Influence of an Introduced Composite of Microorganisms on Infection of Tobacco by *Phytophthora parasitica* var. *nicotianae*

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ABSTRACT

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A composite of organisms that colonized tobacco roots rapidly in field soil was evaluated for its ability to compete with *Phytophthora parasitica* var. *nicotianae* for occupation of sites susceptible to infection within root systems of tobacco. The composite comprised organisms derived from the microbial community within the rhizosphere of tobacco. Numbers of root infections by the pathogen after 2 wk of tobacco growth in infested soil were not reduced within soils amended with the composite composed of propagules of *Trichoderma harzianum*, *Aspergillus carbonarius*, *Aspergillus terreus*, *Penicillium steckii*, and *Pseudomonas putida*. As determined from plate counts, amendment of soils with the composite was associated with increased densities of fungi and fluorescent *Pseudomonas* spp. around tobacco roots as compared to densities around roots in nonamended soils. However, no differences in density or patchiness (assessed by Lloyd's indices) of hyphae on root surfaces were observed. Survival of the pathogen in nonrhizosphere soil was not influenced by the introduced organisms. Amendment of pathogen-infested soils with the composite was associated with significant decreases in mortalities of tobacco after 90 days of plant growth in a glasshouse.

Attempts to control soilborne pathogens through manipulations of single microbial antagonists within plant rhizospheres have met with variable success (1). Selections of these individual isolates often have been based on a limited set of criteria, including expressions of antagonism such as inhibition of pathogen growth or hyperparasitism in vitro. When a selected organism is introduced into soil, the utility and degree of expression of these traits is uncertain.

Greater insight into effective means of manipulations of biological rhizosphere components towards control of a pathogen might be derived from investigations that focus on basic processes of population interactions around root systems of a particular crop species. Of initial importance is the question of how extensiveness and intensity of root system colonization by indigenous microorganisms influence the behavior of a pathogen at sites of importance to its activity. Investigations of these events would provide insight into patterns of population interactions that are characteristic of a particular crop-pathogen system. Such characterizations of population interactions at microsites and their impact on epidemic development also would provide a baseline for evaluations of the efficacy of subsequent manipulations of rhizosphere components towards reduced root infection and disease development. In particular, it would begin to allow for the decomposition of disease control mechanisms into components related to expressions of direct antagonism and population density effects at important microsites.

Investigations of this kind would be a helpful preliminary step to development of biological control of black shank of tobacco (*Nicotiana tabacum* L.), which is incited by *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker. Initial populations of this pathogen in soil are very low and highly aggregated (6,13); the pathogen is capable of little, if any, saprophytic growth. Populations build up rapidly in association with developing tobacco root systems as the result of secondary inoculum production, including sporangia and zoospores, on surfaces of infected roots (8,13). It is within the rhizosphere of tobacco where interactions between populations of *P. p. nicotianae* and microbial antagonists would be most effective in limiting disease.

Studies were established to evaluate colonization, by indigenous microorganisms, of regions within tobacco root systems that are susceptible to infection by *P. p. nicotianae*. Interactions between these two microbial populations were studied in both raw field soil and soil being recolonized after removal of indigenous populations. Additional studies were established to evaluate the relationship between colonization of critical regions when a portion of the indigenous microbial community was enhanced in number and infection by the pathogen.

MATERIALS AND METHODS

A composite of fungi and bacteria known to rapidly colonize developing tobacco root systems in raw field soil was used in all trials. The composite represented a subset of the microbial community encountered around these roots. In a previous series of experiments, fungi, bacteria, and actinomycetes that colonized the rhizosphere and root surfaces of tobacco were quantified by platings on appropriate media on a weekly basis during 4 wk of plant growth in field soil (4). Fungi were ranked in their order of dominance each week and over time. Single, randomly selected isolates of *Trichoderma harzianum* Rifai, *Aspergillus carbonarius* (Bainier) Thom, *A. terreus* Thom, *Penicillium steckii* Zaleski, and *Pseudomonas putida* (Trevisan) Migula were selected as dominant organisms over the course of isolations.

Each fungal isolate was grown on potato-dextrose agar for 2 wk at 25 C and 12 hr of light (300 $\mu E/m^2/sec$) per day. Conidia were washed from surfaces of colonies and concentrations were determined from counts in 20 separate hemacytometer fields. The isolate of *P. putida* was grown in King's medium B (15) for 24 hr at 25 C in the dark; bacterial cells then were pelleted by centrifugation, washed, and resuspended in 0.1 mM $MgSO_4$. Densities of bacteria were determined from optical densities of suspensions and calibrated to absolute numbers of bacteria per milliliter of suspension.

Field soil (Blichton sand) collected from Gainesville was passed through a 1-mm-mesh sieve before use in experiments. Autoclaved soil was treated for 1 hr on each of two successive days and was kept covered until used. Within 24 hr of treatment, propagules of selected organisms were added to autoclaved and nonautoclaved soils. Conidia and bacterial cells were combined in suspension and added to autoclaved or nonautoclaved field soil to establish a

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density of 1×10^5 colony-forming units (cfu) of each isolate per gram of soil.

