SENSITIVITY IN GROWTH RESPONSES OF TREE SEEDLINGS TO VARIATION IN IDENTITY AND ABUNDANCE OF ECTOMYCORRHIZAL FUNGI

by

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Abstract

Interdependent organisms such as trees and ectomycorrhizal fungi are described as coevolved. Partner species in coevolved interactions are expected to be sensitive to intraspecific variation of each partner due to the intimate and interdependent nature of their interactions. In this thesis, I considered specific aspects of variation in each of the ectomycorrhizal partners and how this variation influenced the other partner. In particular, I used experimental and meta-analytical approaches to evaluate 1. how colonization levels, regardless of ectomycorrhizal fungal taxon, correlated to host growth; 2. how ectomycorrhizal fungi differentially influenced growth of different genera of plant hosts, and 3. how variation in growth of a single host species was correlated to the composition of ectomycorrhizal fungal communities in various soil environments. Because controlling for and manipulating ectomycorrhizal fungi on host plants is integral to these questions, I also tested the efficacy of two methods to control colonization by ectomycorrhizal fungi on host plants and found that fungicides and mesh can be effective barriers to colonization. Results from the meta-analysis and experiments indicated that colonization levels did not consistently scale with host growth response, however, suggesting that colonization levels may not be an ecologically useful factor to gauge the growth responses of host plants to ectomycorrhizal fungi. In addition, there was little sensitivity in growth responses of host plants to variation in the identity of ectomycorrhizal fungi. Seedlings across multiple host genera increased in biomass and shoot height when inoculated with ectomycorrhizal fungi regardless of the identity of the fungal associate. When ectomycorrhizas were considered in a multi-specific context (i.e. one host species associated with a community of ectomycorrhizal fungi), variation in host shoot properties was not correlated with species composition of the community of ectomycorrhizal fungi on their roots but rather appeared to be coupled to edaphic conditions. These results indicate that the variation in ectomycorrhizal fungi perceived and selected for by the host plant may be of a discrete (presence/absence of ectomycorrhizal fungi) rather than continuous nature (variation in identity or abundance of ectomycorrhizal fungi).

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Co-authorship Statement

Chapter 2 was co-authored with Drs. Laurie Marczak, Melanie Jones and Roy Turkington. I identified, designed and conducted the research, including data collection, analysis and manuscript preparation. Laurie Marczak assisted with data collection, analysis and manuscript preparation and revision. Melanie Jones and Roy Turkington assisted with manuscript revision.

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1 Introduction

Context

Interdependent species that adapt to changes in each other are described as coevolved. Each partner in the relationship exerts selective pressures on the other, thereby affecting each others' evolution. Coevolution is the process resulting from a close association between the individuals of two, or more, different species (Thompson 1994). Plants and mycorrhizal fungi appear to have had such an interdependent relationship since plants invaded land. The association between plants and mycorrhizal fungi can also be considered symbiotic, defined by de Bary (1878 as cited in Sapp 1994) as "the living together of unlike named organisms".

Ectomycorrhizas are characteristic of tree species within the families Pinaceae, Cupressaceae, Fagaceae, Myrtaceae, Betulaceae, and Salicaceae and coevolution between phyto- and mycobionts from several orders including Agaricales, Gautieriales, Hymenogastrales, Phallales, Lycoperdales, Melanogastrales, Sclerodermatales, Aphyllophorales, Pezizales and Elaphomycetales has been occurring for about 200 million years (Kendrick 2000). An ectomycorrhiza is the physical association of roots and ectomycorrhizal fungi, with the fungus forming a compact layer of hyphae around the roots (mantle) connected to a network of hyphae growing in between root cells (Hartig net). Nutrient transfer (carbon to the mycobiont supplied by the host plant and mineral nutrients via the fungus to roots of the phytobiont), occurs at the interface between the Hartig net and root cells (Smith and Read 1997). Historically, ectomycorrhizas have been categorized as mutualistic because each symbiont was deemed to benefit from the exchange of resources (Sapp 1994).

Ectomycorrhizal symbionts vary in taxonomic identity, morphology, function and abundance, and symbionts may evolve to these characteristics in response to each other. In spite of their intimate interactions with their symbiotic partner, plants and fungi also respond to abiotic and biotic factors external to the symbiosis. Fungi forming ectomycorrhizal associations with roots of a host plant will interact with the biotic (e.g. bacteria, microfauna) and abiotic (e.g. soil solution chemistry, water potential) environment of the soil matrix. For example, soil fauna can consume up to 50% of ectomycorrhizal hyphae (Setala 1995) and up to 55 isolates of bacteria are reported to

occur on ectomycorrhizas formed between a single host-fungus combination (Bending et al. 2002). The ecological amplitude of host plants (measured by height and biomass performance) is clearly dependent on soil properties such as nutrient and moisture availability (Burns and Honkala 1990).Thus, ectomycorrhizas exist in a complex biotic and abiotic milieu, and heterogeneity in either the biotic or abiotic portions of that milieu will be ecologically significant to the association.

Within a forest stand the number of ectomycorrhizal fungal species is an order of magnitude higher than that of host species (Bruns 1995). Dickie (2007) recently showed that total ectomycorrhizal fungal richness is a linear function of the number of ectomycorrhizal plant species; one hundred fungal species are predicted to associate with just 2 host plant species. Within a forest stand, both edaphic conditions (Farley and Fitter 1999, James et al. 2003) and the distribution of ectomycorrhizal fungi are spatially heterogeneous (Jonsson et al. 2000, Lilleskov et al. 2004, Izzo et al. 2005). Root systems of individual trees normally experience temporal and spatial variation both in soil properties and in the taxonomic identity and abundance of ectomycorrhizal fungi present in the soil. As a result, individual trees form mycorrhizas with a diverse community of ectomycorrhizal fungi.

In this thesis I consider specific aspects of variation in each of the ectomycorrhizal partners and how this variation may influence the other partner. Hosts can vary in taxonomic identity, and within a species, hosts vary genetically and phenotypically. Similarly, ectomycorrhizal fungal communities vary in species composition, individual fungi vary in anatomy and physiology, and populations of different fungal species vary in their abundance on root systems and in the soil as inoculum.

We do not yet have a clear understanding of how finely-tuned phyto- and mycobionts are to each other. Statistically expressed, this means that we do not have a sense of the proportion of the total variation in a particular aspect of one partner that is explained by variation in the other. In this thesis, I use experimental and meta-analysis approaches to evaluate:

i. how colonization levels, regardless of ectomycorrhizal fungal taxon, correlate to host growth

ii. how ectomycorrhizal fungi differentially influence growth of different genera of plant hosts, and

iii. how variation in growth of a single host species correlates to the composition of ectomycorrhizal fungal communities in various soil environments.

Integral to these questions, and an issue that is central to my thesis, is how to control for and manipulate ectomycorrhizal fungi on host plants.

In my experiments, I used growth of seedlings as a measure of host response. I did this for two reasons. First, experimenting with adult trees is intractable. Second, while the seedling phase is relatively short in comparison to the entire lifespan of the tree, selection pressures are high at this stage (Harper 1977). It has also been shown that tree species are more strongly adapted to their regeneration niche than to the adult niche (Poorter 2007), thus the conditions influencing seedlings are important for predicting the distribution of adult trees. Because seedlings cannot reproduce, I use growth as my primary measure of performance as is typically done in ectomycorrhizal studies (see those in Chapter 2).

Consideration of variation in both partners of the ectomycorrhizal symbiosis to the growth response of either partner has been investigated over the past few decades of mycorrhizal research. The novelty of this thesis is the evaluation of this variation from multi-specific and coevolutionary perspectives. I use the term multi-specific to denote the situation where a host plant interacts with many species of ectomycorrhizal fungi. Most research has focused on evaluation of host plants inoculated by a single, target fungal species (but see Baxter and Dighton 2001, Kranabetter 2004), yet in nature seedlings are almost always colonized by several ectomycorrhizal fungi concurrently. I consider responses both to different individual fungi and to different communities of fungi. I use the term coevolution in the broad sense meaning "trait-matching" (Bronstein et al. 2006), in contrast to the more restrictive definition meaning reciprocal evolutionary change in interacting species (Thompson 1994). No formal definition of trait-matching exists; however, Gomulkiewicz et al. (2007) illustrate the concept with the example of plant flowering time synchronized to time of pollinator emergence.

Implicit in my thesis is the understanding that host plants and ectomycorrhizal fungi are coevolved. Understanding precisely how variation in either partner of the ectomycorrhizal symbiosis is matched by the other partner allows us to make conclusions about the sensitivity of the growth responses between symbionts. Host plants showing the same growth response to variation in mycobionts, regardless of fungal taxon or extent of colonization, suggests a response of low sensitivity.

Synchronized responses between variation in host plant growth and that present in mycobionts is suggestive of a more sensitive response. Additionally, because each symbiont can also respond independently to its abiotic environment, variation in the abiotic component may alter the association or even supercede the importance of changes in fungi or host plants involved in the symbiosis.

Literature Review

The level of sensitivity between symbiont responses can be viewed as a measure of the generalization or specialization that has occurred as a result of coevolution. For example, if growth responses of a host plant species are independent of variation in taxonomic identity of its ectomycorrhizal fungi, and in nature host plants were found to associate with a very large number different species of ectomycorrhizal fungi, these findings would indicate this particular host plant is a generalist. Conversely, if it were shown that host growth responses were highly sensitive to the identity of the ectomycorrhizal fungus, and that in nature the host plant was found to associate with a narrower range of fungi, this would suggest that host plants are specialized.

Causes of specialization versus generalization

Specialization is a somewhat arbitrary and relative term used to represent the range of resources a species uses. In the context of coevolution of mycorrhizas, this could refer to the number of species with which a particular species interacts. Generalization and specialization are not static categories (Holmes 1977) and evidence has rejected the hypothesis that specialization is a "dead-end". It is clear that switches between each mode over evolutionary time have been frequent (Thompson 1994, Janz et al. 2001, Nosil and Mooers 2005). Additionally, accumulating research suggests that coevolved partners are highly asymmetric in their degree of specialization (Bronstein et al. 2006). For example, in plant-pollinator systems pollinators tend to specialize on a plant species, but a given plant species may be visited by many different species of pollinators (Vázquez and Aizen 2004). Most theories on the causes of specialization invoke the role of variation in the environment or in some attribute of the organisms involved. For example, within trophic groups, specialization is thought to be a response to environmental constancy and the presence of interspecific interactions, most notably competition (Futuyma and Moreno 1988). The degree of specialization across trophic

groups, such as that observed between coevolved organisms is hypothesized to be a result of variation in availability of partners. For example, Stebbins (1970) posited that generalization in plant-pollinator systems is favored when the availability of the most effective pollinator is unpredictable and conversely, specialization is favored when pollinator availability is reliable. Within symbiotic systems, Douglas (1998) reviewed causes of generalization between hosts and symbionts. She suggested that when effectiveness of symbionts varies differentially with environmental conditions, and these conditions are unpredictable relative to host generation time, host specialization should not be favoured. Specialization is also disadvantageous when the abundance of symbionts in free-living condition is low or their spatial distribution is unpredictable.

Specialization versus generalization in ectomycorrhizal associations

The degree of specialization within ectomycorrhizal associations is typically asymmetric between myco- and phytobionts. Host plant species tend to associate with a higher number of fungal species compared to the number of host species with which an ectomycorrhizal fungal species forms associations (Malloch et al. 1980; Borowicz and Juliano 1991). Generally, most ectomycorrhizal fungi form associations with multiple host species (Horton and Bruns 1998, Simard et al. 1997, Massicotte et al. 1999, Kennedy et al. 2003, Nara 2006), with some exceptions: the genera *Rhizopogon* and *Suillus* associate primarily only with *Pseudotsuga menziesii* and *Pinus* spp. There are also several fungal species that are found only in association with *Alnus* spp. (Molina et al. 1992). Hosts appear broadly receptive to different species of ectomycorrhizal fungi, with *Alnus* having a somewhat restricted receptivity. One exception to this pattern is the high specificity observed between plants in the Monotropoideae and their ectomycorrhizal fungi (Bruns et al. 2002, Bidartondo and Bruns 2005).

When considered in a multi-specific context, plant host attributes often structure the composition of ectomycorrhizal fungal communities. For example, the composition of ectomycorrhizal fungal communities varies among clones of *Picea abies* differing in relative growth rate but grown in the same soil (Korkama et al. 2006). In addition, there is a negative relationship between similarity among ectomycorrhizal fungal communities and taxonomic distance among hosts – similarity among ectomycorrhizal fungal communities is higher on hosts of the same genus or family (Ishida et al. 2007). Thus,

variation in host characteristics may be an ecologically important gradient which is partitioned by ectomycorrhizal fungal species.

Ecological material for specialization: what variation is present among ectomycorrhizal fungi to which plant hosts could respond?

Ectomycorrhizal fungi vary in their abundance and spatial and temporal distribution within the soil (Jonsson et al. 2000, Lilleskov et al. 2004, Izzo et al. 2005, Koide et al. 2007). Hence, as roots of an individual host forage through soil, they will encounter different species and genotypes of ectomycorrhizal fungi. Fungal portions of the mycorrhiza, such as mantle and extramatrical hyphae, vary morphologically depending on the fungal species involved (Agerer 1987-1998, Goodman et al. 1996). Functional variation among species of ectomycorrhizal fungi has been reported for carbon demand (Bidartondo et al. 2001), nutrient uptake (Read and Perez-Moreno 2003), and pH (Wallander 2002, Yamanaka 2003, Dunabeitia et al. 2004) and drought tolerance (Parke et al. 1983, Boyle and Hellenbrand 1991, Dixon and Hiol-Hiol 1992). Variation among fungal isolates of the same species has also been reported for nutrient uptake (Cairney 1999, Sawyer et al. 2003, Guidot et al. 2005). Overall colonization levels and hyphal biomass of ectomycorrhizal fungal usually decreases in soils having high nitrogen or phosphorus levels (Treseder 2004), but different species of ectomycorrhizal fungi differ in their sensitivity to changes in nitrogen and phosphorus (Jones et al. 1990, Brandrud and Timmermann 1998, Wallenda and Kottke 1998, Nilsson and Wallander 2003, but see Clemmensen et al. 2006).

Overview of Thesis

In Chapter 2 using meta-analysis, I quantitatively assess which causes more variation in host growth responses to ectomycorrhizas: changes in host or fungal taxonomic identity? In addition, I examine whether colonization levels, regardless of fungal identity, correlate to plant host response. In the meta-analysis, the effect of ectomycorrhizas is based on comparisons of non-inoculated to inoculated seedlings. I highlight limitations to this approach in Chapter 2, but I also review and test the major techniques currently available to create ectomycorrhizal controls in Chapter 3, where the results of implementing physical and chemical barriers to ectomycorrhizal colonization are presented. In Chapter 4, I explore the contribution to seedling growth of

variation in ectomycorrhizal colonization levels, relative to genetic variation, in a host species, lodgepole pine (*Pinus contorta* var. *latifolia*). Finally in Chapter 5, I experimentally examine the response of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) to variation in ectomycorrhizal community composition and in soil fertility and moisture characteristics. The relative importance of variation in the abiotic versus symbiotic environment for both host growth and ectomycorrhizal community is separated statistically using a multivariate approach. Both species used in experiments are common, widely distributed trees in British Columbia. I end the thesis with general conclusions and suggested future research in Chapter 6.

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2 The mutualism-parasitism continuum in ectomycorrhizas: A quantitative assessment using meta-analysis[1]

Introduction

Host plants do not always respond positively to mycorrhizas; thus, defining mycorrhizas as mutualists has been challenged (Francis and Read 1995, Johnson et al. 1997, Brundrett 2004, Jones and Smith 2004). Mycorrhizal fungi benefit from these associations because fungal reproduction is dependent on symbiosis with a plant host (Jones and Smith 2004). Data from large, single studies of arbuscular mycorrhizal associations indicate that host plants have a continuum of positive to negative responses to mycorrhizas (e.g. Klironomos 2003), but data for ectomycorrhizal associations are scattered among many small studies. Hence we have much less understanding of the range of host responses to ectomycorrhizal associations.

The validity of altering the definition of mycorrhizas to remove the requirement for mutualistic responses can be quantitatively evaluated by measuring the mean and variation of host response over many pairwise combinations of host and fungus. The mean indicates whether hosts have a positive, neutral or negative response to mycorrhizas, and variation around the mean indicates whether there is a range of host responses and outcomes are dependent on the context of the association. Variation in host response from positive to negative outcomes would support the continuum concept. The absence of variation around the mean indicates that regardless of the biotic or abiotic environment of the association, host responses are consistent.

Ectomycorrhizal plants, which include many tree species in the northern hemisphere, experience two main kinds of variation in ectomycorrhizal associations: the identity and abundance of fungal species. The manner by which a host responds to variation in mycobiont identity has important evolutionary consequences. If there has been selection for specialization among mycobionts, we predict that growth responses of hosts will depend upon the taxonomic identity of the fungus. Functional variation among taxa of ectomycorrhizal fungi is well documented for characteristics including nutrient uptake (e.g. Abuzinadah and Read 1989, Dighton et al. 1990, Jongbloed et al. 1991, Lilleskov et al. 2002), and drought (Parke et al. 1983, Boyle and Hellenbrand

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1991, Dixon and Hiol-Hiol 1992) and pH tolerance (Wallander 2002, Yamanaka 2003, Dunabeitia et al. 2004).

Each ectomycorrhizal root tip represents a conduit for resource exchange. As such, the extent to which a root system is colonized could also influence response of the phytobiont to mycorrhization. If ectomycorrhizs are mutualisms, we would predict that higher levels of colonization are positively correlated to growth of the host; however, this relationship is not consistent (e.g. Jones et al. 1990, Thompson et al. 1994). As well, Treseder and Allen (2002) predict a unimodal relationship between increasing nutrients in the soil and mycorrhizal biomass, although how this relationship affects host growth is uncertain. Nutrient status of the soil is hypothesized to be a key factor in determining host position on the mutualism-parasitism continuum (Johnson et al. 1997).

The past few decades have generated sufficient individual studies on plant host responses to ectomycorrhizal associations that some generalizations can now be made about the nature of the association (mutualistic to parasitic) across different host-fungus pairings. But, to date there has been no quantitative synthesis that allows us to determine the general direction or magnitude of this phenomenon, or the variation in these responses. Meta-analysis is an increasingly common analytical tool used by ecologists to quantitatively summarize the results of multiple independent studies (e.g. Gurevitch et al. 2000, Treseder 2004, Cardinale et al. 2006, Lortie and Callaway 2006), and is particularly useful when published studies have conflicting results. Meta-analyses have also been used to highlight gaps in the data and to identify common methodological problems or constraints. More importantly, by treating separate empirical studies as independent data points weighted by their replication and precision, meta-analysis allows us to discern general patterns already existing in the data that might not be otherwise evident. We used meta-analysis to determine: 1) how hosts respond to different ectomycorrhizal fungi; 2) if the response is host or fungal specific; 3) if levels of colonization modify the response; 4) if soil nutrient conditions modify host growth responses, and 5) if the perception of mycorrhizas as mutualisms has biased publication of results. We posed two additional questions about the role of experimental conditions in modifying host response: 6) does contamination of controls modify detectable host response to ectomycorrhizas? and 7) does host response change with the length of association between host and fungus (i.e. experiment length)?

Methods

Data collection

We searched ISI Web of Science (1965 – present) using the keyword 'ectomycorrhiza'. Of the 3591 hits, we selected papers written in English reporting either total biomass (g), shoot height (cm) or shoot:root ratio of tree seedlings inoculated with ectomycorrhizal fungi paired with non-inoculated control seedlings. We also checked the "literature cited" section of these papers for additional references. Total biomass is a measure of productivity. Shoot height may be indicative of competitive ability in the seedling establishment phase, where tree seedlings have to compete with rapidly growing herbs. Changes in shoot:root ratio may identify factors that increase seedling survival in nutrient limited environments or that control the potential carbon supply to ectomycorrhizal fungi, the currency mediating the association.

For each study, we recorded the mean, standard deviation and sample size for both inoculated and control seedlings. When necessary, we digitized graphs to obtain this information. When experimental treatments involved several combinations of host species with ectomycorrhizal fungal species or fungal isolates, we treated each combination as a separate study, although not all studies were completely independent. Inclusion of several studies from one paper tends to reduce the overall heterogeneity in effect sizes, but excluding multiple results from a paper could underestimate effect sizes (Gurevitch and Hedges 1999). When results from papers involved inoculation trials in combination with explicit manipulations of the environment, other than nutrient levels (e.g. pH, pathogen abundance, nematode density, salinity, soil moisture, CO_2), we used data from "ambient treatments". For example, we recorded data for inoculated and control seedlings from ambient CO_2 levels while excluding data from treatments featuring elevated CO_2 levels. Among those papers that manipulated fertilizer types and amounts, only the manipulation of inorganic phosphorus levels was reported in a sufficient number of studies to merit further analysis. We converted phosphorus additions to a common unit, mg P kg^{-1} substrate, and treated it as a continuous predictor with values ranging from 0 to 136 mg kg^{-1} substrate. We did not include studies where inoculation resulted in no colonization, or where there were no control data (non-inoculated treatments). When repeated measures were taken in a study, we used data from the last sampling period to capture the maximal length of association between host and fungus.

Host and fungal identity were treated as categorical explanatory variables in the meta-analytic model. We recorded the species of host and, when given, ectomycorrhizal fungus (in some cases the fungus was an unknown isolate, or the species epithet was not provided). We then grouped species into genera for both host and fungus, and when testing for differences among genera, we included only those that were represented by at least 10 studies. Duration of association and colonization level (percent of root tips colonized or percent root length colonized) of inoculated seedlings were investigated as possible continuous explanatory variables in the model. When colonization level was given as a range, we used the median value.

Contamination of non-inoculated seedlings reduces differences in colonization levels between control seedlings and inoculated seedlings. Consequently, the perceived response of hosts to ectomycorrhizal inoculation may be reduced as a result of contamination. We determined the magnitude of contamination by calculating the level of colonization on control seedlings relative to that measured on inoculated seedlings according to the proportion:

$$C_C / (C_C + C_{TR})$$

where C_{TR} is the percent colonization of target fungi on inoculated seedlings, and C_C is the percent colonization of contaminant fungi on control seedlings.

We quantified the duration of the association by recording the number of weeks each experiment ran. This measure was the only consistent proxy to evaluate the influence of experimental duration on host outcome to ectomycorrhizal associations; however, we recognize that extreme differences in growth rates among host species would render absolute length of time irrelevant. We also examined the relationship between time and the variation among effect sizes. To do so, residuals were calculated using the absolute difference of effect sizes from the cumulative mean and weighted by their sample sizes. Residuals were then regressed against duration of association. We performed identical calculations to examine residuals for effects sizes across phosphorus levels.

Data analysis

The effect size of ectomycorrhizal inoculation for total biomass, shoot height and shoot:root ratio was calculated as the natural log of the response ratio of inoculated to control seedlings. The response ratio (R) is the ratio of the mean outcome in the

experimental (inoculated) group to that of the control (non-inoculated) group (Rosenberg et al. 2000). Only 12% of the studies in our analysis reported measures of variation around means. Consequently we weighted values by their sample size instead (Shurin et al. 2002, Lajeunesse and Forbes 2003, Marczak et al. 2006), and while this increases the probability of Type II errors, it avoids underestimating effect sizes (Gurevitch and Hedges 1999). Effect sizes were considered significantly different than zero when 95% confidence intervals did not overlap zero; explanatory variables were considered significant at $\alpha = 0.05$.

Tests for homogeneity of effect sizes were based on the statistic Q_T , with larger values indicating greater heterogeneity in effect sizes among comparisons (Rosenberg et al. 2000). We assessed the importance of publication bias using a non-parametric rank correlation test (Spearman's rho). A significant correlation between effect size and sample size across studies would indicate bias in the publication of extreme effect sizes. Effect sizes for all analyses were not normally distributed, so we relied on randomization tests (4999 iterations) to assess significance levels.

We first tested the null hypothesis that all effect sizes were equal, and if rejected, we examined the categorical (fungal and host genus identity) and continuous (colonization levels, magnitude of contamination, and duration of association) explanatory variables described above. When categorical predictors were significant, we assessed differences among groups based on 95% bootstrapped confidence intervals. We then regressed effect size against all continuous predictor variables. For any significant explanatory variable, we only report those explaining > 5% of the variation in effect sizes as estimated by Q_M/Q_T, where Q_M is the variation in effect sizes that is explained by a particular model (Rosenberg et al. 2000). All data analyses were performed in MetaWin software version 2.1.4 (Rosenberg et al. 2000).

Results

Seedling response to ectomycorrhizal inoculation

Overall we extracted 459 studies of inoculation response of total biomass from 36 papers, 329 studies of shoot height from 24 papers, and 235 studies of shoot:root ratio from 20 papers (Appendix A). Across all growth traits, we assessed the outcome of 21 host genera inoculated with 31 fungal genera; however, these inoculations were not

represented in all possible combinations. The mean age of seedlings at the end of experiments was 23 weeks (range = 10 to 104 weeks).

On average, seedlings increased in total biomass and shoot height, but did not change in shoot:root biomass allocation when inoculated with ectomycorrhizal fungi (mean cumulative effect sizes = 0.208, 0.113, -0.0174, respectively; Fig. 2.1). However, there was significant heterogeneity in the data (Q_T = 10152, df = 458; Q_T = 95389, df = 328; Q_T = 705, df = 234, respectively; all $p < 0.001$) to indicate that further structure existed.

The identity of the host genus was significant in explaining variation in effect sizes for both total biomass ($p = 0.028$, df = 4, 409, $Q_M/Q_T = 0.18$) and shoot:root ratio ($p < 0.001$, df = 3, 191, $Q_M/Q_T = 0.22$). In particular, inoculated seedlings of the genera *Quercus*, *Pseudotsuga* and *Eucalyptus* increased in total biomass more than those of *Pinus* and *Picea* (Fig. 2.2a), while *Picea* seedlings allocated more biomass to shoots than seedlings of *Quercus*, *Pseudotsuga* and *Pinus* when inoculated (Fig. 2.2b). Although there was a positive relationship between total biomass and shoot height ($p < 0.001$, df = 1, 567, $r^2 = 0.37$), neither categorical nor continuous predictors explained variation in effect sizes of shoot height. Fungal genus influenced allocation of biomass to shoots versus roots ($p < 0.001$, df = 5, 199, $Q_M/Q_T = 0.26$), but did not explain variation in effect sizes for total biomass or shoot height. Seedlings inoculated with fungi from the genus *Scleroderma* allocated more biomass to roots than that observed for other genera (Fig. 2.3).

Level of colonization of inoculated seedlings, ranging from 0.5 to 98%, was not important in explaining variation in effect sizes for total biomass ($p = 0.043$ df = 1, 349, $Q_M/Q_T = 0.03$), shoot height ($p = 0.30$, df = 1, 220) or shoot:root ratio ($p = 0.03$, df = 1, 211, $Q_M/Q_T = 0.03$) (note that although level of colonization was significant, $Q_M/Q_T < 0.05$ for both total biomass and shoot:root ratio [see Methods])(Fig. 2.4). Heterogeneity in effect sizes was unrelated to the magnitude of contamination for total biomass ($p = 0.20$, df = 1, 324), shoot height ($p = 0.48$, df = 1, 211) and shoot:root ratio ($p = 0.063$, df = 1, 197)(Fig. 2.5). Contamination levels were highest in those experiments performed in nurseries and in the field, and lowest in those in growth chambers ($p < 0.001$, $F_{3,416} = 76.9$) (Table 2.1).

The average length of experiments was 21 weeks (range = 8 to 104 weeks), slightly less than the average age of seedlings used in experiments. Duration of

association between host plant and fungus did not explain variation in effect sizes for total biomass ($p = 0.86$, $df = 1, 457$) or shoot height ($p = 0.97$, $df = 1, 327$) (Fig.2.6a, b). On average, seedlings allocated more biomass to roots than shoots, with increasing duration of association ($p < 0.001$, $df = 1, 233$, $Q_M/Q_T = 0.06$) (Fig.2.6c). The magnitude of contamination was positively related to duration of experiment ($p < 0.001$, $df = 1, 418$, $r^2 = 0.14$) (Fig. 2.7). Variability among effect sizes decreased with duration of association for both total biomass ($p < 0.001$, $df = 1, 457$, $r^2 = 0.12$) and shoot height ($p < 0.001$, $df = 1, 327$, $r^2 = 0.25$), but was unrelated to duration for shoot:root ratio ($p = 0.033$, $df = 1, 233$ (Fig. 2.8). That is, longer running experiments had effect sizes more similar to the cumulative mean. In particular, for measures of total biomass and shoot height, variation among effect sizes declined to nearly zero (effect sizes converged on the cumulative mean) at approximately 30 weeks (Fig. 2.8). The level of contamination for control seedlings was predicted to increase by 84% for this time period (Fig. 2.7).

There was evidence for significant publication bias in data for total biomass; Spearman's rho for the correlation between effect size and sample size was -0.28 ($p < 0.001$), indicating that there was an over-representation of studies with positive effect sizes at low replication. There was no evidence of publication bias in data for shoot height ($Rs = 0.054$, $p = 0.33$) or shoot:root ratio ($Rs = -0.105$, $p = 0.109$).

Seedling response to ectomycorrhizal inoculation and phosphorus addition

We analyzed 234 studies (6 host and 15 fungal genera) from 10 papers for changes in total biomass of seedlings inoculated with ectomycorrhizal fungi under phosphorus (P) additions ranging from 0 to 136 mg P kg^{-1} (Appendix B). The cumulative effect size was positive (0.0769), but the 95% confidence intervals overlapped zero, indicating there was no average change in total biomass of seedlings inoculated with ectomycorrhizal fungi subjected to manipulated phosphorus levels when all levels of substrate P, including no additions, were included. There was underlying structure in the data ($p < 0.001$, $df = 232$, $Q_T = 1236$); however, of the explanatory variables, only host genus explained a significant amount of variation in effect size ($p < 0.001$, $df = 3, 24$, $Q_M/Q_T = 0.31$). Specifically, seedlings of the genera *Eucalyptus*, *Pinus* and *Larix* had relatively less biomass than those of *Picea* when inoculated, regardless of phosphorus level. There was a negative relationship between the residuals of effect size and amount of phosphorus added, indicating that variation among effect sizes decreased

with levels of phosphorous (Fig. 2.9). Publication bias was also evident in these data ($Rs = 0.31$, $p < 0.001$), i.e. there were a lack of studies with positive effect sizes at low sample sizes.

Discussion

Seedling response to ectomycorrhizal inoculation

Across the studies included in our analysis, it appears that on average, hosts respond positively to ectomycorrhizal inoculation; both total biomass and shoot height are greater in inoculated seedlings. However, when all available studies are considered and weighted by their sample sizes, the evidence in support of positive growth outcomes through ectomycorrhizal inoculation is considerably weaker than many single studies suggest. Additionally, factors unrelated to inoculation *per se* have influenced interpretation of host responses to ectomycorrhizal inoculation, namely publication bias towards large positive effects, the duration of experiments and artificial pairing of host and fungal symbionts. The presence of these factors effectively reduces and distorts the spectrum on which host responses to ectomycorrhizal inoculation are evaluated.

The spectrum is reduced: Publication bias inflates measures of effect sizes

Under a model of no publication bias, estimated effects should be distributed around the unknown true effect, with the spread of the effects representing their variances. As sample sizes increase, the spread of the distribution should decrease resulting in a funnel shaped distribution of effect sizes. Publication bias against studies with negative results will produce a negative correlation between sample size and the magnitude of effect (Begg and Mazumdar 1994) and this inflates the magnitude of overall effect sizes calculated in a meta-analysis. We detected publication bias for measures of total biomass but not for shoot:root ratio or shoot height responses to inoculation. Shoot height increases with ectomycorrhizal inoculation, but it is independent of the identity of host and fungal genus, colonization levels and duration of association. Because the lower limit of the cumulative effect on total biomass is well above zero, there may indeed be a change in seedling biomass upon inoculation.

Among the papers used in this meta-analysis, Dixon et al. (1984) and Hung and Molina (1986) explicitly reported that data had been omitted due to non-significant differences between control and inoculated seedlings. It is unlikely that these particular

omissions alone caused publication bias in our dataset, but they may be symptomatic of bias in the selection of data reported in published papers. At the other extreme, although they did not affect the results of the meta-analysis, host-fungus pairings extracted from Burgess et al. ([1994]; identified as outliers in Fig. 2.4a and b) were irregularities in our dataset, reporting highly positive responses to ectomycorrhizal inoculation by various strains of *Pisolithus*. Due to the tradition of categorizing mycorrhizal fungi as mutualists, such extreme positive results are unlikely to go unpublished. Negative results in mycorrhizal research may be more likely to go unpublished compared to other fields in which no *a priori* expectation exists of the magnitude or direction of the outcome of species interactions.

From a silvicultural perspective, interest primarily in positive growth responses to ectomycorrhizal inoculation may be warranted, but it has hindered our ability to evaluate the full spectrum of responses. Moreover, negative responses are not aberrant outcomes when we consider that hosts are evolutionarily compatible with both mutualistic and parasitic modes of symbioses. For example, the pathways and physiological machinery involved in arbuscular mycorrhizal development are conserved among symbiosis types, including those that are parasitic (Mathesius 2003, Paszkowski 2006). As arbuscular mycorrhizas are considered to be ancestral to all other mycorrhizal types (Wang and Qiu 2006), there is no biological basis to presume that responses to ectomycorrhizal inoculation should be solely positive. Changing our definition of ectomycorrhizas (Johnson et al. 1997, Brundrett 2004, Jones and Smith 2004) will become necessary as evidence accumulates on their evolutionary origins (Hibbett et al. 2000) and on variation in the outcomes of ectomycorrhizal associations (Sachs and Simms 2006). This will also broaden the view of their ecological role.

The spectrum is distorted: Factors that covary with time may cause spurious effects

Not surprisingly, levels of contamination were highest on seedlings grown in either the field or in nurseries, although most of the experiments from which the data were extracted were done in greenhouses. The magnitude of contamination was positively correlated to the duration of the experiment (Fig. 2.7). The problem of increased contamination could be alleviated if measurements were made earlier. This approach, however, is not recommended. Variation among effect sizes for both total

biomass and shoot height significantly declined with the duration of the experiment. Factors such as maternal effects (Weiner et al. 1997), substrate differences, temperature and light conditions may all obfuscate the role of ectomycorrhizas in influencing seedling growth in shorter experiments. Seedlings also vary in the time it takes to develop ectomycorrhizal associations. On roots of *Eucalyptus globulus*, ectomycorrhizas formed by *Pisolithus tinctorius* and *Paxillus involutus* developed in 4 days when in direct contact (Horan et al. 1988). Conversely, colonization was not observed until 4 weeks on roots of *Eucalyptus coccifera* inoculated with *Thelephora terrestris* or *Laccaria bicolor* (Jones et al. 1990). Early measurements (prior to 30 weeks) may preclude detection of a mycorrhizal "signal" as the strength of this signal is likely to be weak compared to other factors influencing seedling growth.

The spectrum is distorted: Effects of crossing hosts and ectomycorrhizal fungi not known to co-occur remain poorly understood

Often inoculation trials are performed using artificial pairings of host and fungus (e.g. Chen et al. 2006) and rely on ectomycorrhizal fungi that are amenable to experimentation. Choosing fungi based upon characteristics that render them easy to work with in laboratory conditions may also have selected for uniformity in other traits. Until techniques become available to represent the diversity of ectomycorrhizal fungi observed in natural systems, interpretations of host response to ectomycorrhizal inoculation will be limited. Moreover, the geographic origin of fungi and hosts used in trials may affect inoculation responses in unpredictable ways. Similar to plants, some but not all species of ectomycorrhizal fungi are cosmopolitan in their distribution. One corollary to this pattern is that not all host and ectomycorrhizal fungal species will interact and that at any given location a host species will encounter a subset of the global pool of ectomycorrhizal fungi. This geographic variation in plant-mycorrhizal community structure has likely resulted in a mosaic of coevolution between plants and mycorrhizal fungi (Thompson 2005), but we still have very few data on the consequences of this mosaic on mycorrhizal inoculation responses (but see Hoeksema and Thompson 2007, Klironomos 2003, Monzon and Azcon 1996, Sylvia et al. 2003). This lack of knowledge of the range of host responses to exotic symbionts also carries over to conservation research; the ecological consequences of mycorrhizal fungal species' introductions are unpredictable (Schwartz et al. 2006).

In our meta-analysis, we could not categorize each host/fungus pairing as "local" or "foreign", as such information was either unavailable, or it was not clear at what scale we should consider a host and fungal species to co-occur (e.g. within a forest stand, region or country). Studies on arbuscular mycorrhizas have shown that crossing local plants and fungi produces a greater range in responses measured by plant biomass than for crosses involving foreign symbionts (Klironomos 2003). Conversely, variation in plant growth was independent of fungal isolates when different geographic populations of 3 host plant species were crossed with 4 populations of the ectomycorrhizal fungus *Rhizopogon occidentalis* (Hoeksema and Thompson 2007). Origin of fungal isolate was also not found to be important in modifying growth of *Eucalyptus globulus* (Thompson et al. 1994). These findings are consistent with our results that variation in fungal identity bears little consequence to variation in shoot height or seedling biomass.

The role of variation in fungal properties in host response to ectomycorrhizal inoculation

The magnitude of effect size for seedling biomass and shoot height for the most part did not covary with variables related to ectomycorrhizal fungi, namely colonization level and genus identity. Our results suggest that colonization levels are not an ecologically useful measure of host response to ectomycorrhizal inoculation (Fig. 2.4). Moreover, we suggest that focus on colonization levels has distracted investigation from other possible mechanisms that may be more critical determinants of host response to ectomycorrhizas.

