Biological Control of Fusarium Crown Rot of Tomato Under Field Conditions

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ABSTRACT

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Conidial suspensions of five fungal antagonists of *Fusarium oxysporum* f. sp. *radicis-lycopersici* were applied to the roots and crowns of tomato transplants at the time of planting. The suspension contained 5×10^5 conidia of each of three isolates of *Trichoderma harzianum*, one isolate of *Aspergillus ochraceus*, and one isolate of *Penicillium funiculosum*. The pathogen was added at the time of planting to soil 10 cm from the transplant as 0, 50, 500, and 5,000 chlamydospores per plant in 20 ml of water. The incidence of disease increased as the inoculum density of the pathogen was increased in fumigated soil not augmented with the antagonists; disease incidence, however, did not increase as the inoculum density was increased in fumigated soils that were augmented with the antagonists. At 5,000 chlamydospores of the pathogen per plant, disease incidence at harvest was 7% in soils augmented with antagonists and 37% in nonaugmented soils. The pathogen population decreased from 600 to 200 propagules per gram in soil augmented with antagonists, but increased from 1,000 to over 5×10^4 propagules per gram in nonaugmented soils. Yield was not affected significantly by treatment or planting date.

Fusarium crown rot of tomato (*Lycopersicon esculentum* Mill.) was first reported in south Florida during the 1974–1975 growing season (13). Attempts to control the disease with chemicals and host resistance were unsuccessful (10). At present, the only effective control measure is the application of a captafol drench to greenhouse beds immediately after steaming (10). The captafol drench selectively inhibits recolonization of the soil by the pathogen, *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis and Shoemaker. When captafol was applied as a preplant or postplant drench to the transplant hole under south Florida field conditions, the estimated yield was slightly higher, but there was a 1 wk delay in plant maturation (14). Furthermore, a complete soil drench of the entire bed is not practical for tomato production in south Florida because a plastic mulch is maintained during the entire growing season.

The possibility of obtaining disease control with biological agents was investigated. It was hypothesized that if soil was augmented with antagonists that decreased the rapid saprophytic development of the pathogen during the early stages of soil recolonization, less infection would occur and the severity of the epidemic would be reduced. In growth-chamber and greenhouse experiments, infection incidence and mean lesion length on tomato plants were reducd when antagonists were added to fumigated soil (7).

The purpose of this study was to test the potential of antagonists to control Fusarium crown rot of tomato under field conditions.

MATERIALS AND METHODS

The field used for the experiments during the 1979–1980 season was located near Delray, FL, and contained Pompano fine sand with a pH of 4.5 (measurement obtained from a 1:2 suspension of soil in 0.01 M $CaCl_2$). The soil was fumigated with methyl bromide-chloropicrin (67:33, v/v) at 1 kg of fumigant to 20 m^2 of soil injected approximately 20 cm below the soil surface. Plastic mulch (0.25 mm thick) was placed over the bed immediately after injection of the fumigant. Each bed was 1 m wide and the beds were separated by 1-m access rows that were not fumigated. Two weeks after fumigation, 5-wk-old tomato (cultivar Walter) transplants were planted approximately 30 cm apart in two rows which were about 50 cm apart on each bed. Subsurface irrigation was maintained at approximately 40 cm below the surface and other cultural practices were similar to those employed in the area.

Tomatoes had not been grown previously in the field, and the pathogen was not detected by soil dilution plating on a selective medium (5) before planting. This situation allowed the establishment of field plots with defined initial inoculum levels of both the pathogen and the antagonists. The pathogen was added to the soil by injecting a total of 20 ml of a suspension of chlamydospores under the plastic mulch at opposite sides of the transplant, 10 cm from the transplant hole. Macroconidia of the pathogen were incubated at 28 C for 4 wk at 10^6 macroconidia per milliliter in sterile deionized water to induce chlamydospore formation (7). The treatments included infestation of soil with pathogen populations of 0, 50, 500, or 5,000 chlamydospores per plant with or without the addition of antagonists.

