BIOLOGICAL CONTROL OF FUSARIUM CROWN ROT OF TOMATO

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1980

ACKNOWLEDGMENTS

The author would like to thank David Mitchell, Eric Moore, Ronald Sonoda, and James Thomas, for this work would not have been so enjoyable, or even possible, without the patience and tolerance they have demonstrated during the course of these studies.

The love, friendship, and expert advice provided by the author's wife, Kathy, have made the difficult times pass with little notice, and the good times treasured forever.

The author especially is grateful towards his parents, for the guidance and support they have provided so consistently.

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June, 1980

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Major Department: Plant Pathology

Fusarium crown rot of tomato was controlled effectively with a composite of several biological agents under growth-chamber, greenhouse, and field conditions. The biological agents were selected for their abilities to proliferate in freshly fumigated soil, to establish high populations in the root zone of the host, and to interact with the pathogen to reduce the incidence of infection or disease. The antagonists selected were three isolates of Trichoderma harzianum, one isolate of Penicillium restrictum, and one isolate of Aspergillus ochraceus.

Chlamydospores of the causal agent, Fusarium oxysporum f. sp. radicis-lycopersici, were formed under axenic conditions so that defined concentrations of specific inocula could be added to freshly fumigated soil. The relationship of inoculum density to incidence of infection was determined under growth-chamber conditions. The inoculum concentrations of the pathogen at which 50% of the plants were infected were 300, 900, and 6500 chlamydospores per gram of soil which had been

fumigated, not fumigated, or fumigated and infested with antagonists, respectively. In greenhouse experiments the mean lesion length on stems increased as the inoculum density was increased in fumigated soil; lesion length, however, did not increase as the inoculum density was increased in fumigated soil with antagonists added. In nonamended soils, the disease incidence and mean lesion length were 70% and 2.22 cm, respectively, at the highest inoculum density, 5×10^4 chlamydospores per pot. At that inoculum density in antagonist amended soils, the incidence of disease and mean lesion length were 44% and 0.96 cm, respectively.

In field experiments, disease incidence increased as the inoculum density of the pathogen was increased in soils that were fumigated but not amended with the antagonists; disease incidence, however, did not increase as the inoculum density was increased in soils that were fumigated and amended with the antagonists. At 5000 chlamydospores of the pathogen per plant, disease incidence at harvest was 7% in soils amended with the antagonists and 37% in nonamended soils. The pathogen population decreased from 600 to 200 propagules per gram of soil during the growing season in the soils amended with the antagonists, but increased from 1000 to over 5×10^4 propagules per gram of soil in nonamended soils.

Control of disease was attributed to the abilities of the antagonists to inhibit the saprophytic activities of the pathogen. The population of the pathogen was stable or decreased in fumigated soil that had been amended with the antagonists, but the pathogen population increased 20-fold in nonamended soils. Microbial interactions which

influenced succession during the early recolonization stages of treated soils explain the observations that the activities of the pathogen were more closely related to the total number of fungal propagules detected in soils than to any single soilborne fungal species. Successful control of Fusarium crown rot of tomato with biological agents was dependent upon the production practices, the biology of the pathogen, and the methods used for selection and application of the antagonists.

SECTION I
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

Introduction

Fusarium crown rot of tomato (Lycopersicon esculentum Mill.), caused by Fusarium oxysporum Schlecht f. sp. radicis-lycopersici Jarvis and Shoemaker, is a disease which is severe when tomatoes are grown in soil treated with biocides (14,15,28). This phenomenon fits Kreutzer's (17) concept of disease trading in which dominant pathogens are controlled by soil treatments but minor pathogens are elevated to major importance because they can recolonize soil in which their competitors and antagonists have been eliminated. The traditional methods of applying fungicides directly to plants growing in previously treated soil have proved ineffective in the control of Fusarium crown rot (27). Rowe and Farley (27), however, controlled the disease with the application of captafol to freshly steamed soil before planting. The successful results were attributed to the selective action of the fungicide which inhibited reinvasion by the pathogen but did not adversely affect the recolonization of the soil by other airborne microorganisms.

Thompson (36), as early as 1929, realized that chemical agents would be useful in disease control mainly where conditions are relatively unfavorable for the pathogen and that biological agents would be more important when the environment is conducive to activity of the

pathogen. In Florida, an environment conducive to the development of Fusarium crown rot is established when plastic mulch is applied during fumigation and maintained during the entire growing season. If antagonists of F. oxysporum f. sp. radicis-lycopersici could be introduced under the plastic before the soil is recolonized by the pathogen, it should be possible to reduce disease. Thus, the nature of the pathogen and tomato production methods provide an excellent system for a quantitative field study of biological control.

Before field studies are undertaken, however, the importance of fumigation and recolonization of soil by antagonists can be evaluated critically by quantitatively determining the relationships of the pathogen and antagonists to incidences of infection and disease in fumigated or nonfumigated soil under growth-chamber and greenhouse conditions. The relationships of inoculum density to disease severity in root rots caused by Fusarium spp. are well documented (1,7,11,31). Baker (3) proposed that biological effects of antagonists on disease could be quantified by the analyses of curves derived by plotting disease severity to inoculum density of the pathogen. Significantly greater inoculum densities of the pathogen should be required to cause a proportionate amount of disease when antagonists are present.

The quantification of inoculum required the development of a procedure in which defined levels of chlamydospores of the pathogen could be established in freshly fumigated soil. The present procedure used in soil density studies with Fusarium spp. involves placing plants in soil with populations of the pathogen established by assaying artificially infested, aged soil with selective media and diluting the assayed infested soil with noninfested soil (11). This procedure is not

applicable to a system which demands freshly treated soil because populations of many microorganisms can become established during the time required for the aging of soil infested with the pathogen. An alternative to this method is to infest soil with chlamydospores produced under axenic conditions and quantified by direct count; a subsequent estimation of the population can be obtained by soil dilution plating.

The objectives of this study were: 1) to determine the relationships of densities of chlamydospores of F. oxysporum f. sp. radicis-lycopersici to the incidence of infection in fumigated and nonfumigated soils, 2) to determine the effects that selected antagonists have on the relationship of inoculum density to the incidence of infection, and 3) to determine the effects of antagonists on disease severity. The techniques and procedures were developed so that they can be applied to any disease which is severe after fumigation due to decreased competitor populations and subsequently increased pathogen populations.

Materials and Methods

The isolate of F. oxysporum f. sp. radicis-lycopersici was obtained from a diseased tomato plant collected in a south Florida field. Cultures were stored in soil tubes according to the method of Toussoun and Nelson (37).

Pompano fine sand was treated with methyl bromide-chloropicrin (67/33% v/v) at the rate of 1 kg of fumigant to 50 kg of soil for 2 days in a sealed container and then allowed to air in the greenhouse for 4 days.

Chlamydospores of the pathogen were used as inoculum to simulate natural conditions in which chlamydospores of Fusarium spp. are the

major survival structure (37). For the production of chlamydospores, macroconidia were washed from 2-wk-old cultures grown on potato dextrose agar (Difco, Detroit, MI 48201) at 25 C under continuous fluorescent light (3000 lux). The macroconidia formed intercalary chlamydospores after 4 wk of incubation at 10^6 macroconidia per milliliter of autoclaved deionized water at 28 C in the dark.

The potential antagonists were isolated from recolonized soils 1 wk after fumigation. A soil dilution of 1 g of air-dried soil in 1 liter of water was plated 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., St. Louis, MO 63178) per liter of medium (PDA-TC). Conidial suspensions of each isolate were obtained by washing 2-wk-old cultures grown on potato dextrose agar at 25 C under 10 hr of fluorescent light (2000 lux) per day. Each suspension then was added to 1 kg of freshly fumigated soil (final water concentration = 10% wt/wt). One-half kilogram of the infested soil then was placed in a plastic container and stored at 25 C for 1 wk after which the population density of each isolate was determined by dilution plating on PDA-TC. The remaining soil that had been infested with an individual isolate was placed in 100-ml polypropylene beakers at 80 g of soil per beaker. Two germinated 'Bonnie Best' tomato seeds were placed in each beaker, and the beakers were moved to growth chambers set at 20 C and 12 hr of light (4000 lux) per day. After 2 wk the roots were washed lightly and plated on PDA-TC. One week later the number of colonies of each potential antagonist growing from the roots was used to evaluate its ability to occupy the root environment. Those isolates

which increased populations rapidly in the freshly fumigated soil and occupied the root environment then were tested for their potential to increase the ratio of inoculum density to infection incidence in preliminary growth-chamber experiments. Of the 26 isolates which fulfilled the first two requirements, the five that were selected for the rest of the tests included three isolates of Trichoderma harzianum Rafai, one isolate of Penicillium restrictum Gilman and Abbott, and one isolate of Aspergillus ochraceus Wilhelm.

Concentrations of conidia of the antagonists and chlamydospores of F. oxysporum f. sp. radicis-lycopersici were determined by counting 40 fields of a standard hemocytometer, and the desired dilutions were added to soil.

The relationships of inoculum density to infection incidence were determined by experiments done in growth chambers. Two germinated 'Bonnie Best' tomato seeds were placed in a 100-ml polypropylene beaker which contained 60 g of infested soil layered over 50 g of autoclaved sand. A range of inoculum densities of the pathogen was used and a composite of the antagonists was added at the constant concentration of 5000 conidia per isolate per gram of air-dried soil. Twenty-four beakers of each inoculum combination then were placed in growth chambers. After 2 wk the soil was washed from the roots; the plants were soaked in 0.6% sodium hypochlorite for 1 min and rinsed in autoclaved deionized water. The roots and lower stem were plated on Komada's (16) selective medium for F. oxysporum and observed after 10 days for colonies of the fungus. Populations of the pathogen in the soil from the beakers also were determined after 2 wk of incubation in the growth chamber. The populations of the pathogen were quantified by

dilution plating of Komada's (16) medium. The pathogenic isolates were identified by the technique of Sanchez et al. (29), 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 and wilt inducing F. oxysporum isolates.

In the greenhouse experiments, 5-wk-old tomato ('Walter') transplants were placed individually in plastic pots (15-cm diameter) containing fumigated soil. A 4-mil-thick plastic film was placed over the soil to simulate the plastic mulch used in production fields. The transplant was planted through a 3-cm hole in the center of the plastic film. Fifty milliliters of a suspension containing 5×10^5 conidia of each antagonist were poured into the planting hole and over the transplant's crown and roots. Ten milliliters of a chlamydospore suspension of the pathogen were injected into the soil at each of two points approximately 7 cm from the plant. The plants were fertilized with half-strength Hoagland's (12) solution every 2 wk and watered when necessary. After 12 wk the root weight, infection incidence, disease incidence, and lesion length were recorded.

The data presented in this section are means of experiments repeated two times. Each replicate consisted of 48 plants per treatment in the growth-chamber studies and 15 plants per treatment in the greenhouse studies.

Results

Percentages of infection, percentages of diseased tomato plants, and mean lesion lengths increased with increasing inoculum levels of the pathogen (Fig. 1, Table 1). In the growth-chamber experiments, the

ratio of inoculum density to infection incidence was lowest in fumigated soils and highest in fumigated soils with the antagonists added (Fig. 1-A).

Slopes of regression lines plotted with data from the growth-chamber experiments of Log_{10} (Log_e 1/1-X), where X is the proportion of infected plants, on Log_{10} inoculum density were 0.82 ($r = 0.98$), 0.98 ($r = 0.97$), and 0.99 ($r = 0.99$) with fumigated soil, nonfumigated soil, and fumigated soil plus antagonists, respectively (Fig. 1-B). The inoculum densities required for 50% infection of plants (ID_{50}) in each soil were interpolated to be approximately 300, 900, and 6500 chlamydospores per gram of air-dried soil in fumigated soil, nonfumigated soil, and fumigated soil plus antagonists, respectively.

When the initial inoculum density of the pathogen was 500 chlamydospores per gram of air-dried soil, populations increased after 2 wk to 4000 propagules per gram of soil in fumigated soil, remained constant in nonfumigated soil, and decreased to 50 propagules per gram of soil that had been fumigated and amended with antagonists.

