AN ABSTRACT OF THE THESIS OF

Carlos Colinas for the degree of Doctor of Philosophy in Forest Science presented on June 17, 1992.

Title: Seedling Survival, Mycorrhiza Development and Rhizosphere Biology of *Pseudotsuga menziesii* Seedlings Outplanted on a Degraded Forest Site in Southwestern Oregon

Signature redacted for privacy.

Abstract approved:

Randy Molina

Inoculation of planting holes with small amounts of soil from a mature forest and a plantation improved survival and growth of *Pseudotsuga menziesii* seedlings in a degraded clear-cut in southwestern Oregon. To determine the component(s) of the transferred soil responsible for survival and growth increases, we treated forest, plantation and clear-cut soil with fertilizer to test for a nutrient effect, with dimethoate and carbofuran to test for microarthropod or nematode effects, with fumagillin to test for protozoa effects, with captan to test for fungal effects, with penicillin and oxytetracycline to test for bacterial effects, with pasteurization to test for effects of active forms of organisms, with tyndallization to test for effects of resting forms of organisms, and with water as controls.

All biocides significantly reduced the populations of target organisms in the transfer soils, except dimethoate-carbofuran which failed to reduce numbers of microarthropods. All biocides also affected some populations of non-target organisms. Inoculation with tyndallized, pasteurized and captantreated soils increased seedling growth and survival. Inoculation with untreated forest or plantation soils failed to increase diversity of mycorrhizal types, although they increased numbers of mycorrhizae. Soil transfers with significant effects on seedling rhizospheres increased the number of *Rhizopogon* and *Thelephora* mycorrhizae and decreased the number of active bacteria.

It is hypothesized that the role of the transfered soil is not to supply a missing key organism from the soil ecosystem, but to provide a safe site for some organisms, already present in the clear-cut or brought in along with the seedlings, to proliferate in an enivironment free from the influence of a deleterious organism sensitive to heat and captan present in the clear-cut soil. Alternative hypotheses are discussed.

Seedling Survival, Mycorrhiza Development and Rhizosphere

Biology of *Pseudotsuga menziesii* Seedlings Outplanted on a

Degraded Forest Site in Southwestern Oregon

by

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A Carlos y a Asun

por su esfuerzo

durante todos estos años.

A Nati

que concibió este sueño

y a mí,

y me enseño a soñar

en tan poco tiempo.

(To Carlos and Asun for their efforts all along.
To Nati that conceived this dream and me, and taught me to dream in those few years.)

Seedling Survival, Mycorrhiza Development and Rhizosphere Biology of *Pseudotsuga menziesii* Seedlings Outplanted on a Degraded Forest Site in Southwestern Oregon

INTRODUCTION

High elevations in the Sisikyou mountains have short growing seasons due to the cold winters and prolonged summer drought. Environmental stresses together with thin sandy soils sensitize this ecosystem particularly to man-caused disturbance (Perry et al. 1989).

Intense timber harvesting of high elevation units in the Siskiyou National Forest during the 1960's has left a legacy of understocked or unreforested sites estimated at 2,500 Ha (Amaranthus, pers. comm.). These sites continue to pose a difficult challenge to restoration ecologists; several attempts to regenerate them have proven unsuccessful. Our understanding of why these sites took a degenerative path instead of rebounding from the disturbance is not yet clear.

One of these sites is Cedar Camp, a non reforested clear-cut in the Galice District of the Siskiyou National Forest in southwestern Oregon. Cedar Camp was logged and burned in 1967 and after four plantings still has negligible conifer regeneration. Adjacent mature mixed stands of *Pseudotsuga menziesii, Abies*

concolor and *A. magnifica* have abundant natural regeneration of *Pseudotsuga menziesii*, *Abies sp.* and *Libocedrus decurrens*.

Numerous studies have been done in Cedar Camp over the last few years (Amaranthus and Perry 1987, Perry et al. 1984, Friedman et al. 1989) laying a solid foundation that encourages more basic research. Amaranthus and Perry (1987) attempted reforestation of Cedar Camp, testing an approach long known to truffle growers in Italy: soil transfers from truffle orchards could induce truffle growth in places that had not produced truffles before. Because conifers require mycorrhizae, it seemed logical to provide the trees with a local source of diverse mycorrhizal inoculum in the form of soil from a neighboring stand. Seedling survival increased on this harsh site following soil inoculation, and they hypothesized that mycorrhiza development and function was a primary determinant of this response. They also suggested that other soil biota and inorganic and organic compounds might be involved. Curiously, they reported that inoculation with pasteurized soil enhanced survival, thereby casting doubt on a soil biological effect.

Both the decline in mycorrhiza formation by seedlings after removal of trees (Parke et al. 1984; Harvey 1980; Perry et al. 1987) and the beneficial effect of inoculating outplanted seedlings with mycorrhizal fungi (O'Dell et al. in press 1992, Castellano and Molina 1990) are well documented. However, inoculations with the ectomycorrhizal fungus *Rhizopogon*

vinicolor did not improve survival at Cedar Camp (Amaranthus unpublished data).

The effects of rhizosphere microorganisms other than mycorrhizal fungi on seedling performance are complex and less well studied (Paulitz and Linderman 1991). Bacterial effects have received the most attention. Bacteria affect plant growth both positively and negatively (Chanway et al. 1991) and directly influence mycorrhiza formation (Garbaye and Bowen 1989). Rhizosphere bacteria also influence seedling growth through production of vitamins (Strzelczyk 1985) and plant growth regulators (Strzelczyk and Pokojska-Burdziej 1984), removing toxic products of fungal secondary metabolism (Duponnois and Garbaye 1990), or through their influence on other rhizosphere organisms (Bowen and Theodorou 1979).

Nematodes and arthropods have been poorly studied in forest soils, but they can also influence seedling performance. They regularly feed on hyphae, mycorrhizae and fruitbodies of mycorrhizal fungi (Vogel p77 arr), and thereby potentially regulate fungal populations. Their role in dispersing propagules of fungi or bacteria is not well known but could be important at the microsite level. For example, Visser et al. (unpublished data cited in Visser 1985) isolated 120 fungal taxa from one species of soil Collembolan, and Ponge (1990) describes how two mycorrhizal fungi grew preferentially along mite tunnels inside dead leaves.

In an attempt to identify the soil organism or organisms in the transferred soils responsible for increasing the seedling growth and survival observed by Amaranthus and Perry (1987), we studied four groups of soil organisms: bacteria, fungi, protozoa and grazers -the latter formed by nematodes and microarthropods. We used selective biocides to remove each group from a given soil transfer and determined the effect of its absence. We also added pasteurized soil, wherein active organisms had been killed, and tyndallized soil. Tyndallization, three pasteurizations at 24 h intervals, eliminates resting forms of organisms by killing them after they become active following the first, or occasionally the second pasteurization. This yields a high degree of sterility without disturbance of autoclaving (Wolf et al. 1989). Amaranthus and Perry (1987) also suggested that the increase in seedling survival may have been due to the release of nutrients from the break down of microorganisms after pasteurization. To test that hypothesis we added an amount of fertilizer to one soil treatment that approximated the amount of nitrogen immobilized in microbial biomass (Hart 1989).

We selected the biocides for this study according to two criteria: their ability to reduce the population of the target organisms, and their reported innocuousness to non-target groups.

Oxytetracycline has been effective against bacteria in soils in previous studies (Ingham 1985 and references therein). To achieve a wider spectrum of bactericidal effect than with a

single antibiotic we used a combination of oxytetracycline and penicillin to remove bacteria.

Captan was used to remove fungi. It affects most but not all groups of fungi (Ingham and Coleman 1984, Ingham 1985). Captan in high concentration effectively suppresses ectomycorrhizal fungi, a key fungal group (Pawuk et al. 1980., Bakshi and Dobriyal 1970.). Other authors have reported no effect of captan on mycorrhiza formation, but they used much lower concentrations than we planned.

Fumagillin was used to remove protozoa. To our best knowledge it is the only selective protozoacide available in agriculture. It is used in apiculture (Szabo and Heikel 1987) and aquiculture (Lauren et al. 1989). This experiment is the first time fumagillin has been used in soil.

Carbofuran and dimethoate were used in combination to remove "grazers", defined here as nematodes and arthropods. Carbofuran is effective against nematodes and arthropods (Ingham 1985) but also affects fungi (Ingham 1984). Dimethoate is regarded as the most selective biocide available against arthropods, although some indirect effects, such as reduction in grass growth, in active bacteria and in ciliates, and increase in fungi have been reported (Ingham 1984).

The term "selective" when applied to biocides is not absolute. Generally, biocides do not completely eliminate target

populations, and usually have some effect on non-target populations. Moreover, effects of biocides on target and non-target organisms vary with soil, vegetation and climatic regime (Ingham et al. 1991). A basic study of the actual effects of the selected biocides in this particular system was needed before using them as research tools. A study was conducted to assess effects of selected biocides on their target group and on the non-target groups of organisms of interest. This study is described in the first chapter.

In the second and third chapters I describe the field study in which seedlings were inoculated with soils treated with the agents mentioned above. Three soils were used in the experiment: soil from the actual clear-cut, soil from the adjacent forest and soil from a nearby plantation. The latter two soils are the same as those used by Amaranthus and Perry (1987).

The second chapter focuses on whether the increase in growth and survival observed by Amaranthus and Perry (1987) was caused by a biotic or an abiotic component of the soil ecosystem, and if it was biotic, to determine in which of the above group(s) the responsible organism(s) belongs.

The third chapter focuses on the effects of the treatments on the number and type of mycorrhizae, number of active bacteria, length of active fungal hyphae, and number of actinomycetes in the seedlings' rhizosphere. Actinomycetes were of especial

interest because this group of bacteria has been linked to regeneration failure in Cedar Camp (Friedman et al. 1989).

The results of a greenhouse experiment referred to in the discussion sections of chapters two and three are presented in Appendix 1. The objective of this greenhouse experiment was to avoid the confounding effects of environmental stresses. *Pseudotsuga menziesii* seedlings were grown in soil from the clear-cut amended with the same soils treated with the same agents (except tyndallization that was substituted for methyl bromide) as in the field study.

CHAPTER 1. EFFECTS OF SELECTED BIOCIDES ON TARGET AND NON-TARGET ORGANISMS IN FOREST SOILS

Abstract

Oxytetracycline-penicillin (combined), captan, fumigillan and dimethoate-carbofuran (combined) were applied to forest soils to test their effects on soil populations of active and total bacteria, active and total fungi, protozoa, nematodes and microarthropods. All biocides significantly reduced the populations of target organisms, except dimethoate-carbofuran, which failed to reduce numbers of microarthropods. All biocides also affected populations of non-target organisms. Oxytetracycline-penicillin reduced active hyphal lengths. Captan reduced nematode and bacterial numbers. Fumagillin reduced nematode and total bacterium numbers and active hyphal lengths. Dimethoate-carbofuran reduced active bacterial numbers and active hyphal lengths. Effects on target and non-target organisms and possible mechanisms of resistance are discussed.

Introduction

Indirect effects of agricultural chemicals are difficult to deduce. They must be determined experimentally, a costly process, but an important one for the health of crops, humans, and other organisms further up the food chain. To compound the problem,

effects of biocides on target and non-target organisms vary with soil, vegetation and climatic regime (Ingham et al. 1991).

Selective biocides provide a valuable research tool to discover the importance of different groups of soil organisms in soil health. For example, Amaranthus and Perry (1987) hypothesiszed that the absence of unknown soil organisms was responsible for regeneration failure on a degraded site in southwestern Oregon. In a case like these, selective biocides can help narrowing down the search for those unknown organisms.

Unfortunately, selective biocides have been poorly studied in forest soils. Therefore, to further investigate the hypothesis put forward by Amaranthus and Perry (1987) we undertook this exploratory study.

The target organisms of interest were bacteria, fungi, protozoa, nematodes and arthropods. Our objective was to determine the effects of groupspecific biocides on target and non-target groups.

Selection of biocides

Biocides were selected according to two criteria: their ability to reduce the population of the target organisms, and their reported innocuousness to non-target groups.

Oxytetracycline was effective in soils (Ingham 1985 and references therein). To achieve a wider spectrum of bactericidal

effect than with a single antibiotic, we used a combination of oxytetracycline and penicillin to remove bacteria.

Captan was used to remove fungi. It affects most but not all groups of fungi (Ingham and Coleman 1984, Ingham 1985). At high concentrations, captan effectively suppresses ectomycorrhizal fungi (Pawuk et al. 1980, Bakshi and Dobriyal 1970.), a key fungal group in forest soils of Oregon. At low concentrations may not affect ectomycorrhiza formation (Marx et al. (1982; Marx and Rowan 1981)

Fumagillin was used to remove protozoa. To our best knowledge it is the only selective protozoacide available.. It is used in apiculture (Szabo and Heikel 1987) and aquiculture (Lauren et al. 1989).

Carbofuran and dimethoate were used in combination to remove "grazers", defined here as nematodes and arthropods. Carbofuran is effective against nematodes and arthropods (Ingham 1985) but also affects fungi (Ingham 1984). Dimethoate is regarded as the most selective biocide available against arthropods, but also reduces grass growth and numbers of active bacteria and ciliates, yet increases fungal growth (Ingham 1884).

Pasteurization was used as a general biocide treatment to kill active forms of bacteria, fungi, protozoa and grazers (Baker and Róistacher 1957, Linderman pers. comm.). Tyndallization (three pasteurizations at 24 h intervals) was used to achieve the

maximum sterility of the soil without alterating soil structure
and chemistry. Autoclaving was not used because of its impact on
the physical properties of the soil. (Wolf et al. 1989)

Methods

Site description

Cedar Camp (42° 4' 45" N, 123° 23' 10" W), an unreforested clear-
cut in the Galice District of the Siskiyou National Forest in
southwestern Oregon, was logged and burned in 1967 and after
four plantings still has negligible conifer regeneration. Adjacent
mature stands of *Pseudotsuga menziesii* (Mirb.) Franco (Douglas
fir), *Abies concolor* (Gord. & Glend.) Lindl. (White fir) and *A.
magnifica* Murr. (Shasta red fir) have natural regeneration of
Pseudotsuga menziesii, *Abies sp.* and *Libocedrus decurrens* Torr.
(Incense-cedar). It is on a 50% slope with SW aspect at an
elevation of ca. 1720 m, and with annual precipitation of 1650
ml, more than half in snow, and experiences a prolonged summer
drought. The soils are sandy and skeletal (mixed Entic
Cryumbrept). Complete soil data are available in Amaranthus and
Perry (1987).

Sampling method

Soils were collected from three sites: Cedar Camp clear-cut,
adjacent mature forest, and a nearby vigorous *Pseudotsuga
menziesii* plantation established in 1974 on a previously burned

clear-cut. All three sites coincide with those described in Amaranthus and Perry (1987). We refer to these soils as clear-cut soil, forest soil and plantation soil. Levels of soil macronutrients and pH did not differ significantly among the three soils (Amaranthus and Perry 1987).

Ten soil samples were taken from each soil type. In the clear-cut and plantation, the first sample was taken at an arbitrarily selected location in the central area; nine additional sample sites were chosen at random directions and distances from the previous sampling site. The first sample from the forest soil was taken at 100 m from the clear-cut edge and the remaining nine as noted above. No samples were taken at less than 65 m from the boundary to avoid edge effects.

Samples were collected by brushing away the litter layer (if present) and then removing soil from an area 20x40 cm to a depth of 10 cm. Samples were sieved through a 1 cm mesh screen to eliminate rocks and debris and composited by site into a single 120 L plastic container.

Experiment 1

Soils were collected on June 4, 1990 while still saturated from spring rains. In order to apply chemicals to the soil as water solutions, water contents were reduced to 50% by spreading soil 2 cm deep on a plastic sheet overnight. Temperature was monitored during the drying process to assure that it had not been

detrimental to soil microorganisms. Maximum temperature reached 8°C during drying, 3°C lower than the soil temperature measured that day in the clear-cut 10 cm below the soil surface. Soils were then returned to the plastic containers and transported to the laboratory to receive treatments.

Pasteurization

Soils were brought to field capacity with distilled water, placed in a tray to a depth of 5 cm, covered with aluminum foil, heated in an oven to 70°C and maintained at that temperature for 3 h. Field capacity was maintained by weighing the trays and adding sterile distilled water as needed. Samples for analysis were taken six hours after pasteurization.

Tyndallization

Tyndallization treatment consisted of pasteurization repeated three times at 24 h intervals. Between pasteurizations the soils were allowed to cool to room temperature ($\approx$18°C). Samples for analysis were taken six hours after last pasteurization.

Chemical agents

Chemical compounds (Table 1) were applied to soils at manufacturer recommended rates (Table 2). They were dissolved in an appropriate amount of distilled water to bring the soils to field capacity (0.27, 0.33 and 0.21 g of water/g dry soil for clear-cut, forest and plantation soils respectively.)

Nine liters of each soil were placed in a 20 L bucket. Vertical holes 1 cm in diameter were made in the soil at 5 cm intervals to facilitate dispersion of solution into the soil. Chemical solution was then added to the soils by pouring it over the surface and allowing it to slowly enter the soil. Soils were incubated at room temperature for 32 h.

Control

Untreated control soils were brought to field capacity with distilled water at the same time that the chemicals were applied to the treated soils.

Sample extraction

At the end of the incubation period, three samples were drawn from each treated soil. Samples were kept at 2°C and the analysis initiated 48 h later.

Experiment 2

Soils were collected on August 9, 1991, carried in coolers on ice to the laboratory, refrigerated at 4°C overnight, sieved and treated with the same agents as in experiment 1 (Table 2). A methyl bromide fumigation was substituted for tyndallization in experiment 2. Soils were prepared for fumigation by placing them in trays 3 cm deep on a plastic sheet outdoors, then covering them with a double layer of plastic sheet held above the soil trays by wood. Plastic sheets were sealed around the edges with

wet sand. A 1 LB (0.45 Kg) can of methyl bromide was placed inside the enclosure and punctured from the outside. After 24 h the plastic cover was removed. The soils were moved to a plastic shelter that allowed adequate air circulation while preventing dust or debris from falling onto the trays. Samples for analysis were taken at the end of the four day aeration period.

All other agents (Table 2) were applied and incubated at room temperature ($\approx 22°C$) for 32 h. The chemical agents were dissolved in the amount of distilled water needed to bring the soils up to field capacity (0.48, 0.62 and 0.35 g of water/g dry soil for clear-cut, forest and plantation soils respectively.). Three samples were drawn from each treated soil at the end of the incubation period, refrigerated at 2°C and the analysis initiated 24 h later.

Measurements

In both experiments we estimated the number of active and total bacteria, and length of active fungal hyphae. Total hyphal length, and numbers of protozoa, nematodes and arthropods were estimated in experiment 1.

Total bacteria

Total numbers of bacteria were determined using FITC (flúorescein isothiocyanate, Sigma Chemical Co.) direct estimations (Babiuk and Paul 1970). A ten-fold dilution series

using an initial 1 g of soil was prepared, and 1 ml of the 10^{-2} dilution was stained by adding 1 ml of FITC solution. This solution was filtered after 3 min. through a 0.2 μm black polycarbonate membrane filter (Nuclepore, Pleasanton, California, USA) held in a 25 mm diameter filter holder (Nuclepore). The soil suspension was then rinsed with 1 ml of 0.5 M sodium carbonate buffer (pH 9.6) and afterwards 1 ml of 5% sodium pyrophosphate. The filters were placed on a microscope slide and the number of fluorescent bacteria in each of ten fields on each filter was counted by epifluorescent microscopy at 1000x magnification with non-fluorescent immersion oil (Cargille Laboratories, Cedar Grove, New Jersey, USA).

Total and active fungi and active bacteria

Active hyphal length was estimated by direct observation using FDA (fluorescein diacetate, Sigma Co.) staining (Ingham and Klein, 1984). A 1 ml aliquot from a 10^{-1} soil solution was stained by adding 1 ml of FDA working solution. After 3 min., 1 ml of molten 1.5% (wt/vol.) agar in phosphate buffer (0.2 M, pH 9.6) was added and approximately 0.1 ml placed on a slide of known area and depth. Length of fluorescent hyphae was determined by direct microscopic observation of the soil-agar film by epifluorescent microscopy at 160x magnification; 90 fields per slide were examined.

Total hyphal length was determined using the same agar film and magnification but with phase contrast microscopy; 10 fields per slide were examined..

Active bacteria were determined on the same agar film by counting fluorescent bacteria in 3 fields using oil immersion epifluorescent microscopy (1000x magnification).

Protozoa

Numbers of protozoa were determined by plating four 0.5 ml aliquots from each ten fold dilution (10^{-1} to 10^{-6}) in tissue culture 24-well plates (Flow Laboratories, McLean, Virginia, USA) containing 0.5 ml of soil extract agar in each well (Darbyshire et al. 1974). Suspensions were incubated at 22°C for 7 days. The most probable numbers of flagellates, amoebae and ciliates were calculated by use of the equations of Halvorson and Ziegler (1933).