Chlamydospores of isolate P-230 of *P. p. nicotianae* were produced axenically in liquid culture by the method of Tsao (28). Spore suspensions were prepared according to the method of Ramirez and Mitchell (24). Immediately after autoclaved and nonautoclaved soils were amended with the composite, chlamydospores were added to establish a density of 50 propagules per gram of soil. Chlamydospores were added to nonamended soils at the same density. Amended soils, infested with the pathogen, were added to 100-ml, polypropylene beakers according to the infested soil layer method (13). Upper, noninfested soil layers were amended with propagules of the composite to match conditions of the center, infested layers. A 2-wk-old seedling of the susceptible tobacco cultivar Hicks was transplanted into the thin, noninfested upper layer of soil in each container. Fifteen seedlings were transplanted in both amended and nonamended soils that had been infested with *P. p. nicotianae*. Control treatments consisted of six seedlings planted singly into beakers containing amended or nonamended soils that were not infested with the pathogen. Transplanted seedlings were maintained in watering trays under clear plastic in a plant growth room at 25 ± 2 C under 16 hr of light (700 $\mu E/m^2/sec$) per day. Plants were watered from below by flooding trays to a depth of 1 cm for about 3 min on alternate days. Trays were maintained at a slight angle to drain excess water.

Patterns of infections of tobacco roots by *P. p. nicotianae* were assessed after 14 days of plant growth. Fifteen asymptomatic plants were removed from each infested soil. Tops of seedlings were removed, and root systems were surface-disinfested in 70% ethanol and rinsed in deionized water. Each root system was dissected according to the classification scheme of the morphometric root analysis system (7), and root segments were plated on selective medium (21). After 48 hr of incubation in the dark, roots were examined for emergence of colonies of the pathogen. Inoculum efficiency was determined from the ratio of the average number of infected roots per plant to the total numbers of chlamydospores in the volume of soil containing each plant (approximately 3,250 chlamydospores). Within autoclaved or nonautoclaved soil, the influences of the introduced composite of organisms on infection and inoculum efficiency were evaluated by F tests.

Colonization of nonrhizosphere soil, tobacco rhizospheres, and root surfaces by organisms making up the composite also was evaluated after 14 days of plant growth in soil amended and not amended with the composite. Ten plants were removed from each soil and were bulked by treatment. Populations of introduced species as well as other fungi and bacteria within rhizospheres, at root surfaces, and in nonrhizosphere soil were estimated as described previously (4). Rhizosphere soil in these experiments was considered to consist of the soil that remained adhered to root systems after gentle shaking.

Six additional infected tobacco root systems from each amended soil infested with the pathogen and three root systems from each noninfested soil were evaluated for root system development as described previously (5). In these experiments, root systems were defined according to the scheme put forth in the morphometric root analysis system described by Fitter (7). Any root that terminates in an apical meristem is defined as a first-order root. Where two first-order roots merge, there begins a second-order root. Where two second-order roots merge, there begins a third-order root and so forth. The union of a particular root element with that of a higher order does not alter the classification of the element of the latter root order. Estimates were made of the average numbers, total lengths, and average lengths of elements within selected root orders as defined in the morphometric root analysis system.

Branching ratios were determined as a measure of total root system complexity (5,7). This parameter describes the number of roots in each order relative to the number of roots in the next higher order. Branching ratio is a function of how root elements within a root system are joined together; it is derived from the linear relationship between the log of numbers of elements and root order number. The antilog of the absolute value of the slope of this line is defined as the branching ratio.

First-order roots of tobacco were found in earlier experiments to be the region of greatest susceptibility to infection by *P. p. nicotianae* (3). Therefore, fungal colonization of these roots was evaluated. Colonization of first-order root surfaces of three of the plants evaluated for growth within each soil was evaluated as described by English and Mitchell (4). Dispersion of fungal hyphae on surfaces was characterized by use of Lloyd's indices of mean crowding and patchiness (19). Influences of the introduced microbial composite on root system development and patterns of root surface colonization were evaluated separately within each soil by F tests. Trials of competition between composite organisms and *P. p. nicotianae* were repeated once.

Interactions between populations of composite microorganisms and *P. p. nicotianae* in nonrhizosphere soil were also examined. Autoclaved or nonautoclaved soil was either amended with 1×10^5 cfu of each composite organism or left nonamended. Fifty chlamydospores of the pathogen were added per gram of each of these soils. One kilogram of each soil was moistened to 15% gravimetric soil moisture content and placed into individual closed plastic containers with small holes to allow air exchange. Containers were weighed daily, and deionized water was added as needed to maintain desired soil moisture. Periodically during a 56-day period, a single soil sample was taken from each treatment combination to a depth of 3 cm with a surface-disinfested cork borer. Samples were diluted appropriately and plated on selective medium (21). After 48 hr of incubation in the dark, plates were examined for colonies of the pathogen. Experiments were conducted four times. At each sampling date, the average density of *P. p. nicotianae* in each treatment was determined over the four experiments. Treatment means at each date were compared by Duncan's multiple range test (18).

The influence of the introduced composite on long-term black shank development was evaluated in glasshouse experiments. Two hundred grams of autoclaved or nonautoclaved field soil was infested with five chlamydospores of the pathogen per gram of soil. Each field soil also had either been amended or not amended with the composite. Each batch of soil was layered over autoclaved builder's sand in a 10-cm-diameter pot. Over this were layered 200 g of autoclaved or nonautoclaved soil amended with 1×10^5 cfu of each composite isolate. One 4-wk-old Hicks tobacco plant was transplanted into each of 15 pots per treatment. Control treatments consisted of 15 Hicks seedlings transplanted in the same manner into autoclaved or nonautoclaved soil, which had not been amended with the composite or infested with the pathogen.

