Introduction

Successful establishment of conifer tree seedlings on reforestation sites often depends on ectomycorrhizal (EM) development to capture scarce site resources (Danielson 1985; Perry et al. 1987). EM fungi can aid seedlings in overcoming moisture and nutrient stress and can decrease transplant shock (Marx 1991), especially on degraded sites such as

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F.P. Teste.[1] Department of Forest Sciences, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
M.G. Schmidt. Department of Geography, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
S.M. Berch. British Columbia Ministry of Forests, Victoria, BC V8W 3E7, Canada.
C. Bulmer. British Columbia Ministry of Forests, Vernon, BC V1B 2C7, Canada.
K.N. Egger. Ecosystem Science and Management Program, University of Northern British Columbia, Prince George, BC V2N 4Z9, Canada.

[1]Corresponding author (e-mail: fteste@interchange.ubc.ca).

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landings (Perry et al. 1987). Landings are relatively small, flat sites that have been compacted by heavy equipment and may have only B and C soil horizons remaining (Plotnikoff et al. 2002). They are characterized by higher bulk density and lower macropore space, lower organic matter and nutrient contents, and poorer water infiltration than that of harvested areas outside of landings (Carr 1987). Compaction on landings reduces soil aeration, which decreases root respiration and microorganism activity (Carr 1987; Page-Dumroese et al. 1998) and typically has detrimental effects on conifer seedlings and EM development.

Commercial nursery managers are often told that inoculating seedlings with appropriate EM inoculants can substantially increase performance of conifers on reforestation sites. However, Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) seedling responses to fungal inoculants are variable. In the Pacific Northwest, inoculation of seedlings with *Pisolithus tinctorius* (Pers.) Coker et Couch, the choice fungal inoculant for reclamation projects (Marx et al. 1992), has not always resulted in increased survival and growth. Inoculation trials using *Laccaria laccata* (Scop.:Fr.) Berk. & Br. increased early survival and growth of Douglas-fir seedlings on certain plantation sites (Perry et al. 1987; Hunt 1992), while no beneficial responses were observed on other sites (Bledsoe 1992; G.A. Hunt, unpublished data). Positive growth results in the Pacific Northwest using Douglas-fir have been observed in seedlings inoculated with *Rhizopogon parksii* Smith and *Rhizopogon vinicolor* (Castellano 1996). *Rhizopogon* spp. are common and often dominate on Douglas-fir seedlings grown in disturbed forest soils (Molina and Trappe 1994). They show strong host specificity with Douglas-fir (Molina and Trappe 1982) and possess rhizo-

(*iv*) adjacent clearcut with undisturbed soil. Burned piles were selected from existing areas where logging debris was disposed of by burning. Clearcut plots were randomly located on sites with similar slope position near the landings.

In the spring of 2000, inoculated and control Douglas-fir seedlings were outplanted in rows on each soil-treatment plot. Control seedlings were planted on all soil-treatment plots. Seedlings inoculated with *L. laccata* and *R. parksii* (OR) were planted on the landing and clearcut plots; seedlings inoculated with *R. parksii* (BC) were planted on the landing and burned pile plots. Each row was made up, on average, of eight seedlings inoculated with the same inoculant. On average, 21, 11, and 9 rows were laid out systematically and dispersed across each landing (shallow- and deep-tilled), clearcut, and burned pile plot, respectively.

Initial measurement of seedling growth and percent EM colonization and final measurement of seedling survival and growth

At time of lifting (i.e., before outplanting), 20 Douglas-fir seedlings per inoculation treatment were subsampled to measure height, root-collar diameter, biomass, and percent EM colonization. Determination of percent EM colonization was based on a modified gridline intercept method (Giovannetti and Mosse 1980) applied after roots were cleared and stained (Phillips and Hayman 1970). Because nursery seedlings often form ectomycorrhizae with little or no mantle, it is necessary to clear and stain roots to detect Hartig net development and to obtain an accurate assessment of percent colonization. The modification to the gridline intersect method was simply to assess each fine feeder root that intersected a gridline and determine whether it had a Hartig net or a mantle. We counted presence or absence of ectomycorrhizae in 200 fine feeder roots. Survival and height were assessed for all seedlings in the fall of 2001 at the end of two growing seasons (16 months) in the field. In September 2001, 15 uninoculated Douglas-fir seedlings were randomly selected and destructively sampled from each clearcut and landing plot to determine EIP and for morphotyping.