Nematodes

Nematodes were extracted from 10 g of soil using a Baerman funnel and an extraction time of 4 days. Numbers of nematodes in extracts were determined by use of a dissecting microscope (50x magnification) and classified as bacterial-, fungal-, plant-, or nematode-feeding nematodes based on oral structures (Freckman and Baldwin 1991).

Arthropods

Microarthropods were extracted from 250 g of each soil in high gradient Tullgren extractors (Merchant and Crossley 1970). Numbers of arthropods in each sample were counted and identified to feeding groups.

Statistical design

The experiment used a randomized block design with agents (7 levels) as treatments and soil type (3 levels) as blocks. Three sub-samples were taken from each treatment and their average considered one observation, with the exception of arthropod determination where only one sub-sample was taken. Analysis of variance was used to test whether the agents had significantly affected organism numbers. Means of treatments were compared to the control with one degree of freedom contrasts (pooled-variance, two-tailed t-tests). Some variables were transformed to meet the assumptions of the statistical tests. Treatment means obtained from transformed data were back transformed to the original scale and reported as medians. Means or medians of treatments are expressed in the text as percent of the control, untreated clear-cut soil. Their absolute values are presented in the figures.. The p-values presented in the figures correspond to tests of mean differences in the transformed scale. Data were analyzed using SPSS for the Macintosh v. 4.0.1 (SPSS Inc., Chicago, Illinois, USA).

Results

Oxytetracycline-Penicillin

The bactericide decreased active bacteria numbers to 20% of the control in experiment 1 and to 15% in experiment 2, and decreased total bacteria numbers to 29% of the control (Fig. 1.1). Treatment with oxytetracycline-penicillin did not significantly affect other organism group in experiment 1 (Fig. 1.1.1), but in experiment 2, the bactericide reduced active fungi to 52% of the control (Fig. 1.2).
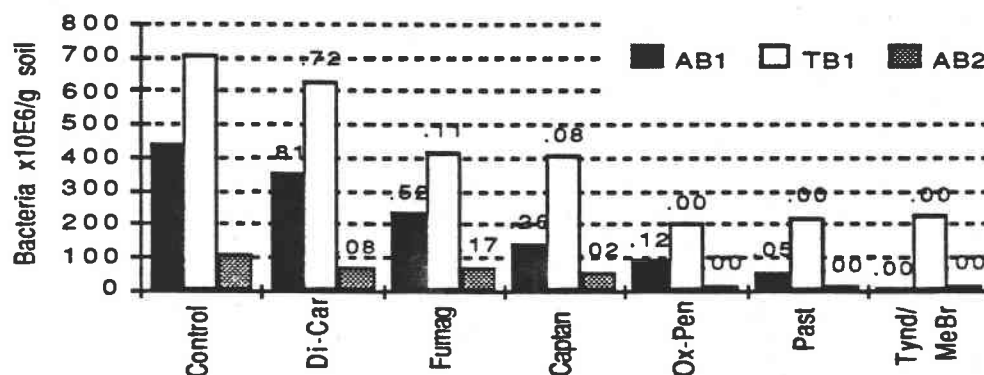
Fig. 1.1. Median number of active bacteria (AB1), and total bacteria (AB2) in experiment 1, and active bacteria (AB2) in experiment 2 per g of dry soil. Di-Car, Dimethoate-Carbofuran; Fumag, Fumagillin; Ox-Pen, Oxytetracyclin-Penicillin; Past, Pasteurization; Tynd/MeBr, Tyndallization in AB1 and TB1 and Methyl Bromide in AB2. The number above the bars is the p-value of the pooled-t-tests comparing treatments to control.

Captan

Captan decreased active hyphal length to 10% of control in both experiments, but did not affect total hyphal length (Fig. 1.2).

Captan reduced the number of active bacteria to 50% of control in the both experiments, but this reduction was only significant in experiment 2. Captan also reduced total bacterial numbers to 57% and bacterial feeding nematode numbers to 30% of control (Fig. 1.1-5) in the first experiment.
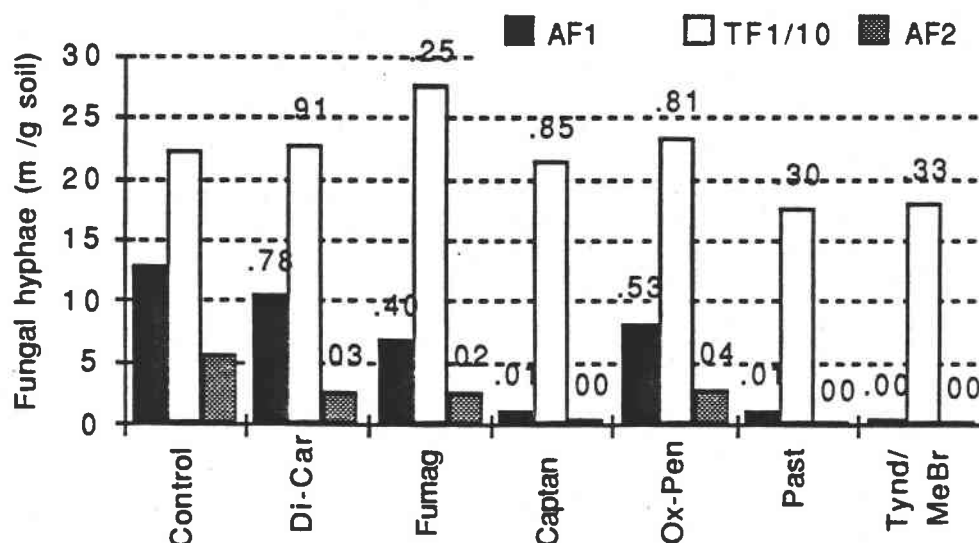
Fig. 1.2. Median length of active hyphae (AF1), and mean length of total hyphae divided by 10 (TF1/10) in experiment 1, and median length of active hyphae (AF2) in experiment 2 per g of dry soil. Di-Car, Dimethoate-Carbofuran; Fumag, Fumagillin; Ox-Pen, Oxytetracyclin-Penicillin; Past, Pasteurization; Tynd/MeBr, Tyndallization in AF1 and TF1 and Methyl Bromide in AF2. The number above the bars is the p-value of the pooled-t-tests comparing treatments to control.

Fumagillin

Fumagillin reduced the numbers of protozoa in the soils by 3 orders of magnitude compared to the control (Fig. 1.3). Numbers of flagellates were greater than ciliates and amoebae. Individuals of the latter two groups were only present in low

numbers and with high variability, making detection of reductions difficult.

Fumagillin reduced nematode numbers to 22%, active fungi to 47% in the second experiment, and total bacteria to 60% of the control (Fig. 1.1-5).
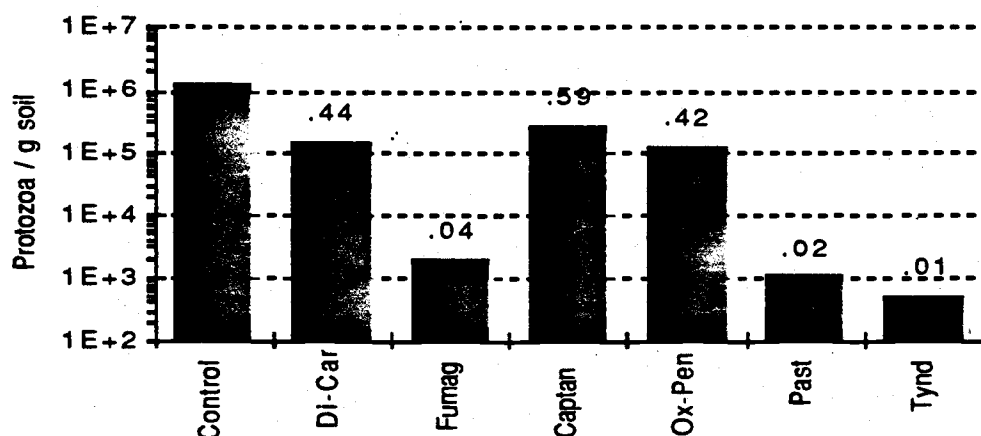
Fig. 1.3. Median number of protozoa per g of dry soil.Di-Car, Dimethoate-Carbofuran; Fumag, Fumagillin; Ox-Pen, Oxytetracyclin-Penicillin; Past, Pasteurization; Tind, Tindallization. The heights of the bars represent the median number of organisms, and the number above them is the p-value of the pooled-t-tests comparing treatments to control.

Dimethoate-carbofuran

Dimethoate-carbofuran reduced nematode numbers to 14% of the control (Fig. 1.4). Median numbers of arthropods were reduced by more than one-half, but results were not statistically significant (Fig. 1.5).

Dimethoate-carbofuran reduced active bacterial numbers and active hyphal length in the second experiment to 61% and 48% of the control (Fig. 1.1-5) respectively.
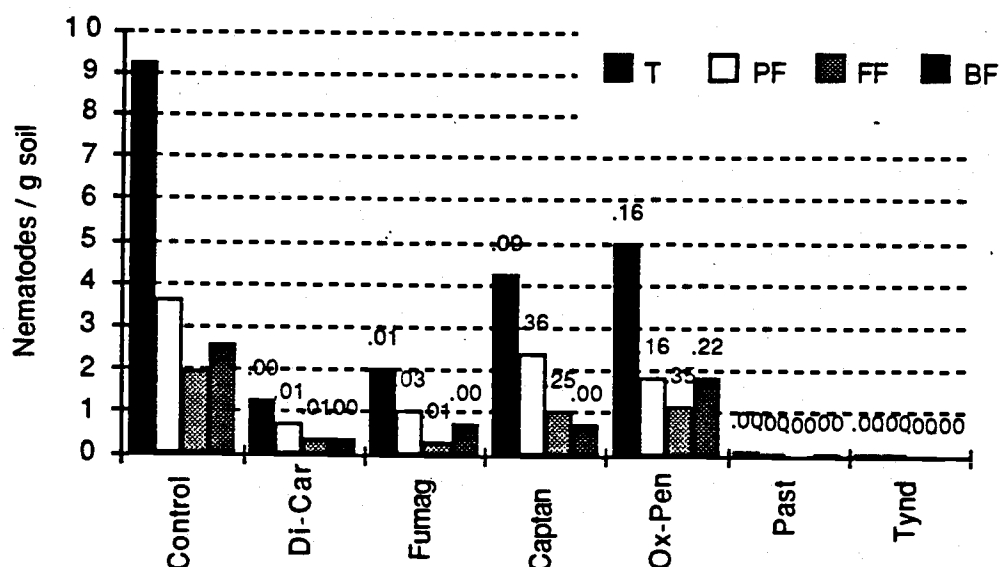
Fig. 1.4. Median number of nematodes (T), plant feeding nematodes (PF), fungi feeding nematodes (FF), bacteria feeding nematodes (BF) per g of dry soil. Di-Car, Dimethoate-Carbofuran; Fumag, Fumagillin; Ox-Pen, Oxytetracyclin-Penicillin; Past, Pasteurization; Tynd, Tyndallization. The number above the bars is the p-value of the pooled-t-tests comparing treatments to control.
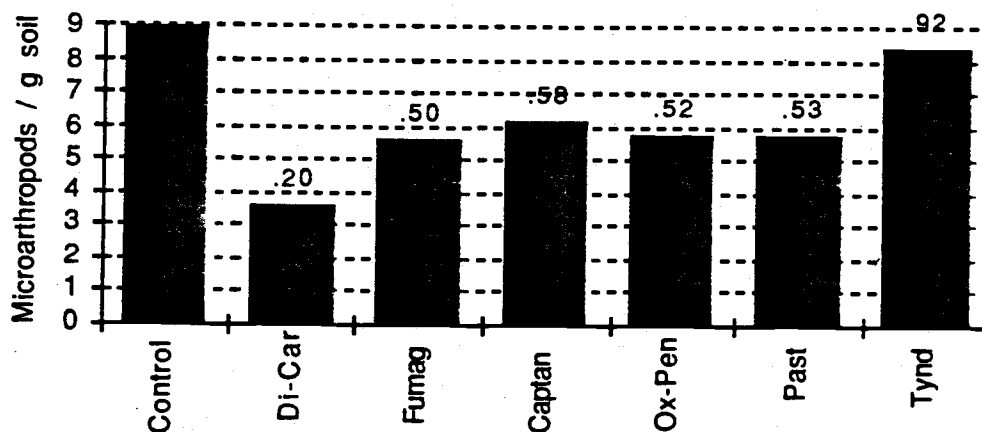
Fig. 1.5. Median number of microarthropods per g of dry soil. Di-Car, Dimethoate-Carbofuran; Fumag, Fumagillin; Ox-Pen, Oxytetracyclin-Penicillin; Past, Pasteurization; Tynd, Tyndallization. The number above the bars is the p-value of the pooled-t-tests comparing treatments to control.

Pasteurization and tyndallization

Pasteurization virtually eliminated protozoa (Fig. 1.1.3) and nematodes (Fig. 1.1.4) from the samples, reduced active bacterial numbers (Fig. 1.1.1) to 10% of the control in both experiments and active hyphal length (Fig. 1.1.2) to 10% in experiment 1 and to 2% in experiment 2.

Pasteurization reduced total bacterial numbers (Fig. 1.1.1) to 30% of the control and did not have significant effects on total hyphal length (Fig. 1.1.2) or microarthropods numbers (Fig. 1.5). Effects of tyndallization and methyl bromide followed the same patterns as pasteurization (Fig. 1.1-5).

Discussion

All biocides strongly reduced or virtually eliminated intended target groups except the grazercide that failed to significantly reduce the number of microarthropods in the soils. All biocides also had some degree of toxicity for other groups they were not intended to harm, ranging from the barely significant reduction of fungal hyphae by the bactericide to the decimation of nematodes by the protozoacide.

Active hyphal length was less than half and bacterial numbers were one third lower in the soils collected for the second experiment than in those collected for the first (p=0.003 and 0.01

respectively). These large drop-offs were likely due to the droughty soil conditions.

Oxytetracycline-Penicillin

The bactericide was effective against the target group without strong effects against other groups. Even though the number of bacteria was reduced to a fifth of the original population, 90 million bacteria were still present in a gram of dry soil. Thus, some groups of bacteria, and hence some functions, may have been depleted while others were enhanced. Although the fast reproduction rate of bacteria would result in rapid recolonization, function could continue to be altered.

Captan

Captan nearly eliminated active fungi from the soils. Its lack of effect on total hyphae was probably due to the lack of time for decomposition of dead hyphae in the 48 h that passed between the end of the incubation period and observation of results (Jenkinson et al. 1976).

Captan affected groups other than the targeted. Active bacteria were reduced in the second experiment, but the scope of this experiment did not allow for a detailed study of which bacterial groups were affected. It would be interesting to assess the impact of captan on beneficial bacteria in agriculture. Captan also reduced nematode numbers, and this effect must be

considered direct, as opposed to a reduction in nematode numbers due to a reduction in their food source as observed by Ingham et al. (1991). Starvation alone probably would not kill so many nematodes in the brief time between chemical application and soil refrigeration 32 h later. Therefore, in these forest soils captan may have a detrimental effect on both fungi and nematodes, as opposed to just fungi in agriculture or grassland soils.

Fumagillin

Fumagillin effectively reduced the numbers of protozoa in the soils, although, as for bacteria, there were still 1,000 protozoa left per gram of dry soil. Fumagillin reduced protozoa numbers to the same level as in pasteurization and tyndallization treatments. This suggests that it was active only against vegetative forms and did not persist in the soil long enough to kill resting forms that may have ecysted during the 32 h incubation period before the samples were refrigerated. Alternatively, the resting forms may have become active later in the plates were soil dilutions were cultured for 7 days to estimate protozoan numbers. Fumagillin reduced nematodes to the same level as the nematicide. Since it also reduced fungal and bacterial populations, it cannot be considered a selective biocide.

Dimethoate-Carbofuran

The "grazercide" treatment was effective against nematodes, but not significantly effective against arthropods due to the large variability. A possible explanation for this is that the soils were collected and treated early in spring, when many arthropods are in inactive forms, and presumably resistant to treatments (Moldenke, pers comm.). This treatment also reduced active fungi and bacteria in the second experiment, so its use as a selective biocide remains questionable.

Pasteurization, Tyndallization and Methyl Bromide

These treatments reduced organisms of most groups more than selective biocides. However, complete removal did not occur. Several explanations are possible.

Total hyphal lengths and bacterial numbers were determined by direct observation. Measurements were done only three and a half days after applying the treatments, and the soils were refrigerated during two of them. There may not have been sufficient time for the cell walls to decompose, and so many organisms included in the total estimates were actually dead (Jenkinson et al. 1976).

The presence of active fungi and bacteria prove that neither heat treatment achieved complete sterility. The question remains how many of the active fungi and bacteria counted were resistant to

these treatments, and how many were the progeny of contaminants or serendipitous survivors (Ridge and Theodorou 1972). The progeny must have grown during the six-hour cooling period following the treatment before the samples were taken and refrigerated, or during the 48 h that the samples were refrigerated before analysis. Therefore, they must be either fast growers or psychrophiles, two scarce traits. On the other hand, fungi or bacteria resistant to heat in their vegetative forms are also uncommon in the soil. More detailed studies are required to identify these organisms taxonomically or functionally. In methyl bromide treated soils some cells may have survived inside aggregates that the gas could not penetrate.

For protozoa, nematodes and arthropods the only available explanation is that their resting forms were able to withstand the treatments. This is supported by the fact that soils for experiment 1 were collected at the end of the winter -there were still patches of snow in the site- and many organisms may still have been in their overwintering resting forms.

Conclusions

Bacteria and active fungi were reduced to some extent by all the treatments that were not directed towards them. This raises again the question of specificity and of the collateral damage that agricultural chemicals cause to organisms other than the target. It is difficult to assess their effects in soil environments

where as many as 99% of bacterial cells do not grow when incubated in the abiotic conditions used for pure culture (Bottomley and Maggard 1990).

Reduction of fungi and bacteria was more severe in the second experiment, August collection, than in the first, June collection. This suggests either a temperature or water mediated effect, or a selective effect on the bacterial groups that composed most of the population in August but were scarce in June. A superimposition of the chemical stress to the natural summer stress could also explain this effect.

Future studies addressing effects of biocides on non-target organisms should not be limited to one observation in time, but span a full year or at least a full growing season.

Treatment effects were not strictly biological. Physical properties were also affected. For example, when replenishing the water lost during pasteurization we observed that the heat made the soils hydrophobic and reduced their water holding capacity. Interestingly, the second pasteurization reduced hydrophobicity while the third resulted in the soils becoming more hydrophilic than they were at the beginning. Towards the end of the first pasteurization, the water formed beads and penetrated the soil very slowly. During the second pasteurization, the water began to penetrate the soil without forming beads. By the end of the third pasteurization, the soil was absorbing water much faster than

before it was first heated. In addition, at the end of each pasteurization treatment, the soil could not hold as much water as at the beginning. Trying to maintain the initial weight by adding water led to standing water in the bottom of the trays. The grazercide also reduced the water holding capacity; standing water was apparent in the bottom of the bucket where the treatment was applied to the soil at the end of the 32 h incubation period. Thus, future research must pay attention to the effects of chemicals on soil physical properties and also on the effects of alteration of soil physical properties on soil biology.

The study was based on measuring total numbers of organisms, without an attempt to identify them taxonomically. This allowed us to explore the effect of the biocides on organisms spanning four kingdoms, but the lack of taxonomic precision prevented us form making strong inferences on the effect of the biocides on ecosystem functions.

In conclusion, the study provided useful data on the capabilities and limitations of selective biocides as research tools in forest soils.

Acknowledgments

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Table 1.1. Description of the chemicals used in these experiments

Product name	Source	Active ingredient, common name	Active ingredient, standard name	% active ingredient	Target organism group
Clean Crop Dimethoate 267 EC Systemic Insecticide	Platte Chemical Co., Inc., Fremont, Nebraska, USA	Dimethoate	O,O-dimethyl- S-(N-methyl-carbamoylmethyl)phosphorodithi oate	30.5%	Arthropods
Furadan 10 G Insecticide	FMC Corporation Agricultural Chemical Group, 2000 Market Street, Philadelphia, PA 19103, USA	Carbofuran	2, 3-dihidro-2,2-dimethyl-7-benzofuranyl methyl carbamate	10%	Nematodes
Captan 50-WP	Stauffer Chemical Company, Westport, CT 06881, USA	Captan Related derivatives	N-(trichlorome thyl) thio] - 4 -cyclo - hexene - 1, 2, - dicarboximide	47.3 % 2.7 %.	Fungi
Fumidil B	Mid-Continent Agrimarketing, Inc., Lenexa, KS 66215, USA	Fumagillin	Bicyclohexammo nium Fumagillin.	2.2%	Protozoa
Oxytetracycline hydrochloride	Sigma Chemical Company, P.O. Box 14508, Saint Louis, MO 63178, USA	Oxytetracy-cline	Oxytetracy-cline hydrochloride		Bacteria
Penicillin-G Potassium salt	Sigma Chemical Company, P.O. Box 14508, Saint Louis, MO 63178, USA	Penicillin	Penicillin-G Potassium salt		Bacteria
Brom-o-gas	Great Lakes Chemical Corporation, West Lafayette, Indiana, USA	Methyl-bromide Chloropicrin	Methyl-bromide Chloropicrin	98% 2%	All organisms

Table 1.2. Concentrations and combinations of chemicals in the treatments.