Characteristics of fungi such as those associated with the development and differentiation of extramatrical mycelium may correlate better to the magnitude of host response as they represent a potential increase to the absorbing surface area of roots (Jones et al. 1990, Agerer 2001). This type of measurement relies on physical mechanisms underlying host benefits of being mycorrhizal. Our results suggest that these benefits may be equally expressed through colonization levels ranging from 0.5% to 98%. It is unlikely that similar resource transfers could occur at low (0.5%) and high (98%) levels of colonization that result in a comparable cumulative positive effect to inoculation among seedlings. Nevertheless, there are many examples of growth response to very low levels of colonization. It is possible that the presence of growth promoting hormones may be responsible for increases in seedling biomass and height

with inoculation. It is well established that plant-associated microorganisms are capable of synthesizing phytohormones that are used for communication between a host and its microflora (Tsavkelova et al. 2006). For example, small amounts of auxins increase shoot elongation and dry weight of wheat inoculated with rhizobacteria (Khalid et al. 2004). Auxins, which are involved in a wide variety of physiological responses that influence growth of woody plants (Kozlowski and Pallardy 1997), are also produced by ectomycorrhizal fungi (Barker and Tagu 2000). Though some research has been conducted on the effects of auxins on ectomycorrhizal development (e.g. Niemi et al. 2002, Rincon et al. 2003), its role at the level of the host has been neglected. Given that positive effects of fungal inoculation are often observed at low levels of colonization for both seedling biomass and shoot height, we suggest that chemical mechanisms may often underlie host responses to ectomycorrhizal inoculation.

We determined that on average, seedlings across multiple host species had more biomass when inoculated with any ectomycorrhizal fungus, regardless of the identity of the fungal associate. This supports findings from research on non-symbiotic interactions; for example, host plants are often generalists with response to different pollinators (Zamora 2000). This result conforms to theory predicting the outcome of multi-specific plant-pollinator systems, i.e. interactions involving many species tend to result in the evolution of generalists because reciprocal specialization is unlikely (Howe 1984). In forest stands, the number of species of ectomycorrhizal hosts is typically an order of magnitude less than that of its fungal symbionts (Bruns 1995). Reciprocal specialization is unlikely in this system due to the changing composition of ectomycorrhizal fungi both spatially (Izzo et al. 2005, Genney et al. 2006, Toljander et al. 2006) and temporally (Izzo et al. 2005, Koide et al. 2007). Thus, hosts may adapt to "landscapes" (*sensu* Howe 1984) of ectomycorrhizal fungi where fungal species diversity diffuses selection from one source.

Nonetheless, we cannot definitively conclude that the identity of the fungus has no role in modifying host response for two reasons. First, although it is evident that inoculation with most fungal genera results in increased biomass allocation to shoots, those fungi from the genus *Scleroderma* are an exception. Seedling allocation to roots increased by almost three times when inoculated by fungi from this particular genus. Diédhiou et al. (2004) concluded that *Scleroderma dictyosporum* has a higher requirement for glucose relative to thelephoroid species, perhaps related to construction

costs of its network-like mycelium (Newton 1991). Plants growing in nutrient-depleted soils allocate more biomass to roots than shoots (Gedroc et al. 1996). If association with fungi from this taxon is perceived by the host as equivalent to growing in nutrient-depleted soils this would explain allocation patterns. Second, there appears to be a difference between those fungi that contaminate seedlings and those used to inoculate seedlings. Because there was no effect of magnitude of contamination on all three growth measures despite a cumulative positive effect, contaminant fungi are likely neutral in their effects. Species of contaminant fungi were for the most part unidentified but included those from the genera *Thelephora* and *Cenococcum*. These fungi are common, widespread, and widely dispersed via airborne spores; whether such characteristics of fungi and magnitude of host response covary should be further studied.

Although a positive growth response was expressed by the most common host genera in our analysis, hosts differed in the magnitude of response. In particular, *Quercus* seedling biomass and biomass allocation to roots ranked highest, and *Picea* lowest, with ectomycorrhizal inoculation. When phosphorus conditions were manipulated (i.e. the subset of studies that explicitly altered phosphorus levels), *Picea* ranked highest in increased seedling biomass with inoculation. We cannot say whether these outcomes are taxon or trait-specific, due to the relatively few genera included in the analysis. For example, mycorrhizal dependency has been hypothesized to relate to various root morphological traits such as root thickness, surface area and incidence of root hairs (Brundrett 2002). In addition, dependency on arbuscular mycorrhizas seems to be higher for hosts that have small seeds or have had seed reserves experimentally reduced (Janos 1980, Allsop and Stock 1995, Siqueira et al. 1998, Zangaro et al. 2003). Our results contrast with those observed for arbuscular hosts; seeds of *Quercus* are generally larger than those of *Picea*, yet are more responsive to ectomycorrhizal inoculation. Root morphology is sensitive to abiotic conditions of the soil, thus its role in determining mycorrhizal dependency is unclear. Going beyond taxonomic correlations with inoculation responses, and identifying those specific host traits that correlate to specific outcomes will enrich our understanding of ectomycorrhizal interactions. In particular, further research within a broad framework, such as that which has developed for leaf traits (Wright et al. 2004), would be especially fruitful to understand trade-offs among plant traits and mycorrhizal responsiveness.

Mycorrhizal associations are predicted to confer most benefit to the host plant in conditions of low nutrients. As such, we would expect a negative relationship between the magnitude of effect size and increasing phosphorus addition but our results do not support this prediction. The range of host responses appears to be environment specific; variation among effect sizes was high for studies with low phosphorus additions. Bougher et al. (1990) have indicated there is an interaction between the effects of fungal taxa and P additions. Specifically, at low P additions (2-12 mg P kg^{-1} soil), differences among *Desoclea maculate*, *Laccaria laccata* and *Pisolithus tinctorius* in host dry mass production are apparent, but these differences are not apparent at greater than 16 mg P kg^{-1} soil. A similar interaction was reported for seedlings colonized by *Laccaria bicolor* or *Thelephora terrestris* along a P gradient (Jones et al. 1990). Our meta-analysis could not detect such an interaction because not all host/fungi combinations were present across the range of P additions. Whether the response to ectomycorrhizal fungi is taxon- or environment-specific (or both) warrants further study as it has implications for the strategies plants may use to maintain ectomycorrhizal associations that confer benefits to the host (Hoeksema and Kummel 2003).

Conclusions and future directions

Publication bias clouds our ability to conclusively determine general principles of host response to ectomycorrhizal inoculation. With recognition that mycorrhizal associations could fall on a continuum of possible outcomes and that this range of responses is ecologically significant, the tendency not to report negative results must be reduced. Our criticism of methods employed to test host response to ectomycorrhizal inoculation is not one of mycorrhizal research in general, but instead reveals the limits of some of the methods used. In particular, there is tension between assessing the response too early when the mycorrhizal signal can be masked, and running the experiment too long and increasing the likelihood of contamination. We see no easy remedy to this problem. The use of mycorrhizal defective mutants, such as those used by Cavagnora et al. (2004) may offer a way to circumvent the issues highlighted with current methods. Even so, the reliance on comparisons between mycorrhizal and non-mycorrhizal individuals, beyond its heuristic purpose, is somewhat artifactual because in nature, non-mycorrhizal phenotypes do not occur, except in very young seedlings. Finally, as recommended in non-symbiotic systems (e.g. Stanton 2003, Strauss and

Irwin 2004), a departure from focusing on pairwise species interactions and moving to consideration of host responses to variation in the composition of ectomycorrhizal fungal communities may encourage a broader perspective on the ecological and evolutionary consequences of ectomycorrhizal associations.

Table 2.1:Means and standard errors (SE) for the influence of location of experiment on the magnitude of contamination.

Source	n	Mean*	SE
Field	43	0.33[a]	0.0251
Nursery	28	0.43[a]	0.0311
Greenhouse	243	0.10[b]	0.0105
Growth chamber	106	0.0026[c]	0.0160

* Means followed by the same letter are not statistically different (Tukey-Kramer HSD, $\alpha = 0.05$).

Figure 2.1: Cumulative mean effect sizes for total biomass, shoot height and shoot:root ratio. Error bars are 95% bootstrapped confidence intervals.

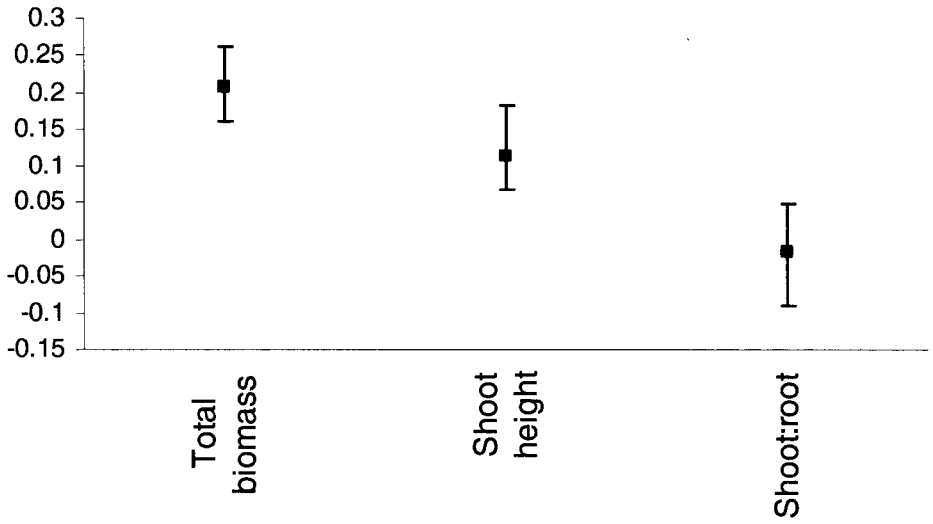


Figure 2.2: Mean effect size for a) total biomass and b) shoot:root ratio by host genus. Means with 95% bootstrapped confidence intervals are shown. Means followed by the same letter are not statisticaly different (95% bootstrapped confidence intervals overlap). For b), positive values indicate allocation of biomass to shoots was higher than allocation to roots.

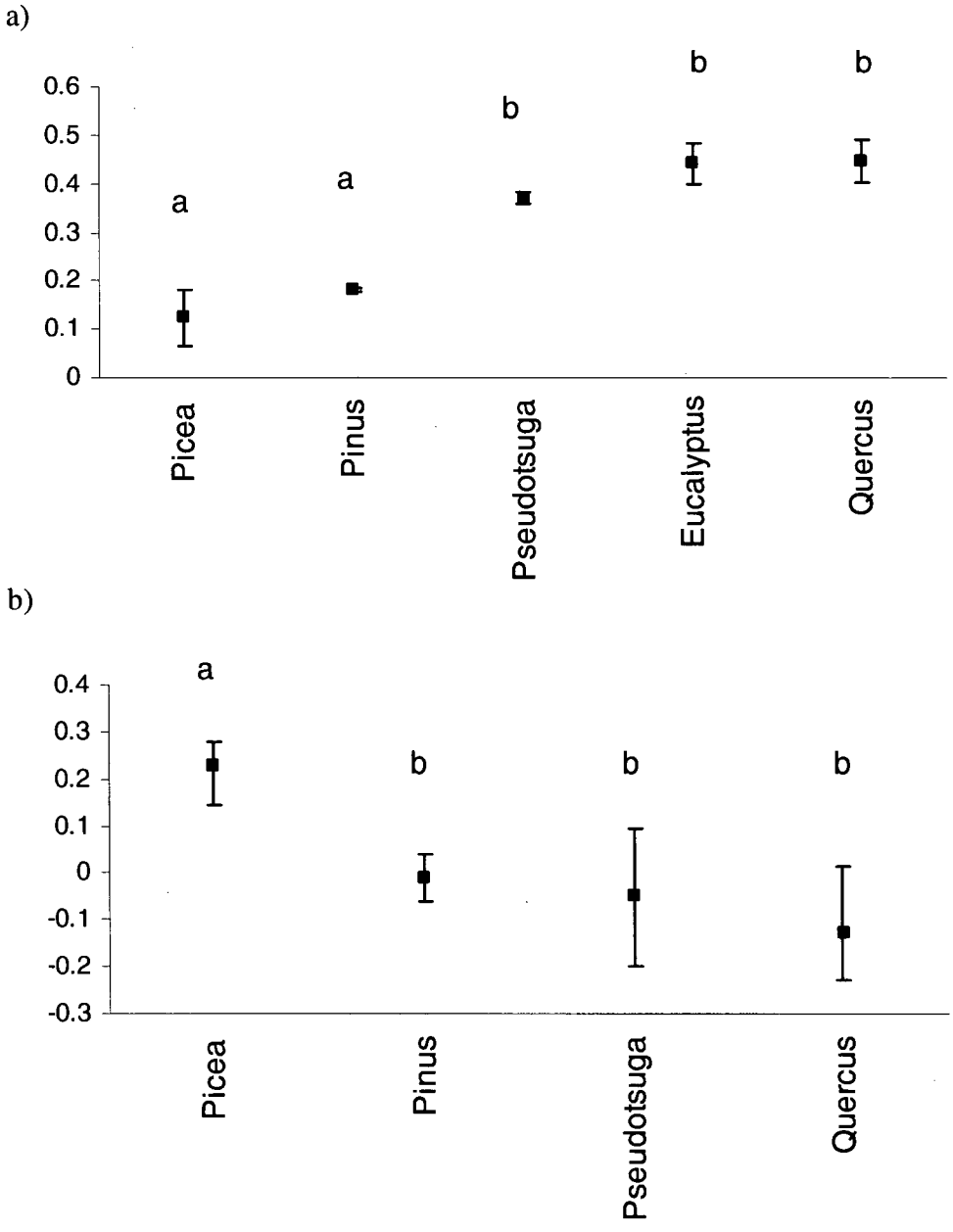


Figure 2.3: Mean effect size for shoot:root ratio by fungal genus. Means with 95% bootstrapped confidence intervals are shown. Means followed by the same letter are not statistically different (95% bootstrapped confidence intervals overlap). Positive values indicate allocation of biomass to shoots was higher than that allocated to roots.

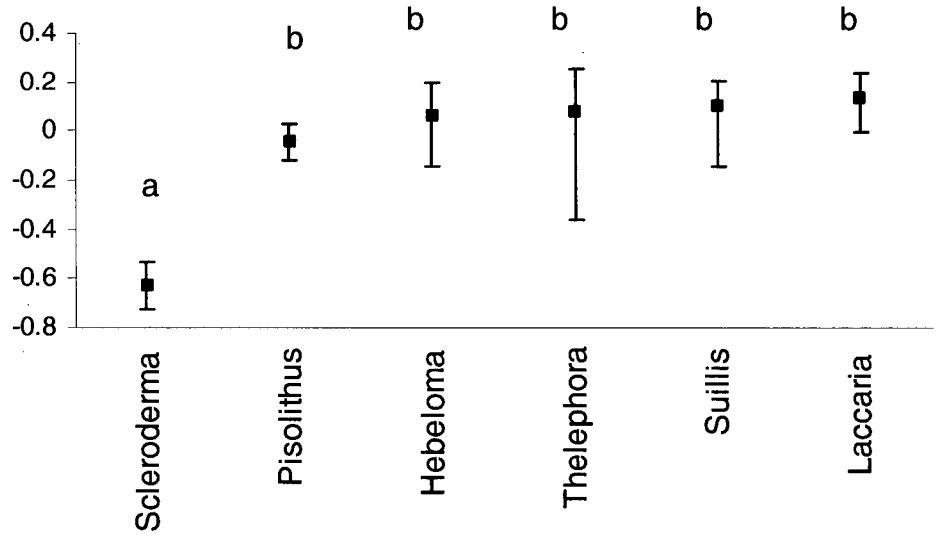
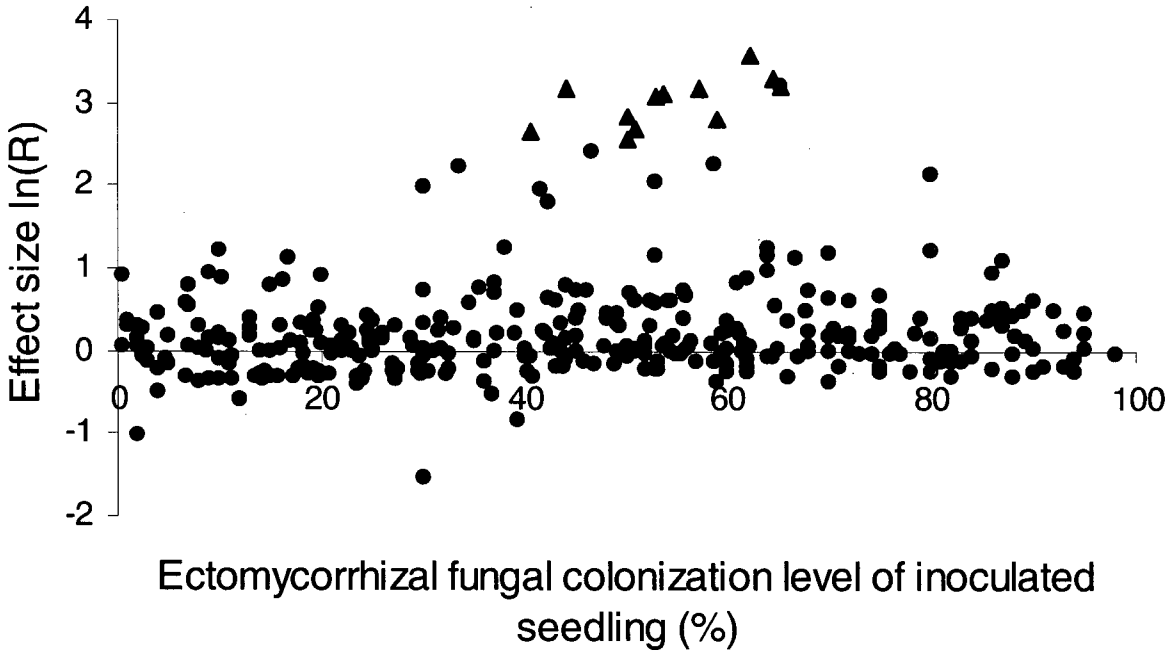
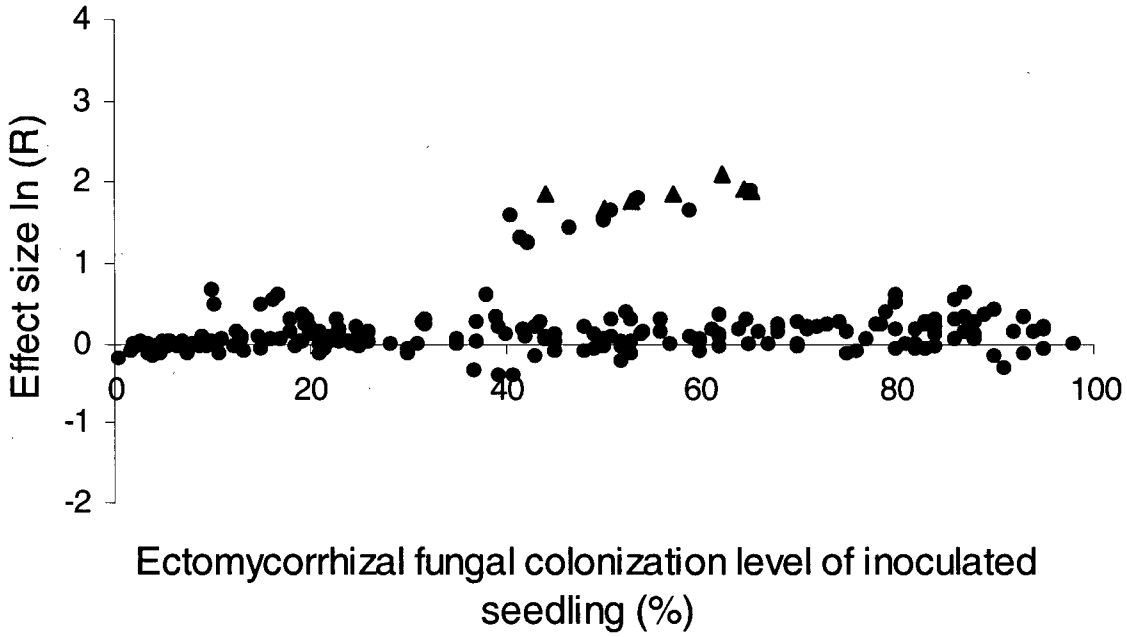


Figure 2.4: Relationship between mean effect sizes and level of ectomycorrhizal fungal colonization of inoculated seedlings for a) total biomass, b) shoot height and c) shoot:root ratio. Outliers (those data points falling above the 97th percentile of the distribution) are indicated as triangles; these were retained in the analysis.

a)

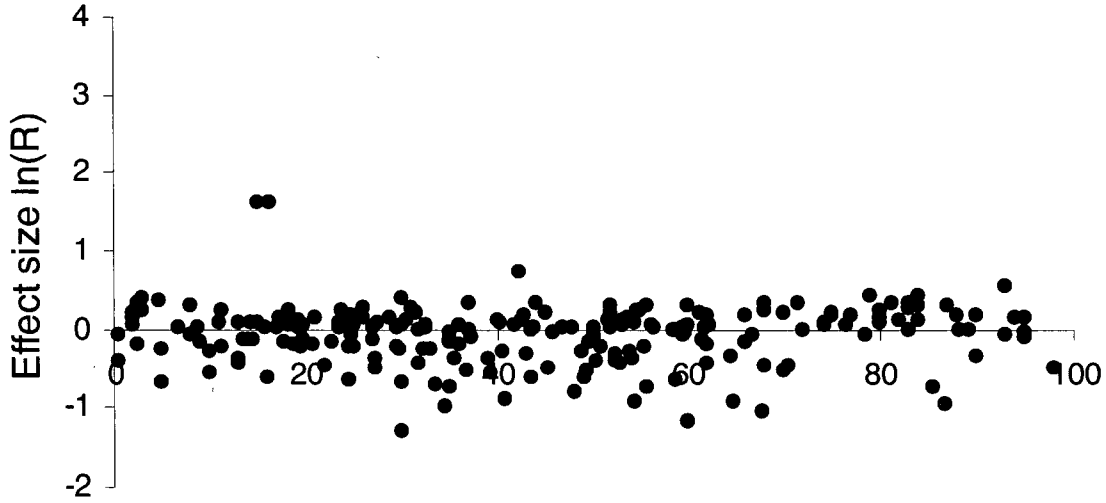


b)



Ectomycorrhizal fungal colonization level of inoculated seedling (%)

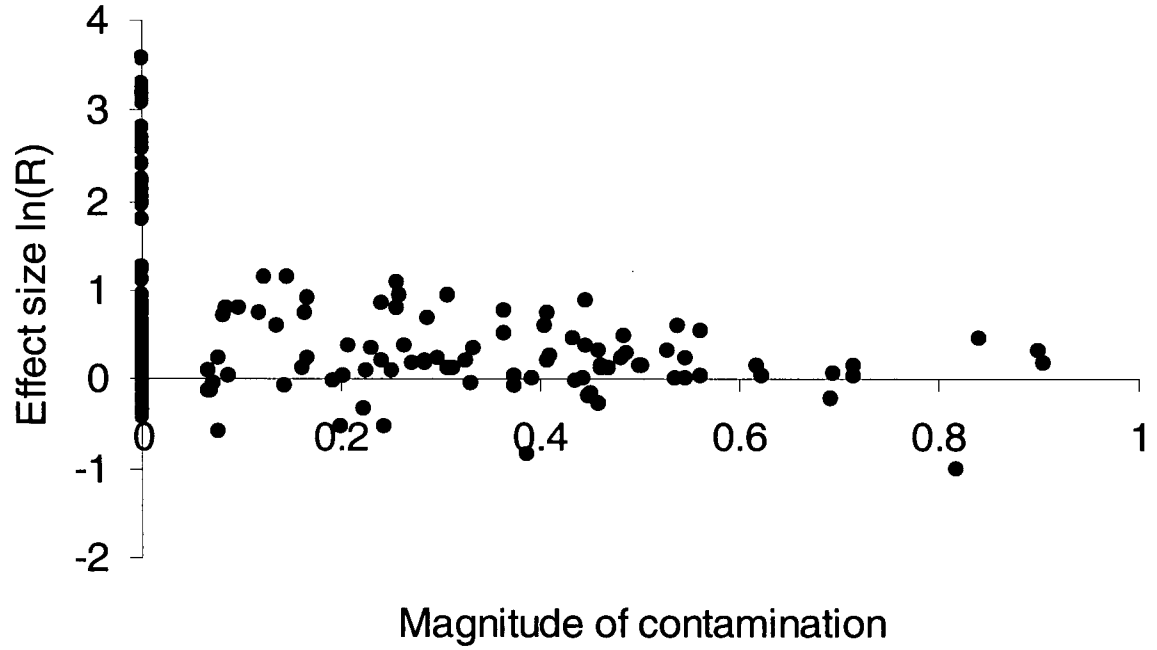
c)



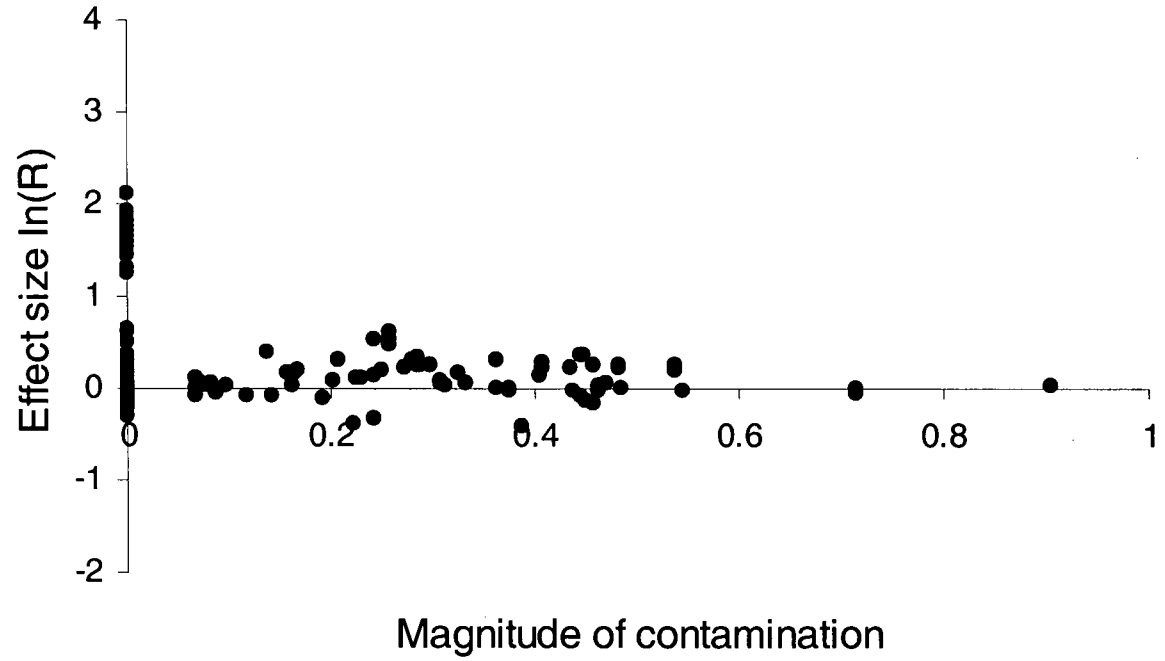
Ectomycorrhizal fungal colonization level of inoculated seedlings (%)

Figure 2.5: Relationship between mean effect sizes and magnitude of contamination for a) total biomass, b) shoot height and c) shoot:root ratio.

a)



b)



c)

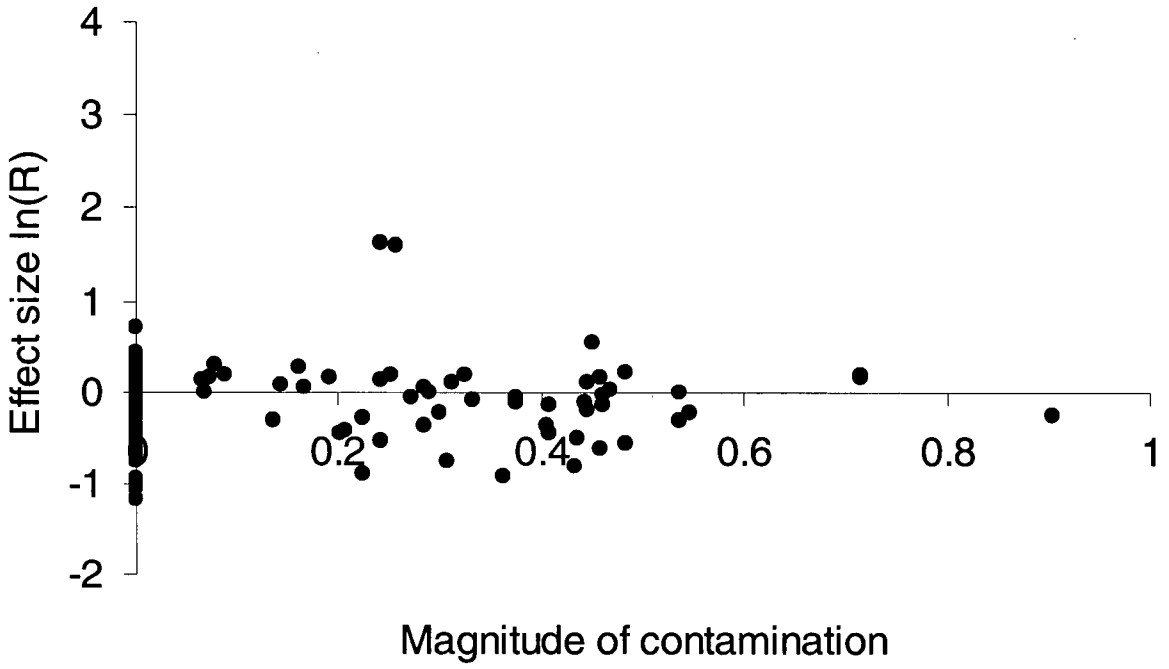
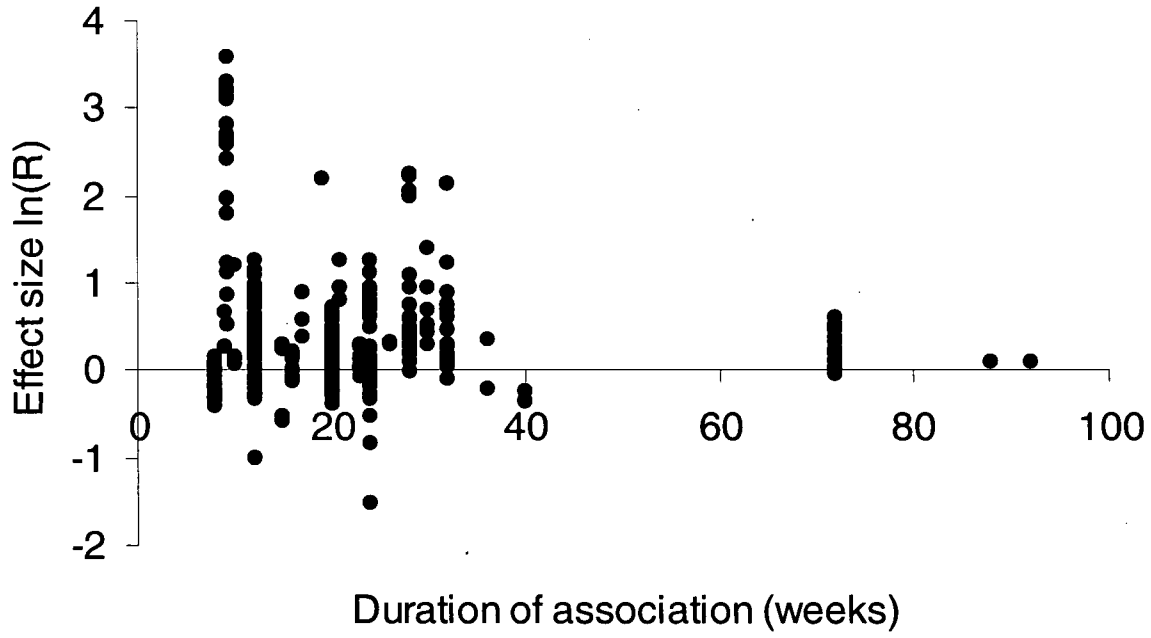
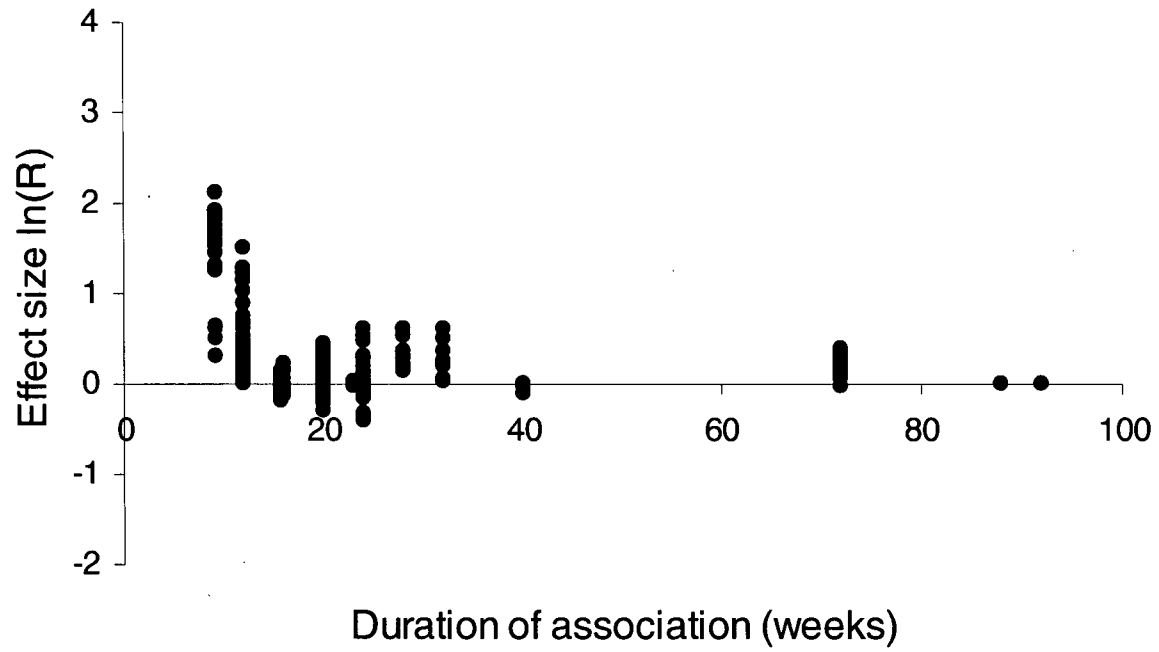

4
3
2
1
0
-1
-2
Effect size ln(R)
0.2
0.4
0.6
0.8
1
Magnitude of contamination

Figure 2.6: Relationship between effect sizes and duration of association of ectomycorrhizal fungus and host for a) total biomass, b) shoot height and c) shoot:root ratio. Q_M/Q_T is the amount of total heterogeneity in the data due to variation in effect sizes explained by the model. Statistics are reported for significant models only.

a)



b)



c)

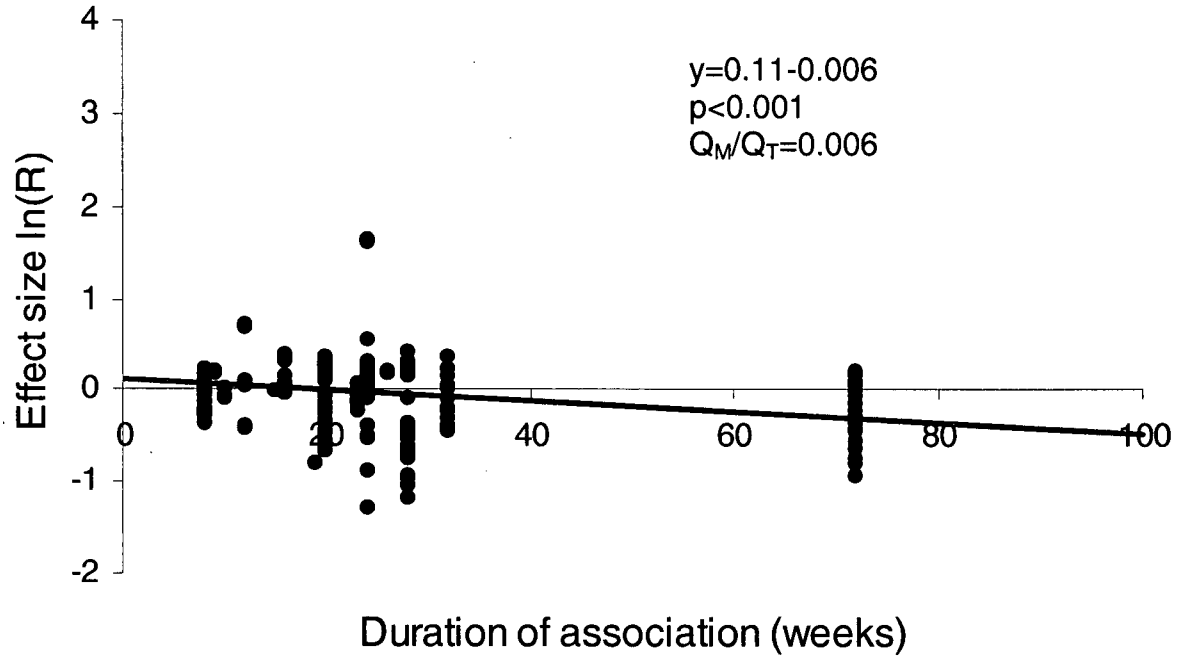

y=0.11-0.006
p<0.001
Q_M/Q_T=0.006
Effect size ln(R)
4
3
2
1
0
-1
-2
0
20
40
60
80
100
Duration of association (weeks)

Figure 2.7: Relationship between magnitude of contamination and duration of association of ectomycorrhizal fungus and host.