Soil sampling and analysis

Soil samples were collected between 28 May and 1 June 2001 by the excavation method (Blake and Hartge 1986), from the top of the mineral layer to a depth of 20 cm at six locations within each plot (following a grid pattern). These were used to determine coarse fragment content, bulk density (Db) of the fine fraction, gravimetric moisture content (θ_m), pH, and nutrient concentrations. For clearcut plots, the forest floor was sampled and placed in separate bags. Mineral soil samples were air dried and sieved through a 2-mm sieve. Soil analyses were carried out by the Analytical Laboratory in Victoria, British Columbia (B.C. Ministry of Forests, Research Branch), using the methods described in the following paragraph.

We determined soil pH in H_2O, total C (Tiessen and Moir 1993), total N (McGill and Figueiredo 1993), mineralizable N (Keeney 1982), available P (Kalra and Maynard 1991), and exchangeable K, Ca, Mg, Fe, and Mn (Kalra and Maynard 1991; Hendershot et al. 1993). Nutrient content per hectare was also determined.

Greenhouse bioassay

Bulk mineral soil was collected from each landing and adjacent clearcut in October 2000 to a depth of 20 cm at random locations and sieved through a screen with a 1-cm mesh. This soil was stored for 18 weeks at 5 °C or colder at the Kalamalka Research Station in Vernon.

Interior Douglas-fir seedlings (seedlot 31851) were grown in styroblocks by Riverside Nursery in Vernon for one growing season. Randomly selected seedlings from the Riverside nursery were cleared and stained before the start of the greenhouse bioassay to determine initial percent colonization. We found that seedlings from the Riverside Nursery had 0% colonization.

In March 2001, 21 seedlings were randomly selected and assigned to soil treatments. A single seedling was planted in each black plastic 3.2-L pot (with six leaching holes per pot), and the pots were randomly placed on greenhouse benches. Perlite (Micronise Ultratech) mixed with collected soil (1:1) was used as the potting medium. Rerandomization of pot location on greenhouse benches was performed once a month.

Seedlings were grown at Simon Fraser University, Burnaby, British Columbia. High-pressure sodium lamps were used with a photoperiod of 15 h per day until the native photoperiod surpassed this value in mid-June. Day and night temperatures were set at 20 °C (±5 °C). Seedlings were watered when volumetric soil moisture reached about <0.1 $m^3 \cdot m^{-3}$, determined using a theta probe (Delta-T Theta Probe Meter(<0R(and)D(er2sgnd)Da

Extraction, amplification, restriction endonuclease digestion, and gel electrophoresis of EM DNA

A subsample of three root tips was randomly collected for each distinct morphotype, placed in 1-mL microvials, and stored at −80 °C until DNA analysis was carried out. All molecular work was carried out in the research laboratory of Keith Egger at the University of Northern British Columbia. We used the procedures outlined in Mah et al. (2001), except that Platinum *Taq* DNA Polymerase (Invitrogen, Burlington, Ontario) was used.

Restriction endonuclease RFLP profiles using *Alu*I, *Hin*fI, and *Rsa*I (Invitrogen) were analyzed with Gene Profiler version 4.05 software (Scanalytics Corp.). Log piecewise linear curve fitting was used to calibrate fragment sizes against a DNA standard (1-kb DNA ladder; Introgen). A database of RFLP patterns generated in the present study was compared with a reference (Egger & Massicotte (E&M)) database containing morphotypes identified in previously published (Mah et al. 2001) and unpublished studies. Matches were first identified by generating a pairwise distance matrix based upon the "PHYLIP Query" (sum of polymorphic bands) distance measure in Gene Profiler. The resulting distance matrix was subjected to neighbor-joining analysis using the "Neighbor" program from the phylogenetic inference software PHYIP (Felsenstein 1996), to identify close matches, and then the patterns were visually compared to determine a final match.

