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Title: Basidiospores of Rhizopogon vinicolor and R. colossus as Ectomycorrhizal Inoculum.

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James M. Trappe

Basidiospores of Rhizopogon vinicolor Smith and R. colossus Smith were inoculated onto container-grown Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings and grown under two levels of soluble fertilizer and one level of slow-release fertilizer. Both fungi formed abundant (54%) ectomycorrhizae under the soluble fertilizer regimes. Slow-release fertilizer greatly reduced percent ectomycorrhizae for both fungi. Stem height was significantly increased under low fertility with all basidiospore application rates of R. colossus and the three lowest application rates of R. vinicolor. High fertility significantly increased ectomycorrhizae at all application rates of R. colossus. The HIGH fertility regime produced plantable Douglas-fir seedlings with abundant ectomycorrhizae of R. colossus and R. vinicolor.

Five different conifers grown in a bareroot nursery were inoculated with three basidiospore rates of seven hypogeous ectomycorrhizal fungi. Two Douglas-fir seed sources were successfully inoculated with both R. vinicolor and R. colossus. For seedlings inoculated with R. vinicolor the HIGH basidiospore rate produced the most ectomycorrhizae on the greatest number of seedlings. For seedlings inoculated with R. colossus the HIGH basidiospore rate (seed source 062) or the MEDIUM rate (seed source 252) produced the most ectomycorrhizae on the greatest number of seedlings. No significant differences in stem heights or diameters could be detected between treatments.

Bareroot nursery grown Douglas-fir noninoculated or inoculated with basidiospores of R. vinicolor were outplanted on a routine reforestation site formerly occupied by alder in southwestern Oregon. After two years, inoculated seedlings had significantly increased survival, stem height, stem diameter, and seedling biomass (PVI). Although new feeder roots of noninoculated and inoculated seedlings were colonized by indigenous fungi, R. vinicolor persisted on the old root system and colonized new feeder roots on inoculated seedlings.

Basidiospores of *Rhizopogon vinicolor* and *R. colossus* as Ectomycorrhizal Inoculum.

by

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INTRODUCTION

All species in the family Pinaceae depend on ectomycorrhizae (from Greek, myco = fungus, rhiza = root) for adequate uptake of nutrients and water. Among other benefits to the host attributed to ectomycorrhiza are; increased adsorption area, longevity of feeder roots, and antagonism of root pathogens. Fungi involved in this association belong to the higher Basidiomycetes, Ascomycetes, and zygosporic Phycomycetes. For an extensive review of the ecology, physiology and practical application of ectomycorrhiza the reader is directed to Marks and Kozlowski (1973) and Trappe (1977).

Inoculation of the host is sometimes required to insure ectomycorrhizal development on the feeder roots, especially for container-grown stock. Inoculation of *Pinus* with the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker & Couch has improved survival and growth of both nursery and container-grown seedlings outplanted in the southeastern United States (Berry 1982, Kais et al. 1981, Marx 1980, Marx et al. 1977, Marx and Artman 1979, Ruehle 1982, Ruehle et al. 1981). Inoculation with *P. tinctorius* has been far less successful on conifers native to the Pacific Northwest (Alvarez and Trappe 1983a, 1983b). Identification of ectomycorrhizal fungi that can be successfully inoculated onto

bareroot conifer seedlings in Pacific Northwest nurseries is needed (Trappe 1977).

Although current research has focused on vegetative mycelium as ectomycorrhizal inoculum (Marx 1980), the use of basidiospores also holds promise (Marx 1980, Mikola 1970, Trappe 1977). Basidiospores of several fungus species have been successfully inoculated onto seedlings: Pisolithus tinctorius (Alvarez and Trappe 1983a, 1983b, Ivory and Munga 1983, Lamb and Richards 1974a, 1974b, Marx 1976, Marx and Barnett 1974, Marx et al. 1976, Marx et al. 1979, Marx et al. 1978, Mullette 1976, Ruehle 1980, Ruehle and Marx 1977), Rhizopogon luteolus Fr. & Nordh. (Donald 1975, Ivory and Munga 1983, Lamb and Richards 1974a, Theodorou 1971, 1980, Theodorou and Bowen 1970, 1973), R. nigrescens Coker & Couch (Ivory and Munga 1983), R. roseolus Corda T. M. Fr. (Lamb and Richards 1974a, 1974b), R. vinicolor Smith (Parke et al. 1983), Scleroderma texense Berk. (Ivory and Munga 1983), Suillus granulatus (L.:Fr.) O. Kuntze (Lamb and Richards 1974a, 1974b), and Thelephora terrestris Fr. (Marx and Ross 1970).

Lack of information on which fungus species can be successfully used for spore inoculation in the Pacific Northwest presently limits development of this technology. Of particular interest in the Pacific Northwest are species of Rhizopogon, the most numerous of hypogeous basidiomycotina, which often associate specifically with

commercially important conifers. Seedling growth enhancement and improved plantation performance have been reported for seedlings inoculated with various Rhizopogon spp. (Donald 1975, Lamb and Richards 1971, 1974C, Momoh 1976, Theodorou 1971, Theodorou and Bowen 1970, Vozzo and Hacskaylo 1971). In the western United States, Rhizopogon spp. are commonly found on newly established seedlings in clearcuts (Parke et al 1984, Pilz and Perry 1984, Schoenberger and Perry 1982). One promising species common in the Pacific Northwest is R. vinicolor, which has recently been successfully inoculated onto Douglas-fir seedlings (Castellano unpublished data, Parke et al. 1983). R. vinicolor is host-specific to Douglas-fir (Molina and Trappe 1982) and has been shown to inhibit in vitro growth of certain root pathogens (Zak 1971). Most importantly, Parke et al. (1983) demonstrated that Douglas-fir seedlings ectomycorrhizal with R. vinicolor could recover faster from drought than nonmycorrhizal seedlings or those ectomycorrhizal with other fungi.

The objectives of this research were to assess the ability of basidiospores of hypogeous ectomycorrhizal fungi to serve as inoculum for conifer seedlings grown in container and bareroot nurseries in the Pacific Northwest and to evaluate the plantation performance of R. vinicolor inoculated Douglas-fir seedlings on a rountine reforestation site.

