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Field performance in northern Spain of Douglas-fir seedlings Inoculated with ectomycorrhizal fungi

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Abstract Experimental plantations were established in northern Spain to determine the effects of different ectomycorrhizal fungi on growth and survival of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) under field conditions. Douglas-fir seedlings were inoculated with *Laccaria bicolor* S238 mycelia in two bareroot nurseries in central France or with spore suspensions of three hypogeous ectomycorrhizal species: *Melanogaster ambiguus*, *Rhizopogon colossus* and *R. subareolatus*, in a Spanish containerised nursery. The effects of ectomycorrhizal inoculation on plant survival after outplanting were limited, being only significant at the Guipuzkoan (Spain) site, when plants inoculated with *L. bicolor* S238 were compared to non-inoculated plants grown in non-fumigated soil. *L. bicolor* S238 had a significant effect on plant growth during the phase of bareroot nursery growth and this difference was maintained after field outplanting. Nursery inoculations with *M. ambiguus*, *R. colossus* and *R. subareolatus* improved plant growth during the first 2 and 3 years after field outplanting. The positive effects of the inoculation treatment on seedling height, root collar diameter and stem volume persisted after 5 years of field growth. Inoculation with these ectomycorrhizal fungi may improve the field performance of Douglas-fir seedlings in northern Spain.

Key words *Pseudotsuga menziesii* · *Laccaria bicolor* · *Melanogaster ambiguus* · *Rhizopogon subareolatus* · *Rhizopogon colossus* · Reforestation

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Introduction

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] has become one of the most important trees for timber production in western Europe, especially in France and Germany (Matthews 1983). The rapid growth of the plantations established 30–70 years ago in northern Spain, together with the adaptability of the species to this region (Toval et al. 1993), make Douglas-fir a prime choice for productive reforestation in this area.

To improve early growth of Douglas-fir seedlings, in the nursery and in the field, controlled ectomycorrhizal inoculations have been tested extensively in many countries (Castellano and Molina 1989; Castellano 1996). Inoculation in container nurseries mostly had no effects on growth (Castellano and Molina 1989), whereas inoculation in bareroot nurseries with fungi such as *Hebeloma cylindrosporum* Romagn. (Le Tacon et al. 1985) or *Laccaria bicolor* (R. Mre.) Orton (Le Tacon and Bouchard 1986) resulted in significantly higher growth. The success of ectomycorrhizal inoculation lies in the correct selection of the mycorrhizal fungus. The selection process must include the establishment of field plantations to evaluate the performance of the inoculated plants (Trappe 1977).

Considerable research on the effects of ectomycorrhizal inoculation on outplanting performance of forest seedlings has been done during the last years. Inoculations of 67 host plant species with 73 fungal species have been reported (Castellano 1996). The majority of the host plant species inoculated where *Pinaceae*, with Douglas-fir involved in 8% of the experiments. The most frequently studied fungus is *Pisolithus tinctorius* (Pers.) Cok. & Couch (42% of cited work). In spite of the high variation in experimental conditions in the field trials, in general Douglas-fir showed a positive growth response when inoculated with host-specific fungi, such as *Rhizopogon vinicolor* A. H. Smith, or when inoculated with other fungi and planted as an exotic in Europe (Castellano 1996). However, inoculation

method, experimental design, and measured parameters differ substantially between these studies and it is difficult to draw firm conclusions or to extrapolate from one situation to another.

Many native ectomycorrhizal fungi present in northern Spain (Álvarez et al. 1993) are able to form mycorrhizas with Douglas-fir under controlled conditions (Parladé et al. 1996a). Some of these are being tested in a programme to select effective candidates for improving field performance of inoculated plants. The present work is part of a series of long-term experiments to assess the benefits of inoculation of conifer seedlings with ectomycorrhizal fungi for survival and growth during reforestation in Spain. The field experiments terminate after 5 years and the results for Douglas-fir in northern Spain are presented here.

