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Effect of fertilization on growth and ectomycorrhizal development of container-grown and bare-root nursery conifer seedlings

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Abstract. The effect of three levels of fertilizer on the growth of three species of containerized-grown conifer seedlings (*Pinus contorta*, *Picea glauca*, and *Picea mariana*) and two species of bare-root conifer seedlings (*Pinus sylvestris* and *Larix sibirica*), and on the colonization of these seedlings by six species of ectomycorrhizal fungi (*Hebeloma longicaudum*, *Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius*, *Rhizopogon vinicolor* and *Suillus tomentosus*), was studied. The growth of the seedlings in both container-grown and bare-root nurseries increased as the levels of fertilizer increased. For better seedling growth and environmental quality it may be possible to reduce the level of fertilizers in commercial nurseries up to 33% by using selected mycorrhizal fungi. Ectomycorrhizal colonization in all seedlings was not affected by fertilizer levels. *Hebeloma longicaudum, L. bicolor, P. involutus*, and *P. tinctorius* formed well-developed ectomycorrhizae, whereas ectomycorrhizal development by *R. vinicolor* and *S. tomentosus* was poor. Native mycorrhizal fungi colonized non-inoculated control seedlings; however, their colonization was always lower than with inoculated fungi.

Introduction

Production of conifer tree seedlings in the nursery requires application of large amounts of chemical fertilizer (Landis 1989). However, high fertility rates often lead to luxury consumption of mineral nutrients in the ideal growing environment of container tree seedling nurseries and affect the formation of some mycorrhizae. Well-developed ectomycorrhizae may improve the growth and survival of outplanting seedlings in the field (Kropp and

Langlois 1990). In many parts of the world, failure in afforestation and reclamation trials and reduction in forest productivity have been attributed to the absence of suitable mycorrhizal fungi (Bjorkman 1970, Mikola 1970, Marx 1980, Delwaulle et al. 1982, Le Tacon 1982, Ruehle 1982, Smith and Reid 1997). In these conditions, the outplanting of seedlings inoculated in the nursery with appropriate strains of ectomycorrhizal fungi is recommended. In other conditions, however, where sufficient and diverse ectomycorrhizal inoculum is present in the soil, good management of native strains may be the best solution (Malajczuk et al. 1994). Seedlings with short shoots and roots but with well-developed mycorrhizae may survive better in the field (Molina 1980, Marx et al. 1982). Both container-grown and bare-root nursery seedlings can be successfully inoculated with beneficial mycorrhizal fungi using either spores or vegetative mycelia, in a vermiculite mixture or as a liquid slurry at the sowing or seedling stage (Ruehle 1980, Cline and Reid 1982, Langlois and Fortin 1982, Marx et al. 1982, Danielson et al. 1984b, Castellano et al. 1985, Le Tacon et al. 1985, Marx and Bell 1985, Hung and Molina 1986). When these inoculated seedlings are outplanted on forest sites harboring high level of naturally occurring ectomycorrhizal fungi, inoculated fungi can be replaced by native fungi. For example, in a study of outplanted Douglas-fir by Bledsoe et al. (1982), the inoculated *Laccaria laccata* (Scop.: fr.) and *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quèl. did not persist and was replaced by native fungi. Other inoculation studies with *Pisolithus* and *Thelephora* have shown no consistent advantage for survival and growth for containerized seedlings (Ruehle et al. 1981, Castellano and Trappe 1991). In contrast, some other studies have shown that *L. bicolor* inoculated on black spruce (Buschena et al. 1992) and Douglas-fir (Le Tacon et al. 1992, Selosse et al. 2000), *P. tinctorius* and other beneficial symbionts inoculated on pines and other tree species (as reviewed by Marx 1991, Brundrett et al. 1996), persisted several years after nursery inoculation, with increased plantation survival and growth.

Although several authors have reported that high fertilizer level decreases mycorrhiza formation (Marx and Barnett 1974, Ruehle and Marx 1977, Ruehle 1980, Ruehle and Wells 1984, Shaw et al. 1982, Gagnon et al. 1987, 1988, Hunt 1988, Reitveld et al. 1989, Chakravarty and Chatarpaul 1990, Le Tacon et al. 1997, Smith and Reid 1997), Molina and Chamard (1983) and Danielson et al. (1984a) reported that ectomycorrhiza formation was not affected regardless of fertilization treatment. In black spruce, exponential fertilization combined with ectomycorrhizal fungal inoculation resulted in both nutrient-loaded and ectomycorrhizally colonized planting stock (Quoreshi and Timmer 1998). According to Trappe (1977) and Marx (1980), different fungal species and ecotypes vary in tolerance to fertilizer

sources and rates of application, therefore; each isolate must be tested on its own merit.

Lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), white spruce [*Picea glauca* (Moench) Voss], and black spruce [*Picea mariana* (Mill.) BSP] are important conifer tree species in western Canada and are widely used for reforestation (Kuhnke 1989). In western Canada's prairie provinces, Scots pine (*Pinus sylvestris* L.) and Siberian larch (*Larix sibirica* Ledeb.) are exotic species used for shelterbelts and landscape plantings. In this study, seedlings of all five tree species were inoculated with six species of ectomycorrhizal fungi (*Hebeloma longicaudum* Pers.: Fr., *Laccaria bicolor* R. Mre., *Paxillus involutus* Batsch: Fr., *Pisolithus tinctorius* (Mich.: Pers.) Coker and Couch, *Rhizopogon vinicolor* A.H. Smith, and *Suillus tomentosus* Kauffman) and were grown as containerized or bare-root seedlings in two forest nurseries to determine the effect of three levels of fertilization on the seedling growth and ectomycorrhizal development.

Methods

Inoculation in containerized and bare-root nurseries

The experiments were conducted in a containerized-grown nursery (Bonnyville Forest nursery, Bonnyville, Alberta) and a bare-root nursery (Shelterbelt Centre, Prairie Farm Rehabilitation Administration, Agriculture Canada, Indian Head, Saskatchewan). Lodgepole pine, white spruce, and black spruce seedlings were grown in the containerized-grown nursery, whereas Scots pine and Siberian larch were grown in the bare-root nursery.

Bonnyville Forest Nursery

Three-week stratified seeds of lodgepole pine (seedlot #MWM9856A 77-23-4-94; germination, 83.8%), white spruce (seedlot #MWM9831A 15-68-9-5-93; germination, 90.8%), and black spruce (seedlot #MWM9827A 68-7-5-96; germination, 89%) were sown in styrofoam blocks (77 cavities) containing 170 ml of peat:perlite (10:1, vol/vol). These styrofoam blocks were purchased from Beaver Plastics in Edmonton, Alberta. The fungal isolates used were *H. longicaudum* (UAMH 9317), *L. bicolor* (NOF 2290), *P. involutus* (NOF 2340), *P. tinctorius* (commercial vegetative inoculum in vermiculite was obtained from Plant Health Care, Inc. PA, U.S.A), *R. vinicolor* (UAMH 6200), and *S. tomentosus* (UAMH 6252). The slurry form of inoculum was produced by growing isolates at 22 °C in liquid MMN (Marx 1969) culture for 2 months. The mycelia were collected and rinsed

with water to remove excess nutrients. Six hundred milliliters of blended mycelia were mixed with 8 liters of water to give a final concentration of 5×10^4 viable propagules/ml for *S. tomentosus* and 5×10^5 viable propagules/ml for the other fungi. Two-week-old seedlings were inoculated by injecting with 5-ml of fungal slurry into the root zone using an analog adjustable-volume dispenser purchased from Fisher Scientific. *P. tinctorius* was applied (1 teaspoon or 5 ml of inoculum) directly below the rooting zone after lifting each seedling. Control seedlings did not receive fungal inoculum. There were three styrofoam blocks per fungal treatment. The blocks were laid on the greenhouse bench in a completely randomized factorial design (six fungal species and one control, three tree species, and three levels of fertilizer). The seedlings were watered and fertilized with N:P:K fertilizer (80:55:160 ppm) using a pump connected to the irrigation system. When seedlings emerged, they were fertilized every 15 days. Later, when seedlings were eight weeks old, they were fertilized once a week. The seedlings were fertilized with every watering. At other times, they were monitored and watered as needed. The temperature in the greenhouse was 25-20 °C (day to night). Eighteen weeks later, 10 random seedlings were harvested from each block. Seedling shoot height, root collar diameter, shoot and root dry weights (oven dried at 75 °C for 24 hours), shoot: root ratio and ectomycorrhizal colonization were recorded. However, our report will focus only on shoot and root dry weights as well as the shoot: root ratios as these are considered being the most sensitive indicators of young seedling response to any treatment (Marx et al. 1994). Ectomycorrhizal colonization was quantified by counting the colonized root tips. Ectomycorrhizal roots were distinguished from non-mycorrhizal root tips by differences in their color, thickness, and texture. Feeder roots (20–25) were also sectioned and stained with trypan blue to observe the fungal mantle and Hartig net to confirm an ectomycorrhizal association. Attempts were also made to re-isolate mycorrhizal fungi from the feeder roots, using standard sterilization techniques of 30% H_2O_2 soaks and water rinses (Molina and Palmer 1982).

