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Title: Management Impacts on the Ectomycorrhizal Associations of Pseudotsuga menziesii var. menziesii Seedlings: Field and Greenhouse Bioassays

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David A. Perry

Field and greenhouse bioassays were used to compare the mycorrhizal associations of Douglas-fir seedlings from undisturbed forests, and nonburned and burned portions of clearcuts on three sites in the west-central Cascades of Oregon. Field soil transfers and greenhouse soil pasteurization and reinoculation were used to investigate soil biology and inoculum potential.

Similar mycorrhizal associations developed in soils from the three regeneration treatments in both field and greenhouse. Regardless of soil origin, proportionately more mycorrhizae developed in clearcuts (especially on site 2); the Brown mycorrhiza type was more frequent in nonburned clearcuts than in other treatments, and the most Cenococcum mycorrhizae formed in burned clearcuts. Rhizopogon species occurred in the clay-silt soil of the low-elevation site only

when that soil was loosened and aerated in the soil transfer. The greatest number of Cenococcum mycorrhizae were found at the high-elevation site, and the greatest number of Brown mycorrhizae on the mid-elevation site.

Major field mycorrhiza types were also observed in the greenhouse. Rhizopogon and Brown types constituted the same proportion of total mycorrhizae on both greenhouse and field seedlings from sites 2 and 3, but not site 1. Greenhouse proportions of Cenococcum and other infrequent mycorrhiza types did not reflect field proportions.

Seedling growth, as well as nonmycorrhizal and total root tip numbers were increased in pasteurized soil. Reinoculation of pasteurized soils reduced nonmycorrhizal and total root tip numbers, albeit not to original levels. A reinoculation ratio of 1 (nonpasteurized soil): 9 (pasteurized soil) produced as many mycorrhizae as entirely nonpasteurized soil.

For these sites, fungal propagule availability or alterations of soil biology and chemistry by timber harvest and slash burning are less important as determinants of first year mycorrhizal associations than above-ground alterations in the seedling environment. Mycorrhiza formation may be impaired by dense or clayey soils. Some soil microbiological factors limit seedling growth.

Silvicultural management of the above-ground environment to ensure prompt regeneration and inoculation of nursery stock with several species of site-specific mycosymbionts should optimize mycorrhizal symbioses of outplanted seedlings.

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Management Impacts on the Ectomycorrhizal Associations of *Pseudotsuga menziesii* var. *menziesii* Seedlings: Field and Greenhouse Bioassays

by

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I dedicate this thesis to my parents for their enduring encouragement of my education.

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INTRODUCTION

Clearcutting and slash burning are widely employed methods of regeneration silviculture in the Pacific Northwest. Both alter a seedling's environment in major ways. Above-ground, surface and air temperatures become more extreme and light levels are increased. Below-ground, available soil moisture and temperatures increase. Organic and mineral soil horizons may be mixed during yarding operations or organic matter may be consumed in hot slash fires. Burning also releases a flush of mineral nutrients, which reduces the acidity of upper soil horizons. Microbial populations shift in response to the loss of nutrient competition from trees, the presence of their decaying root systems, and nutrient inputs from burning (Bisset and Parkinson 1980; Kozlowski and Ahlgren 1974). Brush often competes with seedlings, both above- and below-ground. On harsh sites these alterations may jeopardize timely reforestation.

Among the techniques being developed to enhance plantation success is the inoculation of coniferous nursery stock with

ectomycorrhizal fungi adapted to the conditions created by timber harvest and site preparation. To be effective these fungi must provide significant benefits during the first several years following outplanting, because inoculated fungi are commonly replaced by indigenous fungi as root systems develop (Marks and Foster 1967; Wilcox 1967; Tainter and Walstad 1977).

Fungal species vary in effects on their hosts (Levisohn 1957; Theodorou and Bowen 1970; Lamb and Richards 1971; Robinson 1971; Muttiah 1972; Mason 1974; Trappe 1974); therefore, the mycorrhizal associations which develop under different regeneration conditions may strongly affect seedling survival and vigor.

Many researchers have studied the influence of single environmental variables on growth of mycorrhizal fungi in pure culture and development of ectomycorrhizae in field and laboratory. Excellent summaries of work through 1972 are provided in chapters of the book Ectomycorrhizae: Their ecology and physiology (Marks and Kozlowski 1973). "Soil factors influencing the formation of mycorrhizae"(Slankis 1974) is a concise and shorter review .

Subsequent research has included more field ecology and practical application, e.g. the relationships between ectomycorrhizal development and root growth (Wilcox, 1980), soil moisture (Theodorow, 1978), allelopathic chemicals (Fisher 1980, Rose et al. 1982), soil microorganisms (Bowen and Theodorou 1979,

Bisset and Parkinson 1980), organic matter (Harvey, Jurgensen and Larsen 1978; Alvarez, Rowney and Cobb 1979; Harvey, Larsen and Jargensen 1979; Harvey, Jurgensen and Larsen 1980; Harvey, Larsen and Jurgensen 1980), and colonization of nonmycorrnizal root systems by native fungi (Tainter and Walstad 1977).

Recent greenhouse bioassays at Oregon State University have been used to assess the impact of timber harvest and site preparation on ectomycorrhizal associations and site productivity (Parke 1982, Perry and Rose 1982, Perry, Meyer, Egeland, Rose, and Pilz 1982, Schoenberger and Perry 1981). This research indicates that mycorrhizal populations respond differently to disturbances on various sites. In addition to laying a foundation for further studies, this work highlighted the need for greater understanding of soil inoculum potential (the availability and infectivity of fungal inoculum), how above- and below-ground environmental alterations interact to influence the development of ectomycorrhizal associations, and how accurately greenhouse bioassays represent field mycorrhizal associations.

My studies were designed to address these questions:

1. Do ectomycorrhizal associations differ among three moderately dissimilar sites within the same drainage?
2. Do the associations in soils from nonburned versus burned portions of clearcuts versus undisturbed

forests differ within these three sites?

3. What is the relative importance of the above- and below-ground environments and how do they interact?
   a. Do mycorrhizal associations change when soil from one regeneration treatment is transfered to another?
   b. Does the presence of live tree roots affect the mycorrhizal association which develops?
4. How strongly do the availability and infectivity of fungal inoculum (Soil Inoculum Potential) affect the resultant association?
5. Do greenhouse bioassays of root tips adequately reflect formation of mycorrhizal associations in the field?

Answers will give a better understanding of environmental factors affecting development of indigenous mycorrhizal associations and are needed to improve management guidelines for regeneration and to specify beneficial or widely adapted fungi for seedling inoculation use.

METHODS

Site Characteristics

Three sites were located in the Canyon Creek drainage of the Sweet Home Ranger District, Willamette National Forest, in the Old Cascades of west-central Oregon. All sites had been harvested 2-3 years previously and burned in the year preceding the study (1980). Dry summers and cool, wet winters with about 50 percent of annual precipitation in the snowpack are the norm. Sites were selected to represent a range of soils, elevations, aspects, slopes, and vegetation (Table 1.) Unreplicated measurements of soil pH, bulk density, temperature, and nitrogen fertility are presented in Table 2. Dilution plate counts of fungal, bacterial, and actinomycete populations of field soil collected in August are listed in Table 3.

Regeneration Treatments

Three regeneration treatments were sampled at each site: undisturbed forest, nonburned, and burned portions of adjacent

clearcuts. The undisturbed forest was sampled 3-10 m from the clearcut edge, where light was sufficient for Douglas-fir establishment and potential mycorrhizal inoculum was available from live roots. Burned and nonburned clearcut plots were located at least 20 m inside the clearcut to eliminate forest edge effects. Clearcut plots were no more than 35 m from the undisturbed forest plots.

Sites are designated by numbers as shown in Tables 1, 4, and 5. Regeneration treatments are abbreviated "UF" for undisturbed forest, "NBC" for nonburned clearcuts and "BC" for burned clearcuts.

Soil Treatments

Soil treatments (elaborated in the following procedure sections) were conducted within sites for both field and greenhouse bioassays. Field soil transfers permitted the comparison of the relative importance of above- and below-ground environments in determining which mycorrhizal associations develop on outplanted seedlings. Greenhouse soil pasteurization and reinoculation were used to examine the effect of microorganisms and propagule availability of mycorrhizal fungi on root tip development.