CHAPTER 1

Inoculation of container-grown Douglas-fir with basidiospores of *Rhizopogon vinicolor* and *R. colossus*: interaction of fertility and spore application rate

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SUMMARY

Basidiospores of Rhizopogon vinicolor Smith and R. colossus Smith were inoculated onto container-grown Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings and grown under two levels of soluble fertilizer and one level of slow-release fertilizer. Both fungi formed abundant (54%) ectomycorrhizae under the soluble fertilizer regimes compared to a slow-release fertilizer which reduced percent ectomycorrhizae for both fungi. Stem height was significantly increased under low fertility with all basidiospore application rates of R. colossus and the three lowest application rates of R. vinicolor. High fertility significantly increased ectomycorrhizae at all application rates of R. colossus. The HIGH fertility regime produced plantable Douglas-fir seedlings with abundant ectomycorrhizae of R. colossus and R. vinicolor.

INTRODUCTION

Common cultural practices in container nurseries often inhibit ectomycorrhiza development on tree seedlings. For example, high rates of concentrated soluble fertilizers limit mycorrhiza development (Ruehle 1980). In some experiments (Molina 1979, Shaw and Molina 1980), maximum mycorrhiza development occurred only under reduced fertility. The resulting small seedling size is unacceptable to foresters for planting purposes. Continued research

on the effect of fertility on mycorrhizal inoculation is needed to balance mycorrhiza development with acceptable seedling size.

Research on mycorrhizal inoculations has focused on the use of vegetative mycelium in a vermiculite carrier (Trappe 1977). Basidiospores are a relatively unexplored source of inoculum. Marx (1980) cites several studies in which basidiospores of Pisolithus tinctorius (Pers.) Coker & Couch were used to successfully inoculate bareroot and container-grown seedlings. In the southern hemisphere, basidiospores of Rhizopogon luteolus Fr. and Nordh. (Donald 1975, Theodorou 1971, Theodorou and Bowen 1970, 1973) have been successfully inoculated onto coniferous seedlings with resulting enhancement of seedling growth.

Rhizopogon spp. are of particular interest for inoculation programs in the Pacific Northwest. They are widespread in the region and often associate specifically with commercially important trees. Rhizopogon spp. were also commonly found on newly established seedlings in clearcuts (Parke et al 1984, Pilz and Perry 1984, Schoenberger and Perry 1982). Most importantly, Parke et al (1983) has shown that Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings inoculated with R. vinicolor have improved drought tolerance. Recent studies (Castellano and Trappe unpublished data) indicate that spores of R. vinicolor and R. colossus Smith can be used as mycorrhizal inoculum for bareroot and container-grown Douglas-fir.

Our study was designed to determine the effects of varying

fertilizer sources and levels on mycorrhiza formation and growth of container-grown Douglas-fir seedlings inoculated with basidiospores of R. vinicolor or R. colossus.

MATERIALS AND METHODS

Sporocarps of R. vinicolor (OSC # 40151) were collected on 12 June 1980 near Red Blanket Creek in Jackson county, Oregon USA, at 2700 ft elevation from beneath Douglas-fir. Sporocarps of R. colossus (OSC # 41206) were collected 8 November 1979 near Beaver Creek in Siskiyou county, California USA, from beneath California red fir (Abies magnifica A. Murr.). Voucher specimens are deposited in the Oregon State University Herbarium (OSC). Soon after collection, sporocarps were carefully cleaned with a brush, rinsed with distilled water, cut into pieces (5 mm square), and blended in 1 liter of distilled water at high speed for 90 seconds. Basidiospore concentration of suspensions were determined with a hemacytometer: $7.2 \times 10^{6} ml^{-1}$ for R. vinicolor and $1.3 \times 10^{8} ml^{-1}$ for R. colossus. Suspensions were stored in the dark at $2^{\circ}C$ until used.

Potting substrate consisted of equal volumes of sphagnum peat moss and vermiculite that was steam pasteurized at $80^{\circ}C$ for 30 minutes. Super Cell containers[1/] (165 cc capacity) were filled with substrate. Stratified Douglas-fir seeds were triple sown into

each container and misted twice daily until germination was complete. Germinants were then thinned to one per container.

This was a 2 x 3 x 5 factorial experiment with two fungus treatments, three fertility rates, and five spore concentrations (10^3, 10^4, 10^5, and 10^6 basidiospores/seedling, and one control without inoculum). Each container represented one treatment combination. Containers were arranged in a randomized complete block design with three blocks. Fourteen seedlings were used for each treatment combination giving a total of 1260 (3 x 2 x 3 x 5 x 14) seedlings. Six weeks after germination, we used a pipette machine to inoculate seedlings with the appropiate number of basidiospores.

Three fertility treatments were used: HIGH treatment was approximately the concentration of soluble 20-19-18 NPK (Peter's "Peat-lite special," W. R. Grace & Co., Allentown, Pennsylvania USA) fertilizer recommended by Owston (1974) for operational rearing of container-grown western conifers (biweekly schedule: weeks 4-6, 7 mg/seedling; weeks 8-20, 18 mg/seedling); LOW was one-half the HIGH

1/ Ray Leach "Cone-tainer Nursery," 1787 N. Pine St., Canby, Oregon, USA. Trade or proprietary names are included for information purposes only and do not imply any endorsement by the United States Department of Agriculture.

rate; OSMOCOTE consisted of mixing Osmocote (Sierra Chemical Co., Milpitas, California USA), a slow-release (18-6-12 NPK) fertilizer, into the substrate when the containers were filled at the manufacturers recommended rate for potted plants (7.1 g/liter of substrate). All seedlings received 3.5 mg of chelated iron (Sequestrene Fe 300) dissolved in water, biweekly.

Seedlings were grown in a greenhouse from mid-May through mid-October. Sodium-vapor lamps supplied supplemental light at approximately 11 000 lx for 15-h photoperiod. Seedlings were mist-irrigated three times weekly to saturation.

