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Title: THE NATURE OF FUSARIUM EXCLUSION FROM CONIFEROUS FOREST
SOILS: EVIDENCE OF DIFFERENTIAL MICROBIAL INVOLVEMENT IN
FOREST VS. NURSERY SOILS

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Abstract approved:

Dr. Robert G. Linderman

Fusarium was recovered from only one of fourteen Pacific Northwest coniferous forest soils but from all seven nursery soils tested. Assays using high concentrations of macroconidia added to soil determined that forest soils stimulate macroconidial germination (\bar{x} =12%) often followed by germ tube lysis or the formation of stunted chlamydospores while nursery soils support little macroconidial germination (\bar{x} =1.5%). Treatment of forest soils with aerated steam (minimum of 45° C for 30 min), radiation or propylene oxide reduced the amount of germination in forest soils, suggesting that the forest soil microbiota is involved in the stimulation of macroconidial

germination. Bacteria isolated from Fusarium hyphae placed in forest, field or nursery soils on nylon screens did not significantly influence germ tube lysis when combined with macroconidia in a nutrient broth. Chemical, physical, and nutritional properties of forest soils likely affect the capacity of the forest soil microbiota to influence Fusarium macroconidial germination and germ tube lysis.

The Nature of Fusarium Exclusion
from Coniferous Forest Soils:
Evidence of Differential Microbial Involvement
in Forest vs. Nursery Soils

by

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This thesis is dedicated to my parents, Lois and Lee, and my brother, Lee. Their love strengthens me in all walks of life.

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THE NATURE OF FUSARIUM EXCLUSION FROM CONIFEROUS
FOREST SOILS: EVIDENCE OF DIFFERENTIAL MICROBIAL
INVOLVEMENT IN FOREST VS. NURSERY SOILS

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SUMMARY

Fusarium was recovered from only one of fourteen Pacific Northwest coniferous forest soils but from all seven nursery soils tested. Assays using high concentrations of macroconidia added to soil determined that forest soils stimulate macroconidial germination (\bar{x} =12%) often followed by germ tube lysis or the formation of stunted chlamydospores while nursery soils support little macroconidial germination (\bar{x} =1.5%). Treatment of forest soils with aerated steam (minimum of 45° C for 30 min), radiation or propylene oxide reduced the amount of germination in forest soils, suggesting that the forest soil microbiota is involved in the stimulation of macroconidial germination. Bacteria isolated from Fusarium hyphae placed in forest, field or nursery soils on nylon screens did not significantly influence germ tube lysis when combined with macroconidia in a

nutrient broth. Chemical, physical, and nutritional properties of forest soils likely affect the capacity of the forest soil microbiota to influence Fusarium macroconidial germination and germ tube lysis.

INTRODUCTION

Fusarium spp. cause a variety of economically important diseases in conifer nurseries. Pathogen manifestations include seed rot, pre- and post-emergence damping-off, cotyledon blight and root rot or late damping-off (Bloomberg and Lock 1972). Although Fusarium-caused seedling mortality often reaches epiphytotic levels in the nursery (Bloomberg 1981), the disease is non-existent in coniferous forest soils covered with a needle litter. The absence of Fusarium spp. from forest soil has been known for some time (Morrow 1932; Thornton 1960a; Park 1963). In 1967, Smith showed that Fusarium populations declined rapidly on the roots of sugar pine (Pinus lambertiana) seedlings transplanted to a native pine forest in the Sierra Nevada mountains of California. Toussoun, Menzinger and Smith (1969) implicated water extracts of needle litter in the Fusarium decline since the extracts stimulated Fusarium chlamydospore germination followed by germ tube lysis without the formation of replacement chlamydospores. Purified amino acids (Menzinger 1969) and organic acids (Hammerschlag and Linderman 1975) naturally occurring in needle litter extracts were shown to induce the stimulation-lysis effect on chlamydospores in non-sterile soil.

The importance of the forest soil biota to the survival of Fusarium in conifer forest soil is unknown. Many investigators, however, have linked soil microorganisms to the elimination or sup-

pression of Fusarium in soil. Organisms cited include bacteria (Marshall and Alexander 1960; Huber and Andersen 1966; Huber et al. 1966; Smith and Snyder 1972; Kawamoto and Lorbeer 1976; Smith 1977; Alabouvette et al. 1979; Scher and Baker 1980, 1982), actinomycetes (Meredith 1944; Messiaen et al. 1965; Huber et al. 1966; Komada and Ezuka 1970; Arjunarao 1971a, 1971b), fungi (Rouxel et al. 1979; Lin and Cook 1979; Marois and Mitchell 1981; Marois et al. 1981), and an unidentified biotic complex (Burke 1965). Furthermore, fungi (Bernhauer and Waelsh 1932; Bernhauer and Gorlech 1935) and bacteria (Ishikawa and Oki 1959) can convert shikimic and quinic acids (present in needle leachates, Muir et al. 1964) into phenolic acids such as protocatechuic and gallic acids which can inhibit fungi (Angell et al. 1935; Mukherjee and Kundu 1973).

The purpose of this study was to determine comparatively the extent of Fusarium spp. exclusion from coniferous forest soils and nursery soils in Oregon and Southern Washington, and to compare the effects of microorganisms in forest and nursery soils on the germination response of macroconidia with thoughts toward the potential of forest soil as a source of biocontrol agents.

MATERIALS AND METHODS

Soil Sites and Preparation

Soils from 14 forest sites and 7 nursery sites were sampled at the end of September after seasonal rains had begun for most of the Pacific Northwest. Forest sites were located in the region from Yakima County, Washington, to Benton County, Oregon. Nursery sites were located from Skamania County, Washington, to Douglas County, Oregon. Sites were distributed over a wide range of elevations, soil types and slopes. Dominant conifer species in forest sites included one or more of the following: silver fir (Abies amabilis), grand fir (Abies grandis), Douglas-fir (Psuedotsuga menziesii), ponderosa pine (Pinus ponderosa), lodgepole pine (Pinus contorta), and western hemlock (Tsuga heterophylla). Herbaceous forest floor vegetation was sparse due to preferential selection of sites with closed conifer canopies and well developed needle litter layers. Douglas-fir was the predominant species grown in the nursery sites. At each site, three or four soil samples were collected from the top 0-10 cm of mineral soil, pooled, and refrigerated until processing at Corvallis. All pooled samples were sieved through 2 mm mesh prior to any experimental use.

Soil *Fusarium* Population Assay

One gram of air dry soil from each site was serially diluted using 0.1% water agar and plated on Peptone, PCNB Agar (PPA) (Nash and Snyder 1962). Air drying soil samples reduces bacterial growth on plates and 0.1% water agar allows an even dispersion of propagules in solution and across the agar surface. Fusarium colony counts were made after plates were incubated for 4-5 days at 22-25⁰ C on laboratory benches. There were three replications per soil site and the recovery from each replication was the average of two plate counts.