The composite of antagonists consisted of three isolates of *Trichoderma harzianum* Rifai, one isolate of *Penicillium funiculosum* Thom, and one isolate of *Aspergillus ochraceus* Wilhelm. The antagonists were selected for ability to increase rapidly in freshly fumigated soil, to occupy the root environment of the host, and to increase the ratio of inoculum density to infection incidence under growth-chamber conditions (7). One day before use, conidia of each antagonist were harvested from petri plates containing 14-day-old cultures grown on potato-dextrose agar (Difco, Detroit, MI 48201) at 25 C under 2,000 lux of fluorescent light. The soil was augmented with the antagonists by pouring 25 ml of a suspension containing 10^4 conidia per isolate per milliliter over the roots of each transplant after it was positioned in the transplant hole and then adding another 25 ml over the crown of the transplant immediately after the roots were covered with soil.

Soil-dilution plating techniques were used to monitor populations of the pathogen and antagonists during the growing season. Soil samples were obtained by preparing composite samples of 5-g subsamples from the crown areas of five plants from

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each plot. Komada's (5) medium, which is selective for *F. oxysporum*, was used to isolate the pathogen from soil dilutions of 1:25, 1:100, or 1:1,000 (w/v). The isolates of *F. oxysporum* f. sp. *radicis-lycopersici* from soil dilutions and plant tissue were identified by the technique of Sanchez et al (12), in which the type of lesion on tomato seedlings grown in pathogen-infested water agar is used to differentiate the isolates of the pathogen from nonpathogenic or wilt-inducing *F. oxysporum* isolates. The population densities of the antagonists were monitored by plating soil dilutions of 1:25, 1:100, and 1:1,000 (w/v) in water on potato-dextrose agar which contained 1 ml of Tergitol NPX (Sigma Chemical Co., St. Louis, MO 63178) and 50 mg of chlortetracycline hydrochloride (Sigma Chemical Co.) per liter of medium. Plates were stored at 25 C and 12 hr of fluorescent light (2,000 lux) per day for 7 days before examination for fungal colonies.

A standard hygrothermograph was used to monitor the highest and lowest daily temperatures during the growing season.

The effect of the treatments on yield and disease incidence were determined. Yield data were obtained by harvesting all of the fruit that were past the mature green stage of ripeness each week for 4 consecutive weeks. Fruit weight and number were recorded for each plot. Disease incidence, as determined by the presence of lesions on the crown and lower stems of the plant, was determined at the last harvest date. Sections of the stems at the edges of the lesions were plated on Komada's (5) medium to isolate *F. oxysporum*. The isolates were identified as *F. oxysporum* f. sp. *radicis-lycopersici* by the method of Sanchez et al (13).

A randomized, complete-block design with eight treatments replicated five times was used. There were 20 plants in each plot. The entire experiment was conducted in the field three times at 2-wk intervals.

RESULTS

The incidence of Fusarium crown rot of tomato was affected by the initial inoculum density of *F. oxysporum* f. sp. *radicis-lycopersici*, treatment with antagonists, and the time of planting (Table 1). The increase in disease observed during the third planting as compared to the first and second plantings was associated with cooler prevailing temperatures, a phenomenon reported previously (4). The mean of the lowest temperatures was 12, 11, and 10 C during the first, second, and third planting times, respectively. Yield was not significantly affected by treatments or planting dates.

TABLE 1. Effect of initial inoculum density of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and a composite of five fungal antagonists on the incidence of Fusarium crown rot of tomato under field conditions

Inoculum density (chamydospores per plant)[w]	Antagonists	Plants with lesions (%)			
		Planting date[x]			
		1	2	3	Mean
0	−	0.0	0.0	0.0	0.0
50	−	5.7	8.1	14.7	9.5
500	−	0.0	17.5	24.3	13.9
5,000	−	15.0	42.8	53.9	37.2
Means		5.2 a[y]	17.1 a	23.2 a	15.1 a
0	+[z]	0.0	1.0	1.2	0.7
50	+	5.1	5.2	6.7	5.7
500	+	3.8	1.3	8.6	4.6
5,000	+	1.7	5.4	14.3	7.1
Means		2.6 a	3.2 b	7.7 b	4.5 b

[w] Twenty milliliters of a chlamydospore suspension was injected into the soil 10 cm from the plant.

[x] Planting dates 1, 2, and 3 were 5 September, 19 September, and 13 October 1979.