Shelterbelt Centre Nursery

For each treatment, 1 m × 1 m plots were established in the bare-root nursery and six rows of beds were made for sowing the seeds. For inoculating with *H. longicaudum, L. bicolor, P. involutus, R. vinicolor,* and *S. tomentosus*, vegetative mycelia, prepared using the above method, were mixed with moist peat moss in a cement mixer, applied to the seed bed (0.7 L/m^2) and loosely covered with soil to prevent drying. Commercial vermiculite inoculum of *P. tinctorius* (0.7 liter) was applied to one m^2 of seedbeds in a similar manner. Commercial peat moss was applied to control plots. The plots were laid

Table 1. ANOVA showing the mean square values of growth parameters for container-grown lodgepole pine, white spruce, and black spruce over fertilizer and mycorrhizal fungal species at Bonnyville Forest Nursery

Source	DF	Shoot dry weight	Root dry weight	Shoot:Root ratio	Mycorrhizal short roots
Species	2	4.37**	8.32**	0.91**	1027.15**
Treatment	6	1573.52**	1423.34**	23.50*	5719.22**
Species × Treatment	12	37.59**	17.11**	32.62*	384.79**
Fertilizer	2	3043.93**	1758.67**	50.46*	2.11*
Species × Fertilizer	4	11.85**	5.00**	14.77*	0.72*
Treatment × Fertilizer	12	45.93**	16.09**	18.40*	0.96*
Species × Treatment × Fertilizer	24	8.87**	8.02**	6.19*	1.57*

*significant at $P < 0.05$; **significant at $P < 0.01$.

out in a completely randomized factorial design (six species of fungi and one control, two tree species, and three levels of fertilizers) in three blocks. One week later, open-pollinated (non-stratified) seeds (germination, 90%), collected from Scots pine and Siberian larch trees on site at the Shelterbelt Centre in Indian Head, were sown. When seedlings emerged, plots were fertilized with 33, 67, and 100% of 11 kg of nitrogen (N:P:K, 34:0:0) per 0.405 ha. Fertilizer was applied only once. Eighteen weeks later, 10 random seedlings from each treatment were harvested and evaluated as described above.

Experimental design and data analysis

Data were analyzed using analysis of variance (SAS 1990) and multiple comparisons among means were made using three-factor analysis of variance. The model equation for this design is $Y_{ijkl} = \mu + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \epsilon_{ijkl}$, where Y_{ijkl} is a score for the ith experimental unit in treatment combination $\alpha_j\beta_k\gamma_l$, μ is the overall population mean, α_j is the effect of treated level j, β_k is the effect of treatment level k, γ_l is the effect of treatment level l, the other terms are the joint effects of treatment levels ϵ_{ijkl} is the experimental error. Assumptions of normality and homoscedasticity for the analysis of variance were verified (Steel et al. 1997).

Results

Data from both Bonnyville Forest Nursery and Indian Head Shelterbelt Centre indicated significant interactions in seedlings shoot height, root collar

Table 2. ANOVA showing the mean square values of growth parameters for Scots pine and Siberian larch over fertilizer and mycorrhizal fungal species at the Indian Head Shelterbelt Centre bare-root nursery

Source	DF	Shoot dry weight	Root dry weight	Shoot:Root ratio	Mycorrhizal short roots
Species	1	286.71**	368.72**	506.13**	34.59**
Treatment	6	270.23**	54.31**	76.94**	70.93**
Species × Treatment	6	130.36**	5.72**	62.77**	34.98**
Fertilizer	2	574.34**	122.60**	167.00**	37.87**
Species × Fertilizer	2	136.10**	158.07***	170.47**	6.68**
Treatment × Fertilizer	12	18.34**	6.07**	8.01**	3.02**
Species × Treatment × Fertilizer	12	19.30**	3.43**	9.57**	2.55**

*significant at $P < 0.05$; **significant at $P < 0.01$.

diameter, shoot dry weight, root dry weight, shoot:root ratio, and mycorrhizal short roots with species, treatment, fertilizer, species by treatment, species by fertilizer, treatment by fertilizer, and species by treatment by fertilizer (Tables 1 and 2). With a significant three-factor interaction, the factors, species, treatment, and fertilizers are not independent of one another. The main effects of a factor differ and the magnitude of any main effect depends on the level of the other factor of the interaction. In other words, the difference in measurement between fertilizer levels differs according to the fungal treatments and the species. The significant species by treatment interaction implies that the differences among responses to fungal treatments vary with the tree species. Similarly, the significant species by fertilizer implies that the differences between responses to fertilizer levels vary with the tree species.