Materials and methods

Bareroot Douglas-fir seedlings inoculated with *Laccaria bicolor* S238

Bareroot Douglas-fir seedlings (seed origin WA 422) were grown in the Peyrat-le-Château and Morvan nurseries (central France) in 1986 and 1987. Mycelial inoculum of strain S238 of *Laccaria bicolor* (R. Mre.) Orton, a world-wide ectomycorrhizal fungus for which positive results with Douglas-fir seedlings have been reported in other European countries (Le Tacon et al. 1992), was produced by INRA, Nancy on a peat-vermiculite medium supplemented with nutrient solution. The inoculum was applied, before sowing, by mixing with nursery soil at 2 litres of inoculum per m^2. After 2 years of nursery growth, the inoculated plants were mycorrhizal with *L. bicolor,* with root colonisation ranging from 50% for Peyrat-le-Château seedlings to 30% for Morvan seedlings. Non-inoculated plants became ectomycorrhizal with native contaminants in the nursery, identified as *Thelephora terrestris* (Ehrh.) Fr. or mycorrhizas similar to those formed by *Rhizopogon* spp.

In 1988, two experimental plantations were established at Pontevedra (Galicia, northwestern Spain) in a previous plantation of *Pinus pinaster* Ait. on a brown podzolic soil, pH 5.6 (H_2O), at 550 m elevation and a mean annual precipitation of 1500 mm, and at Girona (Catalonia, northeastern Spain) on a sandy soil, pH 4.9 (H_2O), at 1100 m elevation with a mean annual precipitation of 900 mm, surrounded by *Fagus sylvatica* L. forest with some 40-year-old Douglas-fir plantations. In Pontevedra, inoculated and non-inoculated Douglas-fir seedlings from both French nurseries were compared in a completely randomised design with 4 replicates per treatment, each experimental unit containing 45 plants. At Girona, inoculated and non-inoculated plants grown at Peyrat-le-Château were compared following a completely randomised design. Eight replicates were established for each treatment, containing 15 plants per experimental unit.

In 1989, a third experimental plantation was established at Guipúzkoa (Basque Country, northern Spain) in a pasture field clayey soil, pH 4.9 (H_2O), at 1300 m elevation with a mean annual precipitation of 1200 mm and surrounded by *F. sylvatica* forest and *Larix decidua* Mill. plantations. Bareroot Douglas-fir seedlings grown at Peyrat-le-Château under three different treatments were compared: plants inoculated with *L. bicolor* S238, non-inoculated plants grown in fumigated soil, and non-inoculated plants grown in non-fumigated soil. Experimental units containing 33 plants were installed in a completely randomised design with 6 replicates per treatment.

All experimental plantations were set up with a planting density of 1100 trees per ha (tree spacing 3×3 m), adjusting the sizes of plots to the conditions of each area, and using the local techniques of plantation. During the first 5 years of field growth, data on survival and plant height were taken annually and root collar diameter was measured in the fifth year.

Containerised Douglas-fir seedlings inoculated with hypogeous fungi

Douglas-fir seeds (origin WA 261) were sown in 165-cm^3 containers (Ray Leach "Cone-tainer", Stuewe & Sons, Corvallis, Ore., USA) in a peat (Floratorf, Floragard, Oldenburg, Germany) and vermiculite (Termite grade 2, Asfaltex, Barcelona, Spain) substrate (1:1, v:v) pH 5.5 (H_2O). Seedlings were inoculated with water suspensions of basidiospores of three species of hypogeous Gasteromycetes: *Melanogaster ambiguus* (Vitt.) Tul. & Tul., an ectomycorrhizal fungus collected in association with Douglas-fir in northern Spain, and two Douglas-fir-specific fungi: *Rhizopogon colossus* A. H. Smith and *Rhizopogon subareolatus* A. H. Smith. Spore suspensions were prepared as described in Parladé et al. (1996*b*), adjusted to an inoculation dose of 10^7 spores per container and applied 1 month after seed germination. Seedlings were grown for 1 year and fertilised every 2 weeks with 10 ml per plant of a solution containing 17-6-18 NPK fertiliser and micronutrients; each plant received at each fertilisation 3.65 mg N, 1.29 mg P, 3.87 mg K, 0.35 mg Fe, 0.07 mg Mg, 0.06 mg Mn, 0.06 mg Cu, 0.01 mg Zn, 0.01 mg B, and 0.01 mg Mo. At the end of the growth period, plants with about 50% of mycorrhizal short roots for *M. ambiguus* and *R. colossus*, or 30% for *R. subareolatus* were selected. The percentage of colonised short roots was determined by the method of Parladé et al. (1996b).