[y] Means in same column followed by different leters differ significantly ($P = 0.05$) as determined by t test; data analyzed after transformation to arcsine $\sqrt{x}$.

[z] Conidia from 14-day-old cultures grown on potato-dextrose agar at 25 C under 2,000 lux of fluorescent light of each of five antagonists (three isolates of *Trichoderma harzianum*, one of *Penicillium restrictum*, and one of *Aspergillus ochraceus*) were added to the crown area of the transplant at 5×10^5 conidia of each isolate per plant.

The average yields in nonaugmented soils were 39.1, 39.1, 37.4, and 36.2 kg per 20 plants at the initial inoculum densities of 0, 50, 500, and 5,000 chlamydospores per plant, respectively. The average yields in antagonist-augmented soils were 40.6, 40.7, 39.2, and 35.9 kg per 20 plants at the initial inoculum densities of 0, 50, 500, and 5,000 chlamydospores per plant, respectively.

The addition of antagonists reduced significantly ($P = 0.05$) the mean incidence of disease in the second and third plantings (Table 1). Significant control was not observed in the first planting due to low disease incidence in the controls. The analysis of variance also showed that the inoculum density of the pathogen and the pathogen inoculum density-antagonist interaction significantly affected disease incidence. The mean disease incidence at the highest inoculum density of the pathogen when the antagonists were not added was five times greater than the mean incidence of disease at that inoculum density when antagonists were added.

The population density of the pathogen decreased with time in soils augmented with antagonists and increased with time in nonaugmented soils (Fig. 1). In soils augmented with antagonists in the first planting, the pathogen decreased from 600 propagules per gram of soil 3 wk after planting to 200 propagules per gram of soil 19 wk after planting. In nonaugmented soils the pathogen population increased 1,000 propagules per gram of soil 3 wk after planting to 5.3×10^4 propagules per gram of soil 19 wk after planting. Similar results occurred in each of the plantings.

In augmented or nonaugmented soils, the populations of antagonists and potential antagonists generally decreased until 11 wk after planting, and began to increase about 15 wk after planting (Fig. 2). The increase, however, in augmented soils was approximately four times as great as in nonaugmented soils. The population of *Trichoderma* spp. usually was much higher in augmented soils than in nonaugmented soils. *A. ochraceus* was not isolated from nonaugmented soils, but was present during the entire season in augmented soils.

DISCUSSION

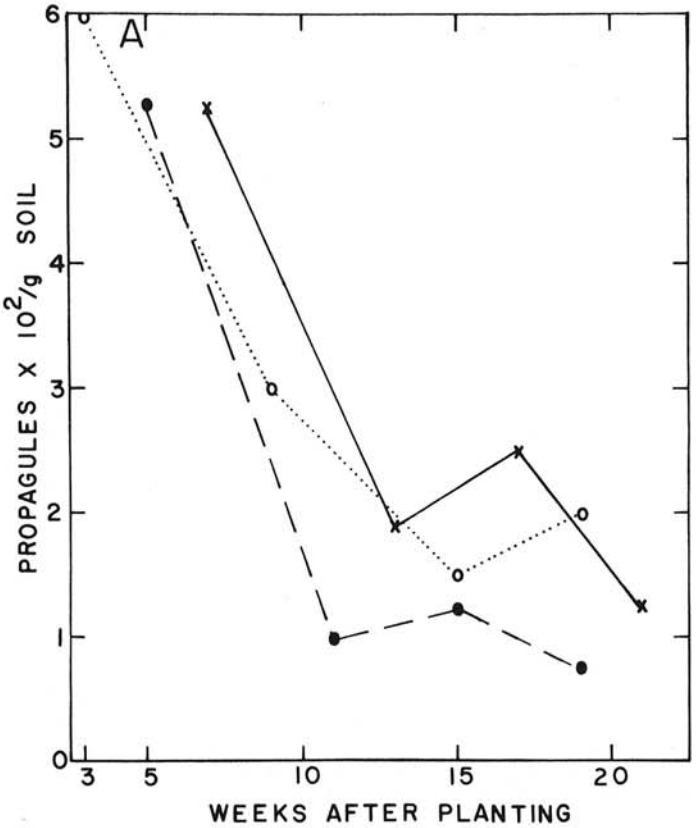
The application of selected antagonists to soil reduced the incidence of Fusarium crown rot of tomato under field conditions. Similar results were obtained in experiments under growth-chamber and greenhouse conditions (7).

