Control of Fusarium Crown and Root Rot of Greenhouse Tomatoes by Inhibiting Recolonization of Steam-Disinfested Soil with a Captafol Drench

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ABSTRACT

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Of six fungicides applied to freshly steamed soil, captafol was most effective in inhibiting recolonization by *Fusarium oxysporum*. Soil drenches of captafol at concentrations as low as 200 μg/ml active ingredient kept the soil pathogen-free for 3 wk. The fungicide remained efficaceous when applied to soil as hot as 90 C but it was inactiviated by autoclaving. Captafol was completely inhibitory to mycelial growth and chlamydospore and microconidial germination of *F. oxysporum* in vitro at concentrations of 25, 2.5, and 0.5 μg/ml, respectively. Field tests in commercial tomato greenhouses demonstrated that application of captafol to freshly steamed soil provided economic control of Fusarium crown and root rot.

A new disease of greenhouse tomatoes (*Lycopersicon esculentum* L.) caused by *Fusarium oxysporum* (Sacc.) Snyd. & Hans. was found in northern Ohio in 1974 (4). The disease has been named Fusarium crown and root rot (4, 6). A similar disease was reported from Japan in 1974 (12) and also from Ontario, Canada in 1975 (3, 6). In 1976, a survey in Ohio revealed that approximately 32 hectares were infested which represented about half of the area grown to tomatoes under glass in the state.

Initial control efforts were oriented toward limiting spread of the organism to other greenhouses by use of sanitation and quarantine procedures; these were unsuccessful. Soil steaming through buried tiles (a standard and generally effective control practice for soilborne pathogens in tomato greenhouses) also failed even though growers increased steaming time and doubled the number of steamings per year (4, 6). Resistant commercial cultivars are not available (4, 5) and the disease has not been controlled by any conventionally applied fungicides.

When aerial microflora were sampled, microconidia of the pathogen were found in infested greenhouses. These propagules rapidly recolonize freshly steamed soil, which accounts for the failure of soil steaming to control the disease (10). Because annual soil steaming is an integral part of disease, insect, and weed control programs throughout the Ohio greenhouse tomato industry, and yet was not capable of controlling the disease alone, research was initiated to investigate the efficacy of fungicide drenches as a means of protecting freshly steamed soil from recolonization by *F. oxysporum*.

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MATERIALS AND METHODS

Fungicide drench experiments.—Six fungicides commonly used on vegetable crops were tested to determine whether any would inhibit recolonization of freshly steamed soil by *F. oxysporum*. The test fungicides were: benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate; Benlate 50 WP]; captafol [cis-*N* (1,1,2,2-tetrachloroethyl) thio-4-cyclohexene-1,2-dicarboximide; Difolatan 4F]; chlorothalonil [tetrachloroisophthalonitrile; Bravo 6F]; dyrene [4,6-dichloro-*n*-(2-chlorophenyl)-1,3,5-triazin-2-amine; Dyrene 50 WP]; fentin hydroxide (triphenyltinhydroxide; Duter 47.5 WP), and mancozeb (a coordination product of zinc ion and manganese ethylenebisdithiocarbamate; Dithane M-45 80 WP). Wooden flats (20 × 40 × 6 cm) filled with 3 liters of moist, sandy-loam soil were covered with aluminum foil and autoclaved for 6 hr to simulate commercial steaming procedures in tomato greenhouses. Flats were incubated in a greenhouse at 20-25 C and allowed to cool overnight before the foil was removed. A 300-ml aqueous suspension (750 μg/ml active ingredient) of each test fungicide then was sprayed with a hand-pumped pressure sprayer on three replicate flats per treatment. One day after treatment, all soils were infested with *F. oxysporum* by atomizing 10 ml of a suspension of microconidia (100 conidia/ml) over each flat. Microconidia were obtained by washing them from the surface of 8- to 10-day-old cultures grown at 22-25 C on potato-dextrose agar (PDA) slants.

Soil populations of *F. oxysporum* were monitored daily, beginning 1 day after infestation. Samples were taken by removing approximately 10 cc of soil with a sterile scoop from each of six to eight scattered locations throughout each flat. After thorough mixing, 10 g were

removed from each sample, added to 90 ml of cold 0.1% water agar and agitated on a Vortex mixer for approximately 1 min. Two additional similarly prepared 10-fold serial dilutions were made, then 1 ml of each dilution was pipetted onto a petri plate containing Komada's medium (7, 8). Plates were dried with the lids ajar on a laminar-flow air bench for about 30 min and incubated for 5 days at 22-25 C. At that time colonies of *F. oxysporum* (3 to 5 mm in diameter) could be easily counted.