Field Procedure

Nonmycorrhizal 1-0 Douglas-fir seedlings grown in Ray Leach fir cells were outplanted in mid-April of 1981. A maximum of five trees were sampled for each Site/Regeneration treatment/Soil treatment combination, but seven were planted to allow for mortality. For the nontransferred soil treatment, seedlings were planted directly in the ground. For the transfers, 8 liter soil plugs were moved between regeneration treatments and one seedling planted in each plug. These soil plugs were isolated from surrounding soil at the new location with muslin on the bottom (to allow drainage) and plastic on the sides. This procedure inevitably resulted in mixed soil horizons, in loosened soil structure, and probably in greater soil aeration. Soil plugs replaced in the same regeneration treatment from which they came served as controls for effects of these alterations. In the UF treatment, the replaced soil plugs tested the consequences of removing live roots as a fungal inoculum source. The arrangement of plugs and planted trees was randomized within each regeneration treatment.

Surviving seedlings were extracted in early November, after autumn rain had induced new root growth. Seedlings were stored at $2^{o}C$ after roots had been washed with a stiff water spray. All trees were examined within a month. Root systems were subsampled by

cutting three 2-cm sections across the entire root system in upper, middle, and lower positions. All root tips in these sections were tallied and summed for each tree. Nonmycorrhizal root tips were classified as either actively growing (New nonmycorrhizal), or inactive but not obviously necrotic (Old nonmycorrhizal). Mycorrhizal tips were counted by types. Types were defined from visual characteristics observable through a binocular microscope at 2 to 5 X (Table 6, pocket). The length of the current season's leader growth was measured and dry weight determined for shoot growth, total shoot (above-ground portion of the plant), total root, and the excised portion of each root system.

Greenhouse Procedure

Twenty subsamples of soil from each regeneration treatment within each site were combined and sifted through a 1 cm^2 sieve for use in the greenhouse bioassay. Sterilized peat and vermiculite were added to all soils in a 2 (soil): 1 (peat): 1 (vermiculite) ratio to prevent soil compaction. Nonpasteurized field soil was simply mixed. Pasteurized soil was mixed after steaming for 30 minutes at 70°C. Three soil treatments consisted of pasteurized soil (9 parts) reinoculated with nonpasteurized soil (1 part). Pasteurized soil from a given site x regeneration treatment was

cross-reinoculated with nonpasteurized soils from each of the regeneration treatments within the same site. Pasteurized soil served to monitor greenhouse contamination and as a control for effects of microorganisms, including mycorrhizal fungi, on root tip development. Reinoculations indicate whether fungal propagule availability limits the abundance of mycorrhizae, and allow the separation of physical and biological effects of pasteurization. Cross-reinoculations reveal whether soils from various regeneration treatments are sufficiently different to induce altered mycorrhizal associations from the same inoculum.

Douglas-fir seeds were surface sterilized with 30% H_2O_2, rinsed with sterile water, and germinated on agar. Aseptic sprouted seeds were planted in Ray Leach pine cells filled with the prepared soils. Seedlings were grown in a greenhouse for four months with a 16 hour light cycle of 1200 lux and watered every second or third day to keep the soil moist but unsaturated. After harvest, all root tips of each seedling were tallied by type. Length of leader growth, and shoot and root dry weights were determined for each seedling.

Root Tip Classification

Visual grouping of mycorrhizae into types (especially the Brown type) probably results in the occasional combining of different fungal species. Identifying the unique mycorrhizae formed by Rhizopogon (Type 1) and Cenococcum (Type 3) was straightforward. Both Rhizopogon and Brown (Type 2) types varied in visual characteristics depending on stage of development. About 1% of the mycorrhizal tips were colonized with two or more fungal species. The type at the extremity was counted.

Experimental Design and Analyses

Tables 4 and 5 present the treatment levels of each factor in the ANOVA and its abbreviation. Soil treatments were conducted for each regeneration treatment within each site. In both field and greenhouse bioassays, sites constitute the blocks of a split-plot design, with regeneration treatment as the other whole unit factor. The site x regeneration treatment interaction was used as the error term for the F-test of their variation. Levels of the sub unit analysis tested by the total error term are soil treatment and the soil treatment x regeneration treatment interaction.

ANOVA was performed on the field and greenhouse growth and root tip variables listed in Table 7. Only three "major" mycorrniza types (Rhizopogon, Brown, and Cenococcum) were sufficiently abundant to be analysed. Duncan's Multiple Range Test was used to test differences between site, regeneration treatment, and soil treatment means. Soil inoculum potential was investigated by using Duncan's test on Rhizopogon, Brown, and Cenococcum means for transferred (field) and reinoculated (greenhouse) soils. Regeneration treatment means for nonpasteurized greenhouse soils were also compared.

T tests of root tip variables comparing UF soil plugs and nontransferred soil in the UF regeneration treatment determined the importance of live tree mycorrhizae as a fungal inoculum source.

Field and greenhouse bioassays were compared by a X^2 Goodness-of-Fit test, in which field mycorrniza frequencies were used to calculate expected mycorrhizal counts for greenhouse seedlings grown in nonpasteurized field soils.

RESULTS

Root Tip Types

Mycorrhiza types:

Mycorrhiza types 1-11 were found on field seedlings and types 1-3, 5, 8, 10, and 12 on greenhouse seedlings (Table 6). One type, the greenhouse contaminant (Type 12), occurred only in the greennouse.

Field and greenhouse mycorrhizae differed little in morphology. Abundant rhizomorphs remained attached to the Rhizopogon mycorrhizae of field seedlings, even after root systems were washed. These rhizomorphs are probably an important portion of root system biomass, when soil conditions permit their development. Rhizopogon rhizomorphs were less common on the young, smaller greenhouse seedlings. Rhizomorphs which did occur grew along main roots and connected Rhizopogon-colonized root tips. This may be an adaptive mechanism for spreading colonization within a root system.

Field Cenococcum mycorrhizae developed a complete mantle and thick, protruding hyphae. In the greennouse, Cenococcum appeared to colonize the base of actively growing feeder roots and had numerous thin hyphal extensions.

Of the minor mycorrhizal types 6, 7, and 9 were infrequent and did not occur in the greenhouse. A dense web of bright yellow fungal rhizomorphs (Piloderma croceum Erikss and Hjortst?) was found in the well-decomposed litter layer of the UF plot on Site 3. Seedlings planted in nontransferred soils of this plot had ten times more yellow (Type 5) mycorrhizae than any others. Most of the remaining yellow mycorrhizae occurred in soils transferred to this plot (i.e. transferred UF, NBC, and BC soils in the UF regeneration treatment of Site 3). Small mammals, soil microfauna, or wind-disseminated spores may have moved inoculum into the soil plugs in spite of our efforts to isolate them. No sporcarps were observed.The mycorrhiza with blue hyphae (Type 8) occurred infrequently on all sites in both the field and greenhouse. In the field, type 8 was found predominantely in UF soils (nontransferred and transferred elsewhere). This trend did not hold in the greenhouse. Mycorrhizal type 10 was found only in Site 1 soils, in both field and greenhouse.

Nonmycorrhizal root tips:

Field and greenhouse nonmycorrhizal root tips were not directly comparable. Field seedlings had spring and autumn flushes of root growth, and when harvested, some nonmycorrhizal roots were

actively growing, whereas others were inactive. Most necrotic root tips were removed with the water spray. Greenhouse nonmycorrhizal root tips were all actively growing.

Field Results

Seedling mortality and growth:

Most seedling mortality occurred during a week of 40-45°C weather in August. Table 8 shows survival percentages for sites (84 seedlings) and regeneration treatments (28 seedlings). Low elevation and SSW exposure contributed to poor survival on Site 1. Root egress from the containerized seedling soil plugs appeared random and well-spaced. Seedlings were nonmycorrhizal upon planting, but developed some mycorrhizae on new roots within the original Ray Leach tube soil plugs. Many mycorrhizae formed in clumps on main lateral roots that grew after planting.

Although leader growth and new shoot weight did not vary among regeneration treatments, total shoot and root weights were greater in clearcuts (Figure 1), the expected result of increased light. Root/shoot ratios were also greater in the clearcuts. Although all measures of growth were largest for Site 2 seedlings, none of the differences was statistically significant.

