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Survival of inoculated *Laccaria bicolor* in competition with native ectomycorrhizal fungi and effects on the growth of outplanted Douglas-fir seedlings

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Abstract

The survival, development and mycorrhizal efficiency of a selected strain of *Laccaria bicolor* along with naturally occurring ectomycorrhizal fungi in a young plantation of Douglas fir was examined. Symbionts were identified and their respective colonization abilities were determined. Eight species of symbiotic fungi, which may have originated in adjacent coniferous forests, were observed on the root systems. Mycorrhizal diversity differed between inoculated (5 taxa) and control (8 taxa) seedlings. Ectomycorrhizal fungi which occurred naturally in the nursery on control seedlings (*Thelephora terrestris* and *Suillus* sp.) did not survive after outplanting. Both inoculated and naturally occurring *Laccaria* species, as well as *Cenococcum geophilum*, survived on the old roots and colonized the newly formed roots, limiting the colonization by other naturally occurring fungi. Other fungi, such as *Paxillus involutus*, *Scleroderma citrinum* and *Hebeloma* sp. preferentially colonized the old roots near the seedling's collar. *Russulaceae* were found mainly in the middle section of the root system. Mycorrhizal colonization by *Laccaria* species on inoculated seedlings (54%) was significantly greater than on controls (13%) which were consequently dominated by the native fungi. Significant differences (up to 239%) were found in the growth of inoculated seedlings, especially in root and shoot weight, which developed mainly during the second year after outplanting. Seedling growth varied with the species of mycorrhizae and with the degree of root colonization. Competitiveness and effectiveness of the introduced strain on improving growth performances of seedlings are discussed.

Introduction

Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) is the main species used for reforestation in France. It is fast growing, capable of adapting to diverse site conditions, prefers a cool climate, high rainfall, middle elevations and deep, acidic soils. Such sites are common in the western part of the Vosgian massif.

Most problems of growth and survival in Douglas-fir plantations occur because of inadequate mycorrhization. Mycorrhizae play a fundamental role in the physiology and ecology of trees, affecting growth, nutrient absorption, water relationships, protection from disease and competition (Kropp and Langlois, 1990). Adequate ectomycorrhizal development is vital for normal Douglas-fir seedling growth (Hall and

Garden, 1985; McComb and Griffith, 1946; Morgan, 1985; Trappe and Strand, 1969). Poorly mycorrhized seedlings are frequently affected by combined drought and nutrient stress during the first year in the field (Bledsoe et al., 1982), limiting root growth in particular. Since new root growth after outplanting has been shown to be essential for seedlings, mycorrhization seems to be a key factor in establishing seedlings on well drained sites (Grossnickle and Reid, 1982; 1983; Parke et al., 1983a). Therefore, artificial inoculation with selected ectomycorrhizal fungi (EMF) is recommended for preventing the usual delay in root growth of noninoculated seedlings until they have been colonized by native EMF.

Evidence of growth stimulation by EMF after outplanting in plantations has been reviewed by Mikola, 1973; Trappe, 1977; Marx, 1977, 1980; Le Tacon, 1982; Molina and Chamard, 1983 and Marx and Cordell, 1988. However, most of these results were achieved with pine and oak species. Field trials concerning Douglas fir were rarely reported in the literature. Bledsoe et al. (1982) observed no positive effects of mycorrhizal inoculation on the growth of Douglas-fir seedlings after outplanting in a dry, burned-over site in eastern Washington, USA, and attributed these unsuccessful results to unsuitable inocula. Enhancement of growth with Douglas-fir seedlings has only been observed in controlled-environment studies and in nursery situations by Sinclair (1971; 1974), Sinclair et al. (1982), Bledsoe and Zasoski (1983), Morgan (1985) and Le Tacon and Bouchard (1986). However, lack of enhancement or negative effects in nurseries have also been mentioned by Trappe (1977), Molina (1982) and Molina and Chamard (1983).

These examples raised the importance of selecting effective fungus-host-site combinations. Effective tree mycorrhization depends on a precise selection of strains adapted to both tree species and forest site type. The essential criterion of selection is the competitiveness of strains as defined by Garbaye (1982). It includes survival, strain tolerance to ecological variations within a site and progressive colonization of new roots after outplanting. Finally, selected fungi must enhance host performance to a greater degree than the native fungi (Danielson, 1988).

Previous studies have shown that *Laccaria laccata* Cooke *sensu lato* is a leading candidate for artificial inoculation of Douglas fir. This broad-range colonizer appears in diverse habitats, produces abundant ectomycorrhizae and sporocarps, is highly active on the root system and is one of the most efficient mycorrhizal associates of Douglas fir in the northwestern USA (Hung and Molina, 1985; Molina, 1980; Molina and Chamard, 1983; Trappe, 1977), as well as in old Douglas-fir plantations of New Zealand (Chu-Chou and Grace, 1981).