Macroconidia Germination Assays

Experiments were performed to determine the extent of Fusarium macroconidial germination in fresh and microbiologically-altered forest and nursery soils. Macroconidia of an isolate of Fusarium oxysporum (F-35) recovered from the roots of a dying nursery-grown Douglas-fir seedling showed moderate germination in initial trials and were used in all germination assays. The isolate was maintained in test tubes of sterile sand stored at 4⁰ C. When needed, the stored isolate was plated onto water agar, single-spored onto V-8 juice agar plates and incubated at 22-25⁰ C, 15 cm below cool-white fluorescent lights (12h photoperiod, 50-100 $\mu\text{E m}^{-2} \text{ sec}^{-1}$) (Toussoun and Nelson 1968). After 14-17 days, macroconidia were harvested,

centrifuged and washed twice in sterile distilled water and added as a concentrated solution (2×10^6 macroconidia/ml) to test soils. One ml of conidial suspension, 2.5 g dry weight equivalent of moist soil and sterile distilled water were mixed in a beaker to give a soil slurry composed of two parts water and 1 part dry soil. A 0.7 ml aliquate of slurries of fresh and pasteurized (65°C /30 min aerated steam) soils from all sites was placed in wells of tissue culture plates (Costar, Cambridge, MA). Plates containing soil slurries were placed in humidity chambers (Fig. 1) and kept in the dark at 21°C for 48 h. Percentage of germination was determined by making a soil smear on a slide, staining it with 0.01% acid fuchsin in lacto-glycerin, and microscopically evaluating 100 macroconidia at a magnification of $\times 400$. The percentage germination reported for each soil is the average of 4 replications.

Following the initial soil survey, four forest soils and one nursery soil were selected for further tests. Each soil site is briefly described below including the predominant conifer species present.

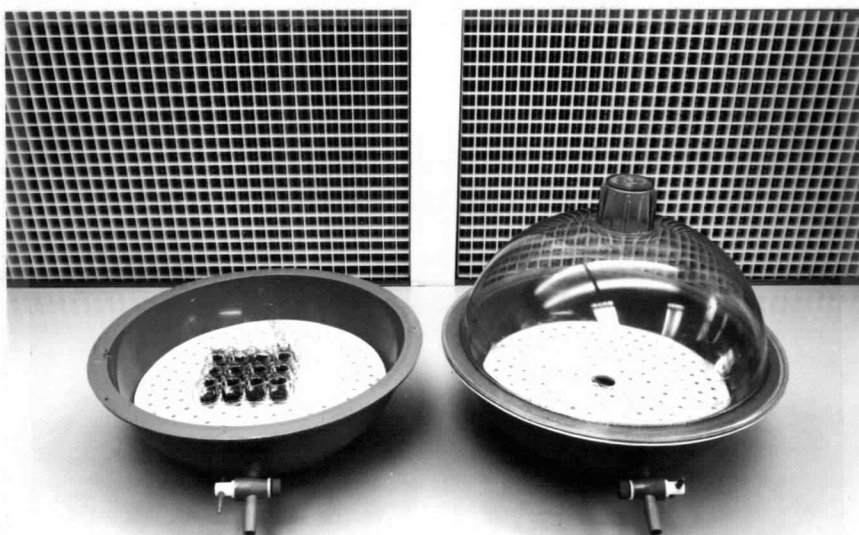


Figure 1. Tissue culture plate in a humidity chamber with lid removed (left), and a humidity chamber with lid in place (right).

Forest

Mary's Peak, Oregon (Soil "4*")- Elev. 1300 m. Mulkey clay loam. Psuedotsuga menziesii, Abies amabilis

Wind River, Washington (Soil "7")- Elev. 360 m. Stabler sandy loam. Psuedotsuga menziesii

Foster Reservoir, Oregon (Soil "10")- Elev. 100 m. Salem clay loam. Psuedotsuga menziesii

Suttle Lake, Oregon (Soil "12")- Elev. 600 m. Lapine very gravelly loamy sand. Pinus contorta

Nursery

Wind River, Washington (Soil "16")- Elev. 360 m. Stabler sandy loam. Psuedotsuga menziesii

*Soil number used in illustrations.

The macroconidial germination assay was used to compare germination of macroconidia in fresh soil with that in soils sterilized by radiation (5.6 megarads minimum over 5 days) or propylene oxide (15 g air-dry soil and 8 ml sterilant placed in a 4 L sterilizing container for 2 days). Fresh soils and soils air-steam pasteurized at 45⁰, 65⁰, and 90⁰ C were also compared.

Data from all macroconidial germination assays was transformed using the arc-sine transformation prior to statistical analysis (Neter and Wasserman 1974).

Microbial Counts, Soil Analysis of 5 Soils

Microbial populations and the chemical and nutritional properties of the five soils were determined to ascertain whether trends of these traits correlated with a soil's capacity to stimulate macroconidial germination. Estimates of populations of bacteria, fungi, and actinomycetes in the soils were made by serial dilutions of 1 g dry weight equivalent of fresh soil with 0.1% water agar and plating 1 ml aliquots on Tryptic Soy Agar (Difco), Tryptic Soy Agar + 100 ppm Rifampicin, and Starch Casein Agar (selective for actinomycetes) (Rose et al. 1980) respectively. Colony counts were made after dark incubation of plates at 23⁰ C for 12 h followed by 24 h at 29⁰ C. There were three replications per soil site and the microbial count of each replication was expressed as the average of two plate counts.

Soils were analyzed at the Oregon State University Soil Testing Laboratory for chemical and nutritional properties.

Macroconidial Conversion to Chlamydospores in Soil Extracts

Five soils described previously and three additional nursery

soils were used to determine whether extracts of forest and nursery soils differ in their capacity to support macroconidial conversion to chlamydospores. Sterile water and fresh soil were combined in a 3:1 water to dry soil ratio, stirred, allowed to stand for 1 h, and stirred again before filtration through Watman #1 paper (Watman Ltd., England). A small amount of a concentrated macroconidial suspension, prepared as previously described, was added to filtrates to give 2×10^4 spores/ml of extract. Three replications of 3 ml of each soil extract-conidia mix were transferred to sterile 12 x 75 mm polypropylene tubes and stored in the dark at 21° C for 10 days. One ml samples of each tube were then serially diluted using 0.1% water agar and plated on PPA after assuring microscopically that macroconidia had either lysed or converted to chlamydospores. Plates were incubated as previously described for the soil Fusarium population assay and Fusarium colonies, resulting from germinated chlamydospores, counted.

Isolation of Possible Antagonists

Sterile nylon twill screens (4 cm²) with 1 mm² holes were placed on the surface of V-8 juice agar plates and single germinated F-35 macroconidia were transferred to the center of each plate. After 10 days, screens covered with hyphae and spores of the fungus were peeled from the agar surface and buried in either forest, field or nursery soils described below.