Site differences in root tip development:

As with growth variables, mycorrnizal, non-mycorrhizal and total root tip numbers did not vary significantly among sites, but all averages were largest for Site 2 seedlings (Figure 2).

When averaged over all soil treatments, there were no differences in the number of Rhizopogon mycorrhizae per seedling among the sites; but on Site 1, there were almost no Rhizopogon mycorrhizae on seedlings grown in nontransferred soil. The number of Brown mycorrhizae per seedling was greater on the mid-elevation site with loamy soil (Site 2) than on the other two sites. More Cenococcum mycorrhizae formed per seedling on Site 3 than on the lower-elevation sites which agrees with observations that Cenococcum develops prolifically on cool, high-elevation sites (Personal communication, J. Trappe).

Effect of above-ground environment (regeneration treatments) on root tip development:

The number of Rhizopogon mycorrhizae per seedling did not vary significantly among regeneration treatments, but more Brown tips per seedling developed in NBC areas than UF or BC, more Cenococcum

mycorrhizae per seedling formed in BC areas than UF or NBC, and proportionately more mycorrhizae developed in the clearcuts than UF areas (Figure 2). Clearcut-grown seedlings on Site 2 had particularly large numbers of mycorrhizae. The number of nonmycorrhizal root tips per seedling was greatest in the BC area of Site 2. These differences among regeneration treatment means encompass all soil treatments; therefore, they must reflect above-ground environmental alterations induced by timber harvest and slash burning.

Effect of below-ground environment (soil treatments) on root tip development:

There were no significant interactions between soil type and the above-ground environment, i.e. soils from the various regeneration treatments did not affect root tip development differently according to the regeneration treatment to which they were transferred.

Fewer nonmycorrhizal tips were counted on seedlings grown in transferred soil plugs than in nontransferred soil. Likewise, fewer nonmycorrhizal tips developed on seedlings grown in soil plugs from UF areas than soil plugs from NBC or BC areas irrespective of the regeneration treatment to which the soil plug was moved.

Fewer Cenococcum mycorrhizae occurred on seedlings grown in soil plugs from BC areas than in soil plugs from UF areas, which contrasts directly with the result (previous section) that more Cenococcum mycorrhizae form on seedlings grown in BC areas.

Otherwise, the soil treatment (soil origin and whether it was transferred or not) had no affect on the mycorrhizal associations of seedlings from sites with loamy soil (2 and 3). However, in the clayey soils of Site 1, more mycorrhizae (especially Rhizopogon) formed in transferred than in nontransferred soils.

Similarity of mycorrhizal associations between seedlings grown in nontransferred soils and replaced soil plugs in the UF regeneration treatment of Sites 2 and 3 indicates that live tree roots (severed in the transfer process) are an unimportant source of inocula on these sites during the first year after outplanting.

Greenhouse Results

Sites and regeneration treatments:

Seedlings grown in soils from the three regeneration treatments did not differ significantly for any greenhouse variable, so all greenhouse data will be presented as site averages.

Shoot weight, root weight, and the number of nonmycorrhizal

root tips were greater for greenhouse seedlings grown in soils from Site 1 than from Sites 2 or 3 (Figures 3 and 4). No other variables differed significantly among sites.

Soil treatments (Pasteurization and reinoculation):

There was no significant soil treatment X regeneration treatment interaction for any greenhouse variable, indicating that soils from the three regeneration treatments do not differ sufficiently to induce divergent mycorrhizal associations or growth responses from a given soil treatment (pasteurization, reinoculation, or the source of inoculum).

In pasteurized soils, Rhizopogon occurred uniformly but infrequently, mycorrhiza type 10 very infrequently, and Cenococcum only once; however, mycorrhiza type 12 (probably the greenhouse contaminant Thelephora terrestris, Marx and Bryan 1970) was common.

Seedlings grown in pasteurized soil had significantly larger leader growth (cm), shoot growth (g), root growth (g), and nonmycorrhizal root tip numbers than seedlings from nonpasteurized soil. Reinoculation of pasteurized soil did not alter seedling growth variables consistently. In general, reinoculation lowered growth averages on Sites 2 and 3 and increased them on Site 1 (Figure 5), but these differences were only occasionally significant.

Reinoculating 9 parts pasteurized soil with 1 part nonpasteurized soil provided enough fungal inoculum for mycorrhizal development comparable to entirely nonpasteurized soils. In contrast, reinoculation lowered nonmycorrhizal and total root tip numbers to levels significantly intermediate between nonpasteurized and pasteurized soils.

When considering which regeneration treatment served as the soil source for reinoculating pasteurized soils, fewer Cenococcum mycorrhizae per seedling formed in pasteurized soils reinoculated with BC soil than with UF or NBC soil. As with field soil transfers, this contrasts with the finding that more Cenococcum mycorrhizae form on seedlings grown in the field BC regeneration treatment (above-ground environment) than in the other two regeneration treatments. Differences among Rhizopogon and Brown means for seedlings grown in reinoculated soils were not significant.

Comparison of Field and Greenhouse Bioassays

Root tip bar graphs for seedlings from nonpasteurized soils are presented in Figure 3. The mycorrhizal sections of these graphs portray observed counts for a X^2 Goodness-of-Fit test used to compare field and greenhouse bioassays of mycorrhizal associations (Table 9). Expected counts for greenhouse seedling mycorrhiza types

were calculated using the percent of total mycorrhizae which each mycorrhiza type constituted on field seedlings. Nonmycorrhizal tips were excluded because field seedlings had inactive nonmycorrhizal root tips, whereas greenhouse seedlings did not.

Proportions of Rhizopogon and Brown types were not significantly different between the field and greenhouse bioassays of Sites 2 and 3. The proportion of Rhizopogon tips greatly increased when Site 1 soils were brought into the greenhouse, while the occurrence of Brown mycorrhizae correspondingly decreased.

Field and greenhouse Cenococcum proportions were identical on Site 2, but greenhouse Cenococcum proportions were greater for Sites 1 and 3. There were consistently fewer "other" minor mycorrhizae in the greenhouse than in the field in spite of the occurrence of the greenhouse contaminant (Type 12). Perhaps homogenization of greenhouse soils and seedling environments eliminated the field microsites requisite to their development.

DISCUSSION

Interaction of the Above-and Below-Ground Environments

In this field bioassay, the below-ground environment consists of soil texture, chemistry, structure, and microorganism populations (including soil inoculum potential), whereas the above-ground environment encompasses the effect that various light, temperature, and precipitation regimes have on seedling physiology and soil conditions.

Results indicate that, in these young clearcuts, a seedling's above-ground environment is a more important determinant of first year mycorrhizal associations than the below-ground environment. The larger numbers of Rhizopogon and total mycorrhizae in the transferred than in the nontransferred soils of Site 1 appears to be an artifact of the transfer procedure (loosening of soil structure and greater aeration?). Other than finding Cenococcum mycorrhizae less often on seedlings grown in plugs of soil from BC areas, the source of transferred field soils did not affect the relative numbers of different mycorrhiza types.

The above-ground environment (regeneration treatment) did alter the average number of mycorrhizae per seedling and the

frequencies of Brown and Cenococcum mycorrhizae. Removal of timber increased soil temperatures and light levels. A blackened soil surface and lack of shade from brush and weeds further increased BC soil temperatures (Table 2) and light levels. Soil temperatures at 10 cm in clearcuts were within the "ideal" range for mycorrhizal development (Parke 1982), whereas UF temperatures were cooler. Mycorrhizal development depends in part on photosynthate production (Hacskaylo 1973). Either light or temperature differences (or both) among above-ground environments may explain the greater number of mycorrhizae which formed on seedlings grown in clearcuts. The disproportionately large number of mycorrhizae found on clearcut grown seedlings of Site 2 may be explained by more favorable moisture, light and temperature regimes than existed on Sites 1 and 3. The physiological mechanisms whereby temperature and light levels imposed on a seedling can alter the frequency of particular mycorrhiza types is unknown.