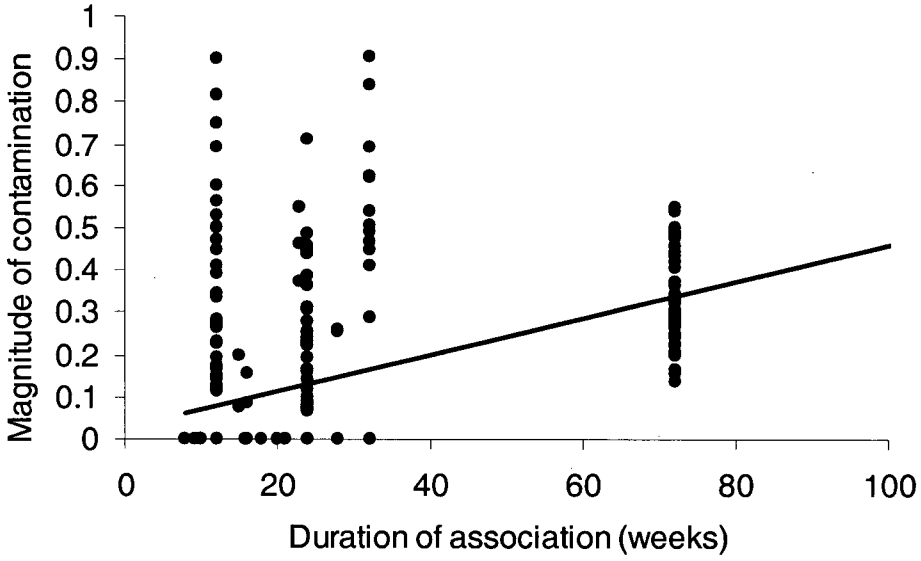
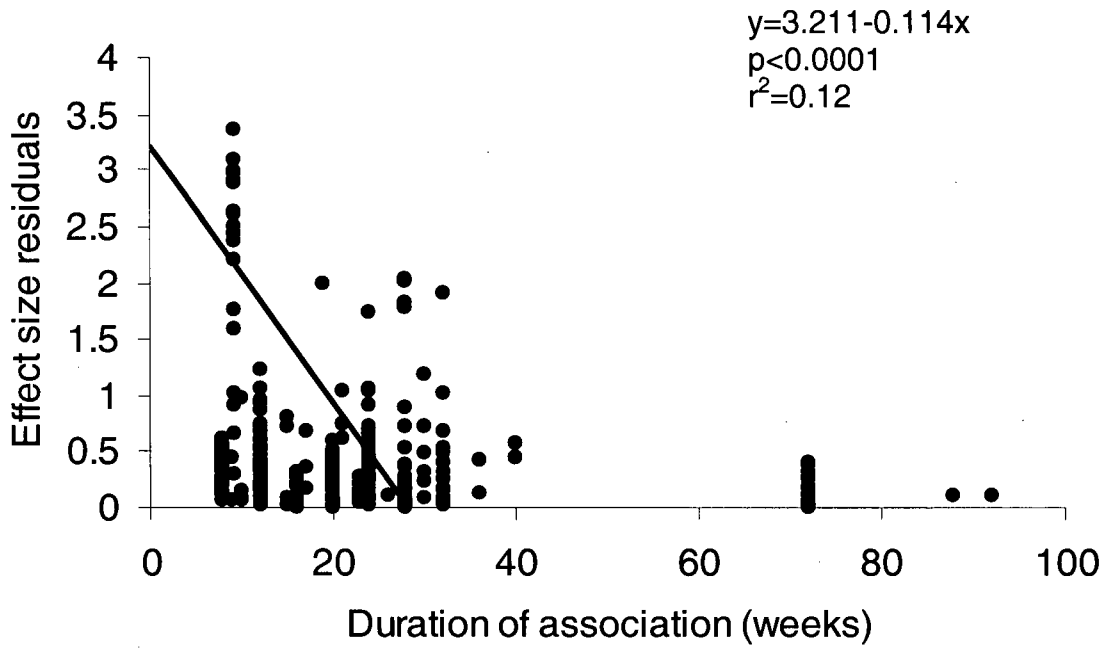
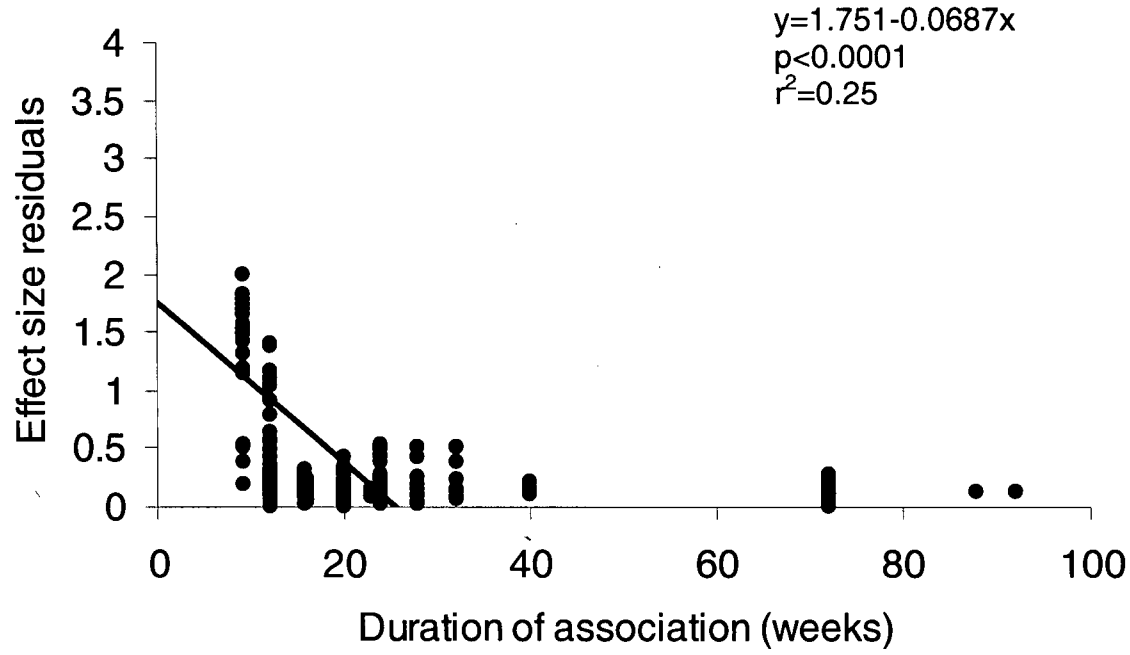


Figure 2.8: Relationship between effect size residuals and duration of association of ectomycorrhizal fungus and host for a) total biomass, b) shoot height and c) shoot:root ratio. Statistics are reported for significant models only.

a)



b)



c)

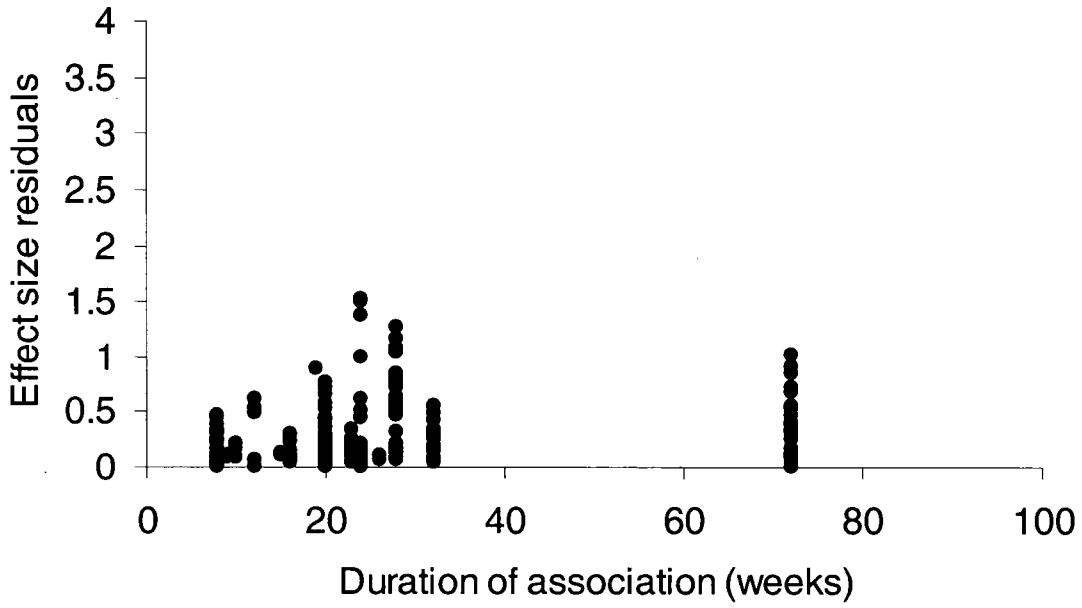
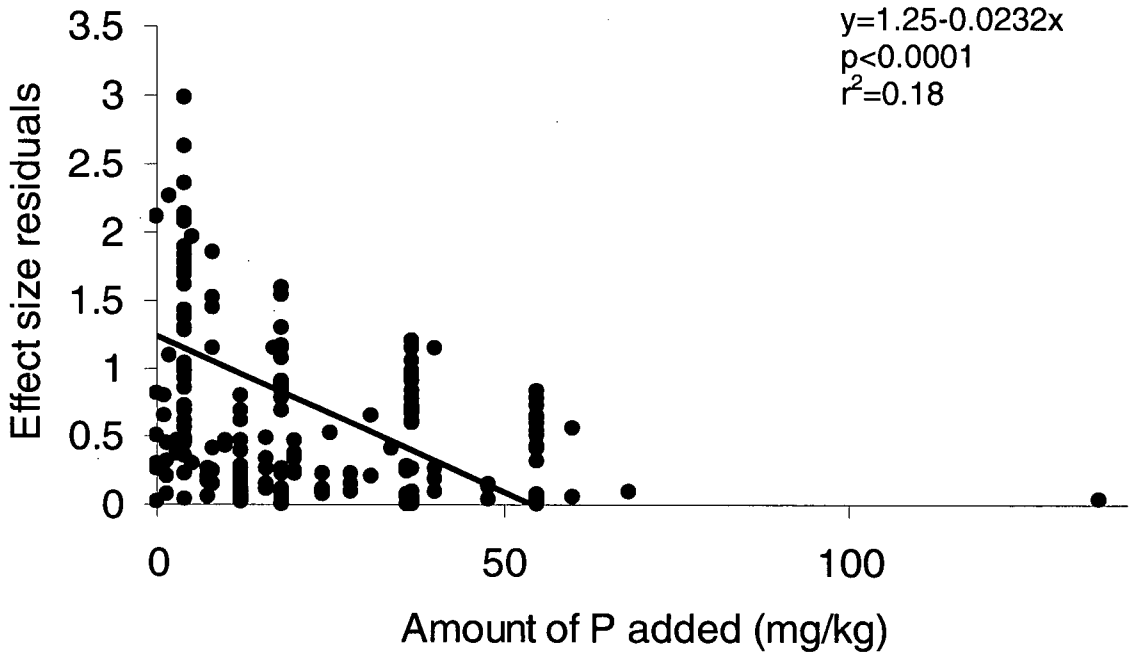

Effect size residuals
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
Duration of association (weeks)

Figure 2.9: Relationship between effect size residuals for total biomass and amount of phosphorus added.



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3 Methods to control ectomycorrhizal colonization: Effectiveness of chemical and physical barriers[1]

Introduction

In mycorrhizal research, evaluation of mycorrhizal effects on plant performance often requires comparisons between mycorrhizal and non-mycorrhizal plants. Creating effective, yet feasible methods to control mycorrhizal colonization in the field has become of utmost importance as there has been a recent demand to increase the ecological relevance of mycorrhizal research (Read 2002). This requires moving away from laboratory based work to experiments conducted in natural environments.

Currently, most studies have obtained non-mycorrhizal plants by employing one of three methods: substrate sterilization (via autoclaving, steam sterilization or gamma irradiation), the creation of mutant plants unable to form mycorrhizas, or the use of fungicides applied to soil around plant roots. Sterilizing soil can result in substantial changes in its chemical and physical properties (Lenis et al.1991, Chambers and Attiwill 1994, Sheremata et al.1997, Shaw et al.1999); moreover, its application in the field is futile because contamination is certain. The development of plants that lack the ability to form mycorrhizas has been limited to a few plant species associating with arbuscular mycorrhizal fungi (AMF) (Marsh and Schultze 2001). More research is also required to determine whether the functioning of mutants is otherwise identical to non-mutant plants (Kahiluoto et al. 2000). Of the fungicides, benomyl has been used effectively to reduce arbuscular mycorrhizal colonization of plants in the field by as much as 80% (Hartnett and Wilson 1999, Wilson et al. 2001, Callaway et al. 2004, Dhillion and Gardsjord 2004). Benomyl, no longer licensed for use in some countries and relatively ineffective against basidiomycetes, is however, not an option to control most ectomycorrhizal fungi. Fungicides have generally not been employed in ectomycorrhizal systems (but see Page-Dumroese et al. 1996, Manninen et al. 1998). Ectomycorrhizal fungal communities are more taxonomically diverse than arbuscular mycorrhizal fungal communities, thus requiring a broad spectrum fungicide to adequately decrease ectomycorrhizal colonization. Of the three methods currently employed to control mycorrhization, the use of fungicides appears the most feasible for field research in

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Two fungicides, Topas® and Senator®, have been suggested by greenhouse managers for control of ectomycorrhizal hyphal growth. Propiconazole, the active ingredient in Topas® (25% a.i.), interferes with ergosterol biosynthesis, which is critical to the formation of fungal cell membranes (Kendrick 2000). The lack of normal sterol production slows or stops the growth of the fungus, effectively preventing further infection and/or invasion of host tissues (Kendrick 2000). Propiconazole incorporated into agar media at 1 ppm or higher inhibited growth of many ectomycorrhizal fungal strains (Zambonelli and Iotti 2001, Laatikainen and Heinonen-Tanski 2002). Colonization of *Pinus sylvestris* roots by ectomycorrhizal fungi decreased by approximately 20%, with some morphotypes affected more than others, when propiconazole was applied for two consecutive years in the field at a rate of 250g l^{-1} every two weeks (Manninen et al. 1998). Thiophanate-methyl, the active ingredient in Senator® (70% a.i.), interferes with the functioning of microtubules, so that treated cells cannot divide. Thiophanate-methyl targets the cells of ascomycetes (Kendrick 2000), but to our knowledge has not been used to control ectomycorrhizal fungi.

Studies of common mycorrhizal networks (CMNs) in plant communities form a unique subset of studies on mycorrhizal effectiveness (Simard and Durall 2004). They require comparisons between plants that are linked with those that are not linked by a CMN (Simard et al. 1997, Booth 2004). In these studies, control plants may be mycorrhizal, but hyphal linkages between plants must be absent. While non-mycorrhizal or non-linked controls are easily established in the laboratory using substrate sterilization techniques, this is more problematic in the field where seedlings are grown in native soils. Mesh barriers constructed of either steel or nylon have been used to prevent formation of ectomycorrhizal connections between plants (e.g. Francis and Read 1984, Schüepp et al. 1992, Booth 2004, Kranabetter 2005), or provide root-free compartments where mycorrhizal hyphae can explore and grow. To restrict penetration of roots and hyphae, mesh with pores 1 µm or smaller has been used (Robinson and Fitter 1999, Johnson et al. 2001, Zabinski et al. 2002, Cardoso et al. 2004), however, given that hyphal width varies (from 1.5 to 9 µm), a mesh with pore sizes larger than 1 µm may restrict penetration of some mycorrhizal fungal species but not others. Consequently, the mesh pore size could alter the ectomycorrhizal fungal community composition. Ectomycorrhizal fungi vary in their ability to absorb and transport nutrients

and water (Simard and Durall 2004); therefore, any alteration of the community may affect transport within the CMN.

The objective of this study was to examine the effectiveness of chemical and physical methods at controlling formation of ectomycorrhizas on Douglas-fir seedlings. We tested the effectiveness of the fungicides, Topas® and Senator®, at various concentrations and application frequencies. We predicted that both fungicides would reduce ectomycorrhizal colonization, however, we expected that colonization of ascomycete fungi would be particularly reduced with the application of Senator®. Thus, the composition of the ectomycorrhizal fungal community would be altered compared to untreated controls. In addition, we tested the effectiveness of nylon mesh with various pore sizes at preventing hyphal penetration, and its effects on ectomycorrhizal community composition of neighboring seedlings. We predicted that percent colonization and similarity of ectomycorrhizal communities between seedlings on opposite sides of the mesh barrier would decrease with decreasing mesh pore size.

Materials and methods

Field soil collection

On August 27-28 of 2003, we collected 600 L of soil from the Black Pines variable retention cut (also known as a green-tree retention cut where some trees are not harvested) and adjacent forest approximately 50 km northwest of Kamloops, British Columbia (120°26'W, 50°42'N). The Black Pines variable retention cut occurs in the dry cool subzone of the Interior Douglas-fir (IDFdk) biogeoclimatic zone (Meidinger and Pojar 1991). It has an elevation of 1180 meters above sea level (masl) and loamy Gray Luvisolic soil (Krzic et al. 2004). The plant community is dominated by residual Douglas-fir *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) trees and advanced regeneration (saplings), with shrub and herbaceous layers dominated by soopolallie (*Sherpherdia canadensis* (L.) Nutt.) and pinegrass (*Calamagrostis rubescens* Buckley), respectively.

We collected forest floor (30 cm x 30 cm) together with mineral soil (to 40 cm depth) from 15 random locations in 1 ha of the Black Pines forest. This soil was used for both experiments. The fifteen samples were combined and thoroughly mixed, then stored at room temperature until needed (see below).

Plant material

Interior Douglas-fir seedlings (seedlot #48520, British Columbia Ministry of Forest Tree Seed Center, Surrey, British Columbia, Canada) were grown at the University of British Columbia (Vancouver, Canada) greenhouse (temperature minimum 20°C, temperature maximum 24°C, average humidity 60%). Seeds were moist-stratified at 4°C for 21 days. Seeds were then sterilized in constantly mixed 3% H_2O_2 for two hours. Styroblock™ 512B trays (Beaver Plastics Ltd., Edmonton, Alberta, Canada) were cut in half horizontally and filled with autoclaved peat and sawdust (3:1, v:v). Three seeds were sown in each cavity and 4 weeks later were thinned to one seedling per cavity. The trays were placed under a mist tent for 12 days and then moved to a greenhouse bench for the remaining time. To improve seedling vigor and discourage mycorrhizal colonization, we applied 1.9 g L^{-1} water soluble Rose Plant Food (Miracle-Gro, Scotts Canada Ltd., Mississauga, Ontario, Canada) (18:24:16 N:P:K) once per week for 4 weeks following germination. Afterwards, we fertilized with 4 ml L^{-1} Peter's solution (Plant-Prod ®, Plant Products Co. Ltd., Brampton, Ontario, Canada) (20:20:20 N:P:K) once per week until the seedlings were transplanted into the treatment pots. For the duration of the two concurrent experiments (five months), natural daylight in the greenhouse was supplemented by 400 W high pressure sodium lamps to maintain an 18 hour photoperiod.

Fungicide experiment

Experimental design and treatments

On September 16, 2003, 14-week-old seedlings were transplanted into 3.2 L pots (175 mm x 180 mm) (Listo Products Ltd., Surrey, British Columbia, Canada) with drainage holes. The pots contained field soil mixed with perlite (3:1, v:v). A 3 x 3 x 3 factorial set of treatments with a separate control group was replicated 10 times in a completely randomized design, where the factors were fungicide type, rate of application, and frequency of application (270 seedlings + 10 controls = 280 total). The three fungicide types were Senator®, Topas®, and a combination of the two fungicides (both from Engage Agro Corporation, Guelph, Ontario, Canada). The three rates of application were: 0.5, 1 or 1.5 ml L^{-1} of Senator®; and 0.5, 1 or 1.5 g L^{-1} of Topas®. Recommended concentrations of Senator® and Topas® are 0.5 ml L^{-1} and 0.5 g L^{-1}, respectively. To our knowledge this is the only study assessing the effect of these

fungicides on ectomycorrhizal fungi thus, we decided as a starting point to use the above rates. The fungicide was mixed with water and added at a constant volume of 600 mL pot^{-1}; therefore, seedlings that were treated with Senator® and Topas® in combination received 300 mL of each fungicide-water mixture. The three frequencies of application were: once at the beginning of the experiment, every two months (three applications total), or every month (five applications in total). For each fungicide application, we drenched the soil around the seedlings, avoiding contact with foliage. Additionally, ten control seedlings were grown in pots to which only water was applied. On September 30, 2003, initial height was recorded for all seedlings. The seedlings were watered as necessary and their locations re-randomized monthly.

Seedling measurements

On February 10, 2004, the height of all surviving seedlings was measured. Shoots were removed, dried at 65° C for 48 hours and weighed. The roots and intact soil of up to seven replicates were stored at 4°C for 45 days before processing. Each root system was soaked in tap water, rinsed clean of soil, and cut into 1 cm fragments. The sample was then divided approximately in half, and one half was dried and weighed. We used this measurement to estimate dry weight of the remaining roots, which were weighed wet, and then cleared and stained following the methodology of Phillips and Hayman (1970) to assess percent ectomycorrhizal colonization. For a given seedling, percent ectomycorrhizal colonization was calculated as:

Percent ectomycorrhizal colonization = (Active ectomycorrhizal root tips / Active ectomycorrhizal root tips + Active non-ectomycorrhizal root tips) x 100

A root tip surrounded by a mantle was classified as mycorrhizal.

In addition to assessing percent colonization, we recorded the abundance and richness of ectomycorrhizal morphotypes in each of the treatments. Root systems of the remaining three replicates from each of the ten treatments were carefully washed under running tap water and then cut into approximately 1 cm pieces. All root fragments were placed in a baking dish containing water and thoroughly mixed. We randomly subsampled and counted up to 100 ectomycorrhizas, or 100 non-ectomycorrhizal root

tips, whichever came first. Generally, ectomycorrhizal tips were turgid and smooth, had emanating hyphae or rhizomorphs (Harvey et al. 1976), and had a Hartig net. A root tip that was dark and wrinkled, or was somewhat hollow and fragmented under minimal pressure was classified as 'dead'. Gross morphology of ectomycorrhizal roots and rhizomorphs were described using a stereomicroscope, while the mantle, cystidia, and emanating hyphae were described using a compound microscope under 400x or 1000x magnification. When possible, mantles were peeled by separating the fungal tissue from the root with forceps and micro-scalpels, and then described. Morphological descriptions were made with reference to Agerer (1985–1998), Ingleby et al. (1990), Goodman et al. (1996), and Hagerman et al. (2001). Morphotyped roots were then dried and weighed.

Mesh barrier experiment

Experimental design and treatments

To test the effect of pore size on penetration by ectomycorrhizal fungi, we grew seedlings in 3.2 L pots divided vertically by nylon mesh barriers with different pore sizes. The pore sizes of the four meshes were: 0.2 µm (catalogue number 25007, polyamide type 250 membrane, Sartorius AG, Goettingen, Germany), 1 µm (catalogue number 03-1/1 Nitex, Sefar America Inc., Depew, NY, USA), 20 µm (catalogue number 03-20/14 Nitex), and 500 µm (catalogue number 06-500/47 Nitex). Control pots were divided by an impermeable acetate sheet to test for ectomycorrhizal contamination through insufficient sterilization, or water and airborne ectomycorrhizal propagules. Each of the five barrier treatments was replicated 12 times in a completely randomized design. The pots were first sterilized in a 20% bleach solution for at least one hour, cut in half vertically, and then reassembled using non-toxic adhesive silicone sealant (catalogue number 3145-Grey-RTV; mil-A-46146, Dow Corning Midland, MI, USA) to attach the mesh and hold the two halves of the pot together. Each pot had two compartments. On August 30, 2003 one compartment was filled with field soil mixed with perlite (3:1, v:v), watered, and planted with14-week-old seedlings (see Plant Material for growth conditions). Three weeks after the seedlings were transplanted into the unsterilized soil, the second compartments were filled with sterilized field soil. Soil was sterilized by autoclaving at 15 p.s.i for 90 minutes, repeated 24 hours later. Uncolonized 17-week-old seedlings were then transplanted into the sterilized soil and

watered. The purpose of transplanting seedlings into the unsterilized field soil 3 weeks prior to the introduction of seedlings into the other half of the pot was to insure that the seedlings were already colonized by ectomycorrhizal fungi when the experiment was started. We refer to the initially transplanted seedlings as "source seedlings". If hyphae from the source seedlings were able to penetrate a mesh of a given pore size, we expected to see mycorrhizal root tips on "recipient" seedlings grown in sterilized field soil.

Once all source and recipient seedlings had been transplanted into the pots, the seedlings were watered as necessary. Just prior to transplanting, we destructively subsampled fifteen source seedlings to quantify ectomycorrhizal colonization following the methodology of Phillips and Hayman (1970). Afterwards, pot location on the greenhouse bench was re-randomized monthly. Initial shoot height was measured shortly after transplanting, on September 30, 2003.

Seedling measurements

At harvest, January 11, 2004, shoot height and biomass (dried at 65°C for 48 hours) were measured. During the harvest, we also inspected mesh barriers for signs of hyphal penetration using a stereomicroscope. We chose to randomly select ten replicates per mesh barrier treatment for morphotyping using similar methods outlined above (5 treatments x 2 seedlings per pot x 10 replicates = 100 seedlings). Three replicate sets of one root tip per morphotype from different seedlings were lyophilized prior to storage for subsequent molecular analysis. On average, 3% of the total roots tips per morphotype examined were sent for molecular analysis. The remainder of the morphotyped roots were dried and weighed with the remainder of the root sample.

Molecular confirmation of ectomycorrhizal fungal species identification

Total genomic DNA was extracted from single ectomycorrhizal tips by pulverizing them for 45 seconds at a speed of 5.0 units using a Bio101 Systems Fast Prep FP120 high frequency shaker (Q-biogene, Carlsbad, CA, USA). DNA was isolated using the procedure of Baldwin and Egger (1996). The final DNA pellet was dried using a speed vacuum concentrator and then re-suspended in 50 μL EDTA-TE buffer.

Following DNA extraction and isolation, the internal transcribed spacer (ITS) region of the fungal nuclear rDNA was specifically amplified by the primers NSI1 and

NLC2 (Martin and Rygiewicz 2005). PCR reactions typically included 1 µL template DNA, 18.6 µL sterile purified water (Barnested Nanopure Diamond water purifier), 0.2 mM deoxyribonucleoties (dNTPs), 2.5 µl 10x PCR buffer, 1.5 mM $MgCl_2$, 0.48 mM each primer, 1.6 mg mL^{-1} bovine serum albumin (BSA), and 0.25 U μL^{-1} AmpliTaq Gold™ (Applied Biosystems, Foster City, CA, USA). Samples were amplified using a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA, USA). A 10 min hot start was followed by PCR cycling as follows: 45 seconds at 94°C followed by 34 cycles of denaturation at 94°C for 45 seconds, annealing at 54°C for 45 seconds, ramping 72°C for 1 minute with a 1 second extension after each cycle, and extension at 72°C for 10 minutes, and then the temperature was held at 4°C. The PCR products were visualized on 1.5% agarose gels using a Gel Logics 440 (Kodak Instruments, Rochester, NY, USA). The PCR product was cleaned using the QIAquick PCR Purification kit (Qiagen Inc., Valencia, CA, USA). Prior to sequencing, the large ITS fragment produced above, was re-amplified in a nested PCR reaction using the primers ITS 1 and ITS 4 (White et al. 1990). PCR products were quantified and then sequenced using a 3730 DNA Capillary Sequencer (Applied Biosystems) at the University of British Columbia Nucleic Acid and Protein Services Unit. All unique morphotypes were sequenced and then aligned using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). Taxonomic matches were based on BLAST results with $\geq$98% sequence similarity.

Statistical analysis

The fungicide experiment examined a 3 x 3 x 3 factorial set with a separate control group of treatments (i.e., separate from the factorial but combined in the layout) in a completely randomized design (Bergerud 1989). We used percent colonization data obtained from the cleared and stained roots and normalized the data with a square root transformation for analysis of variance (ANOVA). We analyzed ectomycorrhizal fungal community data (richness and diversity, relative abundance of morphotypes with >5% of ectomycorrhizal root tips), seedling growth, and square root of percent colonization, first by using the GLM procedure in SAS (SAS Institute Inc. 1999). We then ran a second GLM procedure with a contrast statement to compare the control treatment against all other treatment combinations. Analyses on data collected from cleared and stained roots and morphotyped root tips were done separately, and consequently graphed separately. ANOVA tables were constructed manually to obtain the proper experimental

error terms and degrees of freedom. When significant main treatment effects occurred, we separated means using the Bonferroni multiple comparison test.

For the mesh barrier experiment, the percent colonization and ectomycorrhizal richness for both seedlings per pot were used to calculate the Steinhaus index of ectomycorrhizal community similarity (Legendre and Legendre 1998) and to calculate the difference in morphotype richness (the number of morphotypes on the donor root system minus the number on the receiver root system). The effects of mesh pore size on ectomycorrhizal fungal community data (richness difference and Steinhaus index of similarity), percent ectomycorrhizal colonization and seedling growth (shoot height, biomass and root biomass) were detected with a one-way ANOVA using the GLM procedure in SAS (SAS Institute Inc. 1999). For both percent ectomycorrhizal colonization and seedling growth, the difference in the response variable between source and recipient seedlings within a pot was calculated and used in the analysis. Differences were considered significant at $\alpha = 0.05$. Where significant mesh barrier treatment effects occurred, we separated means using the Bonferroni multiple comparison test. Effects of sterilization on seedling growth and total percent ectomycorrhizal colonization were analyzed using the TTEST procedure for each mesh size (SAS Institute Inc. 1999).

Results

Fungicide treatments

Approximately 30% of the roots of control seedlings (i.e., seedlings receiving only water) were colonized after 21 weeks in the treatment pots. Application of fungicide reduced ectomycorrhizal colonization by up to 50%, depending on fungicide type ($p < 0.0001$) but not application concentration ($p = 0.9$) (Table 3.1). The most effective treatment regime was Topas® applied alone or in combination with Senator® (Fig. 3.1a). Senator® alone was less effective at decreasing ectomycorrhizal colonization, with only a 36% reduction compared with 56% reduction using Topas®. There were no differences associated with different application frequencies (Fig. 3.1b) and there were no significant interactions among any combination of the three treatment factors ($p > 0.05$, Table 3.1). None of the fungicides applied at any concentration or application frequency, affected seedling height or shoot or root biomass (Table 3.1).

A total of eight morphotypes were identified and described (Table 3.2). Two had ≥ 98% sequence matches of their ITS sequences to *Wilcoxina rehmi* and *Thelephora terrestris* accessions in Genbank. DNA from the other six morphotypes either did not amplify or had less than 98% sequence homology with genotypes in Genbank. One morphotype was not identifiable and was classified as undifferentiated. Only the *Rhizopogon/Suillus*-type formed rhizomorphs; the remainder had relatively smooth mantles (Table 3.2).

On average we observed more morphotypes on seedlings that were subject to fungicides than those that were not (Fig. 3.2). However, neither ectomycorrhizal community richness ($p = 0.2$) nor diversity ($p = 0.3$) was significantly affected by the fungicide types. The abundance of *Wilcoxina rehmii* mycorrhizas (the most common ectomycorrhiza) as a percentage of all root tips examined was reduced by Topas® applied alone or in combination with Senator®, when compared to Senator® alone or the control (Fig. 3.3). The abundance of *Cenococcum geophilum*, the other dominant ascomycetous mycorrhiza, was not affected by application of fungicides ($p = 0.6$, data not shown). Similarly, the abundances of *Rhizopogon/Suillus*- and *Tomentella*-type mycorrhizas, the most abundant basidiomycetes, were also not affected by fungicide treatment ($p = 0.7$, $p = 0.8$, respectively, data not shown).

Mesh barrier treatments

Source seedlings had greater shoot height, shoot biomass, root biomass, and ectomycorrhizal colonization than recipient seedlings across all mesh treatments except the 20 µm pore size (Table 3.3), and mesh size did not affect the magnitude of these differences (Table 3.4). Across all mesh sizes, on average, 50 and 21% of roots of source and recipient seedlings were colonized by ectomycorrhizal fungi, respectively. These colonization levels contrast measurements at planting where colonization of source seedlings was less than 1%.

We found six distinct morphotypes on source seedlings (Table 3.2). Most of the six morphotypes were represented in all mesh treatments (Fig. 3.4). *Wilcoxina rehmii* ectomycorrhizas comprised >85% of the community on source and recipient seedlings separated with mesh barriers of 1 µm or larger (> 80%). By contrast, both the 0.2 µm and 1 µm pore-sized meshes blocked the formation of *Rhizopogon/Suillus*-type mycorrhizas on recipient seedlings (Fig. 3.4). This type formed approximately 5% of the

mycorrhizas on source seedlings. MRA-type morphotypes were found on all source seedlings, but were absent from recipient seedlings of all mesh treatments. *Thelephora terrestris* ectomycorrhizas formed an increasingly high proportion of the community on recipient seedlings as mesh size decreased, whereas they were not found on source seedlings. The abundance of *Cenococcum geophilum* mycorrhizas was too low to be useful in detecting mesh effects.

Ectomycorrhizal community similarity, which takes into account richness and relative abundance, between recipient seedlings versus source seedlings increased with mesh pore sizes greater than 0.2 µm ($p < 0.0001$) (Fig. 3.5a). The ectomycorrhizal communities separated by the full barrier (control) or by mesh of pore size 0.2 µm were significantly dissimilar from those separated by mesh with pore sizes 1 µm and larger (Fig. 3.5a). The difference in morphotype richness between source and recipient seedlings was large in the full barrier treatment and generally decreased as mesh size increased ($p = 0.09$) (Fig. 3.5b). When examined under the microscope, we observed hyphae penetrating pore sizes of 1 µm and larger, and roots penetrating only 500 µm pores. Three of the mesh barriers were torn in pots of the 0.2 µm mesh treatment; these replicates were omitted from the analyses.

Discussion

Fungicide effects on ectomycorrhizal colonization

This study shows that fungicides can be used to significantly reduce ectomycorrhizal colonization in controlled experiments. Topas® was more effective than Senator® at reducing ectomycorrhizal colonization levels. The manufacturer's recommended concentration was effective in reducing colonization, and there was no advantage to applying Topas® repeatedly during the course of the experiment. In our study, ectomycorrhizal colonization decreased by as much as 56% compared with the control. Douglas-fir control seedlings in this experiment had relatively low levels of colonization (approximately 30%) but these levels are typical for greenhouse-grown interior Douglas-fir (5-42%) (Hagerman and Durall 2004, Teste et al. 2004). Our results are consistent with another study using propiconazole. Manninen et al., (1998) found that 0.15 g of propiconazole applied to seedlings in the field (versus 9.6 g at the highest application frequency in our study) caused a decrease in ectomycorrhizal colonization of

almost 33% (from 67 to 45% colonization) two years after 2 year-old nursery grown *Pinus sylvestris* seedlings were outplanted.

Although the fungicides did not eliminate ectomycorrhizal colonization altogether, we propose that Topas® reduces colonization to an extent to be useful for field studies. Similar decreases in arbuscular mycorrhizal colonization following benomyl application have resulted in substantial changes in structure of the plant community. For example, reductions in arbuscular mycorrhizal colonization of 60% have changed plant nitrogen and phosphorus concentrations and aboveground community productivity in Boreal grassland communities (Dhillion and Gardsjord 2004). Hartnett and Wilson (1999) found that a 75% decrease in arbuscular mycorrhizal colonization coincided with biomass decreases of dominant C_4 grasses. Callaway et al. (2004) reported that interactions between native grassland species and the invasive *Centaurea maculosa* were substantially altered when experimental plots were treated with benomyl; the fungicide decreased arbuscular mycorrhizal colonization by >80%, resulting in a *C. maculosa* biomass decrease when mixed with *Koeleria cristata* or *Festuca idahoensia*. Assuming reductions in arbuscular and ectomycorrhizal colonization result in similar functional responses in plant communities, we expect that Topas® applied at the recommended rate once every five months will reduce ectomycorrhizal colonization sufficiently to affect seedling performance in the field.