In the past, antagonists usually were selected for ability to inhibit a pathogen under pure culture conditions (1). For several reasons these antagonists failed to reduce disease when applied under field conditions. Of the eight reasons that Baker and Cook (1) presented for such failures, the most important is probably that the environmental conditions in agar are not related to those in the soil. The success of reducing Fusarium crown rot of tomato under growth-chamber and greenhouse conditions was attributed to the formation of a microbial community which inhibited the saprophytic proliferation of the pathogen, rather than to the detrimental interaction of the pathogen with any one species of antagonist (8).

Yield was not affected by the different treatments because of the atypically warm growing season. Fusarium crown rot of tomato is a cool weather disease (3). Production operations in the area reported little problem with the disease during the 1979–1980 growing season when temperatures rarely dropped below 10 C.

The low inoculum densities of the pathogen in augmented soils early in the season probably were responsible for lower disease incidence in augmented than in nonaugmented soils. Rowe and Farley (10) reported that the severity of Fusarium crown rot of tomato is dependent upon the early infection of the tomato plant. The absence of an increase in the population of the pathogen in augmented soils late in the season occurred when populations of antagonists increased. This reduced the amount of inoculum available for infection in succeeding years. The increases in the pathogen population in nonaugmented soils and in the antagonist populations in augmented soils late in the season were attributed to saprophytic proliferation on fresh plant debris.

The high populations of *T. harzianum* in augmented soils, and the failure to detect *A. ochraceus* in nonaugmented soils, indicate



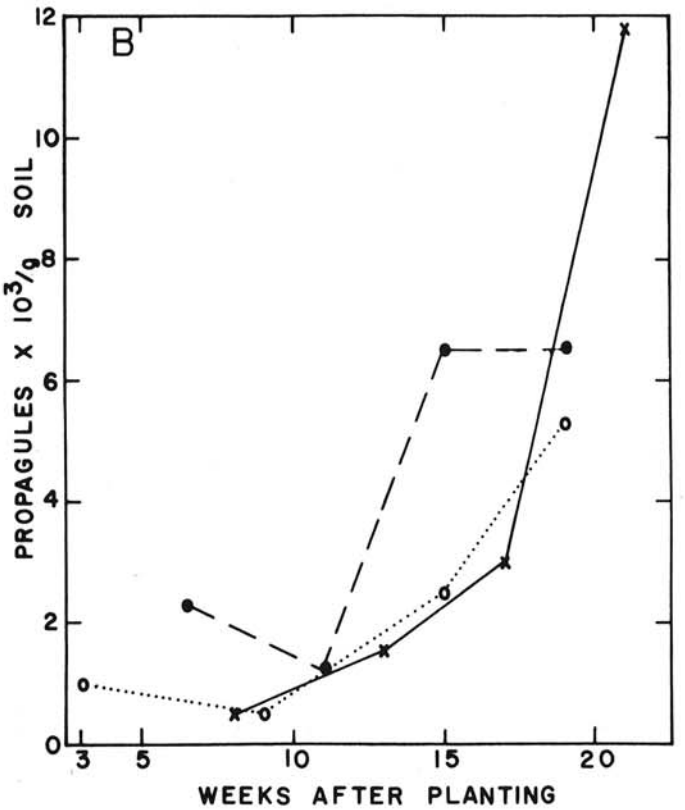
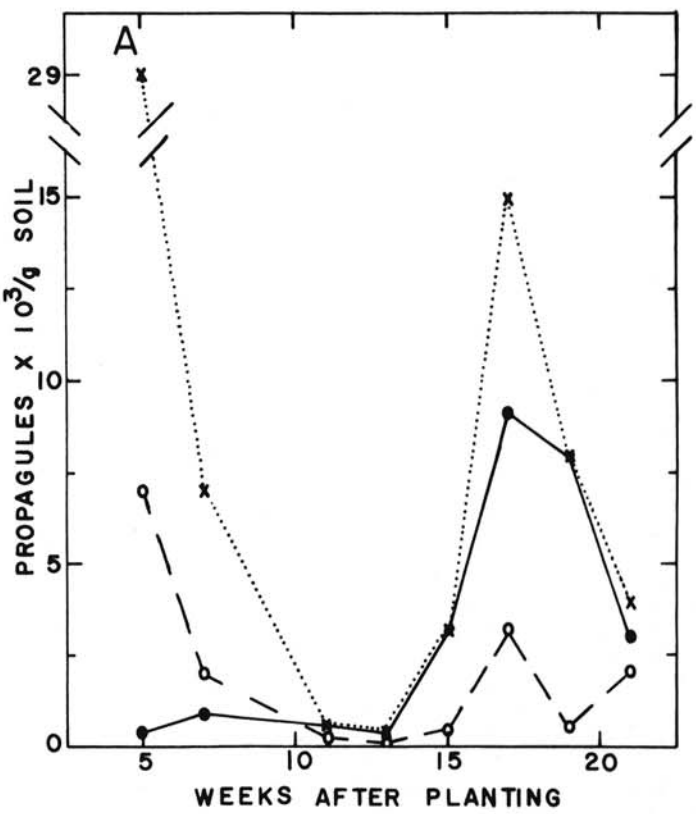