Forest

Sodaville-Waterloo, Oregon (Soil "9")- Elev. 120 m. Olympic clay loam. Psuedotsuga menziesii

Field

Sodaville-Waterloo, Oregon (Soil "18")- Elev. 130 m. Olympic clay loam. Gramineae

Nursery

Brownsville, Oregon (Soil "15")- Elev. 80 m. Wapato silty clay. Psuedotsuga menziesii

The proximity of the forest and field soil sites presumably minimizes soil differences due to soil parent material and climatological effects. Therefore, the major difference between the two soils apparently is the predominant vegetation supported at each site.

After 24 and 48 h, screens were removed from the soils, washed as described by Scher and Baker (1980), and briefly shaken in sterile distilled water. This rinsing method removes most soil and debris from the screens and presumably leaves only microorganisms intimately associated with the pathogen. Screens were placed on potato dextrose agar (PDA) (Difco), V-8 juice agar, plate count agar (PCA) (Difco), and Fusarium mycelium agar (FMA). FMA was prepared as follows: to a 1 L solution of 1.5% noble agar at 45⁰ C was added the filter sterilized soluble contents of approximately 15 g of Fusarium

which was grown for 4 days at 21-23⁰ C in V-8 broth culture, washed and macerated (Virtis blender, 20-30 sec at high speed). The non-soluble component of the macerate was freeze dried, propylene oxide sterilized and also incorporated (1.5 g) in the media.

After 36 h incubation at 21⁰ C, bacterial colonies were abundant around all the screens. Individual colonies from hyphae were streaked on PCA to purify colonies that were subsequently transferred and grown on PCA slants at 21⁰ C for 48 h, harvested with a 30% glycerin solution and stored at -70⁰ C until needed.

Lysis Assay

Forest, field and nursery isolates of bacteria able to grow on FMA were hypothesized to be capable of inciting lysis and were used in the lysis assay. Seven of 10 nursery, 7 of 12 field, and 13 of 18 forest bacterial isolates formed colonies on FMA.

A concentrated macroconidial suspension was added to V-8 juice broth (pH 6.5) to give 6×10^4 spores/ml. V-8 juice broth was prepared as follows: 0.15 L distilled H₂O, 1.36 L V-8 juice (Campbell Soup Co.), and 23 g CaCO₃ were combined, autoclaved (15 min at 121⁰ C), filtered through 4 layers of cheese cloth, autoclaved, and diluted 1:50 with sterile distilled H₂O. For each isolate, 2 ml of the extract-conidia mixture and approximately 10^7 bacterial cells were combined and four 0.3 ml aliquots placed in separate tissue culture well plates. Controls consisted of the conidia suspended in

broth without the addition of bacteria. Plates were incubated in the dark at 21⁰ C until germ tubes from macroconidia in the controls began to autolyse (16 h). One drop samples from all wells were then stained with 0.01% acid fuschin in lactoglycerin and observed microscopically for lysis at x400. Broth from all wells was streaked onto PCA to assure isolate viability and purity at the end of the experiment.

Petri Plate Antagonism Assay

All bacterial isolates recovered from mycelium grown on nylon screens buried in all soils were tested for antagonism to Fusarium on PCA. Bacteria were grown at 21⁰ C on PCA for 48 h. Cells from these cultures were suspended in sterile water (approximately 10⁷ cells/ml) and 10 µL was placed in the center of a PCA plate. After 48 h incubation at 22-25⁰ C, a macroconidial solution was briefly sprayed over the plate. Plates were incubated for 60 h at 22-25⁰ C and then examined for zones of inhibition. The extent of inhibition elicited by each bacterial isolate was determined by averaging 3 replications of 4 measurements of the distance from the edge of a bacterial colony to the edge of Fusarium macroconidial germination and growth.

RESULTS

Soil Fusarium Population Assay

Fusarium was recovered (6.3×10^2 propagules/g soil) from only one of 14 forest soils, but from all 7 nursery soils ($\bar{x}=1.6 \times 10^4$ propagules/g soil) (Table 1).

Macroconidia germination assays

For all 21 sites combined, macroconidia germinated more in fresh than in 65° C pasteurized soils ($P < .05$) (Fig. 2), and more in forest soils than in nursery soils ($P < .01$). Germination in fresh forest soil ($\bar{x}=12\%$) was 8x higher than in fresh nursery soil ($\bar{x}=1.5\%$). Pasteurized forest soil stimulated 7x the amount of germination found in pasteurized nursery soils ($\bar{x}=6\%$, $\bar{x}=.86\%$, respectively). Interestingly, a higher percentage of macroconidial germ tubes appeared distorted or lysed in fresh forest than in fresh nursery soils (Fig. 3). Chlamydospores that formed in fresh forest soil appeared small and poorly formed (Fig. 4).

Elimination of microorganisms from forest soils using radiation or propylene oxide reduced macroconidial germination (Fig. 5) as did air-steam pasteurization. Germination percentages for macroconidia in fresh forest soil ($\bar{x}=25.4\%$) significantly differed from germination in radiation-treated ($\bar{x}=6.5\%$) and propylene oxide-treated

Table 1. Fusarium propagules recovered per gram of forest and nursery soils.

Soils ^a	Propagules/g soil
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	$6.3 \times 10^2 (2.5 \times 10^2)$
14	0
15	$1.3 \times 10^4 (2.3 \times 10^3)$
16	$3.9 \times 10^4 (4.2 \times 10^3)$
17	$5.7 \times 10^2 (1.2 \times 10^2)$
18	$2.1 \times 10^3 (3.1 \times 10^2)$
19	$2.8 \times 10^3 (2.5 \times 10^2)$
20	$5.1 \times 10^4 (5.9 \times 10^3)$
21	$5.4 \times 10^3 (5.5 \times 10^2)$

^aForest soils = 1-14; nursery soils = 15-21. Values are means of 6 plate counts and \pm s.e. (mean).