The specificity of the fungicides for ascomycetes and basidomycetes differed from that expected. Senator® is reported to be more effective against ascomycetes than basidiomycetes, and yet it appeared to have no effect on *Wilcoxina rehmii*, a dominant ascomycete in this study. Manninen et al. (1998) reported that propiconzaole was also more effective at inhibiting ascomycete than basidiomycete symbionts and this is confirmed by Laatikainen and Heinonen-Tanski (2002). The latter found that low concentrations of propiconazole (0.1 ppm) increased growth of *Suillus bovinus* and *S. variegatus* strains grown *in vitro*, and that these fungi were tolerant of concentrations up to 1 ppm. In our study, the effectiveness of propiconazole (Topas®) could not be predicted strictly by taxonomic status. For example, it caused a substantial reduction in colonization by *Wilcoxina rehmii*, but not by *Cenococcum geophilum*, another important ascomycete. Colonization by the basidiomycetes forming *Thelephora terrestris*, *Tomentella*-type, and *Rhizopogon/Suillus*-type mycorrhizas either increased or was not affected by either fungicide however. In our study, Topas® targeted the most abundant

ectomycorrhizal fungi, *Wilcoxina rehmii*, so that the additional application of Senator® provided no further advantage.

Other fungicides have had variable effects on ectomycorrhizal colonization. O'Neill and Mitchell (2000) applied benomyl to *Picea sitchensis* seedlings and found that colonization was reduced from 60% to 20%; however, only one morphotype, *Wilcoxina mikolae*, was observed on the nursery grown seedlings. In another study, the percent of roots colonized by *Thelephora terrestris* or *Laccaria laccata* decreased when 0.3% Dithane M-45 was applied to *Pinus patula* seedlings grown in pouches, and similar reductions in hyphal dry weight occurred when the fungicide was applied to in vitro cultures (Reddy and Natarajan 1995). A wide range of responses were exhibited by 64 strains of ectomycorrhizal fungi grown in vitro and exposed to relatively low concentrations (<10 ppm) of five fungicides (benomyl, chorothalonil, copper oxychloride, maneb and propiconazole) (Laatikainen and Heinonen-Tanski 2002). Conversely, in some other laboratory studies fungicides have increased ectomycorrhizal colonization (Pawuk et al.1980, Marx and Rowan 1981, de la Bastide and Kendrick 1990). This effect is likely due to the selective inhibition of fungi that are competitive towards ectomycorrhizal fungi (Summerbell 1988). In our study, interactions among ectomycorrhizal fungi could have resulted in the increase of basidomyctes observed. *Wilcoxina rehmii,* a rapid colonizer of nursery seedlings (Mikola 1988) was suppressed by the application of Topas®. Removal of this rapid colonizer could have allowed other ectomycorrhizal fungi to colonize seedling root tips. Surveys of the entire fungal community on a large number of replicate seedlings is required to investigate this possibility.

Our results suggest that Topas® should be effective at reducing morphotypes commonly found in greenhouse bioassays of field soils, but there are two caveats. First, we could not assess the effects of fungicides on rare ectomycorrhizal fungal species or those that do not colonize seedlings in greenhouses. Second, Topas® may affect seedling physiology and/or other soil biota. These impacts are more difficult to identify and quantify by short term experiments in a greenhouse setting. Propiconazole has been shown to have growth-regulator effects on plants in the Solanacaeae family (Kendrick 2000), and it has also been shown to affect soil fauna, such as flagellates (Ekelund et al. 2000), as well as soil respiration (Elmholt 1992). Topas® is recommended for prevention of a variety of foliar fungal diseases, and its mode of

action by preventing ergosterol synthesis makes it likely to also affect non-target saprotrophic and parasitic soil fungi. A change in this community would alter potential food substrates of soil fauna. In experiments where reduction of ectomycorrhizal fungi is of primary concern, and side-effects on the soil biota is unimportant, then applications of Topas® can be an effective treatment regime. Given that the active ingredient in Topas® is fungistatic, repeated applications may be required where there is high hyphal turnover, as would happen over a temperate growing season, or where there is high fungal propagule pressure; both of these conditions occur in field situations.

Mesh barrier effects on hyphal penetration

Our study indicates that mesh with pore size 0.2 µm is effective at reducing hyphal penetration and mycorrhizal colonization of neighboring seedlings. However we conclude that the threshold for restricting ectomycorrhizal hyphal penetration lies between 0.2 and 1 µm. Ectomycorrhizal richness tended to increase in sterilized compartments where mesh size equaled or exceeded 1 µm, suggesting hyphae from the source seedlings compartment penetrated the mesh and colonized the recipient seedlings growing in the sterilized compartment. Of even greater significance, ectomycorrhizal community similarity between source and recipient seedlings greatly increased in meshes $\geq$ 1 µm. If the recipient seedlings were mycorrhiza-free, differences in richness alone should have indicated mesh effectiveness at restricting hyphal penetration, regardless of abundance, but the small number of morphotypes may have rendered richness as a measure with little resolving power.

The ectomycorrhizal community observed in our study was typical for interior Douglas-fir seedlings inoculated with field soil and grown in the greenhouse (Jones et al. 1997, Simard et al. 1997, Hagerman and Durall 2004, Teste et al. 2004). The six morphotypes formed on the source seedlings also represented a broad range of mantle types (texture and thickness), width of emanating hyphal forms (width and extension 3 to 7 µm), as well as the presence or absence of rhizomorphs. Their presence allowed us to test the effectiveness of the pore sizes at preventing hyphal penetration by ectomycorrhizal fungi with different characteristics. We might predict, for example, that a mesh with a smaller pore size would be required to prevent penetration of single hyphae, compared to the size required to stop penetration of rhizomorphs. Our findings support this prediction since we found that the rhizomorph-forming *Rhizopogon/Suillus-*

type morphotype was restricted by a mesh size between 1 to 20 µm. We propose that meshes with pore sizes smaller than 1µm would be adequate in field situations.

Although mesh with 0.2 µm pores was the most effective at reducing hyphal penetration, it was very fragile. This characteristic of nylon mesh with pore sizes smaller than 1 µm has been noted previously (Tarafdar and Marschner 1994). Our results suggest that field experiments requiring fine mesh (0.2 µm) should use more durable nylon (i.e. mesh thickness > 115 µm) or metal based mesh.

Our finding that mesh with pore sizes between 0.2 µm and 1 µm are most effective at inhibiting ectomycorrhizal colonization must be interpreted cautiously because some ectomycorrhizas were found in sterilized soils with a 0.2 µm mesh barrier. Within the sterilized compartment of these pots, the ectomycorrhizal community was reduced but not eliminated. For example, *Wilcoxina rehmii* was on the recipient seedlings, regardless of the mesh barrier type, but was not observed in control pots, suggesting that hyphal penetration or spore dispersal may have occurred. We are uncertain why *Wilcoxina rehmii* was not found in the sterilized compartment of the control pots. Further research is warranted on *Wilcoxina rehmii* propagating strategies in nurseries (e.g., hyphal and spore) and morphological plasticity. We also found that *Thelephora terrestris* had colonized root tips in one seedling of the control treatment (i.e. sterilized soil with a full barrier), confirming previous studies that it is a common greenhouse contaminant. Statistical analyses were run without *Thelephora terrestris* (data not shown); however, results were similar, and did not change our conclusions about the hyphal restriction properties of the mesh treatments. MRA-type mycorrhizas were also only observed on source seedlings across all mesh treatments, suggesting that chemical changes induced by autoclaving may have inhibited this particular ectomycorrhizal fungus. Rhizomorphs were completely excluded from sterilized compartments separated by 1 or 0.2 µm mesh.

Conclusions

The use of mesh barriers versus fungicides for controlling ectomycorrhizal colonization depends on the ecological processes that must be maintained and those that can be compromised in the experiment. Future CMN research can benefit from the use of mesh barriers. Mesh barriers with a gradient of pore sizes have the potential to tease out carbon and nutrient pathways (soil-only, hyphal-only, rhizomorph-only, etc.) in

resource sharing CMN studies. However, installing mesh barriers will disrupt soil structure and potentially reduce water flow through small pore sizes. If the purpose of mesh is to exclude mycorrhizal hyphae, and maintain non-mycorrhizal status of the enclosed host, the soil contained in the mesh barrier compartment will require sterilization. Mesh with pore sizes < 1 μm appear to reduce hyphal penetration, however care will be required to exclude fungal propagules arriving via air or water pathways. We suggest that mesh barriers, apart from their disruptive installment, are a more promising method than fungicides to completely exclude fungi.

Table 3.1: Analysis of variance for effect of fungicide type (F), concentration (C), and application frequency (A) on square root percent ectomycorrhizal colonization (%) and size of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings after five months.

		%			Height			Shoot biomass			Root biomass		
Source of variation	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Control vs. all others	1	13.55	9.67	<.0001	0.03	0.00	0.98	0.01	0.01	0.90	0.03	0.32	0.57
Fungicide type	2	9.70	6.92	<.0001	69.40	2.06	0.13	0.29	0.60	0.55	0.08	1.01	0.37
Concentration	2	0.23	0.16	0.85	16.10	0.48	0.63	0.62	1.29	0.28	0.04	0.48	0.62
Application frequency	2	22.81	16.28	<.0001	46.00	1.37	0.26	0.74	1.55	0.22	0.11	1.47	0.23
FxC	4	1.56	1.11	0.35	22.90	0.68	0.61	0.36	0.75	0.56	0.06	0.77	0.55
FxA	4	1.99	1.42	0.23	47.90	1.42	0.23	0.15	0.32	0.87	0.05	0.68	0.61
CxA	4	0.25	0.18	0.95	8.32	0.25	0.91	0.73	1.52	0.20	0.16	2.11	0.08
FxCxA	8	1.44	1.03	0.42	17.90	0.53	0.83	0.43	0.90	0.52	0.02	0.29	0.97
Error	135	1.40			33.70			0.48	0.48		0.08		

Table 3.2: Description of morphological characteristics of ectomycorrhizas observed on Douglas-fir (*Pseudotsuga menziesii* var. *glauca)* seedlings grown in the fungicide (F) and mesh (M) study.

Morphotype and Blast match	Macroscopic description	Mante type(s)	Emanating hyphae	Rhizomorphs	Cystidia
Rhizopogon/Suillus-type (R/S); F and M	Unbranched to subtuberculate silvery white mycorrhiza with rough texture	Outer: felt prosenchyma, hyphae 3-4 µm smooth, and thick-walled; inner: net synenchyma, thin, hyphae 2 µm	3 µm wide; no clamps, crystalline ornamentation, and elbow-like bends	Compact brown with crystalline ornamentation and elbow-like bends	Absent
Thelophora-type (T) Blasted to *Thelephora terrestris*, Accession No. U83486, 619/627 base pairs = 99%; F and M	Unbranched or irregular bright orange to brown (sometimes whitish) mycorrhiza with smooth reflective texture	Outer: net synenchyma, hyphae 3 µm wide; inner: incomplete interlocking irregular synenchyma, hyphae 4-5 µm wide	Rare, 3 µm wide; clamps, smooth with occasional enlarged hyphal junctions	Absent	Common, 40-50 µm long and 3 µm wide with basal clamp

Morphotype and Blast match	Macroscopic description	Mante type(s)	Emanating hyphae	Rhizomorphs	Cystidia
Cenococcum geophilum (Cg); F and M	Unbranched, black mycorrhiza with rough hairy texture	Outer: net synenchyma in a stellate pattern, hyphae 6 µm wide; inner: net synenchyma	5-6 µm wide black, straight	Absent	Absent
Wilcoxina-type (W) Blasted to *Wilcoxina rehmii* Accession No. DQ069001; 510/519 base pairs = 98%	Irregular dark brown to orangish mycorrhiza, often wrinkled, also called E-strain	Outer: not seen; inner: patchy and incomplete net prosenchyma, hyphae 2 µm wide	Absent	Absent	Absent
Mycelium radicis atrovirens-type (MRA); F and M	Unbranched black to brown mycorrhiza with curled hairy or very rough texture	Outer: felt prosenchyma, hyphae 3 µm wide; inner: net synenchyma, hyphae 2-3 µm wide	Rare, 5-7 µm wide, no clamps, smooth but becoming progressively more verrucose away from the mantle	Absent	Absent
Undifferentiated (Undif); F and M	Young orange mycorrhiza with no distinct characters	Barely visible net synenchyma readily turning into Hartig net	Absent	Absent	Absent

Morphotype and Blast match	Macroscopic description	Mante type(s)	Emanating hyphae	Rhizomorphs	Cystidia
Tomentella-type (Tom); F	Swollen dark-brown sandy textured mycorrhiza	Outer: squarish incomplete interlocking irregular synenchyma with thick-walled hyphae; inner: net synenchyma	Absent	Absent	Absent
Piloderma-type (P); F	Yellow coarsely felty mycorrhiza with abundant rhizomorphs	Not determined	Absent	Finely verrucose, septa common, not clamped, approximately 3 µm wide	Absent

Table 3.3: Effect of sterilization on growth and ectomycorrhizal (EM) colonization of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings. A series of t-tests were used to determine differences among source (S) and recipient (R) seedlings grown for each mesh barrier treatment.

Mesh (µm)	Soil	n	Height increment (cm)	SEM*	p	n	Shoot gain (g)	SEM	p	n	Root gain (g)	SEM	p
control	S	10	15	2.6	0.0294	10	0.91	0.137	0.0061	9	0.32	0.056	0.6633
	R	12	10	2.6		12	0.42	0.137		10	0.29	0.056	
0.2	S	12	14	0.8	<.0001	12	0.97	0.084	<.0001	10	0.44	0.064	0.0055
	R	12	9	0.8		12	0.34	0.084		12	0.19	0.064	
1	S	11	20	2.4	0.0195	11	1.55	0.196	<.0001	8	0.61	0.123	0.0360
	R	11	14	2.4		11	0.70	0.196		9	0.31	0.123	
20	S	12	18	3.6	0.3541	12	1.23	0.379	0.4363	10	0.48	0.204	0.4843
	R	8	12	3.6		8	0.86	0.379		5	0.29	0.204	
500	S	12	21	4.1	0.0588	12	1.51	0.319	0.0333	11	0.50	0.074	0.0400
	R	9	11	4.1		9	0.61	0.319		9	0.30	0.074	

*SEM: standard error of the mean. Seedling growth is expressed as height and biomass measured after 5 months.

Table 3.4: (continued) Effect of sterilization on growth and ectomycorrhizal (EM) colonization of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings. A series of t-tests were used to determine differences among source (S) and recipient (R) seedlings grown for each mesh barrier treatment.

Mesh (µm)	Soil	Root: Shoot gain	SEM	p	n	Percent EM colonization (%)	SEM	p
control	S	0.33	0.155	0.0075	9	50	7	<0.0001
	R	0.88	0.155		9	3	7	
0.2	S	0.44	0.056	0.0675	7	43	12	0.0195
	R	0.56	0.056		9	11	12	
1	S	0.49	0.145	0.6007	9	57	8	0.0113
	R	0.41	0.145		9	33	8	
20	S	0.42	0.077	0.5329	10	47	9	0.1308
	R	0.37	0.077		5	29	9	
500	S	0.35	0.083	0.0427	11	51	10	0.0434
	R	0.57	0.083		9	28	10	

*SEM: standard error of the mean. Seedling growth is expressed as height and biomass measured after 5 months.

Table 3.5: Effect of mesh treatment on growth and ectomycorrhizal (EM) colonization of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings. Response differences between source and recipient seedlings were calculated for each pot. This single number was used in the ANOVA for each response variable. Statistically significant mesh treatment effects detected by a Bonferroni multiple comparison test are designated by different letters ($p < 0.05$).

Mesh (µm)	n	Height increment (cm)	SEM*	n	Shoot gain (g)	SEM	n	Root gain (g)	SEM
control	10	6	a ± 2.6	10	0.49	a ± 0.220	9	0.03	a ± 0.088
0.2	12	5	a ± 2.4	12	0.63	a ± 0.201	10	0.23	a ± 0.084
1	10	7	a ± 2.6	10	0.90	a ± 0.220	7	0.33	a ± 0.100
20	8	4	a ± 2.9	8	0.31	a ± 0.247	5	0.16	a ± 0.118
500	9	9	a ± 2.7	9	0.82	a ± 0.232	9	0.18	a ± 0.088

*SEM: standard error of the mean.

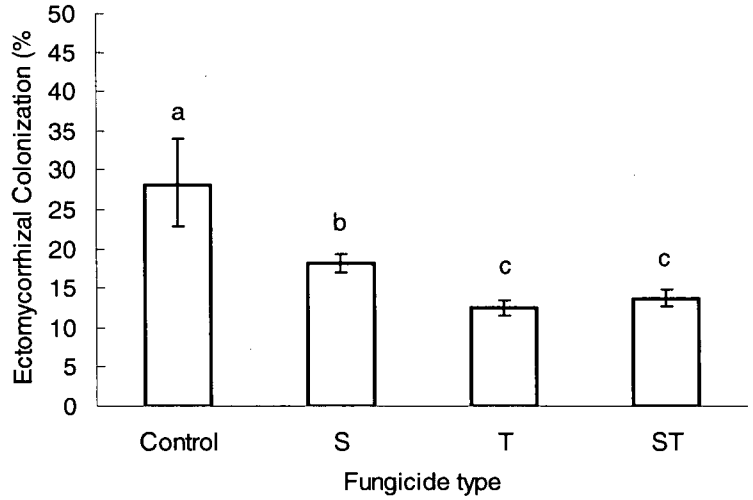
Table 3.6: (continued).Effect of mesh treatment on growth and ectomycorrhizal (EM) colonization of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings. Response differences between source and recipient seedlings were calculated for each pot. This single number was used in the ANOVA for each response variable. Statistically significant mesh treatment effects detected by a Bonferroni multiple comparison test are designated by different letters ($p < 0.05$).

Mesh (µm)	n	Root:Shoot (g)			SEM	n	EM colonization (%)			SEM
control	9	-0.55	a	±	0.105	9	47	a	±	8
0.2	10	-0.12	ab	±	0.100	7	30	a	±	9
1	7	0.08	b	±	0.120	8	26	a	±	9
20	5	0.05	b	±	0.142	5	6	a	±	11
500	8	-0.21	ab	±	0.112	9	29	a	±	8

*SEM: standard error of the mean.

Figure 3.1: Effect of a) fungicide type and b) application frequency on percent ectomycorrhizal colonization (determined by clearing and staining root tips) of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings. Fungicide abbreviations: S = Senator® and T = Topas®. Frequency abbreviations: A = once upon commencement of the experiment, B = every two months, and C = once a month. Statistically significant fungicide treatment effects detected by a Bonferroni multiple comparison test are designated by different letters ($p < 0.05$). Error bars are one standard error of the mean.

a)



b)

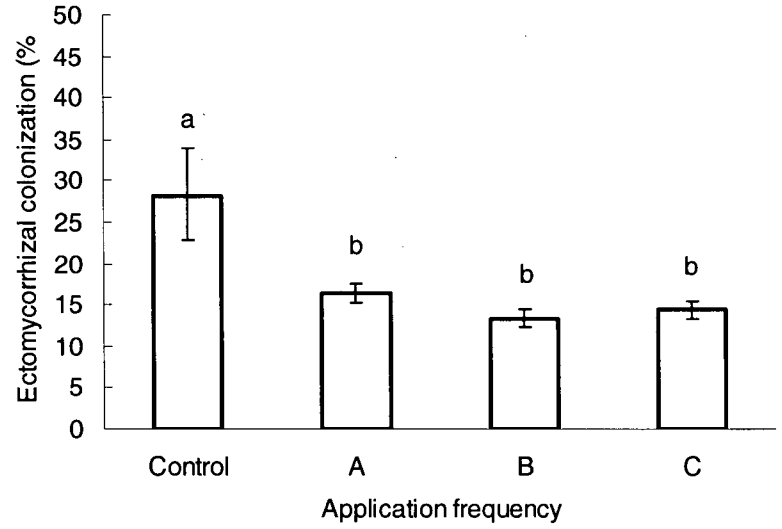


Figure 3.2: Abundance of morphotypes (*Tomentella*-type (Tom) *Thelephora terrestris* (T); *Mycelium radicis atrovirens*-type (MRA); *Wilcoxina rehmii* (W); *Cenococcum geophilum* (Cg); *Rhizopogon/Suillus*-type (R/S); *Piloderma*-type (P) and Undifferentiated (Undif) found on morphotyped Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) root systems grown in soil treated with different a) fungicide types and b) application frequency. Fungicide abbreviations: S = Senator® and T = Topas®. Frequency abbreviations: A = once upon commencement of the experiment, B = every two months, and C = once a month.

a)

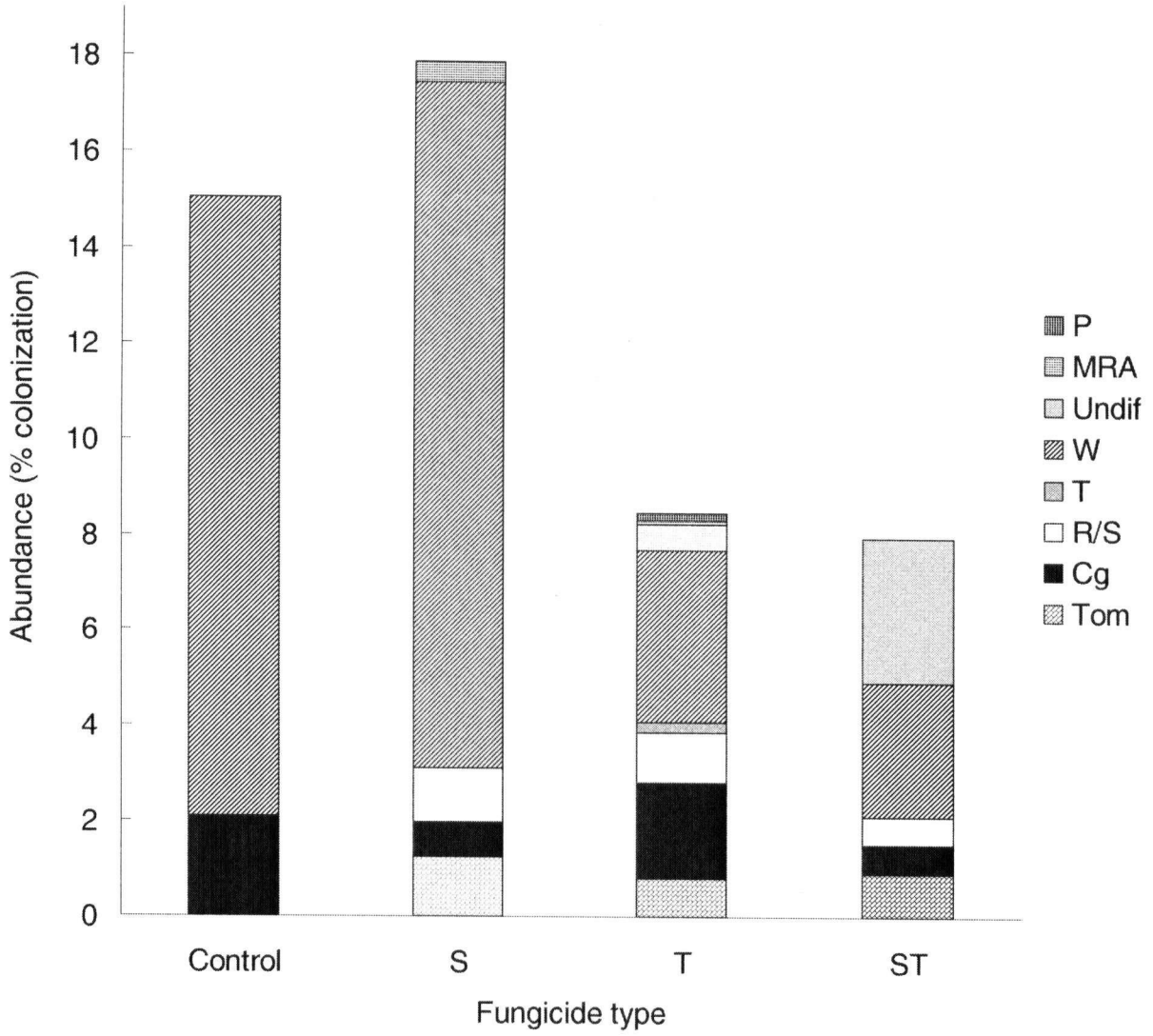


Figure 3.2 (continued): Abundance of morphotypes (*Tomentella*-type (Tom) *Thelephora terrestris* (T); *Mycelium radicis atrovirens*-type (MRA); *Wilcoxina rehmii* (W); *Cenococcum geophilum* (Cg); *Rhizopogon/Suillus*-type (R/S); *Piloderma*-type (P) and Undifferentiated (Undif) found on morphotyped Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) root systems grown in soil treated with different a) fungicide types and b) application frequency. Fungicide abbreviations: S = Senator® and T = Topas®. Frequency abbreviations: A = once upon commencement of the experiment, B = every two months, and C = once a month.

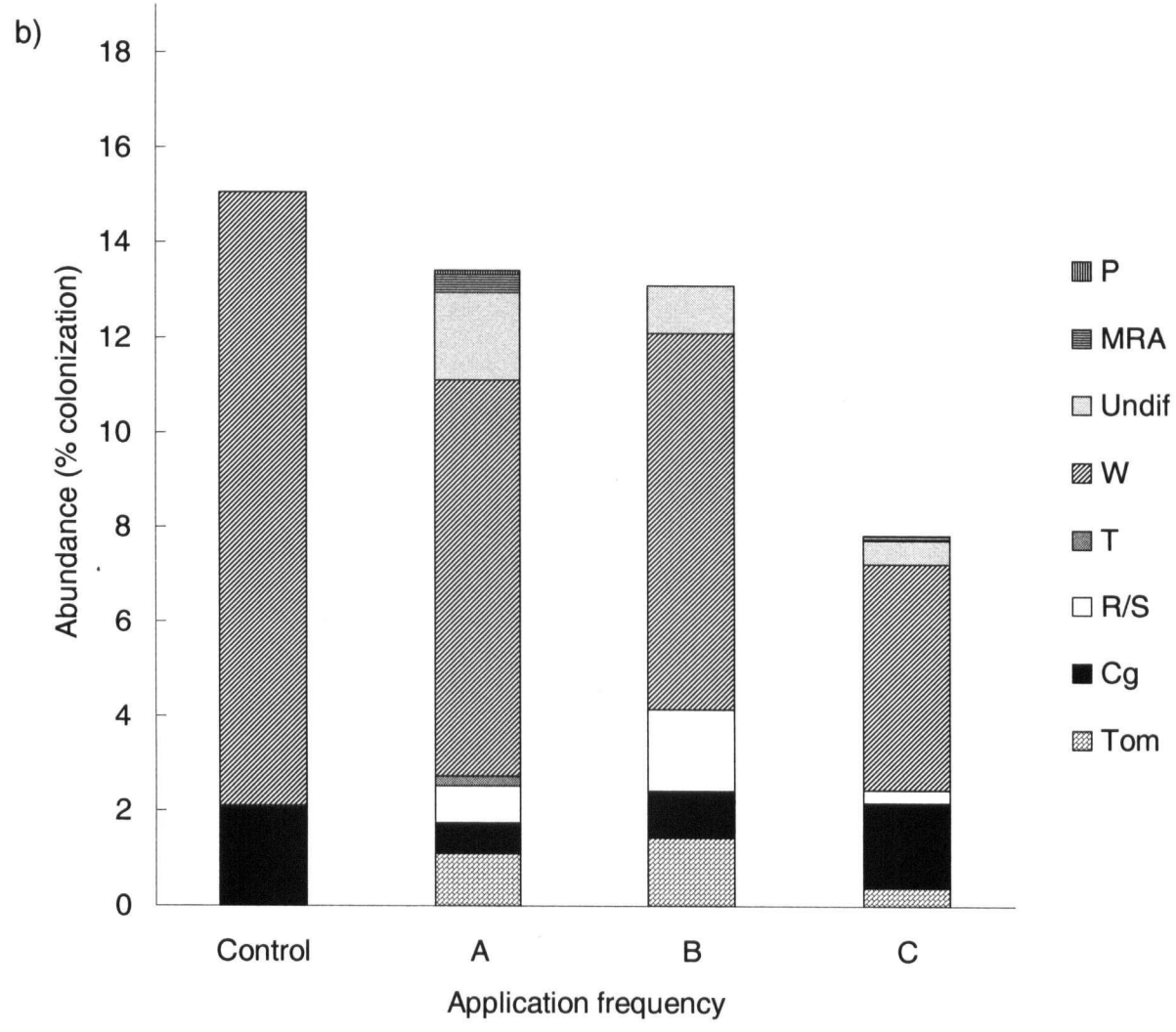


Figure 3.3: Abundance of *Wilcoxina rehmii* ectomycorrhizas, as a percentage of all root tips examined on Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) grown in soil treated with fungicides. Fungicide abbreviations: S = Senator® and T = Topas®. Statistically significant fungicide type treatment effects detected by a Bonferroni multiple comparison test are designated by different letters ($p < 0.05$). Error bars are one standard error of the mean.

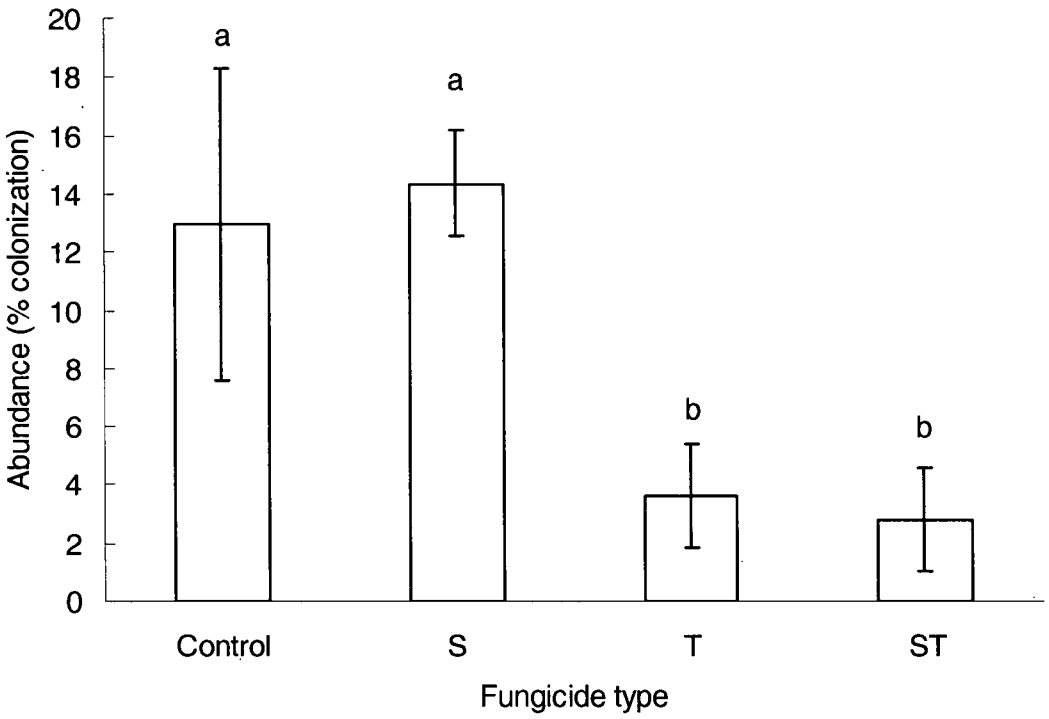


Figure 3.4: Abundance of morphotypes (*Thelephora terrestris* (T); *Mycelium radicis atrovirens*-type (MRA); *Wilcoxina rehmii* (W); *Cenococcum geophilum* (Cg); *Rhizopogon/Suillus*-type (R/S); and Undifferentiated (Undif), as a percentage of all root tips examined on recipient (R) and source (S) soil seedlings separated by a mesh barrier.

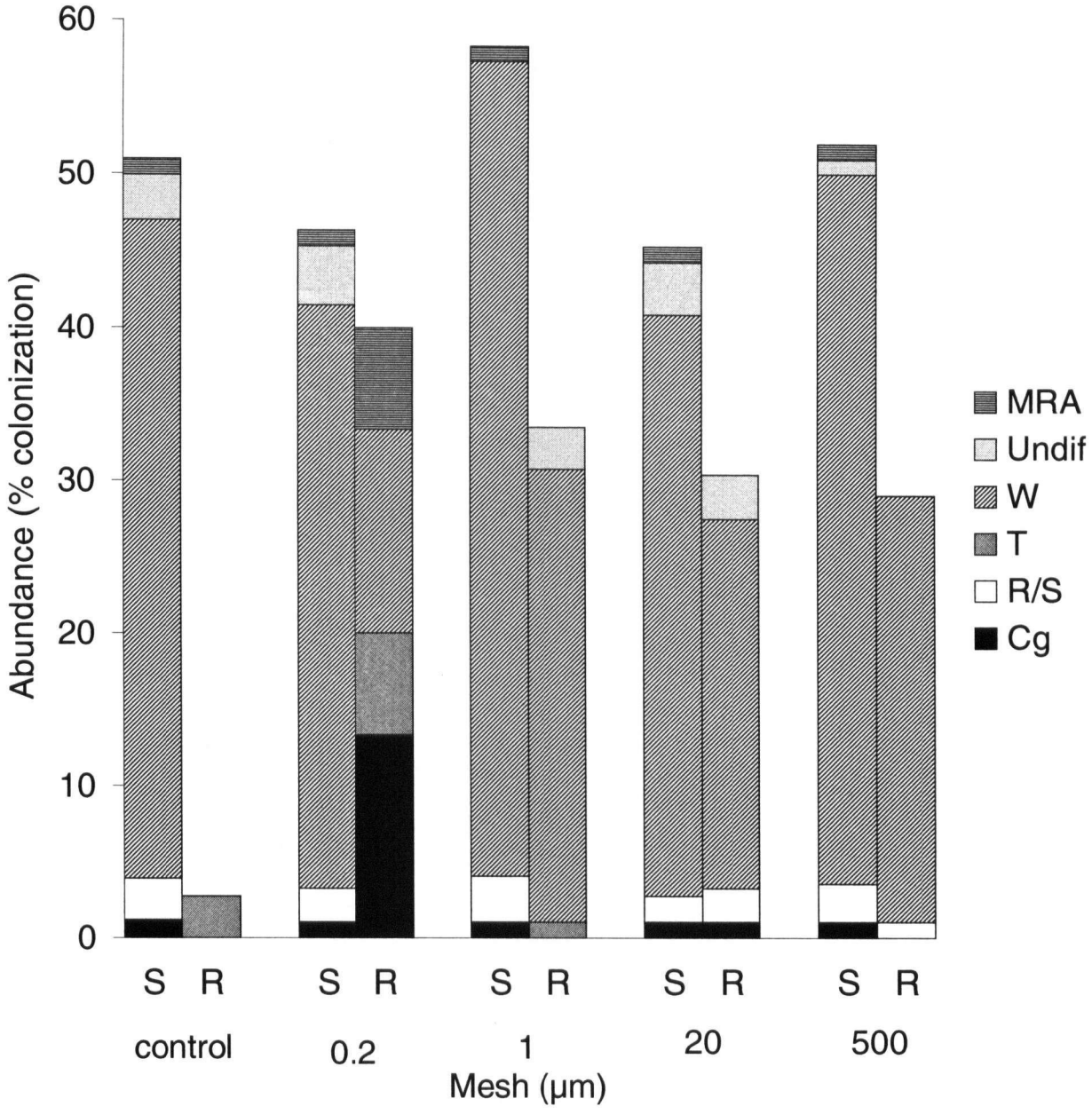
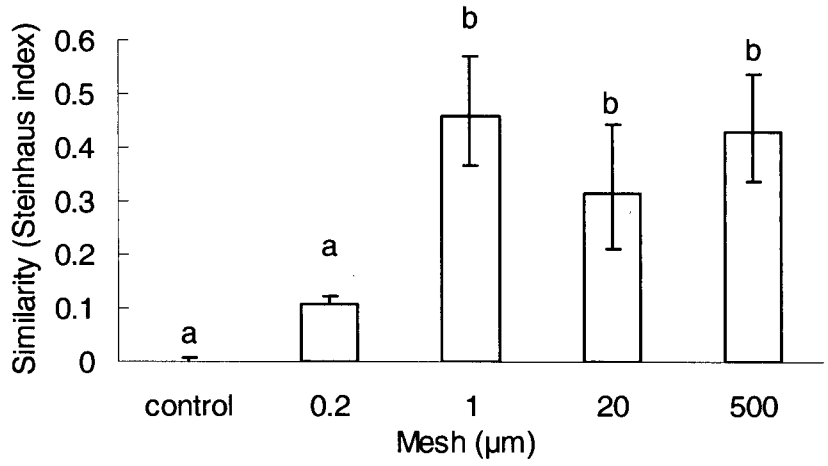
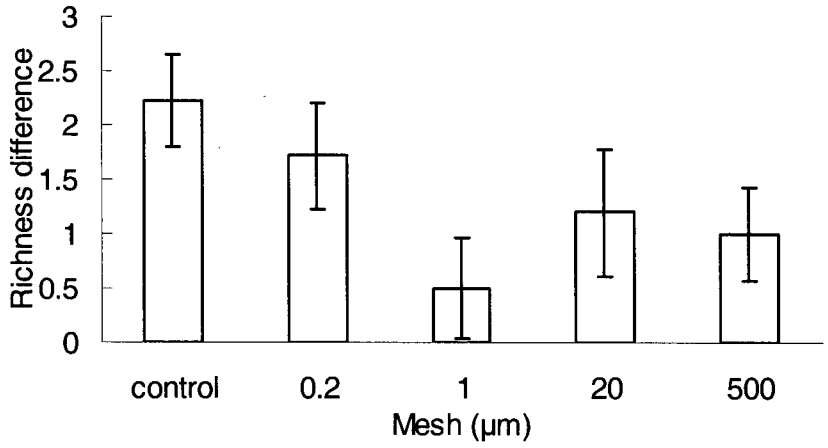


Figure 3.5: Ectomycorrhizal community differences. a) Steinhaus similarity index for ectomycorrhizal communities observed on source and recipient seedlings separated by a mesh barrier. b) Richness difference = number of morphotypes observed on source Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) root systems minus morphotypes present on recipient Douglas-fir separated by a mesh barrier. Statistically significant mesh treatment effects detected by a Bonferroni multiple comparison test are designated by different letters ($p < 0.05$). Error bars are one standard error of the mean.

a)



b)



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4 Ectomycorrhizal colonization and intraspecific variation in growth responses of lodgepole pine[1]

Introduction

Phenotypic variation of any organism is a product of its genotype, environment and the interaction between these components. Both abiotic and biotic factors will be ecologically significant components of an organism's environment. Because many tree species rely on ectomycorrhizal fungi for establishment and survival, variation in the identity and abundance of ectomycorrhizal fungi can impact seedling growth (Dickie et al. 2002). Thus, the presence of ectomycorrhizal fungi in soils is a critical dimension to the biotic environment with which trees will interact.