Plants were maintained for 90 days between 16 and 30 C and were watered from above on alternate days; half-strength Hoagland's solution (10) was added in place of water twice each week. Plants were examined every 5 days for expression of black shank symptoms, including wilting and stem discoloration. To confirm infection by *P. p. nicotianae*, root systems of symptomatic plants were removed from soil, surface-disinfested in 70% ethanol as before, and plated onto selective medium (21). Experiments were conducted three times. For each treatment, the average proportion of mortality at 90 days was determined from the three experiments. Data were transformed by arcsine and treatment effects were evaluated by planned F tests.

RESULTS

Results presented are from one set of experiments. Similar results were obtained when experiments were repeated. Amendment of autoclaved or nonautoclaved field soil with a composite of microorganisms was associated with increases in propagule densities of total fungi and fluorescent *Pseudomonas* spp. within rhizospheres and at root surfaces of Hicks tobacco as compared to corresponding densities in nonamended soils (Table 1; Fig. 1). Large increases in densities of these organisms were noted in nonrhizosphere soils as well. However, densities of introduced fungi and fluorescent *Pseudomonas* spp. in rhizosphere and nonrhizosphere soils were not maintained at the levels initially

established.

After 2 wk of plant growth in autoclaved soil amended with the composite, *T. harzianum* dominated the fungal community in the rhizosphere (Fig. 2). The species dominated the community in nonrhizosphere soil as well. The fungal community associated with root surfaces was not dominated by any single fungal species (Fig. 2). However, *A. carbonarius* occurred at low densities in this region. Within rhizospheres and at root surfaces of plants grown in nonautoclaved soil amended with the composite, *T. harzianum* was one of the least commonly recovered fungal species (Fig. 3). Communities in all regions of this soil were dominated by combinations of other species of the introduced composite.

Amendment of autoclaved or nonautoclaved soil with the composite was not associated with significant alterations ($P > 0.05$) in patterns of fungal colonization of first-order root surfaces (Table 2). However, average hyphal lengths and mean crowding were greater ($P < 0.05$) in association with roots from nonautoclaved soil than from autoclaved soil. Conversely, Lloyd's index of patchiness was greater in association with roots from autoclaved soil than from nonautoclaved soil.

Amendment of autoclaved or nonautoclaved soil with the composite was not associated with significant reductions in numbers of infected roots per infected plant (Table 3). In a similar manner, inoculum efficiency was not altered significantly by amendment of soil with the composite.

Analyses of treatment influences on root growth were restricted to elements within first- and second-order classes as roots within higher orders formed very late during the 14 days of growth after seedling transplant. Root system development was not altered significantly by amendment of soils with the composite. Additionally, in autoclaved or nonautoclaved soil the numbers and total lengths of first- and second-order roots of infected plants and noninfected tobacco plants (from noninfested soil) did not differ significantly (Figs. 4 and 5). The complexity of root system development, as estimated by branching ratios, was not altered significantly by amendment of either autoclaved or nonautoclaved soil with the composite of microorganisms (Table 4). In a similar manner, root system complexity was not altered significantly by infection with the pathogen.

Amendment of autoclaved or nonautoclaved nonrhizosphere soil with the composite did not reduce population densities of *P. p. nicotianae* significantly over a 56-day period (Fig. 6). Mean densities of the pathogen in both amended and nonamended soils