A research program has been underway since 1982 in France to improve Douglas-fir growth and survival by EMF inoculation. The present study was designed (1) to investigate the natural ectomycorrhizal populations of a well drained reforestation site in the Vosges, (2) to determine the survival and competitiveness of a selected strain of *Laccaria bicolor* (Maire) Orton two years after outplanting, (3) to investigate the spatial colonization pattern of *Laccaria bicolor* and the native mycobionts and, finally, (4) to compare their respective influence on the early growth of Douglas-fir seedlings in the field.

Material and methods

Production of ectomycorrhizal seedlings

The selected strain, *Laccaria bicolor* S238 (formerly identified as *Laccaria laccata* before its reclassification by Armstrong et al., 1989), isolated by R Molina and J M Trappe in Oregon beneath *Tsuga mertensiana* (Bong.) Carr., was grown for 1 to 2 weeks on fresh Pachlewski agar (Pachlewski and Pachlewska, 1974). Subsequently, a solid inoculum was grown aseptically in 4-L autoclavable polyethylene bags with a breather strip allowing oxygenation. The bags contained a sterilized peat-vermiculite mixture (2:1), moistened with a 1:10 diluted brewer's malt. Inoculated bags were incubated at 25°C for two months and then stored at 4°C before use. Unpublished results indicated that the malt solution has not to be leached from the inoculated mixture before incorporation to the nursery soil. Former studies (Le Tacon et al., 1983) also showed that the addition of diverse peat-vermiculite media mixtures to fumigated nursery soil does not appear to have significant effects on

the shoot growth and mycorrhization of Douglas-fir seedlings.

Inoculation of Douglas-fir seedlings was carried out at Mieville nursery in central France (Morvan). The natural soil of the nursery was a sandy loam with pH 5.6 (1:5 soil to H_2O), 2.9% organic matter, 0.17% Kjeldahl N and 9.7 meq/100 g exchangeable cations (Metson). All experimental beds were fumigated with methyl bromide ($100\ g\ m^{-2}$) one month before inoculation. Inoculum was then incorporated ($2\ L\ m^{-2}$) into the upper 10 cm of soil with hand tools in May 1986. Douglas-fir seeds from and eastern Washington source (Ashford Zone 422) were sown in inoculated and noninoculated beds at 600 seeds per square meter.

Each year, seedling height was measured. The degree of ectomycorrhizal colonization was estimated on a sample at the end of 1986 and after lifting in 1988. On two-year-old inoculated seedlings, from 70 to 90% of the root tips had Laccaria mycorrhizae and there was no other EMF. On control seedlings, about 20% of the root tips had *Thelephora terrestris* Ehr.: Fr. mycorrhizae. They were also colonized slightly (1 to 5% of the root tips) by an unidentified Suillus-type EMF. In April 1988, the seedlings were harvested.

Field site and outplanting procedure

Seedlings were planted on a recently clear-cut site (4.25 ha) in the western low-Vosges near Brouvelieures. The site (elevation 430 m) is a southeast slope of 25%, slightly convex and composed of a loamy sand developed from the underlying Vosgian-sandstone bedrock. Average annual precipitation is 1170 mm/year; mean annual temperature is 8.4°C. The soil type was podzolic with mor humus (Table 1).

The site is characterized by a mixed pine forest dominated by ectomycorrhizal trees such as *Pinus sylvestris* L. and accompanied by *Abies alba* Mill. and *Fagus sylvatica* L. A part of the original stand was still contiguous to the plantation on the west side. Immediately beside this, on the north slope of the hill, there was an almost pure stand of *Picea abies* Karst. In 1989, the cut area was naturally regenerated by seedlings of *Pinus sylvestris*, *Abies alba*, *Picea abies* and *Betula verrucosa* Ehr. Ericaceous shrubs were locally abundant, especially *Vaccinium myrtillus* L. and *Calluna vulgaris* Hull.

At the end of April 1988, 800 inoculated and 800 noninoculated two-year-old bare-rooted seedlings were outplanted on a regular slope in a part of the reforestation site. This area was divided into eight adjacent plots. Each plot contained three rows of inoculated seedlings followed by three rows of control seedlings on a 3 m spacing. Each row contained thirty seedlings on a 2.5-m spacing and was orientated in the slope direction. Seedlings were hand-planted and the ground was hand-cleared during the summers of 1988 and 1989. Total and annual shoot lengths for a large population of 360 seedlings (10 seedlings per row, randomly selected in 6 plots) were measured on site at the end of each growing season.

Experimental design and data analysis

At the end of the second growing season after outplanting, from October to November 1989, twenty seedlings with ten replicates of each treatment were randomly selected in the eight replicate plots. Seedlings were carefully hand-excavated to retrieve intact root systems. Soil was washed from roots with a fine spray in the laboratory. Growth parameters for each seedling

Table 1. Physical and chemical properties of two horizons in a podzolized soil from the outplanting site

Horizon	Sand (%) (>50μ)	Silt (%) (<50μ)	Clay (%) (<2μ)	pH (H_2O)	Organic matter (%)	Organic C (%)	N total (%)	C:N[a]	CEC (meq/100 g)
A1 (4—10 cm)	77.0	7.7	15.3	4.0	26.1	15.2	0.52	29.1	28.4
A2 (10—20 cm)	85.5	9.7	4.8	4.0	2.9	1.7	0.08	22.5	3.6

[a] Organic carbon/total nitrogen ratio.

