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Title: NURSERY, COLD STORAGE, AND FIELD STUDIES ON WESTERN CONIFERS INOCULATED WITH SPORES OF PISOLITHUS TINCTORIUS

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Dr. James M. Trappe

Inoculations of white fir, Shasta red fir, Douglas fir and ponderosa pine with Pisolithus tinctorius spores when outplanted failed to produce P. tinctorius mycorrhizae at the end of the first growing season. In the third year a few P. tinctorius mycorrhizae were formed on white fir. Inoculations reduced seedling survival in some cases. High rate of spore application may have desiccated roots of the true firs; levels of spore application need careful attention. Soil scarification and ripping significantly promoted growth of white fir seedlings compared to scarification alone.

White fir, Shasta red fir, Douglas fir and ponderosa pine seedlings were inoculated in a bareroot nursery with spores of P. tinctorius. The spores were applied at three rates with vs. without cold-wet pretreatment of 7 vs. 21 days. Pretreatment did not affect their efficiency as inoculum. Inoculated ponderosa pine seedlings grew significantly more than noninoculated. Growth of inoculated Douglas fir, Shasta red, and white fir seedlings did not differ significantly from that of noninoculated. Inoculations in the greenhouse with a wider range of spore application rates revealed that a higher concentration of spores was needed to induce an increase in growth and mycorrhiza formation for Douglas fir than for ponderosa pine. The effective application rates were much higher than those used in nursery inoculations.

Survival and growth of white fir, Douglas fir and ponderosa pine seedlings and survival of mycorrhizal fungi on their roots were assessed after cold storage with or without 5 ppm ethylene in combination with four root treatments: (1) washed, (2) dipped in

Truban solution, (3) dipped in Benlate solution, and (4) no treatment. Ethylene treatment resulted in increased survival, apical bud burst, and new root production when roots were untreated. Root washing and fungicide treatments, however decreased vigor of seedlings, especially that of white fir. P. tinctorius, which formed mycorrhizae with 10-20 percent of the short roots of the seedlings, did not survive cold storage. Thelephora sp. and an ectendomycorrhizal fungus both survived cold storage and rapidly colonized roots newly formed on seedlings planted after cold storage.

NURSERY, COLD STORAGE, AND FIELD STUDIES ON WESTERN CONIFERS INOCULATED WITH SPORES OF *PISOLITHUS TINCTORIUS*

by

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CHAPTER 1

INTRODUCTION

In California the minimum acceptable plantation for timber production has been defined as having 200 live and undamaged trees per acre at the end of 5 years (Schubert and Adams 1971). Spacing varies from 6 X 6 ft to 12 X 12 ft with the most common being 8 X 8 ft. To achieve minimum acceptable stocking density at 8 X 8 ft, 29 percent survival is required at the end of 5 years. Higher stocking density is desirable, especially for white fir (Abies concolor (Gord. et Glend.) Lindl.), red fir (A. magnifica A. Murr.) and Douglas fir (Pseudotsuga menziesii (Mirb.) Franco), because the plantations can be later thinned for Christmas trees to the desired stocking density for timber production.

No reliable survival data are presently available for the fifth year for tree species planted in northern California. Survival values for the first and second years vary widely, depending on site quality, tree species and type of planting stock. Whereas first year survival of 2-0 and 2-1 ponderosa pine (Pinus ponderosa Dougl. ex P. & C. Lawson) can reach 90 to 100 percent, survival of white fir is always much lower irrespective of site and type of planting stock (Adams 1962). Within a given site, first year survival of 1-0 white fir is lower than 2-0 stock. Survival values of 5 percent have been reported for 1-0 stock (Adams 1962) and from 16 to 76 percent (Adams 1961, 1962) for 2-0 stock. Survival of red fir is also low, with values of 55 percent reported for 2-0 stock (Adams 1961).

White fir grows best in moist, cool sites (Tang-Shui Liu 1971). It is susceptible to frost damage (Schubert 1955), less drought resistant than associated species (Stone 1957), and possibly, as other

Abies spp., susceptible to soil compaction (Minore et al., 1969). Red fir is similar to white fir in its site requirements but grows at higher elevations. Research on these species has been limited (Gordon 1970) and needs to be expanded before they can be planted extensively (Adams 1962). Large areas in northern California suitable for white and red fir planting are not being planted because of low survival of these species.

Prompt new root regeneration by transplanted seedlings has always been recognized as important to survival, especially in xeric sites. Initiation of root growth depends on species and weather. The minimum temperature at which roots grow is generally 5-6° C for Pinus spp. and 2-4° C for Abies spp. (Lyr and Hoffmann 1967).

Theophrastus of Lesbos (372-287 B.C.) was the first to observe that roots start growing before shoots in the spring. Today, most authors still agree with his obervations, although exceptions appear to occur among conifers. Root activity in four-year-old Abies alba began 22 days after budbreak whereas in Douglas fir and Norway spruce began 8 to 20 days before bud break (Leibundgut et al. 1963 as cited by Hermann 1977).

Literature on plant hormone action in conifers is scarce and for ethylene practically nonexistent (Zaerr and Lavender 1980). Yet exposure of herbaceous and woody plants to ethylene followed by their transfer to an ethylene free atmosphere induced root initiation (Zimmerman and Hitchcock 1933), and extension of the lateral roots initiated during the ethylene treatment (Crossett and Campbell 1975).

In most trees, the first root growth is made at the expense of reserve materials (Lyr and Hoffmann 1967), which appear to be composed of sugars and starch (Krueger and Trappe 1967). Research by Stone and collaborators over the last 30 years indicate that physiological condition of the seedlings at the time they are lifted is critical to their subsequent survival either for immediate outplanting or for cold storage. Stone (1970) evaluates root regeneration potential (RRP) by lifting the seedlings, removing all white root tips, transplanting into an environment favoring root growth and measuring the length and number of new roots after 28 days. Stone (1955) found not only major

differences in RRP among species but also among individuals of the same species. Identification of distinctive seasonal periodicity in RRP of ponderosa pine (Stone and Schubert 1959a), variation in RRP depending on nursery source of the seedlings (Stone et al. 1963) and on when seedlings were placed in cold storage (Stone and Schubert 1959b, Stone 1970) led to modifications in lifting and cold storage practices of ponderosa pine that greatly improved field survival. Studies on RRP have been extended to include Douglas fir (Stone et al. 1962) and more recently white fir (Stone and Norberg 1979). These studies can potentially improve field survival of white fir.

Mycorrhizae have been widely reported to improve seedling survival and growth (Marx 1980; Mikola 1973; and Shemakhanova 1967). Mycorrhizal fungi selected for inoculation should be ecologically adapted to the habitat in which the seedlings are going to be outplanted (Trappe 1977). In light of these considerations, the studies described in the following chapters were initiated with the objective of establishing mycorrhizae on the seedling by methods that can be easily superimposed on current forestry practices.

To obtain mycorrhizal fungi ecologically adapted to site conditions, young plantations in northern California were examined for sporocarps of mycorrhizal fungi associated with the coniferous species commercially planted in the area, i.e. ponderosa pine, Douglas fir, white fir, and red fir. The fungi associated with ponderosa pine were Rhizopogon abietis Smith, R. idahoensis Smith, R. mutabilis Smith, R. occidentalis Zeller & Dodge, R. sublaterius Smith, R. vulgaris (Vitt) M. Lange, Suillus brevipes (Peck) O. Kuntz, Suillus sp. nov., and Pisolithus tinctorius (Pers.) Coker & Couch. The fungi associated with white fir were R. mutabilis, R. occidentalis, Radügera sp., and P. tinctorius. The fungi associated with red fir were Alpova olivaceotinctus (Smith) Trappe, R. occidentalis, R. sublateritius, R. colossus Smith var. colossus, S. brevipes, and P. tinctorius. P. tinctorius was also collected associated with Douglas fir.

