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Influence of ectomycorrhizal fungal inoculation on growth and root IAA concentrations of transplanted conifers

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Summary We determined whether *in vitro* plant growth regulator production by mycorrhizal fungi is correlated with conifer seedling growth and root IAA concentrations. Container-grown seedlings of interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), lodgepole pine (*Pinus contorta* Dougl.) and ponderosa pine (*Pinus ponderosa* Dougl.) were inoculated at seeding with ectomycorrhizal fungi having a high, moderate or low capacity to produce either IAA or ethylene *in vitro*. Inoculated seedlings were grown for one season in the nursery, harvested in November, cold stored over winter and then transplanted to either a nursery field or a forest site in the spring. Seedling morphology and endogenous IAA in roots were measured immediately after cold storage and again six and 12 months after transplanting. Morphological responses to inoculation varied among different mycorrhizal fungi. Free IAA concentration of roots was increased in some inoculation treatments for all conifer species. In seedlings transplanted to a nursery field, *in vitro* ethylene-producing capacity of the ectomycorrhizal fungi was highly correlated with more morphological features than *in vitro* IAA-producing capacity. Both IAA- and ethylene-producing capacity were significantly correlated with more morphological features in seedlings transplanted to a forest site than in seedings transplanted to a nursery field. One year after transplanting, only *in vitro* IAA-producing capacity was correlated with endogenous IAA concentration of roots of the inoculated seedlings. We conclude that growth responses of conifer seedlings can be partially influenced by IAA and ethylene produced by ectomycorrhizal fungal symbionts.

Keywords: ectomycorrhizae, ethylene, roots, seedling survival.

Introduction

When a seedling is transplanted from the nursery to a forest, survival is highly dependent on its ability to regenerate roots (Stone and Jenkinson 1970, Burdett 1979, Ritchie and Dunlap 1980). One potential method of increasing survival of transplanted seedlings is to exploit mycorrhizal effects on root growth. The beneficial effects of ectomycorrhizal fungal inoculation on transplanted seedlings include increases in root dry weight (Azcon and Barea 1975, David et al. 1983) with concomitant increases in root:shoot ratios (Black 1984, Danielson et al. 1984), and increases in numbers of elongating roots and root length (Dixon et al. 1980, Chilvers and Gust 1982). If mycorrhizal formation increases seedling survival by increasing root growth, some underlying interaction that stimulates root growth must exist between the seedling root system and the mycorrhizal fungus. This influence on root growth must be quantifiable because there are different seedling responses with different fungal species (Slankis 1973, Mosse et al. 1981) and isolates (Trappe 1977).

Root growth is mediated by plant growth regulators (PGRs) (Fabijan et al. 1981, Blake and Reid 1987). Despite the important role PGRs seem to play in forest trees (Zaerr 1967, DeYoe and Zaerr 1976, Zaerr and Lavender 1980), there have been few studies on the synthesis of these compounds by mycorrhizal fungi in symbiosis. Mycorrhizal fungi have been shown to produce auxins (Slankis 1973, Crafts and Miller 1974, Rudawska 1982, Strzelczyk and Polojska-Burdziej 1984, Gay 1986, Frankenberger and Poth 1987) and ethylene (Graham and Linderman 1981, Mosse et al. 1981, Rupp and Mudge 1985, Mitchell et al. 1986). The PGRs produced by mycorrhizal fungi may not only alter the extent of root exudation and carbohydrate allocation within a conifer seedling, but could also influence root morphogenesis and lateral root proliferation (Slankis 1948, 1951, 1973, Gay 1986) and thereby alter the ability of mycorrhizal plants to survive stress and to gain access to resources that ultimately improve fitness.

Although the alleviation of drought stress as a function of PGR-mediated increases in root growth has been indirectly investigated (Parke et al. 1983), the influence of mycorrhizal fungi on PGR-mediated root growth and seedling survival has not been examined. Therefore, we investigated changes in seedling morphology and endogenous root IAA concentrations of seedlings in response to inoculation with ectomycorrhizal fungi with differing capacities for *in vitro* PGR production. Specifically, we tested the hypotheses that: (1) IAA concentration of mycorrhizal conifer roots is influenced by the *in vitro* (IAA and ethylene) production capacity of their associated ectomycorrhizal fungi; and (2) increased concentrations of root IAA cause an increase in root growth resulting in improved seedling growth and survival.

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Materials and methods

Plant material

Seeds of interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (PSME-I) and lodgepole pine (*Pinus contorta* Dougl.) (PICO) were used for the nursery field experiment, and seeds of interior Douglas-fir and ponderosa pine (*Pinus ponderosa* Dougl.) (PIPO) were used for the forest site experiment. Seeds were placed in 55-ml cells in Styroblock containers filled with a 1:1 (v/v) mix of air-steam sterilized vermiculite and peat moss. Two weeks after seeding (April 15, 1988 for the nursery field experiment and April 7, 1988 for the forest site experiment), each Styroblock cell was inoculated with 5 ml of fungal inoculum containing 1.3 colony forming units (cfu) ml^{-1}. The inoculated seedlings were irrigated and fertilized in accordance with standard regimes for the production of nursery stock. All experimental seedlings were lifted on November 20, 1988. Seedlings for the forest nursery experiment were cold stored until May 5, 1989, when they were planted at a 2×2 m spacing in a plowed sandy-loam field in a forest tree nursery. The transplanted seedlings were not irrigated during the growing season. Seedlings for the forest site experiment were cold stored until March 15, 1989, when they were planted at a 2×2 m spacing on a harsh, dry clearcut site located 10 km north of Boston Bar, B.C., Canada. The forest site is a south-facing mid-slope of 20–30° with a sandy-loam dystric brunisol over a thick colluvium. The clearcut site has been described in detail elsewhere (Scagel 1994).