EIP

EM status (colonization, abundance, richness, diversity, and evenness) of the destructively sampled seedlings from the field was assessed and used as an indicator of the EIP of the clearcut and landing soils. Following morphotyping, percent EM colonization was determined based on 300 randomly subsampled root tips. In addition, for the greenhouse bioassay only, 10 seedlings per treatment × block combination (60 seedlings) were subsampled to determine percent EM colonization, by clearing and staining. The number of morphotypes and their relative abundance were also determined. When a morphotype was seen during the initial root system examination but not found in the random 300 root tip count, a value of zero was assigned (i.e., indicating it was present but with negligible colonization). Richness (i.e., number of morphotypes per seedling), diversity (Shannon diversity index (H')), and evenness (Shannon evenness index)

Percent EM colonization of control seedlings was very low (1%), and significantly lower ($p < 0.01$) than that of inoculated seedlings (36%) (Table 1). There were no significant differences in percent EM colonization among seedlings treated with different inoculants (Table 1).

After two growing seasons, there were no significant differences in survival or height increment between any of the inoculation treatments within the different soil treatments (Fig. 1). Nevertheless, *Rhizopogon parksii* (OR) tended to show the greatest height increases (not significant) on all soil treatments. Seedlings growing on clearcuts and burned piles had significantly greater height increments than did seedlings growing on shallow- or deep-tilled landings (Fig. 1). Both shallow- and deep-tilled landings produced very little height increment. Differences between the two tilled soil rehabilitation treatments were not significant, but deep-tilled landings, on average, showed larger height increments.

Soil physical and chemical properties

Soil from shallow-tilled landings had significantly higher bulk density and lower moisture content (in June 2000) than

associated with aging *Suillus–Rhizopogon* morphotypes. It shares a high proportion of fragments with some *Mycelium radicis atrovirens* (MRA) genotypes, but this genotype has not yet been sequenced to confirm a relationship. *Thelephora americana*-like (Ta) also produced three RFLP patterns. Several Ta samples produced RFLP patterns that matched the *Thelephora* genotype 1 pattern in Mah et al. (2001), including the morphotype referred to as "undifferentiated (Undif)". This pattern is close to the pattern described for *Thelephora*-like in Hagerman et al. (1999). A slight variation was found in one Ta type, which matched a *Thelephora* genotype in the E&M databases. One putative *Thelephora* genotype, *Lactarius*-like (Lac), Unknown (flaky yellow) (Fy), and Unknown (caramel jigsaw) (Cj) did not substantially match any patterns in the E&M databases.

EIP

In the greenhouse bioassay, seedlings grown in landing soil had significantly higher EM colonization (14% higher, determined by the clearing and staining method) than seedlings grown in the clearcut soil (Fig. 2). The total number of morphotypes found was greater on seedlings grown on clearcut soil than on landing soil (Fig. 3). *Rhizopogon*-like was the most abundant morphotype to form on seedlings growing in the landing soil (42%). For comparison, five naturally regenerated seedlings were collected from landings, and EM status was determined. Naturally regenerated seedlings growing on the landings had, on average, 37% of their fine root tips colonized by EM fungi (data not shown). Naturally regenerated seedlings were mostly colonized by *Rhizopogon*-like and *Thelephora americana*-like. These results are very similar to what we found with outplanted seedlings (see Figs. 2 and 3).

Morphotype richness and diversity were marginally higher on roots of seedlings grown in clearcut soil (p = 0.13 and 0.12, respectively) than in landing soil (Fig. 4). Root systheir055-1.1sem

Table 3. Nutrient concentrations and contents of soils from landings tilled to a depth of 15 cm (L15cm), landings tilled to a depth of 50 cm (L50cm), clearcuts, and burned piles at Miriam Creek.

