

**A Rapid Technique for Identifying the Clones of
Fusarium oxysporum f. sp. *lycopersici*
Causing Crown- and Root-rot of Tomato**

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ABSTRACT

A petri-plate technique has been developed that will distinguish the race of *Fusarium oxysporum* f. sp. *lycopersici* which causes crown- and root-rot of tomato from those that cause wilt. Isolates of the crown- and root-rot race caused a dark-brown, crown-girdling lesion on seedlings following inoculation of seeds placed on water agar containing either 0.5 ml of Schizophyllum minimal media or propylene-oxide treated carnation leaves. In contrast, the *Fusarium* wilt fungus produced a light-brown discoloration of the entire primary root. These same differences were manifested whether stem pieces from infected tomato plants or spore suspensions from pure cultures of each fungus were used as a source of inoculum.

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A disease of tomato (*Lycopersicon esculentum* L.) caused by an apparently new race of *Fusarium oxysporum* Schlecht f. sp. *lycopersici* (Sacc.) has been described from Japan (5) and southern California (1). The fungus causes crown- and root-rot, and a vascular discoloration of the roots, crown, and basal portion of the stem. Diseased plants became slightly stunted, their leaves became chlorotic, and in terminal stages the plants wilt and die. In Japan, the disease has been reported only on greenhouse tomatoes grown during winter in cold soils (10-15 C). The optimum temperatures for the growth of tomato is between 24 and 31 C. Since soil temperature experiments indicate that the optimum temperatures for disease development is between 10 and 20 C (5), the crown- and root-rot disease probably will be limited to situations in which tomatoes are grown at sub-optimal temperatures. Greenhouse tests in California indicated that, of the tomato cultivars tested that are resistant to races 1 and 2 of the tomato wilt fungus, all are susceptible to the new crown- and root-rot race. This paper reports a rapid laboratory technique to differentiate clones of *F. oxysporum* that cause crown- and root-rot from those that are saprophytic and from those that cause wilt.

Experiments were conducted with isolates of the tomato crown- and root-rot fungus (X-5) and race 1 of the tomato wilt fungus (No. 212) that were grown on V-8 and potato dextrose agar media, respectively. Control seeds were inoculated with either a saprophytic clone of *F. oxysporum* or a pathogenic clone of *F. oxysporum* f. sp. *asparagi* or *F. oxysporum* f. sp. *niveum*. Seeds of Bonny Best tomato were surface sterilized for 30 minutes with a 1.0% solution of sodium hypochlorite. Conidia were

washed in three changes of distilled water and seeds or seedlings were inoculated with either a cell suspension of 1×10^4 conidia/ml, with an agar plug bearing the fungus, or with surface-sterilized stem sections infected with either the wilt or crown rot pathogen. Ten to fifteen seeds were placed in a petri plate containing either sterilized washed sand, water agar (15 ml), water agar plus 0.5 ml Schizophyllum minimal medium (3), or water agar containing two propylene oxide-treated carnation leaves (approximately 2.5 cm long). The carnation leaves were used because Toussoun and Nelson (4) reported that species of *Fusarium* produced their characteristic fruiting structures on that medium, which permitted ready identification. The plates were pre-germinated in the dark in an incubator set at 25 C and then incubated under fluorescent lights [11,102 lx (1,200 ft-c)] in a room where temperatures were 20 ± 2 C.

Three to 7 days after seeding and inoculation of the seedlings the initial symptoms of crown-rot appeared on the root-stem transition region or crown in the form of a dark-brown lesion which enlarged and girdled the entire crown (Fig. 1). The lesion frequently involved a portion of the primary root as well. Occasionally, the fungus caused discrete lesions on the cotyledons and hypocotyl. The saprophytic clone of *F. oxysporum*, and the clones of the wilt-inducing races of *F. oxysporum* which attack asparagus and watermelon, did not cause infection in tomato. However, the clone of the tomato wilt fungus caused a light tan discoloration of the entire primary root in 10-14 days. Both washed and unwashed conidia of the crown-rot fungus behaved similarly, causing a similar amount of infection and disease symptoms. Conidial inoculation of seeds resulted in uniform infection and disease severity of the seedlings. However, inoculation of seeds or seedlings by placing a single source of inoculum at the center of the petri plate resulted in an uneven rate of infection and occasional escapes. This was especially apparent when either sand or water agar was used as a substrate for the seedlings, but not when the water agar was supplemented with either carnation leaves or with Schizophyllum minimal media. The uneven rate of fungal spread and infection was eliminated in the water agar or sand plates if the inoculum was placed at three loci instead of one.