Lodgepole pine

Seedling shoot and root dry weights increased significantly when grown under high (67% and 100%) fertility levels compared to the 33% fertilizer level (Table 3). When inoculated with *H. longicaudum*, no significant differences in shoot and root dry weights were observed at 67% and 100% fertility levels (Table 3). For *P. tinctorius*, no significant difference in shoot dry weight was observed at 67% and 100% fertility levels. We also found no significant difference in root dry weight at 67% and 100% fertility levels for *L. bicolor*. Shoot:root ratio was significantly higher in control seedlings at the 33% and 67% fertility levels as compared with other treatments (Table 3). *H. longicaudum, L. bicolor, P. involutus, P. tinctorius*, and *S. tomentosus*

Table 3. Effect of three levels of fertilizer on the growth and ectomycorrhizal development of lodgepole pine seedlings (Bonnyville Forest Nursery)

Mycorrhizal fungi	Fertilizer level[a]	Shoot dry weight (g)	Root dry weight (g)	Shoot:Root ratio	Mycorrhizal short roots (%)
Control	33%	0.52i	0.17i	3.12a	18.0g
	67%	0.96h	0.29h	3.36a	15.2g
	100%	1.19g	0.59f	2.03b	19.0g
Hebeloma longicaudum	33%	0.99h	0.71d	1.30d	72.4e
	67%	1.91de	0.82c	2.32b	75.4e
	100%	2.00d	0.95bc	2.10b	73.0e
Laccaria bicolor	33%	0.94h	0.68e	1.38d	85.0c
	67%	1.82e	0.84c	2.10b	82.1d
	100%	1.99d	0.90c	2.20b	87.1c
Paxillus involutus	33%	1.07g	0.69e	1.56d	91.1b
	67%	2.10b	1.00b	2.11b	90.1b
	100%	2.51a	1.18a	2.14b	89.8b
Pisolithus tinctorius	33%	1.06g	0.75d	1.41d	93.3ab
	67%	1.99c	0.97bc	2.03b	95.0a
	100%	2.23c	1.22a	1.86c	95.0a
Rhizopogon vinicolor	33%	0.47i	0.21h	2.16b	33.4f
	67%	0.87h	0.40g	2.18b	30.8f
	100%	1.30f	0.62f	2.09bc	31.9f
Suillus tomentosus	33%	0.45i	0.25h	1.85c	82.7d
	67%	1.01g	0.41g	2.47b	85.0c
	100%	1.34f	0.60f	2.23b	85.9c

Values are the means of 10 replicates. Means in a column followed by the same letters are not significantly different ($P < 0.05$).
[a]Percentage of the operational fertilizer level.

formed well-developed ectomycorrhizae and the colonization varied from 72.4% to 95% (Table 3). Mycorrhizal colonization by *R. vinicolor* was poor and only 30.8% to 33.4% of feeder roots were colonized. Naturally occurring mycorrhizal fungi (*Amphinema byssoides* and *Thelephora americana*) also colonized control seedlings and the colonization varied from 15.2% to 19%. No significant differences in mycorrhizal colonization were observed at three levels of fertilizer except for *L. bicolor* and *S. tomentosus* (Table 3).

Table 4. Effect of three levels of fertilizer on the growth and ectomycorrhizal development of black spruce seedlings (Bonnyville Forest Nursery)

Mycorrhizal fungi	Fertilizer level[a]	Shoot dry weight (g)	Root dry weight (g)	Shoot:Root ratio	Mycorrhizal short roots (%)
Control	33%	0.31k	0.18k	1.78d	10.0e
	67%	0.70i	0.33i	2.15bc	11.9e
	100%	1.10g	0.62f	1.82d	9.9e
Hebeloma longicaudum	33%	0.90h	0.40g	2.21c	65.0c
	67%	1.17g	0.88c	1.31e	62.8c
	100%	2.10de	0.97b	2.14bc	64.4c
Laccaria bicolor	33%	1.10g	0.45g	2.30bc	80.0b
	67%	1.99e	0.88c	2.54ab	82.1b
	100%	2.21d	0.95b	2.77a	82.0b
Paxillus involutus	33%	1.82f	0.80d	2.46b	90.1a
	67%	2.50c	0.98b	2.27bc	91.9a
	100%	2.80a	1.01b	2.34bc	90.7a
Pisolithus tinctorius	33%	1.15g	0.77d	2.29bc	91.0a
	67%	2.20d	1.00b	2.54ab	90.0a
	100%	2.66b	1.14a	2.78a	90.0a
Rhizopogon vinicolor	33%	0.44j	0.22j	2.07bc	17.8d
	67%	0.74i	0.35h	2.14bc	17.0d
	100%	1.12g	0.66e	1.70d	18.9d
Suillus tomentosus	33%	0.48j	0.20j	2.45a	10.1e
	67%	0.74i	0.37h	2.04c	10.6e
	100%	1.20g	0.60f	2.01c	13.0e