In the greenhouse experiments, the antagonist amendment reduced significantly ($\underline{P} = 0.05$) the mean lesion length and the incidence of disease (Table 1). The analysis of varience also showed that the inoculum density of the pathogen and the pathogen inoculum density-antagonist interaction significantly affect disease incidence and mean lesion length ($\underline{P} = 0.01$). There were no significant correlations between treatments and root weight or percent infection. All of the treatments, including the controls, had infection incidences of over 90%.

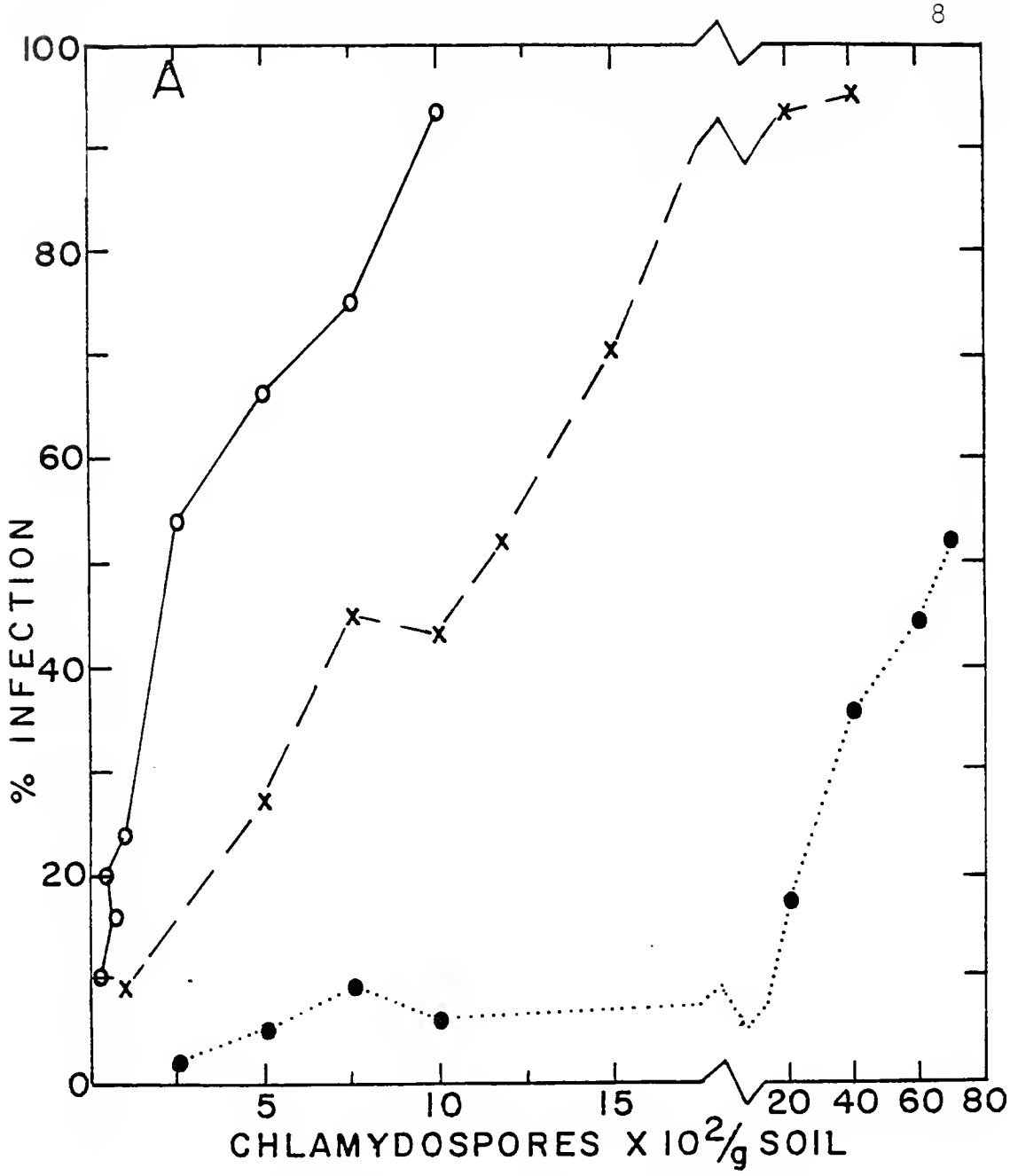

Figure 1 - A. The relationship of percentages of infection of tomato ('Bonnie Best') under growth-chamber conditions to densities of chlamydospores of Fusarium oxysporum f. sp. radicis-lycopersici in fumigated soil (O—O), nonfumigated soil (X— —X), and fumigated soil amended with Trichoderma harzianum, Aspergillus ochraceus, and Penicillium restrictum (●·····●).

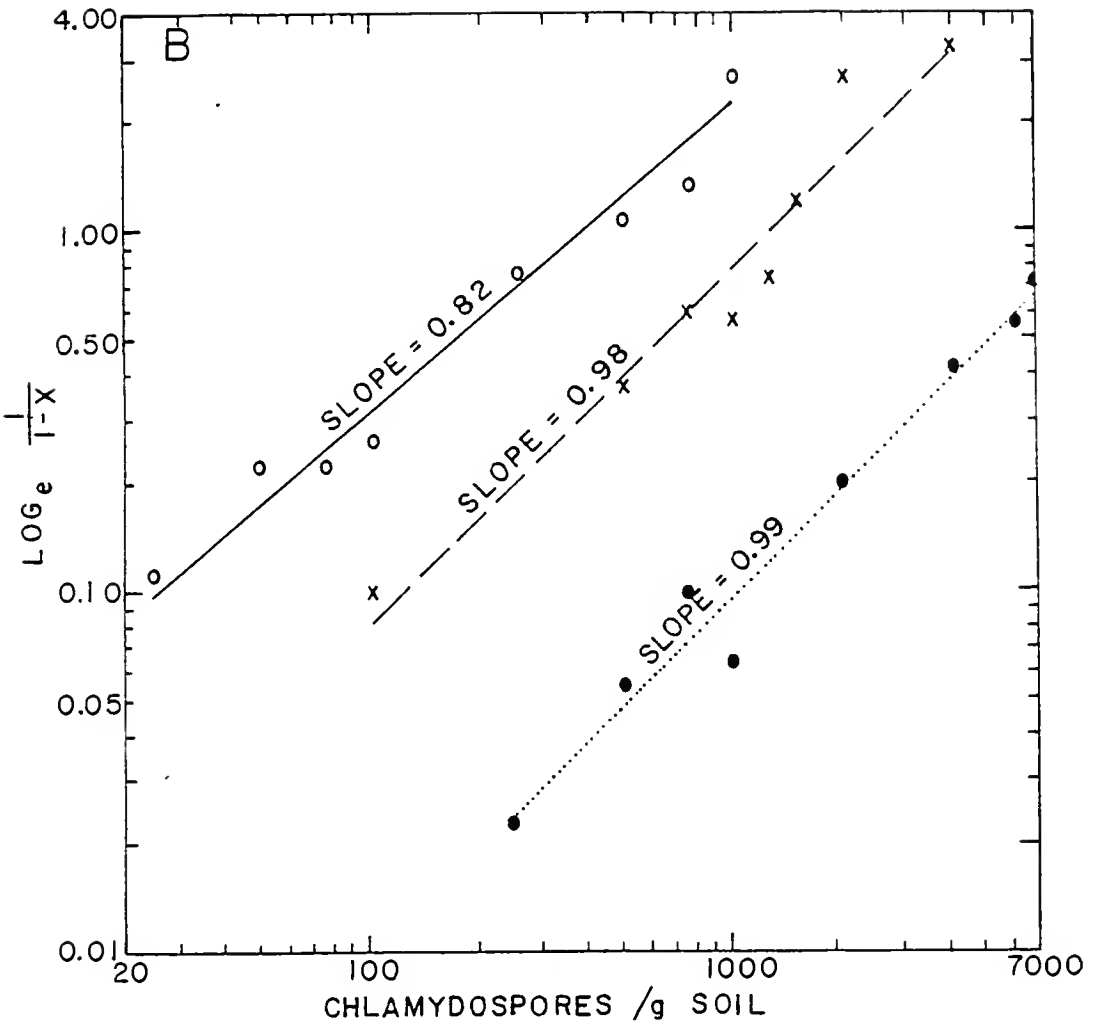

Figure 1 - B. The relationship of percentages of infection adjusted for multiple infections (logarithmic) of tomato ('Bonnie Best') under growth-chamber conditions to densities of chlamydospores (logarithmic) of Fusarium oxysporum f. sp. radicis-lycopersici in fumigated soil (o—o), nonfumigated soil (x– –x), and fumigated soil amended with Trichoderma harzianum, Aspergillus ochraceus, and Penicillium restrictum (●·····●).

Table 1. Effect of initial inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici and a composite of five antagonists on mean lesion length and percentage of plants with lesions under greenhouse conditions.

Inoculum density (chlamydospores per pot)[x]	Antagonists	Mean lesion length (cm)[y]	Percentage of plants with lesions[y]
0	-	0.00	0
500	-	0.98	50
5000	-	1.73	69
50000	-	2.22	70
mean		1.23a	47a
0	+[z]	0.14	4
500	+	0.73	33
5000	+	0.68	33
50000	+	0.96	44
mean		0.63 b	28 b

[x]20 ml of a chlamydospore suspension were injected into the soil 7 cm from the plant.

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

[z]+ = conidia of each of five isolates (three isolates of Trichoderma harzianum, one isolate of Penicillium restrictum, and one isolate of Aspergillus ochraceus) were added to the crown area of the transplant at 5×10^5 conidia of each isolate per pot.

Discussion

The relationships of inoculum density to disease incidence have been applied in the quantification of several soilborne diseases caused by Fusarium spp. (1,7,11). In this study the ID_{50} in nonfumigated soil was 900 chlamydospores per gram of soil, which is similar to the ID_{50}s found in other diseases caused by Fusarium spp. in nontreated soils (7,11). The ID_{50} in the fumigated soil was 300 chlamydospores per gram of soil; however the pathogen population increased from 500 chlamydospores to 4000 propagules per gram of soil during the experiment. Guy and Baker (11) reported a similar ID_{50} when the pathogen population increased due to a chitin soil amendment. High ID_{50}s (2000 or more conidia per gram of soil) were reported by Abawi and Lorbeer (1) in steam-treated and nontreated soils. However, the method for determining disease severity was based on percent emergence rather than on the percentage of infected or diseased tissue used in other investigations (1). Also, conidia were used as the inoculum source rather than chlamydospores, as in other studies. Their data, however, still indicate that the lower ID_{50} occurred in treated soil. The position and slope of the curve derived by the log-log transformation of inoculum density to disease severity affect the ID_{50} of a particular disease system. Guy and Baker (11) found that the addition of organic materials to the soil altered the ID_{50} by shifting the position of the curve rather than changing its slope, which they attributed to the relative changes in infection rates being directly correlated with inoculum density. A similar shift was noted in this study in the soils that were fumigated,

nonfumigated, or fumigated and amended with antagonists. In all soils the slope was approximately 1.0.

The attenuation of isolates of Fusarium spp. grown on artificial media must be considered in quantifying the relationship of inoculum density to disease incidence and severity. As an isolate becomes less virulent, more inoculum will be required to cause disease regardless of the particular treatment. The axenic production of chlamydospores of a pathogen reduces the chances of attenuation because the isolate does not reproduce vegetatively between experiments; thus the possibility of genetic variation is minimized. Changes in virulence of F. oxysporum f. sp. radicis-lycopersici were not observed in any of the experiments. The germination rate of chlamydospores of the fungus on potato dextrose agar was not significantly different from 100% ($P = 0.05$) for up to one year after their formation. French (8) reported that chlamydospores formed from macroconidia of F. oxysporum f. sp. batatas remained virulent for 7 yr when stored in water.

The application of a broad spectrum biocide to soil creates a biological vacuum which disrupts the stability of the soil community. The early recolonization pattern of treated soil involves a shift to an early successional pioneer stage that consists of a few species which occur in large numbers (42). Thus the community is of low diversity and may be readily invaded by new species if the environmental conditions are not severe. In this study the pathogen population remained stable, as determined by dilution plating, in the more advanced seral stages of nonfumigated soils. In the pioneer successional stage of fumigated soils, however, the pathogen was able to compete and increase in population

density. The limited ability to compete as a saprophyte is characteristic of other pathogenic Fusarium spp. (21,24,35).