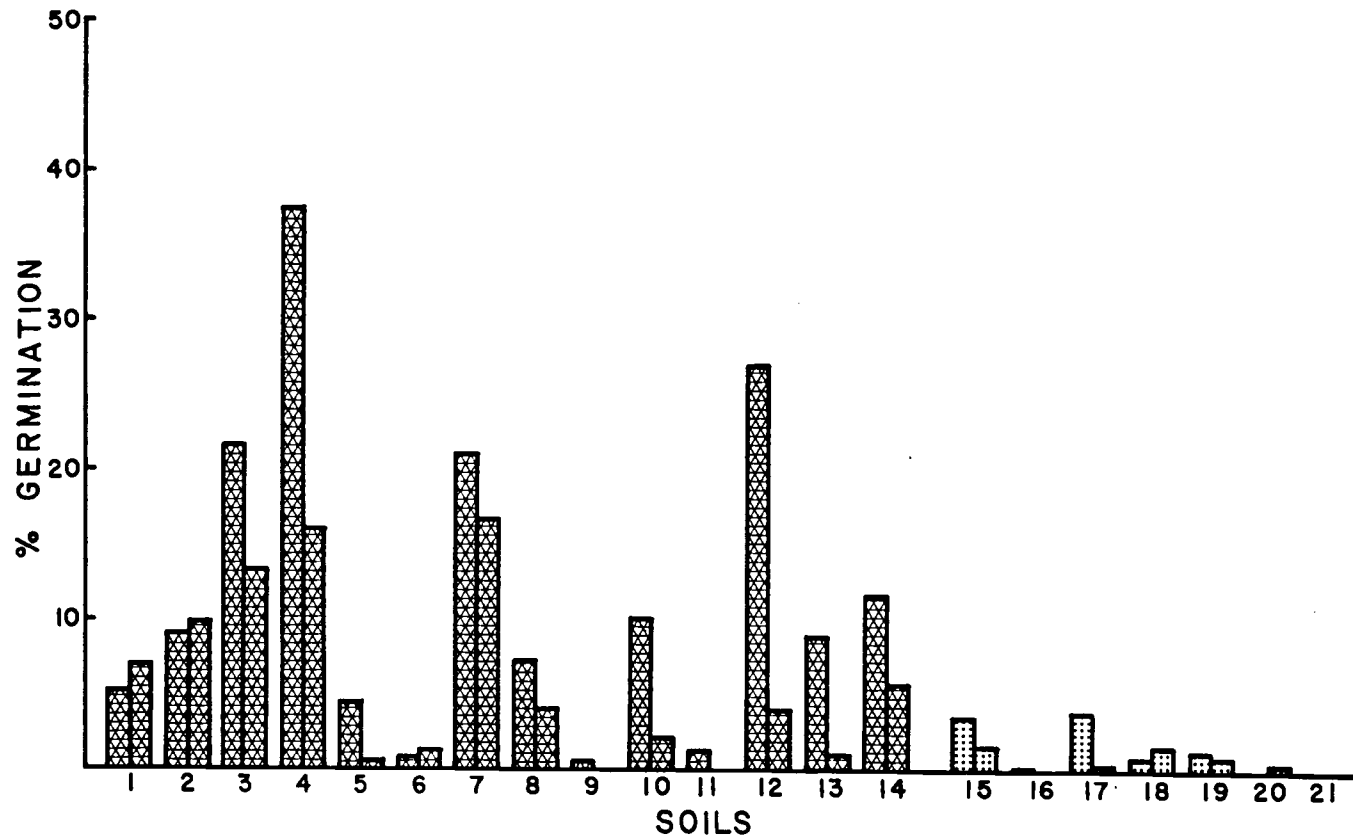


Figure 2. Effect of 65° C aerated steam pasteurization of forest and nursery soils on *Fusarium oxysporum* macroconidial germination (~~xxxx~~ forest soils (1-14), ::::: nursery soils (15-21)). Left-hand column of each pair = fresh soil; right-hand column of each pair = pasteurized soil.



a



b

Figure 3. Representative *Fusarium oxysporum* macroconidium germinated in (a) fresh forest soil (germ tube lysed) and (b) fresh nursery soil (germ tube healthy).

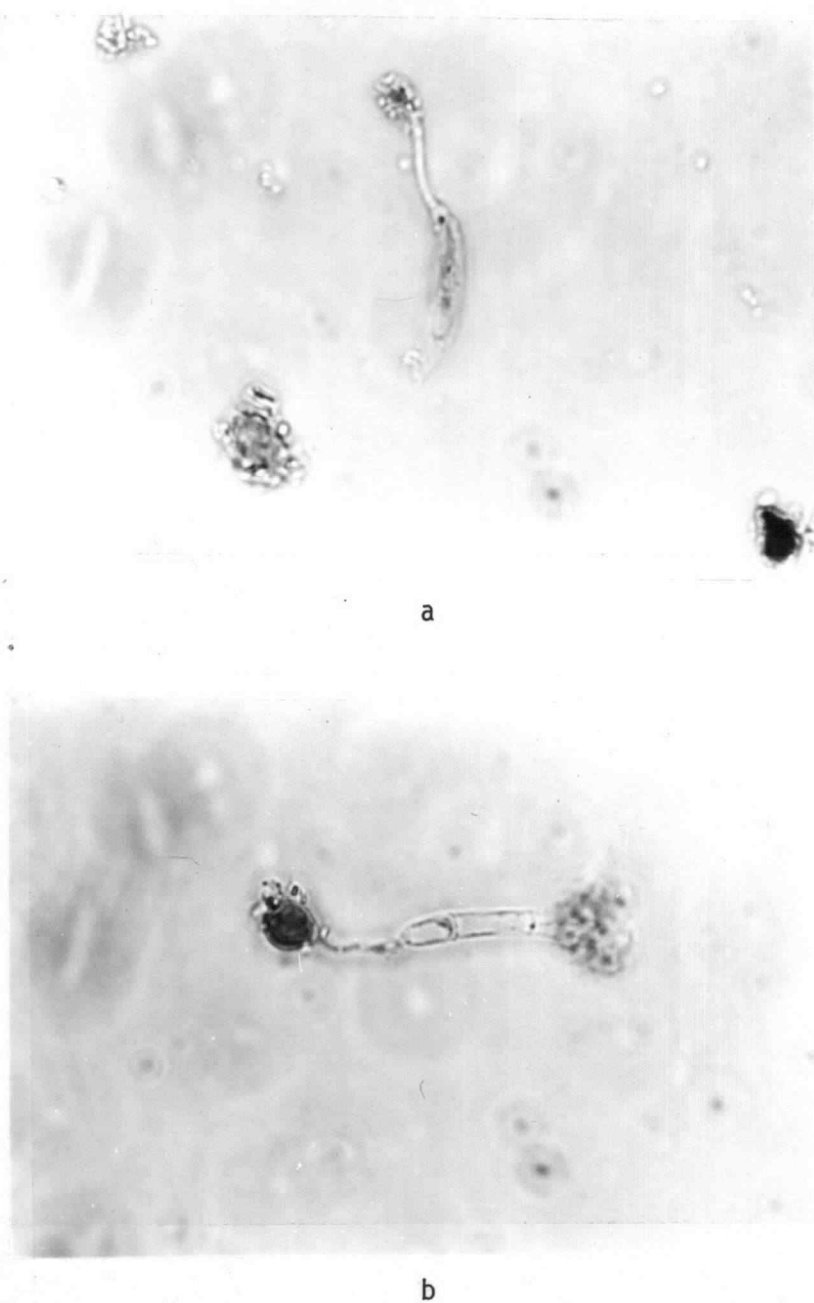


Figure 4. Representative *Fusarium oxysporum* chlamydospore formed in (a) fresh forest soil (chlamydospore small and distorted) and (b) fresh nursery soil (chlamydospore normal).