Selection of mycorrhizal fungi and preparation of inocula

The mycorrhizal fungi used in this study and their plant growth regulator (PGR) characteristics are given in Table 1. Gas chromatography–mass spectrometry analyses were used to determine the concentrations of IAA in mycelia and culture filtrates of the ectomycorrhizal fungi (Scagel 1994, Scagel and Linderman 1998). The mycorrhizal fungal isolates were classified based on the extent and consistency of their *in vitro* PGR production as high (HI; *Laccaria laccata*, Ll-11), moderate (MI; *L. laccata*, Ll-19) and low (LI; *L. laccata*, Ll-7) IAA producers and high (HE; *Rhizopogon vinicolor*, Rv-7, PSME; *L. laccata*, Ll-17, PICO; *Suillus luteus*, Sl-1, PIPO), moderate (ME; *R. vinicolor*, Rv-8, PSME; *L. laccata*, Ll-2, PICO; *S. luteus*, Sl-3, PIPO) and low (LE; *R. vinicolor*, Rv-5, PSME; *L. laccata*, Ll-14, PICO; *S. luteus*, Sl-2, PIPO) ethylene producers. Fungi for inoculation were grown in flasks containing 2 l of semi-solid modified Melin-Norkrans (MMN) medium (Molina and Palmer 1982). Cultures were incubated in the dark at 20 °C and agitated daily. After five weeks of growth, mycelium was harvested by filtration, fragmented with a Waring Blender and diluted (1:10) (v/v) with liquid MMN medium (Molina and Palmer 1982) to give an inoculum concentration of 1.3 cfu ml^{-1}. Five ml of inoculum was injected into each Styroblock cell.

Assessments of seedling morphology and mycorrhiza colonization

On removal from cold storage, total height and root collar diameter of the seedlings were measured. Representative seedlings (five seedlings per species × isolate combination for a total of 60 seedlings and 10 noninoculated controls) were also sampled for root dry weight, shoot dry weight and mycorrhizal colonization at this time.

One growing season after transplanting, all seedlings were harvested (November 3, 1989 for the nursery field experiment, and November 7, 1989 for the forest site experiment) and final height and root collar diameter were recorded. Fresh weights of root systems were determined before subsamples were taken for IAA analysis. Stems and roots not subsampled for IAA analysis were dried at 100 °C for 72 h and their weights determined. Estimated dry weights of the root samples taken for IAA analysis were determined from the fresh weight to dry weight ratio of the remainder of the root system. Total root dry weight was calculated by adding the estimated dry weight of

Table 1. Experimental fungal isolates and their *in vitro* IAA and ethylene production characteristics (see Scagel 1994).

Fungal isolate	Fungal species	PGR Production	Tree species	Isolate culture number	Isolate origin
Ll-7	*Laccaria laccata* (Scop. *ex* Fr.) Berk. and Br.	LI[1]	PSME,PICO,PIPO[3]	S-22	Balco Inc., Kamloops, B.C., Canada
Ll-11	*L. laccata*	HI	PSME,PICO,PIPO	B-101a	USDA-FS, Corvallis, OR
Ll-19	*L. laccata*	MI	PSME,PICO,PIPO	CS-23	CFS, Horse Mtn., B.C., Canada
Ll-2	*L. laccata*	ME[2]	PICO	Lala1	Univ. Wash., Bald Mtn., OR
Ll-14	*L. laccata*	LE	PICO	S-443	USDA-FS, Lost Prairie, OR
Ll-17	*L. laccata*	HE	PICO	505	Balco Inc., Kamloops, B.C., Canada
Rv-5	*Rhizopogon vinicolor* (A.H. Smith)	LE	PSME	CS-31	Reisolated from USDA-FS No. 9428
Rv-7	*R. vinicolor*	HE	PSME	CS-33	Reisolated from USDA-FS No. 9444
Rv-8	*R. vinicolor*	ME	PSME	CS-34	Reisolated from USDA-FS *R. vivi*
Sl-1	*Suillus luteus* (L. Fr.) S.F. Gray	HE	PIPO	CS-42	CFS, Vernon, B.C., Canada
Sl-2	*S. luteus*	LE	PIPO	CS-41	CFS, Horse Mtn., B.C., Canada
Sl-3	*S. luteus*	ME	PIPO	CS-36	CFS, Savona, B.C., Canada

[1] LI, MI, and HI represent low, moderate and high *in vitro* IAA producers, respectively.
[2] LE, ME and HE represent low, moderate and high *in vitro* ethylene producers, respectively.
[3] PSME = Douglas-fir; PICO = lodgepole pine; PIPO = ponderosa pine.

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the root samples taken for IAA analysis to the dry weight of the remainder of the root system.

Growth was measured as described by Hunt (1982) and Ledig (1976). Relative growth rate (RGR) was determined as $RGR = (\ln X_n - \ln X_{n-1})(T_n - T_{n-1})^{-1}$, where X_n is the parameter at time T_n and X_{n-1} is the parameter at time T_{n-1}. The values are reported as percentage increases (% $year^{-1}$).