The ability of other plant pathogens to compete as saprophytes in treated soil also may be reduced with the reestablishment of the microbial community (4,23,30,39). A decrease in the percentage of isolations of Verticillium albo-atrum from seedlings of Senecio vulgaris was attributed to the recolonization of autoclaved soil by airborne propagules of other microorganisms (30). This decrease is similar to that observed by Rowe and Farley (27) with Fusarium crown rot of tomato.

The observed decrease in the population of the pathogen from an initial inoculum density of 500 chlamydospores per gram of fumigated soil amended with antagonists to 50 propagules per gram of soil may have been caused by the production of toxins or by parasitism of the pathogen by the antagonists. The increase in the ratio of inoculum density to infection in the same soil may have been due to the adverse effects of the antagonists on the growth of the pathogen or the successful competition of the antagonists at potential infection sites. The reduction in the ability of the pathogen to infect roots is evident by the comparison of the ID_{50}s of 300 and 6500 chlamydospores per gram of soil in fumigated soil and fumigated soil with antagonists added, respectively. The reduced number of infected seedlings was correlated with the decreased mean lesion lengths when antagonists were added as compared to when the antagonists were not added in the greenhouse experiments. The correlation of increased numbers of infections and the increase in disease severity has been reported in other diseases caused by Fusarium spp. (20,31,32).

The reduced infection in the growth-chamber experiments and the reduced lesion length in the greenhouse experiments were due to the partial restabilization of the treated soil and root environment by the addition of the antagonists. The observation that nearly all of the plants were infected, including the controls, in the greenhouse studies was attributed to the long duration (12 wk) of the experiment and the ability of the pathogen to spread as airborne inoculum. Rowe et al. (28) found that under greenhouse conditions treated soil was rapidly reinfested by the pathogen as airborne microconidia.

The success of reducing the severity of Fusarium crown rot of tomato with biological control is dependent upon the reestablishment in freshly treated soil of a microbial community that impairs the reinvasion by F. oxysporum f. sp. radicis-lycopersici. The host-pathogen model employed in this study allowed the development of a system for the selection of antagonists and the quantification of a biological control procedure under growth-chamber and greenhouse conditions. This information provides qualitative and quantitative bases for the application of antagonists in the field in south Florida. In addition to the field experiments, information is needed on the effects of the addition of antagonists on recolonization by artificially and naturally introduced microorganisms. The interactions of several microbial populations can best be understood at the community level of the ecosystem and therefore the concepts of community stability and succession will be applicable to future studies on the quantification of biological control.

SECTION II
EFFECT OF FUNGAL COMMUNITIES ON THE PATHOGENIC AND SAPROPHYTIC ACTIVITIES OF *FUSARIUM OXYSPORUM* F. SP. *RADICIS-LYCOPERSICI*

Introduction

The causual agent of Fusarium crown rot of tomato (*Lycopersicon esculentum* Mill.), *Fusarium oxysporum* Schlecht f. sp. *radicis-lycopersici* Jarvis and Shoemaker, is able to compete as a saprophyte in freshly treated soils. Fusarium crown rot is associated with the rapid saprophytic proliferation of the pathogen. Naturally and artificially introduced soil recolonizers may reduce the severity of the epidemic by impeding saprophytic proliferation (13,27).

The competitive saprophytic ability of a pathogen is affected by the community of microorganisms which surrounds it. A soilborne plant pathogen that grows poorly or not at all in nontreated soil may grow readily as a saprophyte in soils in which the microbial community has been disturbed recently, as by the application of a broad spectrum biocide. The successional theory of community ecology states that a series of communities, or sere, develop after such a perturbation (22). Specific seral communities have certain characteristics in common, regardless of whether they are plant, animal, or fungal communities. The early successional communities are of low diversity and are dominated by a few species, r-selected species, which grow rapidly and quickly deplete the available resources. The success of a particular r-selected species is dependent upon the absence of competitors (22).

In more diverse communities which develop later, the few r-selected species are replaced by a number of k-selected species, which grow slower and utilize the available resources over a longer period of time. The later successional stages have communities of increased diversity and stability (22). Although k-selected species coexist with other k-selected species, the r-selected species are usually unsuccessful in the presence of the more diverse k-selected communities. Control of an r-selected pathogen may be possible by establishing other r-selected species before the pathogen is introduced or by establishing k-selected species before or after the introduction of the pathogen.

Since the early studies of biological control, biological agents have been selected for their antagonistic properties toward the pathogen in various laboratory tests outside of the soil environment (2). The results of these tests usually have not been repeatable under field conditions. In the present series of experiments, antagonists were selected for their abilities to proliferate in freshly treated soil, to occupy the root environment of the host, and to increase the ratio of inoculum density to infection incidence, as discussed in Section I. All of these factors were evaluated using field soil, rather than agar, as the growth medium. The selection for antagonists that were successful competitors, rather than for those that produce toxins or show hyperparasitism on agar plates, may result in the successful control of other plant diseases in which the epidemic is dependent upon the saprophytic growth of an r-selected pathogen.

The objectives of this study were: 1) to determine the effect of fungal communities on the saprophytic development of F. oxysporum f. sp.

radicis-lycopersici in soil, 2) to monitor the rate of fungal species immigration into soils amended or not amended with the antagonists, and 3) to quantify the effects of fumigation and antagonists on the activity of the pathogen in soil and on the host-pathogen interaction.

Materials and Methods

The ability of F. oxysporum f. sp. radicis-lycopersici to compete as a saprophyte was quantified by monitoring the pathogen populations in soils in the absence of the host. The pathogenic ability of the pathogen in different soil communities was quantified as the percentage of host plants infected after exposure to pathogen infested soil for 2 wk.

An isolate of F. oxysporum f. sp. radicis-lycopersici was obtained from a diseased tomato plant collected in a south Florida field. Cultures were stored in soil tubes according to the method of Toussoun and Nelson (37).

Pompano fine sand was treated with methyl bromide-chloropicrin (67/33% v/v) at the rate of 1 kg of fumigant to 50 kg of soil for 2 days in a sealed container. The soil was aired in the greenhouse for 4 days before further use.

Chlamydospores of the pathogen were formed from macroconidia under axenic conditions and quantified by direct count with a standard hemocytometer. The establishment of defined initial inoculum densities of the pathogen in freshly fumigated soil was accomplished by methods reported in Section I.

The antagonists used in the artificial infestation and recolonization experiments included three isolates of Trichoderma harzianum Rafai, one isolate of Penicillium restrictum Gilman and Abbott, and one isolate

of Aspergillus ochraceus Wilhelm. The antagonists were selected as described in Section I.

Freshly fumigated soil was divided into five allotments of 20 kg each (Table 2). The pathogen was added to three of the allotments at 1000 chlamydospores per gram of air-dried soil. Of the three allotments infested with the pathogen, one was placed in plastic containers to inhibit recolonization by airborne microorganisms, another was left uncovered in the greenhouse to allow natural recolonization to occur, and the third was left uncovered in the greenhouse and infested with the antagonists at 1000 conidia of each of the five fungi per gram of air-dried soil. The conidia were obtained as described in Section I. The two allotments without the pathogen were left uncovered in the greenhouse and one was infested with the antagonists as above. The pathogen was also added at the above concentration to a sixth allotment which consisted of nonfumigated soil. The water content of the soils was maintained at approximately 10% by weight during the experiments.

The competitive saprophytic and pathogenic abilities of the pathogen in the different soil communities were quantified by monitoring its population density in the soils and by determining its ability to infect susceptible tomato seedlings, respectively. Every 7 days 1500 g of soil from each allotment were removed. The population of F. oxysporum f. sp. radicis-lycopersici then was determined in those soils previously infested with the pathogen. The pathogen was added to those soil samples which were not previously infested with the pathogen to determine the saprophytic and pathogenic activities of chlamydospores of the pathogen when introduced to previously recolonized soil. The population of the pathogen in these soils was determined after 2 wk incubation in

Table 2. Treatments applied to 20-kg allotments of soil.

Treatment	Soil fumigated[w]	Pathogen added[x]	Antagonists[y]	Natural recolonization[z]
1	+	weekly	-	+
2	+	weekly	+	+
3	+	initially	-	-
4	+	initially	-	+
5	+	initially	+	+
6	-	initially	-	N/A

[w]+ = soil fumigated with methyl bromide-chloropicrin (67/33% v/v) at 1 kg of fumigant to 50 kg of soil for 2 days and then allowed to air in the greenhouse for 4 days.
- = soil not fumigated

[x]weekly = Fusarium oxysporum f. sp. radicis-lycopersici was added at 1000 chlamydospores per gram of soil to a 1500 g soil sample obtained every 7 days for the duration of the experiment.
initially = Fusarium oxysporum f. sp. radicis-lycopersici was added to the entire soil allotment 4 days after fumigation at 1000 chlamydospores per gram of soil.

[y]+ = conidia of each of five antagonists (three isolates of Trichoderma harzianum, one isolate of Penicillium restrictum, and one isolate of Aspergillus ochraceus) added to the entire soil allotment at 1000 conidia of each isolate per gram of soil 4 days after fumigation.
-= antagonists not added.

[z]+ = soils left uncovered to allow recolonization from naturally occurring airborne inocula.
- = soil placed in plastic containers to inhibit naturally occurring recolonization.

growth chambers. The populations of the pathogen in the soils were quantified by dilution plating on Komada's (16) medium, which is selective for F. oxysporum. Pathogenic isolates were identified by the technique of Sanchez et al. (29), as described in Section I. The ability of the pathogen to infect host plants grown in soil from the different treatments was determined by placing two germinated 'Bonnie Best' tomato seeds in a 100-ml polypropylene beaker which contained 60 g of soil layered over 50 g of autoclaved sand. The beakers then were placed in growth chambers at 20 C and watered every 48 hrs. After 2 wk, the soil was washed from the roots; the roots and lower stem were soaked in 0.6% sodium hypochlorite for 1 min, rinsed in autoclaved deionized water, and plated on Komada's (16) medium. The plates were examined after 10 days at 25 C. The plants were considered to be infected if F. oxysporum f. sp. radicis-lycopersici grew from the crown area of the seedling.

The soil fungal communities were monitored by dilution plating of soil samples 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., St. Louis, MO 63178) per liter of medium. The plates were incubated for 7 days at 25 C and 2000 lux of fluorescent light. Benomyl (Benlate 50% WP, E. I. du Pont de Nemours and Co., Wilmington DE 19898) was added to a replicate set of plates at 2.5 ppm when soil dilutions in water of 1:25 or lower were used to inhibit the fast growing colonies of Trichoderma spp. Dilution series ranged from 1:10 to $1:10^6$ (wt:vol). The particular series of dilutions used was dependent upon the expected populations of fungi.

The experiments were repeated at least twice; 10 petri plates were used for each soil dilution and 48 tomato plants were used to determine the incidence of infection for each experiment.

Results

The infection incidence of tomatoes by F. oxysporum f. sp. radicis-lycopersici was correlated with the inoculum density of the pathogen in nonamended (r = 0.99) and amended (r = 0.86) soils when the pathogen was added to soil samples taken every 7 days during recolonization of fumigated soils (Table 2, Treatment 1,2). Populations of the pathogen and infection incidence were correlated (r = 0.93 and 0.90, respectively) with the natural logarithm of the total number of fungal propagules detected in the nonamended soils. The inoculum density of the pathogen and infection incidence also were correlated (r = 0.89 and 0.94, respectively) with the natural logarithm of the total number of fungal propagules detected in the amended soils. No population of a single genus of antagonists had a correlation greater than r = 0.85 with either the infection incidence or inoculum density of the pathogen.

The proportion of infected tomato plants was directly related to the ability of the pathogen to proliferate in soils which contained different fungal communities (Fig. 2,3). In the recolonization experiments, the highest infection incidence and the highest pathogen populations occurred 4 days after fumigation (Fig. 2,3). The populations of naturally occurring recolonizers were low or not detected in the soil at that time (Table 3,4). In both amended and nonamended soils, saprophytic growth of the pathogen and infection incidence decreased with time after fumigation. The incidence of infection eventually stabilized at approximately 1% in amended and nonamended soils. The rapid decrease in both the population of the pathogen and in the incidence of infection in nonamended soils by 18 days after fumigation

occurred as natural populations of Trichoderma spp. increased 400-fold between 11 and 18 days after fumigation (Table 4).

