Comparison of biomass allocation in ectomycorrhizal and nonmycorrhizal Douglas fir seedlings of similar nutrition and overall size

M.G. DOSSKEY[1], R.G. LINDERMAN and L. BOERSMA
Department of Soil Science, Oregon State University, Corvallis, OR 97331 USA and USDA Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, OR 97330 USA.
[1]*Present address: Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29802, USA*

Abstract

Biomass allocation in 6-month-old ectomycorrhizal Douglas fir seedlings was compared to that in nonmycorrhizal seedlings of the same age, nutrient status and total biomass. Seedlings colonized by *Rhizopogon vinicolor* had the same distribution of biomass between roots, stems and needles, but only 56% of the total length of roots (including mycorrhizal branches) compared to nonmycorrhizal seedlings. *Laccaria laccata* had no effect on distribution of biomass or root length of seedlings. The results for *Rhizopogon* provide direct evidence that the process of ectomycorrhizal colonization can significantly affect plant biomass allocation by one or more mechanisms not directly related to altered nutrition or overall plant size.

Introduction

It is well established that growth of plants often changes in response to colonization by ectomycorrhizal fungi. Colonization of seedlings is frequently associated with higher biomass production, sometimes following an initial period of similar or reduced growth compared to nonmycorrhizal seedlings (see review by Harley and Smith, 1983). Larger plant size has been attributed primarily to mycorrhizal enhancement of nutrient uptake, particularly of P (Bowen, 1973). Changes in biomass allocation also occur. Colonization frequently decreases the proportion of plant biomass in root systems, stimulates fine roots to branch, and alters numbers of short roots (e.g. Alexander, 1981; Danielson et al., 1984).

In their review, Harley and Smith (1983) argued the need for deeper understanding of ec-

tomycorrhizal effects on plant morphology through distinguishing changes due to larger size or better nutrition from those due more directly to ectomycorrhizal colonization and its physiological consequences. For example, lower proportion of plant biomass in root systems can be a normal consequence of larger plant size and of better nutrition (Fitter and Hay, 1987). One way to make this distinction is to compare morphology of mycorrhizal plants with nonmycorrhizal plants of the same overall size and nutrition. We have found only two such published comparisons, both for vesicular-arbuscular mycorrhizal (VAM) citrus, where plant biomass and leaf P concentration were equal to nonmycorrhizal controls (Graham et al., 1987; Graham and Syvertsen, 1985). Their data show that in cases where significant morphological changes occurred, VAM colonization resulted in proportionally more biomass in roots, but reduced root

length per unit root biomass. Similar comparisons for ectomycorrhizas and their hosts have not been previously published.

In this note, we report a comparison of biomass allocation in ectomycorrhizal and non-mycorrhizal Douglas fir seedlings of similar nutrition and overall plant size.

Materials and methods

Seedling inoculation and growing conditions

Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] germinants were transplanted at 8-week age into 21×4 cm tubes containing mineral soil mixed with inoculum of *Laccaria laccata* S238-A or fungus-free substrate. The soil mix consisted of 3 parts pasteurized sandy loam soil (organic matter 0.8%; CEC 19 cmol$_c$ kg^{-1}; P (Bray) 10 mg kg^{-1}; pH 6.5) and 1 part mycorrhizal inoculum substrate (v/v, bulk density 1060 kg m^{-3}). Inoculum substrate of *Laccaria laccata* consisted of mycelium cultures grown for three months on a mixture of 30 parts vermiculite, 1 part peat moss, and 12 parts modified Melin-Norkrans (MMN) solution (v/v/v; Marx and Kenney, 1982). Fungus-free control substrate was identically prepared but contained no fungal culture. Prior to mixing with soil, all substrate was rinsed in cool tap water to remove residual MMN solution. Two hundred seedlings were transplanted into soil mixed with each one of these substrates.