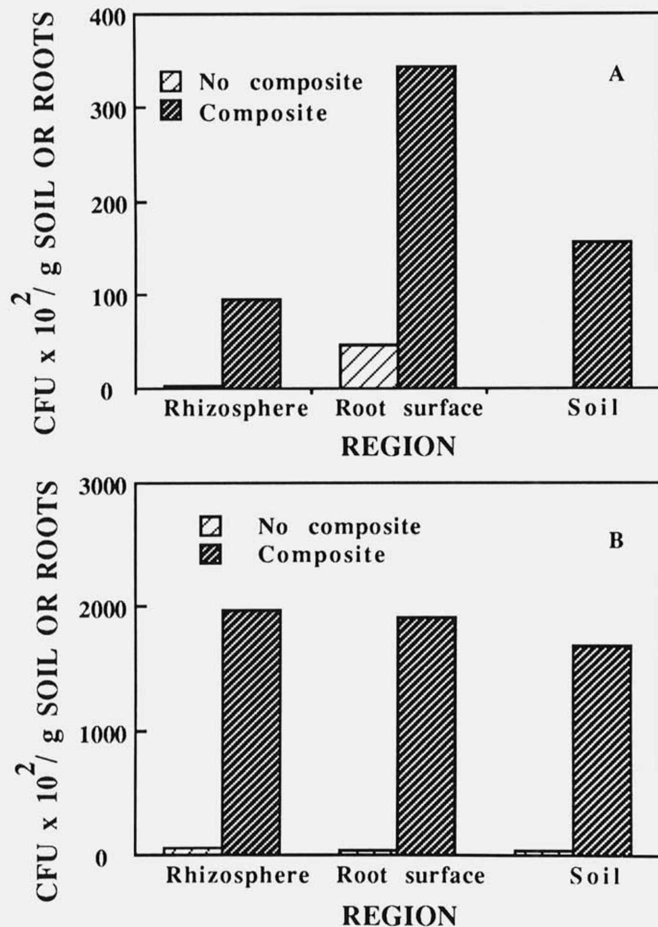

Fig. 1. Population densities of fluorescent *Pseudomonas* spp. around tobacco root systems after 14 days of plant growth in **A,** nonautoclaved or, **B,** autoclaved soil that had or had not been amended with *Trichoderma harzianum*, *Aspergillus terreus*, *A. carbonarius*, *Penicillium steckii*, and *Pseudomonas putida*.

TABLE 1. Influence of an introduced composite of fungi and bacteria on population densities of total fungi associated with the rhizospheres and root surfaces of Hicks tobacco and nonrhizosphere soil after 14 days of plant growth in nonautoclaved and autoclaved soils

		Total fungi (cfu $\times 10^2$/g of soil or roots[a])		
Soil	Composite	Rhizosphere	Root surface	Soil
Autoclaved	–[b]	334.5	4.3	2.4
	+	5335.2	2976.6	4320.0
Nonautoclaved	–	101.5	91.0	209.8
	+	408.6	199.2	1421.5

[a] Colony-forming units $\times 10^2$ per gram of oven-dried soil or roots estimated from soil or root-surface suspensions plated onto potato-dextrose agar amended with chlortetracycline hydrochloride and Tergitol NP-10.

[b] – is composite of organisms not added to soil; + is 10^5 conidia of each of *Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii,* and 10^5 bacterial cells of *Pseudomonas putida* added per gram of autoclaved or nonautoclaved field soil.

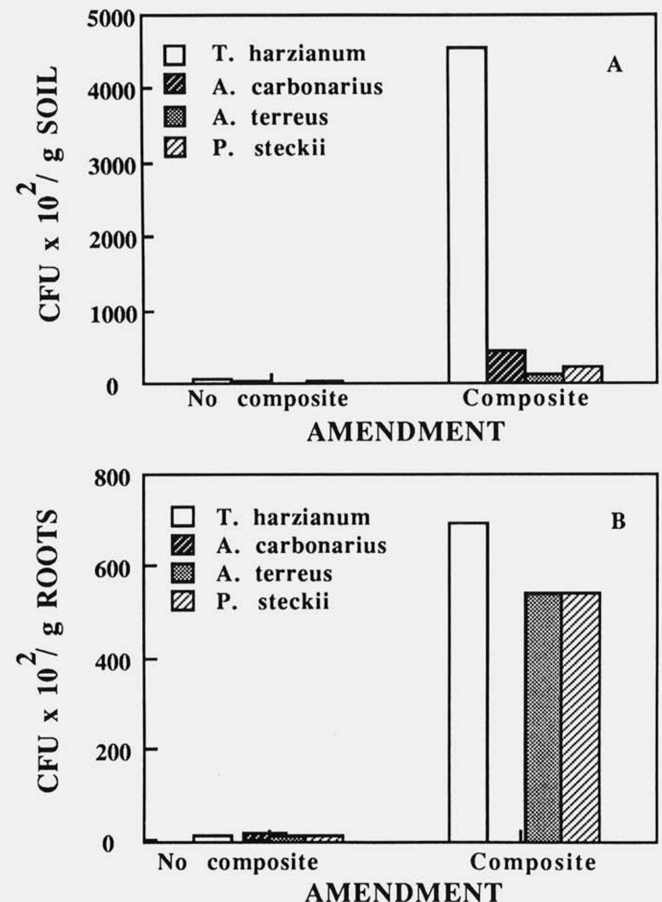

Fig. 2. Densities of introduced fungal species within the **A,** rhizosphere and, **B,** at root surfaces of tobacco after 14 days of growth in autoclaved soil that had or had not been amended with a composite of these four fungal species and *Pseudomonas putida*.

declined steadily over time after brief initial increases. Maximum densities within each treatment were attained between days 2 and 7 and did not differ significantly ($P = 0.05$). After this period of increase, propagule densities declined in all soils. At each sampling date, densities did not differ significantly. Viable propagules of the pathogen remained in each soil after 56 days. At the end of each