All fungi except S. brevipes were tested in pure culture syntheses for ability to form mycorrhizae with ponderosa pine and Douglas fir. Results were positive for all except Radügera sp. and

R. abietis. The fungus chosen for further experimentation was P. tinctorius because of its ability to form mycorrhizae with the four tree species studied, ubiquity of sporocarps and ready availability of spore inoculum, and successful application in reforestation programs (for a review see Marx 1980). The advantages of spore inoculation have been discussed by Trappe (1977) and specifically for P. tinctorius spores by Marx et al. (1979).

The first attempt was to inoculate seedlings when outplanted. Failure to get P. tinctorius mycorrhizae or to improve seedling survival by this method led to inoculations of seedlings in the nursery at different spore application rates. Studies were also conducted on ability of seedlings to regenerate new roots after cold storage in an ethylene atmosphere. The effect of cold storage on survival of P. tinctorius mycorrhizae was also examined.

INOCULATIONS OF *ABIES CONCOLOR* AND OTHER CONIFERS WITH *PISOLITHUS TINCTORIUS* SPORES AT TIME OF OUTPLANTING IN THREE CALIFORNIA FORESTS

CHAPTER 2

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ABSTRACT

Inoculations of white fir, Shasta red fir, Douglas fir and ponderosa pine with *Pisolithus tinctorius* (Pt) spores when outplanted failed to produce Pt mycorrhizae at the end of the first growing season. In the third year a few Pt mycorrhizae were formed on white fir. Inoculations reduced seedling survival in some cases. High rate of spore application may have desiccated roots of the true firs; levels of spore application need careful attention. Soil scarification and ripping significantly promoted growth of white fir seedlings compared to scarification alone.

INTRODUCTION

True firs constitute about one-fourth of the commercial sawtimber in California, but research on them has been fragmentary and often incidental to studies of other species. For many years forest managers considered these species less desirable than other conifers

(Gordon 1970). Commercial acceptance of white fir (Abies concolor (Gord. and Glend.) Lindl.) led to increasing interest in its artificial regeneration and the realization of its erratic performance when outplanted. Literature on field survival of white fir is scarce, but values varying from 16 percent (Adams 1962) to 76 percent (Adams 1961) have been reported for 2-0 stock and 5 percent (Adams 1962) for 1-0 stock. Knowledge of its physiology is limited, so its erratic field performance cannot yet be explained.

Because mycorrhizal fungi improve field survival and growth of outplanted seedlings (for a review, see Marx 1980) our study was undertaken to determine whether a) inoculation of bareroot or containerized seedlings with spores of Pisolithus tinctorius (Pers.) Coker and Couch (Pt) immediately prior to outplanting results in mycorrhiza formation and b) mycorrhizae thus formed affect subsequent survival and growth of white fir seedlings. The study sites included three areas in northern California as well as different types of planting stock and site preparation. In one study area, ponderosa pine (Pinus ponderosa Dougl. ex P. & C. Lawson), Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) and Shasta red fir (Abies magnifica var. shastensis Lemm.) were included in the study.

MATERIALS AND METHODS

The studies were established in plantations of W. H. Beaty & Associates Inc. near Westwood, Lassen County; Champion International Co., in McCloud, Siskiyou County; and Soper-Wheeler Co., in Mooreville, Yuba County. The bareroot seedlings were grown in the H. H. nursery at Sebastopol and the Humboldt nursery at Eureka. The ponderosa pine planted in McCloud and white fir planted in Westwood were 1-0 seedlings from H. H. The remaining seedlings planted in McCloud were 2-0 from Humboldt nursery. The containerized white fir seedlings were 1-0 grown at the Simpson Timber Co. nursery at Korbel. The sites in McCloud and Westwood had been mechanically scarified the previous year, with an estimated 15 cm of top soil removed. In Westwood one site was ripped after scarification. In Mooreville the

planting was done in an area with no site preparation clearcut the previous year. Site quality ranged from poor at Westwood, to intermediate at McCloud and good at Mooreville. Pt spores were extracted from sporocarps collected in the preceding fall in the Siskiyou Mountains and cold-stored until used (Marx 1976).

Prior to treatment roots of 16 groups, each of 16 randomly selected seedlings, were dipped in a slurry of water and vermiculite. Control seedlings were then planted. Seedlings were inoculated by enclosing the slurry-dipped roots of a group of seedlings in a large polyethylene bag containing Pt spores. A cloud of spores, produced by gently tapping the bottom of the bag, coated the roots. When spores had settled, the seedlings were removed and immediately planted in a completely randomized design in the last week of April. Four weeks later, after the soil settled, seedling heights were measured. Seedlings had not broken bud when measured.

Before treatments were applied 20 randomly selected seedlings from each type of planting stock were brought to the laboratory for measurements of stem diameter, total height, crown height, dry weight of the crown, number and length of lateral roots. Presence of mycorrhizae was assessed by rating their occurence in the upper, middle and lower third of the seedling root system.

In November after the first and third growing seasons, seedling survival was recorded and seedlings randomly selected by treatment for laboratory measurements as described above.

The statistical design was completely randomized, with the experimental unit a row of 16 seedlings planted at 2 m spacing. Each treatment was replicated eight times for white fir. Treatments on the remaining three species were replicated four times.

Analysis of variance was done on the survival data. Growth data was processed by analysis of covariance with initial height as the covariate.

RESULTS AND DISCUSSION

Examination of seedlings prior to outplanting revealed that the containerized seedlings lacked mycorrhizae whereas about two thirds of the short roots of bareroot seedlings were colonized by Thelephora spp. When seedlings were examined at the end of the first growing season in the field no Pt mycorrhizae were found. Microscopic examination of the roots revealed large numbers of Pt spores still present on root surfaces. All seedlings were mycorrhizal, presumably with fungi native to the sites. At the end of the third growing season a few Pt mycorrhizae were seen in the Westwood seedlings. These seedlings also had Cenococcum geophilum Fr. mycorrhizae. No Pt mycorrhizae were found in the seedlings examined from the other sites.

Survival was high for all species, specially for the true firs. Spore inoculation negatively affected first year survival of white fir planted in Westwood and red fir planted in McCloud but had no effect on subsequent years (Table 1). Mortality of inoculated white fir seedlings was highest in the poorest site, Westwood (Table 1). Pt spores are hydrophobic, so we suspect that the coating of spores on roots of inoculated seedlings were excessive to the point of inhibiting water uptake by the seedlings, thereby increasing first growing season mortality. The failure of the inoculation to produce mycorrhizae may be due in part to antagonism by resident fungi in the soil and in other part to endogenous inhibitors in spores that limit germination when spores are massed. Marx (1976) reported that Pt mycorrhiza formation is reduced when spore inoculation rate exceeds that which produces the most mycorrhizae.

Growth of inoculated and control seedlings did not differ at Westwood, where the site had been scarified and ripped. However, where the site was scarified only, inoculated seedling had larger total height and crown height than controls (Table 2). This initial growth advantage was not maintained through the third year. Inoculated ponderosa pine seedlings at McCloud had larger stem diameters and crown heights than control seedlings (Table 2). Improved growth may

Table 2.1. First and third year survival (%) of seedlings inoculated with Pisolithus tinctorius spores at time of outplanting. Values are means of 256 seedlings for white fir, 128 seedlings for other species.