Water agar fortified with carnation leaves or with 0.5 ml of Schizophyllum minimal media was finally adopted as the best substrate for the tomato seeds. Tomato seeds rather than tomato seedlings are used as indicator hosts because the tomato seedlings are still very young and vigorous when infection occurs, and because inoculation and seeding of the plate can be accomplished at the same time. The carnation-leaf water agar medium was used to screen isolates for pathogenicity that were recovered from diseased field or greenhouse plants by plating diseased sections on the *Fusarium*-specific media of Nash and Snyder (2). Water agar fortified with the Schizophyllum minimal medium is used to screen for the pathogenicity of chemically-induced mutants and heterokaryons of the crown- and root-rot fungus.

Clones of the fungus that caused a crown- and root-rot of seedlings in the laboratory also caused a crown- and root-rot of tomato plants in the greenhouse when soil temperatures did not exceed approximately 25 C.

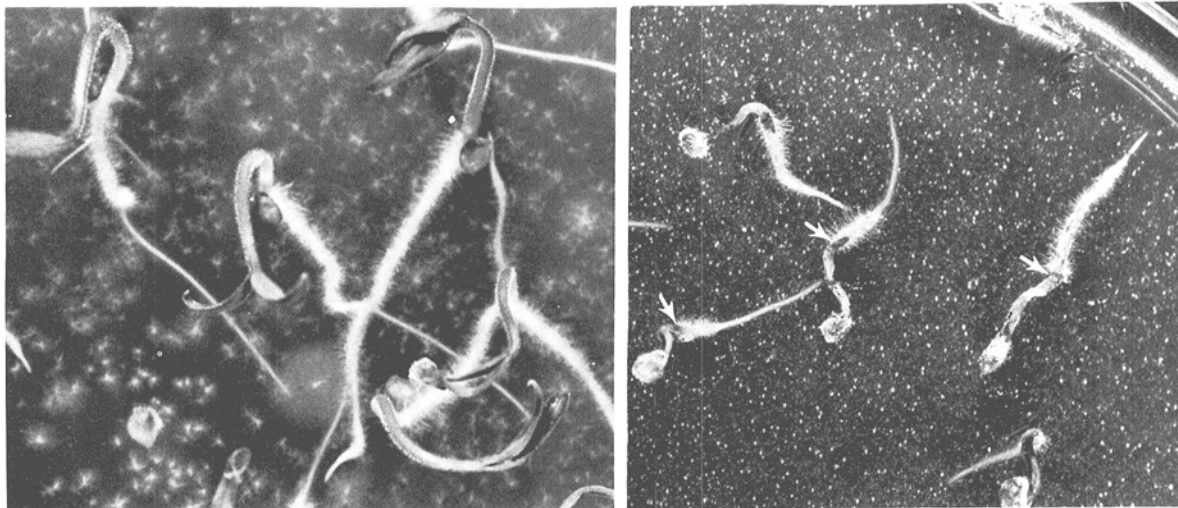


Fig. 1. Healthy (left) and crown- and root-rot affected Bonny Best tomato seedlings 5 days after seeding and inoculation (right). Note the lesion (arrows) affecting the crown and upper portion of the hypocotyl.

Greenhouse plants were grown in a sterile UC mix and were inoculated when 15 to 20 cm tall by injecting 5-10 cc of a spore suspension (1×10^5 cells/ml) cultured on V-8 medium around the crown and roots of the plants. Since the crown- and root-rot fungus characteristically attacks these regions of the tomato seedlings 3 to 7 days after seeding, the test provides a rapid diagnostic screening technique for the new race of the fungus.

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