In spring 1991, three experimental plots, one for each fungal species, were established in Álava (Basque Country, northern Spain) on a set-aside agricultural sandy soil, pH 6.1 (H_2O). The site was located at 790 m altitude on the north slope of the Cantabrian Range, with a mean annual precipitation of 770 mm, surrounded by *Quercus pyrenaica* Willd. forest and some spots of *F. sylvatica*. Treatments were repeated 8 times following a completely randomised design with 20 plants in each experimental unit.

In spring 1992, an additional experimental plot was set up at the same site using non-mycorrhizal and *R. subareolatus* mycorrhizal Douglas-fir with a mean mycorrhizal colonisation of 50%. Seedlings, grown and inoculated as described above, were distributed following a completely randomised design with 7 replicates per treatment and 19 plants in each experimental unit.

All the experimental plantations were set up with a planting density of 1100 trees per ha (tree spacing 3×3 m), adjusting the sizes of plots to the conditions of each area, and using the local techniques of plantation. During the first 5 years of field growth, data on survival and plant height were taken annually. In the fifth year of growth, stem diameters were measured at 25 cm above the ground, and root samples were taken from 6 randomly selected plants in each treatment. To retrieve root systems, soil around the plant was carefully hand-excavated from the seedling collar to 0.5 m distance and 25 cm depth. Samples of lateral roots were taken. Short roots were examined under a microscope for the presence of ectomycorrhizas of the inoculated fungi.

Data analysis

Data on survival at the end of the second year of field growth (arcsine $\sqrt{}$ transformed), and growth parameters (stem height, root collar diameter and stem volume) at the end of the fifth year of field growth were processed by ANOVA analysis and Tukey's test ($P = 0.05$) to determine the significance of treatment differences. Differences in growth rates between inoculated and control treatments were determined by ANOVA and Tukey's test ($P = 0.05$) comparing the annual relative increments in height $[(h_f - h_i) / h_i$; h_f = height at the end of the growing season, h_i = height at the beginning of the growing season].

Results

Bareroot Douglas-fir seedlings inoculated with Laccaria bicolor S238

Pontevedra site

At the time of field outplanting (1988), the *L. bicolor* mycorrhizal seedlings were significantly taller than the non-inoculated plants from Peyrat-le-Château, whereas initial differences in seedling size were not significant for plants grown at Morvan. After 2 years of field outplanting, no differences in plant survival were detected between treatments (Table 1). During the first year of field growth, considering all plants produced in both nurseries, differences in relative growth rates were significant between plants produced Peyrat-le-Château and those produced at Morvan, both inoculated and non-inoculated (Fig. 1B). At the end of the assessment period, plant height differences were not significant when analysed using initial height as a covariant, even though the inoculated plants from Peyrat-le-Château were 50 cm taller than their respective non-inoculated controls (Table 1). Differences in diameter and stem volume were significant between inoculated and non-inoculated plants produced at Peyrat-le-Château. Fructification of *L. bicolor* was never observed at this site.