(shoot length, root collar diameter, shoot weight, number of total root tips and root lengths) were measured. Annual growth in diameter was also determined by observation of growth rings at collar level with a micrometric lens.

Each root system was subdivided into three sections: 0–10 cm, 10–20 cm and more than 20 cm, based on the distance from the seedling collar. After subdivision, roots were cut into 5-cm lengths. Subsamples (20%) were randomly selected and kept overnight in a water-diluted solution of FAA (formol-acetic acid-ethanol). Root units were subsequently examined for identity, survival or colonization of introduced and native mycobionts, with the aid of a stereoscopic microscope. Lastly, the total root system of each sample was dried and root dry weight was measured.

Ectomycorrhizal types were identified by visual characteristics, first including the structure of the fungal sheath and the presence of extramatrical mycelium and mycelial strands, following the classification system of Al-Abras (1985), as well as the colour and branching pattern. Species were identified according to previous observations in the literature. In order to help with identifications of potential mycobionts, a survey of epigeous sporocarps were also carried out in the plantation and in the adjacent forests during the fall of 1989.

Mycorrhizal colonization by each mycobiont was expressed as (1) the total number of mycorrhizal root apices identified as being colonized by a given mycobiont and as (2) this absolute number divided by total root tips × 100 (percent mycorrhization). Necrotic root tips and mycorrhizae were not taken into account in these calculations. Lastly, the frequency of mycorrhizae occurrence was expressed as the absolute presence of each type on ten sample plants of each treatment.

Results

Investigations into the natural ectomycorrhizal populations

Twenty ectomycorrhizal taxa were collected in the study area (Table 2). Thirteen species closely

Table 2. Sporocarps of ectomycorrhizal fungi collected in both pine and spruce forests and in the adjacent clear-cut area during the fall of 1989

Ectomycorrhizal macrofungi	Site		
	Pine forest	Spruce forest	Clear-cut area
1. *Amanita citrina* (Schaeff.) S F Gray	×	·	·
Inocybe hystrix (Fr.) Karst.	×	·	·
Russula cyanoxantha (Schaeff.: Schw.) Fr.	×	·	·
Russula mairei Sing.	×	·	·
Suillus variegatus (Swartz: Fr.) Kuntze	×	·	·
2. *Amanita muscaria* (L.: Fr.) Per.: Hook.	·	×	·
Cantharellus tubaeformis Fr.	·	×	·
Cortinarius sp. 1	·	×	·
Cortinarius sp. 2	·	×	·
Laccaria laccata Cooke	·	×	·
Russula ochroleuca (Pers.: Secr.) Fr.	·	×	·
Russula turci Bres.	·	×	·
3. *Lactarius rufus* (Scop.: Fr.) Fr.	×	×	·
4. *Paxillus involutus* (Batsch: Fr.) Fr.	×	×	×
Xerocomus badius (Fr.) Kühn.: Gilb.	×	×	×
Laccaria proxima (Boud.) Pat.	·	×	×
5. *Dermocybe cinnamomea* (L.: Fr.) Wünsche	·	·	×
Hebeloma crustuliniforme (Bull.) Quél.	·	·	×
Lactarius sp.	·	·	×
Scleroderma citrinum Pers.: Pers.	·	·	×

associated with beech, pine or spruce were exclusive to the closed forests (groups 1, 2 and 3). Most of these species may be designed as late-stage ectomycorrhizal fungi (Dighton and Mason, 1985) and were not found in the plantation. Three species were common to both the forests and the plantation (group 4). They appear to be fungi, able to initiate ectomycorrhizal symbiosis on young seedlings of diverse tree species, and known to maintain associations with older trees. Amongst them, *Paxillus involutus* and *Xerocomus badius* could have originated from the cut pine forest. *Laccaria proxima*, on the other hand, being apparently absent of the pine forest, could have originated from the spruce forest where it is abundant. Four other species, mainly early-stage fungi such as *Dermocybe cinnamomea*, *Hebeloma crustuliniforme* and *Scleroderma citrinum*, were exclusive to the plantation (group 5).

Survival and development of Laccaria bicolor and other ectomycorrhizae

Seventeen months after outplanting, total mycorrhization of seedlings differed very significantly between the treatments (Table 3). Four times as many mycorrhizal root tips per plant were found on the inoculated seedlings than on the control seedlings. This difference may be attributed to the clear dominance of *Laccaria* species on the inoculated seedlings as compared to the controls, causing a 7-fold difference in the number of Laccaria-mycorrhizae between the treatments. However, the percent mycorrhization by *Laccaria* species on inoculated seedlings after outplanting is lower than that formerly observed in the nursery ($>70\%$). This difference may be due to the competition from native mycobionts in the field.