Values are the means of 10 replicates. Means in a column followed by the same letters are not significantly different ($P < 0.05$), using Bonferroni (Dunn) t-tests.
[a]Percentage of the operational fertilizer level.

All six species of ectomycorrhizal fungi were re-isolated from the respective inoculated seedlings.

Black spruce

Both shoot and root dry weights were increased when seedlings were grown under higher (67 and 100%) fertility levels compared to the 33% fertilizer level (Table 4). Shoot:root ratio differed significantly among the treatments; the highest shoot:root ratio was observed with *L. bicolor* and *P. tinctorius*

at 67 and 100% fertilizer levels and *S. tomentosus* at 33% fertilizer level (Table 4). *H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius,* formed well-developed ectomycorrhizae in the feeder roots and the colonization varied from 62.8 to 91.9% (Table 4). Mycorrhizal colonization by *R. vinicolor* and *S. tomentosus* was poor and only 10.1 to 18.9% feeder roots were colonized. Naturally occurring mycorrhizal fungi (*A. byssoides* and *T. americana*) also colonized control seedlings and the colonization varied from 9.9 to 11.9%. No significant differences in mycorrhizal colonization were observed at three levels of fertilizer (Table 4). Except for *S. tomentosus*, other mycorrhizal fungi were isolated from the feeder roots.

White spruce

Both shoot and root dry weights increased as the levels of fertilizer were increased, the greatest shoot and root biomass was observed at 67 and 100% fertilizer levels in all the treatments (Table 5). The highest shoot:root ratio was observed with *L. bicolor* at 67 and 100% fertilizer levels, whereas the lowest shoot:root ratio was observed with *P. tinctorius* (33%) and *S. tomentosus* (100%). *H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius* formed well-developed ectomycorrhizae and the colonization varied from 60.1 to 92.9% (Table 5). Mycorrhizal colonization by *R. vinicolor* and *S. tomentosus* was poor and only 15.0 to 20.3% feeder roots were colonized. Naturally occurring mycorrhizal fungi also colonized control seedlings and the colonization varied from 11.3 to 16.6%. No significant differences in mycorrhizal colonization were observed at the three levels of fertilizer except for the control and *Hebeloma* inoculated seedlings (Table 5). Except for *S. tomentosus*, the other five species of mycorrhizal fungi were re-isolated from feeder roots.

Scots pine

Shoot dry weight was not significantly different at 67% and 100% fertilizer levels in control, *H. longicaudum, P. involutus,* and *P. tinctorius* inoculated seedlings (Table 6). When seedlings were inoculated with *L. bicolor, R. vinicolor,* and *S. tomentosus*, shoot dry weight was significantly higher at the 67% fertilizer level than at 33 and 100% fertilizer levels. No significant differences in root dry weight were observed at the three levels of fertilizer when inoculated with *L. bicolor, P. involutus,* and *P. tinctorius*, whereas significantly higher root dry weight was observed at 67% level of fertilizer in control, *R. vinicolor,* and *S. tomentosus* inoculated seedlings (Table 6). For *H. longicaudum,* the highest root dry weight was observed at 100% fertilizer level. Shoot:root ratio was significantly higher with *P. involutus* and *P. tinc-*

Table 5. Effect of three levels of fertilizer on the growth and ectomycorrhizal development of white spruce seedlings (Bonnyville Forest Nursery)