Minor differences in soil inoculum potential may exist, but fungal propagule availability was not limiting to mycorrhiza formation. Reducing fungal propagules by 90% in reinoculated greenhouse soils did not reduce the number of mycorrhizae which formed, nor was cross-reinoculation (UF, NBC, and BC inoculation of pasteurized UF, NBC, and BC soils) significant. Our results collaborate the finding (Wright and Tarrant 1958) that more

Cenococcum mycorrhizae per seedling form in burned portions of clearcuts than other areas, but as reported elsewhere (Schoenberger and Perry 1982) our greenhouse indices of soil inoculation potential indicate Cenococcum propagules may be less abundant or less able to form mycorrhizae in soils from burned areas. The greater number of Cenococcum mycorrhizae formed on field seedlings grown in soil plugs transferred from UF areas than from BC areas provides further evidence that the above-ground environment may overwhelm differences in Cenococcum inoculum.

Several researchers have reported diminished soil inoculum potential or mycorrhizal development in clearcuts, especially burned or old ones (Parke 1982, Perry et. al. 1982, Perry and Rose 1982, Schoenberger and Perry 1982, Wright and Tarrant 1958). That these changes were not noted on our young clearcuts, suggests that prompt regeneration may be important in order to secure adequate formation of indigenous mycorrhizae. Prompt regeneration may be secured through appropriate silvicultural manipulation of a seedling's above-ground environment (e.g. shelterwoods, shade-blocking, brush control, varied slashburn intensities, etc.) or by using nursery stock designed for specific sites.

Soil Pasteurization and Reinoculation

Large increases in seedling growth and root tip numbers occurred in all soils following pasteurization. Unlike other studies (Perry, et. al. 1982, Perry and Rose 1982), this response did not differ among soils from the various regeneration treatments. Although steam-induced changes in soil chemistry may have increased nutrient availability, increased seedling growth has also been reported when field soils are sterilized with methyl bromide (Klock and Benson 1975). Our reinoculations also provided evidence that changes resulting form soil pasteurization are at least partly a biotic phenomenon. Although reinoculation did not consistently reduce seedling growth, nonmycorrhizal and total root tip numbers were reduced by reinoculation to levels intermediate between nonpasteurized and pasteurized soils. Reduction of nonmycorrhizal root tip numbers (beyond the reduction accounted for by the increase in mycorrnizae numbers following reinoculation) may be mediated through hormone (Slankis 1973) or ethylene (Graham 1979) production by the fungal symbiont of mycorrhizae or by biotic changes in the rhizosphere resulting from the reintroduction of other soil microoorganisms.

Greenhouse Versus Field Bioassays

The greenhouse bioassay accurately reflected the relative proportions of Rhizopogon and Brown mycorrhiza types on Sites 2 and 3, and should, within limits, be considered a useful technique for studying the effects of disturbance on mycorrhizal inocula. The lack of correspondence between greenhouse and field bioassys on Site 1 may reflect the more radical changes in soil structure resulting from the admixture of peat vermiculite to clay-silt, as opposed to loamy soils. The greater proliferation of mycorrhizae in the transferred field soils of Site 1 also indicates that dense soil may alter mycorrhizal associations, especially by limiting the development of Rhizopogon.

Minor components of field mycorrhizal associations were not accurately reflected by greenhouse bioassays. Except for Site 2, Cenococcum proportions increased in the greenhouse. Other greenhouse mycorrhiza types differed from those in the field and did not represent the same percent of total mycorrhizae. The importance of these minor mycorrhiza types to seedling health and vigor is unknown and should be researched.

Selection of Fungi for Nursery Inoculation

Seedlings inoculated with mycorrhizal fungi may have a survival advantage the first year following outplanting even on young clearcuts with sufficient fungal inocula, but mycorrhizal nursery stock may be particularly useful on older or severely burned clearcuts.

The consistent appearance, in this and other studies (Alvarez 1982, Parke 1982), of Rhizopogon as a common field mycorrhizae on Douglas-fir, suggests that it may be useful for nursery inoculation. Its prolofic rhizomorph production may enable root systems colonized with this fungus to more rapidly exploit surrounding soil for water and nutrients upon outplanting. This may not hold true for dense or compacted soils where rhizomorph growth can be impaired. The Brown mycorrhiza type reported in this study and others (Schoenberger and Perry 1982) should be identified for nursery use because it is a major component of our mycorrhizal associations, it may be well adapted to nonburned clearcuts, and if it does consist of several fungal species, this will be clarified. Cenococcum may be a useful fungal symbiont for seedlings intended for burned or high-elevation sites. Inoculation of nursery

seedlings with several fungal species to more closely mimic first year field mycorrhizal associations holds promise for improving the effectiveness of these procedures for enhancing outplanting survival and growth.

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APPENDICES

APPENDIX I

(Figures)

Figure 1. Root and shoot growth of field seedlings as affected by above-ground environment (averaged over soil treatments and sites)

FIELD

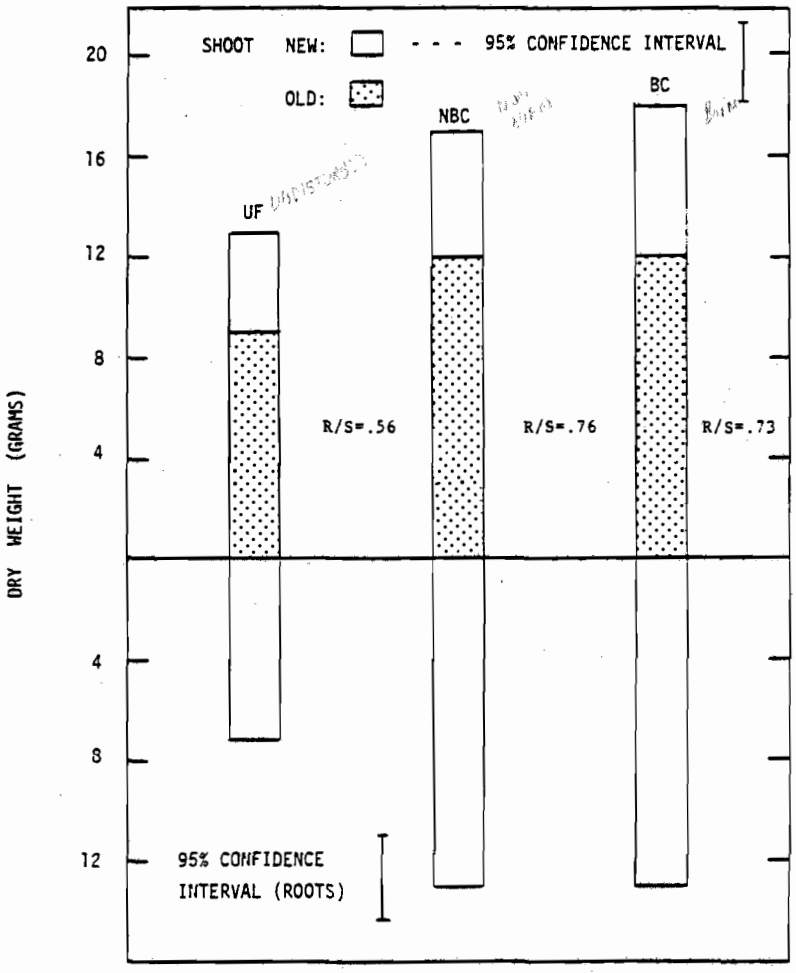

FIGURE 1.

Figure 2. Root tip formation for field seedlings by above-ground environment (averaged over soil treatments)