At the conclusion of the experiment, entire root systems of ten seedlings randomly selected from each treatment in each block were gently washed with tap water to remove the substrate. Each seedling was examined for degree of ectomycorrhiza development. All feeder roots were counted whether colonized by fungi or not. Degree of ectomycorrhiza development is expressed as the percentage of mycorrhizal feeder roots to total feeder roots. Stem height and caliper and ovendry weight of shoots and roots were also measured. All control seedlings used for comparison of growth parameters were nonmycorrhizal. An analysis of variance was used to detect differences between treatments; significant differences between means of treatments were separated by Tukey tests at $P = 0.05$.

RESULTS

MYCORRHIZA DEVELOPMENT

Rhizopogon vinicolor formed abundant (88%) ectomycorrhizae at all basidiospore application rates within both HIGH and LOW treatments. The OSMOCOTE treatment, however greatly reduced colonization of feeder roots at all application rates to less than 10 percent. Ectomycorrhiza morphology was the same as that described by Zak (1971). Degree of ectomycorrhiza development did not differ significantly between basidiospore rates within the LOW treatment or the OSMOCOTE treatment. The lowest (10^3) basidiospore application rate in the HIGH treatment was significantly lower in percent mycorrhizal feeder roots than higher application rates (Table 1.1).

Rhizopogon colossus formed abundant (54%) ectomycorrhizae with all inoculated seedlings within both LOW and HIGH treatments (Table 1.2). Again ectomycorrhiza development was greatly reduced by the OSMOCOTE treatment. Morphology of ectomycorrhizae was the same as that described by Trappe (1967). Within the HIGH treatment, the two highest basidiospore application rates produced significantly greater percent of mycorrhizal feeder roots than the two lowest rates. Also, within the LOW treatment the two highest application rates significantly increased mycorrhiza development over the lowest rate.

Within the OSMOCOTE treatment, mycorrhiza development did not differ significantly between application rates for either fungus

treatment (Tables 1.1 and 1.2).

R. colossus produced a significantly higher percentage of mycorrhizal feeder roots at each application rate within the HIGH treatment compared to the same rate within the LOW treatment (Figure 1.1). Also, ectomycorrhiza percentage was significantly less with the OSMOCOTE treatment than with the LOW or HIGH treatments for both fungi at all spore application rates (Figure 1.1).

SEEDLING GROWTH

In the LOW treatment, all basidiospore application rates of R. colossus significantly increased seedling height over control seedlings; the three lowest application rates of R. vinicolor significantly increased seedling height over control seedlings. Compared to controls, root dry weight significantly decreased for seedlings inoculated with R. vinicolor at all application rates but did not differ significantly for seedlings inoculated with R. colossus. For the most part, shoot dry weight increased significantly for seedlings inoculated with R. colossus, but there was essentially no significant difference for seedlings inoculated with R. vinicolor. Stem caliper did not differ significantly for either fungal treatment regardless of basidiospore application rate.

In the HIGH treatment, seedling height decreased significantly for seedlings inoculated with R. vinicolor at all application rates, whereas seedlings inoculated with R. colossus did not differ significantly from control seedlings. Root dry weight generally decreased significantly with inoculation by either fungus. Shoot

dry weight decreased significantly for seedlings inoculated with R. vinicolor at all basidiospore application rates whereas little significant difference was detected for seedlings inoculated with R. colossus. Stem caliper generally decreased significantly in seedlings inoculated with R. vinicolor, whereas it was either slightly increased or did not differ in seedlings inoculated with R. colossus.

In the OSMOCOTE treatment, no biologically significant differences were observed.

All seedling growth parameters increased from LOW to HIGH to OSMOCOTE treatments (Tables 1.1 and 1.2), respectively.

DISCUSSION

High rates of soluble fertilizer required to produce plantable 1-0 container stock commonly reduce ectomycorrhiza development (Marx and Barnett 1974, Ruehle 1980, Ruehle and Marx 1977, Shaw et al 1982). In our study, the HIGH (near operational) rate of soluble fertilizer actually increased ectomycorrhiza development for R. colossus, and did not affect ectomycorrhiza development of R. vinicolor (Figure 1.1). All inoculated seedlings grown with soluble fertilizers formed abundant ectomycorrhizae (54%). Osmocote, a slow-release fertilizer, limited ectomycorrhiza development to less than 10% of total feeder roots (Figure 1.1). This contrasts with previous research with vegetative inoculum,

showing that slow-release fertilizers allow good mycorrhiza colonization (Crowley et al 1981, Maronek and Hendrix 1979, 1980, Maronek et al 1981, Molina and Chamard 1983). We hypothesize that the relatively constant source of high levels of nitrogen and phosphorus provided by the slow-release fertilizer inhibited germination of spores. The pulsing nature of application of soluble fertilizers perhaps allowed the spores to germinate when fertility levels were low. Once spores had germinated, the fungal mycelium was able to withstand the influx of high levels of fertilizer. Molina and Chamard (1983) suggest that slow-release fertilizers be used for experimental inoculations with fungi that are sensitive to repeated applications of soluble fertilizers. Our data suggest that both fertility regimes be tested in initial inoculation trials.

R. colossus colonized seedling feeder roots within the HIGH treatment more completely than within the LOW treatment; the increased vigor of host seedlings grown under high fertility likely stimulated fungus activity.

Overall seedling growth was best within the OSMOCOTE treatment, followed, in order, by the HIGH and the LOW treatments. Plantable seedlings were produced in both the OSMOCOTE and HIGH treatments (Owston 1974).

R. vinicolor (Zak 1971) and R. colossus (Trappe 1967) produced abundant rhizomorphs on inoculated seedlings, which are thought to function in water transport (Duddridge et al 1980). Parke et al (1983) showed that Douglas-fir seedlings mycorrhizal with

R. vinicolor were more drought tolerant than nonmycorrhizal seedlings or seedlings mycorrhizal with fungi that did not produce rhizomorphs. This is an extremely important consideration when selecting fungi for inoculation of seedlings destined for drought-prone sites. Significant amounts of fungal biomass, particuluraly rhizomorphs, were not included in root dry weight because destructive sampling methods were used. We hypothesize that root dry weight of inoculated seedlings would be significantly larger than for control seedlings if fungal biomass were included.