Quantifying levels of colonization on root tips of host trees is one method to measure the extent of interaction with their ectomycorrhizal fungi; however, the relationship between host growth and colonization level is inconsistent (see Chapter 2). Because experiments on ectomycorrhizas have used different species (both phyto- and mycobiont) and substrates, it is difficult to untangle which factors contribute to the poor overall relationship between colonization level and plant response. Within a host species, however, we might expect the relationship between colonization level and host growth to be less variable, especially in homogenous environments. Furthermore, within more genetically similar groups nested within a species, the relationship between colonization level and host growth is expected to be even less variable.

Research approaches to study mycorrhizal fungi do not allow direct manipulation of the level of colonization of an individual plant. Here we present results from a greenhouse experiment with seedlings from seed families within the species *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm., which naturally varied in ectomycorrhizal colonization levels; that is, colonization levels were not manipulated. We have analyzed the results to test the direction and consistency of the relationship between colonization level and growth responses across seed families. Earlier studies have considered the role of host genotype in determining level of colonization (Tagu et al. 2001, 2005; Gehring et al. 2006) and the composition of ectomycorrhizal fungal communities (Korkama et al. 2006), but to our knowledge this is the first study to test the relationship

[1]A version of this chapter has been accepted by Canadian Journal of Botany as: Karst J, Jones MD, and Turkington R. Intraspecific variation in height of lodgepole pine is minimized with increased ectomycorrhizal colonization.

Methods

Greenhouse experimental set-up

The greenhouse experiment was set up to test the effects of five ectomycorrhizal fungal species, plus a non-mycorrhizal control treatment on the variation in growth responses of ten seed families of lodgepole pine seedlings. Each fungal species x family treatment was replicated twenty times. Seed was produced during controlled pollination trials by the British Columbia Ministry of Forests using trees from seed planning units in the central interior of British Columbia. Seed from within each family was full sib, i.e. genetic similarity was higher within than among families. Relative wood density was the primary trait for which seeds had been selected.

Seeds were soaked 24 hours in distilled water, then sterilized in 30% H_2O_2 for 15 minutes, and 3% H_2O_2 for a further 2 hours. All solutions were mixed constantly. The seeds were dried and kept at 4°C for 28 days. For each fungal species x family replicate, we sowed two seeds into a SC10R Super cell Ray Leach cone-tainer (Stuewe and Sons, Inc., Corvallis, Oregon, USA) measuring 3.8 cm in diameter and 21 cm in length, filled with 3:1 (v:v) autoclaved peat and perlite. All cone-tainers were sterilized previously in a 30% bleach solution for 30 minutes. The cone-tainers were held in RL98 trays (Stuewe and Sons, Inc., Corvallis, Oregon, USA) and randomized monthly. We covered the seeds with 0.5 cm of autoclaved sand and all seeds were watered every 4 days. Two weeks after germination, we thinned seedlings to one per cone-tainer. For the next eight months, we watered the seedlings as required and fertilized once every 2 weeks with ¼ strength Ingestad's solution (Pelham and Mason 1978). Natural daylight in the greenhouse was supplemented by 400 W high pressure sodium lamps for 18 hours daily. The average temperature ranged from 20 to 25°C and the average relative humidity was 53%.

At four weeks and again at four months we inoculated the seedlings with 5 mL of mycelial slurry of one of the five ectomycorrhizal fungi. The species of fungi used were: *Cenococcum geophilum*, *Rhizopogon roseolus*, *Wilcoxina mikolae*, *Hebeloma crustuliniforme* and *Paxillus involutus*. Cultures of these fungi were obtained from the Mycorrhiza Research Group, University of British Columbia Okanagan and maintained on solid modified Melin-Norkrans (MMN) media. To obtain the mycelium, we placed approximately twenty 0.5 cm^3 cubes of actively growing mycelium in each flask of liquid

MMN media. Liquid cultures were grown under sterile conditions and shaken daily. No contamination occurred in liquid cultures. The mycelial slurry used to inoculate seedlings was produced by blending 150 mL of mycelium with 1850 mL of distilled water. We also produced a non-mycorrhizal slurry for control plants from solid MMN media that had not been inoculated.

When seedlings were harvested after nine months, visual observations of morphotypes under a dissecting scope showed that none of the inoculated fungi were present on the roots; however, seedlings were mycorrhizal with other fungi. Hence, we harvested 45 randomly selected seedlings per seed family in order to test the relationships among percent colonization, seed family and growth responses (height and biomass) of lodgepole pine. We measured the height of each harvested seedling and subsequently dried the shoots at 65°C for 72 hours. Roots were refrigerated at 4°C until examined (see below) and then were dried at 65°C for 72 hours.

Assessment of ectomycorrhizal fungal colonization

Seed families having less than 10% of seedlings survive were omitted from the analysis; thus, we examined eight of the initial ten families. Using these 360 seedlings (45 seedlings x 8 families), a power analysis performed in JMP IN 5.1 (Sall et al. 2005) determined that 64 seedlings were required to detect observed differences in height due to seed family, with a 97% probability of achieving a significance of 0.05. Since we could not estimate variation in mycorrhizal colonization in advance, we used variation in height to determine how many seedlings to examine for colonization. Consequently, we sub-sampled eight seedlings randomly from each family for which mycorrhizal colonization was measured. Entire root systems were carefully washed under running water and cut into approximately 1-cm pieces. All root fragments were placed in a baking dish containing water and a random sub-sample was then distributed into a Petri plate. We examined 300 (± 57) root tips per seedling under a stereomicroscope. Tips were classified as mycorrhizal if root hairs were absent. Examination of a sub-set of these roots under high magnification (400x) confirmed that this approach accurately distinguished mycorrhizal from non-mycorrhizal roots. Two morphotypes were distinguished based on the presence or absence of cystidia and on characteristics of the mantle and mycelial strands.

Molecular analyses

Total genomic DNA from three replicate tips of each morphotype identified was extracted by pulverizing the tips for 45 seconds at a speed of 5.0 units using a Bio101 Systems Fast Prep FP120 high frequency shaker (Q-biogene, Carlsbad, CA, USA). DNA was isolated using the procedure of Baldwin and Egger (1996). The final DNA pellet was dried using a speed vacuum concentrator and then re-suspended in 50 µL EDTA-TE buffer.

Following DNA extraction and isolation, the internal transcribed spacer (ITS) region of the fungal nuclear rDNA was specifically amplified by the primers NSI1 and NLC2 (Martin and Rygiewicz, 2005). PCR reactions typically included 1 µL template DNA, 18.6 µL sterile purified water (Barnested Nanopure Diamond water purifier), 0.2 mM deoxyribonucleoties (dNTPs), 2.5 µL 10x PCR buffer, 2.0 mM $MgCl_2$, 0.48 mM each primer, 1.6 mg mL^{-1} bovine serum albumin (BSA), and 0.25 U μL^{-1} AmpliTaq Gold™ (Applied Biosystems, Foster City, CA, USA). Samples were amplified using a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA, USA). A 10 minute hot start was followed by PCR cycling as follows: 45 seconds at 94°C followed by 34 cycles of denaturation at 94°C for 45 seconds, annealing at 54°C for 45 seconds, ramping 72°C for 1 minute with a 1 second extension after each cycle, and extension at 72°C for 10 minutes, and then the temperature was held at 4°C. The PCR products were visualized on 1.5% agarose gels using a Gel Logics 440 (Kodak Instruments, Rochester, NY, USA). The PCR product was cleaned using the QIAquick PCR Purification kit (Qiagen Inc., Valencia, CA, USA).

Prior to sequencing, the large ITS fragment produced above, was re-amplified in a nested PCR reaction using the primers ITS 1 and ITS 4 (White et al. 1990). PCR products were quantified and then sequenced using a 3730 DNA Capillary Sequencer (Applied Biosystems) at the University of British Columbia Nucleic Acid and Protein Services Unit. All unique morphotypes were sequenced and then aligned using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). Taxonomic matches were based on BLAST results with $\geq$97% sequence similarity.

Statistical analyses

We used an analysis of covariance (ANCOVA) to test the effect of seed family on seedling growth responses using level of ectomycorrhizal fungal colonization (% root

tips colonized) as a covariate regressor. We included an interaction term (seed family x % colonization) to determine if colonization interacted with seed family (i.e. whether the slope of the relationship between colonization and a given growth response differed by seed family). To meet ANCOVA assumptions, we ensured that colonization levels did not differ by seed family using an analysis of variance (see Results). We used a reciprocal transformation on shoot height to meet the assumption of homogeneity of variance. As shoot height was the only growth response to show unequal variance across seed families and Burgess and Malajczuk (1989) demonstrated decreases in variation of height among individuals of *Eucalyptus globulus* Labill. with inoculation by ectomycorrhizal fungi, we further explored the effects of colonization level on variation in shoot height. To do so, we used colonization level to predict the residuals in seedling height. Residuals were calculated by taking the absolute value of the deviation of each seedling from its mean family value, standardized by that particular family average:

$$\text{seedling residual} = \left| y_{iF} - X_F \right| / X_F$$

where y_{iF} is the value for the ith seedling from family F and X_F is the mean for that family. We used the family means in contrast to the overall mean because seed family had a significant effect in explaining variation in height among seedlings (see Results). In other words, we removed seed family effects to look at the independent contribution of colonization level on the height response of each individual seedling. All analyses were performed in JMP IN 5.1 (Sall et al. 2005). The relative abundance of each ectomycorrhizal fungal species was calculated as the percentage of the total number of ectomycorrhizal tips that were colonized by a given fungal species.

Results

All seedlings were mycorrhizal. The mean level of colonization was 85% (SD 15%), ranging from 39 to 100% per seedling. Mean colonization levels did not differ by seed family ($df = 7, 56$; $F = 1.08$; $p = 0.39$). The effect of colonization on root and shoot biomass varied by seed family (Table 4.1, Fig. 4.1). In particular, both positive and negative relationships between colonization level and shoot mass were observed, although seedlings in most families did not show any response to colonization levels (Fig. 4.1). For the majority of seed families no relationship was observed between

colonization level and root mass, however, two seed families showed negative relationships (Fig. 4.1). Shoot height differed only by seed family (Tables 4.1, 4.2).

Residual height variation across seedlings was weakly explained by colonization levels. In particular, there was a negative relationship between the magnitude of seedling deviation from its family mean and level of colonization (Fig. 4.2). Thus, increased colonization lessened height differences among seedlings within families. The mean coefficient of variation in height for each seed family was not related to mean colonization level ($df = 1, 6$; $F = 0.0024$; $p = 0.96$). Colonization levels were also not related to the deviations of mean family heights from the overall mean ($df = 1, 6$; $F = 0.03$; $p = 0.87$) indicating that colonization levels did not diminish differences among seed families.

The two morphotypes identified on seedling root tips had ≥97% sequence matches of their ITS sequences to *Thelephora terrestris* and *Rhizopogon vulgaris* accessions in Genbank. Neither fungus had been inoculated onto seedlings, but rather were greenhouse contaminants. *Thelephora terrestris* was the most common ectomycorrhizal fungus to colonize seedlings. It was present on root tips of all seedlings and had a mean relative abundance of 98%. *Rhizopogon vulgaris* colonized roots of 5% of the seedlings, with a mean relative abundance of 60% on those seedlings. *R. vulgaris* was found on seedlings from two seed families (Table 4.2).

Discussion

Seed family effects on the relationship between colonization level and host growth

In this study the role of genetics was clear in determining seedling growth characteristics: seed family affected height and biomass of individual seedlings. Results from provenance trials of lodgepole pine in British Columbia, Canada indicate that differences in height of 20-yr trees are also, to some degree, under genetic control (Rehfeldt et al. 1999); adaptive differences among populations that were related to their climate of origin were demonstrated among populations that had been transplanted to various test sites across British Columbia. In our study, responses in seedling biomass were modified by colonization levels, representing a seed family x ectomycorrhizal colonization interaction. Because ectomycorrhizal fungi are part of the biotic environment, their presence should be viewed as a component within the more general

framework of assessing genotype x environment interactions influencing seedling growth.

Across different host plant species, the relationship between mycorrhizal colonization and host growth parameters varies (Jones et al. 1990, Thompson et al. 1994). Results from our study indicate that this inconsistency can be observed even at an intraspecific level. The environment of the seedlings in our experiment was homogeneous, indicating that the identity of seed family alone can be an important factor determining the relationship of ectomycorrhizal colonization to seedling biomass. Studies from other systems also confirm that genotypic effects can be such that they are as strong as species effects. For example, the effects of genotypic diversity of *Solidago altissima* on arthropod diversity and community structure living on their leaves are comparable to those from studies testing the effects of species diversity manipulations (Crutsinger et al. 2006). Manipulations of the genetic diversity of seagrass (*Zostera marina)* showed that increasing genetic diversity results in increased invertebrate community resilience and decreased recovery time to disturbances caused by goose herbivory (Hughes and Stachowicz 2004); this finding mirrors those reported in experiments manipulating functional (species) diversity (Díaz and Cabido 2001).

Ectomycorrhizal colonization and host phenotypic variation

Ecological processes may be drivers of population differentiation and divergence (Schluter 2001). The role of ecological processes in population convergence has gained more attention with the introduction of neutral theory (Hubbell 2001, 2006); nonetheless, equalizing mechanisms are usually invoked in the context of explaining species coexistence (Chesson 2000). To our knowledge, ours is the first study to show that increases in colonization by ectomycorrhizal fungi tends to reduce intraspecific variability or, in other words, differences in height tend to be equalized among seedlings. Our results indicate that a mycorrhizal signal, albeit a weak one (9% of the variance in seedling height residuals was explained by colonization level), was observed at the seed family level. Such low r^2 values are not unusual given that the mean amount of variance explained in ecological experiments is only 2.5-5.4% (Møller and Jennions 2002).

Our results suggest that those seedlings able to escape ectomycorrhizal fungal colonization could benefit in terms of height gained. Nonetheless, roots of seedlings of

lodgepole pine occurring under natural conditions are heavily colonized by ectomycorrhizal fungi (Bradbury 1998; Kranabetter et al. 1999; Bothwell et al. 2001). We offer two reasons to explain high levels of colonization on seedlings in natural conditions. First, poorly colonized seedlings have an equal likelihood of growing shorter than the average seedlings. Hosts may invest more in the maintenance of ectomycorrhizas as a strategy analogous to insurance that buffers against extreme variation in performance. However, despite some theoretical developments (Kummel and Salant 2006), the amount of control a host has on the composition and abundance of its mycorrhizal fungal partners is uncertain. If host selection of a fungal partner is passive, our results would suggest that, in areas devoid of ectomycorrhizal fungi, stochasticity will influence seedling growth more so than in areas replete with ectomycorrhizal fungal inoculum. Second, there may be no advantage *per se* to reduced intraspecific variation. It may be present only as a byproduct of selection pressures on hosts for ectomycorrhizal colonization, which in turn, is selected for to increase plant survival in low nutrient conditions. Host benefits received from increased ectomycorrhizal colonization in terms of nutrient uptake may outweigh the costs (e.g. carbon) of supporting ectomycorrhizal fungi.

We observed a negative relationship between colonization level and intraspecific variation within, but not among families. Other studies have reported that phenotypic variation among families is minimized in the presence of ectomycorrhizal fungi. For example, thirty open-pollinated families of *Picea abies* grown with or without *Laccaria bicolor* showed striking reductions in variance of shoot and root dry weight among families when ectomycorrhizas were present (Mari et al. 2003). Variation in root architecture, an important trait for nutrient acquisition, also declined when ectomycorrhizas with *Paxillus involutus* were present on *Picea abies* (Boukcim and Plassard 2003). In particular, the number of lateral roots per seedling differed when the two families when non-mycorrhizal, but not when they were mycorrhizal. Our results are an advance over these earlier studies, which considered colonization as a categorical variable only (i.e., presence or absence). Colonization levels of seedlings in the field are more likely to be continuous rather than a discrete property as commonly employed in experiments. As such, results from our study may be more reflective of naturally occurring colonization levels.

Our results differ from experiments that treat ectomycorrhizal colonization level as a response variable. We found that colonization levels did not differ by seed families; a power analysis indicated that at least 220 seedlings would be required to detect significant differences (α=0.05) in colonization levels among families 97% of the time. Progeny obtained from crosses between two species, *Populus deltoides* and *P. trichocarpa* differed in the extent to which they were colonized by *Laccaria bicolor* (Tagu et al. 2001). Hence, Tagu et al. (2005) concluded that the ability to form ectomycorrhizas (measured by colonization levels) is a quantitative trait under polygenic control. The few measures of broad sense heritability calculated for levels of ectomycorrhizal colonization range from 0.09 to 0.81 (Rosado et al. 1994; Tagu et al. 2001, 2005) indicating possibly high involvement of environmental factors in determining the level of colonization, depending on the host and fungal species. The genetic basis to the response by hosts to ectomycorrhizal colonization levels deserves further study.

The prevalence of contamination on seedlings

None of the target fungi were successful in colonizing seedlings. Although we inoculated seedlings twice in the experiment, it is likely that the aggressive colonization abilities of those ectomycorrhizal fungi common to greenhouses facilitated their early establishment on seedlings. Contamination of seedlings used in ectomycorrhizal experiments is not uncommon. Nearly half of all studies extracted from papers used in the meta-analysis (Chapter 2) reported contamination of seedlings. While levels of contamination are lower in experiments performed in growth chambers, the small size of growth chambers necessitates the use of young seedlings, or running experiments for short durations. Clearly, the presence of contamination is problematic in experiments where maintaining non-ectomycorrhizal controls is required.

Conclusions

The role of the environment in determining plant phenotypes is undisputed in ecology. Our results suggest that mycorrhizal fungi should be considered as a component of the environment that can influence the amount of phenotypic variation in a population. Moreover, we highlight the importance of intraspecific differences in determining the sensitivity between symbionts involved in mycorrhizal associations. As

such, models of intraspecific interactions should consider ectomycorrhizal associations when assessing phenotypic variability. Since we are unable to manipulate colonization levels directly, future research should examine the effects of the presence, absence and species of ectomycorrhizal fungi on the variance among seed families screened for high differentiation in growth traits. Additionally, the ecological relevance of decreased intraspecific variation through mycorrhizal colonization deserves further study.

Table 4.1: Analysis of covariance for effects of seed family, percent ectomycorrhizal fungal colonization of root tips (% colonization) and their interaction on growth responses of *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. seedlings.

		Shoot height (cm) †		Shoot mass (g)		Root mass (g)	
Source	df	F	p	F	p	F	p
Family	7	2.20	0.051	1.31	0.26	3.44	0.0046
% colonization	1	0.65	0.43	0.48	0.49	2.42	0.13
Family x % colonization	7	0.70	0.67	2.33	0.039	3.084	0.0091

† A reciprocal transformation was used on shoot height to meet homogeneity of variance assumption.

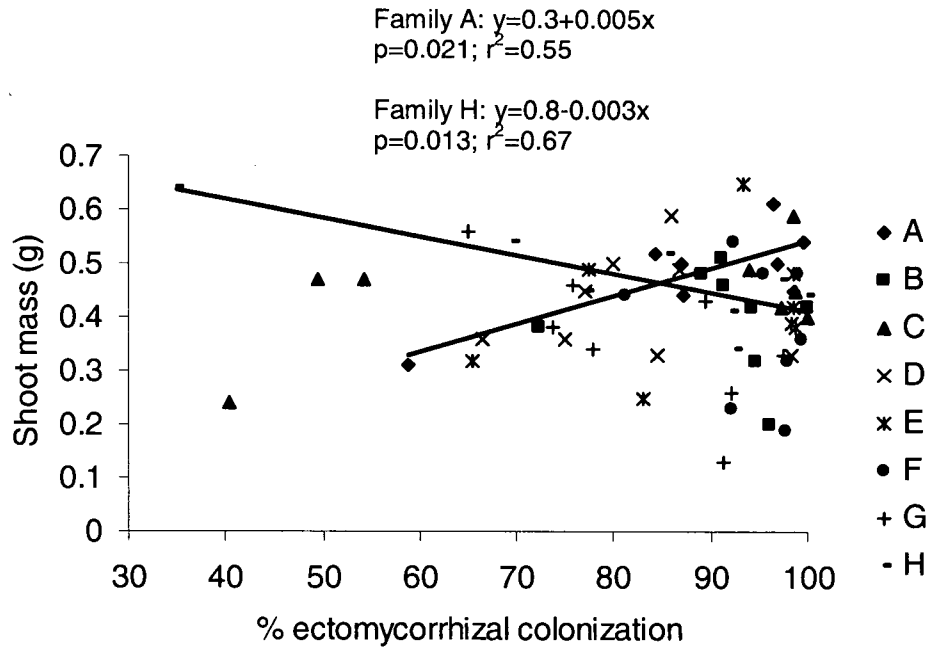
Table 4.2: Mean shoot height of full sib families of *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. Seedlings grown for 36 weeks (n=8). British Columbia Ministry of Forests seed family identification follows in brackets seed family designation.

	Shoot height (cm)	
Seed family	Mean	SD
A (2094 x 2065 CP RD5)	5.3[b]	0.54
B (354 x 468 BV RD2) †	5.2[b]	0.79
C (1659 x 479 BV RD2) †	6.2[a]	1.01
D (268 x 1631 BV RD1)	5.1[b]	0.54
E (1817 x 220 PG RD5)	6.2[a]	0.76
F (253 x 236 PG RD2)	4.8[c]	0.87
G (466 x 502 BV RD2)	5.1[b]	1.24
H (2076 x 1620 CP RD2)	5.3[b]	0.67

* Family effects sharing the same letter are not statistically different ($P < 0.05$ Tukey-Kramer multiple comparison test).

†Root tips of seedlings colonized by *Thelephora terrestris* and *Rhizopogon vulgaris*; all other seedlings colonized by *Thelephora terrestris* only.

Figure 4.1: The effect of ectomycorrhizal fungal colonization by seed family on shoot (top panel) and root mass (bottom panel) of *Pinus contorta* var. *latifolia* seedlings. Regression lines are shown for only those seed families showing a significant relationship between shoot or root mass and level of colonization. See Table 4.2 for British Columbia Ministry of Forests seed family identification.



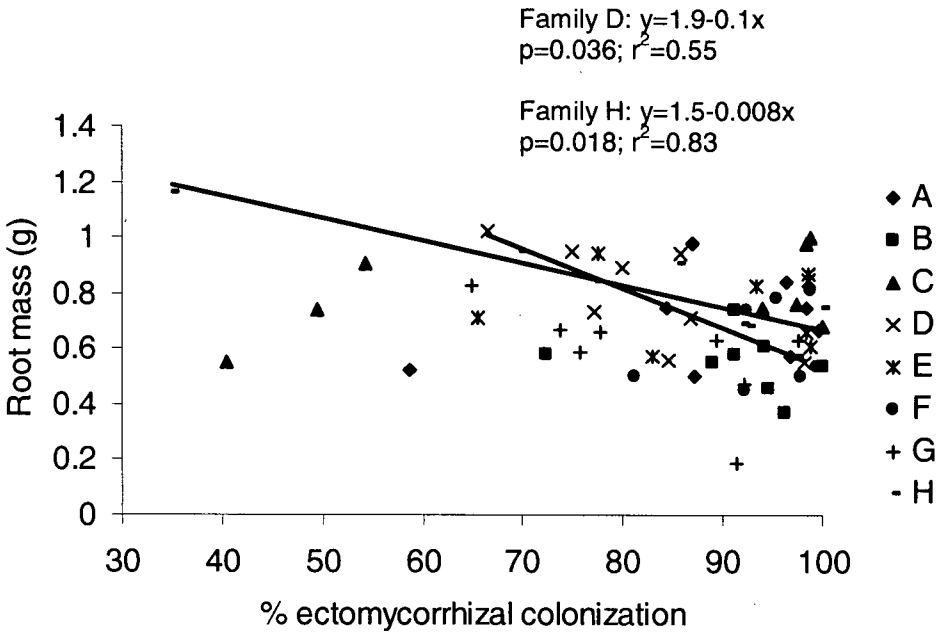
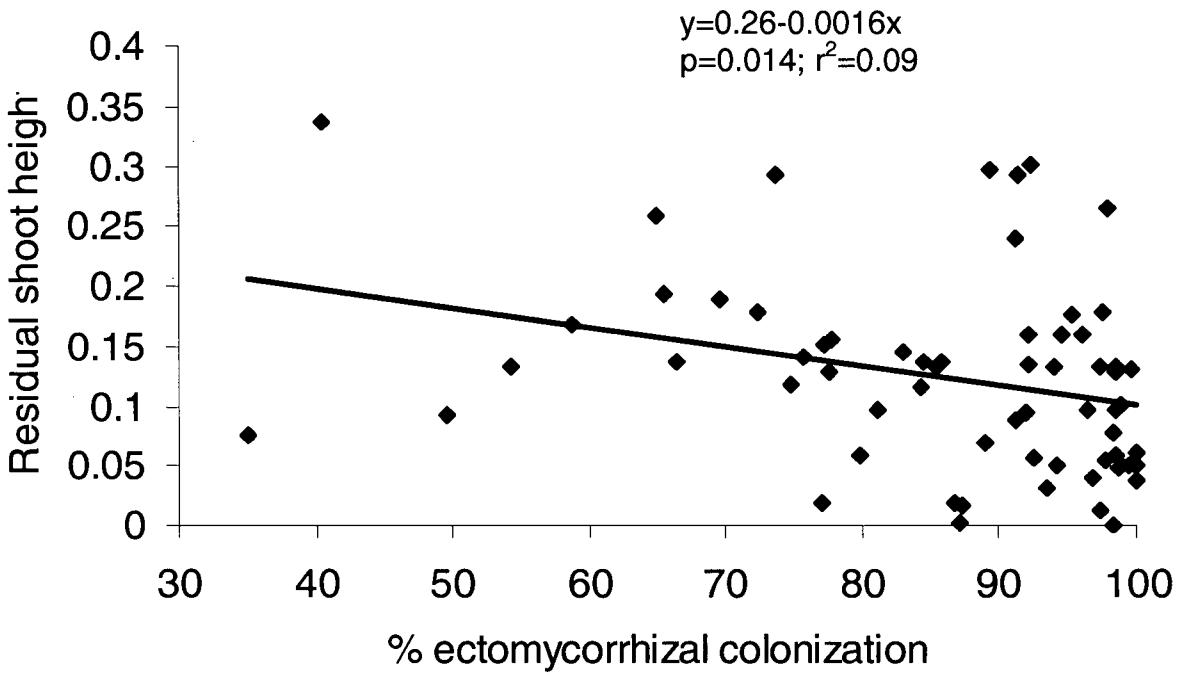


Figure 4.2: The contribution of ectomycorrhizal fungal colonization to height variation in seedlings of *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm., independent of seed family effects.



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5 Interactions among soil characteristics, host intraspecific variation and ectomycorrhizal fungal communities

Introduction

Partner species in coevolved interactions are expected to be sensitive to intraspecific variation of each partner due to the intimate and interdependent nature of their interactions (Thompson 1994). Ectomycorrhizal associations are of particular interest because individual trees host communities of fungi, and whether the composition of these communities is sensitive to intraspecific variation in hosts is poorly understood. Previous research has shown that host individuals provide a range, or gradient, of biotic variation, and that this gradient produces changes in ectomycorrhizal fungal communities. For example, variation among host individuals induced by defoliation (Saikkonen et al. 1999, Cullings et al. 2005a) or varying levels of parasitism will change the composition of ectomycorrhizal fungal communities (Cullings et al. 2005b, Mueller and Gehring 2006). Communities of ectomycorrhizal fungi have also been reported to segregate depending on the associated growth rate of their host (Korkama et al. 2006).

In addition to the variation provided by properties of host species, variation in soil characteristics can structure ectomycorrhizal fungal communities. For example, differences in soil nitrogen (Lilleskov et al. 2002) and nutrient and moisture status (Gehring et al. 1998, Robertson et al. 2006) have been shown to alter the composition of ectomycorrhizal fungal communities. Similarly, the ecological amplitude of host plants (measured by height and biomass performance) is clearly dependent on soil properties such as nutrient and moisture availability (Burns and Honkala 1990). Thus, variation in soil characteristics acts in parallel to influence both the composition of ectomycorrhizal fungal communities and intraspecific variation in hosts. This process alone should create a correlation between the composition of ectomycorrhizal fungal communities and intraspecific variation among hosts, independent of a direct interaction between the two components.

Identification of a correlation between environmentally-induced intraspecific variation in hosts and composition of their associated ectomycorrhizal fungal community is key to understanding how environmental gradients structure ectomycorrhizal fungal

communities. A correlation not only indicates that variation in one symbiont is synchronous with variation in the other, but also that the environmental properties influencing host variation may act indirectly to affect the composition of ectomycorrhizal fungal communities. The possible sources of variation that influence ectomycorrhizal fungal communities would have to be extended to include those that affect host intraspecific variation. Because host and ectomycorrhizal fungal communities are interdependent (Kernaghan 2005), covariance between host intraspecific variation and the composition of the ectomycorrhizal community cannot be used to infer causation. Regardless, the presence of a correlation between the two components offers a way of determining the ecological relevance to ectomycorrhizal fungal communities of variation present within both a host species and the abiotic environment.

The objective of this study was to identify environmental factors that directly influence composition of ectomycorrhizal fungal communities, as well as those that may act indirectly through host intraspecific variation. We characterized "the environment" by measuring variation in soil characteristics related to fertility and moisture. Host intraspecific variation was measured by properties including shoot height, total biomass and root:shoot ratio. The composition of ectomycorrhizal communities was quantified in two distinct ways: 1) categorical; the presence or absence of individual ectomycorrhizal fungal species comprising a community and 2) continuous; the relative abundance of each species.

Materials and methods

Overview

We grew Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) seedlings in pots containing soils that varied naturally in fertility. We also implemented an artificial gradient of soil moisture on replicate subsamples of these soils. Seedlings became colonized with the ectomycorrhizal fungi present in the soils and both the seedlings and fungi were subject to the variation in soil fertility and moisture. We then used multivariate analyses to correlate variation in soil fertility and moisture to variation in host growth and ectomycorrhizal fungal community composition.

Origin of soils

Soil was collected from the Thompson and Okanagan Valleys of the southern interior plateau of British Columbia, Canada. This area has a continental climate, with warm, dry summers and cool winters. In valley bottoms, the average daily minimum for the winter months is -5°C; for the summer months, the average daily maximum is 25°C (Environment Canada 2004). There is a strong elevational gradient in annual precipitation ranging from 300 mm at lower elevations (300-800 masl) to greater than 1000 mm at montane (1200-1400 masl) elevations (Meidinger and Pojar 1991). Open forests of Douglas-fir mixed with Ponderosa pine (*Pinus ponderosa* Dougl. Ex P. & C. Lawa.) and several species of grasses (*Koeleria macrantha* [Ledeb.] J. A. Schultes f., *Poa pratensis* L. and *Calamagrostis rubescens* Buckl.) occur at lower elevations, whereas at higher elevations, Douglas-fir grades into hybrid spruce (*Picea engelmanni* Parry ex Engelm. x *Picea glauca* [Moench] Voss) and lodgepole pine (*Pinus contorta* Dougl. Ex. Loud. var. *latifolia* Engelm.) (Meidinger and Pojar 1991).

Soil collection

Sampling locations were distributed over a distance of 140 km and ranged in elevation from 360 to 1390 masl (Table 5.1). This elevational range coincides with that of Douglas-fir in this region of British Columbia. After removing loose litter or moss, we collected 50 x 50 x 10 cm deep volumes of soil from six locations within the rooting zones of Douglas-fir trees at each of six approximately 400 m^2 sites. We sieved the soil through a 2.5 cm^2 mesh in the field to remove woody debris and stones and afterwards refrigerated the soil at 4°C in plastic tubs. By removing soil from the field, only those fungal species able to survive through resistant propagules will be retained in soil samples. The species pool of ectomycorrhizal fungi evaluated in this assay will be substantially less than what occurs in the field because those species requiring mycelial connections will be absent.

To determine the nutrient status of soils, one subsample of mixed soil from each of the six sites was analyzed (Soilcon Laboratories Ltd., Richmond, British Columbia, Canada) for pH, % organic matter measured by loss on ignition, total organic C, ammonium N, nitrate and nitrite N, total N, available P, and estimated C:N (Table 5.2). Analyses were performed using procedures described in Carter (1993) and McKeague (1978).

Plant material

In mid-November, 2003, non-mycorrhizal Douglas-fir seedlings were grown in a greenhouse at the University of British Columbia, Vancouver, from seeds (seedlot #48520, collected at 850-950 masl) obtained from the BC Ministry of Forest Tree Seed Center (Surrey, British Columbia, Canada). Seeds were moist-stratified at 4°C for 21 days, then sterilized in 3% H_2O_2 and mixed constantly for 2 hours. We sowed the seeds into #1206 bedding inserts (Kora Products, Bramalea, Ontario, Canada) filled with an autoclaved 3:1 (v:v) mixture of peat and perlite. Two seeds were placed into each cavity and covered with 0.5 cm of sterilized sand. The trays were misted each day for six weeks, after which seedlings were transplanted into 1.5 L pots. Just prior to transplanting, a random subsample of twenty seedlings was harvested to determine the initial mass of seedlings. We also cleared and stained roots from fifteen additional seedlings to confirm their non-mycorrhizal status. Throughout the experiment, natural daylight in the greenhouse was supplemented by 400 W high pressure sodium lamps for 18 hours daily. The temperature ranged from 20 to 24°C and the relative humidity was maintained at 60%.

Maintenance of soil moisture

In December 2003, the field soil was removed from cold storage and mixed with perlite (3:1 v:v). Soils from each sampling site were crossed with three levels of watering (10, 20, and 30% volumetric soil moisture). The range in watering levels was based on field measurements of soil at 450 and 1200 masl taken over one week in July 2003 using a CS620 Hydrosense soil moisture probe (Campbell Scientific, Inc., Utah, USA). In total, 200 pots were prepared into which the seedlings were transplanted. One hundred and eighty pots were prepared for the treatments (6 sites x 3 watering levels x 10 replicates = 180) and 20 were prepared to establish allometric relationships between seedling height and biomass to adjust the total pot weight due to increased seedling biomass (based on seedling height) over the course of the experiment. Dry soil was determined to be equivalent to 3% soil moisture using a CS620 Hydrosense soil moisture probe (Campbell Scientific, Inc., Utah, USA). The weight of a pot required to maintain the designated soil moisture levels was then calculated based on this initial measurement.

The seedlings were transplanted on January 6, 2004 and during the 8 months of the experiment we regularly weighed pots and added enough water to bring the pot weight up to the appropriate weight for the watering treatment imposed. It was not feasible to maintain the pots at constant soil moisture; we let the pots dry to 10% below their designated soil moisture level before adding water. This required that the seedlings be watered every three days at the beginning of the experiment and each day by the end of the experiment.

Final harvest

A final harvest was done August 18, 2004. Shoots were dried at 65°C for 48 hours and weighed. Roots were bagged along with their surrounding soil and refrigerated at 4°C. For processing, entire root systems were carefully washed under running water and then cut into approximately 1-cm pieces. All root fragments were placed in a baking dish containing water and a random subsample was then distributed into a Petri plate. We aimed to count at least 100 root tips per individual seedling. In cases where seedlings had fewer than 100 root tips, all tips were counted. Generally, ectomycorrhizal tips were turgid and smooth, had emanating hyphae or rhizomorphs, and a Hartig net. A root tip that was dark and wrinkled, or was somewhat hollow and fragmented under minimal pressure was classified as dead. Gross morphology of ectomycorrhizal roots and rhizomorphs was determined under a stereomicroscope while Hartig net, mantle, emanating hyphae, and other such features were observed with a compound microscope under 400 or 1000x magnification. When possible, mantle peels were made by separating the fungal tissue from the root with fine forceps and micro-scalpels. Morphological descriptions were made with reference primarily to Ingleby et al. (1990) and Goodman et al. (1996). Once processed, roots were dried at 65°C for 48 hours and weighed.

Two root tips representing each morphotype were lyophilized, and total genomic DNA was extracted from single ectomycorrhizal tips following the methods of Teste et al. (2006) (Chapter 3). We were successful in amplifying fungal DNA from only one morphotype out of seven (see Results), possibly due to lyophilization techniques. Thus, we relied on morphological characteristics to differentiate among ectomycorrhizal types.

Statistical analyses

Four response variables were measured or calculated for each seedling at the end of the experiment: percentage of root tips that were mycorrhizal, total dry weight, shoot height, and root:shoot ratios. Data were aggregated to obtain a mean for each site x watering level treatment. We used the actual soil moisture content measured in each pot and treated it as a continuous variable because the soil moisture values of the initially designated categories overlapped.