						Exchangeable					
Soil treatment	Total C	Total N	C:N ratio	Mineralizable N	Available P	K	Ca	Mg	Fe	Mn	Al
Concentration[a]											
L15cm	14.7a	0.49a	28.8a	1.3a	22a	0.13a	5.1a	1.1a	0.002a	0.107a	0.057a
	(6.2)	(0.17)	(0.4)	(0.1)	(8)	(0.01)	(0.4)	(0.1)	(0.001)	(0.018)	(0.017)
L50cm	17.4a	0.54a	29.9a	1.9a	23a	0.17a	5.2a	1.1a	0.005a	0.076a	0.061a
	(10.2)	(0.24)	(1.7)	(1.0)	(12)	(0.02)	(0.4)	(0.2)	(0.002)	(0.011)	(0.015)
Clearcut	27.9a	1.08a	27.0a	21.7b	75a	44.90b	49.0b	45.2b	0.028b	1.078b	0.529b
	(7.6)	(0.35)	(5.0)	(7.6)	(37)	(2.63)	(5.7)	(2.6)	(0.007)	(0.102)	(0.138)
Burn	27.9a	0.68a	38.3a	3.6ab	68a	0.36a	6.6a	1.2a	0a	0.011a	0a
	(10.7)	(0.14)	(4.7)	(1.6)	(18)	(0.04)	(1.5)	(0.2)	(0)	(0.004)	(0)
p	0.47	0.21	0.31	0.03**	0.20	<0.0001***	0.0001***	<0.0001***	0.009***	0.0011***	<0.0001***
Content (kg·ha^{-1})											
L15cm	40 000a	1420a	—	4a	63a	158a	6340a	890a	2.5a	170a	42ab
	(14 000)	(350)	—	(1)	(27)	(4)	(450)	(280)	(1.6)	(64)	(20)
L50cm	34 000a	1150a	—	4a	50a	151a	4950a	690a	6.7a	91ab	35a
	(16 000)	(360)	—	(1)	(23)	(27)	(310)	(260)	(3.9)	(8)	(20)
Clearcut	98 000b	2270a	—	50b	125a	205a	4550a	310a	7.5a	101ab	104b
	(23 000)	(540)	—	(12)	(49)	(8)	(610)	(150)	(1.2)	(18)	(25)
Burn	35 000a	930a	—	5a	107a	209a	3640a	380a	0.0a	9b	0a
	(11 000)	(140)	—	(2)	(45)	(33)	(760)	(20)	(0.0)	(3)	(0)
p	0.02**	0.07*	—	0.006***	0.26	0.32	0.07*	0.15	0.08*	0.04**	0.008***

Note: Concentrations are for mineral soils (0–20 cm depth); total element contents are for mineral soils (0–20 cm depth) plus the forest floor. Values are means, with standard errors in parentheses. *, $p < 0.1$; **, $p < 0.05$; ***, $p < 0.01$. Means in the same column followed by the same letter are not significantly different ($p < 0.05$ was used for Tukey's HSD multiple comparison test).

[a]Nutrient concentrations are in the following units: total C and total N, grams per kilogram; mineralizable N and available P, milligrams per kilogram; exchangeable cations, centimoles per kilogram.

Morphotype	Brief morphotype description[a]
Rhizopogon-like (Rz)	Irregular to subtuberculate, silvery white mycorrhiza; hairy, brown mycelial strands; felt prosenchyma outer mantle; wide, emanating hyphae with elbow-like bends; very similar to *Rhizopogon vinicolor* and *Rhizopogon parksii*
Thelophora americana-like (Ta)	Brown or orange, thin, and sometimes wrinkled mycorrhiza; net synenchyma outer mantle; long cystidia with basal clamp
Cenococcum geophilum (Cg)	Pure black mycorrhiza; net synenchyma in a stellate pattern; large (5 µm wide), black, emanating hyphae at low magnification
Unknown (dark brown) (Db)	Dark brown, felty mycorrhiza; net prosynenchyma outer mantle; large (4.5 µm wide), yellow, emanating hyphae
Unknown (silver-amorphous) (Sa)	Smooth, silver mycorrhiza; interlocking irregular synenchyma outer mantle with mucilaginous matrix
Amphinema byssoides	

organic-matter-poor sites (Page-Dumroese et al. 1990) negatively affect growth of Douglas-fir regardless of EM status.

At time of lifting in the present study, roots of inoculated seedlings were colonized at 36%, compared with 1% for control seedlings. Similarly, Hunt (1992) found inoculated and control interior Douglas-fir seedlings colonized at 52% and 0%, respectively, at time of planting. Other conifers raised under similar nursery conditions in the Pacific Northwest typically have much better colonization at time of planting. For instance, inoculated and control Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and lodgepole pine were both colonized in the 90% range (Hunt 1992). It is not clear why interior Douglas-fir seedlings are often found with low levels of colonization at time of lifting, but it could be that the benefits of EM inoculants may not be attained at these relatively low colonization levels.