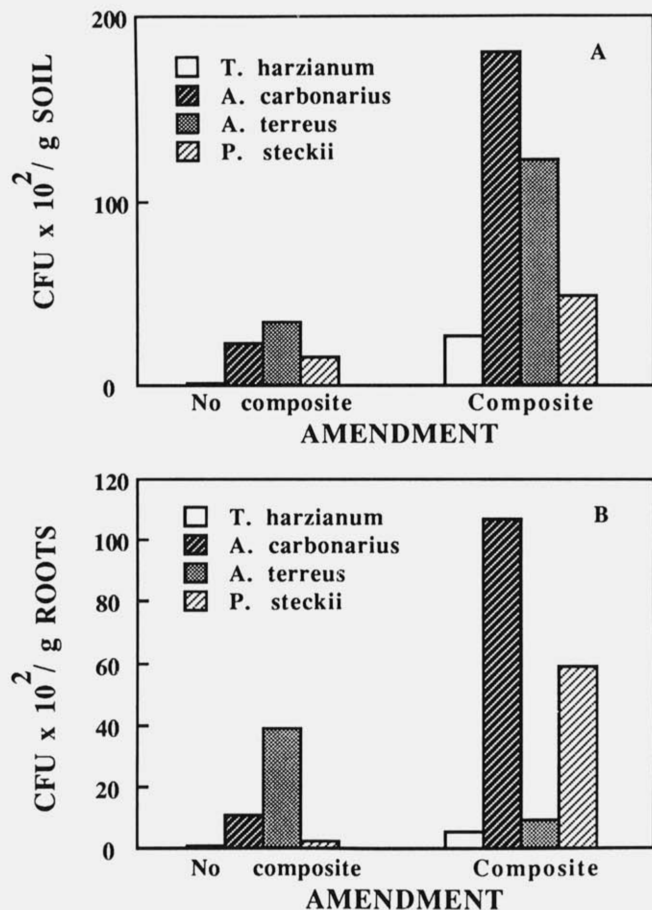

Fig. 3. Densities of introduced fungal species within the **A,** rhizosphere and, **B,** at root surfaces of tobacco after 14 days of growth in nonautoclaved soil that had or had not been amended with a composite of these four fungal species and *Pseudomonas putida*.

TABLE 2. Influence of an introduced composite of fungi and bacteria on fungal colonization of surfaces of first-order roots of Hicks tobacco after 14 days of plant growth

Soil	Composite	Length of hyphae (cm)/ 10 cm of first-order roots[a]	Mean crowding[b]	LIP[c]
Autoclaved	–[d]	0.8[e]	8.9[e]	19.9[e]
	+	0.4	4.1	15.9
Nonautoclaved	–	16.2	55.3	5.7
	+	11.2	51.2	7.6

[a] Average lengths of hyphae were estimated by using the line intersect method of Tennant (27); values are the averages of numbers of hyphal intersects with reticule grid lines in up to 100 microscope fields selected along the lengths of first-order roots of three seedlings.

[b] Mean crowding is $\overset{*}{m} = m + m/k$; where m equals mean number of hyphal intersects with reticule grid lines per examined microscope field and k equals the dispersion parameter as derived from the negative binomial distribution.

[c] LIP is Lloyd's index of patchiness where $\overset{*}{m}/m = 1 + (1/k)$.

[d] – is composite of organisms not added to soil; + is 10^5 conidia of each of *Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii*, and 10^5 bacterial cells of *Pseudomonas putida* added per gram of autoclaved or nonautoclaved field soil.

[e] Within autoclaved or nonautoclaved soil, the influences of the composite on parameters of colonization were not significant ($P > 0.05$).

TABLE 3. Influence of an introduced composite of fungi and bacteria on observed infections caused by *Phytophthora parasitica* var. *nicotianae* on roots of Hicks tobacco and inoculum efficiency after 14 days of plant growth in field soil infested with 50 chlamydospores of the pathogen per gram of soil

		Infected roots[a]					
		Root order					
Soil	Composite	1[b]	2	3	4	Total plant	Inoculum efficiency[c]
Autoclaved	–[d]	5.9	1.0	0.2	0.0	7.1[e]	0.001[e]
	+	4.3	0.8	0.2	0.0	5.3	0.001
Nonautoclaved	–	8.3	2.2	0.4	0.0	10.9	0.003
	+	5.7	0.9	0.3	0.0	6.9	0.001

[a] Numbers of infected roots per root order or individual plant were determined as the average of up to 15 asymptomatic, infected root systems that had been dissected completely by root order and plated onto selective medium (21).

[b] Root orders are as defined in the morphometric root analysis system (7).

[c] Efficiencies were determined from the average of the ratios of numbers of infected roots per seedling to total number of chlamydospores (approximately 3,250 chlamydospores) within the voume of soil containing each seedling.

[d] – is composite of organisms not added to soil; + is 10^5 conidia of each of *Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii*, and 10^5 bacterial cells of *Pseudomonas putida* added per gram of autoclaved or nonautoclaved field soil.

[e] Within autoclaved or nonautoclaved soil, the influence of the composite on total infections per seedling and inoculum efficiency were not significant ($P > 0.05$).