Mycorrhizal fungi	Fertilizer level[a]	Shoot dry weight (g)	Root dry weight (g)	Shoot:Root ratio	Mycorrhizal short roots (%)
Control	33%	0.41k	0.20i	2.13cd	14.4f
	67%	0.70j	0.30h	2.35c	11.3g
	100%	0.93i	0.61f	1.52h	16.6f
Hebeloma longicaudum	33%	0.89i	0.42g	2.12cd	60.1d
	67%	1.90f	0.77d	2.49bc	65.0c
	100%	2.20d	0.81d	2.75b	63.1cd
Laccaria bicolor	33%	0.88i	0.43g	2.06cd	71.1b
	67%	2.15d	0.74e	2.91ab	74.8b
	100%	2.35c	0.80de	3.07a	73.0b
Paxillus involutus	33%	1.30g	0.82d	2.46b	92.6a
	67%	2.71a	0.98c	2.27bc	91.8a
	100%	2.47bc	1.13b	2.34bc	92.9a
Pisolithus tinctorius	33%	1.20h	0.87d	1.37i	90.0a
	67%	2.02e	1.09b	1.86e	92.0a
	100%	2.55b	1.28a	2.00cd	89.9a
Rhizopogon vinicolor	33%	0.43k	0.25hi	1.74f	20.2e
	67%	0.75j	0.40g	1.89e	21.9e
	100%	1.07h	0.64f	1.66g	20.3e
Suillus tomentosus	33%	0.40k	0.22i	2.01d	15.9f
	67%	0.76j	0.38g	1.86e	15.4f
	100%	1.17h	0.63f	1.37i	15.0f

Values are the means of 10 replicates. Means in a column followed by the same letters are not significantly different ($P < 0.05$), using Bonferroni (Dunn) t-tests.
[a]Percentage of the operational fertilizer level.

torius at 67 and 100% fertility levels. Lowest shoot:root ratio was observed at 33% fertility level in control, *P. tinctorius*, and *R. vinicolor* inoculated seedlings (Table 6). All six species of mycorrhizal fungi colonized feeder roots. No significant difference in mycorrhizal colonization was observed among the three levels of fertilizers when inoculated with *L. bicolor, P. involutus,* and *P. tinctorius* (Table 6). When inoculated with *R. vinicolor* and *S. tomentosus*, mycorrhizal colonization was significantly higher at the 67% fertilizer level than the control seedlings. Unidentified native mycorrhizal fungi in the bare-

Table 6. Effect of three levels of fertilizer on the growth and ectomycorrhizal development of Scots pine seedlings (Indian Head Shelterbelt Centre)

Mycorrhizal fungi	Fertilizer level[a]	Shoot dry weight (g)	Root dry weight (g)	Shoot:Root ratio	Mycorrhizal short roots (%)
Control	33%	0.096e	0.081d	1.17f	36.8d
	67%	0.118c	0.084bc	1.41cd	40.0c
	100%	0.110c	0.080d	1.48c	40.2c
Hebeloma longicaudum	33%	0.104d	0.085b	1.22e	37.4d
	67%	0.116c	0.084bc	1.38d	40.5c
	100%	0.113c	0.090d	1.32d	40.3c
Laccaria bicolor	33%	0.080f	0.090a	1.37d	54.3a
	67%	0.121b	0.090a	2.11a	53.54a
	100%	0.116c	0.091a	2.11a	52.22a
Paxillus involutus	33%	0.123b	0.088ab	1.40cd	52.5a
	67%	0.190a	0.092a	2.06a	52.2a
	100%	0.191a	0.091a	2.09a	51.9a
Pisolithus tinctorius	33%	0.113c	0.093a	1.20e	42.0bc
	67%	0.190a	0.092a	2.06a	40.7c
	100%	0.191a	0.092a	2.06a	41.7bc
Rhizopogon vinicolor	33%	0.093e	0.078e	1.18f	34.4e
	67%	0.123b	0.087b	1.41cd	43.2b
	100%	0.114c	0.080d	1.48c	40.8c
Suillus tomentosus	33%	0.080f	0.081d	0.99f	37.0d
	67%	0.121b	0.088ab	1.37d	43.9b
	100%	0.116c	0.080d	1.52b	40.9c

Values are the means of 10 replicates. Means in a column followed by the same letters are not significantly different ($P < 0.05$), using Bonferroni (Dunn) t-tests.
[a]Percentage of the operational fertilizer level.

root nursery also colonized control seedlings with colonization varying from 36.8 to 40.2% (Table 6). All six species of mycorrhizal fungi were re-isolated from feeder roots.