In addition, 400 Douglas fir germinants were transplanted at 6-week age into tubes containing fungus-free substrate. Half of these seedlings were inoculated by injecting 5 mL of a spore suspension of *Rhizopogon vinicolor* FSL 788-5 into each tube. The spore suspension was prepared by macerating sporocarps in distilled water with a blender, making a suspension of approximated 6×10^5 spores mL^{-1}.

Seedlings were maintained in a greenhouse for four more months with a 15-hour light period. Natural light was supplemented with high-pressure sodium-vapor lamps (PAR $\simeq 150$ μmol m^{-2} s^{-1} at crown height) during four-hour periods in the morning and evening. Seedlings were watered as needed to maintain moist soil conditions. With every fifth watering (every two to three weeks) each seedling received 15 ml of nutrient solution containing (in mg kg^{-1} solution) 120 N (7:1 NO$_3^-$:NH$_4^+$), 20 P, 100 K, 100 Ca, 36 Mg, 64 S, and Long-Ashton micronutrients (Hewitt, 1966). This fertilization regime was intentionally low compared to recommended fertilizer rates (Owston, 1974) because high fertility is known to retard mycorrhiza formation in container seedlings (Marx and Barnett, 1975). The locations of seedlings on the greenhouse bench were rearranged biweekly to minimize possible effects of uneven microenvironment.

After this four-month period, shoot and needle expansion had ceased and terminal buds had begun to form in all treatments. Well-developed mycorrhizae had formed on an estimated 36% of root tips on *Rhizopogon*-inoculated seedlings, and on 73% of root tips on *Laccaria*-inoculated seedlings. These growing conditions fortuitously yielded no differences in needle N, P, K, and Ca concentrations, and in plant biomass ($p < 0.05$) between the mycorrhizal seedlings and their non-mycorrhizal controls (see Dosskey et al., 1990). Needle concentrations averaged 0.64% N, 0.07% P, 0.71% K, and 0.32% Ca. These values indicate that N and P were deficient, but otherwise well-balanced, compared to established critical levels for maximum growth of Douglas fir (Kreuger, 1967; Mohren et al., 1986). However, seedling growth was not severely limited. Plant biomass averaged 2.2 g and shoot heights were 10–18 cm, which are similar to other published values for 6-month old, containerized Douglas fir seedlings grown in greenhouses (Molina, 1980; Owston, 1974). Relatively low leaf N and P concentrations may be normal for plants measured near the end of the growing season, and grown under less than full sunlight (Sheriff et al., 1986; Tester et al., 1985).

Measurement of biomass allocation

Sixty plants were randomly selected from each treatment and were carefully washed to remove soil from roots. Each plant was separated into needles, stem, and roots, and then oven-dried (65°C, 48 h) and weighed. Each root system was separated from the stem immediately above the first root branch. Since separating the mycorrh-

iza sheath and intercellular hyphae from root tissue is virtually impossible, these fungal tissues were included with roots. Fine soil hyphae washed off with the soil.

Another random selection of 12 root systems from each treatment were carefully measured for total length of roots, including mycorrhizal branches, using a modified line-intersect method (Newman, 1966). To do this, each root system was cut into several pieces in order to spread roots out evenly into two, 27-cm-dia. dishes. Each dish had a 4-line grid etched to its plexiglas bottom and a matching 4 line grid overlay made of fine thread. Intersections of roots with the plane formed between the matching top and bottom grid lines were determined visually. Water-filled dishes enhanced visibility for counting fine roots and mycorrhiza branches, and the plane-intersect method reduced error which would otherwise be caused by inadvertent shifts in the observer's position.

Plant dry mass (i.e. biomass) and root length parameters of mycorrhizal seedlings were compared to their nonmycorrhizal controls by one-way analysis of variance (STSC Inc., 1986).

biomass, however, might account for some of the root system biomass of mycorrhizal seedlings since sheath and intercellular hyphae could not be removed from roots. Fungal tissue may contribute up to 40% of the biomass of well-developed ectomycorrhizas (see review by Harley and Smith, 1983). If fine, colonized roots make up 20% of the biomass of a seedlings root system (a liberal estimate), then fungal tissue could account for as much as 8% of its root system biomass, or about 3% of total plant biomass. According to this estimate, the proportion of plant biomass in root tissue of mycorrhizal seedlings in this study could be as low as 37% (see Table 1). This reasoning suggests that perhaps there was a small reduction in the biomass of root tissue associated with *Rhizopogon* and *Laccaria* colonization.