The rate of immigration of naturally occurring fungal species was slower in soil amended with antagonists than in nonamended soil (Table 3,4). Thirty-nine days after fumigation, Cladosporium spp. and a wet spored Mucor sp. were the only naturally occurring species isolated from amended soils, whereas ten different naturally occurring species, including Trichoderma sp. and Penicillium sp., were isolated from nonamended soils. It was not possible to determine if the dominating populations in amended soils were from the original introduced antagonists, but the species were the same as those introduced.

The frequency of isolation of any particular species varied with time and treatment (Table 3,4). Aspergillus ochraceus dominated amended soils during the early stages of recolonization, and it accounted for 46% of the total number of propagules isolated 4 days after fumigation. The majority of the isolates from amended soils 11 and 18 days after fumigation were T. harzianum. Beginning 18 days after fumigation, 85% of the total number of colonies were either P. restrictum or T. harzianum. In amended soils, the introduced species always accounted for at least 98% of the total number of fungi isolated.

Some species of fungi were isolated frequently in freshly fumigated soil but occurred less frequently with time. In nonamended soils, Geotrichum sp., Cephalosporium sp., and Cylindrocarpon sp. were not isolated past 18 days after fumigation. Pythium sp., Syncephalastrum sp., Cunninghamella sp., and Rhizopus sp. were not isolated from nonamended soils until 39 days after fumigation (Table 4).

The maximum number of fungal propagules (approximately 2 X 10^5 propagules per gram of soil) in amended and nonamended soils was similar when naturally occurring recolonization was allowed. The population of F. oxysporum f. sp. radicis-lycopersici in fumigated soils which had been infested with the pathogen and placed in plastic containers to inhibit naturally occurring recolonizers was higher than the total fungal populations in any of the other treatments (Fig. 4). Under these conditions the pathogen population increased to 10^6 propagules per gram of soil 25 days after fumigation. By direct observation it was determined that the pathogen population consisted predominantly of microconidia.

When the pathogen was added 4 days after fumigation (Table 2, Treatment 3,4), its population density increased and then decreased with time in nonamended soils (Fig. 4,5). The proportion of infected plants was not related to the population density of the pathogen in nonamended soils. When natural recolonization was inhibited, the pathogen population increased and then decreased as the incidence of infection remained relatively constant at 100% (Fig. 4). When natural recolonization of fumigated soil was not inhibited, infection decreased with time and the population of the pathogen increased and then decreased (Fig. 6).

When the pathogen was introduced into soils that were either nonfumigated or fumigated and amended with the antagonists (Table 2, Treatment 5,6), the pathogen populations remained relatively stable (Fig. 6, 7). In fumigated, amended soils the infection incidence increased to 44% 11 days after fumigation and then decreased to approximately 23% by 39 days after fumigation. In nonfumigated soils, the infection incidence remained relatively stable, and varied from 40 to 50% (Fig. 7).

Table 3. Populations of fungi that recolonized fumigated soils amended with one isolate of Aspergillus ochraceus, one isolate of Penicillium restrictum, and three isolates of Trichoderma harzianum at 1000 conidia of each isolate per gram of soil.

Fungi	Propagules X 10^2/g of soil at days after fumigation[x]						
	4	11	18	25	32	39	46
Penicillium spp.	0.0	0.1	0.0	0.2	0.2	0.1	0.2
P. restrictum	12.9	200.0	130.0	1500.0	950.0	400.0	350.0
Trichoderma spp.	0.1	0.2	0.3	0.2	0.1	1.0	0.4
T. harzianum	7.3	333.0	400.0	1350.0	110.0	723.0	430.0
A. ochraceus	17.4	250.0	27.0	19.0	23.0	213.0	62.0
Cladosporium spp.	0.2	1.2	8.0	14.6	10.0	6.6	9.5
Mucor spp.	0.0	0.0	0.0	0.4	0.5	0.4	0.3
Total	37.9	784.5	565.3	2884.4	1093.8	1344.1	852.4

[x]Propagules X 10^2 per gram of air-dried soil detected in potato dextrose agar which contained 1 ml of Tergitol NPX and 50 mg of chlortetracycline hydrochloride per liter of medium.

Table 4. Populations of fungi that recolonized fumigated, nonamended soils.

Fungi	Propagules X 10^2/g of soil at days after fumigation[x]						
	4	11	18	25	32	39	46
Penicillium spp.	1.0	45.0	40.0	46.6	170.0	170.0	380.0
Trichoderma spp.		5.0	2000.0	1720.0	330.0	1010.0	1380.0
Aspergillus niger		0.1	0.5	0.8	0.3	0.3	4.3
Cladosporium spp.	5.0	12.5	6.5	8.5	9.0	10.0	28.2
Mucor spp.			0.2	0.6	0.8	0.9	0.3
Fusarium roseum			0.1	0.1	0.1	0.2	0.7
Pythium sp.						0.1	0.3
Rhizopus sp.						0.1	0.1
Cunninghamella sp.						0.1	0.1
Geotrichum sp.	0.2	5.0	2.5				
Cephalosporium sp.	0.1						
Cylindrocarpon sp.	0.1						
Fusarium solani			0.2				
Syncephalastrum sp.						0.1	1.0
Total	6.4	67.6	2050.0	1776.6	510.2	1191.8	1795.0

[x]Propagules X 10^2 per gram of air-dried soil detected in potato dextrose agar which contained 1 ml of Tergitol NPX and 50 mg of chlortetracycline hydrochloride per liter of medium.

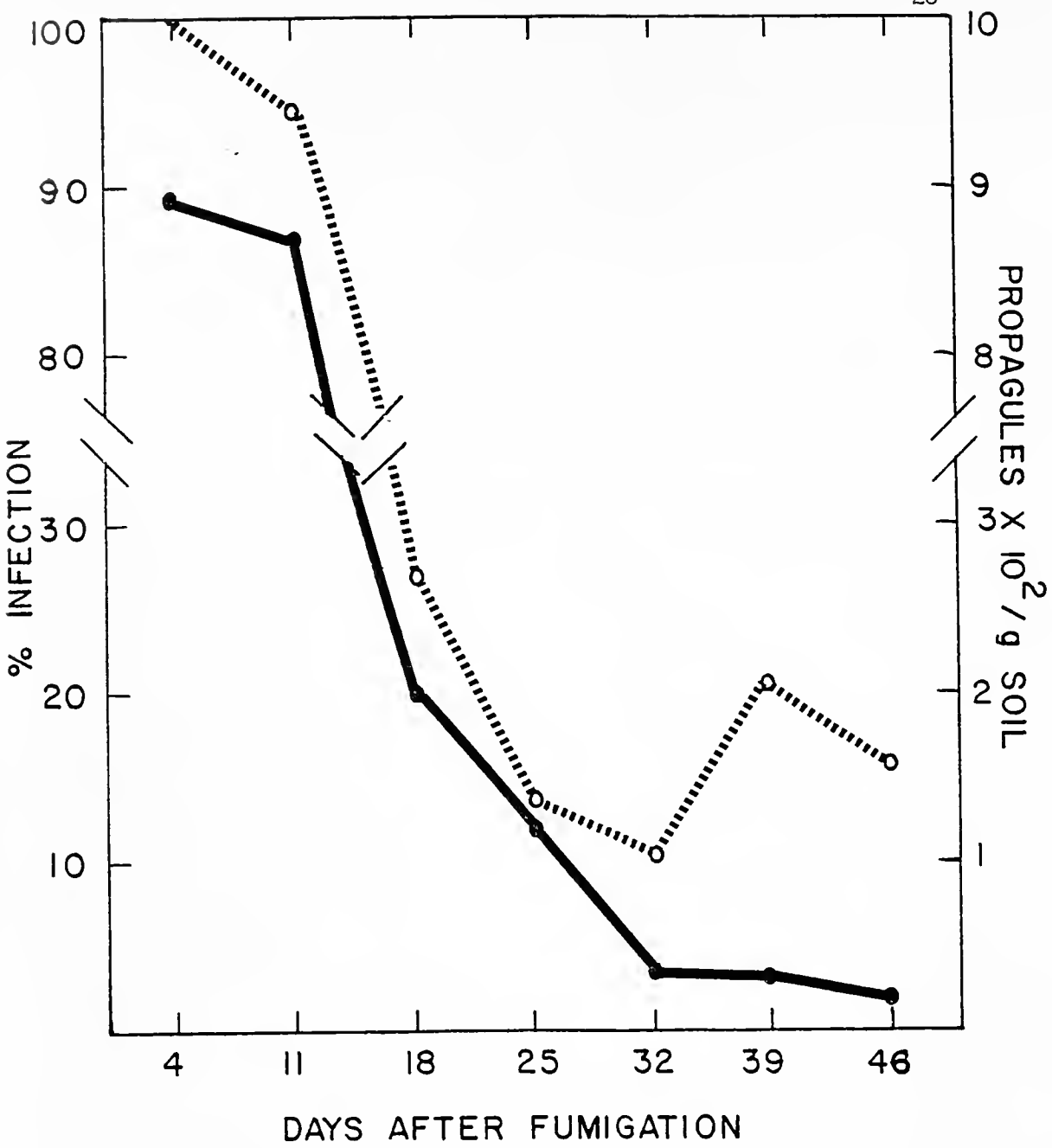

Figure 2. The relationship of percentage of infection of tomato ('Bonnie Best') (●—●) and inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici (o⁞⁞⁞o) to time after fumigation of soils which were allowed to recolonize naturally; the pathogen was added at 1000 chlamydospores per gram to 1500 g of soil every 7 days and tomato plants were maintained in the infested soil for 14 days under growth-chamber conditions before infection incidence and the inoculum density of the pathogen were determined.