Treatment	Chemicals	Active ingredient µg per g dry soil
Grazercide	Dimethoate	200
	Carbofuran	25
Fungicide	Captan	25
Protozoacide	Fumagillin	10
Bactericide	Oxytetracycline	10
	Penicillin (Kunits)	10

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CHAPTER 2. OUTPLANTING PERFORMANCE OF *Pseudotsuga menziesii* SEEDLINGS PLANTED IN A DEGRADED SITE AND INOCULATED WITH DISTURBED AND UNDISTURBED FOREST SOILS PRETREATED WITH SELECTIVE BIOCIDES.

Abstract

Inoculation of planting holes with small amounts of soil from a mature forest and a plantation improved survival and growth of seedlings in a degraded clearcut in Southwestern Oregon. To determine the component(s) of the transferred soils responsible for survival and growth increases we treated forest, plantation and clearcut soil with fertilizer to test for a nutrient effect, with dimethoate and carbofuran to test for microarthropod or nematode effect, with fumagillin to test for protozoa effect, with captan to test for fungi effect, with penicillin and oxytetracicline to test for bacteria, with pasteurization to test for active forms of organisms, with tyndallization to test for resting forms of organisms, and with water for control before inoculating the planting hole with it. Odds of survival were increased by addition of untreated plantation soils, but not by addition of plantation soils that were fertilized or treated with dimethoate+carbofuran, fumagillin, or oxytetracycline+penicillin. Addition of untreated mature forest soil did not increase survival. Regardless of the source of transfer soil, survival odds were increased by captan, pasteurization and tyndallization. Treatments affected seedling dry weights differently than

Treatments affected seedling dry weights differently than survival. Dry weights were increased by both untreated plantation and untreated forest soil transfers, whereas neither soil had that effect when treated with dimethoate and carbofuran to remove microarthropods and nematodes. Dry weights of seedlings given clearcut soil transfers were increased by adding fertilizer, and by pasteurizing and tyndallizing, with the latter two treatments also increasing number of short roots. We hypothesize that soil transfers from the plantation enhance survival because they provided a safe site for beneficial rhizosphere organisms to proliferate free from the influence of deleterious organisms present in the clearcut soil and sensitive to captan and heat. Alternative explanations are also discussed. In contrast to survival, poor growth in clearcuts is related to nutrient supply. However, effects of transfer soils on seedling growth appear to be related to the presence of nematodes and/or microarthropods.

Introduction

Forest practices in the Siskiyou mountains of southwestern Oregon have left a legacy of understocked or unreforested sites estimated at 2,500 Ha. These sites continue to pose a difficult challenge to restoration ecologists; several attempts to regenerate these sites have proven unsuccessful. Our understanding for why these sites took a degenerative path instead of rebounding from the disturbance is not yet clear.

One of these sites is Cedar Camp, an unreforested clear-cut in the Galice District of the Siskiyou National Forest in southwestern Oregon. Cedar Camp was logged and burned in 1967 and after four plantings still has negligible conifer regeneration. Adjacent mature stands of *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir), *Abies concolor* (Gord. & Glend.) Lindl. (White fir) and *A. magnifica* Murr. (Shasta red fir) have natural regeneration of *Pseudotsuga menziesii*, *Abies sp.* and *Libocedrus decurrens* Torr. (Incense-cedar).

Amaranthus and Perry (1987) tested an approach long known to truffle growers in Italy: soil transfers from truffle orchards could induce truffle growth in places that had not produced truffles before. Because conifers require mycorrhizae, it seemed logical to provide the trees with a local source of diverse mycorrhizal inoculum in the form of soil from a neighboring stand. Seedling survival increased on this harsh site following soil inoculation, and Amaranthus and Perry (1987) hypothesized that mycorrhizal development and function was a primary determinant of this response. They also suggested that other soil biota and inorganic and organic compounds might be involved. Curiously, they reported that inoculation with pasteurized soil also enhanced survival thereby casting doubt on a soil biological effect.

Both the decline in mycorrhiza formation by seedlings after removal of trees (Parke et al. 1984; Harvey 1980; Perry et al.

1987) and the beneficial effect of inoculating outplanted seedlings with mycorrhizal fungi (O'Dell et al. 1992, Castellano and Molina 1990) are well documented. However, inoculations with the ectomycorrhizal fungus *Rhizopogon vinicolor* did not improve survival at Cedar Camp (Amaranthus unpublished data).

The effects of rhizosphere microorganisms other than mycorrhizal fungi on seedling performance are complex and less well studied (Paulitz and Linderman 1991). Bacterial effects have received the most attention. Bacteria both positively and negatively affect plant growth (Chanway et al. 1991) and directly influence mycorrhiza formation (Garbaye and Bowen 1989). Rhizosphere bacteria also influence seedling growth through production of vitamins (Strzelczyk and Rozycki 1985) and plant growth regulators (Strzelczyk and Pokojska-Burdziej 1984), or by removing toxic products of fungal secondary metabolism (Duponnois and Garbaye 1990).

Nematodes and arthropods have been poorly studied in forest soils, but they can influence seedling performance too. They regularly feed on hyphae, mycorrhizae and fruitbodies of mycorrhizal fungi (Vogel), and thereby potentially regulate fungal populations. Their role in dispersing propagules of fungi or bacteria is not well known but it could be important at the microsite level. For example, Visser et al. (unpublished data cited in Visser 1985) isolated 120 fungal taxa from one species of soil

Collembolan, and Ponge (1990) describes how two mycorrhizal fungi grew preferentially along mite tunnels inside dead leaves.

In an attempt to identify the soil organism or organisms responsible for increasing the seedling growth and survival observed by Amaranthus and Perry (1987) we studied four groups of soil organisms: bacteria, fungi, protozoa and grazers -the latter including nematodes and microarthropods. We used selective biocides to remove each group from a given soil transfer and determined the effect of its absence. We also added pasteurized soil wherein active organisms had been killed and tyndallized soil that should be as sterile as can be achieved without the disturbance of autoclaving (Wolf et al. 1989). Amaranthus and Perry (1987) also suggested that the increase in seedling survival may have been due to the release of nutrients from the break down of microorganisms after pasteurization. To test that hypothesis we added an amount of fertilizer to one soil treatment that approximated the amount of nitrogen immobilized in microbial biomass (Hart 1989).

The specific objectives of the study were: a) to determine if the increase in growth and survival observed by Amaranthus and Perry (1987) was caused by a biotic or an abiotic component of the soil ecosystem, and b) if it was biotic, to determine to which of the above group(s) the responsible organism(s) belongs.

Materials and methods

Site description

Cedar Camp (42° 4' 45" N, 123° 23' 10" W) is on a 50% slope with SW aspect at an elevation of ca. 1720 m. Annual precipitation averages 1650 mm, more than half in snow. A prolonged summer drought is common. The soil is sandy and skeletal (mixed Entic Cryumbrept). Complete soil data are available in Amaranthus and Perry (1987).

Transfer soils

Soils were collected from three sites: Cedar Camp clear-cut, adjacent mature forest, and a nearby vigorous *Pseudotsuga menziesii* plantation established in 1974 on a previously burned clear-cut. All three sites coincide with those described in Amaranthus and Perry (1987). We refer to these soils as clear-cut soil, forest soil and plantation soil. Levels of soil macronutrients and pH did not differ significantly among the three soils (Amaranthus and Perry 1987).

Ten soil samples were taken from each soil type. In the clear-cut and plantation, the first sample was taken at an arbitrarily selected location in the central area; nine additional sample sites were chosen at random directions and distances from the previous sampling site. The first sample from the forest soil was taken at 100 m from the clear-cut edge and the remaining nine as

noted above. No samples were taken at less than 65 m from the boundary to avoid edge effects.

Samples were collected by brushing away the litter layer (if present) and then removing soil from an area 20x40 cm to a depth of 10 cm. Samples were sieved through a 1 cm mesh screen to eliminate rocks and debris and composited by site into a single 120 L plastic container.

Soils were collected on June 4, 1990 while still saturated from spring rains. In order to apply chemicals to the soil as water solutions, water contents were reduced to 50% by spreading soil 2 cm deep on a plastic sheet overnight. Temperature was monitored during the drying process to assure that it had not been detrimental to soil microorganisms. Maximum temperature reached 8°C during drying, 3°C lower than the soil temperature measured that day in the clear-cut 10 cm below the soil surface. Soils were then returned to the plastic containers and transported to the laboratory to receive treatments.

Agents

The chemical compounds applied to the soils and their sources are described in Table 1. The combinations and concentrations at which they were applied are described in Table 2. Preparation and application were as follows.

Pasteurization

Soils were brought to field capacity with distilled water, placed in a tray to a depth of 5 cm, covered with aluminum foil, heated in an oven to 70°C and maintained at that temperature for 3 h. Field capacity was maintained by weighing the trays and adding sterile distilled water as needed. Planting in the field started seven hours after pasteurization.

Tyndallization

Tyndallization treatment consisted of pasteurization repeated three times at 24 h intervals. Between pasteurizations the soils were allowed to cool to room temperature ($\approx$18°C). Planting in the field started seven hours after the last pasteurization.

Chemical agents

Chemical compounds (Table 1) were applied to soils at manufacturer recommended rates (Table 2). They were dissolved in an appropriate amount of distilled water to bring the soils to field capacity (0.27, 0.33 and 0.21 g of water/g dry soil for clear-cut, forest and plantation soils respectively.)

Nine liters of each soil were placed in a 20 L bucket. Vertical holes 1 cm in diameter were made in the soil at 5 cm intervals to facilitate dispersion of solution into the soil. Chemical solution was then added to the soils by pouring it over the surface and allowing it to slowly enter the soil. Soils were incubated at room

temperature for 32 h. Planting in the field started at the end of the incubation period.

Control

Untreated control soils were brought to field capacity with distilled water at the same time that the chemicals were applied to the treated soils.

<u>Seedling inoculation and data collection</u>

Seedlings were 1-0 container grown *Pseudotsuga menziesii* seed source 491 from the Timberlands nursery (Lebanon, Oregon) approximately 20 to 30 cm tall. The ectomycorrhizal (EM) fungus *Thelephora terrestris* was present on the seedlings' roots. Each seedling was placed in a planting hole to which 150 ml of transfer soil from one of the 24 treatments (3 soils x 8 agents) was added.

All seedlings were carefully excavated in early December 1990 following fall rains and soil-moisture recharge, placed inside air tight plastic bags on ice in the field, and transported to the laboratory where they were stored at 2°C for up to six weeks.

Length of new roots outside the plug was measured by the grid intercept method (Marsh 1971) to the nearest centimeter.

All short roots outside the plug were counted under the dissecting microscope.

Caliper was measured at planting and at the end of the experiment to the nearest tenth of a mm, leader growth was measured to the nearest mm, and weight to the nearest mg. Fresh weights were obtained after gently washing the root systems to remove adhered soil and blotting on a paper towel to remove excess water. Dry weights were obtained after drying for 4 days at 70°C.

Nutrients

Ovendried foliage was removed from twigs and ground to 40 mesh in a Wiley mill. Nitrogen and phosphorous were determined using a Rapid Flow Analyzer (RFA300, Alpkem Co. Clackamas, OR, USA) (Thomas et al. 1967), following Kjeldahl digestion (Nelson and Sommers 1972)

Statistical design and analysis

The experiment followed a randomized block design with soils (3), agents (8) and blocks (8) as factors.

Eight blocks were laid in areas distant at least 2 m from large shrubs. Five seedlings per treatment were planted at random in each block totaling 5x24x8=960 seedlings in the experiment.

Pocket gophers (Thomomys bottae) caused high mortality early in the growing season. Since the purpose of the study was not the impact of small mammals on seedling survival, the seedlings that

were killed by the gophers were removed from the study. This forced the removal of one entire block and 127 seedlings from the other seven blocks. There was no indication of differences in gopher damage among agents (p=0.9) or soils (p=0.4), but they damaged blocks differently (p=0.003).

The independent variable "survival" was analyzed considering the five seedlings that received the same soil transfer in each block as one experimental unit defined as "surviving seedlings out of five planted seedlings". This variable has a binomial distribution and was analyzed using logistic regression in GLIM 3.77 (Royal Statistical Society, London, UK). Survival rates are reported in terms of relative survival —how many seedlings survived per seedling that did not survive—, or odds of survival, in a treatment, using the ratio of the odds of survival to compare a treatment to the control (Agresti 1990).

The independent variables associated with seedling growth and nutrient status were analyzed considering each seedling an experimental unit using standard analysis of variance. Means of treatments were compared to the control with one degree of freedom contrasts (pooled-variance, two-tailed t-tests). All computations were made with SPSS for the Macintosh v. 4.0.1 (SPSS Inc., Chicago, Illinois, USA).

Some variables needed to be transformed to meet the assumptions of the statistical tests. Treatment means obtained

from transformed data were back transformed to the original scale and reported as medians. Means or medians of treatments are expressed in the text as percent of the control, untreated clearcut soil. Their absolute values are presented in the figures.

Results

Seedling survival

The median odds of survival in the control were 0.17 (95% C.I.: 0.08, 0.38). Untreated forest soil did not increase survival over control. Untreated plantation soil increased the odds of survival 1.7 times over the control. Plantation soils that were fertilized or treated with dimethoate-carbofuran, fumagillin, or oxitetracycline-penicillin did not significantly increase survival over controls, however, except for dimethoate-carbofuran, mean odds of survival in these treatments were only slightly less than for seedlings receiving untreated plantation soil.

Seedlings given clearcut soil that had been pasteurized, tyndallized or treated with captan had significantly higher odds of survival than controls. These tretments also increased survival when applied to forest and plantation soils.

Survival

Odds ratio

CC-Contr CC-Fert .61 CC-DiCar .73 CC-Fumag .54 CC-Capt .08 CC-OxPen .55 CC-Past .05 CC-Tynd .02

MF-Contr .49 MF-Fert .67 MF-DiCar .70 MF-Fumag .53 MF-Capt .11 MF-OxPen .52 MF-Past .09 MF-Tynd .04

PL-Contr .01 PL-Fert .18 PL-DiCar .27 PL-Fumag .14 PL-Capt .01 PL-OxPen .13 PL-Past .01 PL-Tynd .00
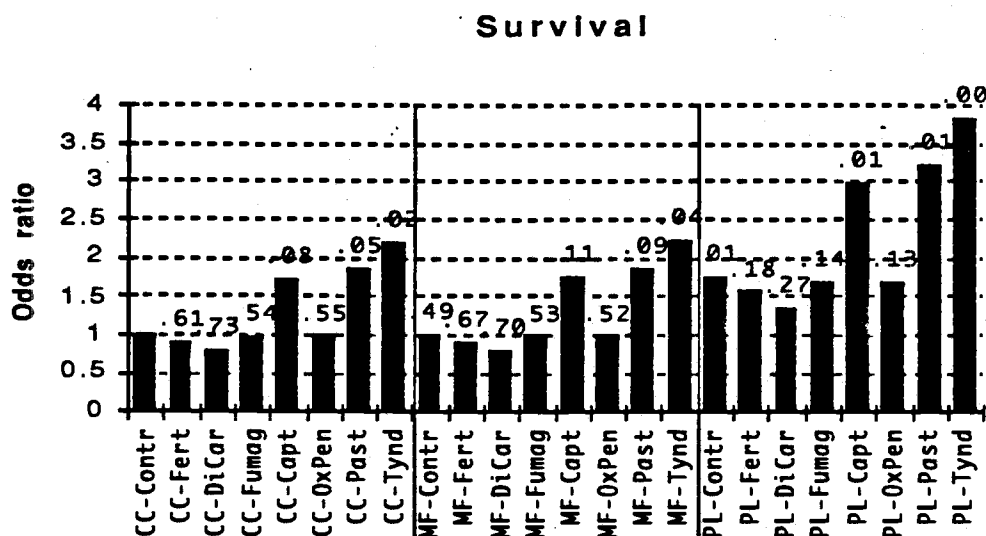
Fig 2.1. Seedling survival. The heights of the bars represents the mean odds ratios of treatments to the control and the numbers above them are the p-values of the onetailed ttests comparing the treatments to the control. Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Seedling size and growth

Seedling dry weight

Three treatments stimulated the growth of seedlings given only clearcut soil: Tyndallization, pasteurization and fertilization (Fig. 2.2). Untreated plantation and forest soils increased seedling dry weight to 180% and 190%, respectively. The stimulating effect of both forest and plantation soils was removed by the grazercide, and in forest soil by tyndallization also.

Root, shoot and needle dry weights followed the same pattern as total seedling dry weight, but were more variable (Figs. 2.3, 2.4, 2.5). The strongest effects occurred on shoot dry weight. There were interactions between soils and agents in all weight variables except in root dry weight. The treatments did not affect shoot/root ratio.

Seedling Dry Weight

Fig. 2.2. Median seedling dry weight in g. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Shoot Dry Weight
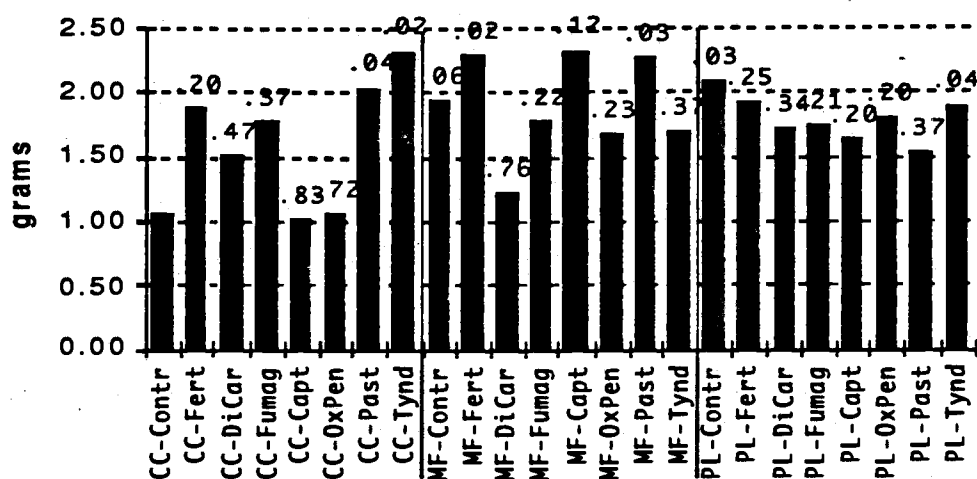
Fig. 2.3. Median shoot dry weight in g. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.
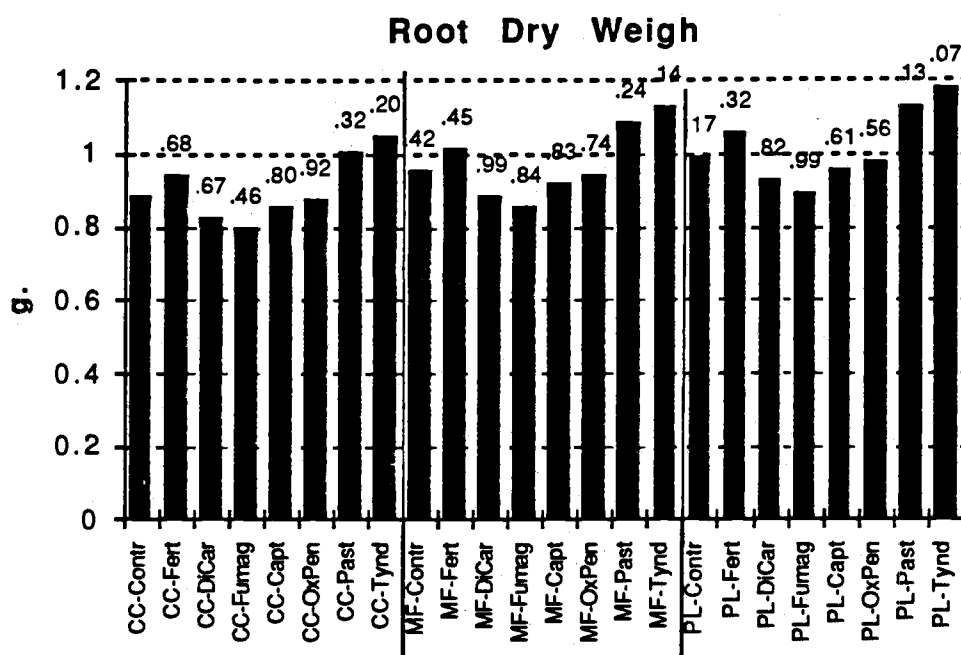
Root Dry Weigh