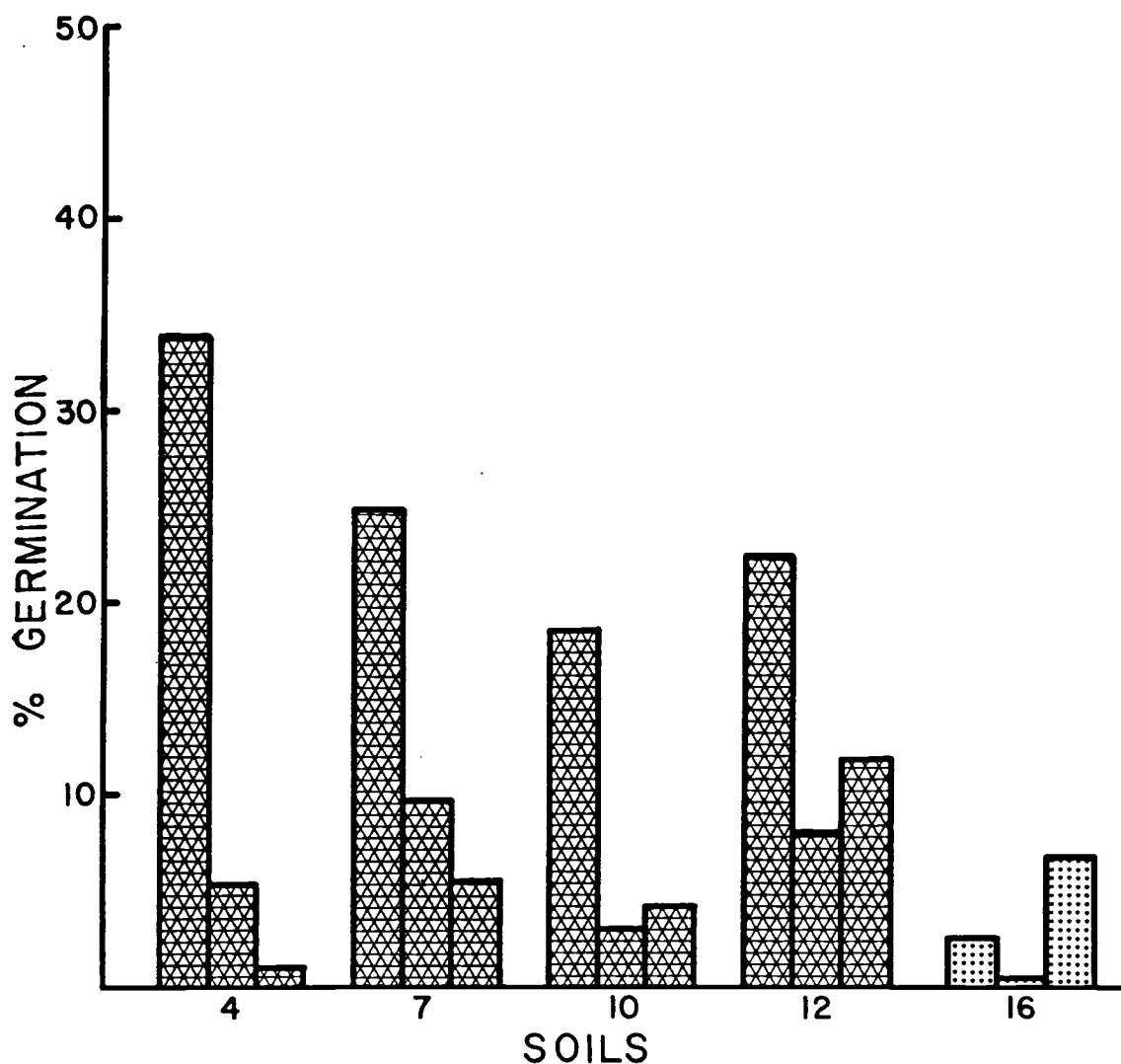


Figure 5. Effect of radiation and propylene oxide treatment of forest and nursery soils on *Fusarium oxysporum* macroconidial germination (~~xxxxx~~ forest soils, ~~xxxxx~~ nursery soil). For each column triplet: left-hand column = fresh soil; center column = radiation treated soil; right-hand column = propylene oxide treated soil.

(\bar{x} =5.6%) soil ($P < .0001$). Student-Newman-Keuls' multiple range test failed to show a significant difference between the effect of radiation and propylene oxide treatment on forest soil. An unexpectedly high percentage of macroconidia germinated in the propylene oxide treated nursery soil (Fig. 5), perhaps due to a residual germination stimulant induced by the propylene oxide treatment.

Increasing pasteurization temperature generally caused decreased spore germination in the 4 forest soils tested (Fig. 6). Germination percentages for macroconidia in fresh forest soil (\bar{x} =27.7%) significantly differed from germination in soils pasteurized at 45° C (\bar{x} =11.2%), 65° C (\bar{x} =7.4%), and 90° C (\bar{x} =4.7%) ($P < .01$). Student-Newman-Keuls' multiple range test failed to show a significant difference between any of the pasteurization treatments.

Microbial Counts and Soil Analysis of 5 Soils

All counts of microbial types were higher in the nursery than in the forest soils (Figure 7) ($P < .0001$ for all types, Wilks multivariate test). One-way analysis of variance found significant differences in the number of bacteria ($P < .0001$), fungi ($P < .01$) and actinomycetes ($P < .0001$) in the 4 forest soils assayed.

Table 2 shows the chemical and nutritional properties of these soils. The Wind River Nursery soil differed markedly from the forest soils in many chemical and nutritional properties, and the forest soils differed markedly from each other in these same properties.