Mycorrhizal formation was determined by counting the number of primary laterals on a seedling and the percentage that were mycorrhizal. Root system volume was measured by water displacement. Random subsamples of half the root system were used to quantify the number of primary laterals per ml of root system. Representative mycorrhizae were examined for Hartig net development and re-isolations of the mycorrhizal fungi were performed on seedlings selected for IAA analysis after the first and second growing seasons.

Analysis of IAA

Samples for IAA analysis were taken from five seedlings per conifer species × mycorrhizal fungus combination. Immediately after harvesting, samples were immersed in liquid nitrogen and stored at −20 °C until extraction by a modification of the method of Cohen et al. (1987) and Miller et al. (1990) as described by Scagel and Linderman (1998). Primary root tips were sampled for endogenous IAA concentrations immediately before cold storage (November 1988) and 6 months after transplanting (November 1989).

Experimental design and statistical analysis

For the nursery field experiment, each tree species was inoculated separately with six mycorrhizal fungal isolates (HI, HE, MI, ME, LI, LE and noninoculated control) and replicated 30 (two blocks of 15) times in a randomized block design. In the nursery field, blocks were represented by rows for a total of 210 trees per species.

For the forest site experiment, each tree species was inoculated separately with six mycorrhizal fungal isolates (HI, HE, MI, ME, LI, LE and noninoculated control) and replicated 25 times in completely randomized designs. At the forest site, the plot layout consisted of seven rows of 25 seedlings for a total of 175 trees per species. For each tree species, seedling treatments were arranged randomly between and within rows.

In both experiments, means were analyzed by tree species as a one-way analysis of variance using the SYSTAT statistical package (Wilkinson 1989). Where significant differences were detected ($P < 0.05$), means were separated using Fischer's Protected Least Significant Difference test. Fungal isolate was not used as a blocking factor in the analysis.

Results

Nursery field experiment

Aboveground responses Stem dry weight, seedling height, root collar diameter and aboveground growth of Douglas-fir and lodgepole pine varied among mycorrhizal inoculation treatments (Table 2). Treatment effects on seedling height and root collar diameter were more pronounced after the second growing season than after the first growing season. Although Douglas-fir and lodgepole pine showed similar height and diameter responses to inoculation treatments (Table 2), the effect of inoculation treatments on shoot dry weight differed between the species (Figure 1). In Douglas-fir, the relative treatment effects on shoot dry weight were similar in the first

Table 2. Effects of mycorrhizal fungal isolates on morphology of Douglas-fir (PSME) and lodgepole pine (PICO) seedlings after one growing season in the nursery (Year 1) and 6 months after being transplanted to a nursery field (Year 2).

Tree species	Fungal isolate[1]	Height (cm)		Root collar diameter (mm)		Growth rate (% $year^{-1}$)		Root:shoot ratio		Colonization (%)	
		Year 1	Year 2	Year 1	Year 2	Height	Diameter	Year 1	Year 2	Year 1	Year 2
Douglas-fir	Control	23.15	33.70	2.76	5.41	37.55	67.30	1.52	0.98	22.3	8.3
	HI	24.16	39.83*[2]	2.94	6.02*	49.90	54.26	1.89	1.06	54.8*	41.8*
	MI	24.39	38.43*	2.81	5.69*	48.04	70.55	1.67	1.03	51.4*	26.0*
	LI	22.58	41.18*	2.86	5.60	60.00*	67.19	1.61	0.93	31.8	24.8*
	HE	26.35*	49.40*	3.15*	7.07*	62.85*	80.85*	1.79	1.52*	73.5*	52.5*
	ME	22.05	45.71*	3.34*	5.66*	72.90*	52.75	2.26*	1.69*	68.6*	31.9*
	LE	22.89	42.95*	3.01	5.90*	92.93*	67.30	3.39*	1.33	15.6	19.5
Lodgepole pine	Control	30.91	42.10	4.31	6.25	37.16	30.90	0.66	0.69	18.9	15.6
	HI	31.74	47.02*	4.46	7.39*	50.50*	39.30	0.44	0.98*	62.5*	53.8*
	MI	30.63	46.22*	4.30	7.64*	57.50*	41.14	0.33	1.11*	82.0*	61.9*
	LI	30.40	44.19	4.17	6.84	49.49	37.41	0.32	1.64*	52.3*	35.1*
	HE	31.56	58.39*	5.91*	8.07*	31.15	61.53*	0.86	0.99*	42.1*	39.0*
	ME	35.42*	60.74*	5.12*	8.00*	44.63	53.93*	0.92*	0.60	27.4	19.4
	LE	37.84*	55.87*	5.27*	7.34*	33.13	38.97	1.02*	0.79	30.5	22.7

[1] Inoculation treatments: C = control; HI = high IAA producing *L. laccata* isolate; MI = moderate IAA producing *L. laccata* isolate; LI = low IAA producing *L. laccata* isolate; HE = high ethylene producing *R. vinicolor* (PSME) or *L. laccata* isolate (PICO); ME = moderate ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate; and LE = low ethylene producing *R. vinicolor* (PSME) or *L. laccata* isolate (PICO).

[2] An asterisk (*) indicates a value significantly greater than the control value ($P < 0.01$).