Inoculation of nursery stock with vegetative inoculum of Rhizopogon spp. common to the Pacific Northwest has not been successful (Molina 1980). For the first time, plantable seedlings have been produced specifically inoculated with basidiospores of R. vinicolor or R. colossus. Marx (1980) concluded that improved plantation performance of seedlings inoculated with P. tinctorius over noninoculated seedlings occurred only when 50% or more of the root system was colonized by P. tinctorius. All spore application rates within HIGH and LOW treatments surpassed this threshold of significant mycorrhiza development.

Studies are now in progress with Douglas-fir seedlings inoculated with basidiospores of R. vinicolor to test their effectivness when planted on hot, dry sites common to southwest Oregon.

TABLE 1.1 Growth and ectomycorrhiza development of container-grown Douglas-fir seedlings inoculated with five rates of Rhizopogon vinicolor basidiospores under three fertility regimes.

FERTILIZER TREATMENT	SPORE RATE	HEIGHT (cm)	STEM CALIPER (mm)	MYCORRHIZAE (%)	DRY WEIGHT SHOOT (g)	DRY WEIGHT ROOT (g)
LOW	10^3	12.84a	2.77a	95.3a	0.73a	0.67a
	10^4	12.97a	2.77a	97.2a	0.75a	0.68a
	10^5	13.32a	2.77a	95.7a	0.80a	0.70a
	10^6	12.66ab	2.77a	89.1a	0.55b	0.69a
	control	12.15b	2.77a	0.0b	0.82a	0.86b
HIGH	10^3	14.63a	2.87a	88.6a	0.98a	0.93a
	10^4	14.43a	2.84a	97.6b	0.95a	0.87a
	10^5	16.91b	2.84a	99.2b	1.01a	0.96a
	10^6	17.31b	2.89ab	98.9b	1.15a	0.98ab
	control	20.03c	2.92b	0.0c	1.48b	1.13b
OSMOCOTE	10^3	29.88a	3.09a	9.6a	2.13a	1.00a
	10^4	30.93a	3.07a	7.5a	2.09a	0.96a
	10^5	31.54a	3.07a	5.9a	2.13a	0.97a
	10^6	31.98a	3.02a	1.4a	1.92a	0.87a
	control	31.25a	3.07a	0.0b	2.06a	0.82a

NOTE: Means within fertility treatments not sharing a common letter are significantly different by Tukey's test at P = 0.05.

TABLE 1.2 Growth and ectomycorrhiza development of container-grown Douglas-fir seedlings inoculated with five rates of Rhizopogon colossus basidiospores under three fertility regimes.

FERTILIZER TREATMENT	SPORE RATE	HEIGHT (cm)	STEM CALIPER (mm)	MYCORRHIZAE (%)	DRY WEIGHT SHOOT (g)	DRY WEIGHT ROOT (g)
LOW	10^3	13.92a	2.82a	54.6a	0.82a	0.81a
	10^4	14.54a	2.84a	67.9ab	0.89b	0.81a
	10^5	14.59a	2.82a	72.9b	0.92b	0.83a
	10^6	14.68a	2.79a	75.1b	0.97c	0.85a
	control	12.15b	2.77a	0.0c	0.82a	0.86a
HIGH	10^3	21.27a	3.02b	76.3a	1.53b	1.24a
	10^4	20.36a	2.97ab	80.1a	1.29a	0.92b
	10^5	20.02a	2.97ab	95.1b	1.48ab	0.91b
	10^6	20.08a	2.97ab	98.2b	1.44ab	0.90b
	control	20.03a	2.92a	0.0c	1.48ab	1.13a
OSMOCOTE	10^3	29.04a	3.00ab	3.7a	1.91a	0.90a
	10^4	29.59a	2.97a	7.8a	1.88a	0.95a
	10^5	29.26a	3.07b	5.6a	1.85a	0.88a
	10^6	31.17a	3.02ab	0.7a	2.11a	0.80a
	control	31.25a	3.07b	0.0b	2.06a	0.82a

NOTE: Means within fertility treatments not sharing a common letter are significantly different by Tukey's test at P = 0.05.