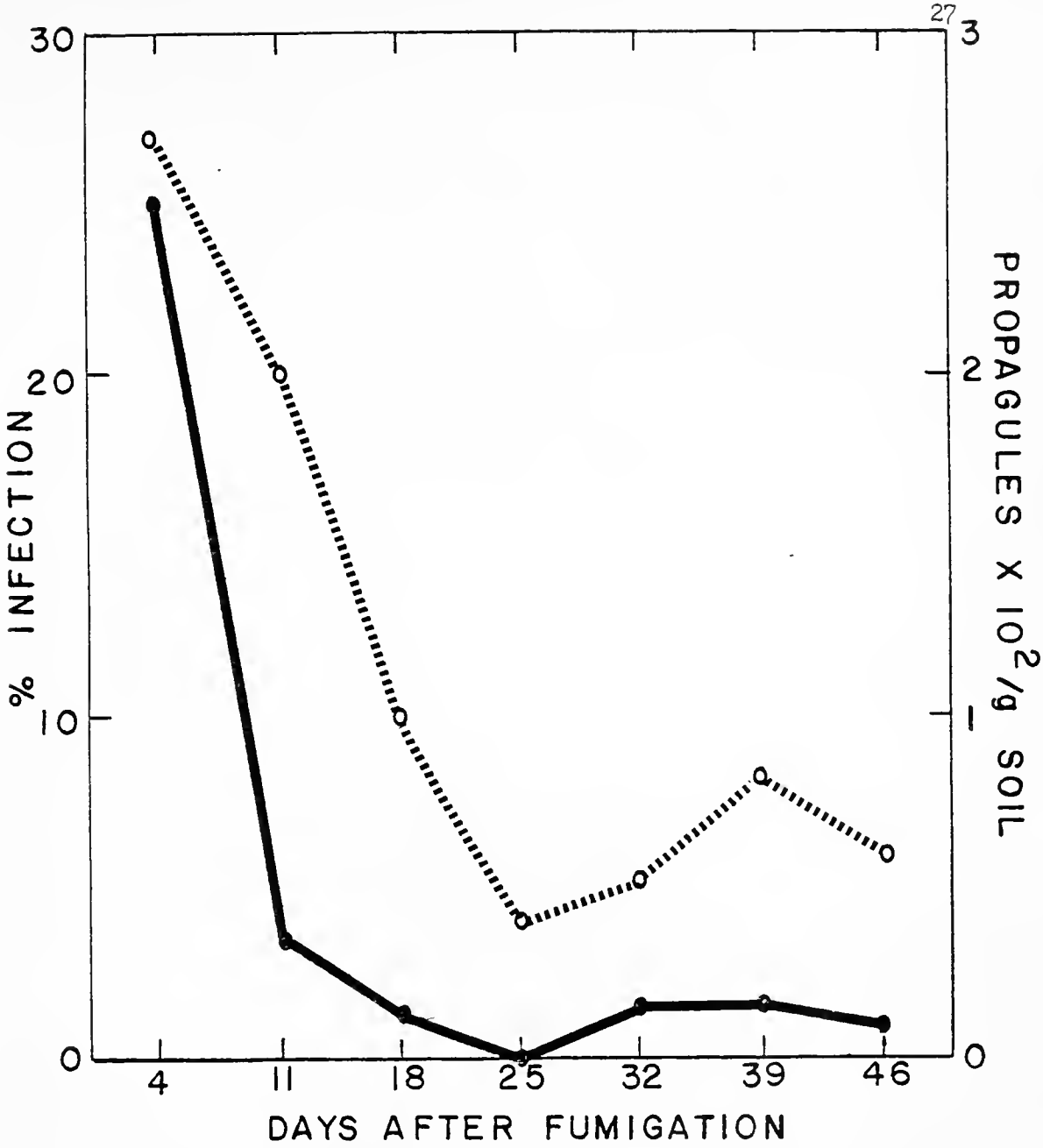

Figure 3. The relationship of percentage of infection of tomato ('Bonnie Best') (●—●) and inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici (o⁞⁞⁞o) to time after fumigation of soils which were amended with three isolates of Trichoderma harzianum, one isolate of Aspergillus ochraceus, and one isolate of Penicillium restrictum at 1000 conidia of each isolate per gram of soil; the pathogen subsequently was added at 1000 chlamydospores per gram to 1500 g of soil every 7 days and tomato plants were maintained in the infested soil for 14 days under growth-chamber conditions before infection incidence and the inoculum density of the pathogen were determined.

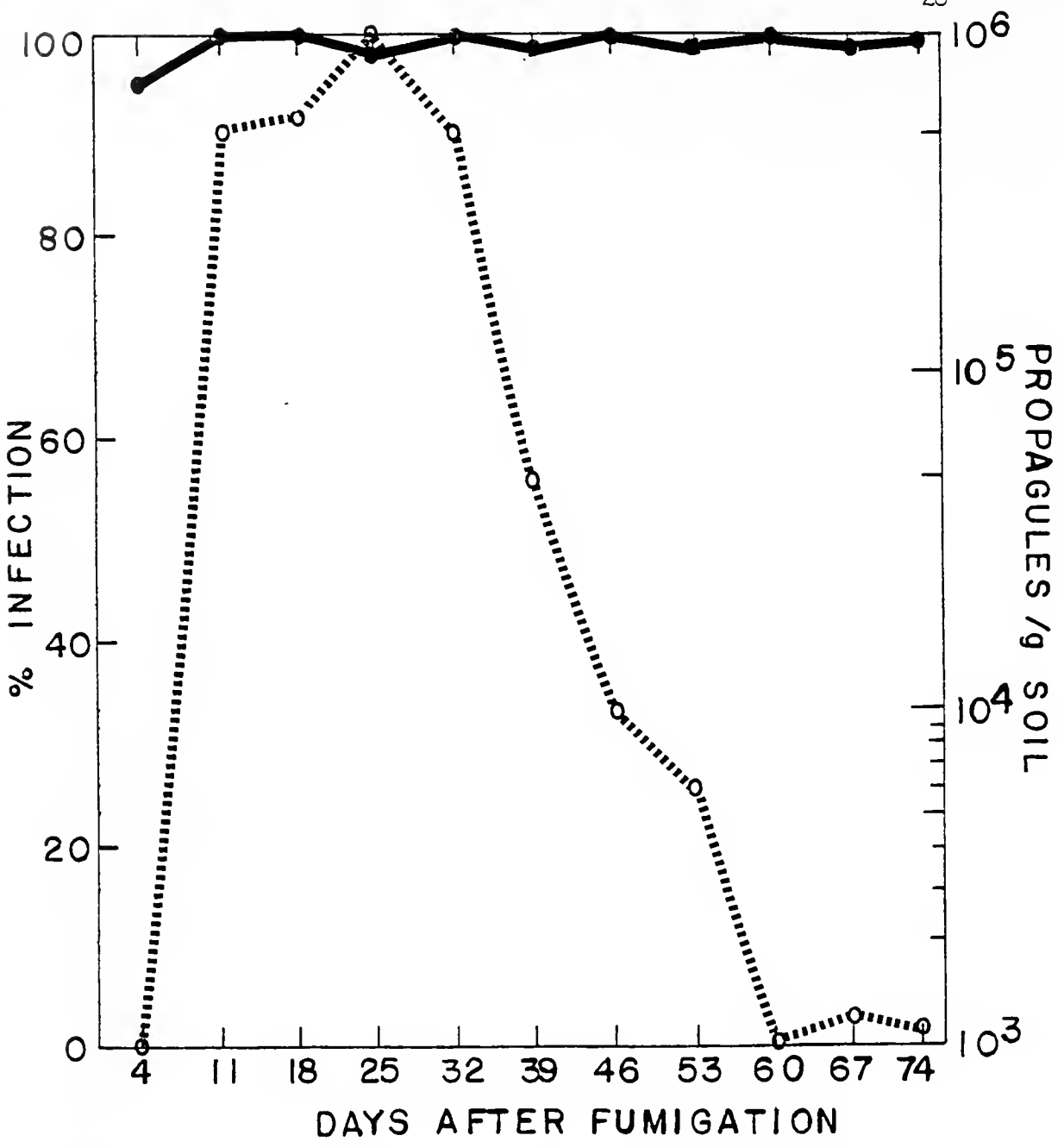

Figure 4. The relationship of percentage of infection of tomato ('Bonnie Best') (●—●) and inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici (○|||||○) to time after fumigation of soils in which recolonization by other microorganisms was inhibited; the pathogen was added 4 days after fumigation at 1000 chlamydospores per gram of soil.

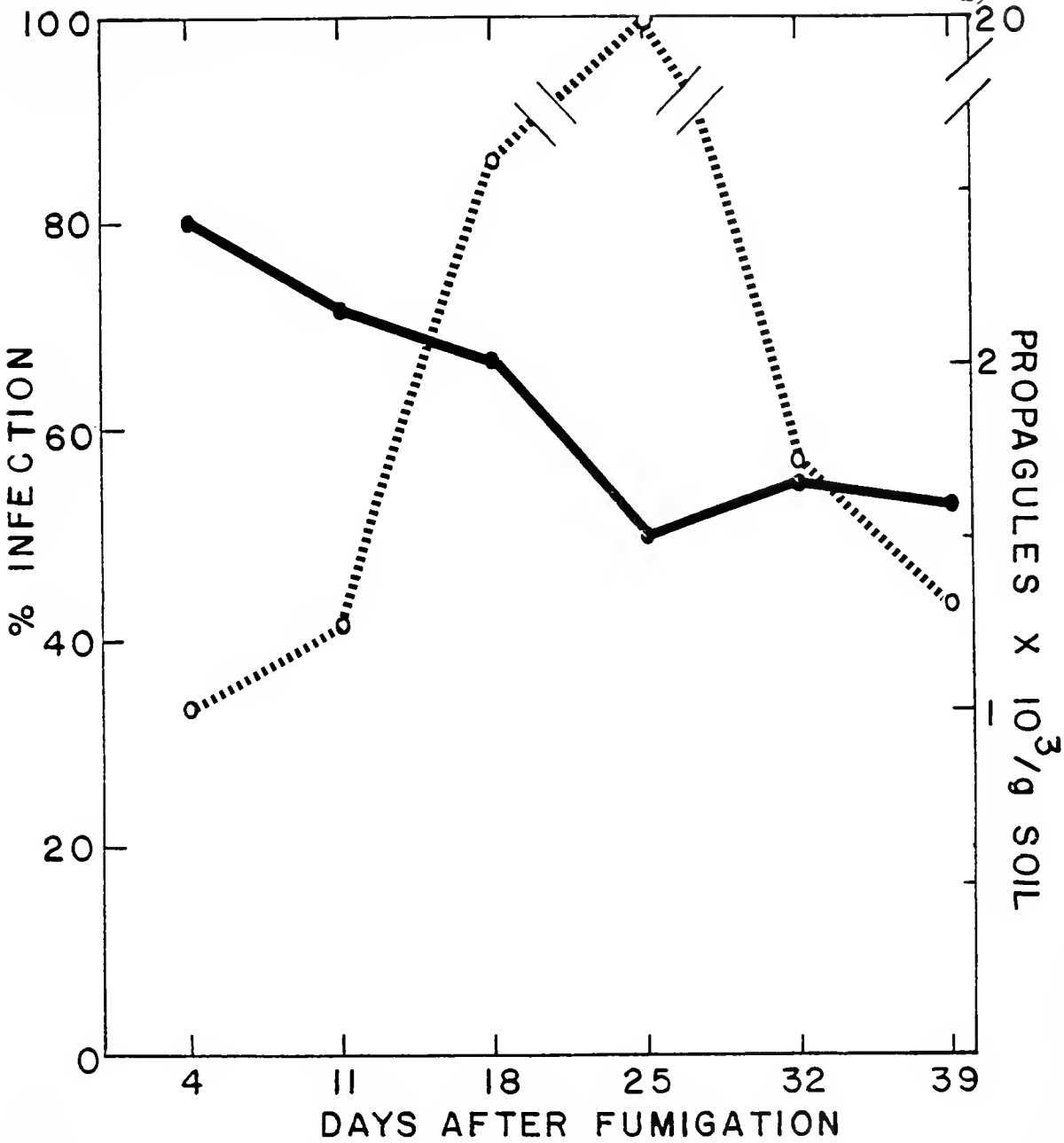

Figure 5. The relationship of percentage of infection of tomato ('Bonnie Best') (●—●) and inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici (○----○) to time after fumigation of soils in which recolonization by other microorganisms was not inhibited; the pathogen was added 4 days after fumigation at 1000 chlamydospores per gram of soil.