We used multivariate analyses to test for correlations among seedling growth, soil characteristics and the ectomycorrhizal fungal community. In particular, canonical correspondence analysis (CANOCO 4 - ter Braak and Smilaurer 1998) was used to correlate variation in (i) seedling growth traits, (ii) soil moisture, and (iii) soil fertility with variation in fungal community composition. Fungal community composition was considered in two, multivariate forms. Columns in each matrix represented individual morphotypes and rows represented soils of the various treatment combinations. We first assessed individual morphotypes in a categorical nature which resulted in a matrix of 0s and 1s indicating presence (1) or absence (0) of each individual morphotype. Next, we assessed the abundance of individual morphotypes; this resulted in a matrix of cells with values ranging from 0 to 100 (% relative abundance). This type of analysis assumes that species have unimodal distributions along environmental gradients. Values for soil moisture and soil fertility were in two separate matrices due to asymmetrical units of replication (soil moisture: $n = 16$; soil fertility: $n = 6$). We also used redundancy analyses, which assume linear relationships between response and explanatory variables, to correlate (i) soil moisture and (ii) fertility with seedling growth traits (total biomass, root:shoot ratio and seedling height). Values for seedling growth traits were centered and standardized. The significance level for all ordinations was determined by Monte Carlo permutation tests (999 permutations).

Results

Soil properties affected shoot growth (Table 5.3). Specifically, 32% of the variance in seedling traits was explained by soil moisture ($p = 0.018$); both height and biomass increased with soil moisture (Fig. 5.1). A substantial amount of variance in seedling traits was explained by the ratio of carbon to nitrogen in the soil ($r^2 = 0.63$, $p = 0.0090$). When examined individually, only height was positively correlated to C:N (Fig.

5.2). On average seedlings were 11.5 (± 3.08 SD) cm tall, weighed 2.2 (± 1.03 SD) g and had root:shoot of 1.2 (± 0.31 SD).

In total, seven morphotypes were identified on the roots of the seedlings (Fig. 5.3) with two *Wilcoxina* morphotypes being the most frequent. Fungal DNA from *Wilcoxina* mycorrhizas with abundant, smooth emanating hyphae matched that of *Wilcoxina mikolae* in a BLAST search of Genbank (99% match; expected = 0.0). A second type of *Wilcoxina* mycorrhiza, which we refer to as *Wilcoxina* II, was clearly distinguishable from the first because it had few, roughly verrucose emanating hyphae. Those matching descriptions of mycorrhizas formed by *Rhizopogon*, *Amphinema*, *Piloderma* spp. as well as *Mycelium radicis atrovirens* (MRA)–type mycorrhizas, as described by Jones et al. (1997) and Hagerman et al. (1999), were less frequent.

The presence or absence of each of the seven morphotypes was independent of variation in shoot growth traits ($p = 0.27$) (Table 5.3). Likewise, variation in soil moisture levels did not explain variation in the presence or absence of the seven ectomycorrhizal fungal morphotypes ($p = 0.34$) (Table 5.3). Of the soil fertility characteristics measured, only total amount of nitrogen explained significant amounts (31%) of the variance in the presence or absence of ectomycorrhizal fungal morphotypes ($p = 0.012$) (Table 5.3). *Piloderma*, *Rhizopogon* and *Amphinema*- type morphotypes were present in low nitrogen soils, and both *Wilcoxina* morphotypes and *Cenococcum geophilum* occurred in soils with mid-range values of nitrogen (Fig. 5.4). The MRA-type morphotype was present only in high nitrogen soils (Fig. 5.4). No other soil fertility variables (i.e. pH, % organic matter, % organic C, mg kg^{-1} of NH_4, NO_3/NO_2 and P, or C:N) correlated with presence or absence of morphotypes (minimum $p \geq 0.41$). Total amount of nitrogen was not correlated to C:N ratio in these samples ($p = 0.084$). Variation in total colonization (i.e., abundance measured by percent colonization of all morphotypes combined) for each site x watering level combination was not correlated to seedling biomass or height (minimum $p = 0.95$), but was positively correlated to root:shoot ratio ($p = 0.048$, $r = 0.52$; Fig. 5.4). Total colonization was not related to the soil moisture level ($p = 0.090$).

While neither soil moisture levels nor variation in seedling growth covaried with the presence or absence of the individual fungal morphotypes, variation in seedling traits was related to the relative abundance of each of the seven morphotypes (Table 5.3). Specifically, *Wilcoxina mikolae* and *Rhizopogon*-type morphotypes were more abundant on tall seedlings compared to other morphotypes ($r^2 = 0.22$, $p = 0.024$) (Fig.

5.5). The abundances of each morphotype, however, were not related to soil moisture ($p= 0.45$) or any of the soil fertility variables (minimum $p \geq 0.074$).

Discussion

In our study, the composition of the ectomycorrhizal fungal community was influenced both directly and indirectly by variation in the soil environment. The presence or absence of each of the seven morphotypes was correlated with total soil nitrogen, but this community metric was not correlated to any host shoot growth responses. In other words, of the pool of morphotypes sampled in our assay, occurrence of each morphotype was influenced by the soil environment. When morphotypes were measured by their relative abundance, we found that the abundance of specific morphotypes did not respond directly to any of the soil variables, but instead was mediated by growth characteristics in the host. Because host growth was affected by soil moisture and C:N ratios, we suggest that the abundance of morphotypes sampled were indirectly affected by soil conditions.

It is to be expected that the presence or absence of individual morphotypes correlated directly to soil nitrogen. Because we did not measure nitrogen status of host individuals, we cannot distinguish whether fungi responded directly to nitrogen levels in the soil or indirectly via nitrogen status of the host (e.g. Nilsson and Wallander 2003). However, there have been numerous studies showing that in culture, ectomycorrhizal fungal species show distinct preferences for different forms and levels of nitrogen (e.g. Lilleskov et al. 2002 and references therein). Niche segregation along nitrogen gradients has also been demonstrated in the field (see reviews by Wallenda and Kottke 1998, Treseder and Allen 2000). Interestingly, those factors that affected the presence or absence of individual morphotypes did not affect seedling growth traits. Nantel and Neumann (1992) also reported that factors affecting fungal species distribution were different from those affecting the distribution of their tree hosts, namely humus characteristics. More recently, Toljander et al. (2006) demonstrated that despite variation in host identity along a nutrient gradient, most variation in the ectomycorrhizal fungal community was attributable to soil characteristics such as extractable ammonium and base saturation. Overall, findings from previous literature suggest that beta diversity in ectomycorrhizal fungal communities is somewhat controlled by the presence or

absence of particular host species, but soil environmental heterogeneity is a more important factor maintaining ectomycorrhizal fungal diversity.

Our results were based on the responses of seedlings grown from open pollinated seeds in which the effect of genetic diversity is expected to be consistent across treatments. Thus, the nearly three-fold difference in seedling heights we observed was probably mostly due to soil environmental variation. The range of soil variation coincides with the elevational range of Douglas-fir in this study area. However, because we had few samples (n = 6) of soils within the study area, the resolving power of seedling sensitivity to variation in soil characteristics is low. Nonetheless, this difference in seedling heights is more than that reported by Korkama et al. (2006) who observed dissimilar ectomycorrhizal fungal communities between fast and slow-growing clones of *Picea abies*. In our study the phenotypic gradient, as measured by variation in shoot biomass, height and root:shoot ratios, was not sufficient to promote partitioning among fungal morphotypes. It is possible that phenotypic variation expressed belowground could be amplified to the extent that the composition of the ectomycorrhizal community would be affected. In particular, variation in quantity and quality of exudates, should be tested as possible determinants of membership within ectomycorrhizal fungal communities.

The environmental gradients did not influence host intraspecific variation sufficiently to determine membership within the fungal community, however they were important in modifying the abundance of morphotypes present. Because it is unlikely that colonization levels of individual morphotypes influence host intraspecific variation to the same extent as variation in soil characteristics (e.g. C:N ratios explained 63% of the variance in seedling growth traits whereas only 22% was related to morphotype abundance), we suggest that C:N ratios and soil moisture levels may act indirectly to modify the abundance of individual morphotypes. Högberg et al. (2007) also reported the importance of indirect effects of soil chemistry (C:N ratio) on abundance of ectomycorrhizal fungi as measured by PLFA biomarkers.

Of the soil characteristics measured, it was surprising how little impact variation in soil moisture had on the ectomycorrhizal fungal community. Soil moisture has been shown to induce changes in the species composition of ectomycorrhizal fungal communities surveyed in intact forests (Shi et al. 2002; Swaty et al. 2004) and in part this is thought to reflect differences in the drought tolerance of ectomycorrhizal fungi

(Parke et al. 1983; Boyle and Hellenbrand 1991). The relationship between ectomycorrhizal colonization and soil moisture has been previously reported but the direction of the response varies (Lodge 1989; Gehring and Whitham 1994; Runion et al. 1997; Nilsen et al. 1998; Swaty et al. 1998; Valdes et al. 2006). The portion of the soil moisture gradient studied clearly influences the response of ectomycorrhizas to soil moisture, but despite a four-fold difference in imposed soil moisture values, ectomycorrhizal fungi did not sort along this particular gradient. Possibly, plasticity of colonization levels present at a fungal species level accommodates variation in soil moisture.

Because we removed soils from the field, the available ectomycorrhizal fungal species pool should have been similar across soils as we sampled only those species with resistant propagules. Results from both the field (e.g. Bidartondo et al. 2001) and greenhouse studies document that the resistant propagule community (sensu Taylor and Bruns [1999]) is often spatially homogeneous. For example, *Wilcoxina* spp. and *Cenococcum geophilum* were reported to be abundant and spatially homogeneous in soils collected from mixed-conifer forest bioassayed with two host species (Izzo et al. 2006). Cline et al. (2005) demonstrated that ectomycorrhizal fungal communities on Douglas-fir seedlings planted at various distances outside mycelium networks were similar to those on greenhouse seedlings grown in field soil. We cannot rule out however, that those fungal species widely distributed via resistant propagules may also be "generalists" when responding to intraspecific host variation and soil moisture.

In conclusion, individuals of a host species and species within their associated ectomycorrhizal fungal community respond to different environmental gradients. While host traits were controlled mostly by variation in soil moisture and C:N ratio, the occurrence of particular ectomycorrhizal morphotypes was structured by levels of total nitrogen. Host variation did not directly affect the presence or absence of individual ectomycorrhizal fungal morphotypes. However, host variaiton was correlated to the relative abundance of each of the ectomycorrhizal fungal morphotypes, suggesting that the abundance of morphotypes may be modified by those gradients affecting intraspecific host variation. At the seedling stage, soil nitrogen and host growth characteristics influence composition of ectomycorrhizal fungal communities.

Table 5.1: Site coordinates and elevation of soil sampling locations.

Site Label	Latitude	Longitude	Elevation (masl)
BB	50°01.918N	119°21.526W	724
BT	50°02.325N	119°15.994W	1318
OB	49°46.737N	119°36.203W	360
OT	49°42.792N	119°36.101W	1396
RB	50°44.477N	120°32.409W	648
RT	50°49.064N	120°42.524W	1387

Table 5.2: Fertility characteristics of soils collected from six sites from the Thompson-Okanagan region of British Columbia. Values are from a composite of 6 samples per site.

Site Label	pH	Organic matter (LOI) (%)	Organic C (%)	NH_4 (mg/kg)	NO_3/NO_2 (mg/kg)	Total N (%)	P (Bray-P1) (mg/kg)	C:N
BB	7.3	8.4	4.2	10	6	0.30	44.3	14
BT	6.0	11.7	5.8	9	0.1	0.15	25.9	40
OB	5.0	16.8	8.4	20	0.1	0.22	86.9	38
OT	4.7	18.2	9.1	19	0.1	0.21	51.9	44
RB	6.2	11.0	5.5	22	16.5	0.19	100.0	29
RT	5.6	9.8	4.9	9	0.5	0.14	57.7	35

Table 5.3: Types of statistical analyses (canonical correspondence analysis [CCA] or redundancy analysis [RA]) used and significance of explanatory variables tested to explain measures of ectomycorrhizal fungal community composition or seedling growth traits. Only those soil aspects of soil fertility found to be significant are reported in table. Numbers in brackets represent percentage of variance explained by each significant explanatory factor.

		Response variable	
Type of analysis	**Explanatory variable***	Presence/absence of individual morphotypes p	Abundance of individual morphotypes p
CCA	Soil moisture	0.34	0.45
	Seedling growth traits∞	0.27	0.024 (22%)
	Soil fertility (% total N)	0.012 (31%)	≥ 0.074†
RA		Seedling growth traits	
	Soil moisture	0.018 (32%)	
	Soil fertility (C:N)	0.0090 (63%)	

* Variables are categorized as explanatory, however it should be recognized that in both analyses, causation cannot be inferred.

† none of the measures of soil fertility (pH, % organic matter, % organic C, % total N, mg kg^{-1} of NH_4, NO_3/NO_2 and P, and C:N) were significant in explaining variation in abundance of individual morphotypes. The minimum p-value across all measures is given.

∞seedling growth traits include shoot height, total biomass and root:shoot

Figure 5.1: Relationships between soil moisture (%) and seedling height, biomass and root:shoot ratio.

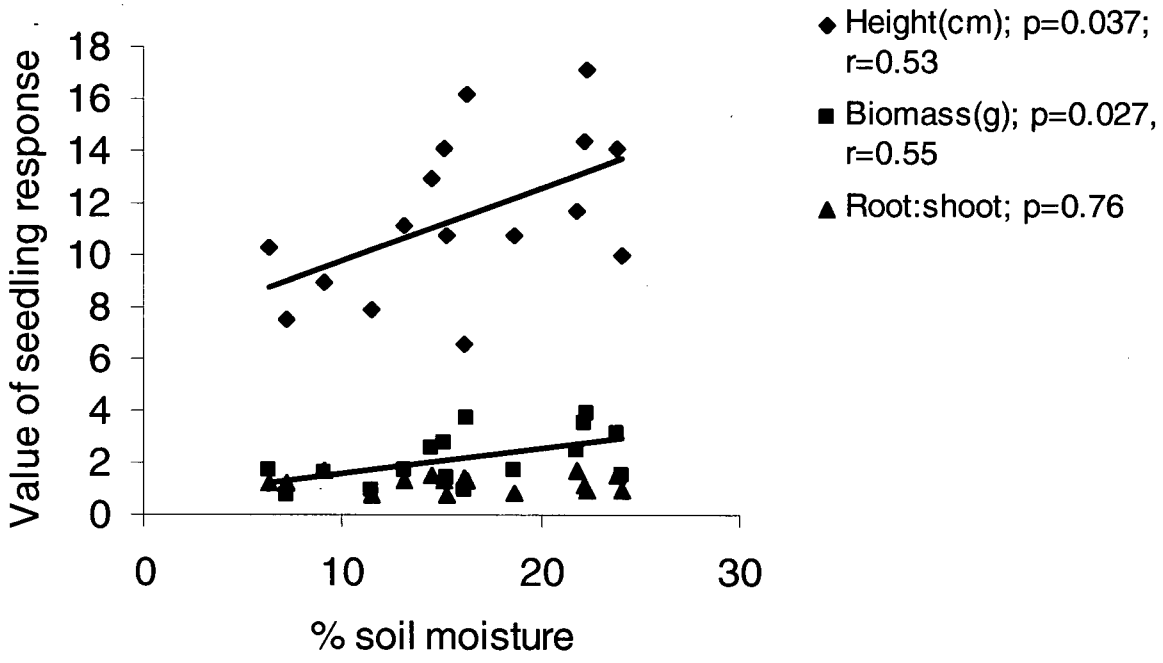


Figure 5.2: Relationships between soil C:N and seedling height, biomass and root:shoot.

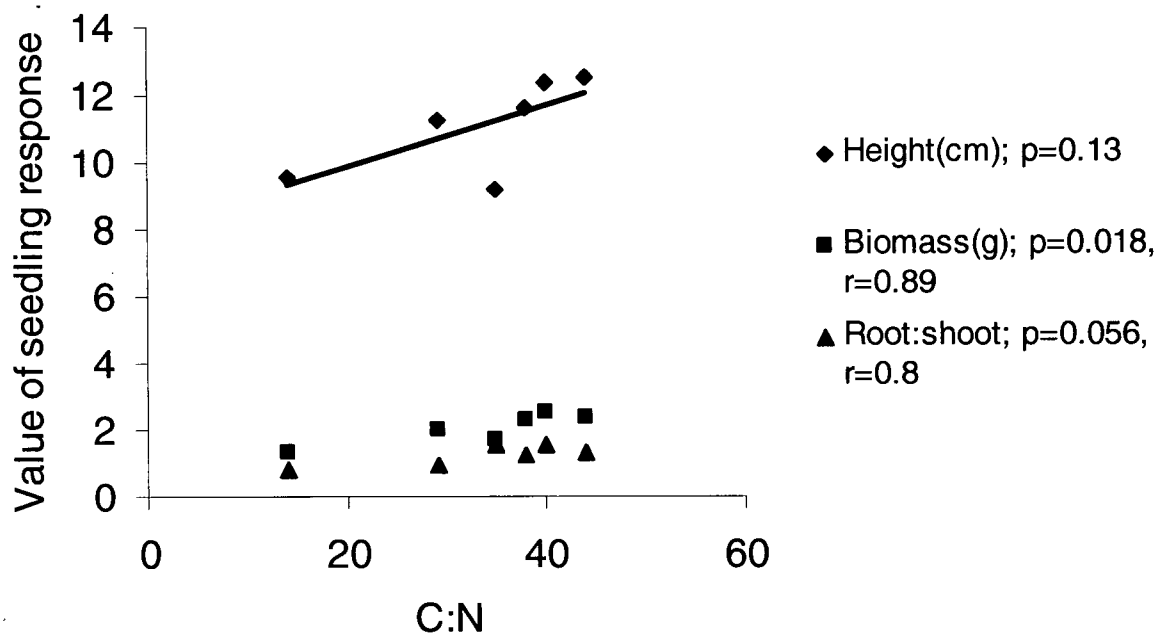


Figure 5.3: Frequency of ectomycorrhizal morphotypes observed across seedlings of *Pseudotsuga menziesii* var. *glauca*.

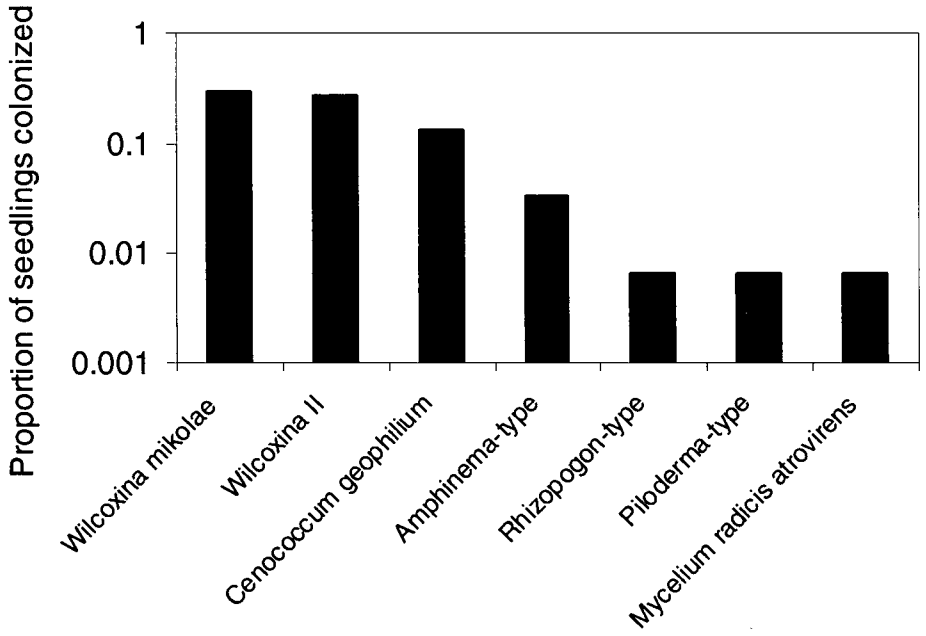


Figure 5.4: Canonical correspondence analysis of ectomycorrhizal morphotypes observed on seedlings of *Pseudotsuga menziesii* var. *glauca* ordinated along gradient of % total soil nitrogen.

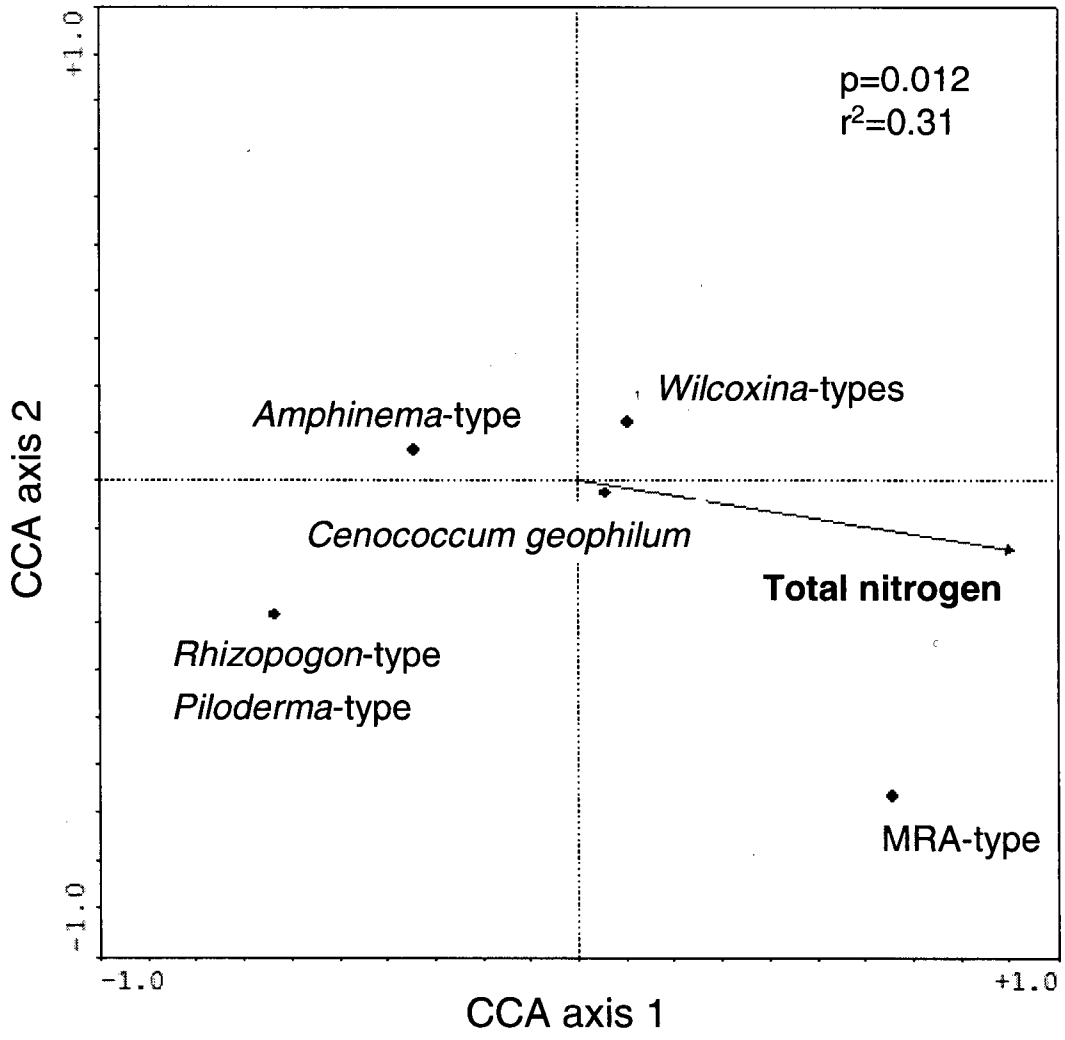


Figure 5.5: Relationship between percent ectomycorrhizal colonization and root:shoot ratio of *Pseudotsuga menziesii* var. *glauca* seedlings.

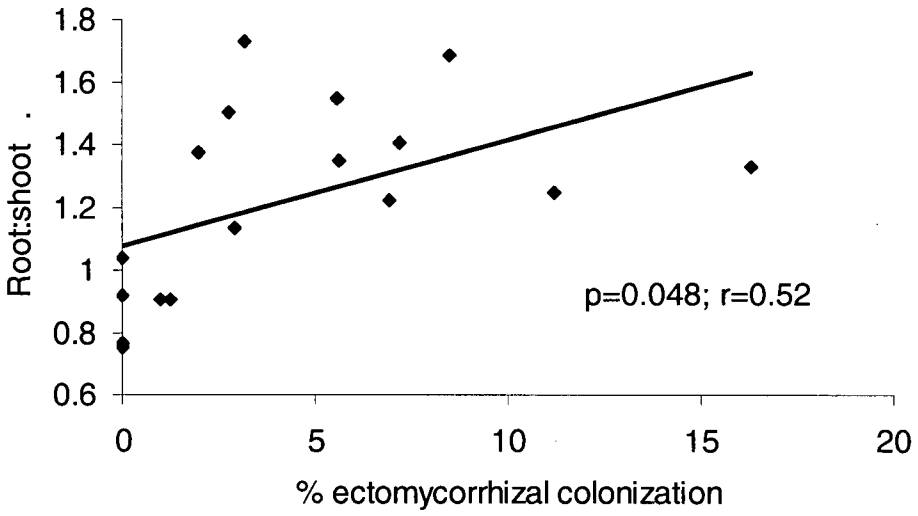
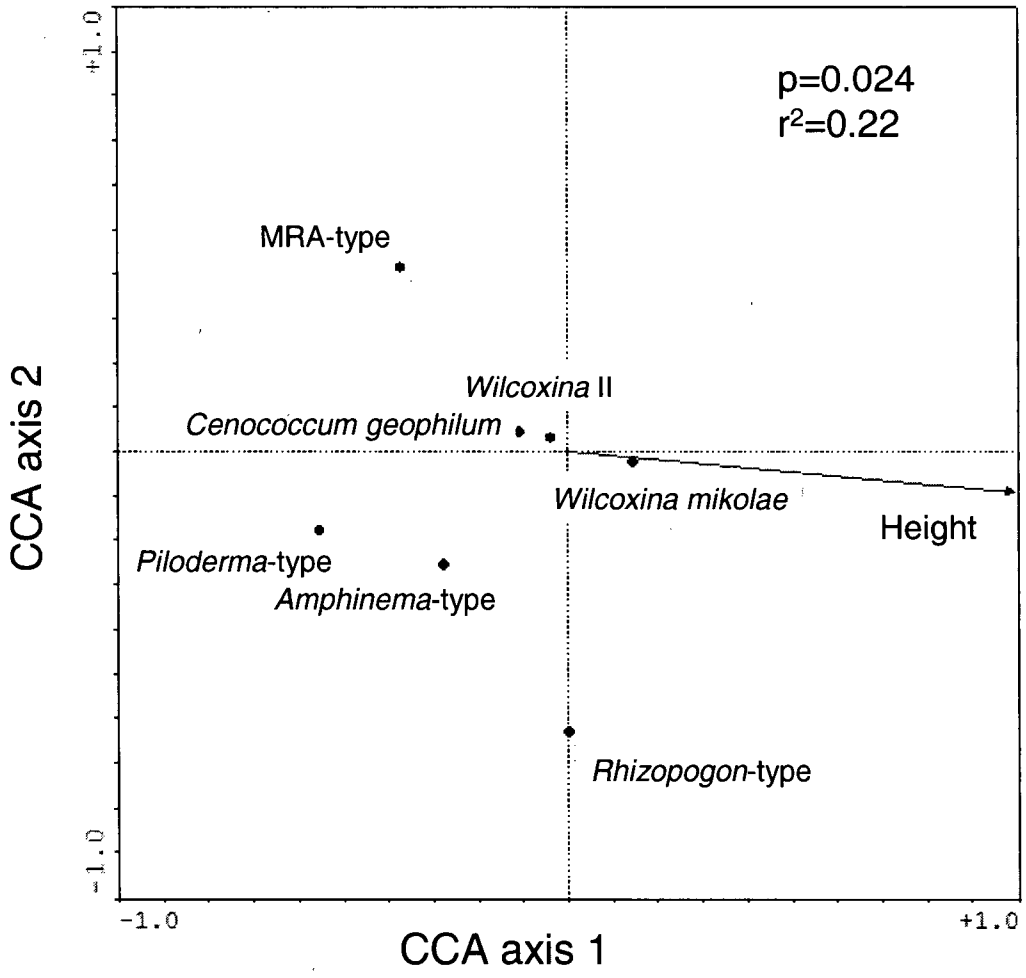


Figure 5.6: Canonical correspondence analysis of ectomycorrhizal morphotypes observed on seedlings of *Pseudotsuga menziesii* var. *glauca* ordinated along gradient of seedling height.



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6 Conclusions

Ectomycorrhizal associations represent interactions among species that are tightly linked, both physically and physiologically. We therefore expect organisms involved in ectomycorrhizal associations to be more sensitive to variation within each partner than organisms involved in free-living associations (e.g. predator-prey relationships). In this thesis, I used meta-analysis and experimental approaches to consider how variation in one partner of the ectomycorrhizal symbiosis affected the other. In particular, my objectives were to evaluate:

i. how colonization levels, regardless of ectomycorrhizal fungal taxon, correlated with host growth
ii. how ectomycorrhizal fungi differentially influenced growth of different genera of plant hosts, and
iii. how variation in growth of a single host species was correlated to the composition of ectomycorrhizal fungal communities in various soil environments.

Because some of my conclusions relied on comparisons of inoculated and non-inoculated seedlings, I also tested the efficacy of two methods to control colonization by ectomycorrhizal fungi on host plants. These results are of practical significance because prior to our experiment, no one had tested whether it was possible to reduce ectomycorrhizal colonization in unsterilized field soil.

My thesis objectives can be distilled into one summary question: to what level of organization of ectomycorrhizal fungi does the growth of host plants respond? I considered several levels of organization: 1. colonization levels regardless of ectomycorrhizal fungal taxon, 2. taxonomic identity of ectomycorrhizal fungi, and 3. communities of ectomycorrhizal fungi. By conducting a meta-analysis on a large body of previously published work, and by applying multivariate analyses to my experimental data, I was able to evaluate the contribution of the three levels of organization to variation in growth responses of host plants. Three main conclusions emerged.

The relationship between colonization level and host growth response is inconsistent

When considering individuals of a particular species of host plant, variation in host growth varied with the abundances of different morphotypes of ectomycorrhizal fungi (Chapter 5). In other words, colonization levels of some fungi increased with seedling height, and decreased for other fungal morphotypes. The relationship between total colonization level and seedling growth differed among seed families of a host species (Chapter 4). When considered across many host genera, I detected no relationship between colonization level and host growth response, regardless of fungal taxon (Chapter 2). These findings suggest that for the most part, host growth response to colonization level is unpredictable.

In order to study the effects of ectomycorrhizal fungi on plants, it is helpful to have control plants that are completely free of ectomycorrhizal contamination. However, this can be challenging in the lab (Chapter 2), and almost impossible in non-sterile field soils (Chapters 3). My experiments demonstrated that fungicides or mesh have the potential to reduce colonization, but this may be of little value if host growth responses do not consistently scale with colonization levels. If plants sometimes respond to very low levels of colonization, this raises the question of whether reductions in colonization levels are meaningful treatments to assess host response to ectomycorrhizas.

There is little sensitivity in growth responses of host plants to variation in the identity of ectomycorrhizal fungi

Seedlings across multiple host genera increased in biomass and shoot height when inoculated with ectomycorrhizal fungi regardless of the identity of the fungal associate (Chapter 2). When ectomycorrhizas were considered in a multi-specific context (i.e. one host species associated with a community of ectomycorrhizal fungi), variation in host shoot properties did not correlate with species composition of the community of ectomycorrhizal fungi on their roots but rather appeared to be more tightly coupled to edaphic conditions (Chapter 5). Thus, the variation a host plant perceives and selects for in ectomycorrhizal fungi may be of a discrete rather than continuous nature, i.e., host plants respond to the presence or absence of ectomycorrhizal fungi but not to variation in their identity. A consequence of the coevolution among organisms in multi-specific systems may be that reciprocal specialization is unlikely, therefore host

plants tend to be generalists in their responses to variation in the identity of ectomycorrhizal fungi.

Publication bias exists in the ectomycorrhizal literature

The meta-analysis investigating the mutualism-parasitism continuum in ectomycorrhizas represents a significant advance in the field of ectomycorrhizal research because it statistically evaluates and summarizes nearly four decades of research on inoculation trials. I demonstrated that publication bias has clouded our ability to determine general principles of host response to ectomycorrhizal inoculation. In the past, mycorrhizas were synonymous with mutualisms, and the tendency to publish results congruent with this perception has resulted in the under representation of studies reporting contrary results demonstrating a more parasitic role for ectomycorrhizas.

Future research directions

The approaches used in my thesis represent initial tests to determine the importance of symbiotic variation to host growth. I suggest several avenues of further research. While the effects of host genotype on colonization level have been documented (Tagu et al. 2001, 2005, Gehring et al. 2006), we are far from understanding host genotype x ectomycorrhiza interactions. Research is necessary to determine the relative importance of host genetics versus the presence, absence and species of ectomycorrhizal fungi on intraspecific variation in growth among individual host plants. This type of research would clarify the importance of interactions between hosts and ectomycorrhizal fungi in influencing seedling growth. I have contributed to this particular topic in Chapter 4 with evidence that the relationship between colonization level and host growth can be positive or negative, depending on plant genotype, within a host species. However, the weakness of this experiment is that the seedlings were not colonized by target fungi. Implementing other inoculation techniques, such as submerging root systems of seedlings in slurries of inoculum or use solid inoculum, may increase the success of inoculation.

It is also critical that future research explores the magnitude of specialization between host taxa and communities of ectomycorrhizal fungi. Multi-specific rather than pairwise interactions have been recognized to be the norm for coevolved organisms

(Stanton 2003, Strauss and Irwin 2004) and those organisms involved in ectomycorrhizal associations are no exception – individual trees frequently host communities of ectomycorrhizal fungi. In other study systems, the role of host plant morphological variation has been shown to be important in determining the composition of dependent communities (Whitham et al. 2006). Until very recently, we have known virtually nothing about how host plants influence the composition of ectomycorrhizal communities. Two pioneering studies have finally addressed this question and found that the relative growth rates (Korkama et al. 2006) and taxonomic identity (Ishida et al. 2007) of hosts alter the composition of their ectomycorrhizal fungal communities, yet much more research is required to adequately assess the sensitivity of host plants to changes in membership within ectomycorrhizal fungal communities and vice versa.

Although the meta-analysis in Chapter 2 is powerful in synthesizing decades of research, it is limited by the features of the studies included in the analysis. In particular, pair-wise host-fungal combinations were the norm, thus conclusions on the sensitivity of hosts to variation in the identity of ectomycorrhizal fungi may change if interactions among species of ectomycorrhizal fungi were to be present. Co-inoculation of hosts by several fungi is challenging and sampling in the field may yield more information on the specificity between hosts and communities of ectomycorrhizal fungi. For example, surveying the taxonomic affinities between various host taxa and their ectomycorrhizal fungal communities, complimented by field experiments that manipulate host characteristics would be a useful initial approach to address this question.

That hosts perceive ectomycorrhizal fungi as functionally redundant, as suggested by the meta-analysis in Chapter 2, may indicate that edaphic conditions are more important than the presence and/or variation in composition of ectomycorrhizal fungal communities in determining seedling growth. In addition, results from Chapter 5 indicate that host growth is coupled to edaphic conditions. Variation in edaphic conditions takes many forms and, compared to descriptions of vegetative variation, our understanding of variation in soil characteristics is poor (but see Bell and Lechowicz 1991, Bell et al. 1993, Farley and Fitter 1999). In particular, the role of spatial structure in the edaphic environment will be critical to understanding the role of ectomycorrhizal associations to host growth given the strong spatial covariance between the composition of ectomycorrhizal fungal communities and soil characteristics. This feature makes it difficult to untangle the ecological importance of either to host plant growth.

The strength of the analytical approach taken in Chapter 5 is that it identifies and statistically parses the variation in host growth due to variation in the composition of fungal communities and soil characteristics – a conceptually novel framework. The weaknesses of the experiment presented in Chapter 5 are that no causation can be inferred and the low number of soils sampled effectively shortens the environmental gradient that might influence host growth and membership within ectomycorrhizal fungal communities. While the contribution of abiotic and symbiotic factors in structuring ectomycorrhizal fungal communities has been evaluated (Nantel and Neumann 1992, Kernaghan et al. 2003, Gehring et al. 2006, Toljander et al. 2006, Hogberg et al. 2007, Taniguchi et al. 2007), it has rarely been posed from a host perspective (but see Dickie et al. 2007). An experimental design for the field that renders changes in the composition of ectomycorrhizal fungal communities independent from variation in soil characteristics remains elusive yet stands as an important challenge in mycorrhizal research.

Final conclusion

Numerous opportunities now exist to investigate the distribution and abundance of plant species in the context of ectomycorrhizal associations. In particular, the degree to which host plants and ectomycorrhizal fungal communities are specialized will be relevant information for forecasts of species' shifts with climate change. As ranges of symbionts are unlikely to change concordantly, it will be crucial to understand the basis and consequences of coevolution between hosts and fungi to predict their future distributions.

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Appendices

A. Identity of host plant and fungal species pairings and effect sizes (Ln R) for seedling biomass, shoot height and shoot:root ratio for each study used in meta-analysis.