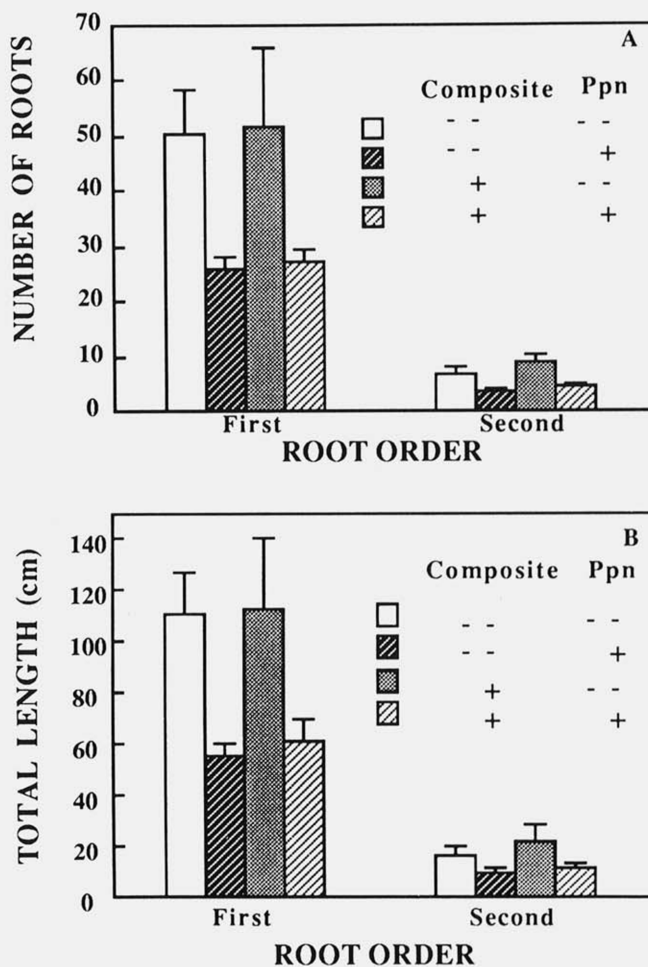

Fig. 4. A, Number and, **B,** total lengths of first- and second-order roots per healthy or infected tobacco plant after 14 days of growth in nonautoclaved soil in the presence (+) or absence (- -) of *Phytophthora parasitica* var. *nicotianae* and in the presence (+) or absence (- -) of the composite of microorganisms. Bars represent standard errors.

experiment, five 2-wk-old Hicks tobacco seedlings were transplanted into each infested soil. All seedlings in all treatments died from black shank.

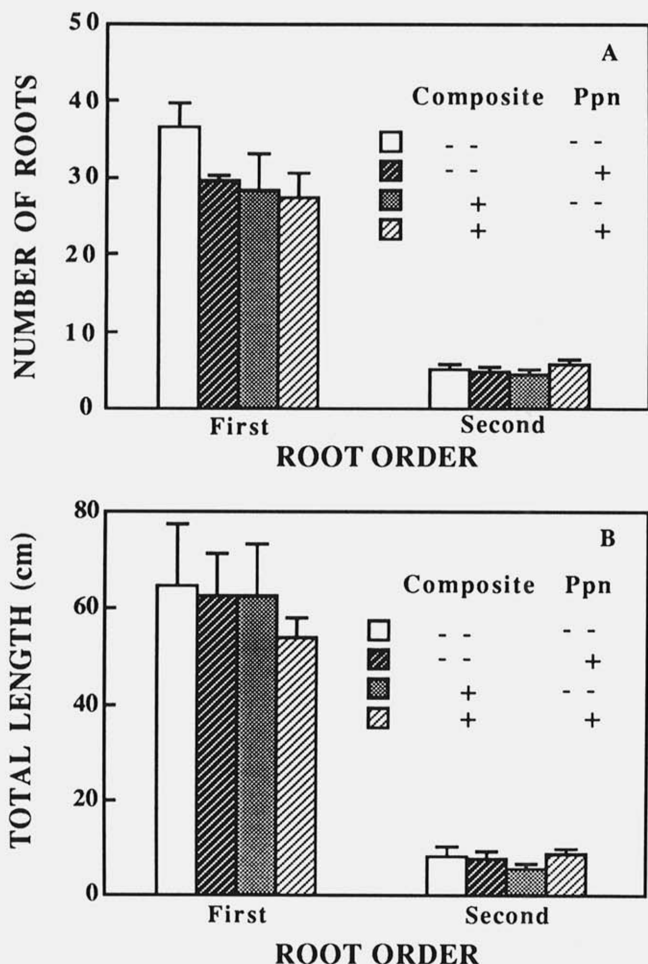

Fig. 5. A, Number and, **B,** total lengths of first- and second-order roots per healthy or infected tobacco plant after 14 days of growth in autoclaved soil in the presence (+) or absence (- -) of *Phytophthora parasitica* var. *nicotianae* and in the presence (+) or absence (- -) of the composite of microorganisms. Bars represent standard errors.

TABLE 4. The influence of an introduced composite of fungi and bacteria on the relationship between infection of Hicks tobacco by *Phytophthora parasitica* var. *nicotianae* and branching ratios after 14 days of plant growth in field soil artificially infested with propagules of the pathogen

Soil	Composite	Chlamydospores/ g of soil	R_b[a]
Autoclaved	–[b]	0	5.8(0.1)[c,d]
		50	5.4(0.4)
	+	0	5.3(0.5)
		50	4.1(0.5)
Nonautoclaved	–	0	5.7(0.9)
		50	5.1(0.2)
	+	0	3.3(0.4)
		50	4.8(0.4)

[a] R_b is branching ratio as defined in the morphometric root analysis system (7).

[b] – is composite of organisms not added to soil; + is 10^5 conidia of each of *Trichoderma harzianum*, *Aspergillus terreus*, *A. carbonarius*, *Penicillium steckii*, and 10^5 bacterial cells of *Pseudomonas putida* added per gram of autoclaved or nonautoclaved soil.

[c] Noninfested soil values are the averages of numbers of elements per root order of three healthy plants; infested soil values are from six infected root systems. Values in parentheses are standard errors.