The identity of native fungi did not differ greatly between inoculated and control seedlings. However, control seedlings had three species more than inoculated ones (8 against 5). No significant differences were found between the treatments in the number of mycorrhizae by native mycobionts, but their proportion was significantly higher on the control seedlings than on inoculated seedlings. On the other hand, the nursery mycobionts *Thelephora terrestris* and the unidentified Suillus-type, which were frequent on control seedlings, were not observed in the field 17 months after outplanting and may be considered as poor competitors.

Table 3 Frequency and colonization of the ectomycorrhizae observed on the root systems of inoculated and control Douglas-fir seedlings, two years after outplanting (fall 1989)

Treatment	Mycobiont	Type of mycorrhizae[a]	Frequency of occurrence[b]	Mean number of mycorrhizal root tips	Mean of percent mycorrhization (%)
Inoculated seedlings	*Laccaria* spp.	B00	10	5467**	54.3**
	Cenococcum geophilum	C23	10	477 ns	4.7 ns
	Paxillus involutus	B1	5	94	1.0
	Scleroderma citrinum	B1	4	95	0.7
	Russulaceae 1	C1	3	71	1.5
	All mycorrhizae	·	10	6278**	64.4**
Control seedlings	*Laccaria proxima*	B00	10	759	13.2
	Cenococcum geophilum	C23	10	255	10.1
	Scleroderma citrinum	B1	5	91	3.7
	Paxillus involutus	B1	4	37	0.6
	Hebeloma sp.	A11	2	17	3.0
	Russulaceae 1	C1	2	218	4.7
	Russulaceae 2	C1	2	55	0.6
	Russulaceae 3	C1	1	93	2.9
	All mycorrhizae	·	10	1566	39.6

[a] Following the Al-Abras (1985) classification system.

[b] Number of occurrence as compared to a constant number of ten replicated seedlings in each treatment.

[c] Mean value of ten data; **highly significant difference between the treatments ($p<0.01$); *significant difference ($p<0.05$); ns: not significant difference ($p>0.05$). Differences of colonization were analysed using only mycobionts with a frequency of 10.

In both treatments, *Laccaria* species and *Cenococcum geophilum* Fr. occurred on all seedlings examined. The Laccaria-mycorrhiza (type B00) on outplanted Douglas fir was characterized by a smooth, brown to honey mantle, with a clear tip. Mycorrhized roots typically showed a series of successive mycorrhizae with ageing, sometimes like knots on a string, giving them a sinuous appearance. Cross sections of young mycorrhizae (current growing season) showed a thin mantle formed by a plectenchymatous layer. Old mycorrhizae (the three preceding years) were characterized by a dark and flat aspect. Cross sections showed the accumulation of brown compounds, presumed to be tannins, in the cortical cells of the host. The flat aspect was caused by the lack of a fungal mantle. As early as the second year, the mantle was formed by only two or three layers of hyphae and completely disappeared by the third year. Nevertheless, a functional Hartig net was still in contact with cortical cells. Similar changes associated with ageing of mycorrhizae were recently described by Al-Abras et al. (1988).

Cenococcum geophilum (type C23) formed short and unbranched mycorrhizae. It was characterized by a jet-black, pseudoparenchymatous mantle with long emerging ornamentations formed by septate hyphae. *Laccaria* spp. and *Cenococcum geophilum* showed a close and dynamic relationship. During dry weeks of 1989, the number of Cenococcum-mycorrhizae clearly increased while Laccaria-mycorrhizae became wrinkled and flat. Close contacts between these two mycobionts on individual fine roots were often observed. Cross sections at the interface of these mycorrhizae showed complex structures with fungal mantles involving *Cenococcum geophilum* as well as Hartig nets characteristic of *Laccaria* species. Typical mycorrhizae of both species were also observed in adjacent sections of the same fine root.

Less frequent species such as *Paxillus involutus* and *Scleroderma citrinum* (type B1) were also observed in both treatments. They were mainly characterized by downy mantles and numerous mycelial strands. The other common mycobiont, Russulaceae 1 was still less frequent. This taxon is characterized by a C1 mycorrhiza-type: coralloid, short and inflated with an orange to light brown shiny mantle. Two other *Russulaceae* were also observed on the control seedlings. Lastly, a *Hebeloma* species (type A11, perhaps *Inocybe* sp.), characterized by a loose and woolly mantle covering many root tips at the same time, was observed on the controls.

Spatial colonization patterns

Observation of excavated root systems showed three different patterns of mycorrhizal distribution: overall colonization, intermediary-section colonization and surface colonization. On the inoculated seedlings, the Laccaria-type act as an overall colonizer and showed no significant differences in their percent mycorrhization within the three sections of the root system (Fig. 1). *Laccaria* species successfully colonized old and new roots, even in the mineral horizons down to 50 cm deep. In both inoculated and control treatments, *Cenococcum geophilum* was the co-dominant species and had an excellent development on newly formed roots. On control seedlings, the latter was less represented in the third section (>20 cm), corresponding to the deep mineral horizons. *Russulaceae* species may be designed as intermediary-section colonizers in both treatments (Fig. 1). They had an optimal development in the second section (10–20 cm) of the root system where they became an important competitor of *Laccaria proxima*. Lastly, the third pattern was shown by type B1 and *Hebeloma* sp. on control seedlings that occurred mainly in the first section corresponding exclusively to surface organic horizons.