Fig. 2.4. Median root dry weight in g. The numbers above the bars are the p values of the contrasts comparing each treatment to the control soil for soils and control agent for agents. Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.
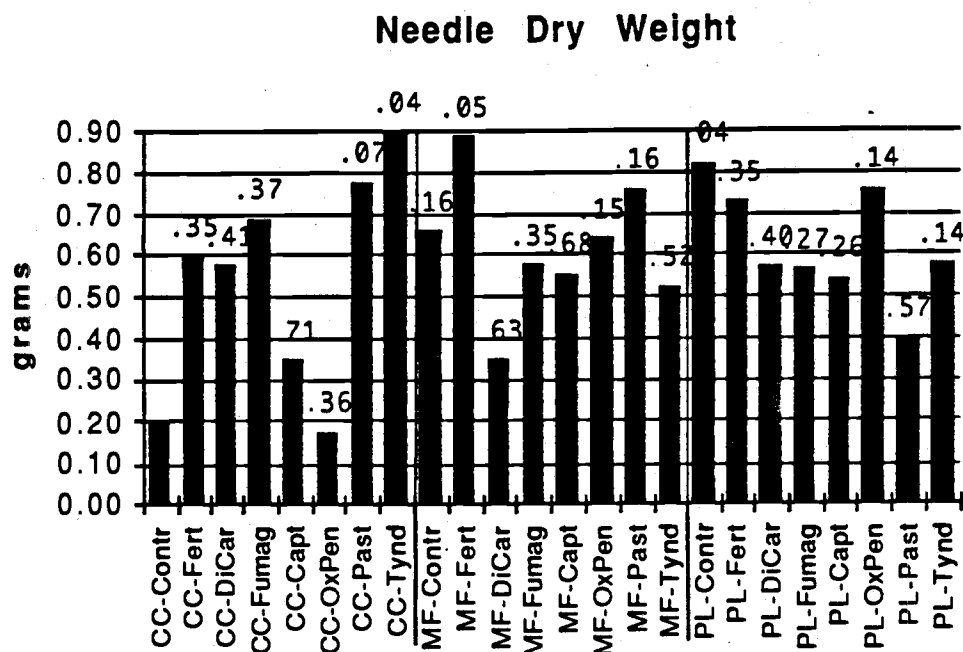
Needle Dry Weight

Fig. 2.5. Median needle dry weight in g. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Basal area increment

Both untreated forest and plantation soils increased basal area increment (Fig 6). In forest soil, the grazercide and the bactericide eliminated the stimulatory effect of the untreated soil, but pasteurization and tyndallization did not. No agent clearly eliminated the stimulatory effects of plantation soil. Fumagillin and pasteurization increased basal area increment in clearcut soil.
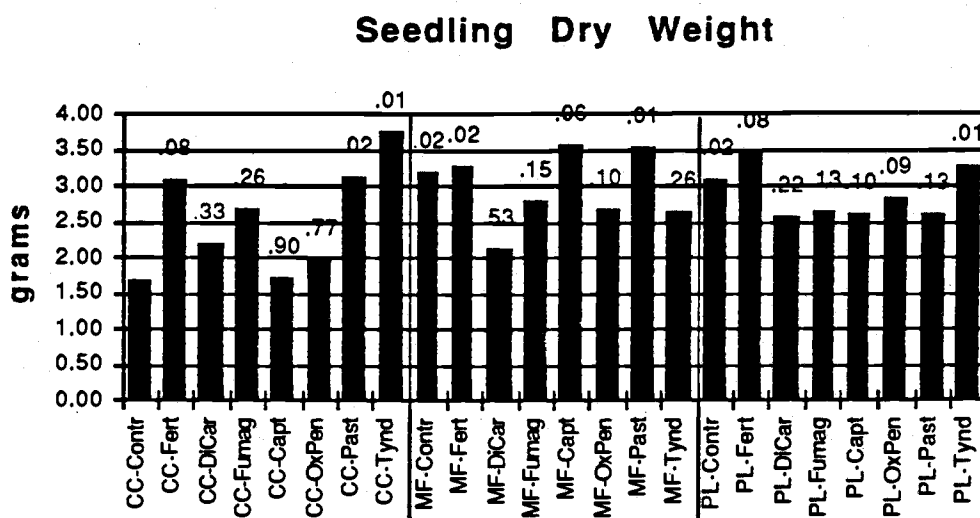
Basal Area Increment
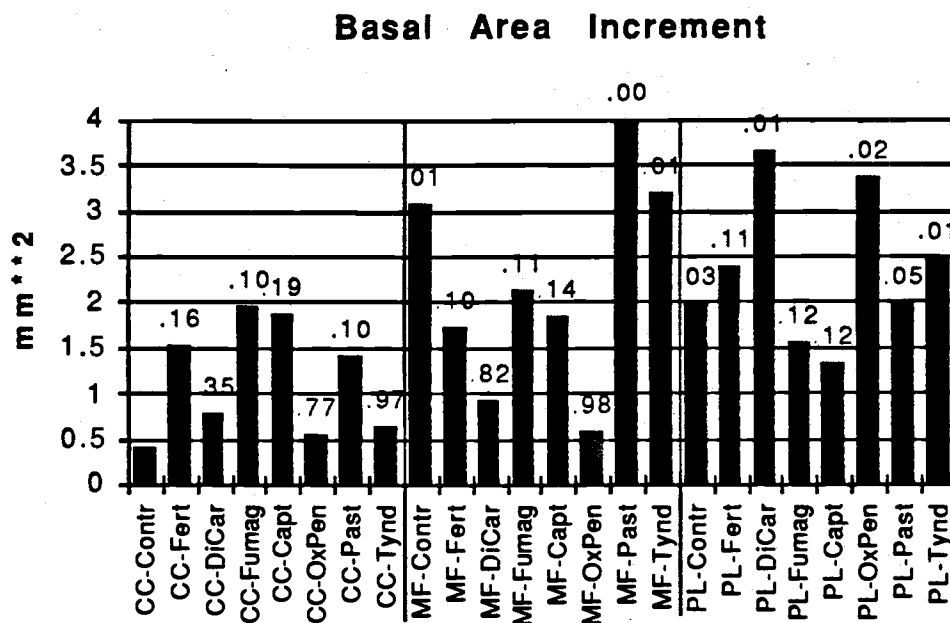
Fig. 2.6. Median basal area increment in mm^2. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Leader growth

Tyndallization was the only agent that increased leader growth in clearcut soil.

Untreated forest and plantation soils failed to increase leader growth, but tyndallization, grazercide and fungicide in forest soil and tyndallization in plantation soil increased leader growth.

Sdlg Leader Growth

Fig. 2.7. Mean leader growth in mm. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Number short roots

High variability limited our ability to distinguish treatment effects on the number of short roots. Pasteurization and tyndallization of clearcut soil increased short root formation. Untreated plantation soil increased the number of short roots. This increase did not occurr when plantation soil was treated with grazercide, protozoacide or fungicide. Untreated forest soil increased the number of short roots.

The number of short roots was positively correlated with the length of the root system ($R^2 = 0.8$).

Number of Short Root
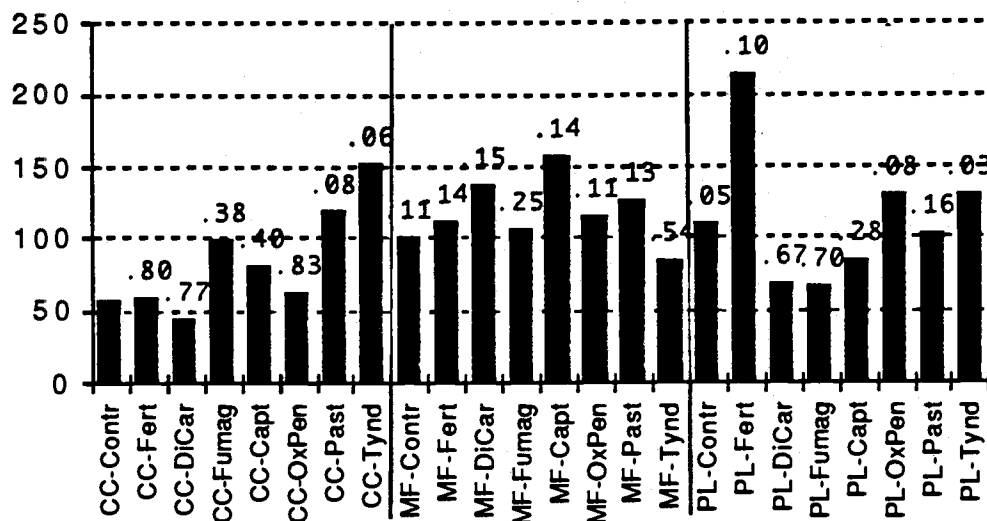
Fig. 2.9. Median number of short roots. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Seedling nutrient status

Phosphorus

Phosphorus concentration in seedling needles was reduced by all the treatments that had a significant effect on it (Fig. 2.10).

Untreated forest and plantation caused small reductions of needle phosphorous concentrations. Tyndallization reduced P concentration when applied to all three soils.

Most treatments increased seedling phosphorus content at least three times, but variability was high and only treatments that increased P content five times or more approached an acceptable significance level. These were tyndallized and pasteurized clearcut soil and fertilized forest soil. P content was correlated with needle dry weight ($R^2 = 0.7$), shoot dry weight ($R^2 = 0.6$) and N content ($R^2 = 0.7$).
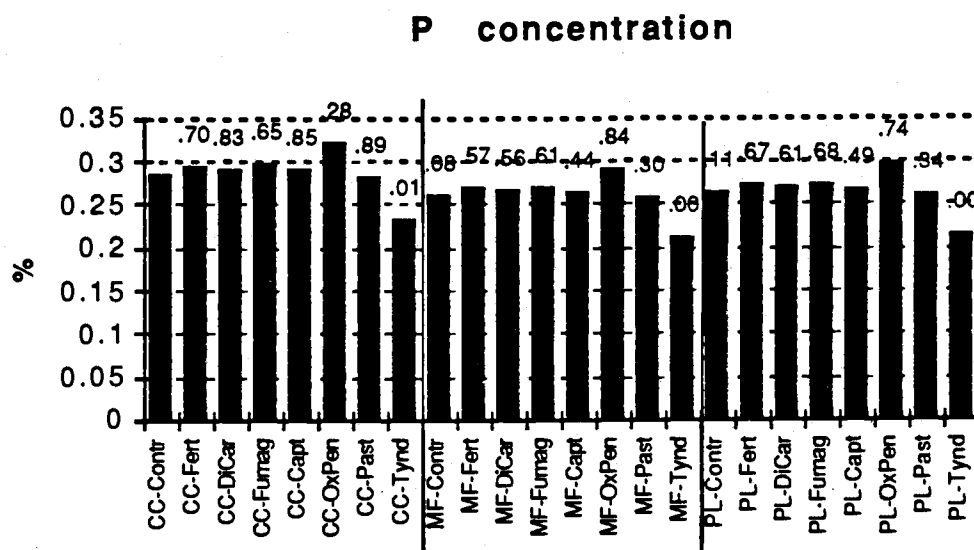
P concentration

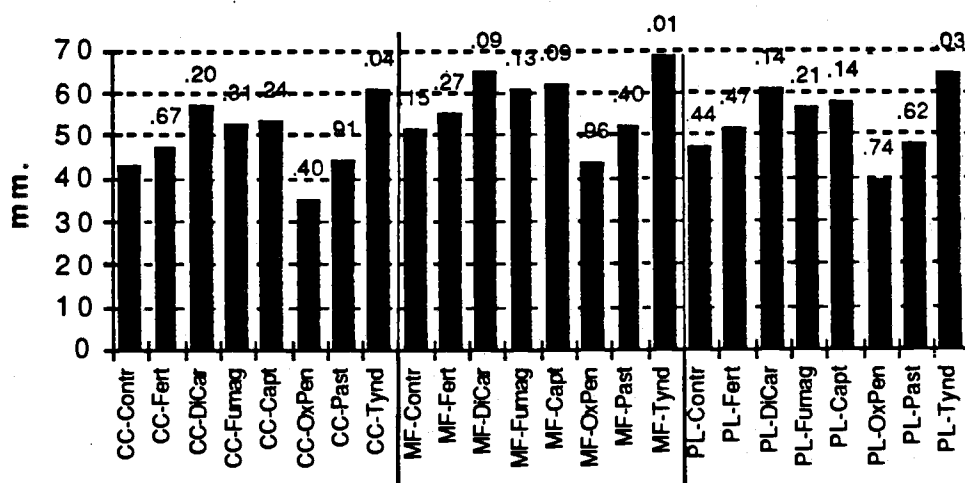Fig. 2.10. P concentration in the needles in % dry weight. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization.

Nitrogen

No treatment had any detectable effect on N concentration or content in the seedlings. The median nitrogen concentration in the needles was 1.61%, and the range extended from 1.37% to 1.88%.

Discussion

The summer of 1990 was unusually dry in Cedar Camp, which we thought would make the differences among treatments in our experiment more clear by imposing additional stress on the seedlings. Moreover, pocket gophers were exceptionally active that summer and we lost one fourth of our seedlings to them. The combined mortality of gopher and drought reduced the number of replicate plants, this resulted in less precise estimates of the variables.

The results obtained with untreated soils closely resemble those obtained by Amaranthus and Perry (1987). Untreated plantation soil improved seedling survival and also increased their growth. Forest soil did not increase survival although it increased growth as much as plantation soil in most of the studied variables. This difference between the effects of treatments on survival and growth was also present in seedlings given clear-cut soil where growth was enhanced by pasteurization and tyndallization while fertilizer increased growth only and fungicide increased survival only. This suggests that factors that increase seedling ability to survive may be different from those that increase seedling ability to grow and may provide future researchers with a system to study independently the effects of survival-promoting or growth-promoting soil organisms. Treatment effects on seedling growth may only manifest in a stressful environment. In unstressed conditions in a parallel greenhouse study (Colinas et

al. in prep. d), amendments of forest and plantation soil to a clearcut soil substrate decreased seedling size.

Alternatively, the different effects of treatments on survival and growth may be an artifact of the study. Biomass was measured only in surviving seedlings. If only seedlings above a certain threshold size survived, the size of the seedlings in treatments with high mortality would have been severely overstimated by considering that the mean of a few outliers at the upper end of the distribution was the mean of the population. In treatments with high survival, the mean constructed with a larger portion of the population, although biassed, would have been more accurate. This possibility could be addressed in future studies by sequential sampling throughout the growing season.

Seedlings given pasteurized, tyndallized or fungicide treated soil had the highest survival. The effect of the fungicide points at harmful fungicide-sensitive organisms preventing seedling survival that can also be eliminated by heat. It can be argued that heat may have altered some toxic soil substance, perhaps humic compounds (Schisler and Linderman 1989), but the soil that received the fungicide treatment did not receive any heat and increased 1.7 times the odds of survival. An alternative possibility, and perhaps the most likely, is that there are several organisms or processes hampering plant regeneration at the site. Heat and captan may prevent some of those processes, although not necessarily the same. Tyndallization and pasteurization

virtually removed all organisms except for microarthopods from the transfer soil (Colinas et al. in prep. a), which still leaves bacteria, fungi, protozoa and nematodes as possible causes of this negative effect. Only 150 ml of soil were trasferred. A very small amount compared with the total volume of soil explored by the roots, and yet capable of increasing seedling survival nearly four times when tyndallized plantation soil was transferred.

Captan virtually eliminated active fungi from the soil (Colinas et al. in prep. a), and also reduced the number of nematodes and bacterial cells in the soils to 50% of the controls. Nematodes were almost eliminated by the grazercide treatment, but that did not increase survival. Similarly, bacteria were reduced by the bactericide treatment to 25% of the control with no subsequent effect on survival. These data suggest that the organisms hampering regeneration in Cedar Camp are fungi, but fall short of proving it. Selective biocides are not completely discriminating (Colinas et al. in prep. a) and there is no reason to believe that the responsible organisms do not belong, for instance, to a bacterial species that was killed by captan and not by the bactericide.

Actinomycetes have been suggested as suppressing regeneration in Cedar Camp (Friedman et al. 1989). The effect of captan on actinomycetes in the transfer soils was not studied. The literature indicates that if captan reduces actinomycete populations it would only be at a concentration more than double the concentration we used (Agnihotri 1971; Wainwright and Pugh

1975; Smiley and Craven 1979). Again, the fact that these researchers could not find significantly lower numbers of total actinomycete propagules in captan treated soils does not mean that entire functional groups of actinomycetes were not removed. At the end of the growing season, the numbers of actinomycetes in the rhizosphere of seedlings that received different treatments were not different (Colinas et al. in prep. c).

Amaranthus and Perry (1987) noted an increase in survival due to pasteurization but they attributed it to chemical factors. Pasteurization, tyndallization and fertilizer removed the detrimental effect of control clearcut soil on seedlings final weight. This would support an explanation of regeneration failure based on poor fertility of the clearcut, but nutrient data do not support that explanation. Plants that received fertilized clearcut soil do not have different concentrations or total contents of P or N in their foliage, and all seedlings had levels of N and P within or above the expected range (Youngberg 1984). Alternatively, the fertilizer may have stimulated rhizosphere organisms that favor seedling weight gain.

The data of this study support more strongly a biologically mediated effect, such as the presence of some organism in the clearcut soil that prevents seedling establishment, since fertilizer did not affect survival. If this were the case the soil transfers might provide a safe site where the beneficial rhizosphere organisms present in the clearcut, or those coming

with the seedlings from the nursery, could grow. This hypothesis still leaves two issues unexplained. The first one is that transfering untreated plantation soil nearly doubled survival of control seedlings, but transfering tyndallized plantation soil nearly doubled survival of seedlings given untreated plantation soil. This would imply a presence in the plantation —or in the forest where tyndallization had a similar effect— of the hypothetical deleterious agent present in the clearcut, although maybe suppressed. The second unexplained issue is why should beneficial and not deleterious organisms proliferate in the soil transfers. Clearly more research is needed to identify the soil organisms responsible for this effects and their mechanisms of action before the observations described in this paper can be fully explained. Future testing of this "safe site" idea should include research on the mechanisms by which such hypothetical organisms might affect seedling survival. Possibilities worth further study include: a) the production of a volatile substance that could permeate the soil and act upon distant roots or microorganisms in their rhizosphere (Stotzky and Schenk 1976; Schisler and Linderman 1989a; Schisler and Linderman 1989b; Schisler and Linderman 1989c; Graham and Linderman 1981), b) the production of substances in the rhizosphere of the roots growing in the transfer soil able to alter the physiology of the whole seedling (Laheurte et al. 1990; Strzelczyk and Pokojska-Burdziej 1984; Strzelczyk and Rozycki 1985), and c) the ability of

the hypothetical beneficial organisms to build up a population in the transfer soil and spread from it following the growing roots.

Seedlings that received untreated forest soil had larger weight and basal area increment than the controls, but if the forest soil was treated with the grazercide, seedlings did not differ from controls. Also, untreated plantation soil increased survival while grazercide treated plantation soil did not. In both cases pasteurized and tyndallized soil —where the number of microarthropods had not been reduced (Colinas et al. in prep. a)— produced significant increases, just as untreated soils. Apparently, the lack of only grazers can revert the positive effect of the untreated forest and plantation soils, although the mean comparison procedure did not allow differences between untreated and treated plantation or forest soils to be tested. Colinas et al. (in prep. d) obtained additional evidence supporting an important role of grazers in a greenhouse study where seedlings growing in clearcut soil amended with grazercide treated forest soil did not have different weights than controls, but seedlings growing in clearcut soil amended with untreated forest soil had lower weights. It seems that the grazercide is able to cancel out the effect of untreated forest soil regardless of whether this effect is to increase or to decrease seedling weight.

The effects of grazercide treated soils on seedling growth can also be explained in the framework of the "safe site" hypothesis.

If the transfer soil were in fact a safe site, it would only be so to organisms able to reach it by their own means or transported by others. In the scale of several mm to a few cm, nematodes and microarthopods are important dispersal agents (Visser 1985). The lack of grazers would limit the access to transfered soils by sessile organisms or propagules that depend on them for transport. Grazers from the site soil may have been prevented to colonize the grazercide treated transfer soil by residual chemicals present, while in heat treated soil this would not be a problem and site grazers would move in the transfer soils quickly. The strong odor of dimethoate persists in the soil for weeks. Whether this is a true deterrent for colonization by soil fauna remains to be tested by an experiment specifically addressing this issue, as well as the precise effect of grazers on the soil. Besides their role as dispersal agents, grazers play an important role increasing nutrient mineralization that results in decreased plant growth in experimental situations when grazers are removed (Ingham et al. 1985; Setäla and Huhta 1991). In our case the decrease in growth caused by the grazercide could not be clearly explained by a decrease in mineralization that would cause nutrient deficiencies in the plants because the concentration of N and P in plants given grazercide treated soil was not lower than in plants that received untreated soil. Neither do the data prove that grazer-increased mineralization was not the key factor since we only studied the concentration of N and P.