Girona site

Plants inoculated with *L. bicolor* S238 were significantly taller than non-inoculated plants when field outplanted in 1988. At the end of the second year after outplanting, no differences in survival between inoculated and non-inoculated trees were recorded (Table 1). Annual relative increases in height were only significantly higher for *L. bicolor* mycorrhizal trees during the second year of field growth (Fig. 2B). In subsequent years, the relative growth of trees did not differ or in some cases non-inoculated seedlings were higher. Plant heights were the same after 5 years of field growth when analysed using the initial heights as a covariant (Table 1). However, at the end of the assessment period plants inoculated with *L. bicolor* S238 were greater in diameter and had a higher stem volume than non-inoculated plants (Table 1). Fructification of *L. bicolor* was never recorded at this site.

Guipuzkoa site

At the time of field plantation (1989), plants mycorrhizal with *L. bicolor* S238 were significantly taller than either control treatment, when grown in fumigated or non-fumigated soil. Plants at the Guipuzkoa site showed significant differences in survival 2 years after outplanting. Inoculated plants showed a 16% higher survival than non-inoculated plants grown in non-fumigated soil in the nursery (Table 1). Although differences in height persisted during the 5 years of field growth, annual relative increments in height were significantly higher than other treatments only for non-inoculated plants grown in fumigated nursery soil during the first year of field growth (Fig. 3B). Thereafter, relative growth rates were similar for all the treatments. At the end of the assessment period, plants inoculated with *L. bicolor* S238 were significantly taller and had greater diameters and stem volume than either control treatments (Table 1). Fruit bodies of *L. bicolor* were regularly found around inoculated trees after the first and second growing seasons.

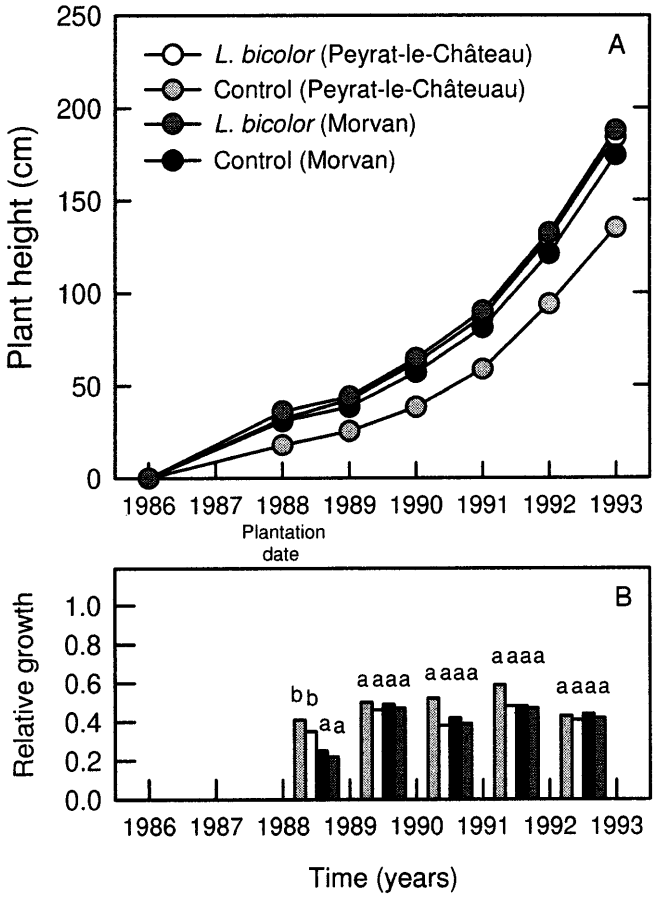

Fig. 1 Growth curves (**A**) and relative annual growth (**B**) of *Laccaria bicolor* S238 inoculated and non-inoculated Douglas-fir plants, produced in the Peyrat-le-Château and Morvan nurseries, after 5 years of field outplanting at Pontevedra. For each year, bars with the same letter are not significantly different ($P = 0.05$)