These patterns were partly issued from differences in the structure of root systems between treatments. Inoculated seedlings had root systems almost twice as developed as controls in length of roots (Table 4). This difference implied that root dry weights were differently distributed between sections: 48, 30 and 22%, respectively, on inoculated seedlings as compared to 59, 30 and 11% on controls. Roots of control seedlings were more concentrated at the surface and became sparsely distributed with increase in depth. The structure of control root systems favored therefore the relative representation of surface-colonizers, well adapted to organic horizons.

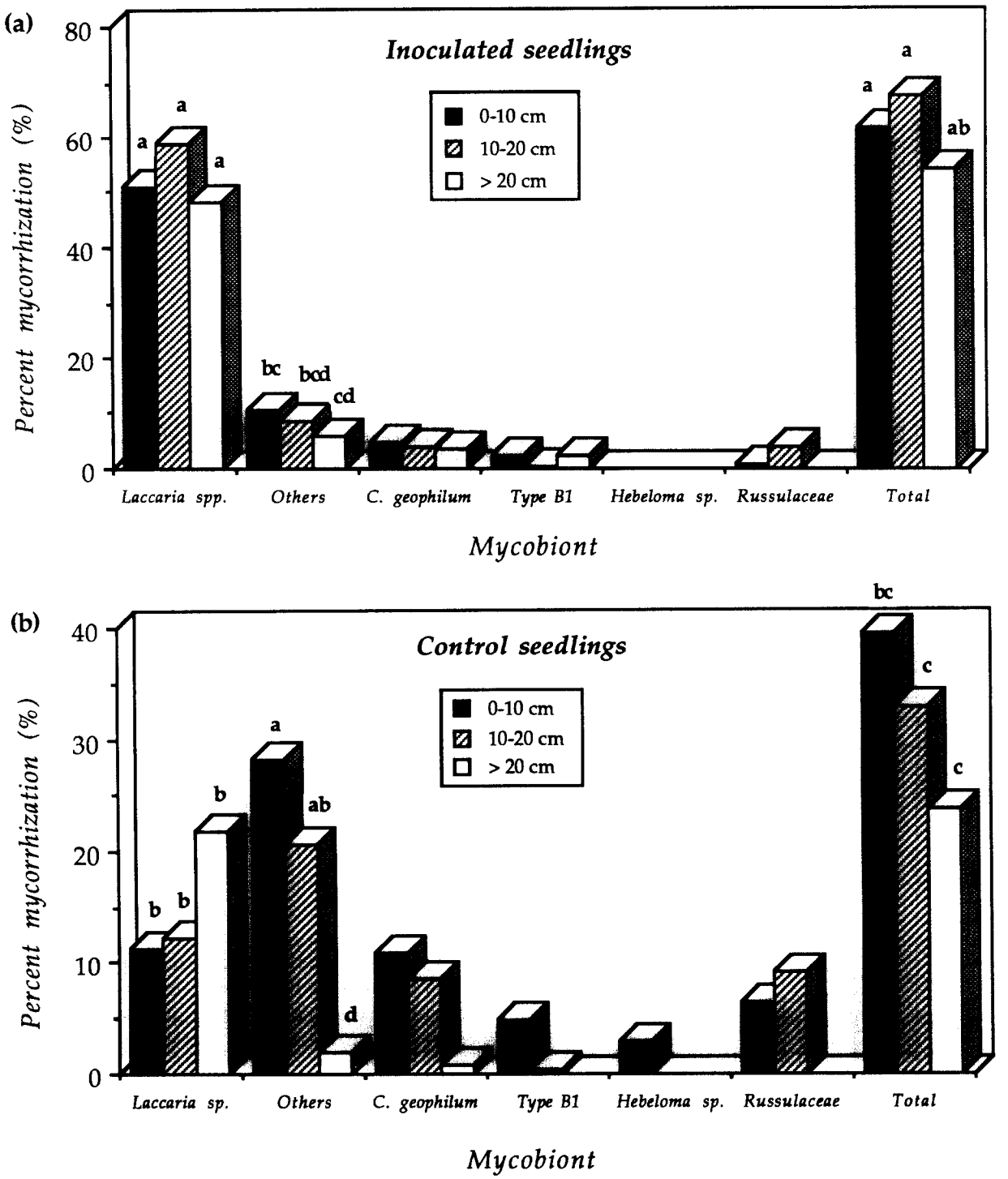

Fig. 1. Percent mycorrhizal development according to the radial distance from the root collar on (**a**) inoculated seedlings and (**b**) control seedlings. For a given mycobiont, mean values with the same letter are not significantly different at the 5% level (*t*-test). The test was performed only for those taxa showing a high frequency in all the sections of the root system. Data were transformed using Value = arcsine (% mycorrhization)$^{-1/2}$ before testing statistical differences. Values in the figure are not transformed. Type B1 includes *Paxillus involutus* and *Scleroderma citrinum* mycorrhizae. Russulaceae-type includes the mycorrhizae of three different taxa. "Others" includes all mycobionts excluding *Laccaria* species.