A. Site 1

B. Site 2

C. Site 3

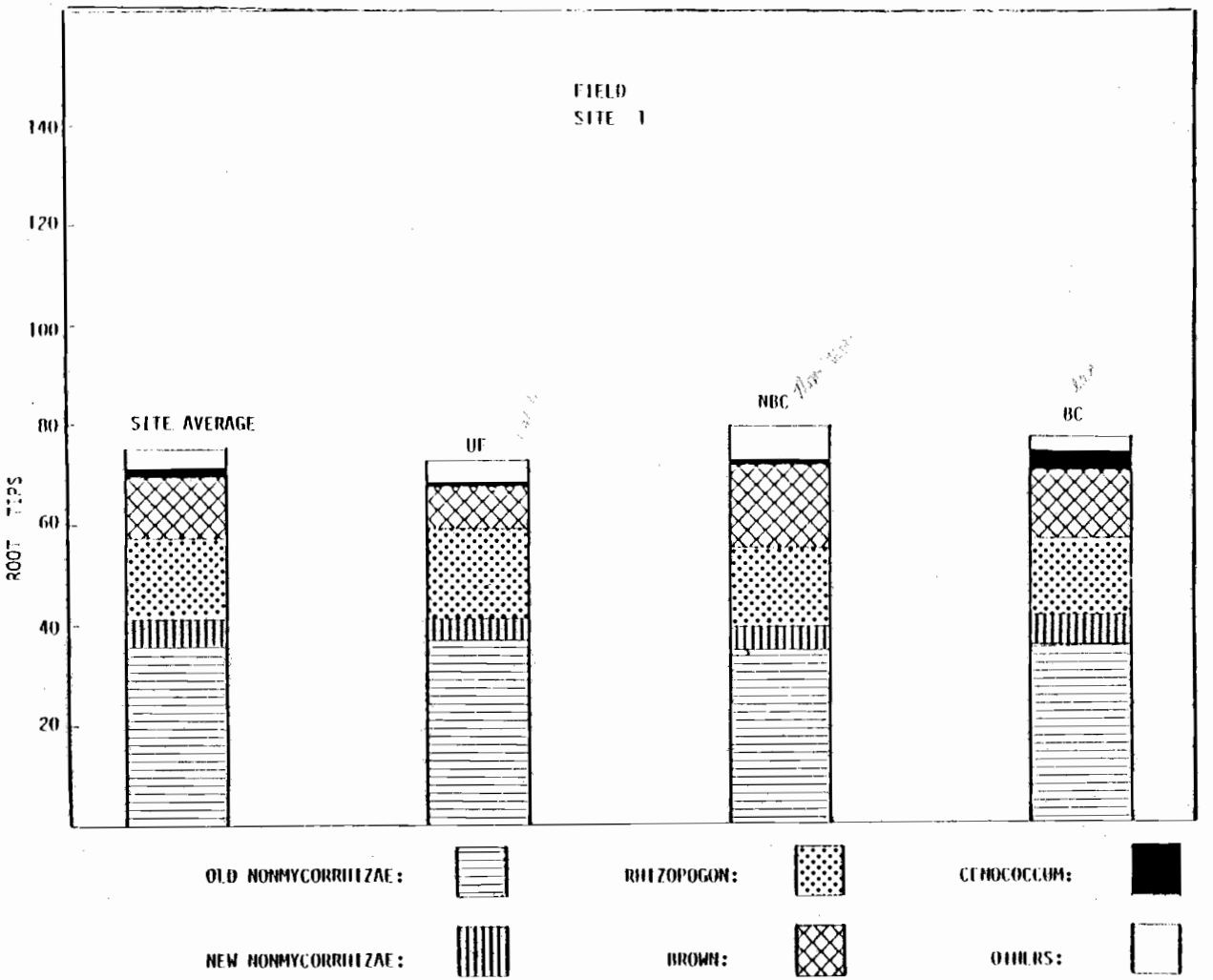
FIELD
SITE 1
ROOT TIPS
140
120
100
80
60
40
20
SITE AVERAGE
UF
NBC
BC
OLD NONMYCORRHIZAE:
NEW NONMYCORRHIZAE:
RHIZOPOGON:
BROWN:
CENOCOCCUM:
OTHERS:

Figure 2 A

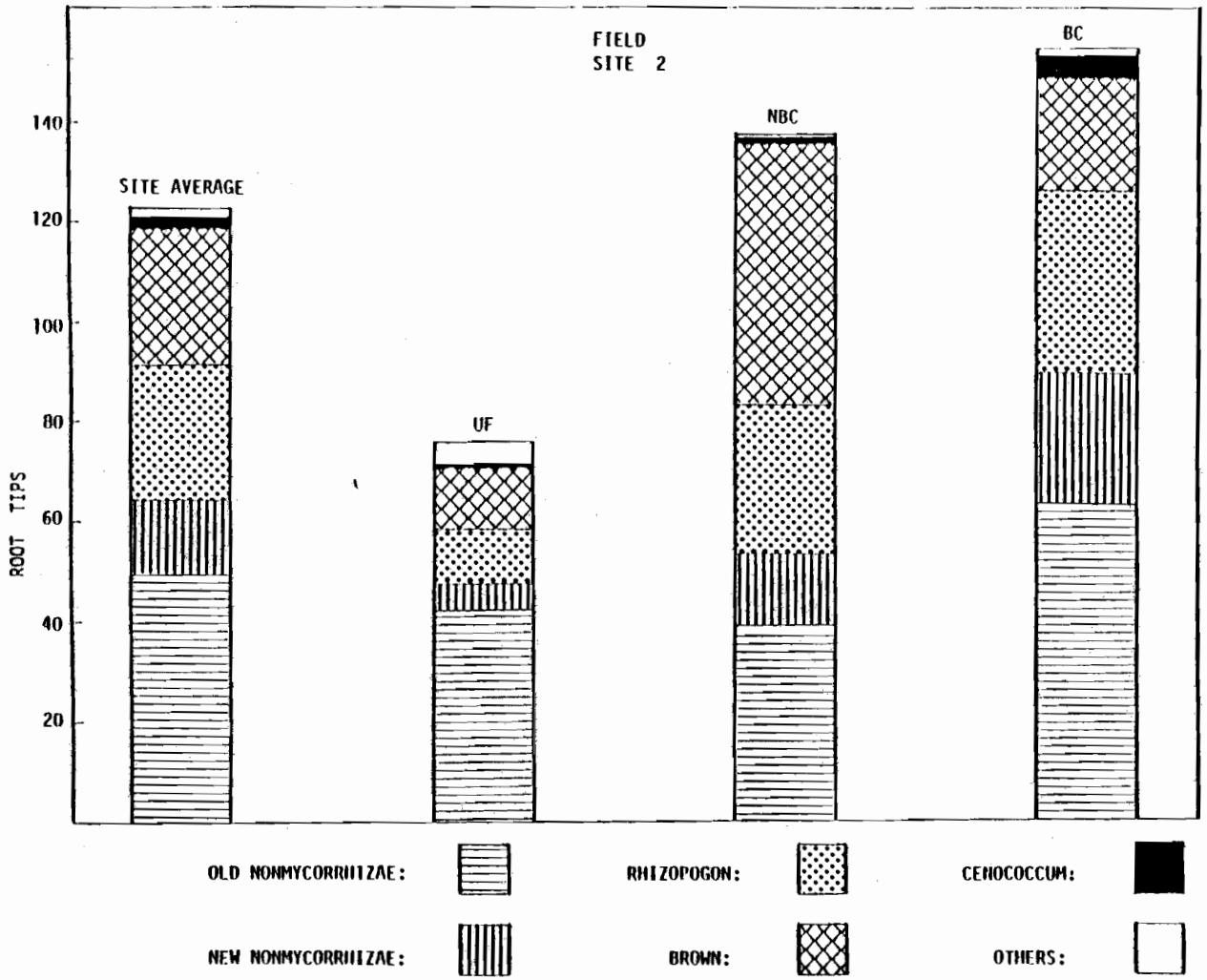
FIELD
SITE 2
ROOT TIPS
140
120
100
80
60
40
20
SITE AVERAGE
UF
NBC
BC
OLD NONMYCORRHIZAE:
NEW NONMYCORRHIZAE:
RHIZOPOGON:
BROWN:
CENOCOCCUM:
OTHERS:

Figure 2 B

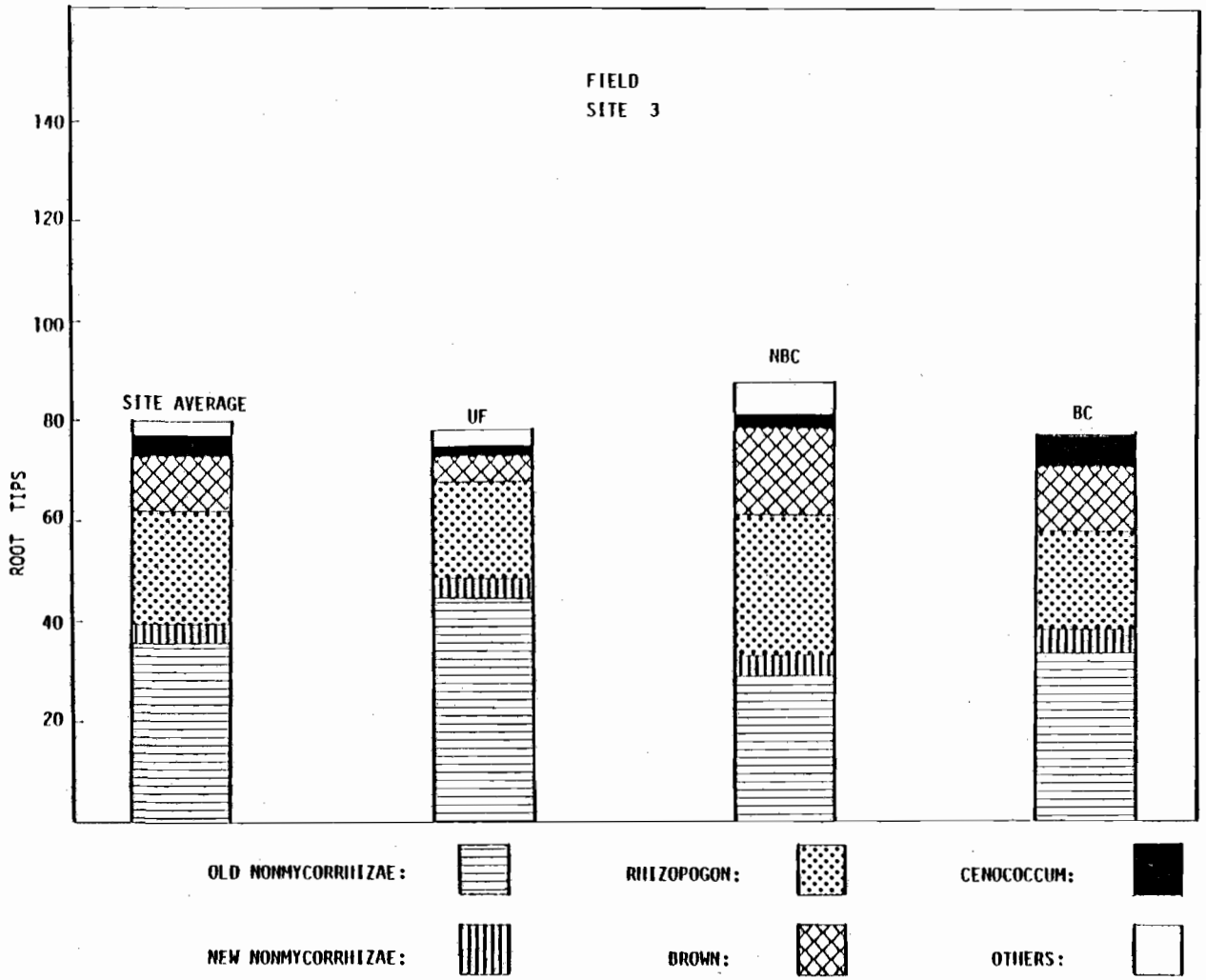
FIELD
SITE 3
ROOT TIPS
140
120
100
80
60
40
20
SITE AVERAGE
UF
NBC
BC
OLD NONMYCORRHIZAE:
NEW NONMYCORRHIZAE:
RHIZOPOGON:
BROWN:
CENOCOCCUM:
OTHERS:

Figure 2 C

Figure 3. Site averages of root tip types for greenhouse seedlings grown in nonpasteurized soils

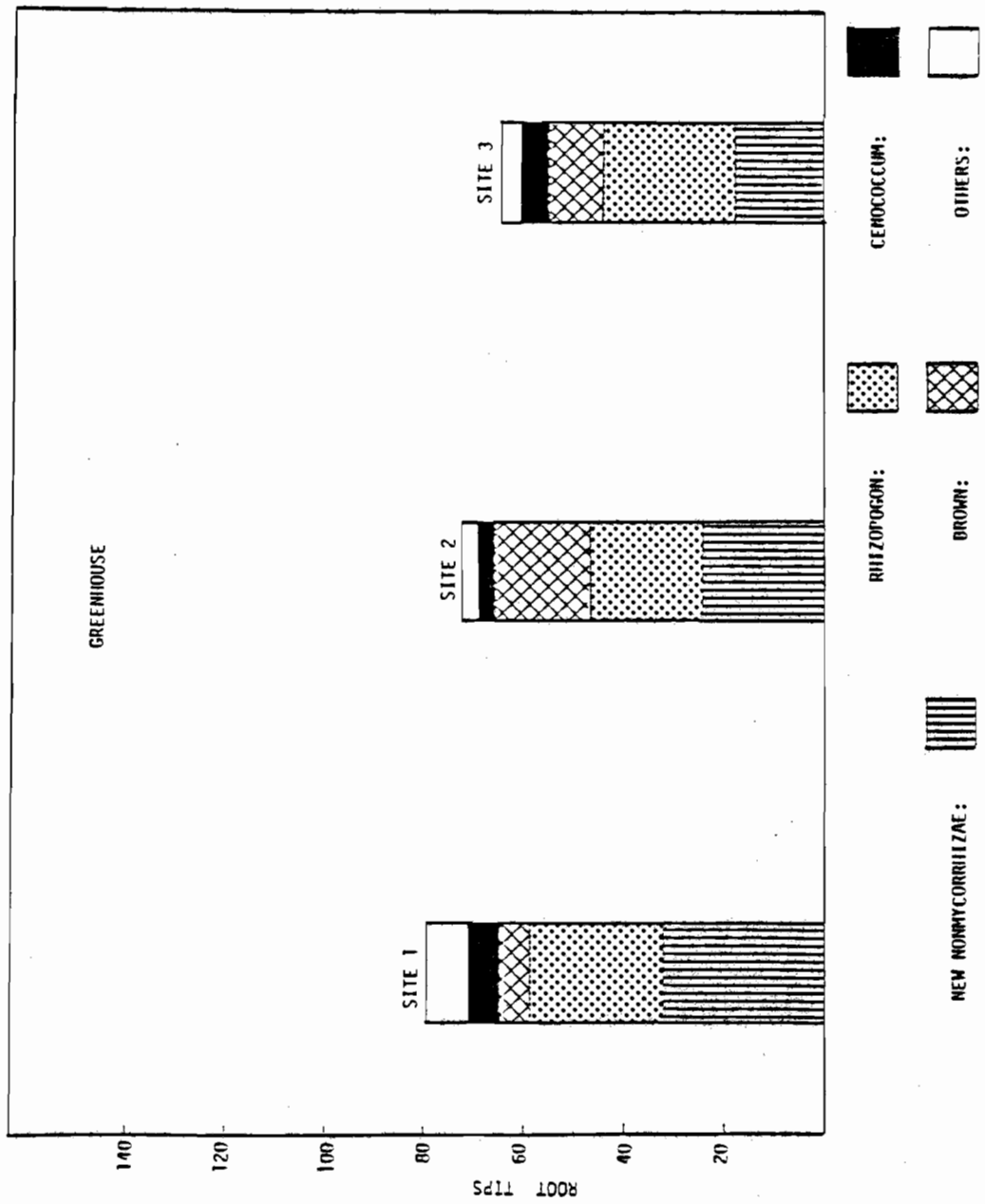
GREENHOUSE
SITE 1
SITE 2
SITE 3
ROOT TIPS
140
120
100
80
60
40
20
NEW NOMAYCORRHIZAE:
BROWN:
RHIZOPOGON:
CENOCOCCUM:
OTHERS:

Figure 3

Figure 4. Mycorrhizal and nonmycorrhizal root tip numbers among greenhouse seedlings grown in nonpasteurized, pasteurized and reinoculated soils