[d] In autoclaved or nonautoclaved soil, neither amendment of soil with the composite nor plant infection by *P. p. nicotianae* influenced R_b significantly ($P > 0.05$).

During 90 days of Hicks tobacco growth in infested soils, black shank developed more slowly in nonautoclaved soil than in autoclaved soil, regardless of amendment (Table 5). Nonautoclaved soil that had not been amended with the composite appeared to be suppressive; mortality of tobacco in this soil was significantly less than tobacco mortality in autoclaved, nonamended soil. Average mortality of tobacco was significantly less in autoclaved or nonautoclaved soil that had been amended with the composite than in corresponding nonamended soils ($P < 0.05$). After 90 days, *P. p. nicotianae* was isolated from all root systems of remaining live plants in amended, autoclaved soil. The pathogen was not isolated from roots of any asymptomatic plants from nonautoclaved soil with or without the composite. Times required to attain 10% plant mortality and to increase from 10 to 50% mortality were similar in autoclaved soils amended or not amended with the composite. The time required for increase in mortality from 10 to 90% in autoclaved, nonamended soil was 53 days; there were insufficient increases in black shank within autoclaved, amended soil to estimate this period of increase. Estimates of these parameters describing disease development could not be developed for tobacco grown in nonautoclaved soils because of insufficient mortality data.

DISCUSSION

As determined from soil plating, densities of total fungi and fluorescent *Pseudomonas* spp. around tobacco roots were increased by the amendment of soil with propagules of a subgroup of the microbial community indigenous to nonautoclaved field soil. In contrast, as determined by direct observation of first-order

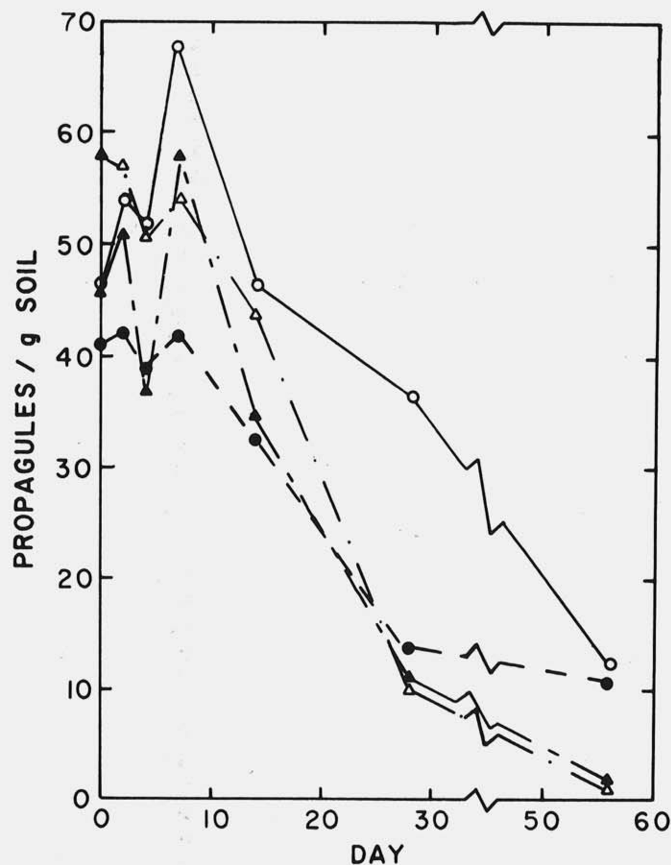

Fig. 6. The relationship of population density of *Phytophthora parasitica* var. *nicotianae* to time in autoclaved field soil not amended (o) and amended (●) with a composite of microorganisms, and in nonautoclaved field soil not amended (Δ) or amended (▲) with the composite. The composite of microorganisms comprised propagules of *Trichoderma harzianum*, *Aspergillus terreus*, *A. carbonarius*, *Penicillium steckii*, and *Pseudomonas putida*. Soil was infested initially with 50 chlamydospores of the pathogen per gram of soil.

root surfaces, amendment of soils with the composite did not alter the intensiveness or extensiveness of fungal colonization of root tissues. However, the species used in the present study were not found to be dominant organisms on root surfaces of various plant species in several earlier studies (9,23,26); rather these species occurred predominantly within rhizosphere soil. Estimates of high densities of fungi derived from platings of root surface suspensions likely resulted from colonies that developed from nongerminated conidia or conidia that had germinated but produced only limited mycelial growth. Contrasts in estimates of populations by the two techniques emphasize the difficulty of interpretation of biological function from population estimates made from plate counts; the forms of organisms must be considered.

The extent of colonization of rhizospheres and root surfaces of Hicks tobacco and of nonrhizosphere soils by introduced competing organisms varied between soil ecosystems. In autoclaved soil, conidia of *T. harzianum* must have germinated readily. Rapid colonization of nonrhizosphere soil and tobacco root systems by this fungus may have restricted proliferation of other introduced fungi and their ability to colonize these regions. Large populations of fluorescent *Pseudomonas* spp. also may have hindered colonization by introduced fungi. Reductions in root surface colonization by soilborne fungi in the presence of high densities of fluorescent *Pseudomonas* spp. have been demonstrated by Kloepper and Schroth (15,16). Although it was impossible to identify fluorescent *Pseudomonas* spp. recovered from soil, it is likely that most were derived from the isolate added initially.