Table 4. Comparison between mean values of growth from a sample of inoculated and control seedlings two years after outplanting

Growth parameter	Inoculated	Control	*t* value[a]	% diff.[b]
Shoot length (cm)	67.2	46.6	3.09**	44
Mean root length (cm)	18.6	12.1	2.24*	54
Diameter (mm)[c]	10.6	6.0	4.66**	77
Number of total root tips	9446	3898	2.91**	142
Root dry weight (g)	12.5	4.8	3.59**	160
Shoot weight (g)	106.2	31.3	4.13**	239

Note: Measurements were performed at the laboratory, immediately after the extraction of seedlings in October and November 1989.

[a] ** very significant difference at the 1% level, * significant difference at the 5% level (*t*-test). The number of observations is ten for each test.

[b] % diff. = (inoculated–control)/control. Growth parameters in the table were arranged according to this increasing difference.

[c] Diameter without bark.

Seedling growth

The difference in shoot length between the two treatments, measured during the entire experiment in the nursery, was not significant at the end of 1986 but was significant at the end of 1987 (Fig. 2). Differences were acquired mainly in 1988, just after outplanting. At this time, stress clearly limited shoot growth of both types of seedlings, but the difference in annual shoot length was greatest due to a drastic decrease in growth rate of the the control seedlings. In 1989, shoot growth was improved in both treatments, but the growth advantage due to inoculation persisted.

The trends observed within this large population of seedlings clearly agreed with the differences measured between the two samples of 10 seedlings in each treatment. All six growth parameters measured were significantly affected by mycorrhizal treatment (Table 4). Inoculated seedlings had greater shoot and root growth than control seedlings. The difference between the inoculated and control seedling values varied from 44 to 239%. Slightest differences were measured in length of shoot and roots, whereas the largest differences were measured in seedling biomass: root dry weight and shoot weight. Influence of inoculation on biomass appeared to be greater on shoots than on roots.

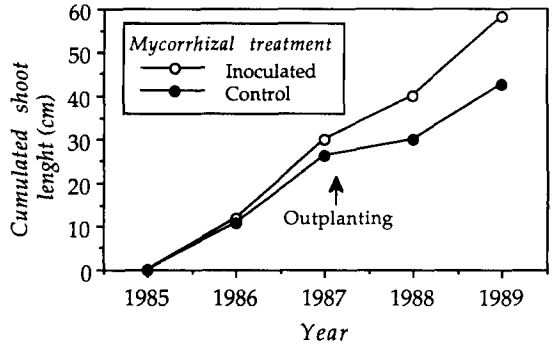

Fig. 2. Comparison of mean values for cumulated shoot length of Douglas-fir seedlings inoculated with *Laccaria bicolor* S238 and uninoculated controls for each growing season. Measurements made on a sample of 360 seedlings.

Significant differences in diameter increment between treatments were slight before outplanting (25 and 67%). Both inoculated and control seedlings then had a geometric growth typical of young seedlings (Fig. 3). By the third year (1988), both seedling types were clearly affected by outplanting stress and the difference in annual diameter growth was reduced again (25%). However, by the fourth year (difference of 138%), inoculated seedlings resumed a typical growth, whereas controls still had a stress-affected growth. Consequently, differences in diameter between treatments were developed mainly during the fourth year, as opposed to height growth which resumed after the third year. This suggests that height growth may be controlled by competition from ground vegetation that induced seedlings to favor height growth over diameter growth in 1989. Total diameters after four years were consequently very different (77%) and constituted, above the ground, a suitable parameter of discrimination between treatments (Table 4).

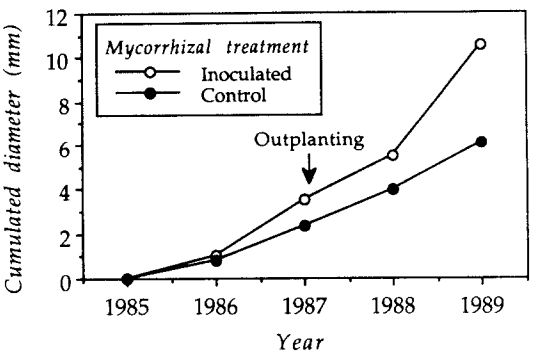

Fig. 3. Mean increase in diameter growth of Douglas-fir seedlings inoculated with *Laccaria bicolor* S238 and uninoculated controls for each growing season. Measured diameters correspond to the annual growth for a sample of 10 seedlings.