Fig. 1. Relationship of population density of *Fusarium oxysporum* f. sp. *radicis-lycopersici* to time after planting under field conditions at planting date one (O···O), planting date two (X——X), and planting date three (●---●), in: **A,** soils augmented with three isolates of *Trichoderma harzianum*, one isolate of *Penicillium funiculosum*, and one isolate of *Aspergillus ochraceus* at 5×10^5 conidia of each isolate per plant; and **B,** nonaugmented soils. The pathogen was added initially at 5,000 chlamydospores per plant.



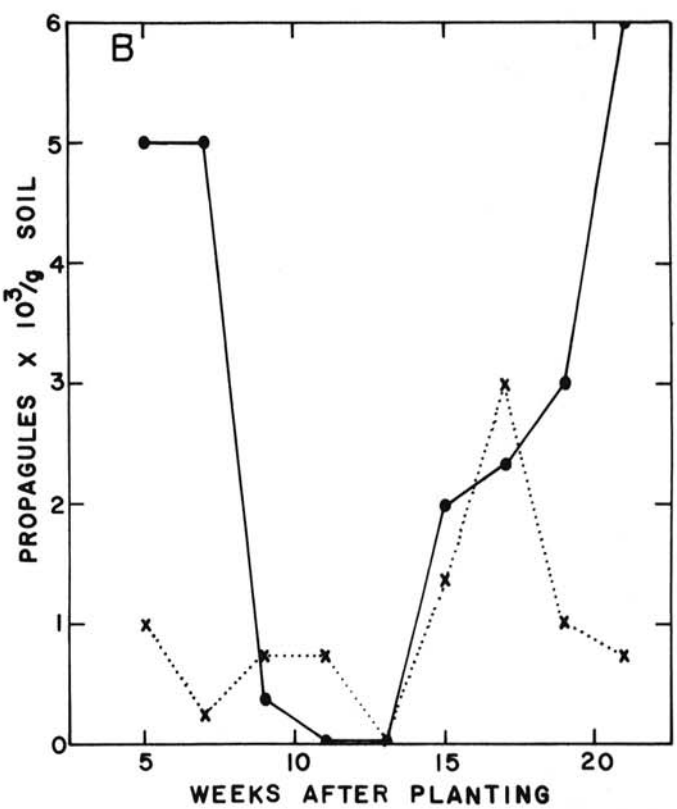


Fig. 2. Relationship of population densities of *Trichoderma* spp. (X···X), *Aspergillus* spp. (O---O), and *Penicillium* spp. (●——●) to time after planting under field conditions in: **A,** soils augmented with three isolates of *Trichoderma harzianum*, one isolate of *Aspergillus ochraceus*, and one isolate of *Penicillium restrictum* at 5×10^5 conidia of each isolate per plant; and **B,** nonaugmented soils.

that the addition of antagonists led to the establishment of populations of the organisms in augmented soils; however, it was not possible to determine if the populations of the specific isolates of the antagonists in augmented soils actually originated from the added antagonists.