Location	Species	Type of planting stock	Survival %[4]			
			Fall 1977		Fall 1979	
			Control	Inoc.	Control	Inoc.
Westwood[1]	White fir	1-0 Bareroot	73	41**	94	94
Westwood[2]	White fir	1-0 Bareroot	74	46**	89	91
Mooreville[3]	Wite fir	1-0 Container	73	69	91	87
McCloud[1]	White fir	2-0 Bareroot	82	80	74	70
McCloud[1]	Ponderosa pine	1-0 Bareroot	100	92	95	94
McCloud[1]	Douglas fir	2-0 Bareroot	85	95	--	--
McCloud[1]	Red fir	2-0 Bareroot	93	80**	--	--

[1]Site scarified, [2]Site scarified plus ripped, [3]Clearcut, no site preparation.

[4]Significant differences between control and inoculated seedlings at $P \leq 0.05$(*) and $P \leq 0.01$(**).

Table 2.2. Growth at the end of the first growing season of seedlings inoculated with spores of *Pisolithus tinctorius* at time of outplanting.

Location	Species	Treatment	Stem diameter (mm)	Total height (cm)	Crown height (cm)	Dry Wt. crown (g)	Total lateral root length (cm)
Westwood	White fir	Control	3.5	10.2	6.9	.79	318
		Inoculated	3.5	13.5**	10.7***	.95	334
McCloud	Ponderosa pine	Control	4.5	18.1	9.0	2.93	508
		Inoculated	5.5*	25.1	15.6*	4.96	696

Means marked with an asterisk are significantly different between control and inoculated seedlings at $P \leq 0.10$ (*), $P \leq 0.05$ (**), or $P \leq 0.01$ (***).

be related to a generally greater length of lateral roots in the inoculated than in the control seedlings.

Analysis of seedling growth parameters pooled by treatment and compared by site preparation at Westwood revealed that stem diameter, total height and crown height of white fir were significantly greater in scarified and ripped ground than in scarified only by the third growing season (Table 3). Ripping may favor seedling growth by encouraging development of deeper root systems (Hermann 1977). Firs may be particularly responsive to ripping, because they are more sensitive to soil compaction than other coniferous species (Minore et al., 1959). Moreover, riprows provide partial shade at the soil line which can prevent heat girdling, a cause of mortality in naturally regenerated fir seedlings in California (Gordon 1970).

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Table 2.3. Growth of 1-0 white fir seedlings at the end of the first and third growing season after outplanting in scarified and scarified plus ripped sites in Westwood.

Site preparation	Fall 1977				Fall 1979[1]			
	Stem diam (mm)	Total height(cm)	Crown height(cm)	Dry wt. crown(g)	Stem diam(mm)	Total height(cm)	Crown height(cm)	Dry Wt. crown(g)
Scarified	3.5	11.8	8.8	.8	4.2	15.2	12.4	3.7
Scarified plus ripped	3.8	12.4	9.1	1.2	5.2*	18.8**	16.3**	4.9

[1]Significant differences between scarified vs. scarified plus ripped at $P \leq 0.05$ (*) $P \leq 0.01$ (**).

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EFFECT OF APPLICATION RATE AND COLD SOAKING PRETREATMENT OF PISOLITHUS BASIDIOSPORES ON EFFECTIVENESS AS NURSERY INOCULUM ON WESTERN CONIFERS

CHAPTER 3

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ABSTRACT

Ponderosa pine, Douglas fir, Shasta red, and white fir seedlings were inoculated in a bareroot nursery with basidiospores of Pisolithus tinctorius. The spores were applied at three rates with vs. without cold-wet pretreatment of 7 vs. 21 days. Pretreatment did not affect their efficiency as inoculum. Inoculated ponderosa pine seedlings grew significantly more than noninoculated. Growth of inoculated Douglas fir, Shasta red, and white fir seedlings did not differ significantly from that of noninoculated. Inoculations in the greenhouse with a wider range of spore application rates revealed that a higher concentration of spores was needed to induce an increase in growth and mycorrhiza formation of Douglas fir than ponderosa pine. These levels were much higher than those used in nursery inoculations.

INTRODUCTION

Outplanted seedlings with Pisolithus tinctorius (Pers.) Coker and Couch mycorrhizae survive and grow better on many sites than seedlings without P. tinctorius mycorrhizae (Marx et al. 1977; Baer and Otta 1981; Dixon et al. 1981; Ruehle et al. 1981). To achieve this enhancing effect with southern pine seedlings at least half of their root systems need to be colonized by P. tinctorius (Marx et al. 1977). Mycelial inoculum has proven more conducive to mycorrhizae formation than spore inoculum in the studies reported so far (Marx et al. 1976).

Millions of seedlings are outplanted each year in the Pacific Coast states. Successful methods for inoculation with vegetative inoculum of P. tinctorius have yet to be developed. The several attempts thus far have all failed. Moreover, the great diversity of sites in mountainous terrain seems likely to require a variety of fungal ecotypes. Spores, however, are readily available. Large sporocarps, some yielding up to 200 g of spores, occur abundantly from early summer to fall in many localities. Sporocarps not collected in the fall often become a mass of bright yellow mycelia by the following early spring. This suggests that spores in this climatic zone may need exposure to cold, wet conditions to overcome dormacy. We undertook this study to determine optimum rate of spore application needed to achieve acceptable levels of P. tinctorius mycorrhizae and to test whether exposure of the spores to cold, wet conditions would alter their potential as inoculum and increase mycorrhiza formation with seedlings in two bareroot nurseries.

MATERIALS AND METHODS

Spore Collection and Storage.

Sporocarps of Pisolithus tinctorius were collected in the Siskiyou Mountains, northern California in fall 1979 from under ponderosa pine (Pinus ponderosa Douglas ex P. & C. Lawson), Douglas fir (Pseudotsuga menziesii (Mirb.) Franco), white fir (Abies concolor (Gord. et Glend.) Lindl.), and Shasta red fir (Abies magnifica var. shastensis Lemm.) at elevations from 3000 to 5000 ft. Spores were

extracted by vacuum and labeled according to host species and elevation at which the sporocarps had been collected. They were stored in the dark at 5°C (Marx 1976) until used. A mixture by host species and elevation was prepared for use in the nursery and greenhouse inoculations. One mg of mixture contained 1.2×10^6 spores.

Nursery Inoculations

The two bareroot nurseries chosen for the study were Tyee at Umpqua, Oregon and H. H. at Sebastopol, California. Tyee was inoculated in late May, at time of sowing. H. H. was inoculated in early June in beds previously sown in late April. Conventional nursery practices were followed in both nurseries throughout the study.

In Tyee 10 treatments were tested: three spore concentrations - 0.05, 0.1, and 0.2 g of spores per ft^2; three durations of soaking of spores at 1.6°C - 0, 7 and 21 days; and control, no spores applied. The experimental unit was a plot 4 ft square. Buffer plots of the same size were left between treatments.

In H. H. three treatments were tested: 0.1 g of spores per ft^2 without and with a 21-day cold soak, and control. The experimental unit was a plot 3 ft square. Buffer plots of the same size were left between treatments.

The spores were weighed to achieve the required concentration per surface area for each plot and suspended in water with a small amount of Tween-20 surfactant. Cold soaking treatments were begun 21 and 7 days, respectively, prior to inoculation. Treatments with no cold exposure were prepared the day of inoculation. All spore suspensions were transported to the nursery in an ice-chest. In the nursery, spore allotments for each plot were suspended in 5 L of water and applied evenly over the plot surface with a watering can.

Seedlings were harvested at the end of the first growing season in October at Tyee, and November at H. H. Fifty seedlings were randomly sampled from each plot, their root systems wrapped in wet paper towels, and transported in plastic bags to the laboratory in ice

chests. After washing, seedlings were examined to remove any with damaged roots. Ten of the remaining seedlings were then randomly chosen and measured for shoot height, stem diameter and percent of feeder rootlets mycorrhizal with P. tinctorius and other fungi. The seedlings were then oven dried and tops and roots weighed.