Positive correlations of the number of ectomycorrhizae with the number of total root tips and root dry weight on both treatments supplied evidence of the interdependence between mycorrhization and root morphology (Table 5). These parameters were nevertheless better correlated on inoculated seedlings which were affected by a less variable ectomycorrhizal flora. On inoculated seedlings, the root collar diameter was also significantly correlated with the number of mycorrhizal root tips. In all cases, the number of Laccaria-mycorrhizae seemed to be responsible for the effect of total mycorrhization. Considering the limited number of replicates, all other correlations measured (with shoot and root lengths, shoot weight and annual diameter growth) were not significant in any treatment.

Discussion

Results suggested that a competitive pressure was exerted on the introduced mycobiont *Laccaria bicolor*. The survey of natural EMF populations indicated that numerous species widely associated with conifers might be living in the plantation site. These species partly originated from the clear-cut stand, probably persisted in the field on seedlings of spruce and fir and spread to the regeneration of pine and birch after clearcutting. On the other hand, early-stage EMF found in the clear-cut area but previously absent from the pine forest might originate mainly through spores from the neighboring forests. Both sources of naturally occurring fungi play a significant role in the colonization of newly formed roots in the field and may have exerted a competitive pressure on the introduced strain of *Laccaria*. Moreover, the observation of successful natural inoculation of Douglas-fir seedlings after harvesting of pine stands (Le Tacon et al., 1984) supports this idea.

The ectomycorrhizal flora observed on the root systems in the present study is more diversified than that mentioned by Le Tacon et al. (1984) on Douglas-fir seedlings in a four-year-old plantation, but has three taxa in common (*Laccaria laccata sensu lato*, *Hebeloma* sp. and *Paxillus involutus*). Mycobionts associated with Douglas fir are also very similar to the nine species reported by Al-Abras (1985) from spruce seedlings four years after outplanting in some Vosgian reforestation sites. However, a difference between the treatments was noted in the ectomycorrhizal diversity. This difference may indicate that the strong dominance of the introduced Laccaria-strain contributes to restricting the number of new colonizers on the root system of inoculated seedlings.

The most important difference observed between the treatments was the number of Laccaria-mycorrhizae on inoculated seedlings which

Table 5. Linear correlations (r^2) between growth parameters two years after outplanting and number of ectomycorrhizal root tips on ten replicates of both inoculated and control Douglas-fir seedlings

Growth parameter	Inoculated seedlings		Control seedlings	
	Laccaria spp.	Total	*Laccaria proxima*	Total
Diameter[a]	0.41*	0.43*	0.20 ns	0.17 ns
Root dry weight	0.60**	0.64**	0.41*	0.45*
Number of total root tips	0.90**	0.87**	0.57*	0.78**

Note: Significant relationship at the 5% *, 1% ** level; ns = not significant. Limits of r^2 are 0.40 and 0.59, respectively.
[a] Annual growth in diameter at the collar level (without bark).

greatly exceeded that of control seedlings. This suggests that the selected *Laccaria bicolor* S238 strain successfully survived after outplanting whereas control seedlings were mainly colonized by native mycobionts such as *Laccaria proxima* which superseded poor competitors from the nursery, such as *Thelephora terrestris* that completely disappeared.

Our results compare very well with those from McAfee and Fortin (1986), in a study of EMF competitiveness on jack pine (*Pinus banksiana* Lamb) in northeastern Canada, who observed a postplanting colonization of 55% by *Laccaria bicolor*, plus 16% by indigenous mycobionts on inoculated seedlings after 2 months in the field. Control seedlings had 29% mycorrhization. In both our studies and those of McAfee and Fortin (1986), native mycobionts had a better colonization on control than on inoculated seedlings. On the contrary, Danielson (1988) in a study of field competitivity of EMF on jack pine seedlings in western Canada found that *Laccaria proxima* and *Thelephora terrestris* had both readily colonized new roots of seedlings after outplanting but were completely superseded by indigenous fungi after one year in the field. In another field study with ectomycorrhizal-inoculated Douglas fir in western North America, Bledsoe et al. (1982) obtained similar results: after 5 months, less than 10% of the original seedling feeder roots were mycorrhizal as compared to 20–50% of *Laccaria laccata* and *Hebeloma crustuliniforme* mycorrhizae just before outplanting. Furthermore, none of the inoculated fungi were present on the newly formed roots at the base of the root system; these were mainly colonized by indigenous fungi.

Among all fungi occurring on outplanted seedlings, we recognized three different patterns of distribution related to root distance from the collar. These spatial patterns suggest a close relationship between EMF and organic-matter content of soil horizons. Moreover, evidences of a strong relationship between organic matter and ectomycorrhizae were shown by Harvey et al. (1979, 1987), Kropp (1982), Parke et al. (1983b) and McAfee and Fortin (1989). Surface-colonizers seem to develop better in humus and litter layers than in mineral horizons whereas, on the contrary, *Laccaria* species and *Cenococcum geophilum* succeed in colonizing the root system along almost its entire length even in horizons with little organic matter. This difference might explain the higher percentage mycorrhization of type B1 and *Hebeloma* species on control seedlings that had a more superficial root system. Similarly, McAfee and Fortin (1986) found that indigenous mycobionts initially colonized lateral roots growing through the surface organic horizons. On the other hand, *Cenococcum geophilum* typically developed in soils with little organic matter (Frankland and Harrison, 1985; McAfee and Fortin, 1989).

