle because of their ability to rapidly recolonize freshly steamed soil. This explains the observation of many growers that the disease often was more severe after steaming, and that the pattern of diseased plants in the greenhouse had no apparent relationship to that observed in the previous crop. Although propagules of *F. oxysporum* buried below the influence of steam treatment also may cause infection, as in the case of Fusarium wilt (6), they probably are not of major importance in this disease. This fungus is not a vascular invader but a root- and crown-rot pathogen that attacks cortical areas. As such, significant infection results from inoculum located in the upper 20-30 cm of soil.

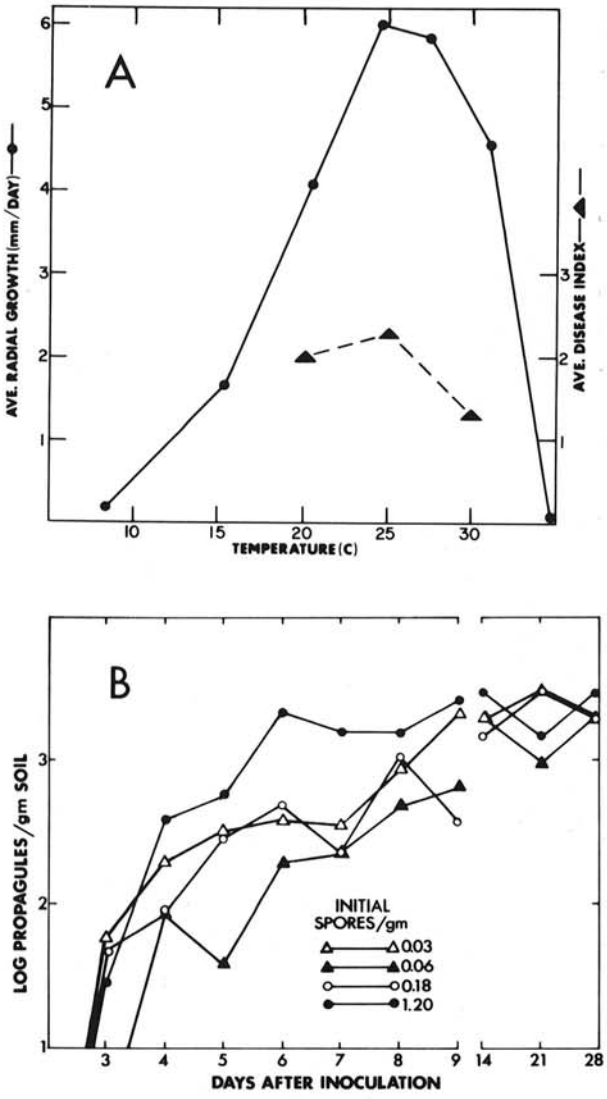

Fig. 2-(A, B). A) Average radial growth rate of *Fusarium oxysporum* (F-1) on potato-dextrose agar and average disease index of Fusarium crown and root rot on Ohio MR-13 tomato at various temperatures. **B)** Propagule concentrations of *Fusarium oxysporum* at 1-day intervals after infestation of autoclaved soil with microconidial suspensions of varying concentrations.

As other workers have reported, *Fusarium* micro- or macroconidia germinate readily on steam-treated soil and grow rapidly on nutrients released during the steaming process (1, 14, 16). When these nutrients are exhausted, the mycelium converts to chlamydospores (1, 8, 16) that then germinate only in the presence of host root exudates or other suitable nutrients (21). Our studies concur with this pattern of development and implicate airborne microconidia formed on organic debris as the cause of reinfestation. Chlamydospores in airborne dust or dispersed mechanically, however, appear ineffective at recolonization of freshly steamed soils. Because of this potential for reinfestation, sanitation immediately after soil steaming is crucial to the control of this disease. Chemical or biological amendments added to soil to prevent recolonization by *F. oxysporum* also may prove useful. This approach is currently being investigated.

The pathogen responsible for crown and root rot of greenhouse tomatoes has been considered a new race of *F. oxysporum* f. sp. *lycopersici* by Yamamoto et al. (29) becuase its host range is limited to tomato and because it is pathogenic to lines resistant to races 1 and 2. In the past, race designation in *F. oxysporum* and other fungi has been based solely on differential cultivar reactions (2, 23, 24). This pathogen, however, has a lower optimal temperature for pathogenicity than f. sp. *lycopersici* and causes a cortical and root rot rather than a vascular wilt. On this basis, we agree with Jarvis et al. (10, 12) that it should be considered a distinct forma specialis from the wilt pathogen and should not be referred to as a race of f. sp. *lycopersici*.

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