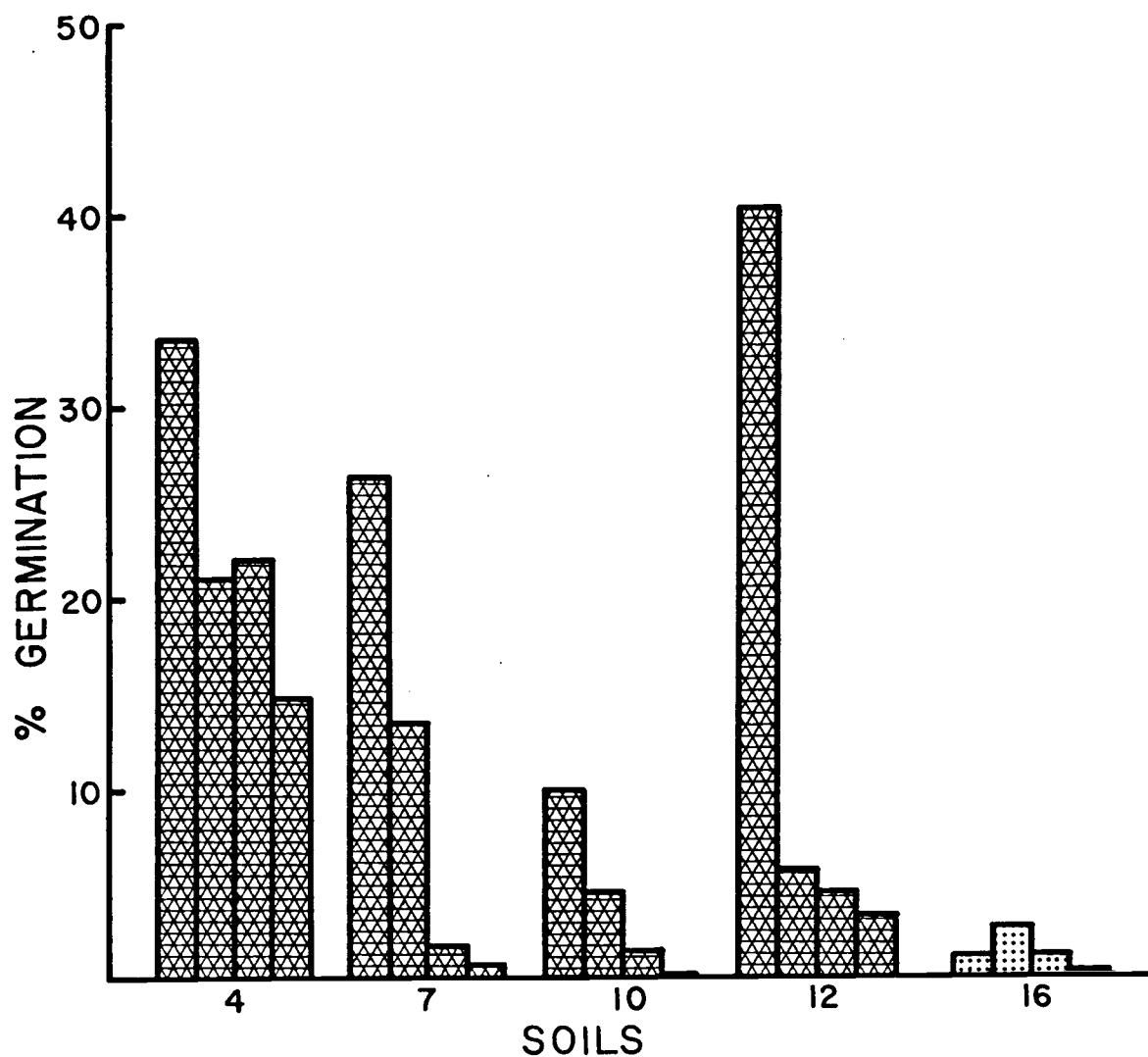


Figure 6. Effect of 45⁰, 65⁰, and 90⁰ C aerated steam pasteurization (P) of forest and nursery soils on *Fusarium oxysporum* macroconidial germination. (XXXX forest soils, : nursery soil). For each column quartet: left-hand column = fresh soil; left-center column = P45⁰ C; right-center column = P65⁰ C; right-hand column = P90⁰ C.

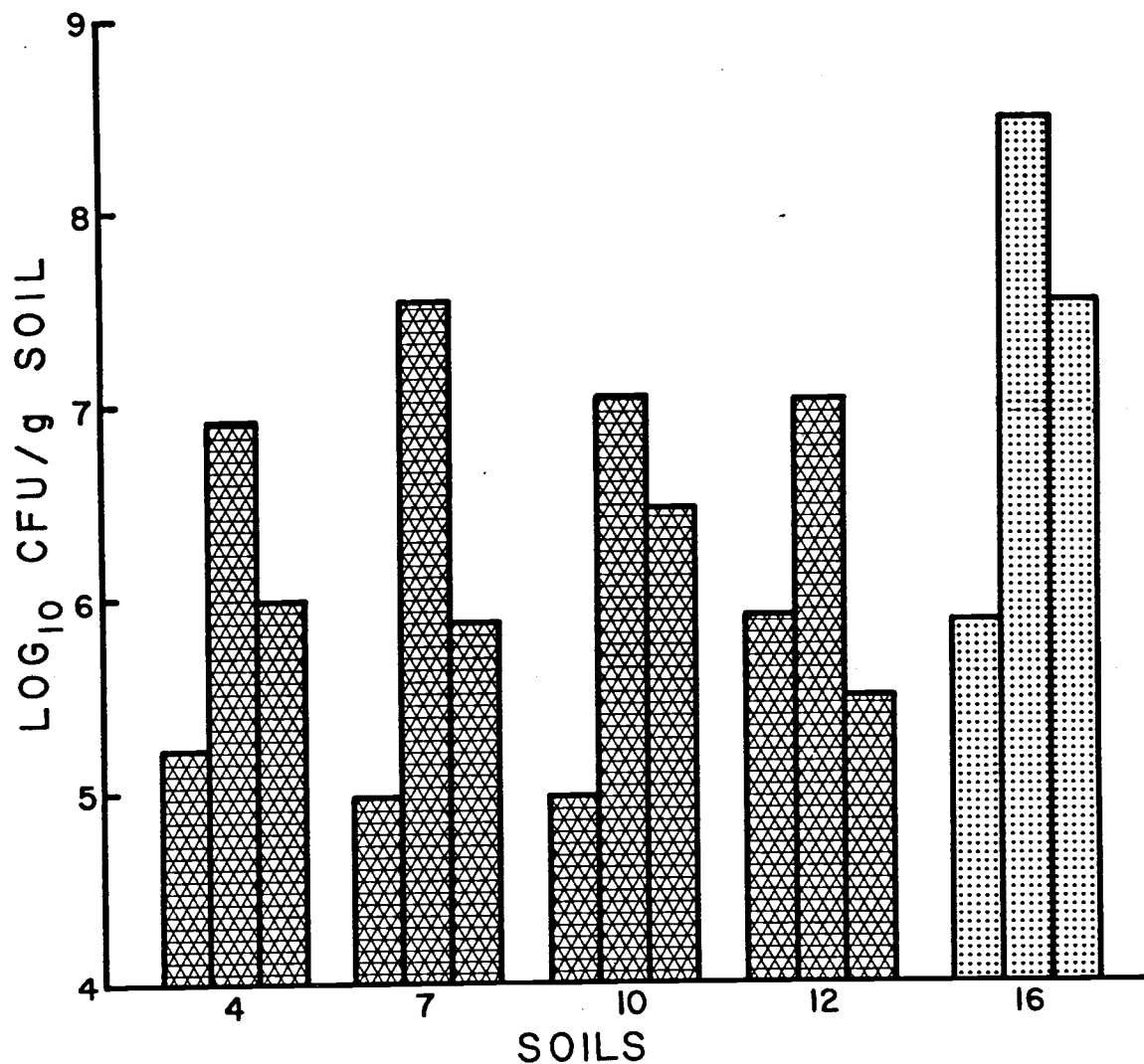


Figure 7. Bacterial, fungal, and actinomycete colony-forming units recovered per gram of forest and nursery soils. (~~xxxx~~ forest soils, nursery soil). For each column triplet: left-hand column = fungi; center column = bacteria; right-hand column = actinomycetes.