The experiments were in a randomized block design with four replications per treatment. Four species were tested in Tyee, only one at H. H. Analysis of variance was performed to test for differences among rates of application, length of cold treatment, their interactions, and control vs. factional for each variable within each species. For variables that differed significantly by treatment, means were compared by Tukey's test ($P \leq 0.05$).

Greenhouse Inoculations.

Soil from Tyee nursery was mixed with coarse vermiculite 1:1 (V:V) and steam pasteurized ($70^{\circ}C$/30 min.). Pots 14 cm in diameter and 12 cm deep were filled with this mixture. Eight stratified seeds of Douglas fir and ten of ponderosa pine were sown in 24 respective pots. Seeds started to germinate two weeks after sowing. Four weeks after sowing seedlings were thinned to four per pot, the height of each seedling recorded and inoculation treatments applied.

The treatments consisted of 0.001, 0.01, 0.1, 1, 10 g of spores per pot, and control. Control treatments received no spores because Marx (1976) found that killed spores did not affect seedling response. The spores were mixed with 40 g of the growing medium and applied as a surface layer. Steam sterilized chicken grit was applied as an additional layer to prevent splashing and drying of the inoculum layer. After inoculation each pot was mist-sprayed to infiltrate spores into the soil mix. The pots were then placed randomly on a greenhouse bench where they remained throughout the experiment. Fertilization was limited to one application of 20-19-18 NPK fertilizer at the rate of 6.1×10^{4} g/cm^{2} mid way through the experiment. Manual watering as needed was done carefully to prevent splashing. The seedlings were harvested 6 months after inoculated.

Total number of short roots were counted as well as estimated from measurements of a subsample of 6 primary laterals sampled in a stratified manner. Dry weight of all primary laterals were recorded for each seedling, and total number of short roots calculated as number short roots in subsample x dry weight of all primary laterals/dry weight of primary laterals in subsample. Percentage of P. tinctorius mycorrhizae was obtained visually for the complete root system as well as calculated from counts of the subsample.

The statistical design was completely randomized. Each treatment was replicated four times with four observations per replication. Each tree species was analyzed separately. Means of variables found to differ significantly in the analysis of variance were compared by Tukey's test ($P \leq 0.05$).

RESULTS

Nursery Inoculations.

For all species in both nurseries, length of cold soaking of spores produced no significant effects, either alone or in combination with application rates. Effects of application rates differed significantly at Tyee, especially with ponderosa pine (Table 1). With all inoculation treatments combined, inoculated pines averaged significantly greater in dry weight of tops, roots, and tops + roots than noninoculated pines. The maximum pine growth was obtained with the lowest rate, which also produced the largest percentage of P. tinctorius and total mycorrhizae (Table 1). For Douglas fir and Shasta red fir, only stem diameter was significantly different among spore application rates. No values for application rates combined differed significantly from controls (Table 1). Growth of white fir was unaffected by inoculation.

Ponderosa pine growth in H. H. was not affected by inoculation. However, the percentage of P. tinctorius mycorrhizae was significantly larger in the inoculated treatment than in the control and twice higher than percentage obtained in Tyee (Table 2).

Table 3.1. Growth and ectomycorrhiza development of 5-month-old Tyee nursery seedlings inoculated with *Pisolithus tinctorius* spores. Values for inoculation treatments are the means of 120 seedlings, for controls, of 40 seedlings.

Species	Application Rate (g/ft^2)	Spores/cm^2 Soil surface	Height (cm)	Stem Diameter (mm)	Dry weight (g)			Mycorrhizae (%)		
					Top	Root	Total	Pt	Other	Total
Pinus ponderosa	.05	64580	5.47a	2.1a	.35a	.17a	.52a	6a	40a	46a
	.1	129170	5.42a	2.1a	.34ab	.16a	.50ab	3a	41a	44a
	.2	258340	4.96a	2.0a	.29b	.15a	.44b	4a	35a	39a
	0	0	5.05	2.0	.27*	.13*	.40*	0	33	33
Pseudotosuga menziesii	.05	64580	5.24a	1.37ab	.20a	.14a	.34a	0a	54a	54a
	.1	129170	5.55a	1.47a	.20a	.13a	.33a	1a	52a	53a
	.2	258340	5.02a	1.34b	.16a	.12a	.28a	0a	57a	57a
	0	0	5.37	1.40	.19	.14	.33	0	49	49
Abies magnifica var. *shastensis*	.05	64580	5.10a	1.50a	.24a	.11a	.35a	1a	6a	7a
	.1	129170	5.30a	1.42b	.23a	.11a	.34a	0a	11a	11a
	.2	258340	5.40a	1.42b	.21a	.10a	.31a	0a	7a	7a
	0	0	5.27	1.45	.20	.11	.31	0	12	12
Abies concolor	.05	64580	3.73a	1.62a	.19a	.10a	.29a	0a	22a	22a
	.1	129170	4.16a	1.70a	.20a	.11a	.31a	1a	38b	39a
	.2	258340	4.30a	1.62a	.20a	.12a	.32a	0a	36b	36a
	0	0	4.23	1.73	.20	.12	.32	0	32	32

Means in a column, within a tree species sharing a common letter do not differ significantly by Tukey's test at $P \leq 0.05$. An asterisk (*) denotes significant differences between inoculated and control treatments based on F -test ($P \leq 0.05$).

Table 3.2. Growth and ectomycorrhiza development of 6-month-old H. H. nursery ponderosa pine seedlings inoculated with Pisolithus tinctorius spores. Values for inoculation treatments are the means of 80 seedlings, for controls, of 40 seedlings.

Application Rate (g/ft^2)	Spores/cm^2 Soil surface	Height (cm)	Stem Diameter (mm)	Dry weight (g)			Mycorrhizae (%)		
				Top	Root	Total	Pt	Other	Total
.1	129170	11.61	3.3	1.62	.82	2.44	15	85	100
0	0	11.93	3.4	1.76	.87	2.63	1*	96	97

An asterisk (*) denotes significant differences between inoculated and control treatments based on F-test ($P \leq 0.05$).

The levels of P. tinctorius mycorrhizae were low for all species and concentrations, well below the minimum levels reported to be needed to increase field survival and growth of seedling when outplanted in the sourtheastern United States (Marx et al. 1977; Ruehle et al. 1981). Seedlings from Tyee nursery had 7 to 57 percent mycorrhizal colonization of short roots, mostly with Thelephora sp. (Table 1). The percentages of mycorrhizae other than with P. tinctorius were much higher in the seedlings grown in H. H., though not statistically significant (Table 2).

Greenhouse Inoculations

Calculated total number of short roots were significantly correlated with the actual total number of short roots. The correlation coefficients were .95 for Douglas fir and .93 for ponderosa pine. The standard errors of estimate were 35 for Douglas fir and 74 for ponderosa pine. Correlation analysis of percentage P. tinctorius mycorrhizae measured visually and calculated gave correlation coefficient values of 0.96 and 0.95 for Douglas fir and ponderosa pine respectively. The standard error of estimate was 5 for both species. Analysis of variance for total number of short roots and percentage of P. tinctorius mycorrhizae measured by the two methods gave similar results. Values listed for total number of short roots and percentage of P. tinctorius mycorrhizae are those obtained from calculations based on subsampling (Tables 3 and 4).

Growth of Douglas fir measured as fresh weight of the top and height increase from time of inoculation to harvest was significantly increased by inoculation with 10 g of spores per pot (Table 3). Two seedlings from the 10 g treatment showed deep blue stain in the meristematic region of their primary roots. The cause, although unknown, seemed physiological rather than microbial. Significantly greatest colonization of roots by P. tinctorius occurred in treatments with 1 g of spores, with percentage of P. tinctorius mycorrhizae ranging from 22 to 75 and a mean of 45.