The competitiveness of the introduced strain was assessed according to definitions by Marx (1980) and Garbaye (1982). Results indicated clearly that (1) *Laccaria bicolor* prevents the development of poor competitors such as *Thelephora terrestris* on inoculated seedlings in the nursery; (2) *Laccaria bicolor* successfully survived on inoculated seedlings and was maintained for four years on old roots as seen by effective Hartig nets; (3) the Laccaria-strain acts as a pioneer fungus and progressively colonized newly formed root tips on all parts of the root system and, lastly; (4) native mycobionts were less diversified and had a lower colonization on seedlings inoculated with *Laccaria bicolor*. Consequently, this strain may be considered as highly competitive on Douglas fir in the studied site.

Because of this capacity to develop on newly formed roots in mineral horizons, *Cenococcum geophilum* appeared to be a major competitor of *Laccaria* species. Moreover, mixed mycorrhizae involving both these species on the same short root were frequently observed during the driest weeks. Such an association was previously described by Marks and Foster (1967). The advantage of *Cenococcum geophilum* during the driest weeks is supported by the fact that it is well recognized as being drought resistant (Trappe, 1977). Other examples suggest that the development of this usually poor early colonizer might, in the field, be favored by the presence of other early colonizers (Garbaye, 1982; Garbaye and Wilhelm, 1984).

Our assertion concerning the high competitiveness of *Laccaria bicolor* S238 might not have been totally useful if we had not considered the effects of this strain on improving growth per-

formances of seedlings. Results indicated that all growth parameters measured in this study were significantly affected by inoculation. The greatest differences were in biomass increase, either in root or shoot weight. This finding disagrees with results reported by Bledsoe et al. (1982) that indicated no significant effects of prior mycorrhizal inoculation on height growth of Douglas-fir seedlings after two years in the field. Moreover, in the latter case mycorrhizal inoculation had a negative effect on weight growth of inoculated seedlings.

Results indicated that diameter and total height of seedlings were closer before than after outplanting. As suggested by Marx and Cordell (1988), field studies may be considered to perpetuate the initial effect of ectomycorrhizae, since size differences at the nursery are part of the response to mycorrhizal inoculation. This idea is supported by a significant relationship between the number of mycorrhizae (especially Laccaria-type) and seedling growth, particularly for root dry weight in both treatments, two years after outplanting. These results suggested that mycorrhizal development is the principal cause of growth stimulation and distinctly influences seedling growth between treatments. Mycorrhizal inoculation permits a fast recovery of normal growth as early as the second growing season following outplanting. Considering also that inoculation intensity of an introduced strain is a decisive factor in fine root colonization (Garbaye, 1983; Hung and Molina, 1985), nursery inoculation appears therefore to be a key factor in seedling growth improvement after outplanting. In this way, improved growth responses clearly depend on the character of the inoculated fungus as well as on its degree of root colonization (Garbaye and Wilhelm, 1984).

In summary, this study not only confirmed the high performance of *Laccaria bicolor* as mycorrhizal colonizer on Douglas fir, but it also established the high competitiveness of this strain on well drained loamy sand after clearcutting of a pine stand, and showed a significant improvement in seedling growth by the use of mycorrhizal inoculation. However, considering that some aspects of this study have not been completely resolved, conclusions should be made cautiously and confirmation must await further research. First, observations should by continued for the next few seasons in order to confirm trends described in this paper.

Secondly, conclusions of this study could apply only to similar sites in the artificial part of the Douglas-fir area of distribution and may not be generalized to plantations of indigenous trees such as Douglas-fir plantations in northwestern North America. The host-fungus-site combination used in this study is not an example of natural ectomycorrhizal association. Both *Laccaria bicolor* S238 and Douglas fir are symbionts introduced from North America where they have long co-evolved and consequently have developed a high compatibility. In spite of their abundance on the study site, native European strains, that have not developed such a compatibility with Douglas fir, might however be at disadvantage in their competition for mycorrhizal colonization. Competitive pressures on the introduced strain might therefore be lower than estimated and comparable to disturbed sites with poor EMF populations. This argument might explain the significant differences between the present results and those of Bledsoe et al. (1982) involving indigenous species.

Present results are very important in view of practical applications of mycorrhizal technology in France. Mycorrhization should thus be considered more and more as an interesting alternative to usual silvicultural techniques in forestry. Contrary to other growth-stimulating operations, such as fertilization and use of phytocides, mycorrhizae are respectful of the environment and have a selective effect on the ground vegetation: positive for inoculated seedlings and neutral for the competitive ground-storey vegetation. This constitutes a decisive argument in favor of using mycorrhizal technology in reforestation practices.

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