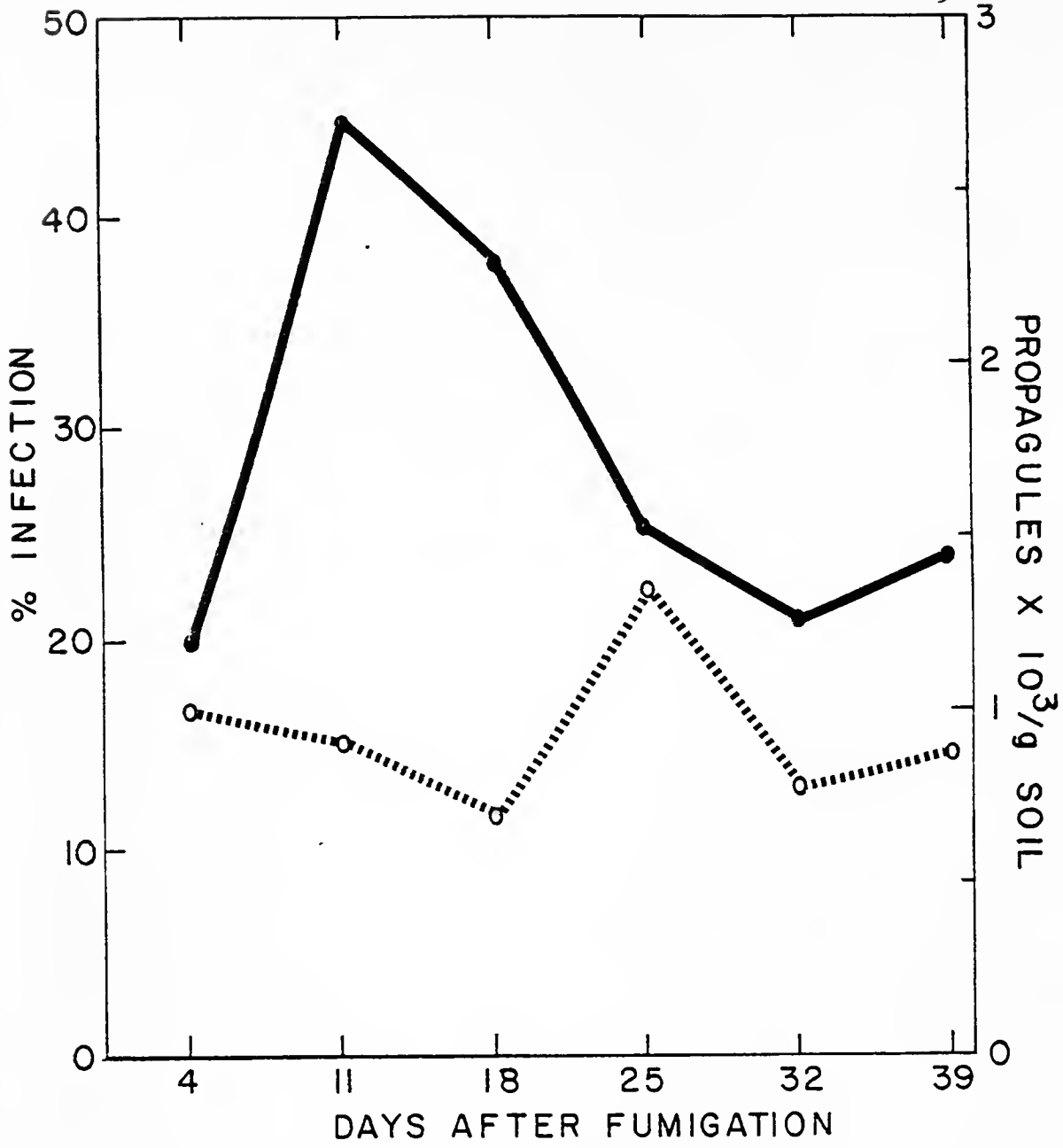

Figure 6. The relationship of percentage of infection of tomato ('Bonnie Best') (●——●) and inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici (○----○) to time after fumigation of soils which were amended with three isolates of Trichoderma harzianum, one isolate of Aspergillus ochraceus, and one isolate of Penicillium restrictum at 1000 conidia of each isolate per gram of soil; the pathogen was added 4 days after fumigation at 1000 chlamydospores per gram of soil.

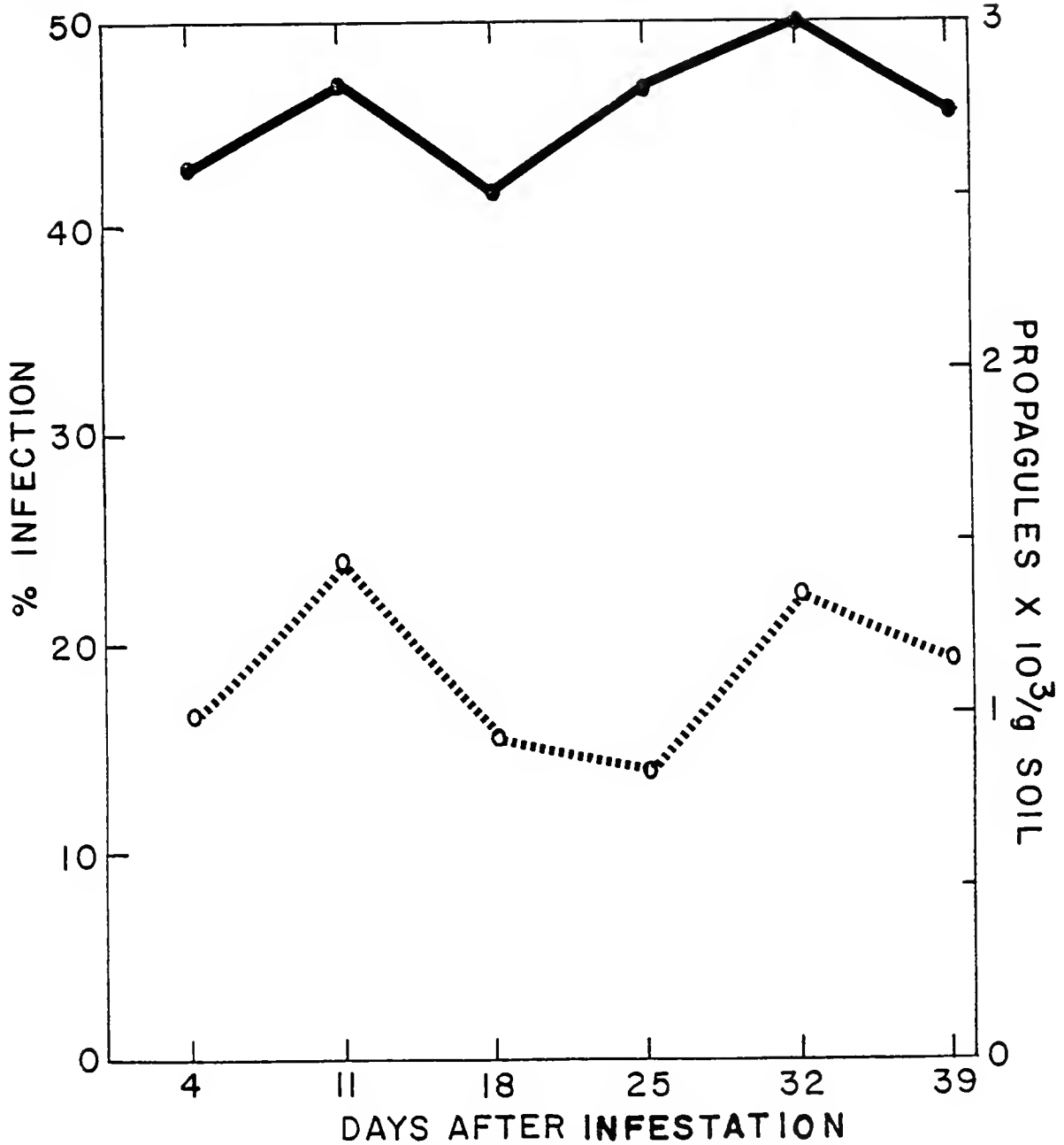

Figure 7. The relationship of percentage of infection of tomato ('Bonnie Best') (●—●) and inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici (o⋯⋯o) to time in nonfumigated soils; the pathogen was added on day 4 at 1000 chlamydospores per gram of soil.

Discussion

Decreases in the saprophytic activity of F. oxysporum f. sp. radicis-lycopersici due to competition from naturally recolonized and artificially amended soils similar to those observed in this study have been reported by other investigators (13,27); however, the compositions of the communities have not been examined. In this study, the fungal recolonization of soils was monitored by soil dilution plating, and the theories of successional mechanisms were invoked to explain why the pathogen population decreased as recolonization continued.

For comparison purposes, the pathogenic and saprophytic activities of the pathogen also were monitored in nontreated soils. Saprophytic proliferation of the pathogen did not occur in nontreated soils. Nash et al. (19) reported similar results when they added chlamydospores of F. solani f. phaseoli to nontreated soils. The lack of chlamydospore germination in natural soils has been attributed to insufficient nutrients and to the presence of inhibitory substances in the soil (41).

The ability of the pathogen to infect the host, as determined by the ratio of inoculum density to infection incidence, was higher in nonfumigated soils than in fumigated soils that had been allowed to undergo recolonization for 25 days. This phenomenon was termed induced antagonism by Welvaert (42). The practical aspect of induced antagonism is that it prolongs the effect of a soil treatment after the treatment is no longer active. The decreases in infection incidence of tomato and saprophytic proliferation of the pathogen in fumigated soils were correlated with the microbial population that formed during recolonization.

The relationship of the inoculum density of F. oxysporum f. sp. radicis-lycopersici to the infection incidence of tomato was influenced

critically by the different soil treatments. In fumigated soils in which the pathogen was added every 7 days and in nonfumigated soils, pathogen population and infection incidence were related. The inoculum density of the pathogen was not related to infection incidence when the pathogen was maintained in fumigated, nonamended soils; in fumigated, amended soils; or in fumigated soils in which recolonization by other organisms was inhibited. The ability of the pathogen to infect the host decreased with time in fumigated, nonamended soils. Although the pathogen population increased, the incidence of infection did not increase. In fumigated, nonrecolonized soils the incidence of infection was nearly 100% during the duration of the experiments. The high infection incidence was attributed to the high efficiency of the pathogen for host infection in the absence of competing organisms. The decrease of the pathogen population in the nonrecolonized soil probably was due to the exhaustion of nutrient sources or to the accumulation of toxic metabolites. At present, it is not possible to explain the results obtained when the pathogen was maintained in fumigated, amended soils. The pathogen population was relatively stable, but the infection incidence increased and then decreased with time. The low incidence of infection 4 days after fumigation, when the pathogen and antagonists were first added, may have been due to antagonisms by A. ochraceus, which was the predominant antagonist at that time. Perhaps the population of T. harzianum, which was dominant when the incidence of infection was high, was not as capable of restricticting the pathogenic activities of the pathogen. The decrease in infection incidence with time may have been due to increased antagonism as the combination of populations of P. restrictum and T. harzianum increased during the later stages of succession.

The total number of fungal populations detected increased rapidly after fumigation and then decreased with time. Kreutzer (17) attributed the rapid increase in populations of recolonizing fungi to the availability of nutrients. Wilson (43) hypothesized that the rapid increase in organisms following fumigation or other severe perturbation was the result of the growth of noninteractive populations, and that the eventual decrease in populations was the result of interactive population growth. In the noninteractive stages of recolonization, competition is low because of the large niche space available. In the interactive stages, however, the populations interact with each other and the effect is an overall reduction in the total number of individuals. Further evidence for the application of the noninteractive model to soil recolonization was obtained when the pathogen was allowed to grow as a saprophyte in the absence of other soil recolonizers. The highest number of total fungal propagules in any of the experiments (10^6 propagules of the pathogen itself per gram of soil) was obtained when the pathogen was maintained in a noninteractive environment by the exclusion of other competing species. The noninteractive model may explain why the highest inoculum density of total fungal propagules was not associated with the lowest inoculum density of the pathogen or with the lowest infection incidence of the host. Conversely, inoculum density of the pathogen and the infection incidence of the host were closely related to the total populations in amended soils; this was expected because the artificially introduced isolates were selected specifically for their antagonistic actions toward the pathogen, as described in Section I.

In fumigated soils to which the pathogen was added every 7 days, there were higher correlations of infection or inoculum density with

the total number of fungi ($r = 0.89$ to 0.94) than with any single species ($r \leq 0.85$). The presence of a specific organism was not as influential on the activity of the pathogen as was the total, composite fungal community. The higher correlations obtained when the activity of the pathogen was compared to total number of detected fungi agrees with Park's (25) conclusion that the probability of an organism coming in contact with a single species is much less likely than the organism interacting with the entire community. The lack of specific antagonism of any one species towards the pathogen was further supported when several isolates of the major recolonizing species did not show antagonism towards the pathogen on nutrient agar (unpublished results). The changes in species composition and dominance in nonamended soils were attributed to different successional stages of recolonization. Fungal succession in treated soil has been the subject of several reviews (4,17, 42). The mechanism of succession usually is similar to that proposed by Garrett (9) for microbial succession on plant debris, in which the available carbon source determines which organisms are dominant. In opposition to reports of fungal succession are instances in which single species remain dominant during the entire exploitation of the substrate (5,10,18,40). This occurs when a species is introduced artificially at high inoculum densities to a substrate prior to colonization by other organisms. Bruehl and Lai (5) reported that the advantage of prior colonization of wheat straw increased the competitive saprophytic ability of several fungi. The importance of prior colonization in soil systems is evident in these studies by the decreased immigration rates by naturally occurring fungal species in amended soils. In addition, when the pathogen was added to freshly fumigated soil, it was able to proliferate

for up to 25 days after fumigation; conversely, when added to fumigated soils which had undergone recolonization for 25 days, it was unable to compete as a saprophyte.