Table 3.3. Growth and ectomycorrhiza development of 6-month-old greenhouse grown Douglas fir seedlings inoculated with Pisolithus tinctorius spores. Each value is the mean of 16 seedlings.

Application Rate (g/pot)	Spores/cm^2 Soil surface	Increment Height (cm)	Fresh Weight (g)	Dry weight (g)		Short Roots	
				Top	Root	Total no.	Pt (%)
0	0	3.93a	.65a	.24ab	.31a	351a	0a
.001	7792	3.29a	.52a	.21a	.32a	423a	11a
.01	7792 x 10	2.70a	.40a	.16a	.25a	432a	8a
.1	7792 x 10^2	2.55a	.39a	.16a	.27a	330a	11a
1	7792 x 10^3	3.60a	.56a	.21a	.26a	328a	45b
10	7792 x 10^4	6.11b	1.09b	.36b	.31a	298a	7a

Means in a column followed by a common letter do not differ significantly by Tukey's test ($P \leq 0.05$).

Table 3.4. Growth and ectomycorrhiza development of 6-month-old greenhouse grown ponderosa pine seedlings inoculated with Pisolithus tinctorius spores. Each value is the mean of 16 seedlings.

Application Rate (g/pot)	Spores/cm^2 Soil surface	Increment Height (cm)	Fresh Weight (g)	Dry weight (g)		Short Roots	
				Top	Root	Total no.	Pt (%)
0	--	3.85a	1.13ab	.40abc	.58ab	662a	23b
.001	7792	4.36a	1.23ab	.42abc	.57a	796a	18ab
.01	7792 x 10	3.39a	1.00a	.37a	.60ab	668a	15ab
.1	7792 x 10^2	4.06a	1.06a	.33ab	.46ab	968a	26b
1	7792 x 10^3	4.86ab	1.43bc	.45bc	.61ab	1001a	9ab
10	7792 x 10^4	6.06b	1.60c	.49c	.73b	805a	2a

Means in a column followed by a common letter do not differ significantly by Tukey's test ($P \leq 0.05$).

The results for ponderosa pine (Table 4) are difficult to interpret due to unexplainable contamination of controls. The trend seems to indicate that lower application rates are needed for mycorrhizal formation in ponderosa pine than in Douglas fir. Growth of ponderosa pine seemed to be favored by higher application rates than those that produced maximum colonization with P. tinctorius.

DISCUSSION

Douglas fir seedlings inoculated with 10 g of P. tinctorius spores per pot in the greenhouse had a mean top growth 1.5 times larger than control seedlings. Increased top growth was not correlated with increased root dry weight, number of short roots or higher colonization by the fungus: highest percentages of mycorrhizae were obtained with the application of 1 g of spores per pot. The high rate of spore application needed to induce either seedling growth or mycorrhiza development in the greenhouse grown seedlings indicate that the application rates used in the nursery were too low to induce a response, especially in Douglas fir, Shasta red, and white fir seedlings.

Mycorrhiza formation in ponderosa pine seemed to require lower rates of spore application than Douglas fir. Higher percentages of mycorrhizae were obtained on ponderosa pine than on Douglas fir for the same rate of spore application up to a threshold value. Above the threshold, mycorrhiza formation in both species appeared to correlate negatively with increasing rates of spore application. A self-inhibitory effect seemed to be present at high spore concentrations, as suggested by Marx (1976). Ruehle (1980) using an application rate of 75000 spores/cm^2, found that, regardless of seedling age at time of inoculum, levels of P. tinctorius mycorrhizae would not exceed 10 percent. Our results are similar but comparisons of different sources of spores must be cautious because spore viability could be different. Germinability of P. tinctorius spores can be low (Lamb and Richards 1974) and probably variable, depending on method of spore extraction from sporocarps, and conditions and time

in storage. Even when spore extraction is limited to the upper portion of the sporocarp where spores are mature, it is practically impossible to distinguish healthy from aberrant spores. The latter are estimated to represent at least 20 percent of the spores (Mims 1980)

A cold soaking of spores for up to 21 days did not increase mycorrhiza formation in our experiments. Given the hypothesis that overwintering of spores in the field takes place prior to germination however, longer cold treatment combined with leaching still merits exploration. Additional research is also needed on the overall ecology and physiology of P. tinctorius spores to increase their efficiency as a source of inoculum for mycorrhiza formation. Conceivably this research will result in a consistent and economical method for nursery inoculation.

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EFFECTS OF ETHYLENE AND FUNGICIDE DIPS DURING COLD STORAGE ON ROOT REGENERATION AND SURVIVAL OF WESTERN CONIFERS AND THEIR MYCORRHIZAL FUNGI

CHAPTER 4

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ABSTRACT

Survival and growth of Douglas fir, ponderosa pine, and white fir seedlings and survival of mycorrhizal fungi on their roots were assessed after cold storage with or without 5 ppm ethylene in combination with four root treatments: (1) washed, (2) dipped in Truban solution, (3) dipped in Benlate solution, and (4) no root treatment. Ethylene treatment resulted in increase survival, apical bud burst, and new root production when roots were nontreated. Root washing decreased vigor of the seedlings, especially white fir. Pisolithus tinctorius, which formed mycorrhizae with 10-20 percent of the short roots of the seedlings did not survive cold storage. Thelephora sp. and an ectendomycorrhizal fungus both survived cold storage and rapidly colonized roots newly formed on seedlings planted after cold storage.

INTRODUCTION

Prompt production of new root growth by transplanted seedlings is a major factor in successful reforestation (Stone et al. 1962; Stone

1970). Growth of roots is governed by an array of complex factors, consisting of the environment, nutritional status of shoot and root, and growth regulators (Hermann 1977). Literature relating plant growth substances to coniferous seedling vigor is scarce and for ethylene nonexistent (Zaerr and Lavender 1980). Research on herbaceous and woody plant responses to an ethylene atmosphere followed by their transfer to an ethylene free environment induced root formation (Zimmerman and Hitchcock 1933) and extension of lateral roots initiated during the ethylene treatment (Crossett and Campbell 1975).

Blake and Linderman (unpublished data) exposed autumn-lifted western hemlock and Douglas fir to a range of ethylene concentrations in cold storage and found that 5 ppm ethylene produced seedlings with more vigorous shoot and root growth after planting than control seedlings.

Extensive losses of cold stored coniferous plants occur when molds develop on the seedlings. The fungi have been identified as those normally present in the soil (Venn 1980).

Truban and Benlate have been reported respectively to not affect and to stimulate mycorrhiza development with Pisolithus tinctorius (Pawuk et al. 1980)

The study reported here was undertaken to determine for winter-lifted seedlings (1) what combination of gas treatment (5 ppm ethylene, air) in cold storage and prestorage root treatment (untreated, washed, dipped in Truban and in Benlate solutions) was more conducive to improve survival and new root production, and (2) how cold storage affects survival of Pisolithus tinctorius (Pers.) Coker and Couch and other mycorrhizal fungi present on seedlings.

MATERIALS AND METHODS

One-year-old ponderosa pine (Pinus ponderosa Dougl. ex P. & C. Lawson) seedlings were obtained from H. H. nursery, Sebastopol, California. Two-year-old Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) and white fir (Abies concolor (Gord. et Glend.) Lindl.) were

grown in Tyee nursery, Umpqua, Oregon. Ponderosa pine seedlings were lifted in January. Douglas fir and white fir seedlings were lifted the first week of February. All seedlings were stored at 0.5° C until the last week of May. The seedlings were screened in the cold room for presence of mycorrhizae and those without mycorrhizae discarded. Twenty four hundred seedlings of each species were used in the study with a group of 100 seedlings for each experimental unit.