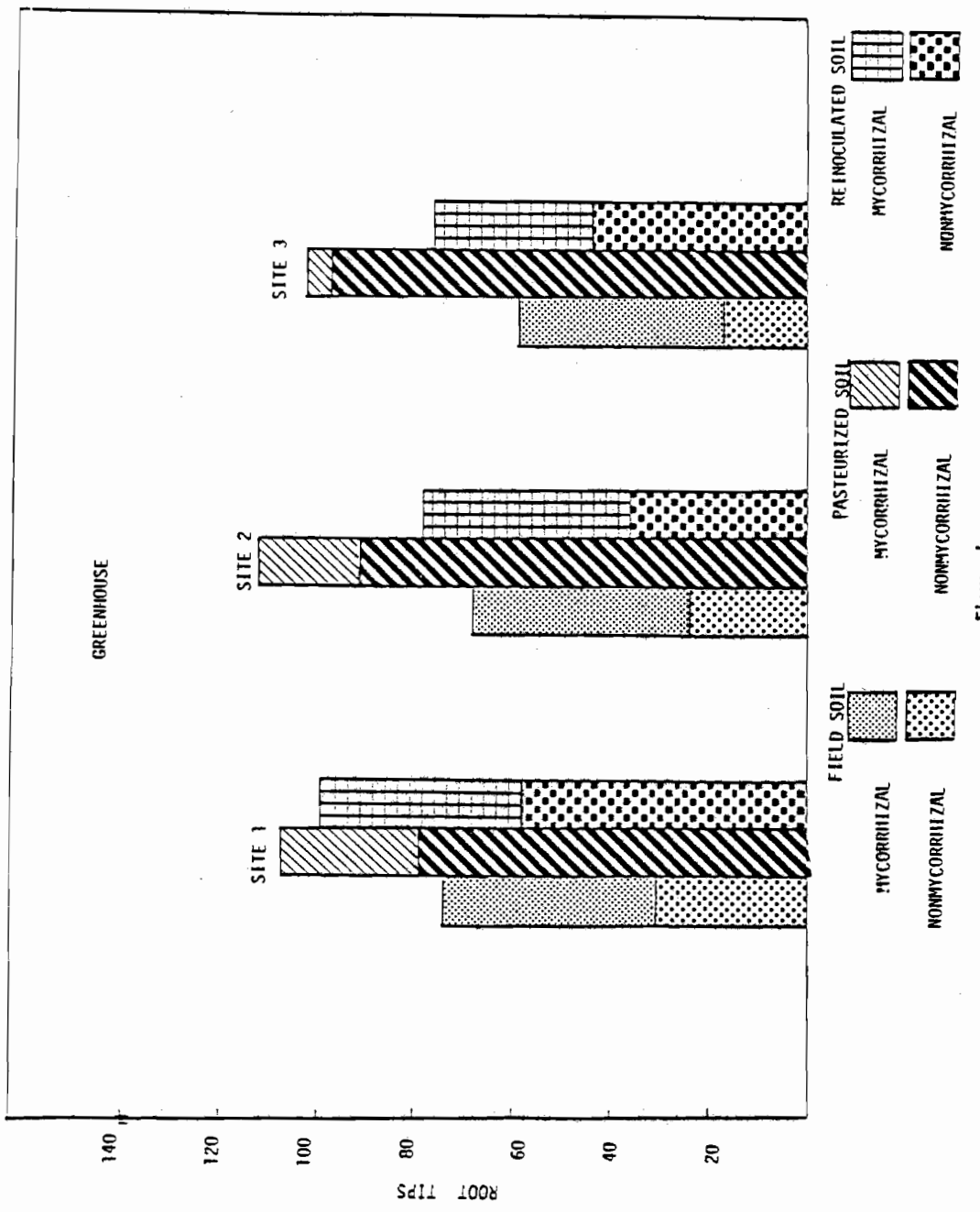
GREENHOUSE
ROOT TIPS
140
120
100
80
60
40
20
SITE 1
SITE 2
SITE 3
FIELD SOIL
MYCORRHIZAL
NONMYCORRHIZAL
PASTEURIZED SOIL
MYCORRHIZAL
NONMYCORRHIZAL
REINOCULATED SOIL
MYCORRHIZAL
NONMYCORRHIZAL

Figure 4

Figure 5. Root and shoot growth among greenhouse seedlings grown in nonpasteurized, pasteurized, and reinoculated soils

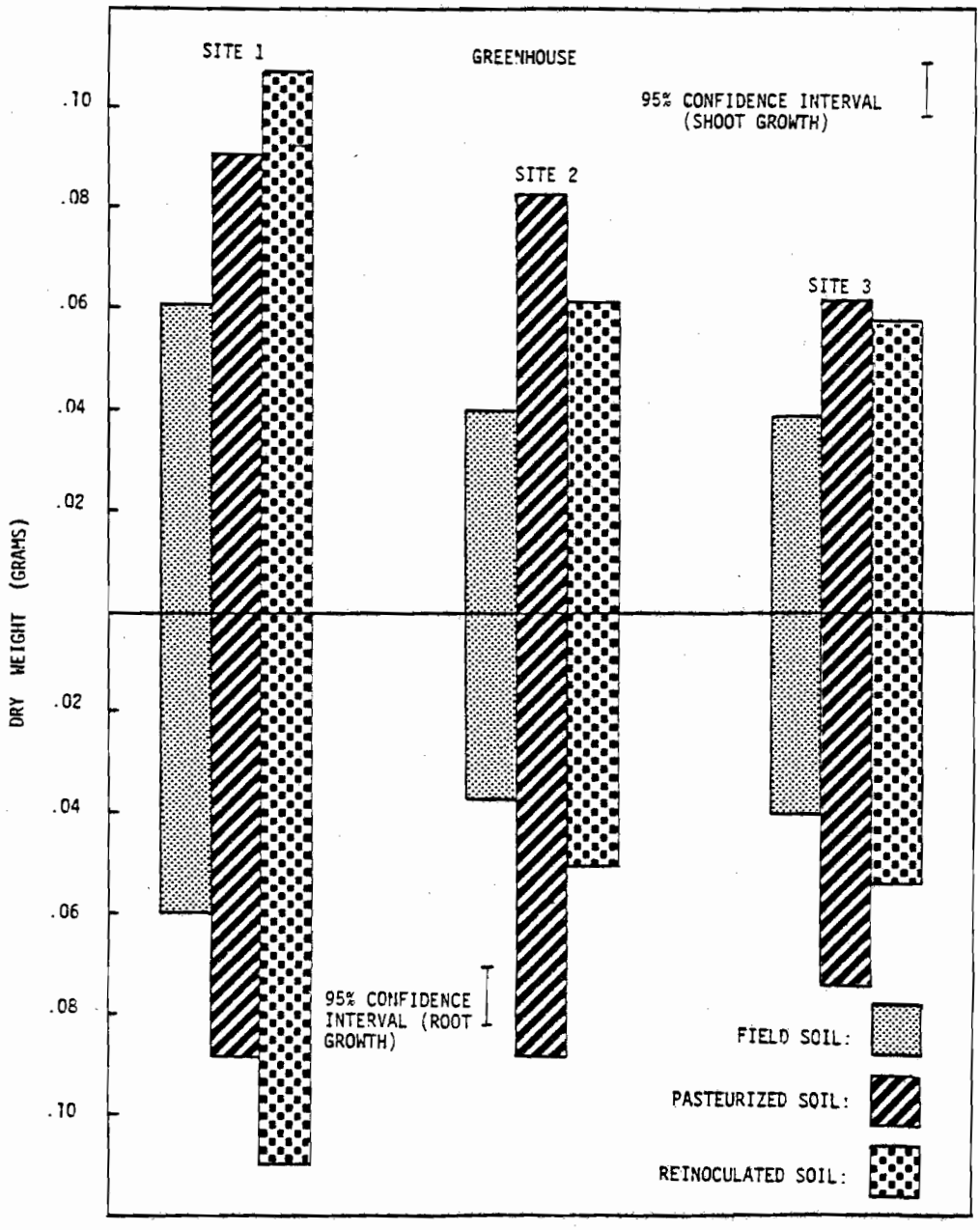
SITE 1
GREENHOUSE
95% CONFIDENCE INTERVAL
(SHOOT GROWTH)
SITE 2
SITE 3
.10
.08
.06
.04
.02
DRY WEIGHT (GRAMS)
.02
.04
.06
.08
.10
95% CONFIDENCE
INTERVAL (ROOT
GROWTH)
FIELD SOIL:
PASTEURIZED SOIL:
REINOCULATED SOIL:

FIGURE 5

APPENDIX II

(Tables)

Table 1

Site Descriptors

	Site 1	Site 2	Site 3
Elevation	500m	800m	900m
Aspect	SSW	Flat (East Shade)	NE (West Shade)
Slope	45%	10%	30%
Soil Description	Dark reddish brown, silty-clay loam	Dark brown clay loam	Grayish brown gravelly loam
Vegetation Community (Undisturbed Forest)	Psme/Acma/ Thpl/Tshe (80 yrs old) Gash/Bene	Psme/Tshe/ Thpl (250 yrs old) Vaccinium/Moss	Psme/Tshe/ Pico/Abpr (200 yrs old) Rhma/Xete/Gash/Bene