One to 2 wk after infestation, ten 2-wk-old tomato seedlings (cultivar Ohio M-R 13) were transplanted into each flat. After 8 wk in the greenhouse at 17-22 C with a 12 hr, 100-200 hlx photoperiod, each plant was uprooted. The tap root was sliced longitudinally and rated for cortical browning on a scale of 0 (no observable symptoms) to 3 (severe discoloration).

In vitro tests with captafol.—The effect of captafol on mycelial growth and microconidial and chlamydospore germination by *F. oxysporum* was tested in vitro. Petri plates were prepared by mixing captafol with PDA (after autoclaving) to concentrations of 0.1-800 μg/ml active ingredient. Agar disks (5 mm diameter) were cut with a No. 2 cork borer from the margins of colonies of *F. oxysporum* grown for 5-10 days on PDA. A single disk was placed in the center of five PDA-captafol plates of each concentration and incubated in the dark in plastic bags at 25 C. Radial growth was measured after 6 days. All experiments were repeated at least three times.

Microconidia of *F. oxysporum* were washed from 8- to 10-day-old PDA slant cultures grown at 22-25 C. Aqueous captafol suspensions (1 ml) were mixed with 1 ml microconidial suspensions (1.4×10^6 conidia/ml) in sterile test tubes. Tubes were incubated for 24 hr at 22-25 C. After incubation, a sample was removed from each tube with a micropipette and examined microscopically at a magnification of ×400. The number of germinated conidia in each of five randomly selected microscopic fields was counted for each sample.

Chlamydospores were produced by infesting autoclaved soil with a conidial suspension of *F. oxysporum* and allowing the soil to air dry slowly in a glass jar on the lab bench for 2-3 mo. The resultant chlamydospore population was determined by dilution-plate assay on Komada's medium (7). Petri plates containing PDA amended with various concentrations of captafol were prepared as before. Soil containing chlamydospores was suspended in 0.1% water agar at concentration of 30-40 chlamydospores/ml. One ml of this suspension was added to five plates of each concentration of captafol. Plates were dried with the lids ajar on a laminar-flow air bench for about 30 min and incubated at 22-25 C for 4-5 days, after which colonies resulting from viable chlamydospores could be counted.

Field trials.—In December of 1976, two commercial growers who experienced heavy losses from the disease the previous season agreed to test the captafol drench treatment in their naturally-infested greenhouses. The previous tomato crop was removed and the soil was rototilled and covered with plastic tarps. Both houses were equipped with steam tiles buried 30-35 cm below the soil surface. Tarped beds were steam-treated for 4-6 hr at 80-85 C. Immediately after tarp removal, captafol was drenched onto the soil surface through permanent overhead irrigation lines at the rate of 56 liters/hectare (20 liters active ingredient/hectare) in 6-12 mm of irrigation water. Approximately 1 hectare was treated at each location. Untreated check plots were not included in these tests because, with the application equipment available, it was impossible to leave small areas undrenched and growers were not willing to leave large areas untreated. Approximately 8-wk-old tomato seedlings (cultivar Ohio M-R 13) were transplanted into the treated soil 1-2 wk after fungicide application. Progress of the crop and the disease was monitored at monthly intervals until July 1977, when approximately 1% of the plants were pulled systematically and root systems were sectioned and examined for cortical browning.

RESULTS AND DISCUSSION

Initial fungicide drench experiments showed that captafol completely inhibited steamed soil recolonization by *F. oxysporum* and kept tomato seedings disease free when subsequently planted into treated soil (Table 1). Two other compounds, dyrene and fentin hydroxide, also provided significant disease control in these tests but allowed some recolonization of soil by the fungus (Table 1). Repeated testing of these fungicides gave similar results leading to further studies with captafol alone. A serial dilution experiment with captafol showed that soil drenches at concentrations as low as 200 μg/ml active ingredient completely inhibited soil recolonization for 3 wk (Fig. 1).

Because of concern expressed by growers as to the practicality of applying a drench treatment after steaming, efficacy of pre-steaming captafol applications was tested. Two identical sets of flats were prepared and treated with captafol drenches ranging from 25 to 800 μg/ml. Soil in one set was treated prior to autoclaving and the soil in the other set was treated after autoclaving

TABLE 1. Effect of fungicide drenches on recolonization of autoclaved soil and on infection of tomato seedlings by *Fusarium oxysporum*

Fungicide drench[a] (750 μg a.i./ml)	Avg. Fusarium propagules/gm soil[b] (log N + 1) (6 days)	Avg. disease index[c] (8 wk)
Benomyl	1.67 bc	1.1 cd
Captafol	0.00 a	0.1 a
Chlorothalonil	2.51 cd	0.7 bc
Dyrene	1.06 b	0.4 ab
Fentin hydroxide	1.83 bc	0.1 a
Mancozeb	3.34 d	3.0 d
Untreated control	3.06 d	3.0 d

[a]Applied with a hand-pumped pressure sprayer to the surface of freshly autoclaved soil at a rate of 300 ml/3,000 cc soil.