A number of other nutrients may have been limiting seedling growth.

There is abundant information in the literature about "dead" soils increasing seedling performance. Klock (1980) observed an increase in survival and growth of *Pinus ponderosa* and *Pseudotsuga menziesii* in fumigated soils in a field experiment. Several authors (Linderman et al. in prep., Henderson and Stone 1970, Hung et al. 1982) report increased growth of seedlings in fumigated soils in nurseries or greenhouse experiments, which in some cases are associated with increased mycorrhizae formation. Perry et al. (1982) found that pasteurizing the soil increased seedling growth in disturbed soils, but not in undisturbed forest soils. Pilz and Perry (1984) found in a greenhouse study that pasteurization increases the total number of root tips, yet decreases the number of ectomycorrhizal root tips; reinoculation of pasteurized soil with unpasteurized soil reverses this effect by lowering the number of total root tips and increasing the number of ectomycorrhizae. It should also be noted that "dead soils" are seldom dead. In our experiment, pasteurized and tyndallized soils had bacteria, fungi, protozoa and nematodes present in them, although in negligible numbers, and microarthropods in numbers not significantly different from the control (Colinas et al. in prep. a).

In our case pasteurized and tyndallized clearcut soil increased the number of short roots. This suggests the presence in the

clearcut soil of some temperature sensitive agent with a negative effect on short root production. Untreated forest and plantation soils also increased short root formation, suggesting that the hypothetical deleterious agent present in clearcut soil is either absent, suppressed or in low concentration in plantation and forest soil. The effect of heat treated forest or plantation soils was comparable to their effect when untreated. This further indicates that the potential deleterious agent was not a problem in either soil, and also makes the response of clearcut soil to heat less likely to be due to physical or chemical characteristics of the soil altered by heat.

The increase in survival and growth caused by soil transfers in Cedar Camp cannot be explained by the addition of an abiotic factor, but cannot be explained just by the addition of a biotic factor either. Survival and growth were not affected by the same factors. Growth of surviving seedlings is presumably related to nutrient deficiency, while low survival appears to be related to certain fungi.

The results of the experiment did not allow us to resolve one of the original objectives of the study: to determine what organisms were missing in the soil ecosystem at Cedar Camp. This objective was based on the probably erroneous assumption that the cause of regeneration failure was the absence of some soil organisms. Our working hypothesis, that the problem at Cedar Camp was the lack of some organism, could not be proven but a new working

hypothesis emerged from the study: the presence of a living entity, perhaps fungi, is at least part of the problem in Cedar Camp soil.

Acknowledgments

Table 2.1. Description of the chemicals used in the experiment.

Product name	Source	Active ingredient, common name	Active ingredient, standard name	% active ingredient	Target organism group
Clean Crop Dimethoate 267 EC Systemic Insecticide	Platte Chemical Co., Inc., Fremont, Nebraska, USA	Dimethoate	O,O-dimethyl- S-(N-methyl-carbamoylmethyl)phosphorodithioate	30.5%	Arthropods
Furadan 10 G Insecticide	FMC Corporation Agricultural Chemical Group, 2000 Market Street, Philadelphia, PA 19103, USA	Carbofuran	2, 3-dihidro-2,2-dimethyl-7-benzofuranyl methyl carbamate	10%	Nematodes
Captan 50-WP	Stauffer Chemical Company, Westport, CT 06881, USA	Captan Related derivatives	N-(trichloromethyl) thio] - 4 -cyclo - hexene - 1, 2, - dicarboximide	47.3 % 2.7 %.	Fungi
Fumidil B	Mid-Continent Agrimarketing, Inc., Lenexa, KS 66215, USA	Fumagillin	Bicyclohexammonium Fumagillin.	2.2%	Protozoa
Oxytetracycline hydrochloride	Sigma Chemical Company, P.O. Box 14508, Saint Louis, MO 63178, USA	Oxytetracy-cline	Oxytetracy-cline hydrochloride		Bacteria
Penicillin-G Potassium salt	Sigma Chemical Company, P.O. Box 14508, Saint Louis, MO 63178, USA	Penicillin	Penicillin-G Potassium salt		Bacteria
Ra-pid-gro	Chevron Chemical Co. San Francisco, CA 94105 USA		Nitrogen Phosphorus Potasium Boron Copper Iron Manganese Zinc	23% 19% 17% 0.02% 0.05% 0.1% 0.05% 0.05%	

Table 2.2. Concentrations and combinations of chemicals in the treatments.

Treatment	Chemicals	Active ingredient µg per g dry soil
Grazercide	Dimethoate	200
	Carbofuran	25
Fungicide	Captan	25
Protozoacide	Fumagillin	10
Bactericide	Oxytetracycline	10
	Penicillin (Kunits)	10
Fertilizer	Ra-pid-gro	60 (Nitrogen)

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CHAPTER 3. ECTOMYCORRHIZA DEVELOPMENT AND RHIZOSPHERE MICROBIAL POPULATIONS OF *PSEUDOTSUGA MENZIESII* SEEDLINGS PLANTED IN A DEGRADED SITE AND INOCULATED WITH DISTURBED AND UNDISTURBED FOREST SOILS PRETREATED WITH SELECTIVE BIOCIDES

Abstract

Inoculation of planting holes with small amounts of soil from a mature forest or a plantation can improve ectomycorrhiza formation of seedlings in degraded clear-cuts in southwestern Oregon. To determine the component(s) of transferred soil responsible for improved ectomycorrhiza formation we treated forest, plantation and clear-cut transfer soils with fertilizer to test for a nutrient effect, dimethoate and carbofuran to test for microarthropod or nematode effect, fumagillin to test for protozoa effect, captan to test for fungi effect, penicillin and oxytetracycline to test for bacteria effect, pasteurization to test for the effect of active forms of organisms, tyndallization to test for the effect of resting forms of organisms, and water for control. We studied the effect of these treatments on number and types of ectomycorrhizae, and length of active mycelium and number of active bacteria in the rhizosphere. Inoculation with untreated forest or plantation soils increased numbers of ectomycorrhizae but did not affect diversity. Most agents had different effects in different soils. Treatments with significant effects increased the number of *Rhizopogon* and *Thelephora*

effects increased the number of *Rhizopogon* and *Thelephora* ectomycorrhizae and decreased the number of active bacteria. We hypothesize that the role of the soil transfer is to provide a rhizosphere environment free from a deleterious organism present in the clear-cut. In this environment, beneficial organisms present in the clear-cut or brought in with the seedling from the nursery can proliferate and form a healthy rhizosphere.

Introduction

In the harsh environment of the high elevations in the Siskiyou mountains of Southwestern Oregon, some forests that were clear-cut and burnt in the late 1960's have proven difficult to regenerate. The total area in this condition approaches 2500 Ha (Amaranthus, pers. comm). One of these sites, Cedar Camp, was clear-cut and burned in 1967, and subsequently planted four times. Conifer regeneration remains poor, even though there is natural regeneration in the adjacent undisturbed stands

Amaranthus and Perry (1987) found that *Pseudotsuga menziesii* seedlings planted on Cedar Camp benefited from inoculation with soil from a nearby vigorous plantation; survival and numbers of ectomycorrhizae increased. They also found that the addition of pasteurized soil, presumably devoid of ectomycorrhizal propagules, increased ectomycorrhizal formation. Colinas et al. (in prep. b) corroborated that soil inoculation increased survival and growth, and found that inoculation with pasteurized or fungicide treated soil also increased survival. This suggests that the removal of some deleterious agent present in the soil, or the creation of unoccupied habitat for beneficial organisms to proliferate, may have been involved.

Relationships of soil biota with plants and among themselves are complex and not well understood (Coleman 1985). Soil organisms can interact directly with the plant (Alstrom 1987; Friedman et

al 1989; Strzelczyk and Pokojska-Burdziej 1984; Chanway and Holl 1992), indirectly through the ectomycorrhizae (Bowen and Theodorou 1979; Garbaye et al. 1990; Garbaye and Bowen 1989.; Duponnois and Garbaye 1990; Duponnois and Garbaye 1991; Summerbell 1987; Sutherland and Fortin 1968; Chakraborty et al. 1985), or through other rhizosphere organisms (Bowen and Theodorou 1979). In a previous study (Colinas et al. in prep. b) we addressed the effects of certain groups of soil organisms on seedling growth and survival. In this study we focus on two potential mediators of the effect of soil organisms on seedlings: ectomycorrhizae and rhizosphere fungi and bacteria.

We inoculated outplanted seedlings with soils previously treated with selected biocides to remove grazers (nematodes and microarthropods), protozoa, fungi and bacteria. We also added pasteurized soil wherein active organisms had been killed, tyndallized soil to remove resting forms of organisms, and fertilized soil. This allowed us to study the roles of these organisms by observing the effects of their absence rather than their presence

Our specific objectives were to determine the effects of the soil transfers on the number and type of ectomycorrhizae, number of active bacteria, length of active fungal hyphae, and number of actinomycetes in the seedlings' rhizosphere. We were especially interested in actinomycetes because this filamentous bacteria

had been linked to regeneration failure in Cedar Camp (Friedman et al. 1989).

Materials and Methods

Site description

Cedar Camp (42° 4' 45" N, 123° 23' 10" W) is on a 50% slope with SW aspect at an elevation of ca. 1720 m. Annual precipitation averages 1650 mm, more than half in snow. A prolonged summer drought is common. The soil is sandy and skeletal (mixed Entic Cryumbrept). Complete soil data are available in Amaranthus and Perry (1987).

Transfer soils

Soils were collected from three sites: Cedar Camp clear-cut, adjacent mature forest, and a nearby vigorous *Pseudotsuga menziesii* plantation established in 1974 on a previously burned clear-cut. All three sites coincide with those described in Amaranthus and Perry (1987). We refer to these soils as clear-cut soil, forest soil and plantation soil. Levels of soil macronutrients and pH did not differ significantly among the three soils (Amaranthus and Perry 1987).

Ten soil samples were taken from each soil type. In the clear-cut and plantation, the first sample was taken at an arbitrarily selected location in the central area; nine additional sample sites were chosen at random directions and distances from the previous sampling site. The first sample from the forest soil was

taken at 100 m from the clear-cut edge and the remaining nine as noted above. No samples were taken at less than 65 m from the boundary to avoid edge effects.

Samples were collected by brushing away the litter layer (if present) and then removing soil from an area 20x40 cm to a depth of 10 cm. Samples were sieved through a 1 cm mesh screen to eliminate rocks and debris and composited by site into a single 120 L plastic container.

Soils were collected on June 4, 1990 while still saturated from spring rains. In order to apply chemicals to the soil as water solutions, water contents were reduced to 50% by spreading soil 2 cm deep on a plastic sheet overnight. Temperature was monitored during the drying process to assure that it had not been detrimental to soil microorganisms. Maximum temperature reached 8°C during drying, 3°C lower than the soil temperature measured that day in the clear-cut 10 cm below the soil surface. Soils were then returned to the plastic containers and transported to the laboratory to receive treatments.

Agents

The chemical compounds applied to the soils and their sources are described in Table 1. The combinations and concentrations at which they were applied are described in Table 2. Preparation and application were as follows.

Pasteurization

Soils were brought to field capacity with distilled water, placed in a tray to a depth of 5 cm, covered with aluminum foil, heated in an oven to 70°C and maintained at that temperature for 3 h. Field capacity was maintained by weighing the trays and adding sterile distilled water as needed. Planting in the field started seven hours after pasteurization.

Tyndallization

Tyndallization treatment consisted of pasteurization repeated three times at 24 h intervals. Between pasteurizations the soils were allowed to cool to room temperature ($\approx$18°C). Planting in the field started seven hours after the last pasteurization.

Chemical agents

Chemical compounds (Table 1) were applied to soils at manufacturer recommended rates (Table 2). They were dissolved in an appropriate amount of distilled water to bring the soils to field capacity (0.27, 0.33 and 0.21 g of water/g dry soil for clear-cut, forest and plantation soils respectively.)

Nine liters of each soil were placed in a 20 L bucket. Vertical holes 1 cm in diameter were made in the soil at 5 cm intervals to facilitate dispersion of solution into the soil. Chemical solution was then added to the soils by pouring it over the surface and allowing it to slowly enter the soil. Soils were incubated at room

temperature for 32 h. Planting in the field started at the end of the incubation period.

Control

Untreated control soils were brought to field capacity with distilled water at the same time that the chemicals were applied to the treated soils.

Seedling inoculation and data collection

Seedlings were 1-0 container grown *Pseudotsuga menziesii* seed source 491 from the Timberlands nursery (Lebanon, Oregon) approximately 20 to 30 cm tall. The ectomycorrhizal (EM) fungus *Thelephora terrestris* was present on the seedlings' roots. Each seedling was placed in a planting hole to which 150 ml of transfer soil from one of the 24 treatments (3 soils x 8 agents) was added. All seedlings were carefully excavated in early December 1990 following fall rains and soil-moisture recharge, placed inside air tight plastic bags on ice in the field, and transported to the laboratory where they were stored at 2°C for up to six weeks. Rhizosphere soil was collected by gently removing the clumps of soil attached to the roots and discarding it, and then shaking the roots briskly inside a bag. Soil particles removed in this manner were considered "rhizosphere soil."

Active fungi and bacteria

Measurements of fungi and bacteria in the rhizosphere were made 48 h after the seedlings were lifted.

Active hyphal length was estimated by direct observation using FDA (fluorescein diacetate, Sigma Co.) staining (Ingham and Klein, 1984). A 1 ml aliquot from a 10^{-1} soil solution was stained by adding 1 ml of FDA working solution. After 3 min., 1 ml of molten 1.5% (wt/vol.) agar in phosphate buffer (0.2 M, pH 9.6) was added and approximately 0.1 ml placed on a slide of known area and depth. Length of fluorescent hyphae was determined by direct microscopic observation of the soil-agar film by epifluorescent microscopy at 160x magnification; 90 fields per slide were examined.

Active bacteria were determined on the same agar film by counting fluorescent bacteria in 3 fields using oil immersion epifluorescent microscopy (1000x magnification).

Actinomycetes

Actinomycete populations were estimated by counting colony forming units (CFU) in a selective growth medium in petri dish. The medium was based on Davies and Williams (1970) and contained: Bacto Actinomycete Isolation Agar, 22 g.; Glycerol 5 g; Actidione (Sigma) 5 mg., Nystatin (Sigma) 5 mg., Polymixin B

Sulfate (Sigma) 0.5 mg., Sodium Benzil Penicillin (Sigma) 0.5 mg. Antibiotics were added after autoclaving for 15 min.

A volume of 0.1 ml of a 10^{-2} and 10^{-3} soil dilution was plated. Two replicate plates were prepared per sample per dilution, and both plates in one dilution were counted after three weeks of culture at 25 °C. The average of those two plates was considered one observation.

Ectomycorrhizae

Seedlings were examined for numbers and types of ectomycorrhizae within three weeks of retrieval from the field. The entire root system of each seedling was examined under the dissecting microscope and all ectomycorrhizae on the newly emergent roots were counted and classified by types according to Roth (1990) and Massicotte (1990 unpublished data). Questionable ectomycorrhizae were examined under the compound microscope for presence of Hartig net.

Statistical design and analysis

The experiment followed a randomized block design with soils (3), agents (8) and blocks (8) as factors.

Eight blocks were laid in areas distant at least 2 m from large shrubs. Five seedlings per treatment were planted at random in each block totaling 5x24x8=960 seedlings in the experiment.

Some variables were transformed to meet the assumptions of statistical tests. Treatment means obtained from transformed data were back transformed to the original scale and reported as medians. Means or medians of treatments are expressed in the text as percent of the control, untreated clear-cut soil. Their absolute values are presented in the tables.

For active fungi, bacteria, and actinomycetes, rhizosphere soil from all live seedlings was pooled by block and treatment in one sample and considered one experimental unit. When no seedling survived in a treatment in a block, the rhizosphere soil of all five dead seedlings was pooled in one sample and considered one experimental unit. An extra indicator variable (live/dead) was added to identify experimental units where all seedlings were dead. Two analyses were performed, one for all experimental units and another for experimental units with live seedlings.

For active fungi and bacteria, only the four blocks with the most surviving seedlings were assayed totaling 96 observations in the analysis of all seedlings and 57 in the analysis of live seedlings. For actinomycetes the living seedlings in all 7 blocks were assayed and included in the analysis of "live seedlings" with a total of 72 observations

Rhizopogon type and *Thelephora* type ectomycorrhizae counts were analyzed with each live seedling as an experimental unit totaling 137 observations.

Analyses of variance were performed in SPSS for the Macintosh v. 4.0.1 (SPSS Inc., Chicago, Illinois, USA.). All treatments were compared to the control using contrasts with one degree of freedom. The p-values of these contrasts are reported in the graphs.

Cenococcum ectomycorrhizae were infrequent and analyzed as presence/absence using logistic regression in GLIM 3.77 (Royal Statistical Society, London, UK).

Results

Ectomycorrhizae

Only 3 types of ectomycorrhizae were found in this experiment. The most abundant coincided with Type 1 of Roth (1990) and Type "Thel" of Massicotte (1990 unpublished data). Both authors report the fungal symbiont in this type as *Thelephora terrestris* Ehrenb.: Fr. The second type coincided with Type 2 of Roth (1990) who identified the fungal symbiont as *Rhizopogon vinicolor* A.H.Smith, and Type "rhizvill" of Massicotte (1990 unpubl.) who identified the fungus as belonging to the genus *Rhizopogon* subgenus *Rhizopogon* section *villosuli*. The third type coincided with Type 4 of Roth (1990) and Type "ceno" of Massicotte (1990 unpubl.). Both authors report the fungal symbiont in this type as *Cenococcum geophillum* Fr.

Rhizopogon EM

Seedlings given clear-cut soil that had been pasteurized, tyndallized, or treated with fumagillin averaged 4 to 5 times more *Rhizopogon* EM than the control (Fig. 3.1). Untreated forest soil increased *Rhizopogon* EM, an effect that was not seen when forest soil was tyndallized. Untreated plantation soil did not effect *Rhizopogon* EM, but it stimulated *Rhizopogon* EM formation when fertilized, pasteurized, tyndallized, or treated with fungicide or bactericide.
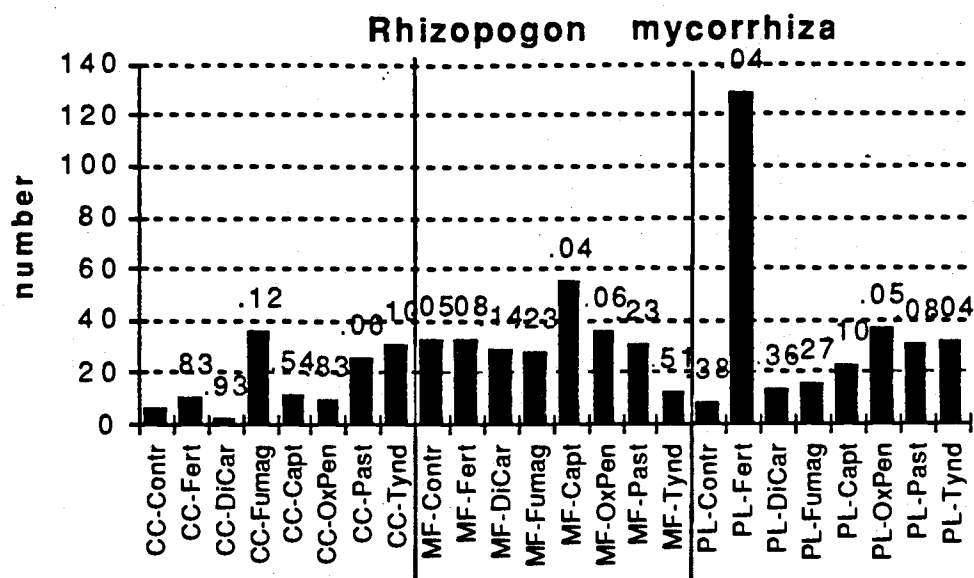
Rhizopogon mycorrhiza

Fig. 3.1. Median number of Rhizopogon type ectomycorrhizae. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Thelephora EM

Seedlings given clear-cut soil that had been pasteurized or tyndallized, had more than twice the number of Thelephora EM of the control (Fig. 3.).