Siberian larch

Both shoot and root dry weights increased as the levels of fertilizers were increased in control and inoculated seedlings. In control seedlings, the greatest biomass was observed at 100% fertilizer levels, whereas no signifi-

Table 7. Effect of three levels of fertilizer on the growth and ectomycorrhizal development of Siberian larch seedlings (Indian Head Shelterbelt Centre)

Mycorrhizal fungi	Fertilizer level[a]	Shoot dry weight (g)	Root dry weight (g)	Shoot:Root ratio	Mycorrhizal short roots (%)
Control	33%	0.101c	0.081d	1.24b	32.6d
	67%	0.114b	0.090c	1.26b	39.2b
	100%	0.122a	0.097c	1.26b	42.0b
Hebeloma	33%	0.110b	0.086d	1.24b	37.7c
longicaudum	67%	0.122a	0.093c	1.31a	42.2b
	100%	0.130a	0.099c	1.25b	44.0a
Laccaria	33%	0.121b	0.090c	1.34a	39.3a
bicolor	67%	0.133a	0.096c	1.37a	45.4a
	100%	0.114a	0.118a	1.15c	44.4a
Paxillus	33%	0.112b	0.089d	1.25b	41.8b
involutus	67%	0.130a	0.097c	1.33a	42.0b
	100%	0.131a	0.116a	1.14c	45.3a
Pisolithus	33%	0.110b	0.088d	1.24b	42.3b
tinctorius	67%	0.130a	0.097c	1.31a	43.4a
	100%	0.130a	0.102b	1.23b	43.6a
Rhizopogon	33%	0.101c	0.081d	1.23b	39.2b
vinicolor	67%	0.112b	0.090c	1.23b	42.0b
	100%	0.125a	0.102b	1.22b	43.8a
Suillus	33%	0.110b	0.080d	1.24b	39.5b
tomentosus	67%	0.116b	0.089d	1.29a	40.1b
	100%	0.124a	0.102b	1.22b	43.3a

Values are the means of 10 replicates. Means in a column followed by the same letters are not significantly different ($P < 0.05$).
[a]Percentage of the operational fertilizer level.

cant differences were observed at 67 and 100% fertilizer levels with *H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius* (Table 7). Root dry weight was significantly greater at 100% fertilizer level when inoculated with *L. bicolor, P. involutus, P. tinctorius, R. vinicolor,* and *S. tomentosus*, whereas no significant difference was observed between 67 and 100% fertilizer levels in *H. longicaudum* and control seedlings (Table 7). Shoot:root ratio was significantly higher at the 67% fertilizer level when seedlings were inoculated

with *H. longicaudum, L. bicolor, P. involutus, P. tinctorius,* and *S. tomentosus,* whereas no significant differences were observed at all levels of fertilizer in control and *R. vinicolor* inoculated seedlings (Table 7). All six species of mycorrhizal fungi formed ectomycorrhizae and the colonization varied from 39.3 to 45.5% (Table 7). Unidentified naturally occurring mycorrhizal fungi also colonized feeder roots of control seedlings and the colonization varied from 32.6 to 42% (Table 7).

Discussion

Production of seedlings for afforestation, reclamation and sometimes reforestation needs not only favorable shoot and root sizes but also well-developed mycorrhizae (Marx et al. 1982). In the nursery, high levels of fertilizers are used to promote initial seedling growth. At the Bonnyville container-grown nursery, shoot and root biomass of lodgepole pine, black spruce, and white spruce seedlings significantly increased when inoculated with *H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius* at both 67 and 100% fertilizer levels. There is evidence that mycorrhizal fungi produce hormones and other growth regulators, which change root physiology, growth, development, and morphogenesis (Hacskaylo 1973, Slankis 1973, Graham and Linderman 1980, Ek et al. 1983, Ho 1984, Smith and Reid 1997). In general, ectomycorrhizal colonization by *R. vinicolor* and *S. tomentosus* and plant dry weights were lower compared with other four fungi. Using jack pine seedlings, Gagnon et al. (1987) also reported poor ectomycorrhizal colonization by *Rhizopogon* sp.

Evidence from our study suggests that similar size of the seedlings can be obtained by application of the lower 67% fertilizer rate and selected mycorrhizal fungi rather than of 100% fertilizer rate without mycorrhizal fungi. Shoot and root dry weights of lodgepole pine seedlings were not significantly different when inoculated with *H. longicaudum* at 67% and 100% fertilizer levels. In white spruce seedlings, no significant differences in root dry weight was observed at 67% and 100% fertilizer levels when inoculated *H. longicaudum* and *L. bicolor.* In black spruce and white spruce seedlings, *S. tomentosus* was not recovered from the feeder roots. It failed to enhance growth and had low ectomycorrhizal colonization. This suggests that *S. tomentosus* either does not form ectomycorrhizal association with these two conifer species or that it could not compete with native mycorrhizal fungi. Although native mycorrhizal fungi colonized control seedlings, overall seedling growth was always better with four mycorrhizal fungi (*H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius*) at either 67% or 100% of the operational level of fertilizer. This supports the importance of screening

of mycorrhizal fungi in the nursery and inoculation of seedlings with selected and beneficial mycorrhizal fungi.