Table 2. Chemical and nutritional properties of forest and nursery soils.

Soil	pH	P ppm	K ppm	Ca meq 100 g	Mg meq 100 g	Na meq 100 g	B ppm	OM%	CEC meq 100 g	NH ₄ ppm	NO ₃ ppm
4	5.0	7	172	2.0	1.0	0.19	1.04	58.7	50.2	20.7	2.7
7	5.7	41	121	2.8	0.6	0.11	0.28	7.0	17.9	10.5	1.3
10	6.3	70	710	15.2	3.4	0.09	0.49	7.4	33.8	7.9	7.2
12	5.4	10	117	4.3	1.5	0.09	0.86	19.5	17.1	55.7	0.5
16	6.0	165	1022	12.8	4.2	0.44	1.30	10.4	23.1	16.1	515

Forest soils = 4, 7, 10, and 12; nursery soil = 16.

Macroconidial Conversion to Chlamydospores in Soil Extracts

Extracts of fresh nursery and forest soils varied in their capacity to induce macroconidial conversion to chlamydospores (Fig. 8) but the average number of colony-forming chlamydospores in nursery ($\bar{x}=4.7 \times 10^4$) and forest ($\bar{x}=5.4 \times 10^4$) extracts did not differ significantly. Thick-walled, apparently healthy chlamydospores were produced in both nursery and forest extracts.

Lysis Assay and Petri Plate Antagonism

All bacterial isolates used in the lysis assay were recovered and were free from contaminants at the end of the experiment. Few isolates differed from others in the ability to lyse Fusarium hyphae and no isolates differed significantly from the control (Fig. 9).

Only 1 of 10 nursery isolates, 2 of 12 field isolates, and 1 of 18 forest isolates exhibited appreciable antagonism in vitro against Fusarium growth on PCA. The zone of antagonism averaged 1.9 mm and 4.3 mm for the bacterial isolates from nursery and forest soils, respectively, while the zones averaged 3.5 mm for each of the field soil isolates.

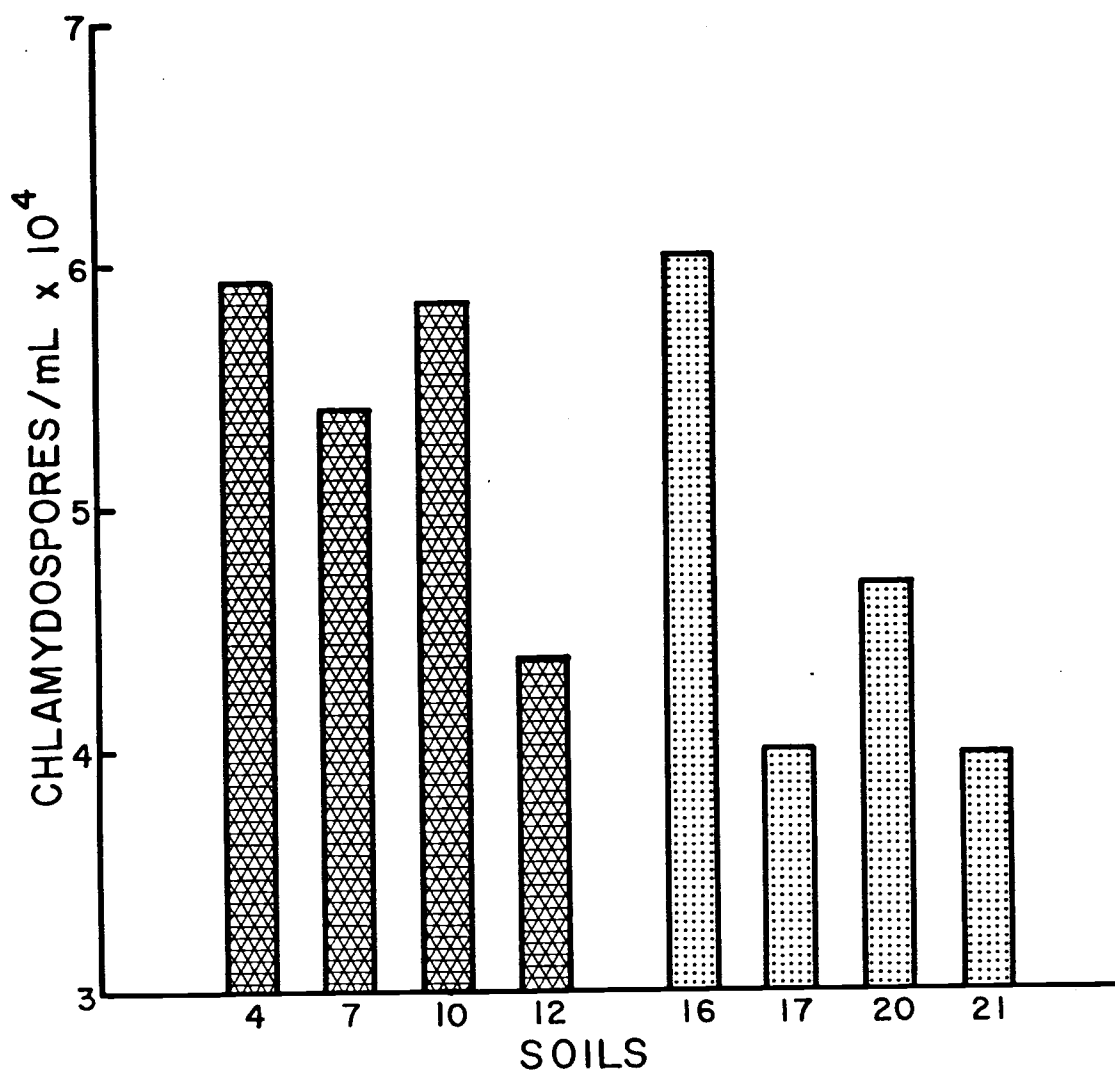


Figure 8. Effect of fresh forest and fresh nursery soil extracts on the production of chlamydospores by *Fusarium oxysporum* (~~xxxxx~~ forest soils, nursery soils).