Four treatments were applied to the root systems prior to further cold storage; (1) washing in 5 L of water followed by shaking to remove excess water, (2) dipping in 1.66 g of Truban (30% active ingredient) or (3) 1 g of Benlate (50% active ingredient) in 5 L of water each also followed by removal of excess water, and (4) no treatment. Following each treatment the 100-seedling units were placed in diffusion-proof Maraflex bags (American Can Corp.) which were then heat-sealed. The Maraflex bags were fitted with tubing and serum septa that permitted removal of gas samples.

In April all the above treatment bags were evacuated and then re-inflated with 25 L of either air or 5 ppm ethylene in air. The seedlings were then stored for one additional month in these atmospheres at 0.5° C.

Levels of ethylene were assayed by extraction of 0.5 ml gas samples, which were immediately analyzed for C_2H_4 with a Hewlett Packard 5830 A gas chromatograph fitted with a 2 m 80-100 mesh Poropak R column at 70°C. N_2 at 40 ml/min. served as the carrier gas, delivering the sample to a flame ionization detector. Ethylene was identified by comparison with a known ethylene-in-air standard. Levels of ethylene were measured prior to and following gas treatment application as well as at the end of cold storage.

After cold storage the 100-seedling units were partitioned into three groups for each species; (1) 30 to be planted in the greenhouse for root studies of seedling growth and vigor, (2) 20 for studies of rhizoplane microbial populations and survival of mycorrhizal fungi, and (3) 50 to be outplanted for field survival studies.

Seedling Growth and Vigor.

Seedlings were planted in a pasteurized (70° C/30 min) mixture of coarse vermiculite: river sand (2:1, v:v), watered immediately, placed randomly on a greenhouse bench, and watered on alternate days. The seedlings were grown under high pressure sodium-vapor lamps (minimum 200 $E.m^{-2}.\ sec^{-1}$ at 400-700 nm) set on a 16 hr photoperiod. The temperature in the greenhouse fluctuated from 25°C during the day to 15° C at night.

At 20 and 40 days after planting survival and apical bud burst were recorded. Ten seedlings were randomly selected from each treatment and replication. Following gentle washing of the roots, total root weight, weight of new roots, number of roots with new growth, and length of new roots was determined. New root growth included growth of new roots and new growth on roots already present.

The statistical design was completely randomized, 2 X 4 factorial for each species with each treatment combination replicated three times. Each replication had 30 seedlings. Data were analyzed with initial root weight as the covariate. Treatments were compared by orthogonal contrasts.

Rhizoplane Microbial Populations and Survival of Mycorrhizal Fungi.

Randomly selected lateral roots of approximately 16 seedlings from each treatment were cut in 1 cm sections. One gram of roots was dried at 60° C for 24 hrs and weighed. Another 1 g was placed in 100 ml of sterile, doubly distilled H_2O and shaken for 20 minutes.

Dilution series were made and 1 ml aliquots of each pipetted onto Petri plates containing twenty ml of sodium albumenate or peptone dextrose with rose bengal and streptomycin (Johnson et al. 1959) agar media. The plates were incubated at 20°C in the dark for 5 days. Bacterial and actinomycete colonies were counted on the sodium albumenate plates. Fungal counts were obtained from peptone dextrose-rose bengal plates.

Each treatment was replicated three times with two observations per replication. Data were analyzed as a 2 X 4 factorial for each species, with treatments compared by orthogonal contrasts.

For survival of mycorrhizal fungi, forest soil from Jaynes Canyon, Siskiyou Mountains, was mixed with coarse vermiculite 1:1 (v:v). Half of the mixture was pasteurized (70° C/30 min). Ponderosa pine seedlings with 10-20% of their short roots colonized with Pisolithus tinctorius were planted in both pasteurized and nonpasteurized soil mix.

Three days later eight stratified seeds of ponderosa pine were sown around each seedling and covered with steam sterilized chicken grit to minimize evaporation and prevent splashing. Three weeks later germinants were thinned to four per pot. Seedlings were watered on alternate days for the duration of the study. The pots were randomly placed on a greenhouse bench. There were eight treatments replicated three times in each of two soil types. Data were not processed statistically because no P. tinctorius mycorrhizae were observed at the end of the experiment.

An additional experiment was done on survival of mycorrhizal fungi during cold storage. The remaining lateral roots of the 16 seedlings were cut in 2.5 cm sections. Half of the roots were autoclaved at 121° C for 1 hr. The root sections were placed as a layer 2.5 cm deep in single celled containers filled with pasteurized (70° C/30 min) river sand. Four seeds of ponderosa pine were sown and later thinned to two germinants per container. The design was completely randomized. Each treatment was replicated three times, with two observations per replication. Data were not processed statistically because no P. tinctorius mycorrhizae were observed at the end of the experiment.

Field survival

All seedlings were outplanted in Hilt Forest, Siskiyou Mountains, California. The sites had been mechanically scarified and ripped in fall 1979. Ponderosa pine seedlings were planted in Jaynes Canyon, at

1520 m elevation on a site that faces North-West and has a 40% slope. Douglas fir seedlings were planted in Upper Dutch Creek at 1520 m elevation on a site that faces South-Southeast and has a 30% slope. White fir seedlings were planted in Middle of Hell Creek at 1670 m elevation on a site that faces East and has a 35% slope.

Plots were completely randomized at each site. Each of the eight treatments were replicated three times with 50 seedlings per plot. Treatments were compared by orthogonal contrasts.

RESULTS

No moldiness was observed on seedlings after 4-5 months in cold storage. Initial and final levels of ethylene inside the Maraflex bags are provided in Table 1. Orthogonal contrast comparisons are presented in Table 2.

Seedling Growth and Vigor

Following planting in the greenhouse the foliage of ponderosa pine seedlings showed bronzing symptomatic of moisture stress, probably during transportation from the nursery to the laboratory. Seedlings recuperated but production of new root growth was delayed.

After 40 days growth in the greenhouse ethylene-stored ponderosa pine seedlings had significantly more new root growth than seedlings stored in air (Tables 2 and 4). For both gas treatments the best survival through time was obtained with seedlings that had no root treatment (Tables 3 and 4). Ponderosa pine seedlings stored in ethylene and with no root treatment had significantly higher survival and bud burst at 20 and 40 days after planting than seedlings with treated roots, and at 40 days new root growth was significantly higher for all variables measured in untreated than treated root systems (Tables 3 and 4). Irrespective of gas treatment, ponderosa pine seedlings with roots only washed in water survived less well than seedlings treated with aquous solutions of either Truban or Benlate. The difference was statistically significant for ponderosa pine

Table 4.1. Levels of ethylene in ppm at the beginning and end of the one month treatment period. Each value represents 12 experimental units with 100 seedlings per unit.

Species	Gas Treatment	Initial Level	Final Level
Ponderosa pine	Air	0	.66 ± .61
	Ethylene	4.37 ± .09	2.94 ± 1.96
Douglas fir	Air	0	.43 ± .04
	Ethylene	4.15 ± .03	3.07 ± 1.24
White fir	Air	0	.96 ± .33
	Ethylene	4.28 ± .06	4.27 ± 1.57

Table 4.2. Matrix of orthogonal contrasts used to compare treatments.