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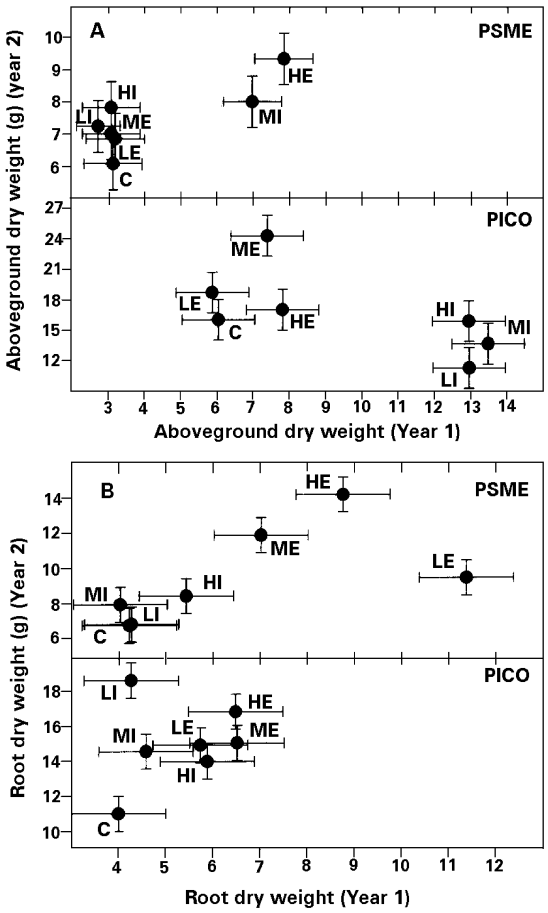

Figure 1. Effects of mycorrhizal fungal isolates on aboveground dry weights (A) and root dry weights (B) of Douglas-fir (PSME) and lodgepole pine (PICO) seedlings after one growing season in the nursery (Year 1) and 6 months after being transplanted to a nursery field (Year 2). Inoculation treatments: C = control; HI = high IAA producing *L. laccata* isolate; MI = moderate IAA producing *L. laccata* isolate; LI = low IAA producing *L. laccata* isolate; HE = high ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate; ME = moderate ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate; and LE = low ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate. Bars represent Fischer's Protected LSD ($P < 0.01$).

and second growing seasons. In contrast, seedlings of lodgepole pine inoculated with fungi characterized by their *in vitro* IAA production had significantly higher shoot dry weights after the first growing season and significantly lower shoot dry weights after the second growing season compared with control seedlings and seedlings inoculated with fungi characterized by their *in vitro* ethylene production. Lodgepole pine inoculated with HI, MI and LI (*L. laccata*; Ll-11, Ll-19, Ll-7) isolates also had 41% more prolepsis growth than other lodgepole pine seedlings after the first growing season.

Belowground responses At the end of the first growing season, root dry weights of lodgepole pine and Douglas-fir showed trends similar to root collar diameter. Root dry weights of Douglas-fir and lodgepole pine seedlings inoculated with isolates characterized by their ethylene production capacity were significantly greater than root dry weights of control seedlings (Figure 1). One year after transplanting, however, all inoculated seedlings had greater root dry weights than control seedlings, except Douglas-fir inoculated with isolate LE (*R. vinicolor*, Rv-5).

Inoculation with mycorrhizal fungi had little effect on the root:shoot ratios of Douglas-fir and lodgepole pine seedlings at the end of the first growing season. Root:shoot ratios of inoculated seedlings were only significantly greater than those of control seedlings when inoculated with isolates ME (*R. vinicolor*, Rv-8, Douglas-fir; *L. laccata*, Ll-2, lodgepole pine) and LE (*R. vinicolor*, Rv-5, Douglas-fir; *L. laccata*, Ll-14, lodgepole pine) (Table 2). At the end of the second growing season, root:shoot ratios were significantly increased in Douglas-fir inoculated with fungi characterized by their *in vitro* ethylene production. All of the fungal isolates except ME and LE (*L. laccata*, Ll-2 and Ll-14) increased root:shoot ratios of the lodgepole seedlings by the end of the second growing season.

The extent of mycorrhizal fungal colonization of the experimental seedlings was isolate dependent (Table 2). Control seedlings of Douglas-fir and lodgepole pine had a background colonization of *Thelephora terrestris* L. before transplanting that was almost eliminated one year after transplanting.

Root IAA responses Four of the six mycorrhizal fungal isolates tested increased free IAA concentrations in roots of Douglas-fir seedlings after both the first and second growing seasons (Figure 2). All of the fungal isolates except LI (*L. laccata*, Ll-7) and LE (*R. vinicolor*, Rv-5) increased the IAA conjugate (ester) concentrations of Douglas-fir roots by the end of the first growing season.

All of the fungal isolates except LE (*L. laccata*, Ll-14) increased free IAA concentration of lodgepole pine roots at both sampling times (Figure 2). Auxin conjugates of lodgepole pine roots were increased by all fungal isolates except LE and LI (*L. laccata*, Ll-14 and Ll-7) after one growing season, and by all isolates except ME (*L. laccata*, Ll-2) after the second growing season.