The applicability of the addition of antagonists in production systems is realized when one considers that three 15-cm-diameter petri plate cultures of each antagonist grown on potato-dextrose agar produced sufficient inoculum to infest approximately 10^4 tomato plants at the prescribed rate of 5×10^5 conidia of each isolate per plate. The application of the antagonists by a drench either before or after planting could be effective in controlling the disease.

Increased disease severity following the application of a broad-spectrum biocide to soils has been reported (2,6,9,15). The usual explanation for this phenomenon is that the disturbance of the microbial community increases the ability of a pathogen to proliferate as a saprophyte during the early stages of recolonization. The application of antagonists in these types of disease situations should be successful in controlling the diseases, if the antagonists are properly selected and administered.

In this study, the success of the biological control agents was dependent upon several factors. The severity of an epidemic of Fusarium crown rot of tomato is dependent upon the rapid proliferation of the pathogen in treated soils (11). When the antagonists were applied to soils before recolonization by the pathogen could occur, they were able to effectively occupy the ecological niche created by the fumigation procedures (7). The preoccupied ecological niche then was rendered unavailable to the pathogen. The decrease in the saprophytic development of the pathogen was due to its inability to compete in soils recolonized by antagonists. Severe disease expression requires infection of the host early in the season (14); therefore, antagonists need to be established mainly around the crown and roots of the transplant. Successful control of Fusarium crown rot of tomato with biological agents was dependent upon production practices, the biology of the pathogen, and the methods used for selection and application of the antagonists.

LITERATURE CITED

1. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman and Company, San Francisco. 433 pp.
2. Bollen, G. J. 1974. Fungal recolonization of heat treated glasshouse soils. Agro-Ecosyst. 1:139-155.
3. Jarvis, W. R., Dirks, V. A., Johnson, P. W., and Thorpe, H. J. 1977. No interaction between root-knot nematode and Fusarium foot and root rot of glasshouse tomato. Plant Dis. Rep. 61:251-254.
4. Jarvis, W. R., and Shoemaker, R. A. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679-1680.
5. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.
6. Kreutzer, W. A. 1960. Soil treatment. Pages 431-476 in: J. G. Horsfall and A. E. Dimond, eds. Plant Pathology, An Advanced Treatise. Vol. 3. Academic Press, Inc., New York. 675 pp.
7. Marois, J. J., and Mitchell, D. J. 1981. Effects of fumigation and antagonistic soil fungi on the relationships of inoculum density to infection incidence and disease severity in Fusarium crown rot of tomato. Phytopathology 71:167-170.
8. Marois, J. J., and Mitchell, D. J. 1981. Effects of fungal communities on the pathogenic and saprophytic activities of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Phytopathology 71:000-000.
9. Rodriguez-Kabana, R., Beute, M. K., and Backman, P. A. 1979. Effect of dibromochloropropane fumigation on the growth of *Sclerotium rolfsii* and on the incidence of southern blight in field-grown peanuts. Phytopathology 69:1219-1222.
10. Rowe, R. C., and Farley, D. J. 1978. Control of Fusarium crown and root rot of greenhouse tomatoes by inhibiting recolonization of steam-disinfested soil with a captafol drench. Phytopathology 68:1221-1224.
11. Rowe, R. C., Farley, J. D., and Coplin, D. L. 1977. Airborne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouses. Phytopathology 67:1513-1517.
12. Sanchez, L. E., Endo, R. M., and Leary, J. V. 1975. A rapid technique for identifying the clones of *Fusarium oxysporum* f. sp. *lycopersici* causing crown- and root-rot of tomato. Phytopathology 65:726-727.
13. Sonoda, R. M. 1976. The occurrence of a Fusarium root rot of tomatoes in south Florida. Plant Dis. Rep. 60:271-274.
14. Sonoda, R. M., Marois, J., and Augustine, J. J. 1978. Fusarium crown rot of tomato in Florida. Proc. Fla. State Hortic. Soc. 91:284-286.
15. Vaartaja, O. 1964. Chemical treatment of seedbeds to control nursery diseases. Bot. Rev. 30:1-91.