	Gas Treatments							
	Root treatment				Root treatment			
Contrast	Untreated	Water	Truban	Benlate	Untreated	Water	Truban	Benlate
1. Air vs. Ethylene	1	1	1	1	-1	-1	-1	-1
2. Untreated vs treated (Air)	3	-1	-1	-1	0	0	0	0
3. Water vs. Truban, Benlate (Air)	0	2	-1	-1	0	0	0	0
4. Truban vs. Benlate (Air)	0	0	1	-1	0	0	0	0
5. Untreated vs. treated (Ethylene)	0	0	0	0	3	-1	-1	-1
6. Water vs. Truban, Benlate (Ethylene)	0	0	0	0	0	2	-1	-1
7. Truban vs. Benlate (Ethylene)	0	0	0	0	0	0	1	-1

Table 4.3. Percentage survival, bud burst and root growth of 1-0 ponderosa pine seedlings grown in the greenhouse for 20 and 40 days following 5 months storage at 0.5° C including 1 month in 25 L of air or 5 ppm ethylene.

	20 Day						40 Day					
				New Root Growth						New Root Growth		
Treatment	Survival (%)	Bud burst (%)	Total Root Wt. (g)	Wt (g)	No.	Length (cm)	Survival (%)	Bud burst (%)	Total Root Wt (g)	Wt (g)	No.	Length (cm)
Air												
Roots untreated	97	31	2.10	.02	38	4	96	44	2.73	.08	69	21
Roots washed	73	14	2.42	.00	12	0	53	22	2.29	.05	43	11
Roots Truban	84	18	2.15	.00	15	5	71	34	2.25	.07	49	22
Roots Benlate	92	39	2.49	.00	32	1	78	55	2.28	.07	29	18
Ethylene												
Roots untreated	100	48	2.11	.00	19	1	98	72	2.63	.16	122	53
Roots washed	84	23	2.10	.00	16	0	70	49	1.85	.03	31	12
Roots Truban	91	9	2.52	.00	20	1	71	33	2.17	.05	20	14
Roots Benlate	90	17	2.25	.00	14	1	77	43	2.30	.11	60	38

Table 4.4. Null hypothesis probabilities of the comparisons of percent survival, bud burst and new root growth parameters of ponderosa pine seedlings grown in the greenhouse for 20 and 40 days, by orthogonal contrasts of the treatments.

	20 Day						40 Day					
				New Root Growth						New Root Growth		
Contrast	Survival (%)	Bud burst (%)	Total Root Wt. (g)	Wt (g)	No.	Length (cm)	Survival (%)	Bud burst (%)	Total Root Wt (g)	Wt (g)	No.	Length (cm)
1	NS	NS	NS	NS	NS	.04	NS	NS	.05	.05	.01	.02
2	.007	NS	.0005	.0005	.008	.04	.0002	NS	NS	NS	NS	NS
3	.004	NS	NS	NS	NS	NS	.003	.04	NS	NS	NS	NS
4	NS	NS	NS	NS	NS	.01	NS	NS	NS	NS	NS	NS
5	.01	.002	NS	NS	NS	NS	.0006	.008	.005	.005	.00009	.003
6	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	.04	NS

NS = Not significant

seedlings stored in air (Tables 3 and 4). The percentage of seedlings that broke bud by the 40th day was significantly lower for those with washed roots than for seedlings treated with fungicides (Tables 3 and 4).

Douglas fir seedlings with untreated roots stored in ethylene had higher survival than seedlings with treated roots. At 40 days new root growth was significantly higher for untreated than for treated root systems (Tables 5 and 6). Douglas fir seedlings stored in ethylene after root washing only had lower survival, new root production and incidence of bud burst than seedlings treated with fungicides (Tables 5 and 6). The Benlate treatment had an initial positive effect on new root production but the effect did not persist (Tables 5 and 6).

Survival of white fir seedlings was not significantly affected by gas treatment, but the percentage of seedlings that broke bud 20 days after planting was significantly higher for seedlings stored in ethylene than in air (Tables 7 and 8). Irrespective of gas treatment, root washing reduced survival and bud burst of white fir seedlings (Tables 7 and 8).

Rhizoplane Microbial Populations and Survival of Mycorrhizal fungi.

Root washing resulted in higher levels of actinomycetes than other root treatments (Table 9). For ponderosa pine seedlings stored in ethylene and Douglas fir seedlings stored in air the populations of actinomycetes on washed roots were significantly higher than on roots treated with fungicides (Tables 9 and 10). For white fir seedlings stored in air, actinomycetes and populations of bacteria were lower on nontreated than on treated roots (Tables 9 and 10). Roots of Douglas fir seedlings stored in ethylene had higher bacteria populations than seedlings stored in air (Tables 9 and 10). Fungal populations were unaffected by either root or gas treatments.

After 5 months growth of new ponderosa pine germinants grown with treated seedlings in the greenhouse, no P. tinctorius mycorrhizae had

Table 4.5. Percentage survival, bud burst and root growth of 2-0 Douglas fir seedlings grown in the greenhouse for 20 and 40 days following 4 months storage at 0.5° C including 1 month in 25 l. of air or 5 ppm ethylene.

Treatment	20 Day						40 Day					
				New Root Growth						New Root Growth		
	Survival (%)	Bud burst (%)	Total Root Wt. (g)	Wt (g)	No.	Length (cm)	Survival (%)	Bud burst (%)	Total Root Wt (g)	Wt (g)	No.	Length (cm)
Air												
Roots untreated	86	86	2.15	.13	99	28	84	95	4.02	.76	279	296
Roots washed	91	72	2.14	.19	125	31	87	91	4.54	.77	244	253
Roots Truban	82	65	2.32	.13	101	27	81	89	6.30	1.23	379	443
Roots Benlate	84	80	2.90	.20	134	50	83	96	3.96	.65	176	242
Ethylene												
Roots untreated	96	89	2.87	.16	146	37	94	93	5.81	1.15	502	389
Roots washed	86	58	2.51	.11	69	18	78	84	4.02	.70	302	180
Roots Truban	89	94	2.81	.14	134	30	88	99	4.80	.97	308	298
Roots Benlate	86	74	3.79	.30	147	70	82	99	4.42	.71	189	232

Table 4.6. Null hypothesis probabilities of the comparisons of percent survival, bud burst and new root growth parameters of Douglas fir seedlings grown in the greenhouse for 20 and 40 days, by orthogonal contrasts of the treatments.

Contrast	20 Day						40 Day					
				New Root Growth						New Root Growth		
	Survival (%)	Bud burst (%)	Total Root Wt. (g)	Wt (g)	No.	Length (cm)	Survival (%)	Bud burst (%)	Total Root Wt (g)	Wt (g)	No.	Length (cm)
1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	.02
5	.03	NS	NS	NS	NS	NS	.03	NS	NS	NS	.05	.01
6	NS	.01	NS	NS	.01	.01	NS	.001	NS	NS	NS	NS
7	NS	NS	.05	.05	NS	.005	NS	NS	NS	NS	NS	NS

NS = Not significant

Table 4.7. Percentage survival, bud burst and root growth of 2-0 white fir seedlings grown in the greenhouse for 20 and 40 days following 4 months storage at 0.5° C including 1 month in 25 L of air or 5 ppm ethylene.

Treatment	20 Day						40 Day					
				New Root Growth						New Root Growth		
	Survival (%)	Bud burst (%)	Total Root Wt. (g)	Wt(g)	No.	Length (cm)	Survival (%)	Bud burst (%)	Total Root Wt (g)	Wt(g)	No.	Length (cm)
Air												
Roots untreated	88	38	2.67	.04	31	10	70	64	4.07	.65	141	171
Roots washed	60	10	3.66	.01	19	5	33	67	4.18	.68	161	161
Roots Truban	86	40	2.59	.09	36	16	72	72	4.61	.75	220	204
Roots Benlate	99	63	2.00	.10	52	25	98	76	3.88	.64	257	215
Ethylene												
Roots untreated	90	57	2.66	.08	50	26	83	74	4.17	.81	146	205
Roots washed	43	23	2.45	.03	22	8	34	31	4.90	.57	350	209
Roots Truban	99	74	2.97	.08	48	21	94	91	4.15	.70	278	241
Roots Benlate	100	66	1.97	.08	34	17	92	90	3.63	.71	300	261

Table 4.8. Null hypothesis probabilities of the comparisons of percent survival, bud burst and new root growth parameters of white fir seedlings grown in the greenhouse for 20 and 40 days, by orthogonal contrasts of the treatments.