The apparent contradiction in the literature over the presence of succession may be explained by examining different models of the mechanisms of succession. Connell and Slatyer (6) have proposed three different mechanisms of succession following a perturbation. In all three models the earlier species cannot invade or grow after the site is fully occupied by the same or later occurring species. In the facilitation model, later species can become established only after earlier inhabitants have suitably modified the environment. This model explains why secondary invaders are detected only after a plant pathogen has invaded the healthy tissues of the host. According to the tolerance model, which is similar to the model proposed by Garrett (9), the later species can establish themselves because they can utilize nutrients at lower levels than earlier recolonizers. According to the inhibition model, later species cannot grow in the presence of earlier species and their establishment is dependent upon their ability to survive longer and gradually replace the earlier species. The results of this study and the literature indicate that succession in artificially infested substrates follows the inhibition model, but that in nonamended substrates succession follows the tolerance model.

A basic principle of community ecology is that the success of a species, such as a plant pathogen, is dependent upon its ability to interact successfully with the abiotic and biotic factors of its surrounding environment. Because fumigation practices create an environment conducive to the proliferation of the pathogen, a possible means of

control is to establish a community which is inhibitory to the pathogen in the treated soil. Fusarium oxysporum f. sp. radicis-lycopersici is an r-selected species, as indicated by the rapid increase and then decrease of its population density in the absence of other organisms; therefore, its ability to compete as a saprophyte is dependent upon the total propagule density of all interacting microorganisms in the soil (22). The saprophytic proliferation of the pathogen was controlled by adding r-selected antagonists to freshly fumigated soil before the re-invasion of the pathogen could occur. By the application of the basic concepts of community ecology to the control of plant disease and the utilization of composites of antagonists, the success of biological control investigations may increase, and disease control in the field may be realized more frequently.

SECTION III
BIOLOGICAL CONTROL OF FUSARIUM CROWN ROT OF TOMATO UNDER FIELD CONDITIONS

Introduction

Fusarium crown rot of tomato was first reported in south Florida during the 1974-1975 growing season (33). Attempts to control the disease with chemicals and host resistance have been unsuccessful (27). At present, the only effective control measure is the application of a captafol drench to greenhouse beds immediately after steaming (27). The captafol drench selectively inhibits recolonization of the soil by the pathogen, Fusarium oxysporum Schlecht f. sp. radicis-lycopersici Jarvis and Shoemaker (15). When captafol was applied as a preplant or post-plant 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 (34). Furthermore, a complete soil drench of the entire bed is not practical to 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 selected soil antagonists could decrease 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 reduced when antagonists were added to fumigated soil

(Section I). 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, Florida, 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 via three chisels. 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 ('Walter') transplants were planted 30 cm apart in two rows which were 50 cm apart on each bed. Subsurface irrigation was maintained at approximately 40 cm below the bed surface. 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 (16) before planting. This situation allowed the establishment of field plots with defined initial inoculum densities of both the pathogen and the antagonists. The pathogen was added to the soil by injecting 10 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 ml in autoclaved deionized water to induce chlamydospore formation (Section I).

The population of antagonists consisted of three isolates of Trichoderma harzianum Rafai, one isolate of Penicillium restrictum Gilman and Abbott, and one isolate of Aspergillus ochraceus Wilhelm. The antagonists were selected for their abilities 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 (Section I). The antagonists were applied at a concentration of 5×10^5 conidia per isolate per plant. One day before use, conidia of each antagonist were harvested from petri plates containing 14-day-old cultures grown on potato dextrose agar at 25 C under 2000 lux of fluorescent light. The antagonists were added to the soil by pouring 25 ml of a conidial suspension 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. The treatments included infestation of soil with pathogen populations of 0, 50, 500, or 5000 chlamydospores per plant with or without the addition of antagonists.

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 each plot. Komada's (16) medium, which is selective for F. oxysporum, was used to isolate the pathogen from soil dilutions of 1:25, 1:100, or 1:1000 (wt:vol). The pathogen was identified further by a technique developed by Sanchez et al. (29) and discussed in Section I. 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., St. Louis, MO 63178) per liter of medium was used to monitor the antagonist populations. Soil dilutions in water of 1:25, 1:100, and 1:1000 (wt:vol) were employed and plates were stored at 25 C and 2000 lux of 12 hr of fluorescent light per day for 7 days before examination for fungal colonies.

A standard hygrothermograph was used to monitor the high and low 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 (16) medium to confirm the presence of the pathogen.

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

Results

The incidence of Fusarium crown rot of tomato was affected significantly ($P = 0.05$) by the initial inoculum density of *F. oxysporum* f. sp. *radicis-lycopersici*, the antagonist amendment, and the time of planting (Table 5). The increase in disease observed during the later planting time as compared to earlier planting times was associated with cooler prevailing temperatures. The mean low temperature during the first and

last planting times were 12 and 10 C, respectively. The mean high temperature during the first month after planting was 34 C in the first planting time, 32 C in the second planting time, and 30 C in the third planting time.

Amendment with the antagonists reduced significantly (P 0.05) the mean incidence of disease (Table 1). The analysis of varience 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 amended with antagonists and increased with time in nonamended soils (Fig. 8). In soils amended 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 nonamended soils the pathogen population increased from 1000 propagules per gram of soil 3 wk after planting to 53000 propagules per gram of soil 19 wk after planting. Similar results occurred in each of the plantings.

In both amended and nonamended soils, the populations of antagonists decreased until 11 wk after planting, and began to increase 15 wk after planting (Fig. 9). The increase, however, in amended soils was approximately four times as great as in nonamended soils. The population of Trichoderma spp. usually was much higher in amended than in nonamended soils. Aspergillus ochraceus was not isolated from nonamended soils, but was present during the entire season in amended soils.

Table 5. Effect of initial inoculum density of Fusarium oxysporum f. sp. radicis-lycopersici and a composite of five antagonists on the incidence of disease under field conditions.

Inoculum density (chlamydospores per plant)[x]	Antagonists	Percentage of plants with lesions[y] Planting date 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
5000	-	15.0	42.8	53.9	37.2
mean		5.2a	17.1a	23.2a	15.1a
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
5000	+	1.7	5.4	14.3	7.1
mean		2.6a	3.2 b	7.7 b	4.5 b

[x]20 ml of a chlamydospore suspension were injected into the soil 10 cm from the plant.

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

[z]Conidia of each of five antagonists (three isolates of Trichoderma harzianum, one isolate of Penicillium restrictum, and one isolate of Aspergillus ochraceus) were added to the crown area of the transplant at 5 X 10^5 conidia of each isolate per plant.

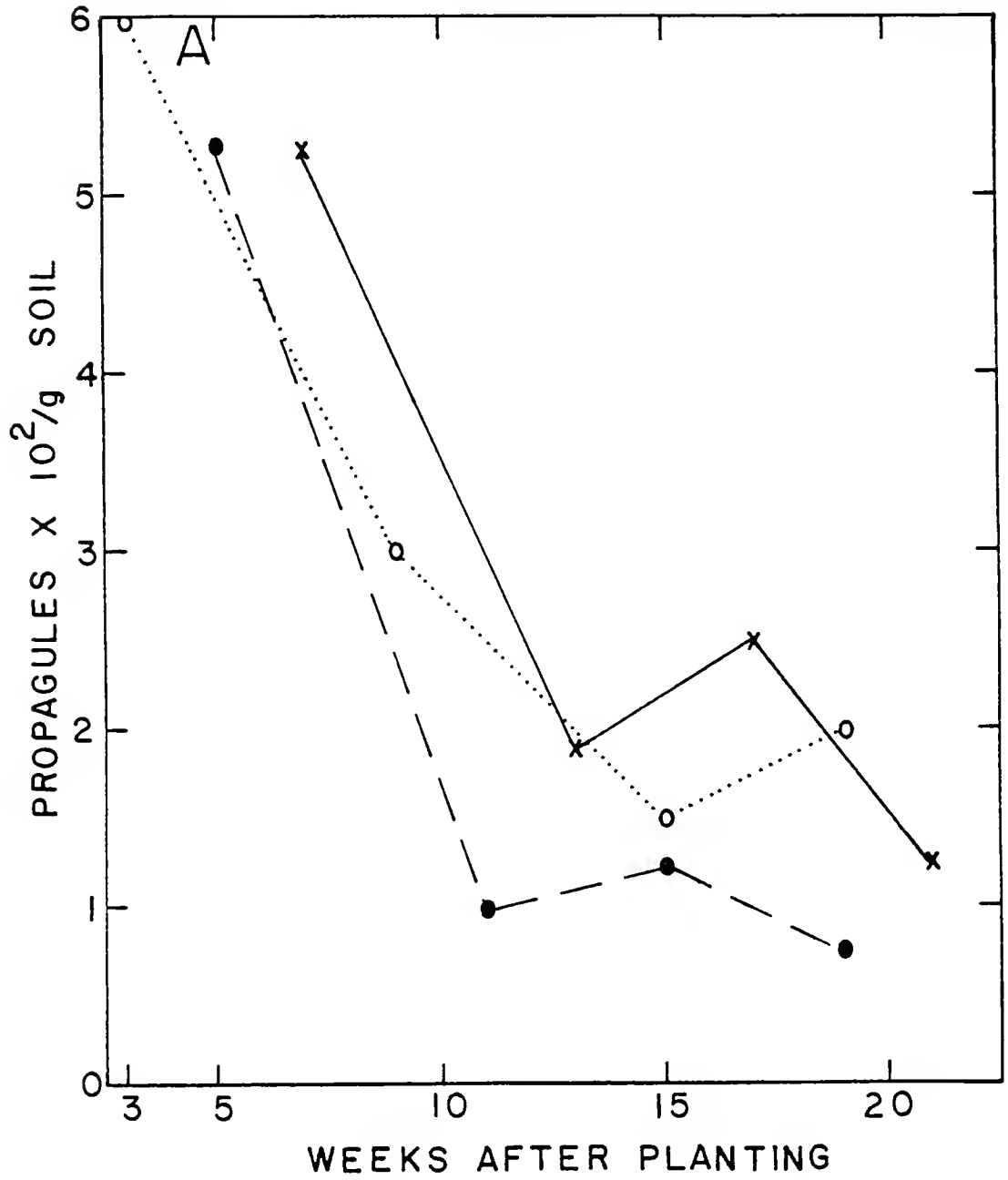

Figure 8 - A. Relationship of population density of Fusarium oxysporum f. sp. radicis-lycopersici to time after planting in soils amended with three isolates of Trichoderma harzianum, one isolate of Penicillium restrictum, and one isolate of Aspergillus ochraceus at 5 X 10^5 conidia per isolate per plant under field conditions at planting date one (o·····o), planting date two (x——x), and planting date three (●— —●). The pathogen was added initially at 5000 chlamydospores per plant.