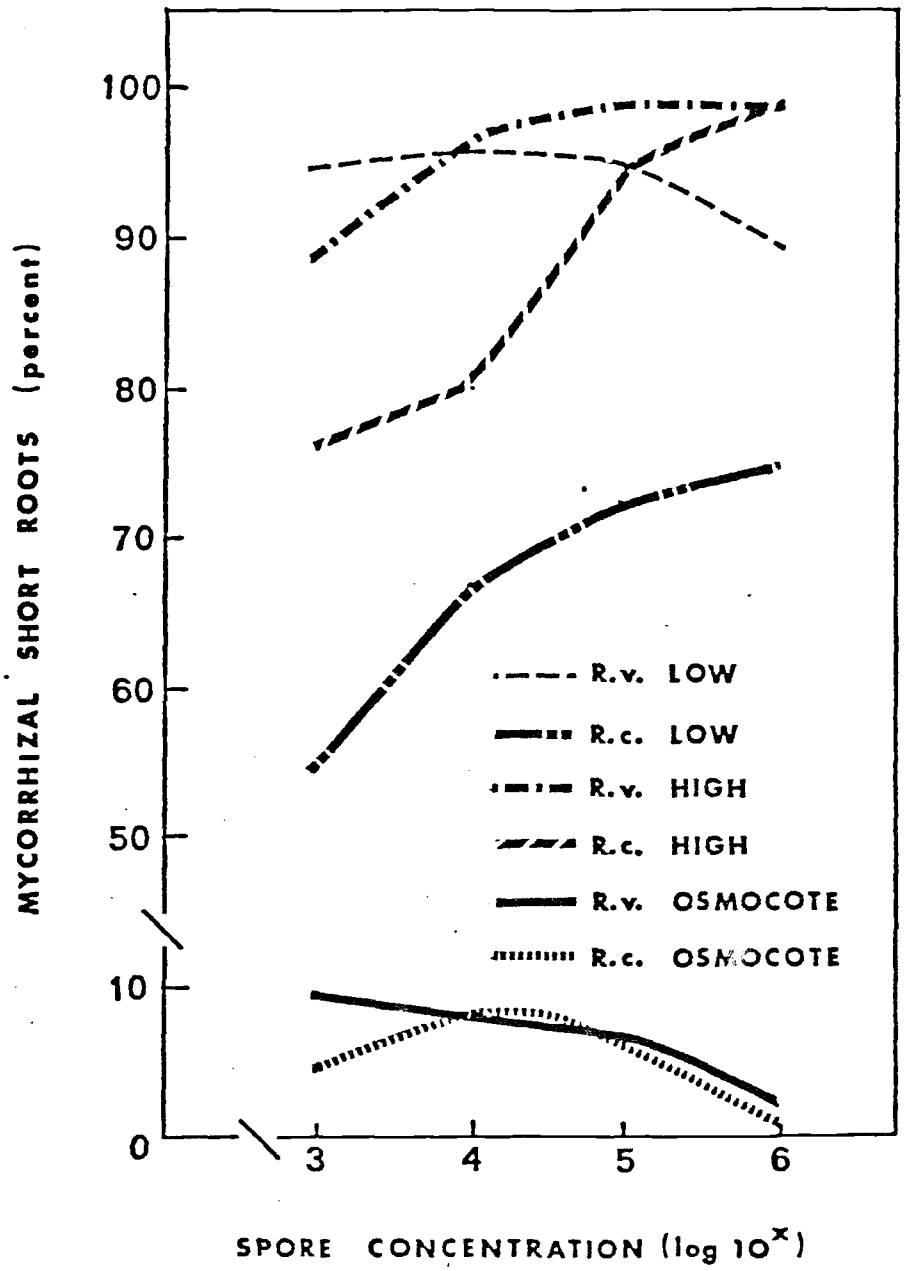

FIG. 1.1 Interaction of basidiospore application rate and fertility. R.c. = Rhizopogon colossus, R.v. = Rhizopogon vinicolor.

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CHAPTER 2

Inoculation of conifer seedlings grown in a bareroot nursery with basidiospores of hypogeous ectomycorrhizal fungi

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SUMMARY

Five different conifers grown in a bareroot nursery were inoculated with three basidiospore rates of seven hypogeous ectomycorrhizal fungi. Seedlings of two Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seed sources were successfully inoculated with both Rhizopogon vinicolor Smith and R. colossus Smith. For seedlings inoculated with R. vinicolor, the HIGH basidiospore rate produced the most ectomycorrhizae on the greatest number of seedlings. For seedlings inoculated with R. colossus, the HIGH basidiospore rate (seed source 062) and the MEDIUM rate (seed source 252) produced the most ectomycorrhizae on the greatest number of seedlings. No significant differences in stem height or diameter could be detected between treatments and controls.

INTRODUCTION

Inoculation of conifer seedlings with ectomycorrhizal fungi can significantly improve outplanting performance (Marx 1980). Although current research has focused on vegetative mycelium as ectomycorrhizal inoculum (Marx 1980), the use of basidiospores also holds promise (Marx 1980, Mikola 1970, Trappe 1977). Basidiospores of several fungus species have been successfully inoculated onto seedlings: Pisolithus tinctorius (Pers.) Coker & Couch (Alvarez and Trappe 1983, Ivory and Munga 1983, Lamb and Richards 1974a, 1974b,

Marx 1976, Marx and Barnett 1974, Marx et al. 1976, Marx et al. 1979, Marx et al. 1978, Mullette 1976, Ruehle 1980, Ruehle and Marx 1977), Rhizopogon luteolus Fr. & Nordh. (Donald 1975, Ivory and Munga 1983, Lamb and Richards 1974a, Theodorou 1971, 1980, Theodorou and Bowen 1970, 1973), R. nigrescens Coker & Couch (Ivory and Munga 1983), R. roseolus Corda T. M. Fr. (Lamb and Richards 1974a, 1974b), R. vinicolor (Castellano et al. 1984, Parke et al. 1983), Scleroderma texense Berk. (Ivory and Munga 1983), Suillus granulatus (L.:Fr.) O. Kuntze (Lamb and Richards 1974a, 1974b), and Thelephora terrestris Fr. (Marx and Ross 1970).

Lack of information on which fungus species can be successfully used for spore inoculation presently limits development of this technology. Of particular interest in the Pacific Northwest are species of Rhizopogon, the most numerous of hypogeous basidiomycotina. Seedling growth enhancement and improved plantation performance have been reported for seedlings inoculated with various Rhizopogon species (Donald 1975, Lamb and Richards 1971, 1974C, Momoh 1976, Theodorou 1971, Theodorou and Bowen 1970, Vozzo and Hacskaylo 1971). Parke et al. (1983) demonstrated that Douglas-fir seedlings inoculated with R. vinicolor basidiospores could recover faster from drought than nonmycorrhizal seedlings or those inoculated with other ectomycorrhizal fungi. R. vinicolor has also been shown to inhibit in vitro growth of root pathogens (Zak 1971).

Inoculation of conifer seedlings with mycelial inoculum of

Rhizopogon spp. common to the Pacific Northwest has not been successful (Molina 1980). However, basidiospores of R. vinicolor and R. colossus can be effective ectomycorrhizal inoculum for container-grown Douglas-fir (Castellano et al. 1984). This study was designed to evaluate the success of nursery inoculation of Douglas-fir, western hemlock (Tsuga heterophylla (Raf.) Sarg.), white fir (Abies concolor (Gord. & Glend.) Lindl.:Hild.), and sugar pine (Pinus lambertiana Dougl.) with basidiospores of Rhizopogon vinicolor, R. colossus, and R. ochraceorubens Smith; Gauteria monticola Harkn.; Hymenogaster parksii Zeller & Dodge; and Hysterangium separabile Zeller and Hysterangium sp..

MATERIALS AND METHODS

Sporocarps were collected fall, 1979 through spring, 1980 (Table 2.1). Voucher specimens were deposited in the Oregon State University Herbarium. The fresh sporocarps were carefully brushed, rinsed with distilled water, cut into small pieces, and blended in distilled water at high speed for approximately 90 seconds. A hemacytometer was used to prepare three separate spore concentration suspensions for each fungal collection; low = 10^5 spores/ml, medium = 10^6 spores/ml, and high = 10^7 spores/ml. Suspensions were stored in the dark at $2^{\circ}C$ until used.