Untreated forest soil increased the number of Thelephora EM. Untreated plantation soil increased the number of Thelephora ectomycorrhizae. The stimulating effect of plantation soil was removed by grazercide and protozoacide.
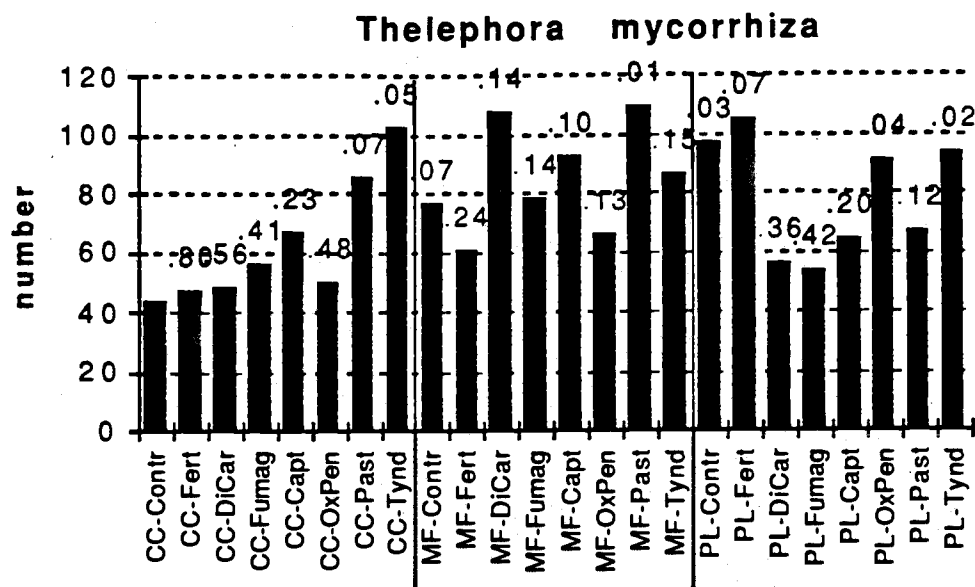
Thelephora mycorrhiza

Fig. 3.2. Median number of *Thelephora* type ectomycorrhizae. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Cenococcum

Cenococcum occurred on only thirty-one seedlings and formed few ectomycorrhizae. From the almost 20,000 ectomycorrhizae counted, less than 200 were formed by *Cenococcum*. No treatment effects were detected.

Active fungi

Analysis of live and dead seedlings

In clear-cut soil, grazercide increased active hyphal length (Fig. 3.3). Untreated forest soil did not affect active hyphal length, but

fertilized and tyndallized forest soil increased active hyphal length. Untreated plantation soil did not increase active hyphal length, but grazercide treated plantation soil increased active hyphal length and pasteurized plantation soil decreased active hyphal length. Dead seedlings had 170% more active hyphae in their rhizospheres than living seedlings (P=0.003).

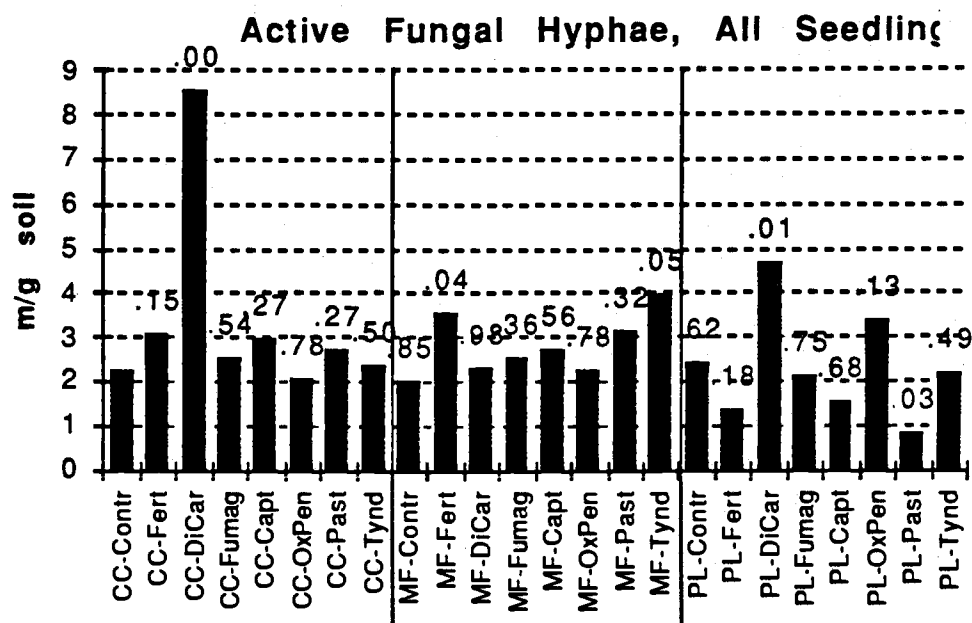**Active Fungal Hyphae, All Seedling**

Fig. 3.3. Median length of active fungal hyphae per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Analysis in living seedlings

Large variability prevented differences among treatment means from being statistically significant. The range of active hyphal

lengths measured was between 0.48 and 3.5 m/g of dry rhizosphere soil (Fig. 3.4).
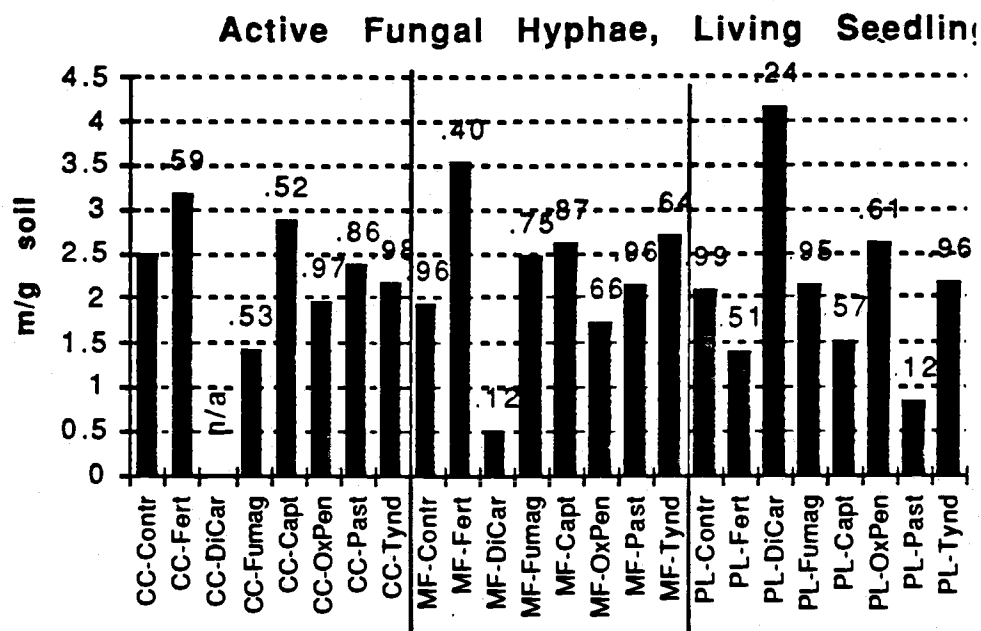
Active Fungal Hyphae, Living Seedlin

Fig. 3.4. Median length of active fungal hyphae per g of dry soil in the rhizosphere of living seedlings. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Active bacteria

Analysis of live and dead seedlings

Seedlings given clear-cut soil treated with protozoacide, bactericide and tyndallization had lower numbers of active bacteria in their rhizospheres (Fig. 3.5). Untreated forest soil decreased the number of active bacteria in the rhizosphere of seedlings .The negative effect of forest soil on number of

bacteria was removed by fertilizer and grazercide. Untreated plantation soil did not effect active bacterial numbers, but all agents except grazercide caused highly significant reductions in plantation soil. Dead seedlings had 50% more active bacteria in their rhizosphere than live seedlings (p=0.025).
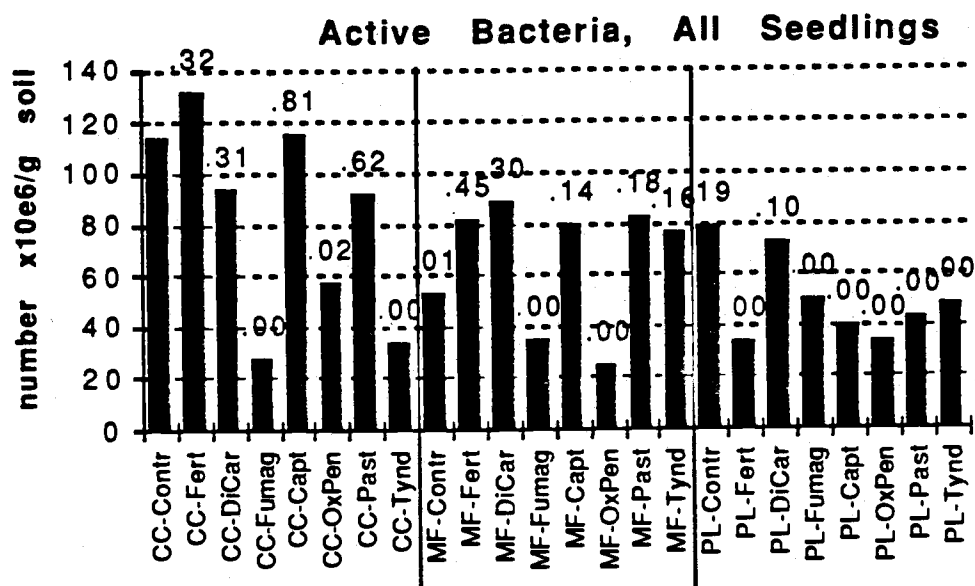

Fig. 3.5. Median number of active bacteria per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Analysis in living seedlings only

Active bacteria in live seedlings revealed the same pattern as when studied over all seedlings, but higher variability prevented differences from being statistically significant (Fig. 3.6). Rhizospheres of live seedlings that received the control soils had

the same median number of active bacteria as dead seedlings in the same treatment.
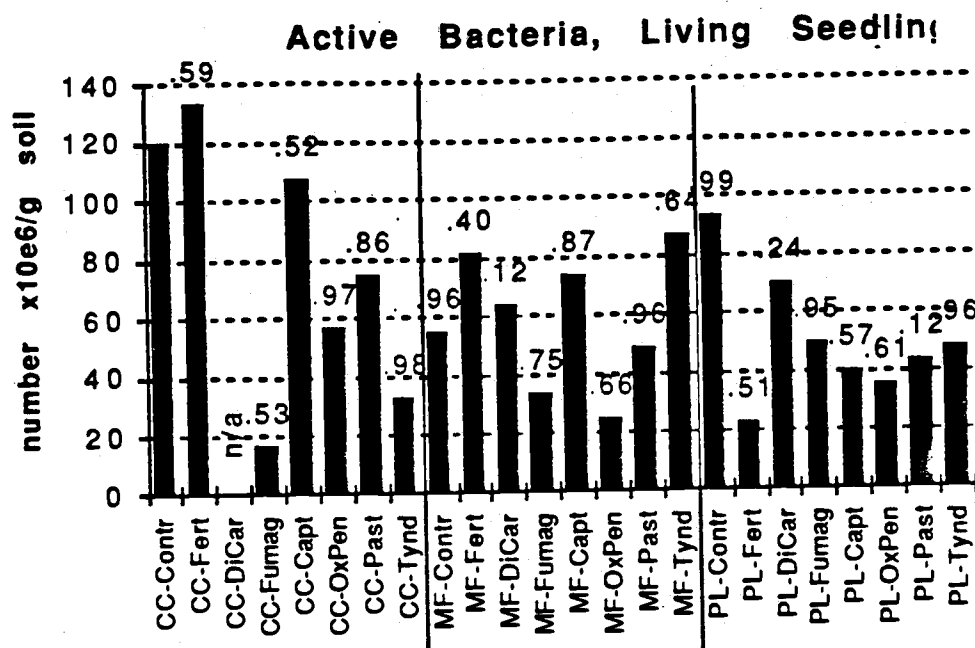

Fig. 3.6. Median number of active bacteria per g of dry soil in the rhizospheres of living seedlings. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Actinomycetes

No treatment affected the number of actinomycetes in' the rhizosphere of seedlings (Fig. 3.7). Live and dead seedlings did not differ in the number of actinomycetes in their rhizosphere. The grand mean was 250×10^3 colony forming actinomycete units per g dry soil and the range was 147×10^3 in tyndallized plantation soil to 355×10^3 in tyndallized clear-cut soil.
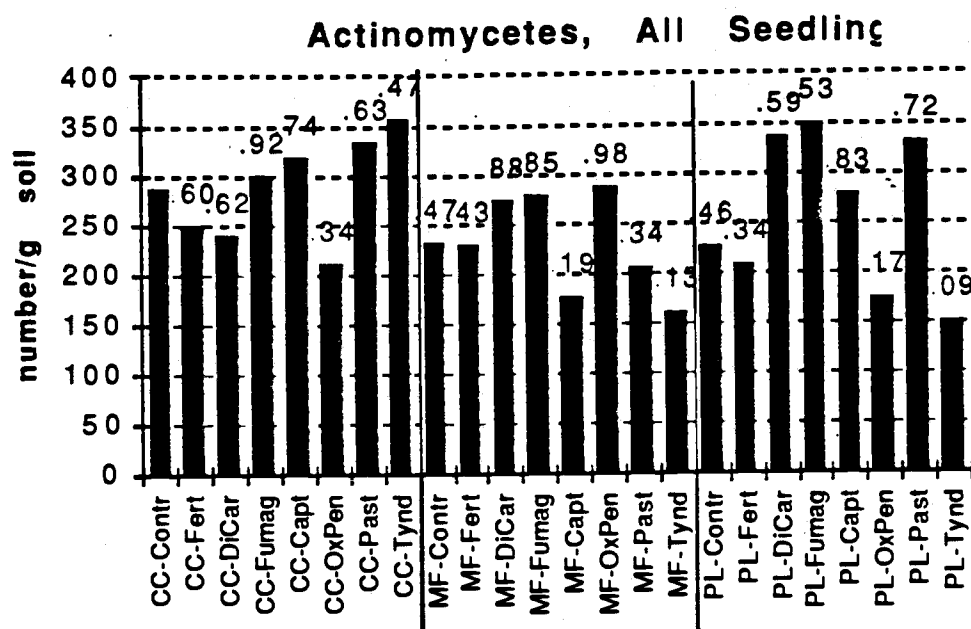
Actinomycetes, All Seedling

Fig. 3.7. Median number of actinomycetes per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (CC-contr). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; Tynd, Tyndallization; CC, clear-cut soil; MF, forest soil; PL, plantation soil.

Discussion

The summer of 1990 was unusually dry in Cedar Camp, which combined with a year of high pocket gopher (Thomomys bottae) activity, caused high seedling mortality. This high mortality reduced the number of replicate plants available, caused uneven replication and resulted in less precise estimates of the variables than we had anticipated.

Ectomycorrhizal diversity was low. We only encountered three types of ectomycorrhizae, even though all newly emergent short roots were ectomycorrhizal. One of these types, Cenococcum, occurred on less than 1% of the short roots and only 20% of the seedlings. In a greenhouse experiment, Colinas et al. (in prep. d) grew seedlings in containers with soil from the same clear-cut amended with plantation or forest soil and likewise retrieved the same three types that we observed in the field. Our data agree with those of Borchers and Perry (1990) collected in a similar understocked clear-cut. They found only four types and approximately 90% of total feeder roots were ectomycorrhizal. Three of their types coincide with ours.

But Massicotte and Molina (1990 unpublished data) working in a nearby forest at slightly lower elevation recovered 8 ectomycorrhizal types in a greenhouse bioassay study from soils from a regenerating clear-cut, 8 from a plantation and 12 from a mature adjacent forest. Given the proximity and similarity

between their study site and ours, we could assume that our transfer soils had a similar diversity of ectomycorrhizal propagules. Yet inoculation with these soils did not increase diversity in our seedlings, neither in the field nor in the greenhouse. This suggest the presence of a factor in the clear-cut soil that prevents propagules of some fungi from forming ectomycorrhizae. Environmental conditions and amount of inoculum are alternative hypotheses that should be addressed in future studies.

Forest and plantation soils increased total number of mycorrhizae corroborating the results of Amaranthus and Perry (1987). But this does not mean that the soil transfers added critical EM inoculum. The positive effect of soil transfers on the number of mycorrhizae can be explained by an effect on the plant increasing its number of short roots. Soil transfers were able to increase survival and growth of seedlings (Colinas et. al in prep. b) despite not contributing new EM components to the system, thus adding evidence against the original hypothesis proposed by Amaranthus and Perry (1987) that lack of regeneration at Cedar Camp was caused by lack of ectomycorrhizal inoculum. The fact that ectomycorrhizal formation by the two major types present in the clear-cut was enhanced by inoculation with pasteurized or tyndallized clear-cut soil suggests that it was not the addition of a biological entity that increased ectomycorrhizal formation. This supports the hypothesis proposed by Colinas et. al (in prep. b)

that the regeneration failure is caused by the presence of a deleterious organism rather than the absence of a beneficial one, or a combination of both. This would also explain why *Rhizopogon vinicolor* inoculated seedlings planted in Cedar Camp did not have better survival than non ectomycorrhizal seedlings (Amaranthus unpubl. data).

Dead seedlings had 70% more active hyphae and 50% more active bacteria in their rhizospheres than living seedlings, possibly because decomposers had started to utilize the organic substances leaking from decaying roots. The data suggest a possible bacterial involvement in soil degradation. The three treatments that increased survival most -tyndallized, pasteurized and captan treated plantation soil- (Colinas et al. in prep. b) also had low number of active bacteria on their rhizosphere, even if counted on living seedlings only. This study cannot address whether increase in survival and decrease of active bacteria are cause or effect of each other. Future studies addressing this cause and effect relationship would clarify the role of bacterial populations on seedling survival.

The addition of forest soil decreases bacteria in the rhizosphere, perhaps because of a negative interaction between some forest soil organism and clear-cut rhizosphere bacteria. This effect was removed by both heat treatments, fertilizer, grazercide and fungicide, making grazers and fungi targets for future studies addressing the antagonism between forest soil and clear-cut

rhizosphere bacteria. Fertilizer did not have any effect on the numbers of organisms in the studied groups (Colinas unpubl. data) . A qualitative change in some populations may have been responsible for its effect.

Bactericide reduced the number of active bacteria in the seedling rhizospheres at the end of the growing season in all three soils and increased ectomycorrhizae in forest and plantation soil, but not in clear-cut soil. Yet, this increase in ectomycorrhizal formation did not translate into increased survival (Colinas et. al. in prep. b). We did not test for the permanence of biocide activity in the transfer soils, but this seems of little relevance because seedling root systems had been growing mostly in site soil for six months when the analysis were performed. Former studies of bacterial effects on ectomycorrhizae show complex positive, negative and mediated interactions (De Oliveira and Garbaye 1989; Garbaye et al. 1990; Duponnois and Garbaye 1991). A negative influence of bacteria on ectomycorrhizal formation emerges from this study, but conclusions based on numbers of bacteria without any breakdown by function are limited.

The treatments did not affect the number of actinomycete colony forming units (CFU) in seedling rhizospheres. This contradicted our working hypothesis that the presence of high levels of actinomycetes would hamper survival and mycorrhization (Friedman et al. 1989). In our case there were substantial differences in survival (Colinas et al. in prep. b) and in number of

ectomycorrhizae among seedlings receiving different treatments but no differences in the numbers of actinomycete CFU. This still does not rule out the possibility that an actinomycete species that represents only a small fraction of the population can be causing the effect and its presence or absence could not be detected by the present method.

The number of actinomycete CFU that we found was lower than the number found by Friedman et al. 1989 on the same site, 250,000 CFU/g dry soil in our experiment versus 350,000 or 420,000 CFU/g dry soil depending on the growth medium they were using. This can be explained by the source of soil samples. In our case the soil came from seedling rhizospheres. In their study it was bulk soil from the top 1-3 cm.

Captan increased *Rhizopogon* ectomycorrhizae while decreasing active bacteria in both plantation and forest soil stressing the connection between those two variables. Captan removed active fungal hyphae from the transfer soils almost completely (Colinas et al. in prep. a). It is difficult to think that any viable propagules were added with this soil, but we did not test for it. Airborne EM fungi propagules should be equally present in all three sites given their proximity. Resting forms of the fungi present only in the plantation or forest soil and not sensitive to captan may have germinated after captan had become inactive in the soil. If so, no difference between untreated and captan treated soil in *Rhizopogon* ectomycorrhizae would likely occur. That does not

seem to be the case, but the mean separation procedure did not allow us to test this difference. Alternatively, captan may have removed a key bacterial group thereby allowing ectomycorrhizal development (Garbaye et al. 1990), or a group of fungi inhibitory of ectomycorrhizal development (Summerbell 1987).

Some amoebae are able to reduce *Rhizopogon* colonization of *Pinus radiata* roots (Chakraborty et al. 1985). In our case, a reduction of three orders of magnitude in the number of protozoa in the transfer soils (Colinas et al. in prep. a) did not have any significant effects on ectomycorrhizae formation or on active fungal hyphae.

Fumagillin strongly reduced active bacteria in all three soils against our expectations because most protozoa are bacterial feeders (Bamforth 1988). We tested the effect of fumagillin on its target group, protozoa, and on all the non-target groups in the transfer soils (Colinas et al. in prep. a) and we found no effects on active bacteria; but it severely reduced the numbers of nematodes in the transfer soils. Protozoa and nematodes are two of the most mobile groups of organisms in the soil ecosystem. The pattern of strong reduction of active bacteria in all three soils seems too strong to be due to spurious variation. Possibly water fluxes in the soil pore space are not enough to transport bacteria from the bulk soil to the root and along the root as it grows. This would make mobile soil organisms that are able to

disperse bacteria a key factor in the development of a healthy rhizosphere (Visser 1985).