Correlations between in vitro PGR production by mycorrhizal fungi and seedling responses In general, more Douglas-fir and lodgepole pine seedling morphological features were significantly correlated with the *in vitro* ethylene production capacities of mycorrhizal fungi than with the *in vitro* IAA production capacities of mycorrhizal fungi (Table 3). Although *in vitro* IAA production was significantly correlated with root IAA concentration in Douglas-fir, *in vitro* ethylene production was highly correlated with root IAA concentration in lodgepole pine. Root IAA concentrations of Douglas-fir seedlings were poorly correlated with most morphological features measured, whereas root IAA concentrations of lodgepole pine were significantly correlated with *in vitro* ethylene production capacity, height growth rate and mycorrhizal colonization.

Forest site experiment

Aboveground responses One year after inoculation, only Douglas-fir inoculated with isolates ME (*R. vinicolor*, Rv-8) , LI (*L. laccata*, Ll-7) and LE (*R. vinicolor*, Rv-5) showed

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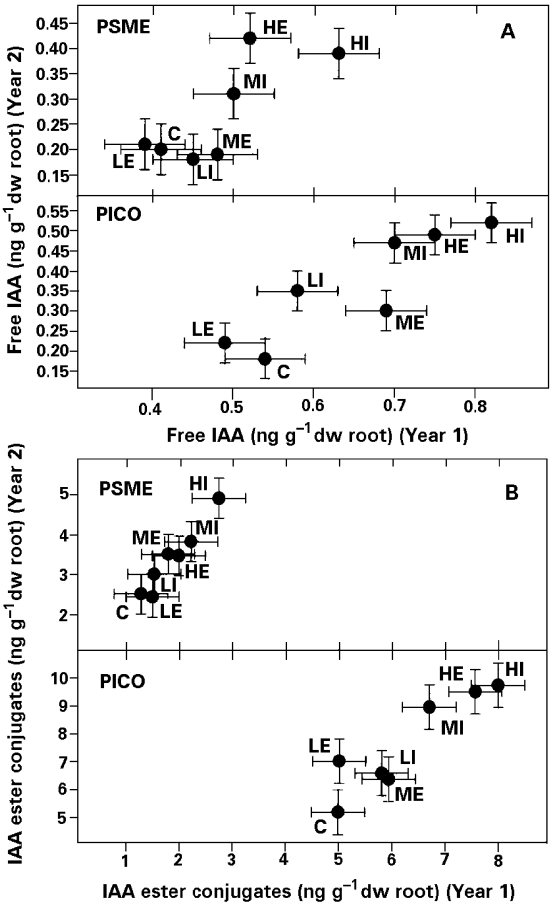

Figure 2. Effects of mycorrhizal fungal isolates on concentrations of free IAA (A) and IAA conjugate (B) of roots of Douglas-fir (PSME) and lodgepole pine (PICO) seedlings after one growing season in the nursery (Year 1) and 6 months after being transplanted to a nursery field (Year 2). Inoculation treatments: C = control; HI = high IAA producing *L. laccata* isolate; MI = moderate IAA producing *L. laccata* isolate; LI = low IAA producing *L. laccata* isolate; HE = high ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate; ME = moderate ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate; and LE = low ethylene producing *R. vinicolor* (PSME) or *L. laccata* (PICO) isolate. Bars represent Fischer's Protected LSD ($P < 0.01$).

significantly greater total height, shoot dry weight and root collar diameter than control seedlings (Figure 3 and Table 4). One year after inoculation with isolates ME (*S. luteus*, Sl-3), LI (*L. laccata*, Ll-7) and LE (*S. luteus*, Sl-2), ponderosa pine seedings had significantly greater height, shoot dry weight and root collar diameter than control seedlings. Among the fungal isolates, only HE (*S. luteus*, Sl-1) significantly increased height and shoot dry weight of ponderosa pine seedlings.

At the end of the second growing season, all inoculated Douglas-fir seedlings were taller than control seedlings, and all of the fungal isolates tested except LI (*L. laccata*, Ll-7) increased root collar diameter (Table 4).

Belowground responses At the end of the first and second growing seasons, Douglas-fir inoculated with some mycorrhizal fungal isolates had greater root dry weights and root:shoot ratios than control seedlings (Figure 3). Root growth responses to inoculation also varied with isolate and time of sampling in ponderosa pine. One year after inoculation, mycorrhiza colonization was significantly greater for inoculated Douglas-fir and ponderosa pine seedlings than for control seedlings (Table 4), whereas two years after inoculation, some Douglas-fir and ponderosa pine seedlings inoculated with mycorrhizal fungi had significantly less mycorrhizal colonization than control seedlings (Table 4).

Root IAA responses More than 50% of inoculated Douglas-fir and ponderosa pine seedlings had significantly higher endogenous root IAA concentrations than noninoculated seedlings (Figure 4). All of the HI and MI (*L. laccata*, Ll-11 and