ISOLATE NUMBER

14	a	HIGH ↑
17	ab	
26	abc	
5	abc	
8	abc	
19	abc	
11	abc	
16	abc	
9	abc	
18	abc	
21	abc	
6	abc	
13	abc	
25	abc	
10	abc	
2	abc	
3	abc	
22	abc	
12	abc	
0	abc	
7	abc	
27	abc	
23	abc	
15	abc	
1	abc	LOW
20	bc	
4	c	
24	c	

Figure 9. Relative ability of forest, field and nursery isolates of bacteria to affect the lysis of Fusarium oxysporum macroconidial germ tubes. 0 = control; 1-7 = nursery isolates; 8-14 = field isolates; 15-27 = forest isolates. Values in the column not followed by same letters are significantly different, $P = .05$ (Student-Neuman-Keuls' multiple range test).

DISCUSSION

Results from these studies indicate for the first time that microorganisms are partially responsible for stimulating Fusarium macroconidial germination followed by germ tube lysis in forest soils. The greater macroconidial germination exhibited in fresh forest soils compared to germination in aerated steam-, propylene oxide- and radiation-treated forest soils (Figs. 2, 5, 6) strongly suggests that the forest soil's microbiota is partially responsible for the stimulation of macroconidial germination. Apparently other factors contribute to the stimulative effect of forest soils since germination rates in treated forest soils were often greater than those found in untreated nursery soils (Figs. 2, 5, 6). These factors could include amino acids (Menzinger 1969) and organic acids (Hammerschlag and Linderman 1975) dissolved in needle litter extracts or chemically altered forms of these extract components due to microbial activity. The expression of a soil's suppressiveness to Fusarium disease, presumably due to stimulation of propagule germination followed by germ tube lysis or the formation of small replacement chlamydospores, has also been reported for bean root rot suppressive soils (Burke 1965).

The widespread absence of recoverable Fusarium populations in coniferous forest soils is well documented (Morrow 1932; Miller et al. 1957; Thornton 1960a, 1960b; Park 1963; Smith 1967; Barrow-Broadbush and Kerr 1981). Our studies confirm this phenomenon in

Pacific Northwestern U.S. forest soils, while showing an abundance of recoverable Fusarium propagules in all of the nursery soils assayed, even when the nursery was adjacent to a sampled forest soil. Coniferous forest soils, therefore, exclude nearly all fusaria, both pathogenic and saprophytic species or strains. In other Fusarium suppressive soil systems, only pathogenic fusaria are suppressed (Nash and Snyder 1965; Komada and Ezuka 1970; Smith and Snyder 1972; Alabouvette et al. 1979). We agree with Toussoun's (1975) assessment that the mechanisms and factors involved in Fusarium exclusion from forest soils are distinct from those involved in soils suppressive only to pathogenic fusaria.

The overall low germination of macroconidia in the soil assays likely resulted from the assay's requirement for high concentrations of macroconidia ($2 \times 10^6/\text{g}$). High concentrations of Fusarium macroconidia are reported to have absolute requirements for exogenous carbon and nitrogen to germinate (Cochrane et al. 1963; Griffin 1970a). Yet, the nursery soil generally had higher carbon and nitrogen levels than forest soils (Table 2) and stimulated less macroconidial germination than did the forest soils. We hypothesize therefore that fresh forest soils contain biotically produced compounds which partially overcome the autoinhibition of Fusarium macroconidial germination. A biotically produced germination stimulant could explain the tendency of microbiologically reduced forest soils to support more macroconidial germination than fresh

nursery soils (Figs. 2, 5, 6), and the tendency of the forest soil stimulation of macroconidial germination to drop in summer months (Schisler, unpublished results) when soil moisture and biotic activity are low. Furthermore, the inability of Fusarium mycelium to convert to chlamydospores in forest soils could result from a germination stimulant counteracting Fusarium chlamydospore-inducing compounds reportedly produced by Fusarium and other soil microbes (Venkat Ram 1952; Ford 1969; Ford et al. 1970; Griffin 1970a, 1970b, 1981).

Results from the microbial population assay show significant differences between the estimated numbers of microorganisms in nursery and forest soils (Fig. 7). However, qualitative rather than quantitative differences in microbial populations of the nursery and forest soils likely account for forest soil stimulation of Fusarium macroconidial germination since (1) macroconidial germination percentages were consistently higher in forest soils than nursery soils though the forest soils differed significantly within the group in all microbial counts (Fig. 7), and (2) macroconidia germinated infrequently in both fresh (high microbial counts) and aerated steam pasteurized (low microbial counts) nursery soils (Figs. 2, 6).

The loss of much of a forest soil's capacity to stimulate spore germination when pasteurized at 45° C (Fig. 6) suggests that non-spore-forming bacteria or heat sensitive fungi (Baker and Cook 1974) are partially involved. The lysis and petri plate antagonism assays

of bacterial isolates recovered from forest, field and nursery soils, however, failed to demonstrate that forest bacterial isolates exhibit greater lytic (Fig. 9) or antagonistic activity than field and nursery isolates. Perhaps forest bacterial isolates, in this case, were unable to produce lytic or antibiotic compounds due to the form and concentration of nutrients used in these assays. The importance of nutrition to the bacterial production of compounds that stimulate or inhibit fungi has been demonstrated by Gilbert and Linderman (1971) who showed a single bacterial isolate produced compounds stimulatory, neutral, and inhibitory to Sclerotium rolfsii depending on the nutrients supplied in the assay medium. Organic or amino acid derivatives unique to forest soils may be necessary precursors in the production of lytic compounds by forest bacterial isolates. Fungi are often important in biocontrol systems as possession competitors of substrates during a pathogen's host-free period (Baker and Cook 1974). Competition from heat sensitive fungi for nutrients in forest soils may contribute to starvation induced lysis of Fusarium mycelium.

Although macroconidia germinated in forest soils infrequently formed stunted, abnormally shaped chlamydospores in soil assays, many robust chlamydospores were formed from macroconidia in both forest and nursery soil extracts (Fig. 8). The dilution of lytic organisms and lytic compound precursors by the soil extraction may partially account for the loss of the forest soils' detrimental effect on

chlamydospore formation. Also, the breakdown of soil aggregates during liquid extraction could alter the physiology and metabolism of microorganisms within aggregates (Stolzy and Van Gundy 1968).

Our results suggest that differential microbial activity in forest vs. nursery soil contributes to the exclusion of Fusarium from forest soils. Additional studies are needed to clarify the effect of the chemical, physical and nutritional environment in forest soils on the soil biota's capacity to influence Fusarium survival. Furthermore, though mycorrhizae (Marx 1969; Sinclair et al. 1982) and volatiles produced by mycorrhizae (Krupa and Fries 1971), the soil microbiota (Hora and Baker 1972; Fries 1973; Gupta and Tandon 1977) and the decomposition of plant residues (Linderman 1970; Linderman and Gilbert 1975) can be important in pathogen suppression, their role in Fusarium exclusion from forest soils is virtually unknown. Elucidating these unknowns will help determine whether portions of the forest soil biota could be practically employed in Fusarium biocontrol systems.

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