	20 Day						40 Day					
				New Root Growth						New Root Growth		
Contrast	Survival (%)	Bud burst (%)	Total Root Wt. (g)	Wt.(g)	No.	Length (cm)	Survival (%)	Bud burst (%)	Total Root Wt (g)	Wt.(g)	No.	Length (cm)
1	NS	.0007	NS	NS	NS	NS	NS	NS	NS	NS	.04	NS
2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	.04	.00003	NS	NS	NS	NS	.004	NS	NS	NS	NS	NS
4	NS	.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	.003	NS
6	.001	.00001	NS	NS	NS	NS	.001	.0001	NS	NS	NS	NS
7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant

Table 4.9. Rhizoplane microorganisms of ponderosa pine, Douglas fir and white fir seedlings after 4-5 months storage at 0.5° C including 1 month in 25 L of air or 5 ppm ethylene. Values are given per 1 g dry weight of roots.

Treatment	Ponderosa pine			Douglas fir			White fir		
	Bacteria	Actino.	Fungi	Bacteria	Actino.	Fungi	Bacteria	Actino.	Fungi
	x10^6	x10^6	x10^4	x10^6	x10^6	x10^4	x10^6	x10^6	x10^4
Air									
Roots untreated	100	8	147	183	13	20	53	10	18
Roots washed	118	30	88	420	37	26	193	56	50
Roots Truban	83	4	79	186	17	29	382	39	30
Roots Benlate	582	17	115	196	15	40	203	28	15
Ethylene									
Roots untreated	619	74	92	545	27	30	46	14	24
Roots washed	911	101	127	476	28	31	196	34	39
Roots Truban	195	15	168	481	15	33	93	18	15
Roots Benlate	195	15	122	573	15	26	294	15	12

Table 4.10. Null hypothesis probabilities of the comparisons of bacteria, actinomycetes and fungi populations on the rhizoplane of ponderosa pine, Douglas fir and white fir roots by orthogonal contrasts of the treatments.

	Ponderosa pine			Douglas fir			White fir		
	Bacteria	Actino.	Fungi	Bacteria	Actino.	Fungi	Bacteria	Actino.	Fungi
Contrast	$x10^6$	$x10^6$	$x10^4$	$x10^6$	$x10^6$	$x10^4$	$x10^6$	$x10^6$	$x10^4$
1	NS	NS	NS	.01	NS	NS	NS	NS	NS
2	NS	NS	NS	NS	NS	NS	.03	.05	NS
3	NS	NS	NS	NS	.006	NS	NS	NS	NS
4	NS	NS	NS	NS	NS	NS	NS	NS	NS
5	NS	NS	NS	NS	NS	NS	NS	NS	NS
6	.01	.05	NS	NS	NS	NS	NS	NS	NS
7	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant

formed. However, both treated seedlings and new germinants had abundant ectendomycorrhizae. In several pots, sporocarps of Anthracobia sp. appeared and were attached to ectendomycorrhizae. The seedlings growing in nonpasteurized forest soil additionally had a Rhizopogon type of mycorrhiza that occurred on the distal end of the ectendomycorrhizae. It appeared that ectendomycorrhizae had predominated on the root system of the stored seedlings, and that the ectendomycorrhizal fungus had initially colonized the short roots of the germinants. The Rhizopogon mycorrhizae apparently developed later.

When ponderosa pine seedlings inoculated with untreated and autoclaved root sections were compared similar results were obtained regarding survival of mycorrhizal fungi. Seedlings inoculated with untreated root sections developed ectendomycorrhizae and Thelephora sp. ectomycorrhizae. Seedlings inoculated with autoclaved root sections remained mostly nonmycorhizal although occasionally Thelephora sp. mycorrhizae were observed, probably due to greenhouse contamination. Ectendomycorrhizae were not observed in seedlings inoculated with autoclaved roots.

Field Survival.

Douglas fir seedlings stored in air had higher field survival than seedlings stored in ethylene (Table 11). White fir seedlings with washed roots had significantly lower field survival than seedlings treated with fungicides (Table 11). No differences by either root or gas treatment were found for ponderosa pine seedlings.

DISCUSSION

Cold storage of ponderosa pine, Douglas fir and white fir seedlings in 5 ppm ethylene seemed to favorably affect survival, early bud burst and new root growth, especially when the root system of the seedlings was not treated. The beneficial effect of ethylene was more pronounced for the 1-0 ponderosa pine than for the 2-0 Douglas fir and

Table 4.11. Percentage survival of outplanted ponderosa pine (1-0), Douglas fir (2-0) and white fir (2-0) seedlings after 4-5 months storage at 0.5° C including 1 month in 25 L of air or 5 ppm ethylene. Each value is the mean of 150 seedlings.

Treatment	Ponderosa pine	Douglas fir	White fir
Air			
Roots untreated	46	50	15
Roots washed	40	44	2
Roots Truban	37	56	12
Roots Benlate	65	45	24
Ehtylene			
Roots untreated	54	38	25
Roots washed	58	19	7
Roots Truban	37	32	23
Roots Benlate	47	32	27

Significant contrasts were C-1 for Douglas fir ($F = .009$) and C-6 for white fir ($F = .04$).

white fir. It appears that cold storage in ethylene is more effective in promoting seedling vigor for autumn-lifted seedlings than for winter-lifted seedlings. Mortality of Douglas fir was reduced from 63% to 7% for seedlings lifted in the fall (Blake cited by Zaerr and Lavender 1980) whereas no such dramatic difference was observed in our study of winter-lifted seedlings. The manner in which ethylene affects seedling vigor and whether this effect is direct or in interaction with other plant growth regulators is unknown. Ethylene may improve seedling vigor by correcting physiological disturbances related to improper completion of the seedling dormancy cycle.

Treatment of the root systems was less desirable than leaving roots untreated. Dipping of roots in fungicides was not significantly detrimental to seedling survival and growth whereas washing of the roots was, especially for white fir. Washing of the roots was associated with higher levels of actinomycetes on the rhizoplane than any other treatment. The negative effects of this treatment were not related to mold, because none developed during storage. Studies on microbial interactions and root nutritional status prior to and following cold storage may shed light on this phenomenon.

Survival of seedlings in the field was low for all species and no doubt related to the dry weather conditions prevalent during the growing season.

After 5 months in the greenhouse no P. tinctorius mycorrhizae survived on ponderosa pine. Prior to storage 10-20% of the short roots of these seedlings were colonized by P. tinctorius. Shortleaf pine seedlings with initial levels of 70% P. tinctorius mycorrhizae were reported to survive cold storage for 4 months (Mark 1979). In our study, death of P. tinctorius mycorrhizae may have been exacerbated by physiological disturbances of the ponderosa pine seedlings manifested by delayed production of roots when planted following storage. However, survival of ectendomycorrhizal fungi was not affected. Ectendomycorrhizae formed on new roots produced by the seedlings that had been in cold storage and the fungus grew equally well through pasteurized and nonpasteurized forest soil to colonize young seedlings grown around them. Ponderosa pine seedlings collected

in the field in the fall also had ectendomycorrhizae, although much of the new root growth was colonized by Rhizopogon spp. native to the forest site. Thelephora mycorrhizae also survived 4 months of cold storage. Thelephora sp. frequently colonized the new growth of existing Thelephora mycorrhizae on Douglas fir as early as 20 days after planting.

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