Authors	Host species	Fungal species	Ln R		
			Biomass	Height	Shoot:root
			n=459	n=329	n=235
Baum et al. 2000	*Populus trichocarpa*	*Laccaria bicolor*	-0.518	-0.334	-0.522
Baum et al. 2000	*Populus trichocarpa*	*Laccaria bicolor*	0.856	0.529	1.627
Baum et al. 2000	*Populus trichocarpa*	*Paxillus involutus*	-0.319	-0.401	-0.895
Baum et al. 2000	*Populus trichocarpa*	*Paxillus involutus*	0.797	0.485	1.600
Baum et al. 2002	*Populus trichocarpa*	*Laccaria laccata*		0.305	
Baum et al. 2002	*Populus trichocarpa*	*Laccaria laccata*		0.101	
Baum et al. 2002	*Populus trichocarpa*	*Laccaria laccata*		0.005	
Baumann et al. 2005	*Pinus sylvestris*	*Paxillus involutus*	0.251		-0.198
Baumann et al. 2005	*Pinus sylvestris*	*Paxillus involutus*	-0.111		0.358
Baumann et al. 2005	*Pinus sylvestris*	*Paxillus involutus*	0.064		-0.087
Baumann et al. 2005	*Pinus sylvestris*	*Paxillus involutus*	0.066		-0.399
Baumann et al. 2005	*Pinus sylvestris*	*Paxillus involutus*	0.292		0.048
Baumann et al. 2005	*Pinus sylvestris*	*Paxillus involutus*			
Beyeler & Heyser 1997	*Fagus sylvatica*	*Lactarius subdulcis*	0.272		
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	0.894		
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	0.368		
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	0.575		
Browning & Whitney 1991	*Pinus banksiana*	*Pisolithus tinctorius*	-0.031		0.236
Browning & Whitney 1991	*Pinus banksiana*	*Cenococcum geophilum*	0.000		0.086
Browning & Whitney 1991	*Pinus banksiana*	*Laccaria proxima*	-0.014		0.114
Browning & Whitney 1991	*Pinus banksiana*	*Hebeloma cylindrosporum*	-0.030		-0.150
Browning & Whitney 1991	*Pinus banksiana*	*Tricholoma pessundatum*	-0.065		0.233

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Browning & Whitney 1991	*Pinus banksiana*	*Thelephora terrestris*	-0.046		0.231
Browning & Whitney 1991	*Pinus banksiana*	*Suillus granulatus*	-0.058		0.242
Browning & Whitney 1991	*Pinus banksiana*	*Hebeloma cylindrosporum*	0.126		-0.022
Browning & Whitney 1991	*Pinus banksiana*	*Pisolithus tinctorius*	0.163		-0.004
Browning & Whitney 1991	*Pinus banksiana*	*Laccaria proxima*	0.198		0.034
Browning & Whitney 1991	*Pinus banksiana*	*Tricholoma pessundatum*	0.138		0.143
Browning & Whitney 1991	*Pinus banksiana*	*Cenococcum geophilum*	0.129		-0.056
Browning & Whitney 1991	*Picea mariana*	*Laccaria bicolor*	-0.099		0.186
Browning & Whitney 1991	*Picea mariana*	*Laccaria proxima*	0.037		0.307
Browning & Whitney 1991	*Picea mariana*	*Pisolithus tinctorius*	-0.092		0.313
Browning & Whitney 1991	*Picea mariana*	*Tricholoma pessundatum*	-0.020		0.336
Browning & Whitney 1991	*Picea mariana*	*Hebeloma cylindrosporum*	-0.007		0.275
Browning & Whitney 1991	*Picea mariana*	*Thelephora terrestris*	0.030		0.374
Browning & Whitney 1991	*Picea mariana*	*Cenococcum geophilum*	0.020		0.306
Browning & Whitney 1991	*Picea mariana*	*Suillus granulatus*	0.020		0.234
Browning & Whitney 1991	*Picea mariana*	*Laccaria bicolor*	-0.104		0.153
Browning & Whitney 1991	*Picea mariana*	*Hebeloma cylindrosporum*	-0.005		0.329
Browning & Whitney 1991	*Picea mariana*	*Pisolithus tinctorius*	0.010		0.058
Browning & Whitney 1991	*Picea mariana*	*Laccaria proxima*	0.172		0.320
Browning & Whitney 1991	*Picea mariana*	*Tricholoma pessundatum*	-0.005		0.015
Burgess & Malajczuk 1989	*Eucalyptus globulus*	*unknown*	0.813		
Burgess & Malajczuk 1989	*Eucalyptus globulus*	*unknown*	0.957		
Burgess & Malajczuk 1989	*Eucalyptus globulus*	*unknown*	1.253		
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	0.511	0.303	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	0.863	0.493	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	1.111	0.614	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	1.215	0.650	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	1.804	1.249	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	1.956	1.308	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	2.408	1.434	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	2.576	1.531	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	2.650	1.590	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	2.696	1.632	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	2.802	1.646	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	2.824	1.686	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.086	1.762	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.116	1.809	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.173	1.843	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.173	1.842	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.189	1.886	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.202	1.896	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.305	1.928	
Burgess et al. 1994	*Eucalyptus grandis*	*Pisolithus sp*	3.566	2.099	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*Laccaria laccata*	0.489	0.143	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*Hebeloma crustulinforme*	0.032	0.000	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*Pisolithus tinctorius*	0.614	0.208	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*unknown*	0.643	0.268	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*Laccaria laccata*	0.489	0.143	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*Hebeloma crustulinforme*	0.032	0.000	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*Pisolithus tinctorius*	0.614	0.208	
Chakravarty & Unestam 1987	*Pinus sylvestris*	*unknown*	0.643	0.268	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.000	0.248	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.000	0.686	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.000	0.598	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Shoot:root
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.000	1.152	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.000	1.152	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	0.000	0.092	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	0.000	0.430	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	0.000	0.213	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	0.000	1.021	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	0.000	1.510	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	0.000	0.666	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	0.000	0.759	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	0.000	1.275	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	0.000	1.214	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	0.000	0.319	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	0.000	0.093	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	0.000	0.378	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	0.000	1.491	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	0.000	0.903	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	0.000	0.325	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma albidum*	0.034	0.198	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma albidum*	0.128	0.168	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma areolatum*	0.128	0.104	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma areolatum*	-0.116	0.059	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma areolatum*	0.476	0.329	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	-0.065	0.021	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.049	0.238	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma cepa*	0.311	0.104	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	-0.112	0.104	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma citrinum*	0.171	0.168	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma flavidum*	0.153	0.147	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma flavidum*	0.430	0.329	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma paradoxum*	0.125	0.389	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma sp*	0.220	0.247	
Chen et al. 2006	*Eucalyptus globulus*	*Scleroderma verrucosum*	0.155	0.168	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma albidum*	0.714	0.294	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma albidum*	0.042	0.216	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma areolatum*	0.300	0.152	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma areolatum*	0.005	0.184	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma areolatum*	0.123	0.205	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	-0.079	0.108	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	0.612	0.275	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma cepa*	-0.165	0.085	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	-0.214	-0.013	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma citrinum*	0.327	0.426	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma flavidum*	0.419	0.375	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma flavidum*	0.750	0.483	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma paradoxum*	0.451	0.393	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma sp*	0.559	0.536	
Chen et al. 2006	*Eucalyptus urophylla*	*Scleroderma verrucosum*	0.507	0.331	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma albidum*	0.400	0.270	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma albidum*	0.253	0.277	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma areolatum*	-0.102	0.178	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma areolatum*	-0.150	0.109	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma areolatum*	0.302	0.034	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma cepa*	-0.221	0.079	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma cepa*	0.270	0.134	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Chen et al. 2006	*Pinus elliottii*	*Scleroderma cepa*	-0.326	0.100	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma citrinum*	-0.312	-0.007	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma citrinum*	0.558	0.205	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma flavidum*	0.438	0.297	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma flavidum*	0.236	0.170	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma paradoxum*	0.224	0.146	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma sp*	0.224	0.170	
Chen et al. 2006	*Pinus elliottii*	*Scleroderma verrucosum*	-0.262	0.021	
Chen et al. 2006	*Pinus radiata*	*Scleroderma albidum*	0.959	0.342	
Chen et al. 2006	*Pinus radiata*	*Scleroderma albidum*	1.260	0.302	
Chen et al. 2006	*Pinus radiata*	*Scleroderma areolatum*	1.134	0.164	
Chen et al. 2006	*Pinus radiata*	*Scleroderma areolatum*	0.895	0.146	
Chen et al. 2006	*Pinus radiata*	*Scleroderma areolatum*	0.794	0.312	
Chen et al. 2006	*Pinus radiata*	*Scleroderma cepa*	1.080	0.165	
Chen et al. 2006	*Pinus radiata*	*Scleroderma cepa*	0.830	0.255	
Chen et al. 2006	*Pinus radiata*	*Scleroderma cepa*	0.959	0.176	
Chen et al. 2006	*Pinus radiata*	*Scleroderma citrinum*	0.391	0.070	
Chen et al. 2006	*Pinus radiata*	*Scleroderma citrinum*	0.717	0.213	
Chen et al. 2006	*Pinus radiata*	*Scleroderma flavidum*	0.935	0.435	
Chen et al. 2006	*Pinus radiata*	*Scleroderma flavidum*	0.717	0.266	
Chen et al. 2006	*Pinus radiata*	*Scleroderma paradoxum*	0.830	0.400	
Chen et al. 2006	*Pinus radiata*	*Scleroderma sp*	0.747	0.422	
Chen et al. 2006	*Pinus radiata*	*Scleroderma verrucosum*	0.623	0.198	
Choi et al. 2005	*Pinus densiflora*	*Pisolithus tinctorius*			
Choi et al. 2005	*Pinus densiflora*	*Pisolithus tinctorius*			
Conjeaud et al. 1996	*Pinus pinaster*	*Hebeloma cylindrosporum*			
Diedhiou et al. 2005	*Afzelia africana*	*Scleroderma dictyosporum*	0.303		-0.518

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Diedhiou et al. 2005	*Afzelia africana*	*Scleroderma verrucosum*	0.175		-0.535
Diedhiou et al. 2005	*Afzelia africana*	*Pisolithus sp*	0.288		-0.417
Diedhiou et al. 2005	*Afzelia africana*	*Thelephora sp*	0.281		-0.461
Diedhiou et al. 2005	*Afzelia bella*	*Scleroderma dictyosporum*	0.571		-0.976
Diedhiou et al. 2005	*Afzelia bella*	*Scleroderma verrucosum*	0.595		-0.939
Diedhiou et al. 2005	*Afzelia bella*	*Pisolithus sp*	0.360		-1.182
Diedhiou et al. 2005	*Afzelia bella*	*Thelephora sp*	0.358		-0.973
Diedhiou et al. 2005	*Anthonotha macrophylla*	*Scleroderma dictyosporum*	0.081		0.181
Diedhiou et al. 2005	*Anthonotha macrophylla*	*Scleroderma verrucosum*	-0.010		0.214
Diedhiou et al. 2005	*Anthonotha macrophylla*	*Pisolithus sp*	0.199		-0.088
Diedhiou et al. 2005	*Anthonotha macrophylla*	*Thelephora sp*	0.186		0.235
Diedhiou et al. 2005	*Cryptosepalum tetraphylum*	*Scleroderma dictyosporum*	0.741		-0.380
Diedhiou et al. 2005	*Cryptosepalum tetraphylum*	*Scleroderma verrucosum*	0.302		-0.510
Diedhiou et al. 2005	*Cryptosepalum tetraphylum*	*Pisolithus sp*	0.474		-0.492
Diedhiou et al. 2005	*Cryptosepalum tetraphylum*	*Thelephora sp*	0.489		-1.063
Diedhiou et al. 2005	*Paramacrolobium coeruleum*	*Scleroderma dictyosporum*	0.456		-0.624
Diedhiou et al. 2005	*Paramacrolobium coeruleum*	*Scleroderma verrucosum*	0.426		-0.650
Diedhiou et al. 2005	*Paramacrolobium coeruleum*	*Pisolithus sp*	0.375		-0.749
Diedhiou et al. 2005	*Paramacrolobium coeruleum*	*Thelephora sp*	0.358		-0.744
Diedhiou et al. 2005	*Uapaca somon*	*Scleroderma dictyosporum*	2.209		-0.726
Diedhiou et al. 2005	*Uapaca somon*	*Scleroderma verrucosum*	2.035		-0.434
Diedhiou et al. 2005	*Uapaca somon*	*Pisolithus sp*	2.242		-0.642
Diedhiou et al. 2005	*Uapaca somon*	*Thelephora sp*	1.984		-0.688
Dixon et al. 1981	*Quercus velutina*	*Pisolithis tinctorius*	1.244	0.598	
Dixon et al. 1981	*Quercus velutina*	*Pisolithis tinctorius*	-0.850	-0.405	
Dixon et al. 1983	*Quercus velutina*	*Pisolithus tinctorius*		0.162	
Dixon et al. 1983	*Quercus velutina*	*Pisolithus tinctorius*		-0.015	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Dixon et al. 1984	*Quercus robur*	*Pisolithuis tinctorius*	0.711	0.397	-0.443
Dixon et al. 1984	*Quercus robur*	*Pisolithus tinctorius*	0.657	0.451	-0.335
Dixon et al. 1984	*Quercus robur*	*Suillus granulatus*	0.249	0.323	-0.074
Dixon et al. 1984	*Quercus robur*	*Thelephora terrestris*	0.601	0.420	-0.357
Dixon et al. 1984	*Quercus robur*	*Suillis luteus*	0.477	0.307	-0.206
Dixon et al. 1984	*Quercus robur*	*Cenococcum geophilum*	0.601	0.411	-0.267
Dixon et al. 1984	*Quercus velutina*	*Pisolithuis tinctorius*	0.443	0.209	0.129
Dixon et al. 1984	*Quercus velutina*	*Pisolithus tinctorius*	0.246	0.222	0.352
Dixon et al. 1984	*Quercus velutina*	*Suillus granulatus*	0.413	0.265	0.163
Dixon et al. 1984	*Quercus velutina*	*Thelephora terrestris*	0.443	0.162	-0.042
Dixon et al. 1984	*Quercus velutina*	*Suillis luteus*	0.282	0.241	0.311
Dixon et al. 1984	*Quercus alba*	*Pisolithuis tinctorius*	0.288	0.211	0.208
Dixon et al. 1984	*Quercus alba*	*Pisolithus tinctorius*	0.201	0.305	0.154
Dixon et al. 1984	*Quercus alba*	*Suillus granulatus*	0.065	0.141	0.312
Dixon et al. 1984	*Quercus alba*	*Thelephora terrestris*	0.105	0.148	0.087
Dixon et al. 1984	*Quercus alba*	*Suillis luteus*	0.201	0.205	0.154
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.095	0.136	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.000	0.109	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.501	0.331	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.071	0.134	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	-0.025	0.080	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.476	0.305	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.048	0.152	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.024	0.138	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.491	0.352	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.000	0.088	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.000	0.111	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.243	0.211	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.023	0.080	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.190	0.095	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.171	0.203	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.023	0.088	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.151	0.095	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.190	0.196	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.223	0.010	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.377	-0.095	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.583	0.310	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.298	0.049	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.391	-0.106	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.649	0.303	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.189	0.010	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.318	-0.062	
Dixon et al. 1987	*Pinus taeda*	*Pisolithus tinctorius*	0.606	0.307	
Dunabeitia et al. 2004	*Pinus radiata*	*Rhizopogon luteolus*		0.075	
Dunabeitia et al. 2004	*Pinus radiata*	*Rhizopogon roseolus*		0.206	
Dunabeitia et al. 2004	*Pinus radiata*	*Scleroderma citrinum*		0.079	
Dunabeitia et al. 2004	*Pinus radiata*	*Rhizopogon luteolus*		0.023	
Dunabeitia et al. 2004	*Pinus radiata*	*Rhizopogon roseolus*		0.143	
Dunabeitia et al. 2004	*Pinus radiata*	*Scleroderma citrinum*		0.175	
Duponnois et al. 2000	*Acacia holosericea*	*Pisolithus tinctorius*	0.621		0.715
Garbaye et al. 1988	*Eucalyptus urophylla x E. kirtoniana*	*Pisolithus tinctorius*		0.215	
Garbaye et al. 1988	*Eucalyptus urophylla x E. kirtoniana*	*Scleroderma texense*		0.148	
Garbaye et al. 1988	*Eucalyptus urophylla x E. kirtoniana*	*Scleroderma aurantium*		0.000	
Garbaye et al. 1988	*Eucalyptus urophylla x E. kirtoniana*	*Hebeloma cylindrosporum*		-0.041	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Garbaye et al. 1988	*Eucalyptus urophylla x E. kirtoniana*	*Scleroderma dictysporum*		-0.083	
Garbaye et al. 1988	*Eucalyptus urophylla x E. kirtoniana*	*Pisolithis tinctorius*		-0.083	
Grandcourt et al. 2004	*Dicorynia guianensis*	*unknown*	0.342		
Grandcourt et al. 2004	*Eperua falcata*	*unknown*	-0.209		
Heinrich et al. 1988	*Eucalyptus pilularis*	*Pisolithus tinctorius*	1.188		
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.352	0.154	0.158
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.388	0.373	0.412
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.385	0.270	0.312
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.384	0.194	0.297
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.277	0.223	0.260
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.390	0.305	0.420
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.298	0.134	0.283
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	-0.039	0.046	0.162
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.104	0.097	0.121
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	-0.137	0.000	0.119
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	-0.136	-0.074	0.001
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	-0.193	-0.133	0.539
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	-0.266	-0.166	0.163
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	-0.029	-0.017	-0.511
Hung & Molina 1986	*Pseudotsuga menziesii*	*Laccaria laccata*	0.021	-0.082	-0.106
Ivory & Munga 1983	*Pinus caribaea*	*Pisolithis tinctorius*		-0.130	
Ivory & Munga 1983	*Pinus caribaea*	*Rhizopogon nigrescens*		-0.130	
Ivory & Munga 1983	*Pinus caribaea*	*Scleroderma bovista*		-0.109	
Ivory & Munga 1983	*Pinus caribaea*	*Scleroderma texense*		0.048	
Ivory & Munga 1983	*Pinus caribaea*	*Thelephora terrestris*		0.065	
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.261		-0.129
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.061		-0.294

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.361		-0.294
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.319		0.133
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.334		-0.217
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.013		-0.224
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.410		0.096
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.022		-0.121
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.280		-0.197
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.335		-0.370
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.242		0.072
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.277		-0.198
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.076		0.035
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.300		-0.254
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.247		-0.229
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.061		0.033
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.230		-0.167
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.270		-0.121
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.337		-0.166
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.141		0.079
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.220		0.084
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.329		-0.143
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.149		-0.279
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.166		0.025
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.172		0.026
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.053		0.139
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.364		-0.066
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.346		0.095
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.194		0.043

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.337		0.093
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.276		-0.142
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.321		0.010
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.119		0.004
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.222		0.120
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.098		0.006
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.136		-0.045
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.170		0.011
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.232		0.021
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.058		0.017
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.018		-0.009
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.268		0.059
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.332		0.067
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.312		0.032
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.067		0.108
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.029		0.072
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.130		0.057
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.002		0.238
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.269		0.073
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.131		-0.040
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.039		-0.213
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.051		0.051
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.050		0.054
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.010		0.103
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.144		0.058
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.370		-0.199
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.235		0.008

Authors	Host species	Fungal species	Ln R		
			Biomass	Height	Shoot:root
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.337		0.093
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.276		-0.142
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.321		0.010
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.119		0.004
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.222		0.120
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.098		0.006
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.136		-0.045
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.170		0.011
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.232		0.021
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.058		0.017
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.018		-0.009
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.268		0.059
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.332		0.067
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.312		0.032
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.067		0.108
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.029		0.072
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.130		0.057
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.002		0.238
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.269		0.073
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.131		-0.040
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.039		-0.213
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.051		0.051
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.050		0.054
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	0.010		0.103
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.144		0.058
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.370		-0.199
Lamhamedi et al. 1990	*Pinus pinaster*	*Pisolithus arhizus*	-0.235		0.008

Authors	Host species	Fungal species	Ln R Biomass	Height	Shoot:root
Lu et al. 1998	*Eucalyptus globulus*	*Hydnangium submellatum*		-0.026	
Lu et al. 1998	*Eucalyptus globulus*	*Hdynotrya sp*		-0.042	
Lu et al. 1998	*Eucalyptus globulus*	*Hydnum repandum*		-0.030	
Lu et al. 1998	*Eucalyptus globulus*	*Laccaria lateritia*		-0.002	
Lu et al. 1998	*Eucalyptus globulus*	*Laccaria lateritia*		0.000	
Lu et al. 1998	*Eucalyptus globulus*	*Laccaria laccata*		-0.007	
Lu et al. 1998	*Eucalyptus globulus*	*Laccaria sp*		0.021	
Lu et al. 1998	*Eucalyptus globulus*	*Leucopaxillus lilacinus*		-0.023	
Lu et al. 1998	*Eucalyptus globulus*	*Mesophellia*		-0.144	
Lu et al. 1998	*Eucalyptus globulus*	*Mesophellia*		-0.023	
Lu et al. 1998	*Eucalyptus globulus*	*Paxillus muelleri*		-0.014	
Lu et al. 1998	*Eucalyptus globulus*	*Paxillus sp*		-0.062	
Lu et al. 1998	*Eucalyptus globulus*	*Pisolithus albus*		-0.055	
Lu et al. 1998	*Eucalyptus globulus*	*Pisolithus microcarpus*		-0.035	
Lu et al. 1998	*Eucalyptus globulus*	*Pisolithus tinctorius*		-0.199	
Lu et al. 1998	*Eucalyptus globulus*	*Pisolithus sp*		-0.007	
Lu et al. 1998	*Eucalyptus globulus*	*Pisolithus sp*		-0.030	
Lu et al. 1998	*Eucalyptus globulus*	*Scleroderma cepa*		-0.026	
Lu et al. 1998	*Eucalyptus globulus*	*Scleroderma cepa*		0.016	
Lu et al. 1998	*Eucalyptus globulus*	*Scleroderma cepa*		-0.112	
Lu et al. 1998	*Eucalyptus globulus*	*Scleroderma sp*		-0.123	
Lu et al. 1998	*Eucalyptus globulus*	*Scleroderma sp*		-0.125	
Lu et al. 1998	*Eucalyptus globulus*	*Tricholoma sp*		0.016	
MacFall & Slack 1991	*Pinus resinosa*	*Thelephora terrestris*			
MacFall & Slack 1991	*Pinus resinosa*	*Pisolithus tinctorius*			
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*			
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.398	0.070	-0.425

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.171	0.050	-0.386
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.398	0.050	0.086
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.023	0.017	0.038
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.134		-0.013
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.266		0.042
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.294		0.045
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.132		0.058
MacFall & Slack 1991	*Pinus resinosa*	*Pisolithus tinctorius*	0.149		-0.112
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.069		-0.072
MacFall & Slack 1991	*Pinus resinosa*	*Pisolithus tinctorius*	0.233		-0.024
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.285		-0.017
MacFall & Slack 1991	*Pinus resinosa*	*Pisolithus tinctorius*	0.304		0.163
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*	0.318		0.199
MacFall & Slack 1991	*Pinus resinosa*	*Pisolithus tinctorius*			
MacFall & Slack 1991	*Pinus resinosa*	*Hebeloma arenosa*			
MacFall et al. 1991	*Pinus resinosa*	*Hebeloma arenosa*	2.187		-0.802
Marx et al. 1976	*Pinus clausa*	*Pisolithus tinctorius*	0.284		
Marx et al. 1976	*Pinus clausa*	*Pisolithus tinctorius*	0.464		
Marx et al. 1976	*Pinus taeda*	*Pisolithus tinctorius*	0.035		
Marx et al. 1976	*Pinus taeda*	*Pisolithus tinctorius*	0.063		
Marx et al. 1976	*Pinus elliottii*	*Pisolithus tinctorius*	0.144		
Marx et al. 1976	*Pinus elliottii*	*Pisolithus tinctorius*	0.165		
Marx et al. 1976	*Pinus strobus*	*Pisolithus tinctorius*	0.693	0.261	0.000
Marx et al. 1976	*Pinus strobus*	*Pisolithus tinctorius*	0.118	0.044	0.028
Marx et al. 1976	*Pinus taeda*	*Pisolithus tinctorius*	0.877	0.348	-0.208
Marx et al. 1976	*Pinus taeda*	*Pisolithus tinctorius*	0.603	0.206	-0.321
Marx et al. 1976	*Pinus virginiana*	*Pisolithus tinctorius*	0.732	0.231	-0.455

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Marx et al. 1976	*Pinus virginiana*	*Pisolithus tinctorius*	0.177	0.022	-0.245
Mason et al. 2000	*Eucalyptus globulus*	*Laccaria fraterna*			
Mason et al. 2000	*Eucalyptus globulus*	*Laccaria fraterna*			
Mason et al. 2000	*Eucalyptus globulus*	*Pisolithus tinctorius*			
Morte et al. 2001	*Pinus halapensis*	*Suillus mediterraneansis*	0.105	-0.006	
Morte et al. 2001	*Pinus halapensis*	*Suillus mediterraneansis*	0.103	-0.006	
Muhsin & Zwiazek 2002	*Picea glauca*	*Hebeloma crustuliniforme*	0.125		0.017
Nylund & Wallander 1989	*Pinus sylvestris*	*Hebeloma crustuliniforme*	-0.515		
Nylund & Wallander 1989	*Pinus sylvestris*	*Laccaria laccata*	-0.599		
Osonubi et al. 1991	*Acacia auriculiformis*	*Boletus suillus*	0.726		0.303
Osonubi et al. 1991	*Albizia lebbeck*	*Boletus suillus*	-1.526		-1.282
Osonubi et al. 1991	*Leucaena leucocephala*	*Boletus suillus*	1.127		-0.062
Osonubi et al. 1991	*Gliricidia sepium*	*Boletus suillus*	0.683		-0.404
Repac 1996	*Picea abies*	*Suillus bovinus*	0.000	-0.032	-0.238
Repac 1996	*Picea abies*	*Suillus bovinus*	0.112	0.023	-0.136
Repac 1996	*Picea abies*	*Suillus bovinus*	-0.070	-0.040	-0.107
Repac 1996	*Picea abies*	*Suillus bovinus*	0.050	0.012	-0.076
Repac 1996	*Picea abies*	*Inocybe lacera*	0.157	-0.018	-0.153
Repac 1996	*Picea abies*	*Inocybe lacera*	0.136	0.021	-0.037
Riffle & Tinus 1982	*Pinus ponderosa*	*Rhizopogon roseolus*	0.936	0.095	
Riffle & Tinus 1982	*Pinus ponderosa*	*Suillis granulatus*	0.771	0.013	
Riffle & Tinus 1982	*Pinus ponderosa*	*Thelephora terrestris*	0.736	-0.076	
Riffle & Tinus 1982	*Pinus ponderosa*	*Pisolithus tinctorius*	0.805	0.020	
Riffle & Tinus 1982	*Pinus ponderosa*	*Cenococcum geophilum*	0.906	0.204	
Riffle & Tinus 1982	*Pinus ponderosa*	*unknown*	0.794	0.052	
Riffle & Tinus 1982	*Pinus ponderosa*	*unknown*	0.724	0.026	
Riffle & Tinus 1982	*Pinus sylvestris*	*Suillus cothurnatus*	0.039	-0.055	0.147

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Riffle & Tinus 1982	*Pinus sylvestris*	*Rhizopogon roseolus*	0.136	0.026	0.256
Riffle & Tinus 1982	*Pinus sylvestris*	*Suillus granulatus*	0.122	0.037	0.088
Riffle & Tinus 1982	*Pinus sylvestris*	*Thelephora terrestris*	-0.028	-0.124	0.146
Riffle & Tinus 1982	*Pinus sylvestris*	*Pisolithus tinctorius*	-0.082	-0.083	0.060
Riffle & Tinus 1982	*Pinus sylvestris*	*Cenococcum geophilum*	0.146	-0.011	0.196
Riffle & Tinus 1982	*Pinus sylvestris*	*unknown*	0.039	-0.055	0.190
Riffle & Tinus 1982	*Pinus sylvestris*	*unknown*	0.230	0.016	0.283
Riffle & Tinus 1982	*Pinus ponderosa*	*Rhizopogon roseolus*			
Riffle & Tinus 1982	*Pinus ponderosa*	*Suillis granulatus*			
Riffle & Tinus 1982	*Pinus ponderosa*	*Thelephora terrestris*			
Riffle & Tinus 1982	*Pinus ponderosa*	*Pisolithus tinctorius*			
Riffle & Tinus 1982	*Pinus ponderosa*	*Cenococcum geophilum*			
Riffle & Tinus 1982	*Pinus ponderosa*	*unknown*			
Riffle & Tinus 1982	*Pinus ponderosa*	*unknown*			
Riffle & Tinus 1982	*Pinus sylvestris*	*Suillus cothurnatus*			
Riffle & Tinus 1982	*Pinus sylvestris*	*Rhizopogon roseolus*			
Riffle & Tinus 1982	*Pinus sylvestris*	*Suillus granulatus*			
Riffle & Tinus 1982	*Pinus sylvestris*	*Thelephora terrestris*			
Riffle & Tinus 1982	*Pinus sylvestris*	*Pisolithus tinctorius*			
Riffle & Tinus 1982	*Pinus sylvestris*	*Cenococcum geophilum*			
Riffle & Tinus 1982	*Pinus sylvestris*	*unknown*			
Riffle & Tinus 1982	*Pinus sylvestris*	*unknown*			
Rincon et al. 2001	*Pinus pinea*	*Hebeloma crustulinforme*	-0.125	-0.008	
Rincon et al. 2001	*Pinus pinea*	*Hebeloma crustulinforme*	0.000	-0.058	
Rincon et al. 2001	*Pinus pinea*	*Hebeloma crustulinforme*	-0.092	-0.071	
Rincon et al. 2001	*Pinus pinea*	*Hebeloma crustulinforme*	-0.030	0.039	
Rincon et al. 2001	*Pinus pinea*	*Hebeloma crustulinforme*	-0.061	-0.049	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Shoot:root
Rincon et al. 2001	*Pinus pinea*	*Laccaria laccata*	0.000	0.166	
Rincon et al. 2001	*Pinus pinea*	*Laccaria laccata*	0.000	-0.070	
Rincon et al. 2001	*Pinus pinea*	*Laccaria laccata*	0.027	0.119	
Rincon et al. 2001	*Pinus pinea*	*Laccaria laccata*	0.154	0.005	
Rincon et al. 2001	*Pinus pinea*	*Laccaria laccata*	0.027	0.027	
Rincon et al. 2001	*Pinus pinea*	*Pisolithus tinctorius*	-0.121	-0.020	
Rincon et al. 2001	*Pinus pinea*	*Pisolithus tinctorius*	-0.154	0.044	
Rincon et al. 2001	*Pinus pinea*	*Melanogaster ambiguus*	-0.208	-0.311	
Rincon et al. 2001	*Pinus pinea*	*Melanogaster ambiguus*	-0.043	-0.100	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon luteolus*	-0.262	0.134	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon luteolus*	-0.230	0.064	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon luteolus*	-0.108	-0.012	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon luteolus*	-0.080	-0.012	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon luteolus*	-0.080	0.160	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon luteolus*	-0.026	0.248	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon roseolus*	-0.241	0.180	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon roseolus*	-0.241	0.222	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon roseolus*	-0.241	0.155	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon roseolus*	-0.304	0.166	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon roseolus*	-0.182	0.158	
Rincon et al. 2001	*Pinus pinea*	*Rhizopogon roseolus*	-0.304	0.121	
Rincon et al. 2001	*Pinus pinea*	*Pisolithus tinctorius*	-0.211	-0.120	
Rincon et al. 2001	*Pinus pinea*	*Pisolithus tinctorius*	-0.182	-0.159	
Rincon et al. 2001	*Pinus pinea*	*Pisolithus tinctorius*	-0.049	-0.209	
Rincon et al. 2001	*Pinus pinea*	*Scleroderma verrucosum*	-0.267	-0.046	
Rincon et al. 2001	*Pinus pinea*	*Scleroderma verrucosum*	-0.384	0.080	
Rincon et al. 2001	*Pinus pinea*	*Scleroderma verrucosum*	-0.187	0.126	

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Rincon et al. 2001	*Pinus pinea*	*Scleroderma verrucosum*	-0.239	0.028	
Rincon et al. 2005	*Pseudotsuga menziesii*	*Rhizopogon luteolus*	-0.363	-0.038	
Rincon et al. 2005	*Pseudotsuga menziesii*	*Rhizopogon roseolus*	-0.363	0.000	
Rincon et al. 2005	*Pseudotsuga menziesii*	*Scleroderma verrucosum*	-0.245	-0.099	
Rouhier & Read 1998	*Pinus sylvestris*	*Paxillus involutus*	-0.067		0.320
Rouhier & Read 1998	*Pinus sylvestris*	*Suillus bovinus*	-0.120		0.380
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Laccaria laccata*	0.236	0.167	0.051
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Laccaria laccata*	0.217	0.131	0.138
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Laccaria laccata*	0.090	0.200	0.182
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Rhizopogon vinicolor*	0.612	0.382	-0.305
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Rhizopogon vinicolor*	0.390	0.305	-0.428
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Rhizopogon vinicolor*	0.242	0.243	-0.234
Scagel & Linderman 1998	*Pinus contorta*	*Laccaria laccata*	0.084	0.111	-0.277
Scagel & Linderman 1998	*Pinus contorta*	*Laccaria laccata*	0.032	0.093	-0.447
Scagel & Linderman 1998	*Pinus contorta*	*Laccaria laccata*	0.136	0.048	-0.756
Scagel & Linderman 1998	*Pinus contorta*	*Laccaria laccata*	0.215	0.327	-0.373
Scagel & Linderman 1998	*Pinus contorta*	*Laccaria laccata*	0.370	0.367	0.102
Scagel & Linderman 1998	*Pinus contorta*	*Laccaria laccata*	0.218	0.283	-0.155
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Laccaria laccata*	0.530	0.296	-0.931
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Laccaria laccata*	0.485	0.216	-0.573
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Laccaria laccata*	0.007	0.244	-0.019
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Rhizopogon vinicolor*	0.599	0.132	-0.377
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Rhizopogon vinicolor*	0.457	0.210	-0.809
Scagel & Linderman 1998	*Pseudotsuga menziesii*	*Rhizopogon vinicolor*	0.319	0.263	-0.630
Scagel & Linderman 1998	*Pinus ponderosa*	*Laccaria laccata*	0.167	0.262	0.042
Scagel & Linderman 1998	*Pinus ponderosa*	*Laccaria laccata*	0.192	0.225	-0.070
Scagel & Linderman 1998	*Pinus ponderosa*	*Laccaria laccata*	0.245	-0.016	-0.227

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Scagel & Linderman 1998	*Pinus ponderosa*	*Laccaria laccata*	0.240	0.260	0.212
Scagel & Linderman 1998	*Pinus ponderosa*	*Laccaria laccata*	0.194	0.172	0.198
Scagel & Linderman 1998	*Pinus ponderosa*	*Laccaria laccata*	-0.037	0.050	-0.082
Schier & McQuattie 1995	*Pinus strobis*	*Pisolithus tinctorius*	0.304	0.109	0.700
Schier & McQuattie 1996	*Pinus rigida*	*Pisolithus tinctorius*	0.654		0.193
Schier & McQuattie 1996	*Pinus rigida*	*Pisolithus tinctorius*	0.270		0.171
Tam & Griffiths 1994	*Castanopsis fissa*	*Pisolithis tinctorius*	0.117		-0.480
Tam & Griffiths 1994	*Castanopsis fissa*	*Cenococcum geophilum*	0.256		-0.256
Tam & Griffiths 1994	*Castanopsis fissa*	*Thelephora terrestris*	-0.094		-0.561
Tam & Griffiths 1994	*Castanopsis fissa*	*Hymenogaster*	0.033		-0.623
Tam & Griffiths 1994	*Castanopsis fissa*	*Sclerodema sp*	-0.158		-0.674
Thomson et al. 1994	*Eucalyptus globulus*	*Protubera*	-0.223		
Thomson et al. 1994	*Eucalyptus globulus*	*unknown*	0.148		
Thomson et al. 1994	*Eucalyptus globulus*	*Chondrogaster*	0.336		
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*	-0.174		
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*			
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*			
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*			
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*			
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*			
Thomson et al. 1994	*Eucalyptus globulus*	*Cortinarius*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hysterangium*	0.039		
Thomson et al. 1994	*Eucalyptus globulus*	*Hysterangium*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hysterangium*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hysterangium*			
Thomson et al. 1994	*Eucalyptus globulus*	*Amanita sp*	-1.022		
Thomson et al. 1994	*Eucalyptus globulus*	*Amanita sp*			