We, as did G. Xiao and S.M. Berch (unpublished data), showed that uninoculated interior Douglas-fir can readily form ectomycorrhizae within months in well-aerated soil, without fertilization and excess watering (Fig. 2). Under these conditions, we obtained higher colonization levels (40%–50%) than with uninoculated seedlings (1%) used as controls in the field. Nursery conditions for the seedlings used in our study may not have favored high colonization levels. Perhaps current nursery conditions could be modified to permit higher colonization of interior Douglas-fir at time of planting. EM colonization might be improved by maintaining soil pH between 4.0 and 6.0, keeping N and P fertilization rates at a modest level, promoting timely irrigation, allowing adequate substrate aeration (Amaranthus et al. 1996), and using selected fungicides, such as fermate, captan, and benomyl, to stimulate EM development (Linderman 1987; Marx and Cordell 1987).

Influence of soil treatments on soil properties and seedling survival and growth

We found no significant differences in physical and chemical soil properties between shallow- and deep-tilled landings. Clearcuts and burned piles had lower soil bulk densities and higher moisture contents than shallow-tilled landings. Soil bulk densities and moisture contents were in the same range as those found by Plotnikoff et al. (2002) on landings in the interior of British Columbia. High bulk density and low moisture content can inhibit conifer seedling root development (Conlin and van den Driessche 1996) and EM growth (Skinner and Bowen 1974; Amaranthus et al. 1996).

Total N, available P, and K contents on landings were in the same range reported by Carr (1987). Total C content and mineralizable N concentration and content were significantly lower for both shallow- and deep-tilled landings than clearcuts. Microbial activity was probably low in mineral soils on landings because of the lack of organic matter. Microbial activity is positively correlated with total C and mineralizable N (Ballard and Carter 1985; Myrold 1987).

Seedlings grown in clearcuts and burned piles had much greater height increments than shallow- and deep-tilled landing-grown seedlings. Douglas-fir seedlings and EM fungi usually encounter growth problems in compacted soil (Skinner and Bowen 1974; Wert and Thomas 1981). Early growth of Douglas-fir may be impaired on landings because of compaction, deficiencies in N, and harsh summer climatic conditions. Douglas-fir seedlings growing on shallow- and deep-tilled landings were chlorotic and had very few live buds. In these conditions, interior Douglas-fir may not overcome transplant shock and is perhaps more sensitive than other commercial species, such as lodgepole pine, to compacted and nutrient-deficient soils on landings (Plotnikoff et al. 2002).

EIP

Fourteen distinct morphotypes made up the EM community in this study. Landings had four morphotypes (with relative abundance >5%) and average EM colonization of 55% at time of sampling. EIP was not lower on landings compared with clearcuts, since richness and diversity of morphotypes were not significantly different on seedlings growing in landing soil as compared with seedlings growing on clearcuts. Berch and Roth (1993) reported 13 morphotypes for coastal Douglas-fir on clearcuts. On more disturbed sites resembling our landings, Parke et al. (1984) and Perry et al. (1982) only found one or two morphotypes and low percent EM colonization. Positive inoculation treatment effects may be more likely when site EIP is low to nonexistent and outplanted seedlings are heavily colonized by inoculated EM fungi.

High percent colonization (≈90%) of coastal Douglas-fir seedlings outplanted in clearcut soils has been reported after one growing season (Borchers and Perry 1990; Roth and

Berch 1992). Two years after outplanting, we found that interior Douglas-fir seedlings had a relatively low percent colonization (30%–45% on clearcuts and 40%–55% on landings). The seedlings in the studies mentioned above were colonized at time of outplanting, while those in our study were not. In our study, a qualitative analysis revealed high fine root mortality on both landing- and clearcut-grown seedlings, indicating that a certain portion of the root systems died off to give rise to new fine roots. Under this scenario, ectomycorrhiza formation would be limited until new roots were established. Also, such a process would present a high carbon demand for the development of these new roots. Douglas-fir seedlings growing on harsh landings may be at a disadvantage compared with other conifer species such as lodgepole pine that are EM at time of outplanting (G. Xiao and S.M. Berch, unpublished data) and apparently do not go through this process. Lodgepole pine is commonly outplanted on degraded sites and does reasonably well on rehabilitated landings (Plotnikoff et al. 2002). It is possible that high percent EM colonization of lodgepole pine at time of outplanting plays a role in its success on degraded sites. Physiological traits of lodgepole pine, such as faster early growth and more plastic response to light and soil conditions, are also likely responsible (Lotan and Perry 1983).

We found *Rhizopogon*-like readily formed ectomycorrhizae and dominated Douglas-fir seedling roots grown on landing soil. Our findings corroborate those in Molina et

may have been one of the factors responsible for the poor growth response of seedlings growing on landings.