Lack of increases in densities of introduced fungi and bacteria in all regions of nonautoclaved soil is difficult to interpret. In nonrhizosphere soil fairly constant population densities of introduced fungal species may be related to fungistasis and antagonistic interactions with other microorganisms (20). Similar mechanisms may have limited increases in populations of fluorescent *Pseudomonas* spp. Steiner and Lockwood (25) reported inverse correlations between sizes of fungal spores and sensitivities to fungistasis. The relatively small conidia produced by fungal species used in this study likely would have been quite sensitive to such influences. Further support of this probability comes from investigations by Papavizas et al (22) and Lewis and Papavizas (17) in which proliferation of *Trichoderma* spp. and other fungal genera introduced into soil as conidia was limited by a lack of available nutrients. In contrast, chlamydospores and mycelium of various fungi added to soil with an attached food base were much more successful in proliferating within nonautoclaved soil amidst an established microbial community.

Whether amendment of soil with nutrients would have improved the efficacy of introduced organisms in reducing infections of roots by *P. p. nicotianae* is uncertain. The goal in the present investigation was not to establish high densities of antagonists in nonrhizosphere soil; rather the intention was to establish a stable community of microorganisms of high density in close association with tobacco roots. It has been suggested that fungistasis might be reduced within the rhizosphere in response to nutrients released from roots (2,20). Only a portion of introduced conidia need have germinated under such influences for introduced fungi to have become established around roots. Similarly a proportion of the population of introduced bacterial cells may have become active under the influences of released nutrients in the vicinity of roots. Intense antagonistic interactions within this region may have limited development of mycelium, further spore germination, and bacterial cell division and thus may have limited increases in densities of detectable fungal or bacterial propagules.

Increases in population densities around tobacco roots were not associated with significant reductions in root infection by *P. p. nicotianae*. Significant reductions in root infection might have been attained either by reducing the initial inoculum density of the pathogen or by increasing the period of plant growth in infested soil. Extension of plant growth period, however, would produce difficulties associated with the logistics of handling much larger root systems and maintaining adequate replications.

Numbers and lengths of roots of infected and healthy plants also did not differ significantly. Again, differences may have become consistently significant with longer periods of plant growth as infected roots became necrotic.

In contrast to growth room experiments, suggestions of enhanced root system colonization brought about by the introduced composite and reduced infection of tobacco were derived from competition trials in a glasshouse. Amendment of nonautoclaved and autoclaved soils that were infested with fewer propagules of *P. p. nicotianae* than in growth room experiments was associated with consistent, significant reductions in tobacco mortality. Even further reductions in mortality may have been attained by colonization of roots with the composite before transplant of seedlings into infested soils. In a preliminary field trial, colonization of tobacco roots by this composite of organisms before outplant to an infested field was associated with significant reductions in mortality at the end of the growing season (English and Mitchell, *unpublished*).

The composite of fungi and bacteria evaluated within these experiments comprised organisms indigenous to field soil. These organisms were selected only for rapid colonization of tobacco root systems. In greenhouse trials amendment of soil with these organisms increased microbial populations around tobacco roots and reduced tobacco mortality in soils infested with *P. p. nicotianae*. Whether this implies that more complete coverage of important microsites was brought about, thus excluding the pathogen, is not certain. It remains to repeat such experiments with other subgroups of organisms selected from the soilborne community capable or incapable of colonizing tobacco roots. It will also be important to repeat these experiments with individual and combined isolates that made up the composite evaluated in the present investigation.

Microsite occupation is a criterion of prime importance to selections of antagonists; it is a criterion that must be satisfied before selections for expressions of direct antagonism. In regards to this particular composite, it is not known what antagonistic traits, if any, characterized these isolates. Improvements in performance of a composite might be attained by further screening of selected isolates for other traits of antagonism or by selections of isolates known to colonize additional regions of soil ecosystems critical to pathogen activity. Additionally, colonization of root systems by introduced organisms might be improved by use of more appropriate propagules of these organisms.

TABLE 5. Influence of an introduced composite of fungi and bacteria on development of black shank in Hicks tobacco grown in soil infested initially with five chlamydospores of *Phytophthora parasitica* var. *nicotianae* per gram of soil[a]

Soil	Composite	Disease development[b] (days)			Mortality at harvest (%)[c]
		t_{10}	t_{10-50}	t_{10-90}	
Autoclaved	–[d]	25	27	53	100[f]
	+	26	28	–[e]	69
Nonautoclaved	–	42	–	–	16
	+	–	–	–	7

[a] Data reported are based on means of three trials.
[b] Days required to attain 10% plant death (t_{10}) or for mortality to increase from 10 to 50% (t_{10-50}) or from 10 to 90% (t_{10-90}) as interpolated from linear regression analyses.
[c] Plant mortality with 15 plants per treatment 90 days after planting.
[d] – is soil not amended with the composite of organisms; + is 10^5 conidia of each of *Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii*, and 10^5 bacterial cells of *Pseudomonas putida* were added per gram of autoclaved or nonautoclaved field soil.
[e] – is insuffcient disease to estimate value.
[f] Within autoclaved or nonautoclaved soil, amendment with the composite reduced mortality ($P < 0.05$).

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