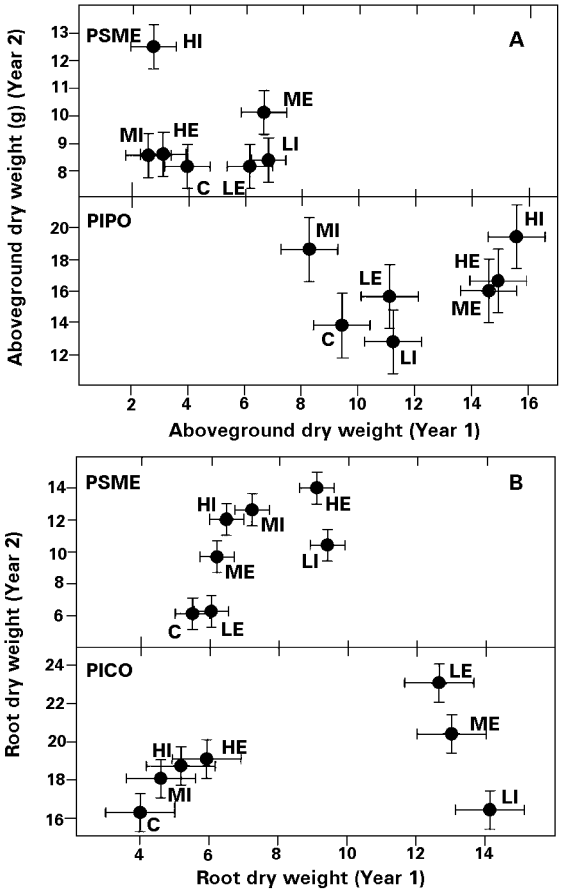

Figure 3. Effects of mycorrhizal fungal isolates on relationship between aboveground dry weights (A) and root dry weights (B) of Douglas-fir (PSME) and ponderosa pine (PIPO) seedlings after one growing season in the nursery (Year 1) and 6 months after being transplanted to a clearcut site (Year 2). Inoculation treatments: C = control; HI = high IAA producing *L. laccata* isolate; MI = moderate IAA producing *L. laccata* isolate; LI = low IAA producing *L. laccata* isolate; HE = high ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate; ME = moderate ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate; and LE = low ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate. Bars represent Fischer's Protected LSD ($P < 0.01$).

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Table 3. Correlation coefficients ($P < 0.01$) between *in vitro* production of plant growth regulators by mycorrhizal fungi and IAA concentration of roots and seedling responses of Douglas-fir and lodgepole pine after one growing season in the nursery (Year 1) and 6 months after being transplanted to a nursery field (Year 2).

Parameter	Douglas-fir		Lodgepole pine		
	In vitro ethylene production	*In vitro* IAA production	*In vitro* ethylene production	*In vitro* IAA production	IAA concentration of roots
Height (Year 1)	0.84	ns[1]	ns	ns	ns
Height (Year 2)	0.70	ns	0.53	ns	ns
Shoot weight (Year 1)	0.71	ns	ns	ns	ns
Shoot weight (Year 2)	0.83	0.69	ns	0.68	ns
Diameter (Year 2)	0.79	0.67	0.83	ns	ns
Root weight (Year 1)	ns	ns	0.69	ns	ns
Root weight (Year 2)	0.82	ns	ns	ns	ns
Root:shoot ratio (Year 1)	ns	0.67	−0.71	0.64	ns
Root:shoot ratio (Year 2)	0.57	0.65	ns	−0.77	ns
Height growth rate	ns	ns	0.84	ns	0.51
Diameter growth rate	ns	ns	ns	0.56	ns
Colonization (Year 1)	0.84	ns	ns	0.65	0.61
Colonization (Year 2)	0.78	ns	ns	0.72	0.77
IAA concentration of roots (Year 1)	ns	0.41	0.81	ns	–
IAA concentration of roots (Year 2)	ns	ns	0.66	ns	–

[1] ns = Nonsignificant correlations.

Ll-19) inoculated Douglas-fir and ponderosa pine seedlings had higher endogenous root IAA (free and ester conjugates) concentrations than control seedlings after both the first and second growing seasons.

Correlations between in vitro PGR production by mycorrhizal fungi and seedling responses One year after inoculation of Douglas-fir seedlings, *in vitro* IAA production capacity by mycorrhizal fungi was significantly ($P < 0.01$) correlated with the concentration of endogenous root IAA conjugates (Table 5). Two years after inoculation of Douglas-fir seedlings, *in vitro* IAA production capacity by mycorrhizal fungi was significantly ($P < 0.01$) correlated to height, mycorrhiza colonization and the endogenous concentration of free IAA in roots (Table 5). *In vitro* ethylene production capacity was significantly ($P < 0.01$) correlated to height, shoot dry weight and

Table 4. Effects of mycorrhizal fungal isolates on morphology of Douglas-fir (PSME) and ponderosa pine (PIPO) seedlings after one growing season in the nursery (Year 1) and 6 months after being transplanted to a clearcut site (Year 2).