A randomized block experiment was established at the International Paper bareroot conifer nursery at Lebanon, Oregon.

Five conifer hosts were inoculated with seven ectomycorrhizal fungi (Table 2.1). Spore concentration treatments consisted of; CONTROL = 0 spores/ft^2, LOW = 10^5 spores/ft^2, MEDIUM = 10^6 spores/ft^2, and HIGH = 10^7 spores/ft^2. Each host x fungus x spore concentration combination was replicated three times within each of three blocks. Each treatment replication plot was 2 x 4 ft. All treatment replications were separated by at least 2 linear feet of nursery bed. Seed was machine sown in 4 rows at 25 seed/linear ft of nursery bed. In the nursery, spore allotments for each plot were suspended in 2 l of water and applied evenly to the soil surface with a watering can.

After the first and second growing seasons, twenty seedlings were carefully excavated from the center of each treatment replication and transported to the laboratory in plastic bags. Root systems of ten seedlings randomly chosen from each treatment replication were gently washed with tap water to remove adhering soil. Each seedling root system was examined with a stereo microscope (8x) and degree of ectomycorrhiza formation assessed by visually estimating, to the nearest ten percent, percentage of total feeder roots colonized. Stem height and diameter were recorded. Most control seedlings were mycorrhizal with two common nursery fungi, Thelephora and Inocybe sp.. Analysis of variance was used to detect differences between treatments; significant differences between treatment means were separated by Tukey's test at P = 0.05.

RESULTS

Of the seven fungi tested, only Rhizopogon vinicolor and R. colossus formed ectomycorrhizae and only with Douglas-fir (Table 2.2).

Stem heights or diameters did not differ significantly between inoculated and noninoculated seedlings after either the first or second growing seasons. After the second growing season heights averaged 25.1 cm (± 4.5) and stem diameters 4.7 mm (± 1.2).

Rhizopogon vinicolor x Douglas-fir (Table 2.2)

First Year

For seed sources 252 and 062, the HIGH spore treatment produced a significantly higher percentage of both ectomycorrhizal feeder roots and number of seedlings colonized than the MEDIUM treatment. The LOW spore treatment did not produce Rhizopogon ectomycorrhizae.

Second Year

After two years, nearly all seedlings were successfully inoculated in the HIGH spore treatment. For seed source 252, the HIGH spore treatment produced a significantly higher percentage of both ectomycorrhizal feeder roots and number of seedlings colonized than the MEDIUM treatment. The LOW spore treatment did not produce Rhizopogon ectomycorrhizae. For seed source 062, all seedlings inoculated with the HIGH spore treatment were mycorrhizal and

averaged 44.4% of feeder roots colonized. Rhizopogon ectomycorrhizae detected the first year in the MEDIUM treatment were not detected the second year; LOW treatment did not produce Rhizopogon ectomycorrhizae.

Rhizopogon colossus x Douglas-fir (Table 2.2)

First Year

For seed source 252, the MEDIUM spore treatment produced a significantly higher percentage of both ectomycorrhizal feeder roots and number of seedlings colonized than the LOW or HIGH spore treatments. For seed source 062, the HIGH and LOW spore treatments produced a significantly higher percentage of both ectomycorrhiza formation and seedlings colonized than the MEDIUM spore treatment; the HIGH spore treatment also produced significantly more inoculated seedlings than the LOW spore treatment.

Second Year

The same significant differences occurred in the second year except that for seed source 062 the number of ectomycorrhizal seedlings no longer differed significantly between HIGH and LOW treatments.

DISCUSSION

Ectomycorrhizal inoculation of nursey grown seedlings is highly desirable for foresters trying to maximize reforestation success.

Delay or lack of ectomycorrhiza formation in the nursery can lead to stunted, phosphorus deficient seedlings (Mitchell et al. 1937, Trappe and Strand 1969) or excessive loss of seedlings to root rot (Marx 1972, Sinclair et al. 1975, Stack and Sinclair 1975). Inoculation of conifer seedlings with vegetative inoculum of Rhizopogon spp. common to the Pacific Northwest has not been successful (Molina 1980). Rhizopogon is the largest genus of hypogeous basidiomycetes and is an important ectomycorrhizal fungus of Pinaceae throughout the world (Molina and Trappe unpublished data). Our study demonstrates that basidiospores of Rhizopogon vinicolor and R. colossus can be effective inoculum for Douglas-fir seedlings grown in nursery beds. Nearly half of the feeder roots of most of the seedlings from both seed sources became ectomycorrhizal with R. vinicolor when inoculated at the HIGH basidiospore rate. Abundant (48.6%) R. colossus ectomycorrhizae were produced on all Douglas-fir seedlings from seed source 062 inoculated at the HIGH basidiospore rate, whereas, the MEDIUM rate produced the most ectomycorrhizae on the greatest number of seedlings for Douglas-fir seed source 252. The differing response of Douglas-fir seed sources to inoculation levels is puzzling and as of yet, unexplained. It does serve as a reminder that different fungus-host combinations can sometimes respond differently.

Why other fungi used in our experiment failed is unclear. Basidiospore viability of ectomycorrhizal fungi is difficult to determine because the requirements for germination are poorly

understood. Perhaps basidiospores of noneffective fungi were not viable or had specific germination requirements that were not met under these nursery conditions with these hosts.

Mikola (1970) proposed that a host-specific fungus may benefit its particular host more than a fungus that is a host generalist. Parke et al. (1983) have shown that Douglas-fir seedlings mycorrhizal with R. vinicolor were more tolerant of drought than were either nonmycorrhizal seedlings or those mycorrhizal with host-generalist fungi or a native fungus. R. vinicolor (Molina and Trappe 1982) and R. colossus (Molina and Trappe unpublished data) are specific to Douglas-fir. We believe these fungus-host combinations can be important in successfully reforesting hard-to-reforest sites, e.g. the hot and droughty sites common in southwest Oregon. Although problems remain in developing the technology of spore inoculation, use of basidiospores as ectomycorrhizal inoculum remains a viable alternative to the use of pure culture mycelium to establish specific fungus-host combinations. Additional research is needed to identify those fungus-host combinations that can be successfully established with basidiospores and the benefit gained from such inoculations in both nursery and plantation.