In spite of a small, if any, change in root tissue biomass, *Rhizopogon* significantly reduced total length of roots of seedlings (including mycorrhizal branches) to 56% of that of nonmycorrhizal controls, and thus reduced root length per unit root system biomass ($p < 0.01$; Table 1). *Laccaria* showed no effect in this regard.

Results

In the absence of nutrition and overall size differences, neither *Rhizopogon* nor *Laccaria* had an effect on distribution of biomass between needles, stems and root systems compared to nonmycorrhizal seedlings (Table 1). Fungal

Discussion

Our results for *Rhizopogon* provide direct evidence of changes in plant biomass allocation caused by ectomycorrhizal colonization which are not attributable to altered plant nutrition or overall plant size. Under growing conditions

Table 1. Biomass distribution and length of roots of mycorrhizal and nonmycorrhizal Douglas fir seedlings having similar total biomass and leaf nutrient concentration

Fungus treatment	Biomass distribution(%)			Root length (m)	Root length/root biomass (m g $^{-1}$)
	Needles	Stem	Roots		
	Group 1				
Nonmycorrhizal	37 ± 1a	22 ± 1a	41 ± 1a	16.4 ± 1.6a	22 ± 1a
Rhizopogon vinicolor	37 ± 1a	22 ± 1a	41 ± 1a	9.2 ± 0.7b	15 ± 1b
	Group 2				
Nonmycorrhizal	37 ± 1a	23 ± 1a	40 ± 1a	15.5 ± 1.1a	21 ± 1a
Laccaria laccata	38 ± 1a	22 ± 1a	40 ± 1a	15.5 ± 1.5a	19 ± 2a

n = 60 for biomass distribution; n = 12 for root length and root length/root biomass.
Values are means ± 1SE.
For comparison of fungal treatments, values within each group not followed by the same letter are significantly different at $p < 0.01$.

which yielded similar nutrition and overall size of mycorrhizal and nonmycorrhizal seedlings, *Rhizopogon* colonization greatly reduced total length of roots and root length per unit biomass.

Reduced root length is consistent with ultrastructure studies of *Fagus* and *Eucalyptus* ectomycorrhizas showing reduced cell axial elongation and rate of cell division compared to uncolonized roots (Chilvers and Pryor, 1965; Clowes, 1951, 1981). Clowes (1981) speculated that reduced length growth of ectomycorrhizas might be caused by resistance to cell elongation against a well-developed apical sheath, and by diversion of plant carbohydrates to the fungus and away from root cell metabolism. Carbohydrate diversion might also decrease root cell turgor pressure for elongation by reducing cell solute concentration. Dosskey et al. (1991) found significantly lower turgor in needles of Douglas fir seedlings when colonized by *Rhizopogon vinicolor*, but not by *Laccaria laccata*, and provided evidence that it was related to the strength of the fungal carbohydrate sink. Reduced length growth might also result from altered hormone relations in roots, since some general morphological features of ectomycorrhizas appear similar to features which can be induced by exogenous application of certain plant growth regulators (Slankis, 1973). Clearly, there are several possible physiological mechanisms which may contribute to ectomycorrhizal colonization-induced changes in plant morphology beyond normal plant responses to improved nutrition and increased size.

Our results for *Rhizopogon* agree with Graham and Syvertsen's (1985) observations of VAM citrus where colonization reduced root length per unit biomass, but not with proportionally larger biomass in VAM root systems. The dissimilarity of effect between the two types of mycorrhizas suggest at least some difference in the causal mechanisms. Lack of effect of mycorrhizal colonization in some cases, such as for *Laccaria* in this study, indicate that the intensity of causal mechanisms may not always be significant.

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