Tree species	Fungal isolate[1]	Height (cm)		Root collar diameter (mm)		Relative growth rate (% year^{-1})		Root:shoot ratio		Mycorrhizal colonization (%)	
		Year 1	Year 2	Year 1	Year 2	Height	Diameter	Year 1	Year 2	Year 1	Year 2
Douglas-fir	Control	18.47	31.80	3.17	5.32	53.8	51.4	1.29	0.57	12.4	36.9
	HI	21.14	42.77*[2]	4.11	6.35*	71.2*	43.7	2.50*	1.22*	72.6*	64.7*
	MI	21.35	39.45*	3.97	5.73	59.3	37.3	3.26*	1.44*	81.4*	39.4
	LI	34.52*	40.58*	5.66*	6.12*	18.1	9.3	1.15	0.54	60.5*	31.8
	HE	19.54	36.29	4.20	6.08	58.4	39.2	2.43*	1.64*	63.9*	54.1*
	ME	31.91*	39.24*	5.19*	6.17*	23.7	15.6	0.87	0.61	52.8*	48.1*
	LE	33.16*	41.35*	4.82*	5.84	25.1	20.5	1.25	0.58	68.4*	43.7*
Lodgepole pine	Control	31.34	46.24	4.39	7.81	39.5	56.6	0.31	0.57	7.3	29.4
	HI	36.42	60.07*	5.23*	8.68*	52.7*	51.3	0.33	1.22*	91.7*	74.2*
	MI	35.17	57.91*	4.54	8.02	47.8*	57.2	0.70*	1.44*	81.2*	78.4*
	LI	42.52*	45.52	6.54*	7.41	7.9	14.8	1.59*	0.54	69.5*	24.5
	HE	40.13*	59.97*	4.64	8.22	42.7	59.1	0.37	1.64*	63.8*	31.5
	ME	46.76*	54.92	7.54*	8.75*	17.8	13.8	1.56*	0.61	73.4*	61.2*
	LE	43.60*	48.61	6.19*	7.19	11.7	15.3	2.67*	0.58	75.0*	59.3*

[1] Inoculation treatments: C = control; HI = high IAA producing *L. laccata* isolate; MI = moderate IAA producing *L.laccata* isolate; LI = low IAA producing *L. laccata* isolate; HE = high ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate; ME = moderate ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate; and LE = low ethylene producing *R. vinicolor* (PSME) or *S. Luteus* (PIPO) isolate.

[2] An asterisk (*) indicates a value greater than the control value ($P < 0.01$).

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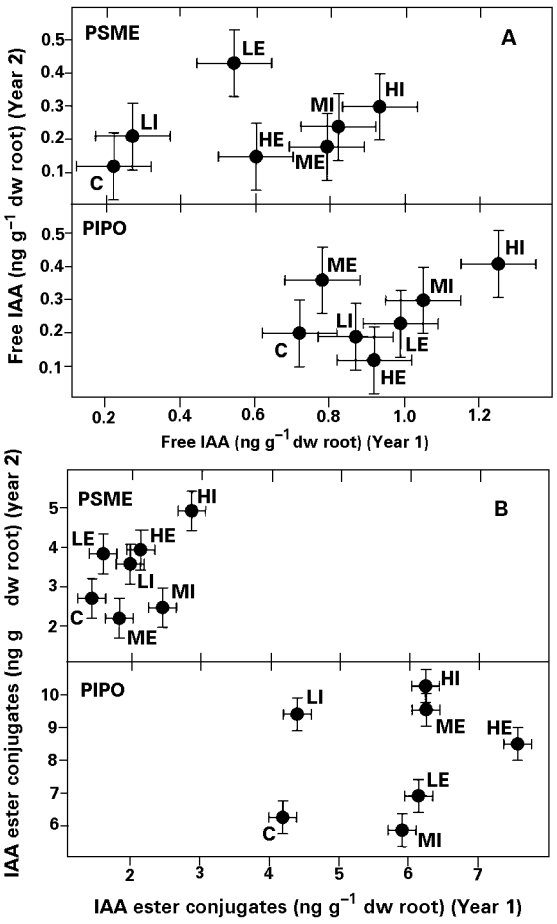

Figure 4. Effects of mycorrhizal fungal isolates on concentrations of free IAA (A) and IAA conjugates (B) in roots of Douglas-fir (PSME) and ponderosa pine (PIPO) seedlings after one growing season in the nursery (Year 1) and 6 months after being transplanted to a clearcut site (Year 2). Inoculation treatments: C = control; HI = high IAA producing *L. laccata* isolate; MI = moderate IAA producing *L. laccata* isolate; LI = low IAA producing *L. laccata* isolate; HE = high ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate; ME = moderate ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate; and LE = low ethylene producing *R. vinicolor* (PSME) or *S. luteus* (PIPO) isolate. Bars represent Fischer's Protected LSD (P < 0.01).

root:shoot ratio of Douglas-fir seedlings at the end of the first growing season and it was significantly ($P < 0.01$) correlated with root:shoot ratio, root collar diameter and root dry weight at the end of the second growing season. The endogenous concentration of free IAA in roots of Douglas-fir was significantly ($P < 0.01$) correlated to several morphological features and to mycorrhizal colonization frequency.

In vitro IAA production capacity by mycorrhizal fungal isolates was significantly ($P < 0.01$) correlated with height, shoot dry weight, extent of mycorrhizal colonization and the endogenous concentration of free IAA in roots of ponderosa pine at the end of the second growing season (Table 5). *In vitro* ethylene production capacity was significantly ($P < 0.01$) correlated only with height, shoot dry weight, and root collar diameter two years after inoculation.

Discussion

Inoculation with ectomycorrhizal fungi enhanced the growth of Douglas-fir, lodgepole pine and ponderosa pine seedlings. Similar findings have been reported elsewhere for ponderosa pine seedlings (e.g., Theodorou and Bowen 1970, LeTacon and Bouchard 1986, Stenström 1990). Different fungi affected the growth of transplanted seedlings differently (cf. Harley and Smith 1983, Stenström 1990). Other studies have reported neutral and negative responses to mycorrhizal inoculation (Bledsoe and Bair 1981, Harley and Smith 1983, Molina and Chamard 1983), although frequently these effects disappeared after transplanting.