In the Indian Head bare-root nursery, both *P. involutus* and *P. tinctorius* stimulated shoot and root biomass and *L. bicolor* increased only root biomass in Scots pine seedlings. Root dry weight of Scots pine was not significantly different at the three levels of fertilizer used when inoculated with *L. bicolor, P. involutus,* and *P. tinctorius*. As suggested above, similar size of root system of Scots pine and siberian larch can be obtained using 67% fertilizer rate and selected mycorrhizal fungi instead of the normal 100% fertilizer level without mycorrhizal fungi. In Siberian larch, root dry weight was significantly increased when inoculated with *L. bicolor, P. involutus, P. tinctorius, R. vinicolor,* and *S. tomentosus* at the regular rate (100%) of fertilizer. These results clearly indicated that different strains of mycorrhizal fungi behave differently at a given rate of fertilizer in the same host plant.

Our study showed that ectomycorrhizal colonization by *H. longicaudum, L. bicolor, P. involutus, P. tinctorius, R. vinicolor,* and *S. tomentosus* was not affected in either container-grown or bare-root nursery regardless of fertilizer treatment. Similarly, *P. tinctorius* forms ectomycorrhizae under a wide range of fertilizers (Marx and Barnett 1974, Beckjord et al. 1980, Dixon et al. 1981, Maronek et al. 1981). Danielson et al. (1984a) also stated that many members of the Agaricales and the Aphyllophorales might well behave differently and tolerate broader or require a narrower range of fertilization. According to Trappe (1977), ecotypes of mycorrhizal fungi vary in cultural characteristics, growth rates, tolerance to chemicals, and nutrient uptake. Therefore, different strains of mycorrhizal fungi can tolerate various rates of fertilizer quite differently within the same host plant. Molina and Chamard (1983) used both slow-release and soluble fertilizers in Douglas-fir and Ponderosa pine seedlings and reported that *L. laccata* formed ectomycorrhiza on more than 90% of short roots when grown under near-operational levels of fertilizer. Although colonization was in general similar at the three fertilizer levels (33%, 67% and 100%) for all the tested fungi, the overall growth of seedlings was better, in most of the cases, at 67% and 100% fertilizer levels. The differences between the 67% and 100% levels were so small that they can be attributed to chance alone (Kirk 1996). Therefore, it may be recommended that for better seedling growth and environmental quality the 67% fertilizer level should be used along with selected mycorrhizal fungi such as *H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius*.

In this study, the native ectomycorrhizal fungi colonized seedlings in both container-grown and bare-root nurseries. However, the colonization of control seedlings by the native ectomycorrhizal fungi, was always lower (less than 50%) than in inoculated seedlings in the container-grown nursery.

In the bare-root nursery, *H. longicaudum, L. bicolor, P. involutus,* and *P. tinctorius* formed excellent ectomycorrhizae with similar mycorrhizal colonization at the three fertilizer levels, whereas *R. vinicolor* and *S. tomentosus* colonized poorly, similar to native mycorrhizal fungi whose level was very low. The ultimate objective of ectomycorrhizal fungal inoculation is higher quality of nursery stock and improved field performance by the seedlings, meaning increased survival, growth and probably wood quality (Hunt 1992). Inoculated fungi must be multi-stage mycorrhizal fungi and should be able to compete with native mycorrhizal fungi in the nursery and in the field. *Hebeloma longicaudum, L. bicolor, P. involutus,* and *P. tinctorius* formed excellent ectomycorrhizae on lodgepole pine, black spruce, white spruce, Scots pine, and Siberian larch. These fungi competed well with native mycorrhizal fungi both in the container-grown and bare-root nurseries. Their colonization was significantly higher than native mycorrhizal fungi and formed abundant ectomycorrhizae at the three fertilizer levels used by both nurseries. Because of the fungal strain by tree seedlot interaction (See among others Castellano and Trappe 1985, Marx 1991, Brundrett et al. 1996), our results apply only to the specific ectomycorrhizal strain and seedlot combinations used in this study. However, the results reported herein are valid for these widely used tree seedlots for operational plantings in the Canadian prairie provinces. These fungal strains also improved shoot and root dry weights of their host plants. These characteristics make them ideal candidates for large-scale inoculation in both container-grown and bare-root nursery set ups. These selected ectomycorrhizal fungi could enable the reduction of inorganic fertilizers (up to 33% less than the operational level). Further field studies are now underway to determine the early growth responses and survival of these inoculated and control seedlings.

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