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Thomson et al. 1994	*Eucalyptus globulus*	*Amanita sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Amanita sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hydnangium*	0.336		
Thomson et al. 1994	*Eucalyptus globulus*	*Hydnangium*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hydnangium*			
Thomson et al. 1994	*Eucalyptus globulus*	*Zelleromyces*	0.307		
Thomson et al. 1994	*Eucalyptus globulus*	*Zelleromyces*			
Thomson et al. 1994	*Eucalyptus globulus*	*Zelleromyces*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hymenogaster*	0.278		
Thomson et al. 1994	*Eucalyptus globulus*	*Hymenogaster*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hymenogaster*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hymenogaster*			
Thomson et al. 1994	*Eucalyptus globulus*	*Thaxterogaster sp*	0.542		
Thomson et al. 1994	*Eucalyptus globulus*	*Thaxterogaster sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Scleroderma sp*	0.000		
Thomson et al. 1994	*Eucalyptus globulus*	*Scleroderma sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Scleroderma sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Scleroderma sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Scleroderma sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Setchelliogaster sp*	0.732		
Thomson et al. 1994	*Eucalyptus globulus*	*Pisolithus sp*	0.307		
Thomson et al. 1994	*Eucalyptus globulus*	*Pisolithus sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Pisolithus sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Pisolithus sp*			
Thomson et al. 1994	*Eucalyptus globulus*	*Laccaria*	0.365		
Thomson et al. 1994	*Eucalyptus globulus*	*Laccaria*			
Thomson et al. 1994	*Eucalyptus globulus*	*Laccaria*			

Authors	Host species	Fungal species	Ln R Biomass	Ln R Height	Ln R Shoot:root
Thomson et al. 1994	*Eucalyptus globulus*	*Laccaria*			
Thomson et al. 1994	*Eucalyptus globulus*	*Hebeloma*	1.138		
Thomson et al. 1994	*Eucalyptus globulus*	*Descolea*	1.151		
Thomson et al. 1994	*Eucalyptus globulus*	*Descolea*			
Turjaman et al. 2005	*Shorea pinanga*	*Pisolithus arhizus*	1.099	0.619	
Turjaman et al. 2005	*Shorea pinanga*	*Scleroderma sp*	0.930	0.533	
Wallander 2000	*Pinus sylvestris*	*Suillus variegatus*	0.288		
Wallander 2000	*Pinus sylvestris*	*Suillus variegatus*	0.431		
Wallander 2000	*Pinus sylvestris*	*Suillus variegatus*	0.693		
Wallander 2000	*Pinus sylvestris*	*Suillus variegatus*	0.526		
Wallander 2000	*Pinus sylvestris*	*unknown*	1.386		
Wallander 2000	*Pinus sylvestris*	*unknown*	0.932		
Wallander et al. 1997	*Pinus sylvestris*	*Piloderma croceum*			
Wallander et al. 1997	*Pinus sylvestris*	*Paxillus involutus*			
Wallander et al. 1997	*Pinus sylvestris*	*Suillus variegatus*			
Wallander et al. 1997	*Pinus sylvestris*	*Suillus variegatus*			
Yazid et al. 1994	*Hopea odorata*	*Pisolithus tinctorius*	2.122	0.617	0.155
Yazid et al. 1994	*Hopea helferi*	*Pisolithus tinctorius*	1.215	0.496	0.239

Full citation of each study used in meta-analysis (excluding studies involving manipulations of nutrients)

Study	Full citation
Baum et al. 2000	Baum C, Schmid K, Makeschin F. 2000. Interactive effects of substrates and ectomycorrhizal colonization on growth of a poplar clone. Journal of Plant Nutrition and Soil Science 163: 221-226
Baum et al. 2002	Baum C, Stetter U, Makeschin F. 2002. Growth response of *Populus trichocarpa* to inoculation by the ectomycorrhizal fungus *Laccaria laccata* in a pot and a field experiment. Forest Ecology and Management 163: 1-8
Baumann et al. 2005	Baumann K, Schneider BU, Marschner P, Huttl RF. 2005. Root distribution and nutrient status of mycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings growing in a sandy substrate with lignite fragments. Plant and Soil 276: 347-357
Beyeler & Heyser 1997	Beyeler M, Heyser W. 1997. The influence of mycorrhizal colonization on growth in the greenhouse and on catechin, epicatechin and procyanidin in roots of *Fagus sylvatica* L. Mycorrhiza 7: 171-177
Bougher et al. 1990	Bougher NL, Grove TS, Malajczuk N. 1990. Growth and phosphorus acquisition of Karri (*Eucalyptus diversicolor* F-Muell) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. New Phytologist 114: 77-85

Study	Full citation
Browning & Whitney 1991	Browning MHR, Whitney RD. 1991. Responses of jack pine and black spruce seedlings to inoculation with selected species of ectomycorrhiza fungi. Canadian Journal of Forest Research 21: 701-706
Burgess & Malajczuk 1989	Burgess T, Malajczuk N. 1989. The effects of ectomycorrhizal fungi on reducing the variation of seedling growth of *Eucalytpus globulus*. Agriculture Ecosystems and the Environment 28: 41-46
Burgess et al. 1994	Burgess, T, Dell B, Malajczuk N. 1994. Variation in mycorrhizal development and growth stimulation by 20 *Pisolithus* isolates inoculated on to *Eucalyptus grandis* W Hill Ex Maiden. New Phytologist 127: 731-739
Chakravarty & Unestam 1987	Chakravarty P, Unestam T. 1987. Differential influence of ectomycorrhizae on plant-growth and disease resistance in *Pinus sylvestris* seedlings. Journal of Phytopathology 120: 104-120
Chen et al. 2006	Chen YL, Dell B, Malajczuk N. 2006. Effect of *Scleroderma* spore density and age on mycorrhiza formation and growth of containerized *Eucalyptus globulus* and E-Urophylla seedlings. New Forests 31: 453-467
Choi et al. 2005	Choi DS, Quoreshi AM, Maruyama Y, Jin HO, Koike T. 2005. Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of *Pinus densiflora* seedlings grown under elevated CO2 concentrations. Photosynthetica 43: 223-229

Study	Full citation
Conjeaud et al. 1996	Conjeaud C, Scheromm P, Mousain D. 1996. Effects of phosphorus and ectomycorrhiza on maritime pine seedlings (*Pinus pinaster*). New Phytologist 133: 345-351
Diedhiou et al. 2005	Diedhiou AG, Gueye O, Diabate M, Prin Y, Duponnois R, Dreyfus B, Ba AM. 2005. Contrasting responses to ectomycorrhizal inoculation in seedlings of six tropical African tree species. Mycorrhiza 16: 11-17
Dixon et al. 1981	Dixon RK, Wright GM, Garrett HE, Cox GS, Johnson PS, Sander IL. 1981. Container-grown and nursery-grown black oak seedlings inoculated with *Pisolithis tincortius*- growth and ectomycorrhizal development during seedling production period. Canadian Journal of Forest Research 11: 487-491
Dixon et al. 1983	Dixon RK, Pallardy SG, Garrett HE, Cox GS, Sander IL. 1983. Comparative water relations of container-grown and bare-root ectomycorrhizal and nonmycorrhizal *Quercus velutina* seedlings. Canadian Journal of Botany 61: 1559-1565
Dixon et al. 1984	Dixon RK, Garrett HE, Cox GS, Marx DH, Sander IL. 1984. Inoculation of 3 *Quercus* species with 11 isolates of ectomycorrhizal fungi. 1. Inoculation success and seedling growth relationships. Forest Science 30: 364-372
Dixon et al. 1987	Dixon RK, Garrett HE, Stelzer HE. 1987. Growth and ectomycorrhizal development of Loblolly pine progenies inoculated with 3 isolates of *Pisolithus tinctorius*. Silvae Genetica 36: 240-245 1987

Study	Full citation
Dunabetia et al. 2004	Dunabeitia MK, Hormilla S, Garcia-Plazaola JI, Txarterina K, Arteche U, Becerril JM. 2004. Differential responses of three fungal species to environmental factors and their role in the mycorrhization of *Pinus radiata* D. Don. Mycorrhiza 14: 11-18
Duponnois et al. 2000	Duponnois R, Founoune H, Ba A, Plenchette C, El Jaafari S, Neyra M, Ducousso M. 2000. Ectomycorrhization of *Acacia holosericea* A. Cunn. ex G. Don by *Pisolithus* spp. in Senegal: Effect on plant growth and on the root-knot nematode *Meloidogyne javanica.* Annals of Forest Science 57: 345-350
Garbaye et al. 1988	Garbaye J, Delwaulle JC, Diangana D. 1988. Growth response of *Eucalyptus* in the Congo to ectomycorrhizal inoculation. Forest Ecology and Management 24: 151-157
Grandcourt et al. 2004	de Grandcourt A, Epron D, Montpied P, Louisanna E, Bereau M, Garbaye J, Guehl JM . 2004. Contrasting responses to mycorrhizal inoculation and phosphorus availability in seedlings of two tropical rainforest tree species. New Phytologist 161: 865-875
Heinrich et al. 1988	Heinrich PA, Mulligan DR, Patrick JW. 1988. The effect of ectomycorrhizas on the phosphorus and dry-weight acquisition of *Eucalyptus* seedlings. Plant and Soil 109: 147-149
Hung & Molina 1986	Hung LLL, Molina R. 1986. Use of the ectomycorrhizal fungus *Laccaria laccata* in forestry. 3. Effects of commercially produced inoculum on container-grown Douglas-fir and ponderosa pine seedlings. Canadian Journal of Forest Research 16: 802-806

Study	Full citation
Ivory & Munga 1983	Ivory MH, Munga FM. 1983. Growth and survival of container grown *Pinus caribaea* infected with various ectomycorrhizal fungi. Plant and Soil 71: 339-344
Lamhamedi et al. 1990	Lamhamedi MS, Fortin JA, Kope HH, Kropp BR. 1990. Genetic variation in ectomycorrhiza formation by *Pisolithus arhizus* on *Pinus pinaster* and *Pinus banksiana*. New Phytologist115: 689-697
Lu et al. 1998	Lu XH, Malajczuk N, Dell B. 1998. Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. Mycorrhiza 8: 81-86
MacFall & Slack 1991	MacFall JS, Slack SA. 1991. Effects of *Hebeloma arenosa* on growth and survival of container-grown red pine seedlings (*Pinus resinosa*). Canadian Journal of Forrest Research 21: 1459-1465
MacFall et al. 1991	Macfall J, Slack SA, Iyer J. 1991. Effects of *Hebeloma arenosa* and phosphorus fertility on growth of red pine (*Pinus resinosa*) seedlings. Canadian Journal of Botany 69: 372-379
Marx et al. 1976	Marx DH, Brysan WC, Cordell CE. 1976. Growth and ectomycorrhizal development of Pine seedlings in nursery soils infested with fungal symbiont *Pisolithus tinctorius*. Forest Science 22: 91-100
Mason et al. 2000	Mason PA, Ibrahim K, Ingleby K, Munro RC, Wilson J. 2000. Mycorrhizal development and growth of inoculated *Eucalyptus globulus* (Labill.) seedlings in wet and dry conditions in the glasshouse. Forest Ecology and Management 128: 269-277

Study	Full citation
Morte et al. 2001	Morte A, Diaz G, Rodriguez P, Alarcon JJ, Sanchez-Blanco MJ. 2001. Growth and water relations in mycorrhizal and nonmycorrhizal *Pinus halepensis* plants in response to drought. Biologia Plantarum 44: 263-267 2001
Muhsin & Zwiazek 2002	Muhsin TM, Zwiazek JJ. 2002. Colonization with *Hebeloma crustuliniforme* increases water conductance and limits shoot sodium uptake in white spruce (*Picea glauca*) seedlings. Plant and Soil 238: 217-225
Nylund & Wallander 1989	Nylund JE, Wallander H. 1989. Effects of ectomycorrhiza on host growth and carbon balance in a semi-hydroponic cultivation system. New Phytologist 112: 389-398
Osonubi et al. 1991	Osonubi O, Mulongoy K, Awotoye OO, Atayese MO, Okali DUU. 1991. Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of 4 leguminous woody seedlings. Plant and Soil 136: 131-141
Repac 1996	Repac I. 1996. Inoculation of *Picea abies* (L) Karst seedlings with vegetative inocula of ectomycorrhizal fungi *Suillus bovinus* (L: Fr) O. Kuntze and *Inocybe lacera* (Fr) Kumm. New Forests 12: 41-54
Riffle & Tinus 1982	Riffle JW, Tinus RW. 1982. Ectomycorrhizal characteristics, growth, and survival of artificially inoculated ponderosa pine and Scots pine in a greenhouse and plantation. Forest Science 28: 646-660

Study	Full citation
Rincon et al. 2001	Rincon A, Alvarez IF, Pera J. 2001. Inoculation of containerized *Pinus pinea* L. seedlings with seven ectomycorrhizal fungi. Mycorrhiza 11: 265-271
Rincon et al. 2005	Rincon A, Parlade J, Pera J. 2005. Effects of ectomycorrhizal inoculation and the type of substrate on mycorrhization, growth and nutrition of containerised *Pinus pinea* L. seedlings produced in a commercial nursery. Annals of Forest Science 62: 817-822
Rouhier & Read 1998	Rouhier H, Read DJ. 1998. Plant and fungal responses to elevated atmospheric carbon dioxide in mycorrhizal seedlings of *Pinus sylvestris*. Environmental and Experimental Botany 40: 237-246
Scagel & Linderman 1998	Scagel CF, Linderman RG. 1998. Influence of ectomycorrhizal fungal inoculation on growth and root IAA concentrations of transplanted conifers. Tree Physiology 18: 739-747
Schier & McQuattie 1995	Schier GA, McQuattie CJ. 1995. Effect of aluminum on the growth, anatomy, and nutrient content of ectomycorrhizal and nonectomycorrhizal eastern white pine seedlings. Canadian Journal of Forest Research 25: 1252-1262
Schier & McQuattie 1996	Schier GA, McQuattie CJ. 1996. Response of ectomycorrhizal and nonmycorrhizal pitch pine (*Pinus rigida*) seedlings to nutrient supply and aluminum: Growth and mineral nutrition. Canadian Journal of Forest Research 26: 2145-2152

Study	Full citation
Tam & Griffiths 1994	Tam PCF, Griffiths DA. 1994. Mycorrhizal associations in Hong Kong *Fagaceae*. 6. Growth and nutrient uptake by *Castanopsis fissa* seedlings inoculated with ectomycorrhizal fungi. Mycorrhiza 4: 169-172
Thomson et al. 1994	Thomson BD, Grove TS, Malajczuk N, Hardy GESJ. 1994. The effectiveness of ectomycorrhizal fungi in increasing the growth of *Eucalyptus globulus* Labill in relation to root colonization and hyphal development in soil. New Phytologist 126: 517-524
Turjaman et al. 2005	Turjaman M, Tamai Y, Segah H, Limin SH, Cha JY, Osaki M, Tawaraya K. 2005. Inoculation with the ectomycorrhizal fungi *Pisolithus arhizus* and *Scleroderma* sp improves early growth of *Shorea pinanga* nursery seedlings. New Forests 30: 67-73
Wallander 2000	Wallander H. 2000. Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. Plant and Soil 218: 249-256
Wallander et al. 1997	Wallander H, Wickman T, Jacks G. 1997. Apatite as a P source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Plant and Soil 196: 123-131
Yazid et al. 1994	Yazid SM, Lee SS, Lapeyrie F. 1994. Growth stimulation of *Hopea* spp (*Dipterocarpaceae*) seedlings following ectomycorrhizal inoculation with an exotic strain of *Pisolithus tinctorius*. Forest Ecology and Management 67: 339-343

B. Identity of host plant and fungal species pairings with associated effect sizes (Ln R) for seedling biomass.

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	0	0.894
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	0	0.368
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	0	0.575
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	2	1.170
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	2	1.170
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	2	2.335
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	4	2.197
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	4	2.147
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	4	3.050
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	8	1.603
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	8	1.518
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	8	1.937
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	12	0.360
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	12	0.754
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	12	0.873
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	16	0.182
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	16	0.416
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	16	0.341
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	20	-0.259
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	20	-0.288
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	20	-0.386
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	28	-0.062
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	28	-0.023

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	28	-0.150
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	36	-0.168
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	36	-0.002
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	36	0.013
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	48	-0.056
Bougher et al. 1990	*Eucalyptus diversicolor*	*Descolea maculata*	48	0.047
Bougher et al. 1990	*Eucalyptus diversicolor*	*Laccaria laccata*	48	-0.064
Browning & Whitney 1992	*Picea mariana*	*Laccaria bicolor*	1.5	0.275
Browning & Whitney 1992	*Picea mariana*	*Laccaria bicolor*	1.5	0.389
Browning & Whitney 1992	*Pinus banksiana*	*Laccaria bicolor*	1.5	0.512
Browning & Whitney 1992	*Pinus banksiana*	*Laccaria bicolor*	1.5	0.144
Browning & Whitney 1992	*Picea mariana*	*Laccaria bicolor*	7.2	-0.089
Browning & Whitney 1992	*Picea mariana*	*Laccaria bicolor*	7.2	-0.131
Browning & Whitney 1992	*Pinus banksiana*	*Laccaria bicolor*	7.2	0.331
Browning & Whitney 1992	*Pinus banksiana*	*Laccaria bicolor*	7.2	0.134
Burgess et al. 1993	*Eucalyptus globulus*	*Cortinarius globuliformis*	4	0.560
Burgess et al. 1994	*Eucalyptus globulus*	*Paxillus muelleri*	4	0.560
Burgess et al. 1995	*Eucalyptus globulus*	*Hysterangium inflatum*	4	0.118
Burgess et al. 1996	*Eucalyptus globulus*	*Hysterangium inflatum*	4	0.629
Burgess et al. 1997	*Eucalyptus globulus*	*Thaxterogaster sp*	4	0.694
Burgess et al. 1998	*Eucalyptus globulus*	*Amanita xanthocephala*	4	0.755
Burgess et al. 1999	*Eucalyptus globulus*	*Hymenogaster zeylanicus*	4	0.694
Burgess et al. 2000	*Eucalyptus globulus*	*Hymenogaster viscidus*	4	1.057
Burgess et al. 2001	*Eucalyptus globulus*	*Hymenogaster zeylanicus*	4	1.355
Burgess et al. 2002	*Eucalyptus globulus*	*Setchelliogaster sp*	4	1.099
Burgess et al. 2003	*Eucalyptus globulus*	*Descolea maculata*	4	1.771
Burgess et al. 2004	*Eucalyptus globulus*	*Hydnangium carneum*	4	1.682

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Burgess et al. 2005	*Eucalyptus globulus*	*Laccaria laccata*	4	1.505
Burgess et al. 2006	*Eucalyptus globulus*	*Laccaria laccata*	4	1.853
Burgess et al. 2007	*Eucalyptus globulus*	*Sclerodema verrucosum*	4	1.771
Burgess et al. 2008	*Eucalyptus globulus*	*Pisolithis tinctorius*	4	2.421
Burgess et al. 2009	*Eucalyptus diversicolor*	*Cortinarius globuliformis*	4	0.516
Burgess et al. 2010	*Eucalyptus diversicolor*	*Paxillus muelleri*	4	0.921
Burgess et al. 2011	*Eucalyptus diversicolor*	*Hysterangium inflatum*	4	0.307
Burgess et al. 2012	*Eucalyptus diversicolor*	*Hysterangium inflatum*	4	0.429
Burgess et al. 2013	*Eucalyptus diversicolor*	*Thaxterogaster sp*	4	0.997
Burgess et al. 2014	*Eucalyptus diversicolor*	*Amanita xanthocephala*	4	0.544
Burgess et al. 2015	*Eucalyptus diversicolor*	*Hymenogaster zeylanicus*	4	1.119
Burgess et al. 2016	*Eucalyptus diversicolor*	*Hymenogaster viscidus*	4	0.806
Burgess et al. 2017	*Eucalyptus diversicolor*	*Hymenogaster zeylanicus*	4	1.444
Burgess et al. 2018	*Eucalyptus diversicolor*	*Setchelliogaster sp*	4	1.365
Burgess et al. 2019	*Eucalyptus diversicolor*	*Descolea maculata*	4	1.805
Burgess et al. 2020	*Eucalyptus diversicolor*	*Hydnangium carneum*	4	1.371
Burgess et al. 2021	*Eucalyptus diversicolor*	*Laccaria laccata*	4	1.959
Burgess et al. 2022	*Eucalyptus diversicolor*	*Laccaria laccata*	4	2.208
Burgess et al. 2023	*Eucalyptus diversicolor*	*Sclerodema verrucosum*	4	1.914
Burgess et al. 2024	*Eucalyptus diversicolor*	*Pisolithis tinctorius*	4	2.714
Burgess et al. 2025	*Eucalyptus globulus*	*Cortinarius globuliformis*	12	0.055
Burgess et al. 2026	*Eucalyptus globulus*	*Paxillus muelleri*	12	0.042
Burgess et al. 2027	*Eucalyptus globulus*	*Hysterangium inflatum*	12	0.002
Burgess et al. 2028	*Eucalyptus globulus*	*Hysterangium inflatum*	12	0.017
Burgess et al. 2029	*Eucalyptus globulus*	*Thaxterogaster sp*	12	0.045
Burgess et al. 2030	*Eucalyptus globulus*	*Amanita xanthocephala*	12	0.005
Burgess et al. 2031	*Eucalyptus globulus*	*Hymenogaster zeylanicus*	12	-0.008

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Burgess et al. 2032	*Eucalyptus globulus*	*Hymenogaster viscidus*	12	0.034
Burgess et al. 2033	*Eucalyptus globulus*	*Hymenogaster zeylanicus*	12	-0.007
Burgess et al. 2034	*Eucalyptus globulus*	*Setchelliogaster sp*	12	-0.034
Burgess et al. 2035	*Eucalyptus globulus*	*Descolea maculata*	12	0.023
Burgess et al. 2036	*Eucalyptus globulus*	*Hydnangium carneum*	12	0.089
Burgess et al. 2037	*Eucalyptus globulus*	*Laccaria laccata*	12	-0.061
Burgess et al. 2038	*Eucalyptus globulus*	*Laccaria laccata*	12	0.065
Burgess et al. 2039	*Eucalyptus globulus*	*Sclerodema verrucosum*	12	0.012
Burgess et al. 2040	*Eucalyptus globulus*	*Pisolithis tinctorius*	12	0.040
Burgess et al. 2041	*Eucalyptus diversicolor*	*Cortinarius globuliformis*	12	-0.317
Burgess et al. 2042	*Eucalyptus diversicolor*	*Paxillus muelleri*	12	-0.380
Burgess et al. 2043	*Eucalyptus diversicolor*	*Hysterangium inflatum*	12	-0.379
Burgess et al. 2044	*Eucalyptus diversicolor*	*Hysterangium inflatum*	12	-0.178
Burgess et al. 2045	*Eucalyptus diversicolor*	*Thaxterogaster sp*	12	-0.124
Burgess et al. 2046	*Eucalyptus diversicolor*	*Amanita xanthocephala*	12	-0.106
Burgess et al. 2047	*Eucalyptus diversicolor*	*Hymenogaster zeylanicus*	12	-0.541
Burgess et al. 2048	*Eucalyptus diversicolor*	*Hymenogaster viscidus*	12	-0.156
Burgess et al. 2049	*Eucalyptus diversicolor*	*Hymenogaster zeylanicus*	12	-0.007
Burgess et al. 2050	*Eucalyptus diversicolor*	*Setchelliogaster sp*	12	-0.119
Burgess et al. 2051	*Eucalyptus diversicolor*	*Descolea maculata*	12	-0.101
Burgess et al. 2052	*Eucalyptus diversicolor*	*Hydnangium carneum*	12	0.097
Burgess et al. 2053	*Eucalyptus diversicolor*	*Laccaria laccata*	12	-0.205
Burgess et al. 2054	*Eucalyptus diversicolor*	*Laccaria laccata*	12	-0.065
Burgess et al. 2055	*Eucalyptus diversicolor*	*Sclerodema verrucosum*	12	-0.081
Burgess et al. 2056	*Eucalyptus diversicolor*	*Pisolithis tinctorius*	12	-0.001
Chen et al. 2000	*Eucalyptus globulus*	*Laccaria lateritia*	5	0.368
Chen et al. 2000	*Eucalyptus urophylla*	*Laccaria lateritia*	5	2.048

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Conjeaud et al. 1996	*Pinus pinaster*	*Hebeloma cylindrosporum*	0	
Grandcourt et al. 2004	*Dicorynia guianensis*	*unknown*	0	0.342
Grandcourt et al. 2004	*Eperua falcata*	*unknown*	0	-0.209
Grandcourt et al. 2004	*Dicorynia guianensis*	*unknown*	8	1.228
Grandcourt et al. 2004	*Eperua falcata*	*unknown*	8	0.312
Grandcourt et al. 2004	*Dicorynia guianensis*	*unknown*	40	1.226
Grandcourt et al. 2004	*Eperua falcata*	*unknown*	40	0.344
Khasa et al. 2001	*Pinus contorta*	*Hebeloma longicaudum*	18	0.902
Khasa et al. 2001	*Pinus contorta*	*Laccaria bicolor*	18	0.853
Khasa et al. 2001	*Pinus contorta*	*Paxillus involutus*	18	0.936
Khasa et al. 2001	*Pinus contorta*	*Pisolithis tinctorius*	18	0.964
Khasa et al. 2001	*Pinus contorta*	*Rhizopogon vinicolor*	18	-0.015
Khasa et al. 2001	*Pinus contorta*	*Suillis tomentosus*	18	0.014
Khasa et al. 2001	*Picea glauca*	*Hebeloma longicaudum*	18	0.764
Khasa et al. 2001	*Picea glauca*	*Laccaria bicolor*	18	0.852
Khasa et al. 2001	*Picea glauca*	*Paxillus involutus*	18	1.246
Khasa et al. 2001	*Picea glauca*	*Pisolithis tinctorius*	18	1.222
Khasa et al. 2001	*Picea glauca*	*Rhizopogon vinicolor*	18	0.109
Khasa et al. 2001	*Picea glauca*	*Suillis tomentosus*	18	0.016
Khasa et al. 2001	*Picea mariana*	*Hebeloma longicaudum*	18	0.976
Khasa et al. 2001	*Picea mariana*	*Laccaria bicolor*	18	1.152
Khasa et al. 2001	*Picea mariana*	*Paxillus involutus*	18	1.677
Khasa et al. 2001	*Picea mariana*	*Pisolithis tinctorius*	18	1.366
Khasa et al. 2001	*Picea mariana*	*Rhizopogon vinicolor*	18	0.298
Khasa et al. 2001	*Picea mariana*	*Suillis tomentosus*	18	0.328
Khasa et al. 2001	*Pinus sylvestris*	*Hebeloma longicaudum*	18	0.066
Khasa et al. 2001	*Pinus sylvestris*	*Laccaria bicolor*	18	-0.040

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Khasa et al. 2001	*Pinus sylvestris*	*Paxillus involutus*	18	0.176
Khasa et al. 2001	*Pinus sylvestris*	*Pisolithis tinctorius*	18	0.152
Khasa et al. 2001	*Pinus sylvestris*	*Rhizopogon vinicolor*	18	-0.034
Khasa et al. 2001	*Pinus sylvestris*	*Suillis tomentosus*	18	1.615
Khasa et al. 2001	*Larix sibirica*	*Hebeloma longicaudum*	18	0.074
Khasa et al. 2001	*Larix sibirica*	*Laccaria bicolor*	18	0.148
Khasa et al. 2001	*Larix sibirica*	*Paxillus involutus*	18	0.099
Khasa et al. 2001	*Larix sibirica*	*Pisolithis tinctorius*	18	0.084
Khasa et al. 2001	*Larix sibirica*	*Rhizopogon vinicolor*	18	0.027
Khasa et al. 2001	*Larix sibirica*	*Suillis tomentosus*	18	0.043
Khasa et al. 2001	*Pinus contorta*	*Hebeloma longicaudum*	37	0.781
Khasa et al. 2001	*Pinus contorta*	*Laccaria bicolor*	37	0.755
Khasa et al. 2001	*Pinus contorta*	*Paxillus involutus*	37	0.908
Khasa et al. 2001	*Pinus contorta*	*Pisolithis tinctorius*	37	0.862
Khasa et al. 2001	*Pinus contorta*	*Rhizopogon vinicolor*	37	0.016
Khasa et al. 2001	*Pinus contorta*	*Suillis tomentosus*	37	0.128
Khasa et al. 2001	*Picea glauca*	*Hebeloma longicaudum*	37	0.982
Khasa et al. 2001	*Picea glauca*	*Laccaria bicolor*	37	1.061
Khasa et al. 2001	*Picea glauca*	*Paxillus involutus*	37	1.281
Khasa et al. 2001	*Picea glauca*	*Pisolithis tinctorius*	37	1.135
Khasa et al. 2001	*Picea glauca*	*Rhizopogon vinicolor*	37	0.140
Khasa et al. 2001	*Picea glauca*	*Suillis tomentosus*	37	0.131
Khasa et al. 2001	*Picea mariana*	*Hebeloma longicaudum*	37	0.688
Khasa et al. 2001	*Picea mariana*	*Laccaria bicolor*	37	1.025
Khasa et al. 2001	*Picea mariana*	*Paxillus involutus*	37	1.217
Khasa et al. 2001	*Picea mariana*	*Pisolithis tinctorius*	37	1.134
Khasa et al. 2001	*Picea mariana*	*Rhizopogon vinicolor*	37	0.057

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Khasa et al. 2001	*Picea mariana*	*Suillis tomentosus*	37	0.075
Khasa et al. 2001	*Pinus sylvestris*	*Hebeloma longicaudum*	37	-0.010
Khasa et al. 2001	*Pinus sylvestris*	*Laccaria bicolor*	37	0.044
Khasa et al. 2001	*Pinus sylvestris*	*Paxillus involutus*	37	0.334
Khasa et al. 2001	*Pinus sylvestris*	*Pisolithis tinctorius*	37	0.334
Khasa et al. 2001	*Pinus sylvestris*	*Rhizopogon vinicolor*	37	0.039
Khasa et al. 2001	*Pinus sylvestris*	*Suillis tomentosus*	37	0.034
Khasa et al. 2001	*Larix sibirica*	*Hebeloma longicaudum*	37	-0.585
Khasa et al. 2001	*Larix sibirica*	*Laccaria bicolor*	37	-0.522
Khasa et al. 2001	*Larix sibirica*	*Paxillus involutus*	37	-0.531
Khasa et al. 2001	*Larix sibirica*	*Pisolithis tinctorius*	37	-0.531
Khasa et al. 2001	*Larix sibirica*	*Rhizopogon vinicolor*	37	-0.648
Khasa et al. 2001	*Larix sibirica*	*Suillis tomentosus*	37	-0.618
Khasa et al. 2001	*Pinus contorta*	*Hebeloma longicaudum*	55	0.505
Khasa et al. 2001	*Pinus contorta*	*Laccaria bicolor*	55	0.485
Khasa et al. 2001	*Pinus contorta*	*Paxillus involutus*	55	0.729
Khasa et al. 2001	*Pinus contorta*	*Pisolithis tinctorius*	55	0.662
Khasa et al. 2001	*Pinus contorta*	*Rhizopogon vinicolor*	55	0.076
Khasa et al. 2001	*Pinus contorta*	*Suillis tomentosus*	55	0.086
Khasa et al. 2001	*Picea glauca*	*Hebeloma longicaudum*	55	0.670
Khasa et al. 2001	*Picea glauca*	*Laccaria laccata*	55	0.716
Khasa et al. 2001	*Picea glauca*	*Paxillus involutus*	55	0.849
Khasa et al. 2001	*Picea glauca*	*Pisolithis tinctorius*	55	0.911
Khasa et al. 2001	*Picea glauca*	*Rhizopogon vinicolor*	55	0.105
Khasa et al. 2001	*Picea glauca*	*Suillis tomentosus*	55	0.156
Khasa et al. 2001	*Picea mariana*	*Hebeloma longicaudum*	55	0.579
Khasa et al. 2001	*Picea mariana*	*Laccaria bicolor*	55	0.608

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Khasa et al. 2001	*Picea mariana*	*Paxillus involutus*	55	0.795
Khasa et al. 2001	*Picea mariana*	*Pisolithis tinctorius*	55	0.793
Khasa et al. 2001	*Picea mariana*	*Rhizopogon vinicolor*	55	0.034
Khasa et al. 2001	*Picea mariana*	*Suillis tomentosus*	55	0.045
Khasa et al. 2001	*Pinus sylvestris*	*Hebeloma longicaudum*	55	0.066
Khasa et al. 2001	*Pinus sylvestris*	*Laccaria bicolor*	55	0.086
Khasa et al. 2001	*Pinus sylvestris*	*Paxillus involutus*	55	0.395
Khasa et al. 2001	*Pinus sylvestris*	*Pisolithis tinctorius*	55	0.398
Khasa et al. 2001	*Pinus sylvestris*	*Rhizopogon vinicolor*	55	0.021
Khasa et al. 2001	*Pinus sylvestris*	*Suillis tomentosus*	55	0.031
Khasa et al. 2001	*Larix sibirica*	*Hebeloma longicaudum*	55	0.045
Khasa et al. 2001	*Larix sibirica*	*Laccaria bicolor*	55	0.058
Khasa et al. 2001	*Larix sibirica*	*Paxillus involutus*	55	0.120
Khasa et al. 2001	*Larix sibirica*	*Pisolithis tinctorius*	55	0.058
Khasa et al. 2001	*Larix sibirica*	*Rhizopogon vinicolor*	55	0.036
Khasa et al. 2001	*Larix sibirica*	*Suillis tomentosus*	55	0.031
MacFall et al. 1991	*Pinus resinosa*	*Hebeloma arenosa*	0	2.187
MacFall et al. 1991	*Pinus resinosa*	*Hebeloma arenosa*	17	1.222
MacFall et al. 1991	*Pinus resinosa*	*Hebeloma arenosa*	34	0.488
MacFall et al. 1991	*Pinus resinosa*	*Hebeloma arenosa*	68	0.175
MacFall et al. 1991	*Pinus resinosa*	*Hebeloma arenosa*	136	0.105
Tyminski et al. 1986	*Pinus sylvestris*	*Laccaria laccata*	1	-0.566
Tyminski et al. 1986	*Pinus sylvestris*	*Hebeloma crustliniforme*	1	-0.714
Tyminski et al. 1986	*Pinus sylvestris*	*Laccaria laccata*	3.1	-0.392
Tyminski et al. 1986	*Pinus sylvestris*	*Hebeloma crustliniforme*	3.1	-0.287
Tyminski et al. 1986	*Pinus sylvestris*	*Laccaria laccata*	10	-0.349
Tyminski et al. 1986	*Pinus sylvestris*	*Hebeloma crustliniforme*	10	-0.392

Authors	Host species	Fungal species	P addition mg kg -1	Ln R Biomass
Tyminski et al. 1986	*Pinus sylvestris*	*Laccaria laccata*	31	-0.128
Tyminski et al. 1986	*Pinus sylvestris*	*Hebeloma crustliniforme*	31	-0.566
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	0	0.053
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	0	0.063
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	8	-0.075
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	8	-0.328
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	12	-0.038
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	12	0.167
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	16	-0.413
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	16	-0.064
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	20	-0.145
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	20	0.321
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	24	-0.037
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	24	-0.147
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	24	-0.019
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	24	0.000
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	36	-0.197
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	36	0.071
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	40	-0.103
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	40	0.174
Walker 2001	*Pinus lambertiana*	*Pisolithis tinctorius*	60	0.012
Walker 2001	*Pinus jeffreyi*	*Pisolithis tinctorius*	60	0.629

Full citation of each study used in the meta-analysis examining the effects of phosphorus addition on the outcome of ectomycorrhizal associations.

Study	Full citation
Bougher et al. 1990	Bougher NL, Grove TS, Malajczuk N. 1990. Growth and phosphorus acquisition of Karri (*Eucalyptus diversicolor* F-Muell) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. New Phytologist 114: 77-85
Browning & Whitney 1992	Browning MHR, Whitney RD. 1991. Responses of jack pine and black spruce seedlings to inoculation with selected species of ectomycorrhiza fungi. Canadian Journal of Forest Research 21: 701-706
Burgess et al. 1993	Burgess TI, Malajczuk N, Grove TS. 1993. The ability of 16 ectomycorrhizal fungi to increase growth and phosphorus uptake of *Eucalyptus globulus* Labill and *E. diversicolor* F. Muell. Plant and Soil 153: 155-164
Chen et al. 2000	Chen YL, Brundrett MC, Dell B. 2000. Effects of ectomycorrhizas and vesicular-arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. New Phytologist 146: 545-556
Conjeaud et al. 1996	Conjeaud C, Scheromm P, Mousain D. 1996. Effects of phosphorus and ectomycorrhiza on maritime pine seedlings (*Pinus pinaster*). New Phytologist 133: 345-351

Study	Full citation
Grandcourt et al. 2004	de Grandcourt A, Epron D, Montpied P, Louisanna E, Bereau M, Garbaye J, Guehl JM . 2004. Contrasting responses to mycorrhizal inoculation and phosphorus availability in seedlings of two tropical rainforest tree species. New Phytologist 161: 865-875
Khasa et al. 2001	Khasa PD, Sigler L, Chakravarty P, Dancik BP, Erikson L, McCurdy D. 2001. Effect of fertilization on growth and ectomycorrhizal development of container-grown and bare-root nursery conifer seedlings. New Forests 221: 179-197
MacFall et al. 1991	Macfall J, Slack SA, Iyer J. 1991. Effects of *Hebeloma arenosa* and phosphorus fertility on growth of red pine (*Pinus resinosa*) seedlings. Canadian Journal of Botany 69: 372-379
Tyminski et al. 1986	Tyminski A, le Tacon F, Chadoeuf J. 1986. Effect of three ectomycorrhizal fungi on growth and phosphorus uptake of *Pinus silvestris* seedlings at increasing phosphorus levels. Canadian Journal of Botany 64: 2753-2757
Walker 2001	Walker RF. 2001. Growth and nutritional responses of containerized sugar and Jeffrey pine seedlings to controlled release fertilization and induced mycorrhization. Forest Ecology and Management 149: 163-179