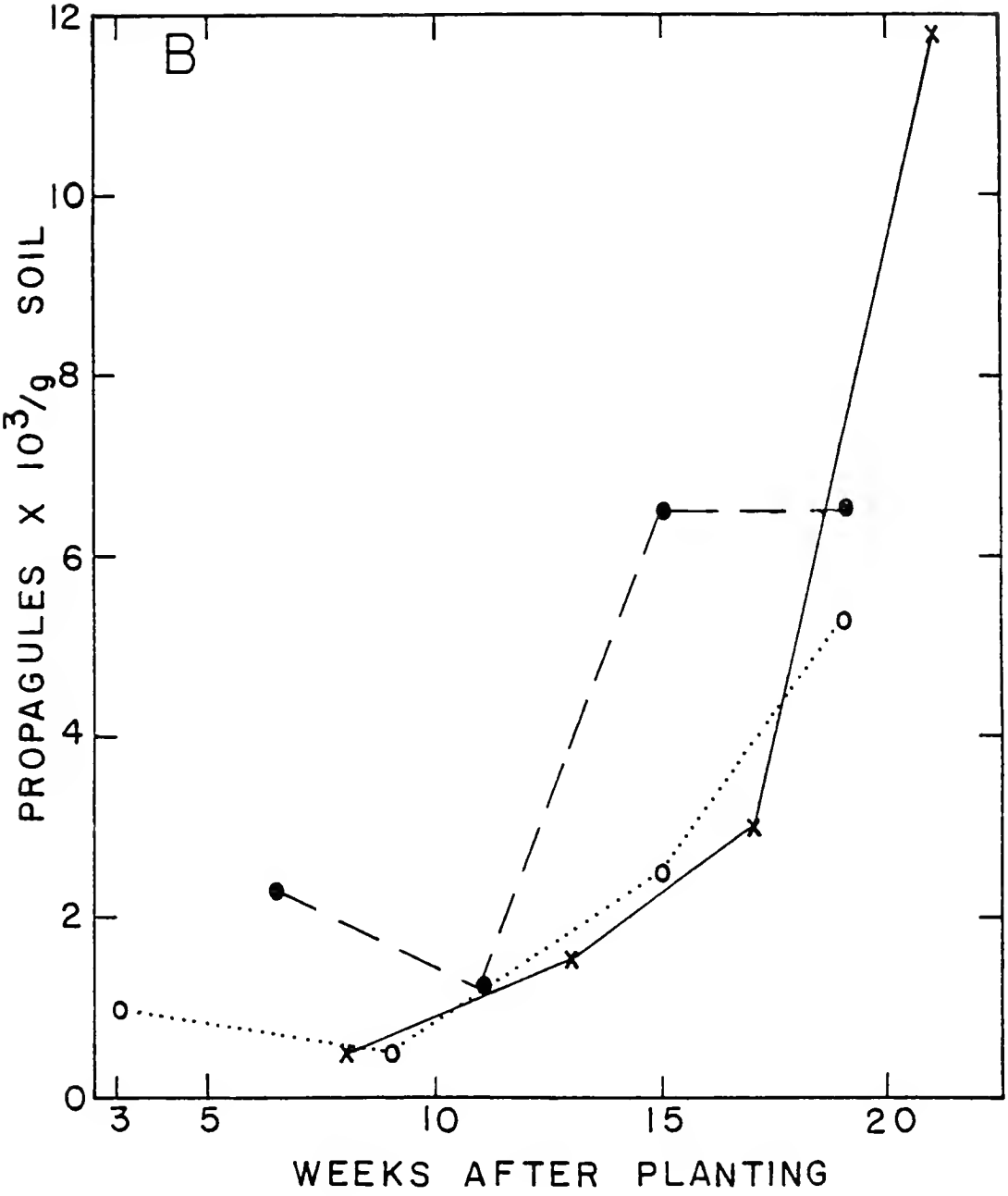

Figure 8 - B. Relationship of population density of Fusarium oxysporum f. sp. radicis-lycopersici to time after planting in nonamended soils under field conditions at planting date one (o·····o), planting date two (x——x), and planting date three (●— —●). The pathogen was added initially at 5000 chlamydospores per plant.

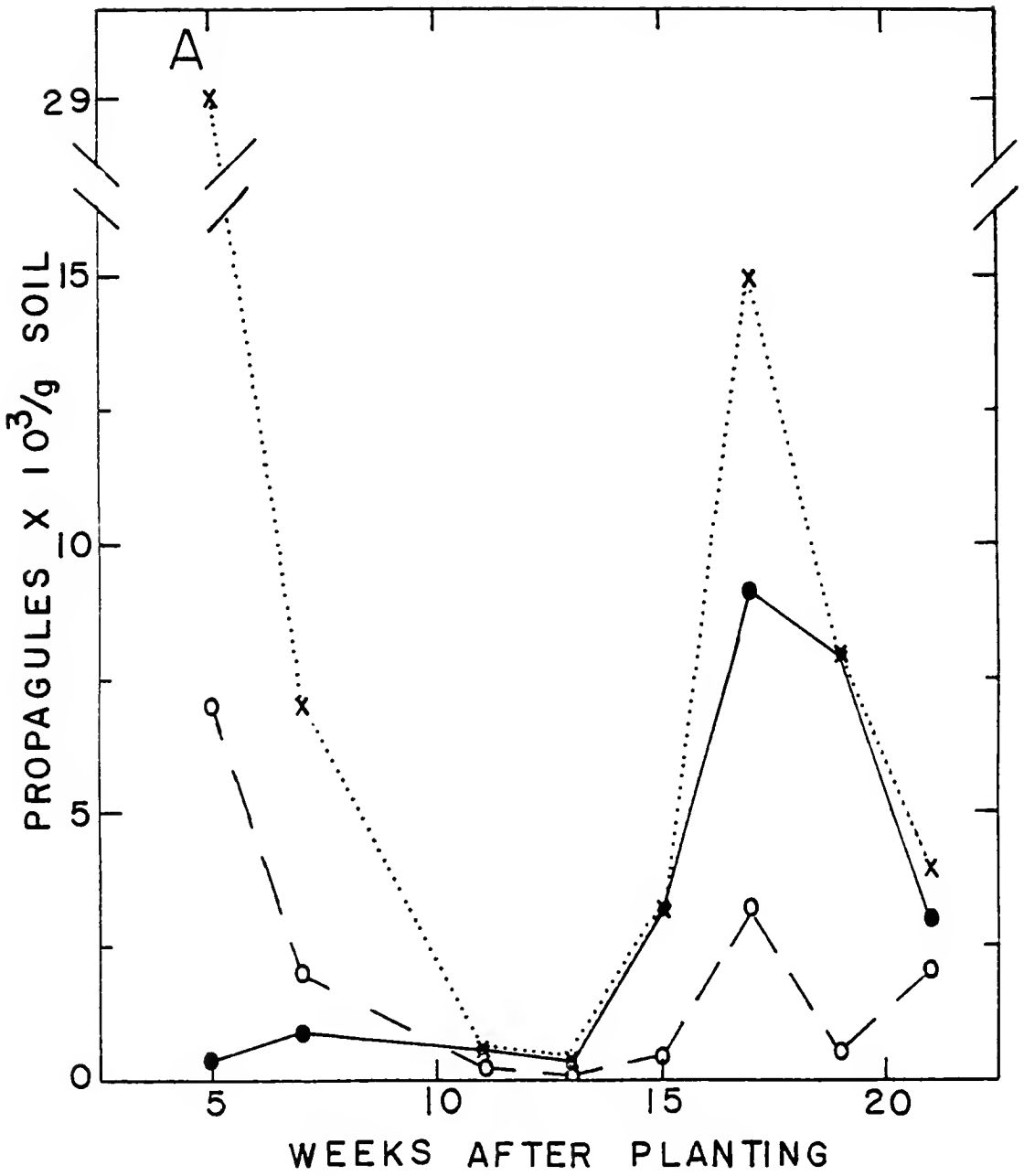

Figure 9 - A. Relationship of population density of Trichoderma spp. (x·····x), Aspergillus spp. (o— —o), and Penicillium spp. (●——●) to time after planting in soils amended with three isolates of T. harzianum, one isolate of A. ochraceus, and one isolate of P. restrictum at 5 X 10^5 conidia per isolate per plant under field conditions.

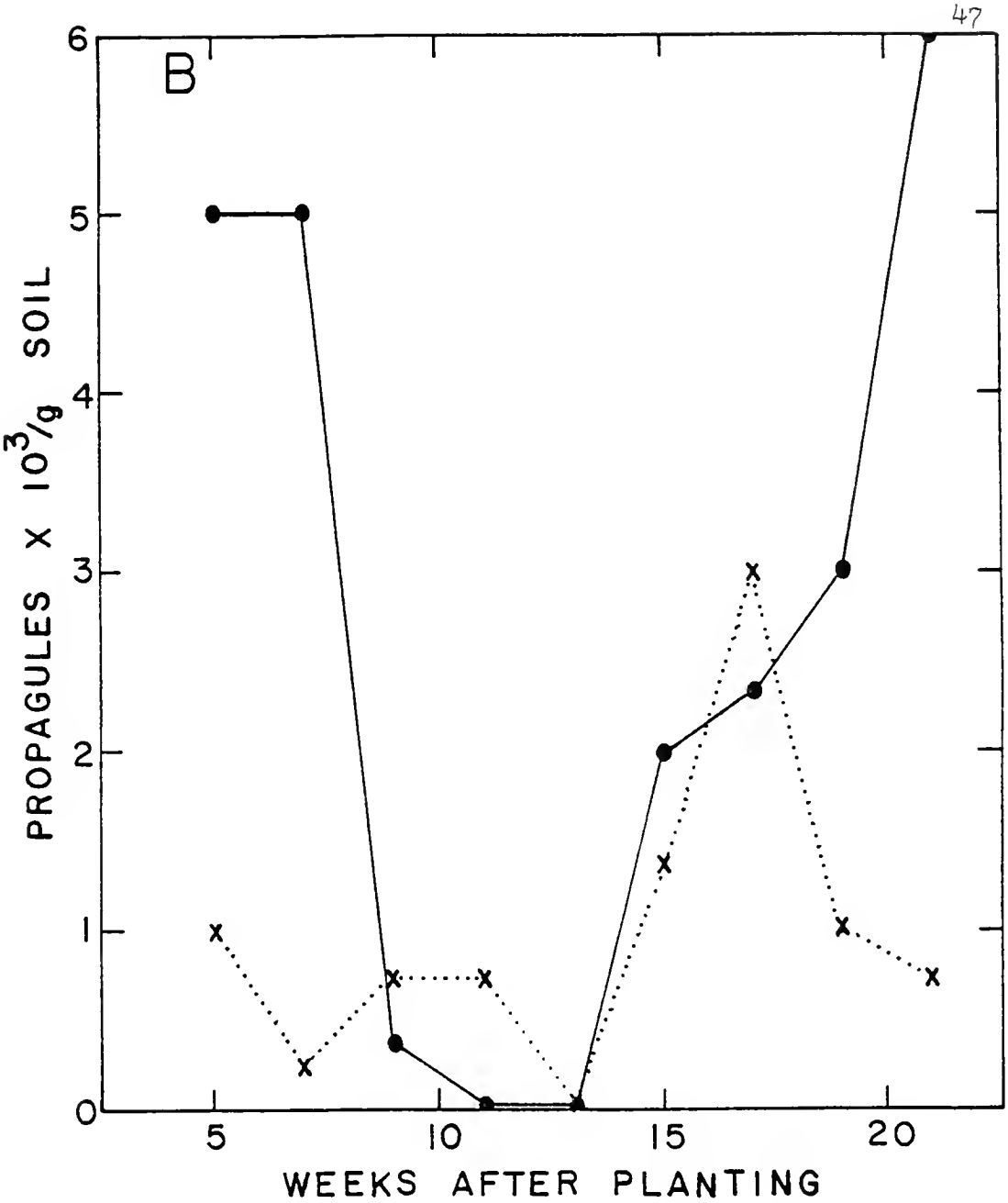

Figure 9 - B. Relationship of population density of Trichoderma spp. (x·····x), and Penicillium spp. (●——●) to time in nonamended soils under field conditions.

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 done under growth-chamber and greenhouse conditions (Section I).

In the past, antagonists usually have been selected for their ability to inhibit a pathogen under pure culture conditions (2). For several reasons these antagonists failed to reduce disease when applied under field conditions. Of the eight reasons that Baker and Cook (2) presented for such failures, the most important is probably the fact that the environmental conditions in agar are unrelated 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 (Section I, II).

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 (14). 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 lower inoculum densities of the pathogen in amended soils early in the season probably were responsible for the reduction of disease severity. Rowe and Farley (27) 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

amended soils late in the season may have been due to increases in populations of antagonists, which were greater in amended than in non-amended soils. The increase in the pathogen population in nonamended soils late in the season may have been due to sporulation on the above-ground lesions or to saprophytic proliferation of the pathogen on fresh plant debris. The lack of an increase in pathogen populations in amended soils also may be important in reducing the amount of inoculum available for infection in succeeding years.

The high populations of T. harzianum in amended soils and the failure to detect A. ochraceus in nonamended soils indicate that the addition of antagonists led to the establishment of populations of the organisms in amended soils; however, it was not possible to determine if the populations of the specific isolates of the antagonists actually originated from the added antagonists.

The applicability of the antagonist amendments to production systems is realized when one considers that three 15-cm 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 plant. The application of the antagonists by a drench either before or after planting could be effective in controlling the disease. It is important, however, that antagonists recolonize the soil before reinfestation by the pathogen occurs.

The increase in disease severity that follows the application of a broad spectrum biocide to soils has been reported (4,17,26,38). 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 (28). When the antagonists were applied to soils before recolonization by the pathogen could occur, they were able to effectively occupy the niche space created by the fumigation procedures (Section II). The preoccupied niche space 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 (15); therefore, antagonists need to be estabilshed 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.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptible standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Associate Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptible standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptible standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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