Acknowledgements

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Table 2.1 Ectomycorrhizal fungi and conifer host combinations.

Fungus Treatment	Herbarium Number	Year of Collection	Location (county, state)
	Pseudotsuga menziesii (seed source 252 & 062)		
Rhizopogon vinicolor	5786	1980	Jackson, Oregon
Rhizopogon colossus	5671	1979	Skamania, Wash.
Gauteria monticola	5782	1980	Jackson, Oregon
Hysterangium separabile	5740	1980	Benton, Oregon
Hysterangium sp.	5742	1980	Benton, Oregon
Hymenogaster parksii	5691	1980	Linn, Oregon
	Pinus lambertiana		
Rhizopogon ochraceorubens	5590	1979	Linn, Oregon
	Abies concolor		
Gauteria monticola	5782	1980	Jackson, Oregon
	Tsuga heterophylla		
Hysterangium separabile	5740	1980	Benton, Oregon
Rhizopogon ochraceorubens	5590	1979	Linn, Oregon

Table 2.2 First and second year ectomycorrhizal development on two Douglas-fir seed sources inoculated with basidiospores of *Rhizopogon vinicolor* and *Rhizopogon colossus*.

Fungus	Seed Source	Spore Rate	Year 1 Seedlings with Mycorrhizae (%)	Year 1 Mycorrhizae per Seedling (%)	Year 2 Seedlings with Mycorrhizae (%)	Year 2 Mycorrhizae per Seedling (%)
R.vinicolor	062	LOW	0.0a	0.0a	0.0a	0.0a
	062	MEDIUM	6.7b	10.0b	0.0a	0.0a
	062	HIGH	60.0c	47.5c	100.0b	44.4b
	252	LOW	0.0a	0.0a	2.0a	0.4a
	252	MEDIUM	73.3b	27.3b	66.7b	44.8b
	252	HIGH	90.0c	45.3c	93.3c	62.1c
R. colossus	062	LOW	40.0b	44.4b	80.0b	25.6b
	062	MEDIUM	3.3a	10.0a	8.3a	2.0a
	062	HIGH	100.0c	59.3b	100.0b	48.6b
	252	LOW	23.3a	17.1a	10.0a	2.4a
	252	MEDIUM	90.0b	40.0b	80.0b	37.9b
	252	HIGH	6.7a	15.0a	33.3a	10.9a

NOTE. Treatment means within fungus x seed source combinations not sharing a common letter are significantly different by Tukey's test at P = 0.05.

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CHAPTER 3

Plantation performance of Douglas-fir seedlings inoculated with basidiospores of *Rhizopogon vinicolor*

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SUMMARY

Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings grown in a nusery bed noninoculated or inoculated with basidiospores of the ectomycorrhizal fungus Rhizopogon vinicolor Smith were outplanted on a routine reforestation site in southwestern Oregon fomerly occupied by alder. After two years, inoculated seedlings had significantly increased survival, stem height, stem diameter, and seedling biomass (PVI) compared to noninoculated seedlings. Although new feeder roots of noninoculated and inoculated seedlings were colonized by indigeous fungi, R. vinicolor persisted on the old root system and colonized new feeder roots on inoculated seedlings.

INTRODUCTION

Successful inoculation of Pinus spp. with the ectomycorrhizal fungus Pisolithus tinctorius (Pers.) Coker & Couch and their subsequent improved plantation performance in the southeastern United States is well documented (Berry 1982, Kais et al. 1981, Marx 1980, Marx et al. 1977, Marx and Artman 1979, Ruehle 1982, Ruehle et al. 1981). Inoculation with P. tinctorius has been far less successful on conifers native to the Pacific Northwest (Alvarez and Trappe 1983a, 1983b). Identification of ectomycorrhizal fungi that can be successfully inoculated onto conifer seedlings grown in Pacific Northwest bareroot nurseries is needed (Trappe 1977). One

promising species is Rhizopogon vinicolor, a hypogeous ectomycorrhial basidiomycete which has recently been successfully inoculated onto Douglas-fir seedlings (Castellano and Trappe 1984, Castellano et al. 1984, Parke et al. 1983). R. vinicolor is host-specific to Douglas-fir (Molina and Trappe 1982) and has been shown to inhibit in vitro growth of certain root pathogens (Zak 1971). Most importantly, Parke et al. (1983) demonstrated that Douglas-fir seedlings ectomycorrhizal with R. vinicolor could recover faster from drought than nonmycorrhizal seedlings or those ectomycorrhizal with other fungi.

This study was designed to compare the plantation performance of 2-0 bare-root Douglas-fir seedlings inoculated with R. vinicolor to that of seedlings ectomycorrhizal with fungi common to bareroot conifer nurseries in the Pacific Northwest.

MATERIALS AND METHODS

Bareroot 2-0 Douglas-fir seedlings were grown at the International Paper Co. nursery in Lebanon, Oregon. Seedlings were inoculated with spores of R. vinicolor 6 weeks after sowing as detailed in Castellano and Trappe (1984). One hundred-sixty each of inoculated and noninoculated seedlings were lifted, graded to uniform stem heights and diameters and examined for amounts and types of ectomycorrhizae. Seedlings were stored in plastic-coated paper bags at $5^{o}C$ for 3 1/2 months prior to planting.

The planting site was formerly occupied by red alder (Alnus rubra Bong.) which was clearcut and broadcast-burned in 1981. It is located in the Oregon Coast Range, 12 air miles southeast of Coos bay at 500 feet elevation with a westerly aspect and slope of less than 7 percent.

Seedlings were planted 13 May 1982 in a randomized block design containing two, twenty seedling rows per treatment in each of four blocks arranged parallel to the contour. Seedlings were planted with shovels at 4 x 4 foot spacing with blocks separated by 12-foot nonplanted strips.