The reason for high concentrations and contents of foliar Fe and Al are unclear. In this study, pH differences (not significant, $p = 0.77$) (Table 2) do not account for the differences in Fe and Al availability, because the availability of

Table 7. Pearson correlations of percent ectomycorrhizal colonization (PEC) with height and diameter increment at time of planting, dry biomass, and foliar element concentrations for each seedling.

			Biomass[a]		Foliar element concentration										
	Height	Diameter	Root	Shoot	N	P	K	Ca	Mg	S	Fe	Mn	B	Zn	Cu	Al
PEC	–0.20	–0.19	–0.03	–0.13	**0.36**	**0.33**	0.24	0.04	0.03	**0.32**	**0.37**	**0.32**	0.10	**0.33**	**0.36**	0.01
p (n = 90)	0.06	0.07	0.80	0.22	<0.01	<0.01	0.02	0.69	0.75	<0.01	<0.01	<0.01	0.37	<0.01	<0.01	0.90
PEC on landings	0.10	0.08	0.22	0.23	**0.53**	**0.47**	**0.54**	0.28	–0.22	**0.53**	**0.43**	**0.53**	0.25	**0.47**	**0.53**	–0.01
p (n = 45)	0.53	0.60	0.14	0.13	<0.01	<0.01	<0.01	0.07	0.16	<0.01	0.01	<0.01	0.11	0.004	<0.01	0.97
PEC on clearcuts	–0.15	–0.11	0.09	–0.07	0.16	0.31	0.14	–0.01	–0.04	0.12	0.27	0.12	0.06	0.31	0.08	0.04
p (n = 45)	0.32	0.47	0.55	0.64	0.30	0.1	0.37	0.94	0.81	0.44	0.08	0.44	0.69	0.05	0.62	0.81

Note: Coefficients >0.25 with $p < 0.05$ are in bold.
[a]Dry biomass gain.

tions and contents of Fe and Al, as mycorrhizae have been shown to increase uptake of heavy metals to toxic levels (Wilkins and Hodson 1989; Gadd 1993). Even though landing and clearcut seedlings were colonized by the same fungi, efficient uptake of Fe and Al may have been greater on landings, given that fungi do not aid the host plant the same way in different soil environments (Allen 1991; Perry et al. 1987).

High levels of exchangeable Al in the mineral soil could not be the reason for high concentrations and contents of foliar Al in landing seedlings, because concentrations and contents of exchangeable Al in the mineral soil were actually significantly higher in clearcuts than in landings. Landings were devoid of organic horizons, so it is possible that Al was more available for uptake, since organic matter complexes Al in nonexchangeable forms (De la Fuente-Martínez and Herrera-Estrella 1999). Macdonald et al. (1998) suggested that seedlings rooting in mineral soil could be more at risk of toxic levels of Al than seedlings rooting in some organic horizons.

Conclusions

Commercially available EM inoculants did not increase survival or growth of interior Douglas-fir seedlings on landings, burned piles, or clearcuts. One of the reasons for a lack of growth response to inoculation may be that EM inoculants applied to interior Douglas-fir seedlings did not produce highly colonized seedlings under current nursery conditions. We suggest that research be carried out to determine the nursery conditions that can be favorable to both EM development and growth of inoculated interior Douglas-fir seedlings.

We found that landings were compacted and had low mineralizable N concentrations, regardless of soil tilling treatments. Deep tilling of landings did not significantly increase survival and height growth compared with shallow tilling. However, while deep tilling probably does not provide an immediate benefit for Douglas-fir seedlings, it may provide a benefit in the long term. The EIP of landings was not as low as expected. Four distinct morphotypes readily formed on seedlings growing in landings 2 years after outplanting. EM percent colonization, richness, and diversity were similar for landings and clearcut-grown seedlings. We suspect that, to some degree, the relatively high native EIP accounted for the lack of growth differences between inoculation treatments. Douglas-fir seedlings and their EM fungi growing on landings were probably stressed by a combination of factors, such as inadequate porosity, high soil temperatures and drought during the summer, possible deficiencies in N, and toxicities of Fe and Al. Soil compaction, low organic matter contents, and Fe and Al toxicity could be addressed by combining tilling with the addition of organic matter.

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