[b]Assayed by dilution plate method on Komada's selective medium (7, 8) [KOMADA, H. 1975. Rev. Plant Protect. Res. (Tokyo) 8:114-125; KOMADA, H. 1976. Proc. Am. Phytopathol. Soc. 3:221 (Abstr.), respectively]. Small letters within a column indicate Duncan's multiple range groupings of treatments that do not differ significantly ($P = 0.05$).

[c]Cortical rot rated on a scale of 0 = no disease to 3 = severe discoloration.

as a control. The ability of captafol to prevent soil recolonization by *F. oxysporum,* as measured by soil dilution plate assay, was eliminated by autoclaving, thus precluding pre-steaming use. Another test was performed to determine if soil temperature at the time of post-steaming application affected efficacy. Captafol drenches (750 μg/ml active ingredient) were applied as before to flats of freshly autoclaved soil cooled to 50, 70, 80, and 90 C. In all cases the fungicide remained efficaceous as indicated by complete inhibition of recolonization of the treated soil by *F. oxysporum.*

To evaluate toxicity of captafol to tomato seedlings, flats were drenched with captafol at concentrations up to 8,000 μg/ml active ingredient.

Then 2-wk-old tomato seedlings (cultivar Ohio M-R 13) were transplanted into each flat and observed under greenhouse conditions. After 5-8 wk, plants at concentrations of 1,000 μg/ml or greater were somewhat stunted as compared with control plants. No phytotoxicity was detected at concentrations of less than 1,000 μg/ml. A similar experiment was performed comparing captafol-treated soil with a commercial peat-vermiculite mix (Jiffy Mix, Jiffy Products of America, W. Chicago, IL 60185) treated in the same manner. No differences in phytotoxicity reactions were seen between the two growth media.

In vitro studies showed that captafol was highly inhibitory to mycelial growth as well as to microconidial and chlamydospore germination (Fig. 2). Microconidia, the main propagule involved in recontamination of soil by this pathogen (10), failed to germinate at captafol concentrations of 0.5 μg/ml active ingredient or higher.

Field tests with the captafol drench technique were successful at both locations. Growers experienced no difficulties in fungicide application after steaming, nor any adverse effects on plant growth. Wilted plants were not observed until April 1977, when a few plants at each location showed typical Fusarium crown and root rot symptoms. In both cases, most wilting plants were in distinct groups, probably indicating inadequate steaming or fungicide application in those areas, or perhaps survival of the pathogen in thick, woody tap roots remaining in the soil. To test the latter possibility, heavily infected tap roots were buried in greenhouse soil beds at depths from 3-30 cm. The soil then was tarped and steamed with the temperature at 15- and 30-cm depths

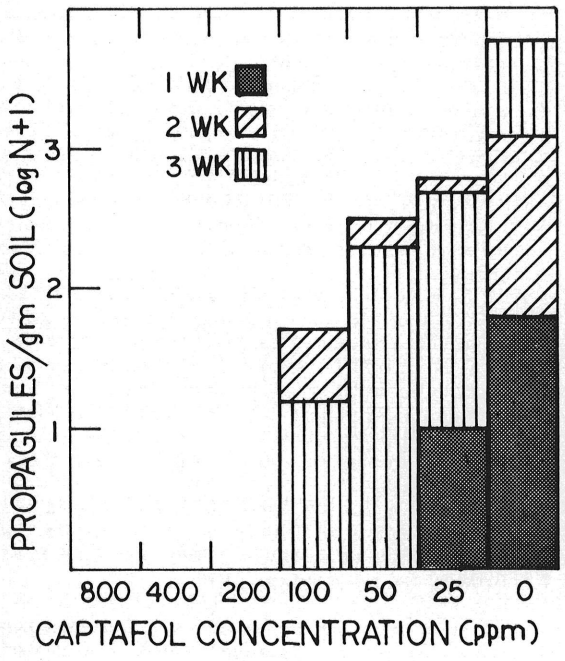

Fig. 1. Effect of captafol drench rates in autoclaved soil on recolonization of the soil by *Fusarium oxysporum* as measured weekly after introduction of the fungus.