Inoculation with ectomycorrhizal fungi significantly increased the concentration of endogenous IAA (both free and conjugate forms) in roots of seedlings transplanted to a nursery field. Increases of a similar magnitude were reported by Mitchell et al. (1986) and Scagel and Linderman (1998), whereas Wallander (1992) observed a much smaller increase, perhaps indicating that the effect is influenced by fungal and conifer species, seedling age, soil microbiological and nutritional conditions, and time of year. Concentrations of IAA conjugates in roots of lodgepole pine were significantly greater when sampled in the fall than when sampled in the spring (Figure 2), indicating that IAA conjugates play a role in the storage of IAA in plant tissue.

Although it is not known whether the host or fungal symbiont (or both) was the source of the increased IAA concentration in the conifer roots, there was a high correlation between the IAA concentration of roots and the *in vitro* IAA production capacity of the fungi. Differences among mycorrhizal fungi in their capacity to produce PGRs (Graham and Linderman 1981, Ho 1986, Gay and Debaud 1988, Scagel 1994, Scagel and Linderman 1998) could explain the different effects exerted on the host plants by the different mycorrhizal fungal isolates.

Ethylene-producing fungi also induced increases in root IAA; however, because the ethylene-producing fungi also synthesized IAA, no conclusion about the source of IAA in the roots can be reached. Furthermore, fungi with differing capacities for *in vitro* ethylene production were equally effective in enhancing root IAA concentrations, indicating that high-ethylene producers did not induce more endogenous IAA in roots than low-ethylene producers.

The presence of mycorrhizae affected the allocation of carbon from photosynthesis, as indicated by increased root:shoot ratios. Similarly, Dosskey et al. (1992) observed that mycorrhizal fungi altered carbon partitioning in needles of drought-stressed plants. Although the mechanisms underlying the hormonal control of carbon partitioning are unknown, these data suggest involvement of IAA or ethylene, or both, produced by or induced by ectomycorrhizal fungi.

Although conditions in the production nursery were conducive to ectomycorrhizal formation as indicated by the extent of colonization after the first growing season, some inoculated seedlings formed fewer mycorrhizae than the control seedlings, which were colonized by *Thelephora terrestris*. We conclude that either the inoculation of some fungi was ineffective or the growth conditions in the nursery were not conducive

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Table 5. Correlation coefficients ($P < 0.01$) between *in vitro* production of plant growth regulators by mycorrhizal fungi and IAA concentration of roots and seedling responses of Douglas-fir and ponderosa pine after one growing season in the nursery (Year 1) and 6 months after being transplanted to a clearcut site (Year 2).

Parameter	Douglas-fir				Ponderosa pine			
	In vitro [C_2H_4]	*In vitro* [IAA]	Root [IAA]		*In vitro* [C_2H_4]	*In vitro* [IAA]	Root [IAA]	
			Free	Ester			Free	Ester
Height (Year 1)	0.47	ns[1]	ns	ns	ns	ns	ns	ns
Height (Year 2)	ns	0.69	0.82	0.73	0.68	ns	0.65	ns
Shoot weight (Year 1)	0.52	ns	0.48	ns	0.57	ns	0.79	0.61
Shoot weight (Year 2)	ns	ns	0.79	ns	0.65	0.75	0.65	ns
Diameter (Year 2)	0.57	ns	0.87	ns	0.75	ns	ns	0.50
Root weight (Year 1)	ns	ns	ns	ns	ns	ns	ns	ns
Root weight (Year 2)	0.83	ns	0.67	ns	ns	ns	0.60	ns
Root:shoot ratio (Year 1)	0.54	ns	0.55	ns	ns	ns	ns	ns
Root:shoot ratio (Year 2)	0.85	ns	0.52	ns	ns	0.62	ns	ns
Colonization (Year 1)	ns	0.52	0.67	ns	ns	0.61	0.74	ns
Colonization (Year 2)	ns	ns	0.73	ns	ns	0.56	0.75	ns
Free [IAA] of roots (Year 1)	ns	ns	–	ns	ns	0.82	–	ns
Free [IAA] of roots (Year 2)	ns	0.84	–	0.49	ns	0.68	–	0.63
[IAA ester] in roots (Year 1)	ns	0.74	ns	–	ns	ns	ns	–
[IAA ester] in roots (Year 2)	ns	0.67	0.49	–	ns	ns	0.58	–

[1] ns = Nonsignificant correlations.

to ectomycorrhizal formation by the particular fungi with which plants were inoculated. In several cases, the extent of colonization was correlated with *in vitro* IAA or ethylene production capacity of the fungus and the IAA concentration of the roots, indicating a possible relationship between relative capacity for IAA or ethylene production and mycorrhizal formation (Slankis 1958, Gogala 1991). However, Scagel (1994) and Scagel and Linderman (1998) did not find a strong relationship between relative capacity for PGR production and formation of mycorrhizae in greenhouse studies with young inoculated seedlings.

Because all of the experimental seedlings survived, we were unable to determine the effects of inoculation with ectomycorrhizal fungi on seedling survival. However, many growth parameters measured were significantly correlated with mycorrhizal fungus inoculation and endogenous root IAA concentrations, especially in the seedlings planted at the forest site. These results partially support the hypothesis that mycorrhizal fungi can stimulate increases in root IAA that can affect growth of roots and shoots after transplanting.

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