Survival, current year's leader growth and stem caliper of all seedlings were measured following the first and second growing seasons. Persistence and development of R. vinicolor ectomycorrhizae on inoculated and noninoculated seedlings were evaluated at the end of the second growing season. R. vinicolor forms readily distinguishable ectomycorrhiza characterized by tuberculate mycorrhiza with abundant thick rhizomorphs (Zak 1971). Five seedlings per treatment per block were carefully excavated with a shovel and assessed with a stereomicroscope for morphological ectomycorrhiza types.

Survival and growth data were integrated into a plot volume index (PVI) by multiplying mean seedling volume by number of surviving seedlings per block following the first and second growing seasons (Marx et al. 1977).

Analysis of variance was used to detect diffences between

treatments; significant differences between treatment means were separated by Tukey's test at P = 0.05.

RESULTS

At time of lifting, feeder roots of inoculated seedlings averaged 46% ectomycorrhizal with R. vinicolor; feeder roots of noninoculated seedlings averaged 60% ectomycorrhizal with Thelephora and/or Inocybe spp. After two years all seedling parameters of inoculated seedlings were significantly larger than those of noninoculated seedlings (Table 3.1). Eleven percent more inoculated seedlings survived than noninoculated seedlings (significant at P = 0.05). Stem height and diameter of inoculated seedlings were significantly larger than noninoculated seedlings by 3.17 cm and .8 mm, respectively. Plot volume index of inoculated seedlings was 34% greater than noninoculated seedlings.

Although both noninoculated and inoculated seedlings were colonized by indigenous fungi, R. vinicolor persisted on the original root system of inoculated seedlings and also colonized feeder roots that formed after outplanting. After two growing seasons, R. vinicolor still comprised more than half of the total ectomycorrhizae of each excavated inoculated seedling.

DISCUSSION

Inoculation of Douglas-fir with basidiospores of R. vinicolor shows considerable promise. Successful inoculation of this host-specific ectomycorrhizal fungus (Molina and Trappe 1982) has been demonstrated for both container-grown (Castellano et al. 1984) and nursery-grown (Castellano and Trappe 1984) Douglas-fir seedlings. R. vinicolor ectomycorrhizae are characterized by prolific production of rhizomorphs which emanate into the surrounding substrate (Zak 1971). Rhizomorphs of ectomycorrhizal fungi have been shown to function in water and nutrient transport (Bowen 1973, Brownlee et al. 1983, Duddridge et al. 1980). Parke et al. (1983) reported that Douglas-fir seedlings inoculated with R. vinicolor recovered from drought faster and to a higher photosynthetic level than seedlings inoculated with mycorrhizal fungi that did not form rhizomorphs or those that were not inoculated.

Plantation performance of inoculated seedlings is deemed as the critical proof of the value of mycorrhizal inoculations (Marx 1980, Trappe 1977). In this study, seedlings ectomycorrhizal with common nursery fungi did not perform as well as seedlings inoculated with a host-specific fungus. The observed increase in survival (11%) and plot biomass (34%) may be economically significant over the rotation. If 11% less seedlings could be planted on average reforestation sites a substantial savings in seedling and planting

costs would be realized. If R. vinicolor proves to increase survival on severely stressed sites, the savings would be even more striking. The cost-benefits of mycorrhizal inoculation techniques need further exploration.

Improved plantation performance of Douglas-fir seedlings inoculated with R. vinicolor contrasts with results reported by Bledsoe et al. (1982). They showed a decrease in first year biomass increment of container-grown Douglas-fir seedlings inoculated with the non-host-specific Laccaria laccata (Scop.:Fr.) Berk. and Br. or Hebeloma crustuliniforme (Bull.:St. Am.). Similarly, Black et al. (unpublished data) found no difference in growth or survival of container-grown Douglas-fir and western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings inoculated with P. tinctorius and outplanted on routine reforestation sites in Oregon. If plantation performance of seedlings on routine reforestation sites in the Pacific Northwest is improved by inoculation with host-specific fungi as compared to host-generalists, then current mycorrhizal research directions need rethinking. Instead of identifying host-generalist fungi for wide-scale inoculation programs in the Pacific Northwest (as is the case of Pisolithus tinctorius in the southeast United States), identification of more host-specific fungi that can be successfully manipulated may prove desirable. Mikola (1970) suggests that host specific fungi confer special physiological advantages to their hosts as compared to host-generalists. The genus Rhizopogon is a good candidate for testing this hypothesis. It contains some 150

species that are commonly associated with Pinaceae, including both host-specific and host-generalist species (Molina and Trappe unpublished data). Some studies of mycorrhiza-inoculated seedlings have reported the colonization of outplanted seedlings by indigenous Rhizopogon species (Black et al. (unpublished data), Pilz 1982, Riffle and Tinus 1982, Tainter and Walstad 1977, Navatil et al. 1981). Rhizopogon spp. are easily cultured from sporocarps and many grow rapidly in culture (Molina and Palmer 1982). Failure of past inoculation attempts with mycelial inoculum (Molina 1980) may be overcome by modification of techniques. Alternatively, basidiospores of R. vinicolor, R. colossus Smith (Castellano et al. 1984, Castellano and Trappe 1984), R. parksii Smith, R. fuscorubens Smith, R. ochraeorubens Smith, and R. subgelatinosus Smith (Castellano unpublished data) are effective ectomycorrhizal inoculum. Fruiting bodies of some of these are often locally abundant in season and could be collected for operational nursery use.

More experimental data are needed to determine if indeed host-specific fungi improve plantation performance of seedlings more than do host-generalist fungi on given sites or over a greater diversity of sites.

Table 3.1 Second year plantation survival and growth of Douglas-fir seedlings inoculated in the nursery with basidiospores of *Rhizopogon vinicolor* or noninoculated. 1/

Treatment	Seedling Height (cm)	Stem Caliper (mm)	Seedling Survival (%)	PVI 2/ (cm^3)
Inoculated	61.74a	14.2a	93.6a	46.61a
Noninoculated	58.57b	13.4b	82.9b	34.87b

1/ Treatment means within each column not followed by the same letter are significantly different by Tukey's test at P = 0.05.

2/ PVI = (root collar diameter)^2 x stem height x number of surviving trees/plot.

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