Clear-cut and plantation soil treated with the grazercide increased active fungal hyphae in the seedling rhizospheres, but this did not translate into increased numbers of ectomycorrhizae in either clear-cut, plantation or forest soil. Plantation soil treated with the grazercide also reduced active bacteria in the seedling rhizospheres. The grazercide did not have any significant effects on active bacteria or active fungal hyphae in the transfer soils (Colinas et al. in prep. a), so this effect was not likely direct. The grazercide did not reduce microarthropods significantly. The effect on active fungal hyphae can be explained based on predator-prey relationships between nematodes and fungi: the lack of grazers causes an increase in fungal prey. The effect on active bacteria could be explained by a mechanism homologous to that of the lack of protozoa on active bacteria. If grazers are a key dispersing agent for non motile bacteria, then the lack of grazers will cause lower bacterial colonization in the seedling rhizospheres (Visser 1985).

Amaranthus and Perry (1987) proposed that inorganic nutrients or organic compounds in pasteurized forest soil may be responsible for the increase in ectomycorrhizae formation. To clarify the first, we introduced a fertilizer treatment designed to provide roughly the same amount of nutrients as would be released by killing all the soil biomass (Hart 1989).

Fertilized clear-cut soil did not increase ectomycorrhizae formation nor havve significant effects on any variables. This weakens the hypothesis that the increase in ectomycorrhizal development caused by soil pasteurization is due to the nutrients released by heat.

Fertilized forest soil increased *Rhizopogon* ectomycorrhizae but so did untreated forest soil. And fertilized plantation soil increased *Thelephora* ectomycorrhizae by the same factor as untreated plantation soil, supporting the hypothesis of no fertilizer effect. Yet, fertilized plantation soil behaved differently from untreated plantation soil in number of *Rhizopogon* ectomycorrhizae and active bacteria. Probably different mechanisms are operating for the different soils.

A soil transfer of 150 ml of the transfer soil added to the planting hole is not enough to surround a seedling root system so that the seedling would only grow on treated soil. The roots were growing predominantly in the site soil. Yet, the presence of treated soil was able to alter the rhizosphere of the roots in contact with the site soil even when the transfer soil was tyndallized. Key organisms must then be already present in the clear-cut but suppressed by hypothetical deleterious organisms, or come with the seedling from the nursery. In either case they would only be able to grow in the presence of some transfer soils that would act as "safe sites". The influence of these hypothetical beneficial organisms must spread throughout the hole root

system in order to affect mycorrhiza formation and populations of soil microorganisms. Future studies should address possible mechanisms by which these hypothetical beneficial organisms might exert their influence. These include the production of volatiles that would impregnate the soil gas phase (Stotzky and Schenk 1976; Schisler and Linderman 1989a; Schisler and Linderman 1989b; Schisler and Linderman 1989c; Graham and Linderman 1981), the production of hormone-like substance able to alter the physiology of the seedling (Laheurte et al. 1990; Strzelczyk and Pokojska-Burdziej 1984; Strzelczyk and Rozycki 1985), and a direct effect of an organism able to grow along with the new root growth and be present in the rhizosphere of all roots instead of just the rhizosphere of roots growing in transfer soil.

The study was able to further explore the mechanisms by which soil transfers improve seedling survival in sites hard to regenerate. Soil transfers facilitate formation of EM probably in an indirect way by modifying rhizosphere biology instead of directly by providing inoculum; one of these positive modifications being to lower the number of active bacteria in the rhizosphere. We hypothesize that the role of the soil transfer is to provide an environment favorable for some organisms already present in the system instead of to bring in some organisms from outside.

Acknowledgements

Table 3.1. Description of the chemicals used in the experiment.

Product name	Source	Active ingredient, common name	Active ingredient, standard name	% active ingredient	Target organism group
Clean Crop Dimethoate 267 EC Systemic Insecticide	Platte Chemical Co., Inc., Fremont, Nebraska, USA	Dimethoate	O,O-dimethyl- S-(N-methyl-carbamoylmethyl)phosphorodithi oate	30.5%	Arthropods
Furadan 10 G Insecticide	FMC Corporation Agricultural Chemical Group, 2000 Market Street, Philadelphia, PA 19103, USA	Carbofuran	2, 3-dihidro-2,2-dimethyl-7-benzofuranyl methyl carbamate	10%	Nematodes
Captan 50-WP	Stauffer Chemical Company, Westport, CT 06881, USA	Captan Related derivatives	N-(trichlorome thyl) thio] - 4 -cyclo - hexene - 1, 2, - dicarboximide	47.3 % 2.7 %.	Fungi
Fumidil B	Mid-Continent Agrimarketing, Inc., Lenexa, KS 66215, USA	Fumagillin	Bicyclohexammo nium Fumagillin.	2.2%	Protozoa
Oxytetracycline hydrochloride	Sigma Chemical Company, P.O. Box 14508, Saint Louis, MO 63178, USA	Oxytetracy-cline	Oxytetracy-cline hydrochloride		Bacteria
Penicillin-G Potassium salt	Sigma Chemical Company, P.O. Box 14508, Saint Louis, MO 63178, USA	Penicillin	Penicillin-G Potassium salt		Bacteria
Ra-pid-gro	Chevron Chemical Co. San Francisco, CA 94105 USA		Nitrogen Phosphorus Potasium Boron Copper Iron Manganese Zinc	23% 19% 17% 0.02% 0.05% 0.1% 0.05% 0.05%	

Table 3.2. Concentrations and combinations of chemicals in the treatments.

Treatment	Chemicals	Active ingredient μg per g dry soil
Grazercide	Dimethoate	200
	Carbofuran	25
Fungicide	Captan	25
Protozoacide	Fumagillin	10
Bactericide	Oxytetracycline	10
	Penicillin (Kunits)	10
Fertilizer	Ra-pid-gro	60 (Nitrogen)

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APPENDIX

RESULTS OF THE GREENHOUSE EXPERIMENTS MENTIONED IN THE DISCUSSIONS OF CHAPTERS 2 AND 3.

These experiments are fully described in Colinas et al. in prep. d and Colinas et al. in prep. e.
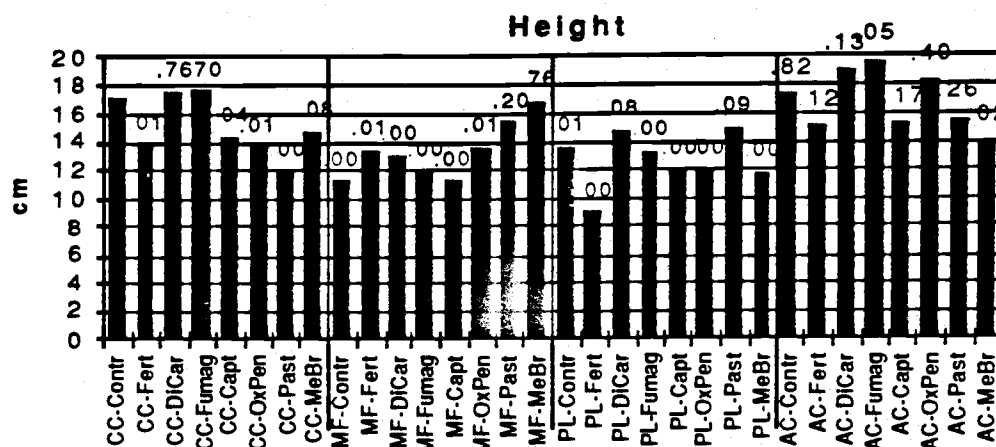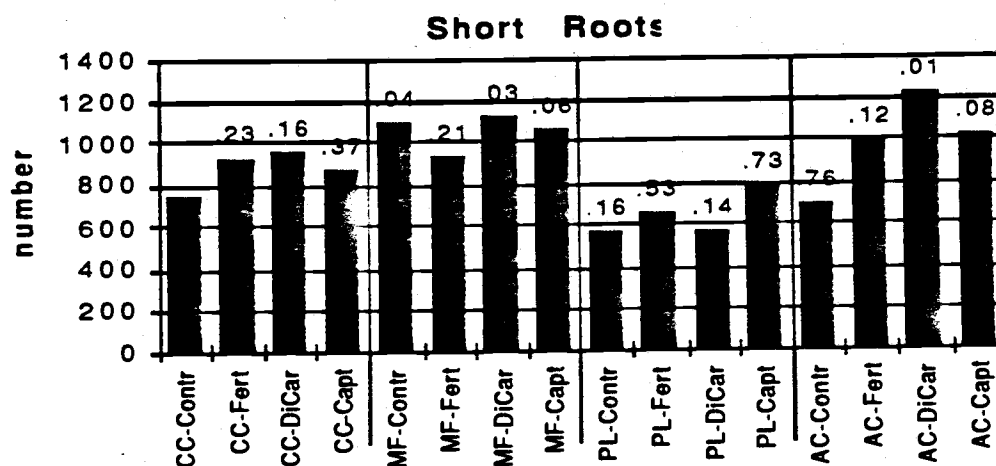

Fig. 4.1. Mean seedling height in cm. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; MeBr, Methyl bromide; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
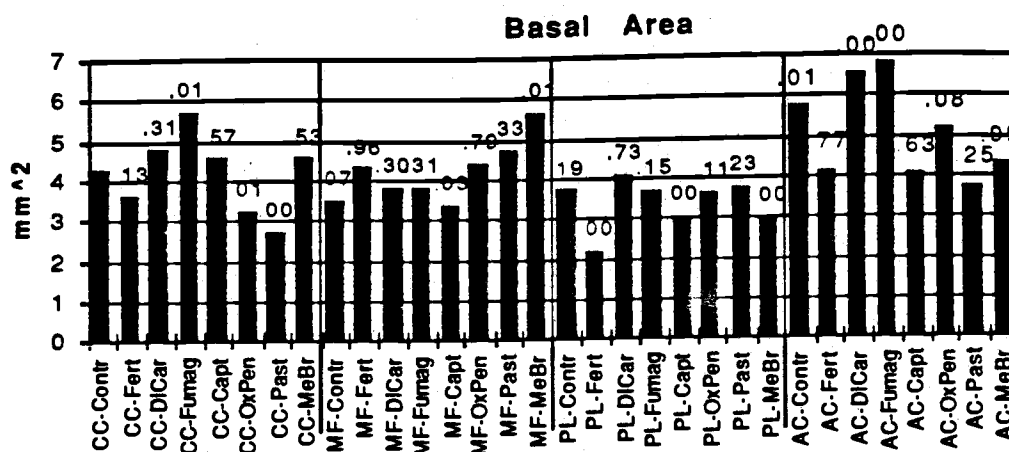
Basal Area

Fig. 4.2. Mean seedling basal area in mm2. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; MeBr, Methyl bromide; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
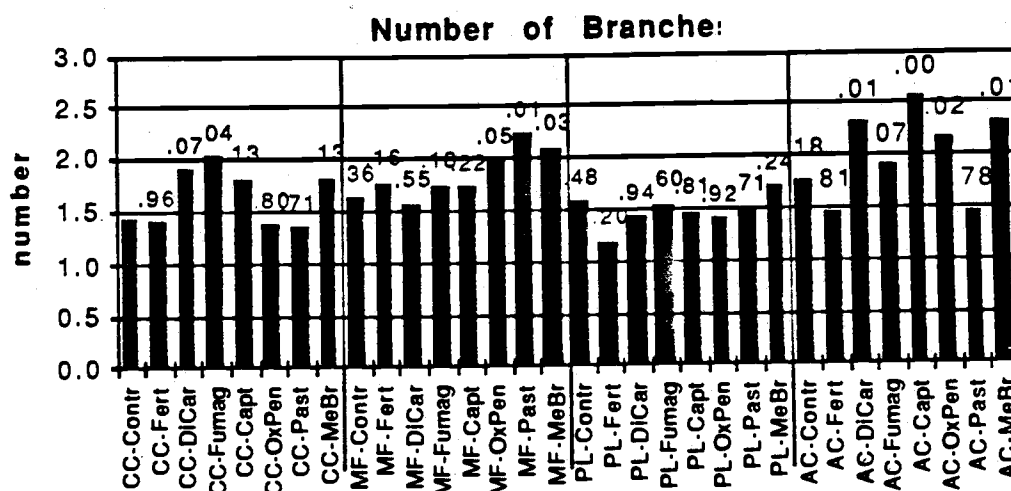
Number of Branches

Fig. 4.3. Mean seedling number of branches. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; Ox-Pen, Oxytetracycline-Penicillin; Past, Pasteurization; MeBr, Methyl bromide; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
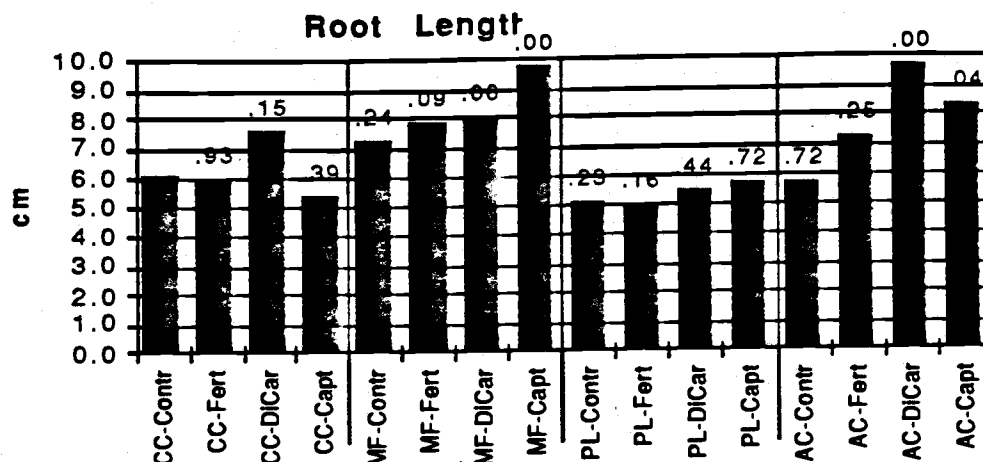
Root Length

Fig. 4.4. Mean seedling root length in cm. The numbers above the bars
are the p values of the contrasts comparing each treatment to the
control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer;
DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC,
clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved
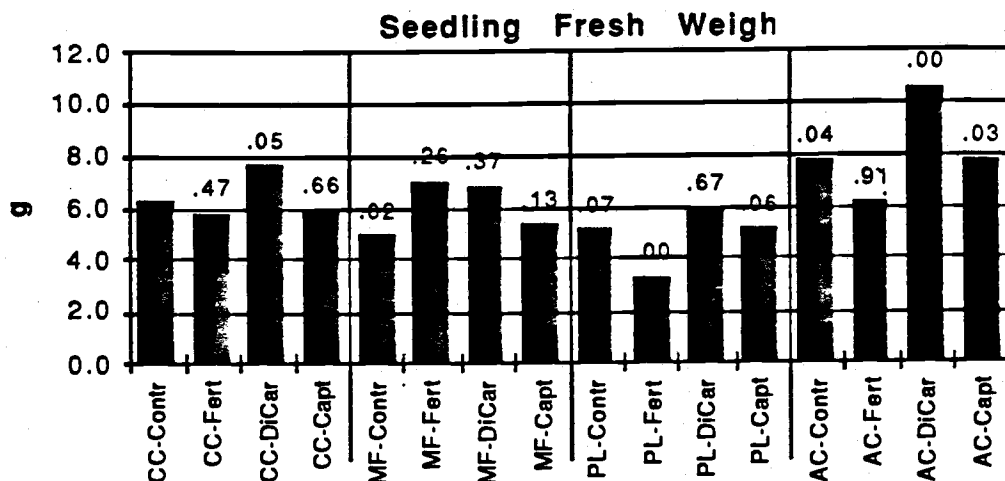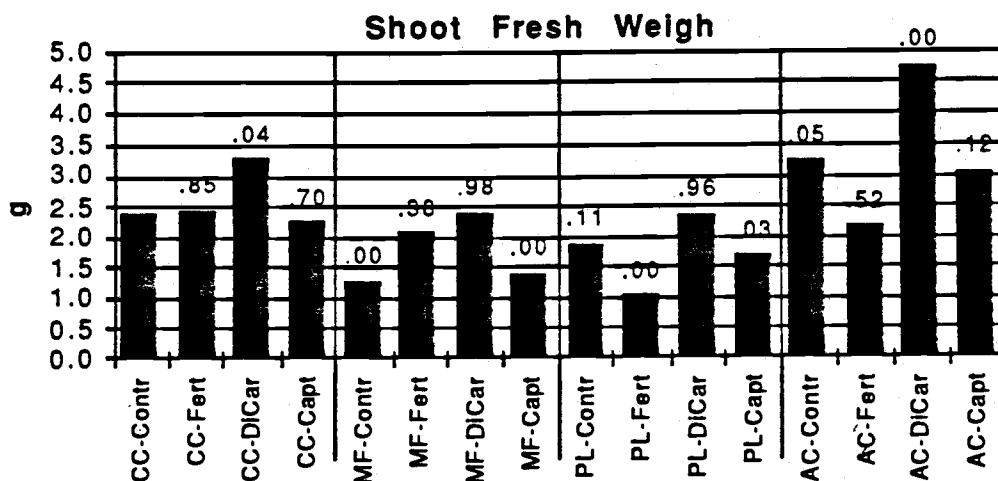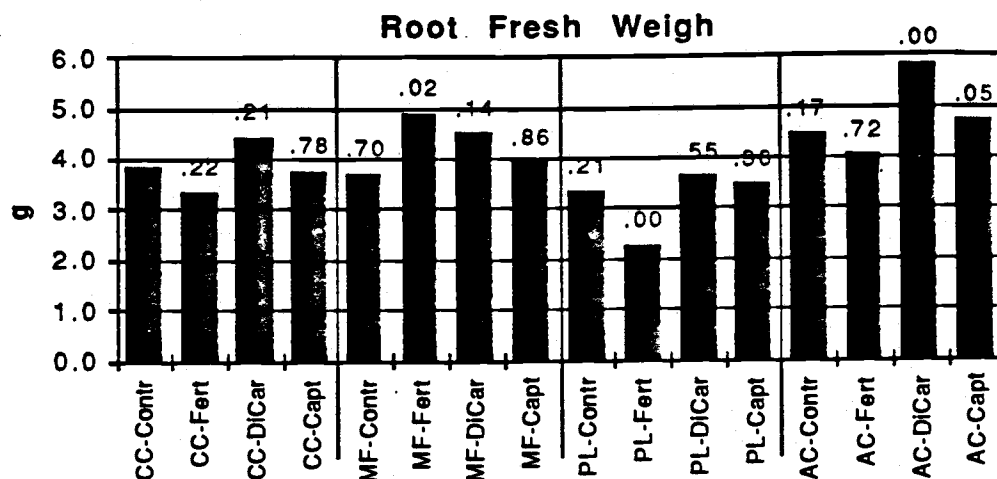clearcut soil.

Short Roots

Fig. 4.5. Mean seedling number of short roots. The numbers above the
bars are the p values of the contrasts comparing each treatment to the
control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer;
DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC,
clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved
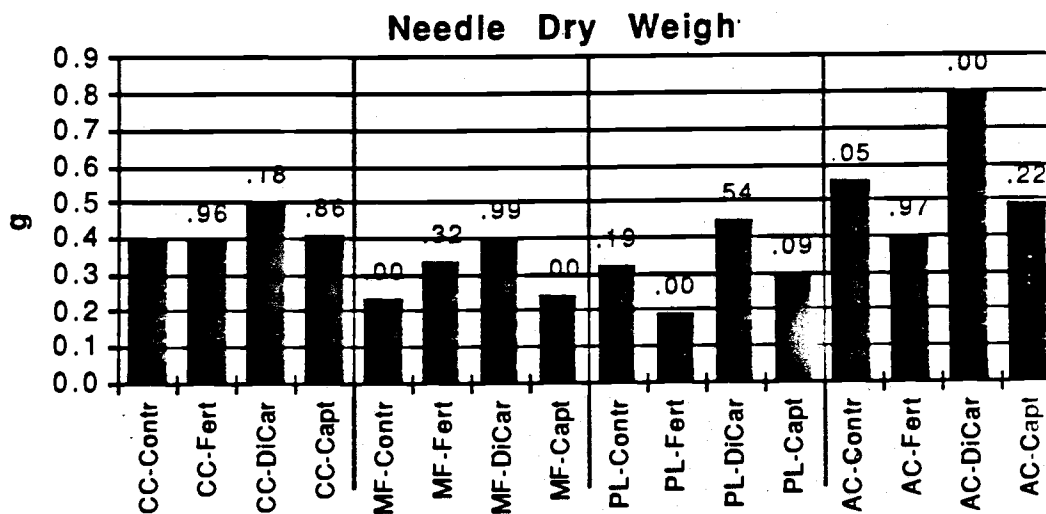clearcut soil.