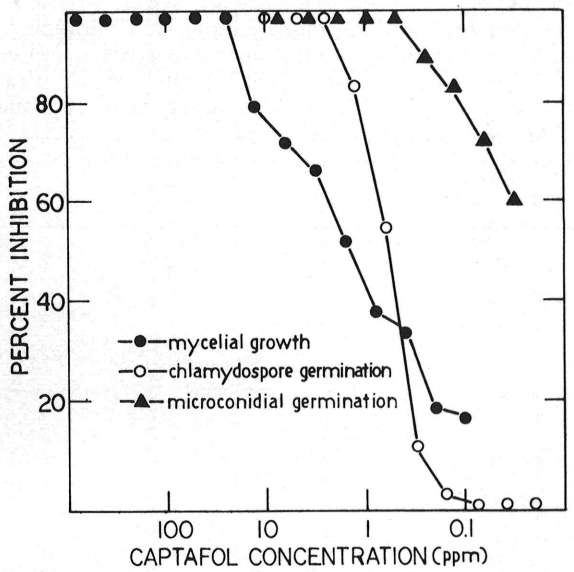

Fig. 2. Effect of the fungicide captafol on in vitro growth and spore germination by *Fusarium oxysporum.*

TABLE 2. Comparison of captafol drench treatment (1977) and no treatment (1976) on the effects of Fusarium crown and root rot (caused by *Fusarium oxysporum*) on tomato production in two commercial greenhouses in Cleveland, Ohio

Location	Visibly affected plants observed in May		Root infection at end of season (July)		Total yield	
	1976 (%)	1977 (%)	1976 (%)	1977 (%)	1976 (kg/m²)	1977 (kg/m²)
A	90	5	99	30	6.9	18.2
B	85	5	85	40	12.7	15.3

remaining above 98 C for over 6 hr. Although most root systems were sterile after steaming, the pathogen was isolated occasionally, indicating a potential source of recontamination.

Approximately 30% of plants pulled at the end of the season in July had some cortical discoloration of the tap root. Although the disease was not completely prevented by the treatment, commercially acceptable tomato yields were harvested at both locations (Table 2). The yield at location B, however, was still somewhat low due to horticultural factors. Growers often are unaware that their tomato plants are infected until early spring when plants first wilt under a heavy fruit load on sunny days. Infection begins months earlier, however, probably soon after transplants are set into infested soil. The captafol drench treatment apparently keeps the initial inoculum concentration in treated soil at levels low enough to allow transplants to develop vigorous root systems that can support heavy fruit loads. Although some infection of roots still may occur later in the season, the plant has become well established by this time.

The problem of recolonization of steam-disinfested or fumigated soils is commonly recognized (2, 9, 11). Attempts to control recolonization by the addition of soil amendments or antagonists have been reported (1, 2, 9). The principle of the treatment described in this report is the use of a fungicide as a chemical "screen" on freshly steamed soil, allowing much natural recolonization to occur but inhibiting re-establishment of a pathogen (Fig. 3). Three to 4 wk after treatment, many bacteria as well as fungi, such as *Mucor* sp., *Neurospora* sp., and *Trichoderma* sp. were easily isolated from commercially treated soil. Captafol has shown little or no activity against bacteria (L. E. Heidrick, Chevron Chemical Co., San Francisco, CA 94119 *personal communication*), so should not directly affect recolonization by that component of the soil microflora. It has a wide range of activity against fungi, however, and its effect against nontarget fungi in this case is not known. The treatment provides economic control of Fusarium crown and root rot in greenhouse tomatoes and has no adverse effects on crop development. This principle may also have application in other situations where it is necessary to protect steam-treated or fumigated soil from recolonization by pathogenic microorganisms.

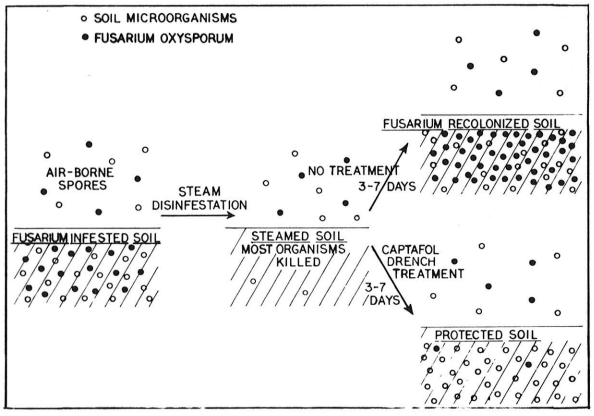

Fig. 3. Schematic outline of the principle of control of Fusarium crown and root rot (caused by *Fusarium oxysporum*) using a captafol drench treatment on freshly steamed soil.

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