Table 2

Abiotic Field Soil Conditions

Site Number	Regeneration Treatment	pH	Bulk Density	Soil Temperatures (°C Surface/10cm)	Kjeldahl (% Dry Weight)	Soil Nitrogen Exchangeable NH_4+ (PPM Dry Weight)	Mineralizable NH_4+ (PPM Dry Weight)
	UF	4.8	.94	32/16	.107	8.23	23.2
1	NBC	4.9	.95	52/18	.101	9.44	31.48
	BC	4.6	.95	52/19	.128	8.9	27.02
	UF	4.3	.55	29/15	.073	4.93	15.66
2	NBC	4.2	.76	52/18	.132	35.27	17.09
	BC	4.7	.78	52/20	.186	7.61	16.70
	UF	4.1	.88	32/16	.127	3.46	5.5
3	NBC	4.3	.89	38/16	.156	8.56	20.35
	BC	4.3	.78	40/17	.150	23.8	18.65

Table 3

Field Soil Microorganism Bioassays

(Thousands of organisms per gram (dry weight) of soil)

(Three samples each: averages and (standard deviations))

Site	Harvest	Fungi	Bacteria	Actinomycetes
	UF	182 (7.8)	3232 (2690)	1995 (789)
1	NBC	153 (23.5)	3595 (949)	2178 (241)
	BC	233 (34.8)	1929 (322)	1628 (276)
	UF	167 (32.8)	1859 (364)	320 (309)
2	NBC	157 (9.5)	1947 (224)	885 (354)
	BC	210 (23.8)	5362 (664)	1217 (398)
	UF	222 (9.1)	3903 (1772)	1766 (346)
3	NBC	157 (22.7)	3280 (795)	793 (79)
	BC	164 (36.1)	1000 (144)	389 (48)

Table 4

FIELD TREATMENTS

SITES

500 METERS SOUTH EXPOSURE CLAYEY SILT LOAM (1)	800 METERS FLAT EXPOSURE LOAM (2)	900 METERS EAST EXPOSURE ROCKY LOAM (3)

REGENERATION TREATMENTS

UNDISTURBED FOREST (UF)	NONBURNED CLEARCUT (NBC)	BURNED CLEARCUT (BC)

SOIL TREATMENTS

UNTRANSFERRED SOIL	SOIL PLUG FROM UF REGENERATION TREATMENT	SOIL PLUG FROM NBC REGENERATION TREATMENT	SOIL PLUG FROM BC REGENERATION TREATMENT

Table 5

GREENHOUSE TREATMENTS

SITES (SOIL ORIGIN)

500 METERS SOUTH EXPOSURE CLAYEY SILT LOAM (1)	800 METERS FLAT EXPOSURE LOAM (2)	900 METERS EAST EXPOSURE ROCK LOAM (3)

REGENERATION TREATMENTS (SOIL ORIGIN)

UNDISTURBED FOREST (UF)	NONBURNED CLEARCUT (NBC)	BURNED CLEARCUT (BC)

SOIL TREATMENTS

NONPASTEURIZED SOIL	PASTEURIZED SOIL	PASTEURIZED, REINOCULATED WITH (UF) SOIL	PASTEURIZED, REINOCULATED WITH (NBC) SOIL	PASTEURIZED, REINOCULATED WITH (BC) SOIL

ECTOMYCORRHIZA TYPES

NUMBER	MANTLE		FORM		ATTACHED MYCELIUM		OCCURRENCE, POSSIBLE I.D.
	COLOR	TEXTURE	INDIVIDUAL TIPS	BRANCHING PATTERN	HYPHAE	RHIZOMORPHS	
1.	White to brown to black	Granular or crystalline	Moderate to long, swollen, usually straight	Single to pinnate to clumped ramiform to tuberculate	Thick dark brown mat enclosing clumps	Abundant, thick, dense, white to brown attached to base of mycorrhizae	Field Greenhouse, *Rhizopogon* species
2.	Light golden brown	Smooth	Short to long, straight to curved, swollen	Single to pyramidally pinnate, loose to moderate spacing	When present, light grey, fine, felty	Absent	Field Greenhouse, (Brown Type)
3.	Jet Black	Crusty	Short to moderate, straight	Usually single, occasionally pinnate, widely spaced	Black coarse, stiff, sparse to dense	Absent	Field Greenhouse. *Cenococcum* species
4.	Pure white	Smooth to slightly granular	Short, bulbous. straight	Single	Absent to abundant. short, coarse, white	Occasional. thick, white. attached to mantle	Field
5.	Bright yellow, occasional greenish-blue or golden patches	Granular	Moderate length, slightly swollen, straight to curved	Single to forked. moderately spaced	Absent	Sparse yellow attached to mantle	Field, (*Piloderma croceum*?)
6.	White to slatey blue-grey	Granular, does not cover entire root surface, criss-cross pattern	Moderately long, unswollen. straight to bent	Well spaced pinnate to massive tight clusters	Fine, fuzzy, white to grey to blue-grey	Occasional, dull white, coalescing from mantle pattern	Field
7.	Ghostly white hints of grey	Very smooth	Moderately long, swollen	Single to ramiform to clumped	Absent	Absent	Field
8.	No evident mantle		Swollen straight to curved	Single to pinnate. loose to moderate spacing	Abundant slate blue-grey, thin, long,	None	Field Greenhouse
9.	White, staining bright blue	Felty	Small	Single	Matted, coarse extension of fluffy mantle	Big, abundant, color as mantle	Field
10.	Dark golden brown or reddish brown to near black	Smooth	Moderately large, swollen, straight	Pyramidally pinnate to ramiform	Patches of silvery white appressed hyphae	Brown, thin. loosely woven. attached to mantle	Field Greenhouse
11.	Black	Smooth shiny	Short swollen	Single or when multiple. tips-blunted into stubs	Rare completely transparent	Absent	Field
12.	Very light brown to white	Smooth	Swollen	Pinnate	Absent	Rare, white. tightly appressed to root	Greenhouse Contaminant, (*Thelephora terrestris*)

Table 7

QUANTIFIED VARIABLES

FIELD	GREENHOUSE
1. New nonmycorrhizal root tips	1. Total nonmycorrhizal root tips
2. Old nonmycorrhizal root tips	2. Total mycorrhizal tips
3. Total nonmycorrhizal root tips	3. Total root tips
4. Total mycorrhizae	4. Ratio: (Mycorrhizal/Total root tips)
5. Total root tips	5. Ratio: (Mycorrhizae/Root weight)
6. Ratio: (Mycorrhizal/Total root tips)	6. Rhizopogon
7. Ratio: (Mycorrhizae/Sample root weight)	7. Brown
8. Rhizopogon	8. Cenococcum
9. Brown	9. Leader growth
10. Cenoccocum	10. Shoot growth
11. Leader growth	11. Root growth
12. New shoot weight	12. Root/Shoot ratio
13. Total shoot weight	
14. Total root weight	
15. Root/Shoot ratio	

Table 8

Field Seedling Survival (%)

(84 seedlings/site, 24 seedlings/regeneration treatment)

Site	1	2	3
Site Average	53.6	65.5	91.7
Undisturbed Forest	67.9	67.9	91.7
Nonburned Clearcut	32.1	57.1	100.
Burned Clearcut	60.7	71.4	83.3

Table 9

Field versus Greenhouse Mycorrhizal Associations
(Goodness of Fit X^2)

Site 1					
	Rhizopogon	Brown	Cenococcum	Others	Sums
Proportion of total mycorrhizae (field)	.388	.387	.092	.133	
Expected	454	453	109	156	
Observed	729	177	178	88	1172
Deviation/X^2	-275/167	276/168	-69/44	68/30	X^2=409

Site 2					
	Rhizopogon	Brown	Cenococcum	Others	Sums
Proportion of total mycorrnizae (field)	.448	.430	.063	.059	
Expected	505	483	71	66	
Observed	554	487	70	14	1125
Deviation/X^2	-49/5	-4/.03	1/.01	52/41	X^2=46

Site 3					
	Rhizopogon	Brown	Cenococcum	Others	Sums
Proportion of total mycorrhizae (field)	.572	.264	.086	.078	
Expected	703	323	105	96	
Observed	746	335	143	3	
Deviation/X^2	-43/3	-12/.4	-38/14	93/90	X^2=107