Seedling Fresh Weigh

Fig. 4.6. Mean seedling fresh weight. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
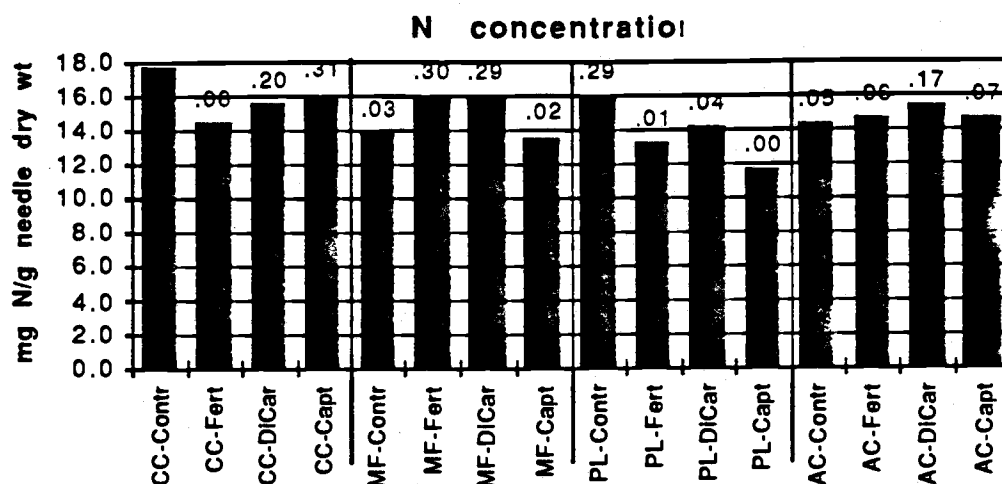
Shoot Fresh Weigh

Fig. 4.7. Mean seedling shoot fresh weight. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.

Root Fresh Weigh

Fig. 4.8. Mean seedling root fresh weight. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
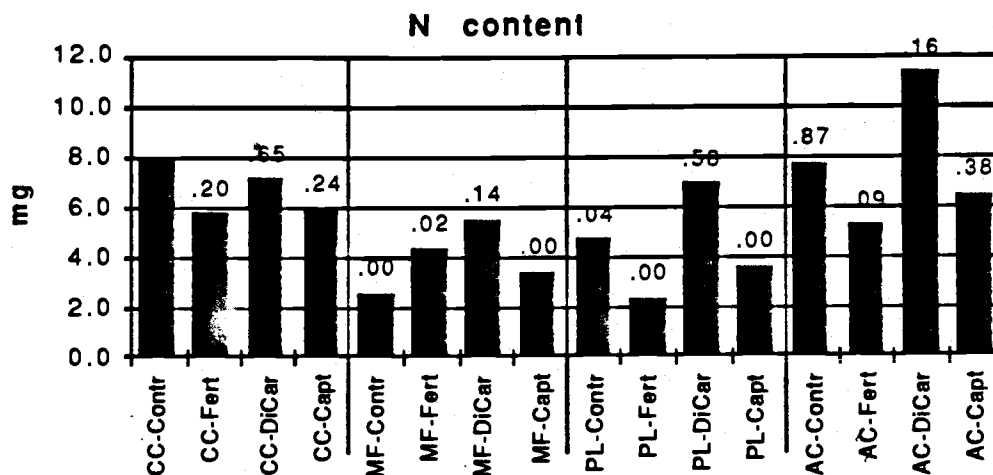
Needle Dry Weigh

Fig. 4.9. Mean seedling needle dry weight. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
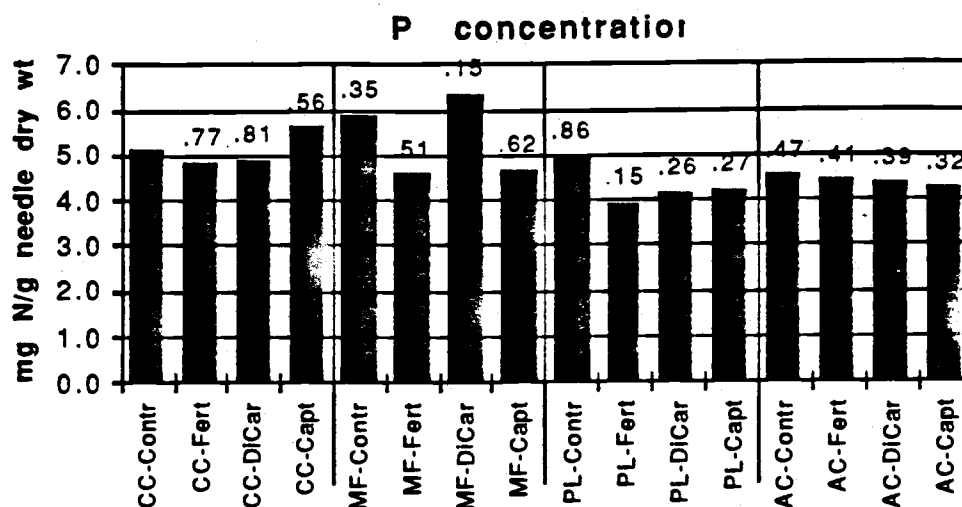
N concentration

Fig. 4.10. Mean seedling nitrogen concentration. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
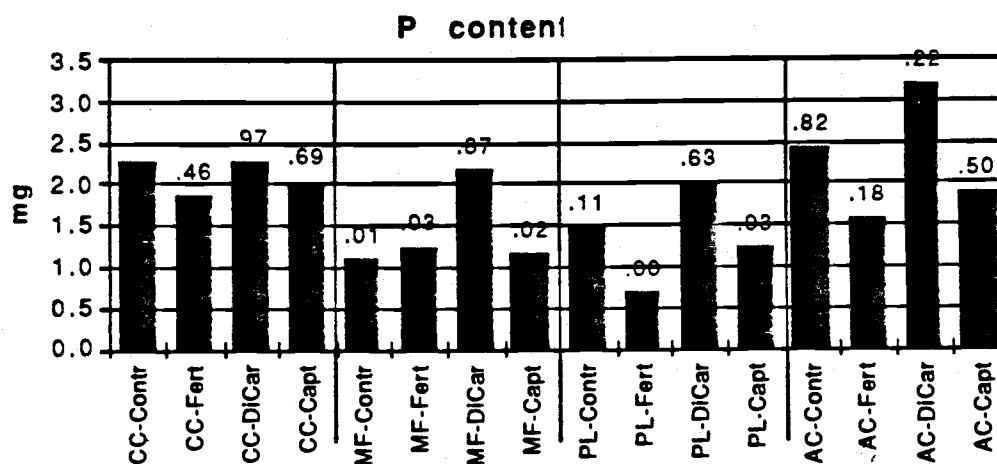
N content

Fig. 4.11. Mean seedling nitrogen content. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
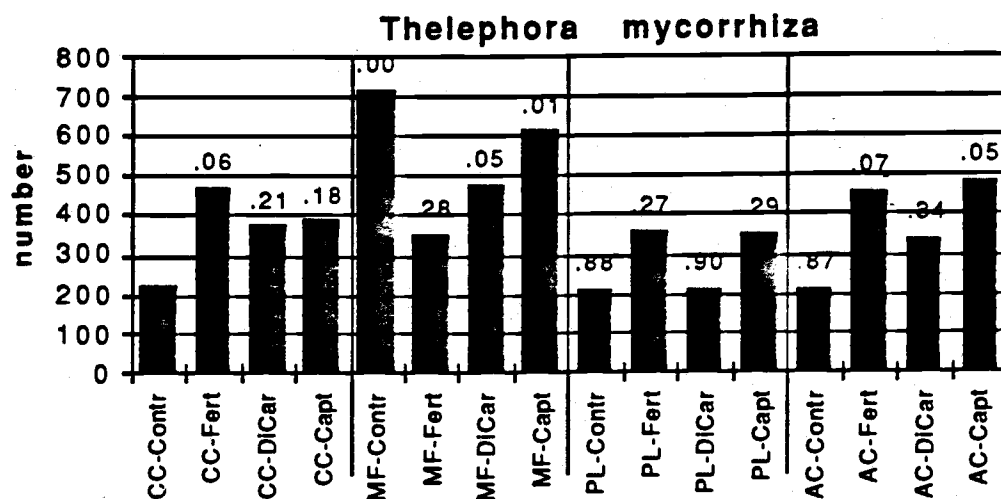
P concentration

Fig. 4.12. Mean seedling phosphorous concentration. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.

P content

Fig. 4.13. Mean seedling phosphorous content. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
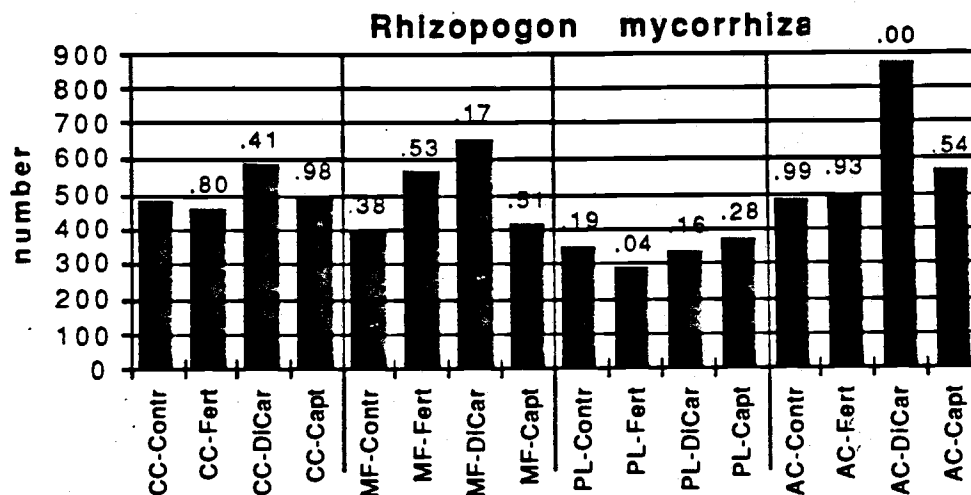
Thelephora mycorrhiza

Fig. 4.14. Median number of *Thelephora* mycorrhizae. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
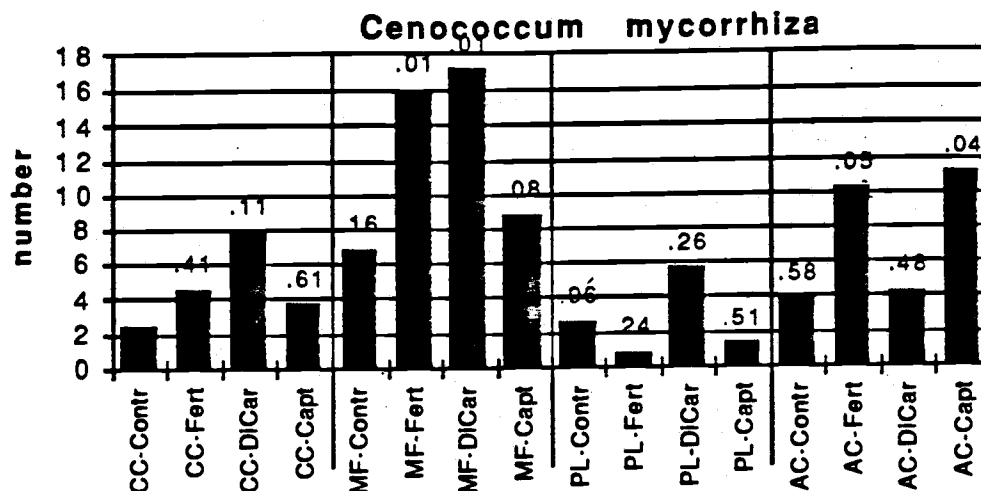
Rhizopogon mycorrhiza

Fig. 4.15. Median number of *Rhizopogon* mycorrhizae. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
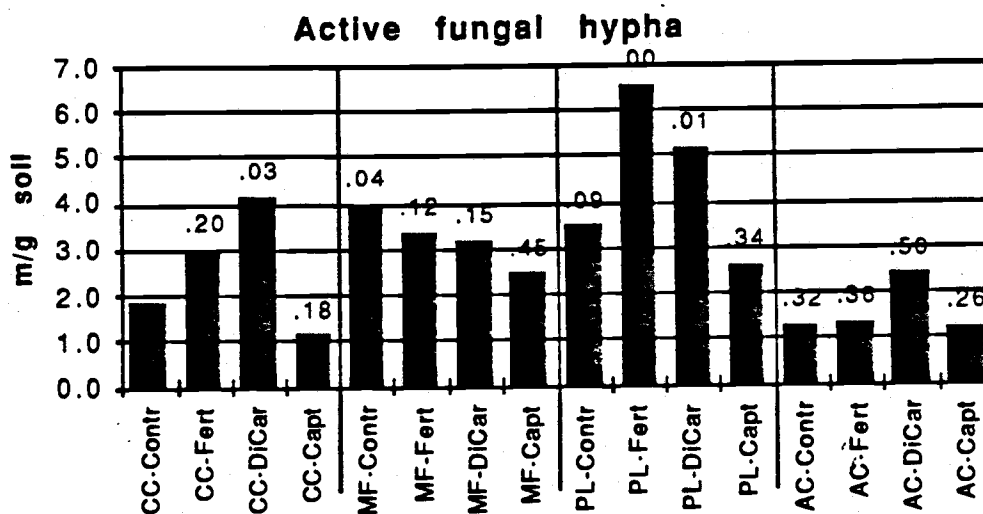
Cenococcum mycorrhiza

Fig. 4.16. Median number of *Cenococcum* mycorrhizae. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
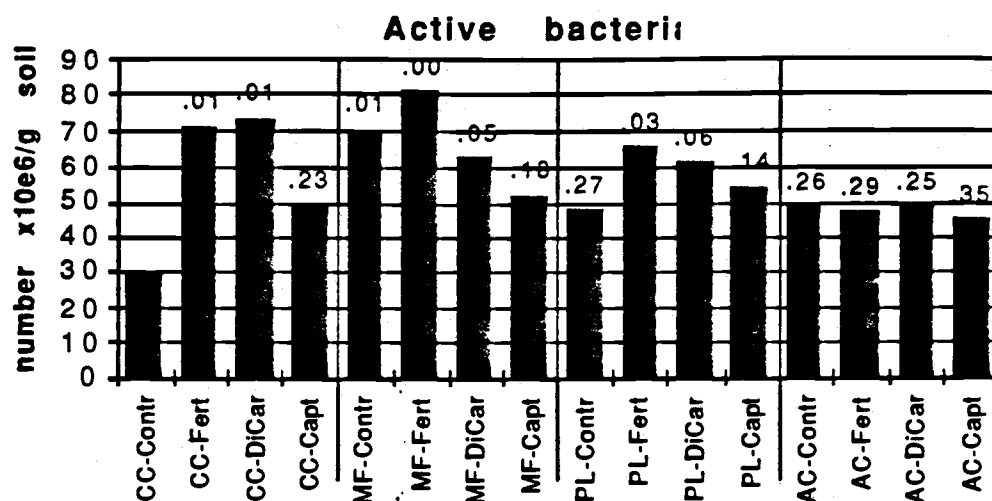
Active fungal hypha

Fig. 4.17. Median length of active fungal hyphae per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.

Active bacteria

Fig.4. 18. Mean number of active bacteria per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
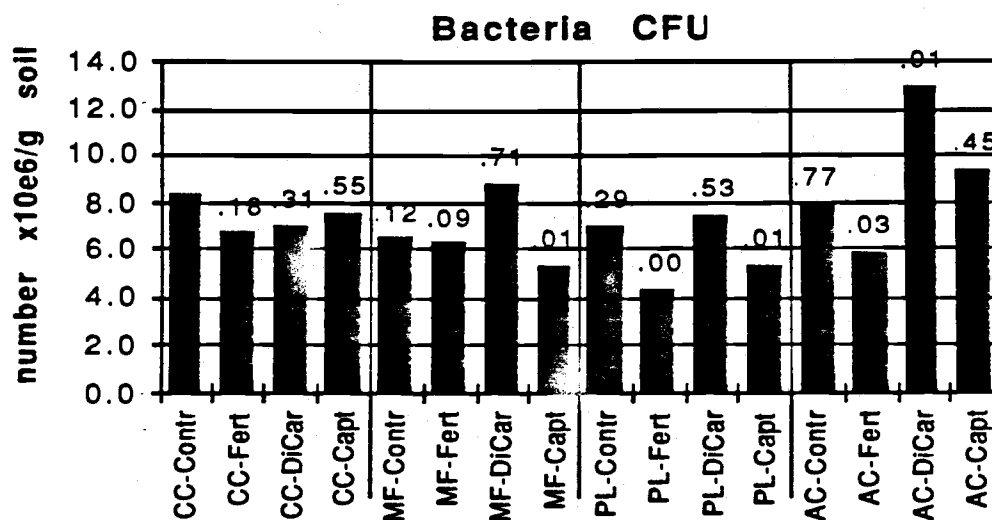
Bacteria CFU

Fig. 4.19. Median number of bacteria CFU per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
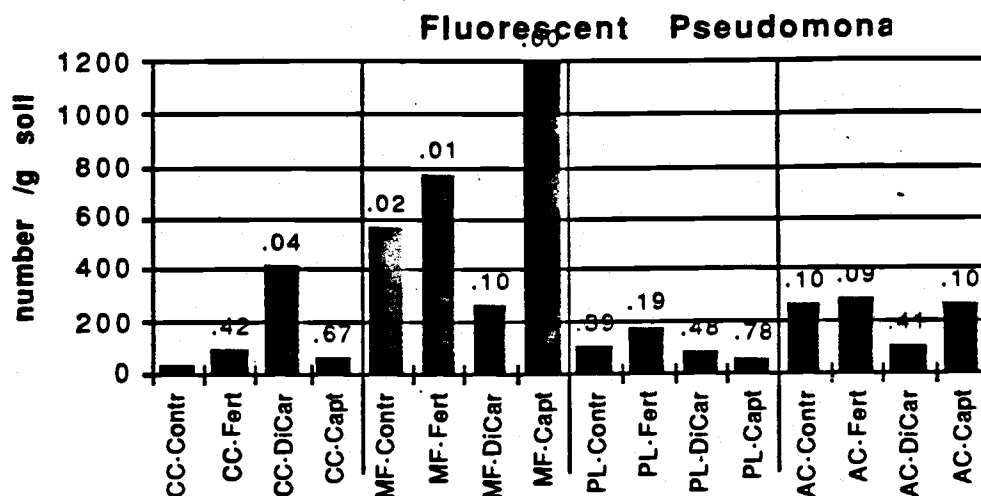
Fluorescent Pseudomona

Fig. 4.20. Median number of fluorescent pseudomonads per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.
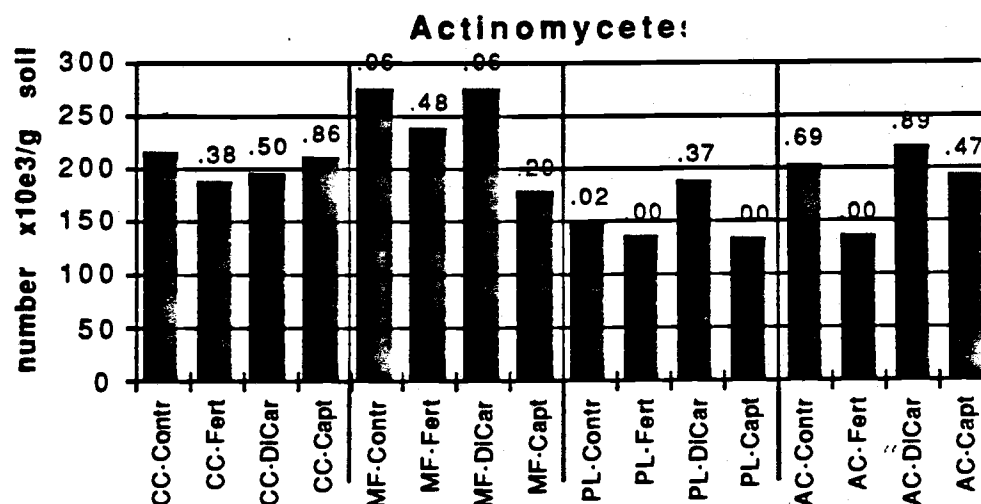
Actinomycete

Fig. 4.21. Median number of actinomycetes per g of dry soil. The numbers above the bars are the p values of the contrasts comparing each treatment to the control (untreated clearcut soil). Contr, untreated; Fert, Fertilizer; DiCar, Dimethoate-Carbofuran; Fumag, Fumagillin; Capt, Captan; CC, clearcut soil; MF, forest soil; PL, plantation soil; AC, autoclaved clearcut soil.