Containerised Douglas-fir seedlings inoculated with hypogeous fungi

The heights of inoculated and non-inoculated seedlings, planted at the Álava sites, were not significantly differ-

Table 1 Douglas-fir seedling survival after 2 years of field growth, and height, root collar diameter and stem volume of nursery inoculated and non-inoculated (control) seedlings 5 years after field outplanting. For each field site and nursery of origin, means in the same column followed by a common letter are not significantly different according to Tukey's test $P = 0.05$

Field site	Nursery of origin	Inoculation treatment	2nd year survival (%)	Initial height (cm)	5-year height (cm)	Root collar diameter (RCD) (mm)	Stem volume[a] (dm^3)
Pontevedra	Peyrat-le-Château	Control	76 a	18 a	135 a	19.6 a	0.58 a
(1988)	Peyrat-le-Château	*Laccaria bicolor* S238	71 a	32 b	184 a	29.8 b	1.93 b
	Morvan	Control	69 a	31 a	174 a	27.8 a	1.41 a
	Morvan	*L. bicolor* S238	70 a	36 a	188 a	30.0 a	1.70 a
Girona	Peyrat-le-Château	Control	82 a	22 a	163 a	27.6 a	1.23 a
(1988)	Peyrat-le-Château	*L. bicolor* S238	89 a	32 b	195 a	36.2 b	2.61 b
Guipuzkoa	Peyrat-le-Château	Control, non-fumigated soil	72 a	19 a	178 a	22.1 a	0.58 a
(1989)	Peyrat-le-Château	Control, fumigated soil	82 ab	18 a	180 a	25.0 a	0.80 a
	Peyrat-te-Château	*L. bicolor* S238	88 b	29 b	215 b	31.6 b	1.52 b
Álava	Cabrils	Control	100 a	20 a	149 a	35.6 a	1.97 a
(1991)	Cabrils	*Melanogaster ambiguus*	90 a	17 a	210 b	49.9 b	5.38 b
	Cabrils	Control	80 a	22 a	134 a	31.1 a	1.38 a
	Cabrils	*Rhizopogon colossus*	86 a	20 a	177 b	42.5 b	3.26 b
	Cabrils	Control	91 a	21 a	140 a	34.6 a	2.05 a
	Cabrils	*Rhizopogon subareolatus*	98 a	22 a	144 a	34.7 a	2.11 a
Álava	Cabrils	Control	86 a	22 a	175 a	18.3 a	0.61 a
(1992)	Cabrils	*Rhizopogon subareolatus*	84 a	24 a	194 b	23.2 b	1.06 b

[a] Calculated as $(RCD)^2 \times$ height (Marx et al. 1991)

ent at planting time. Two years after planting, no statistically significant differences in survival were found between treatments for any of the inoculated fungi (Table 1).

During the 5 years of field growth, plants mycorrhizal with *M. ambiguus* (Fig. 4A) and *R. colossus* (Fig. 5A) were significantly higher than their respective non-inoculated controls. Annual relative growth increments were also significant for both inoculation treatments during the first 2 (Fig. 4B) and 3 years of field growth (Fig. 5B). At the end of the 5-year assessment period, plants inoculated with *M. ambiguus* or *R. colossus* were taller and their diameters and stem volumes greater than their respective non-inoculated controls (Table 1).

In the experimental plot established in spring 1991, significant differences in relative growth between *R. subareolatus*-inoculated plants and non-inoculated controls were detected for the first 2 years of field growth (Fig. 6B). Thereafter, the annual growth of both treatments was equal or was even higher for control plants, and no differences in height were recorded in successive years. At the end of the assessment period, no differences were found in height, diameter or stem volume between non-inoculated controls and inoculated plants (Table 1).

In the experimental plot established in spring 1992, differences in height between non-inoculated and *R. subareolatus* inoculated seedlings were recorded all through the 5 years of assessment (Fig. 7A). Differences in relative annual growth were significant in the first and second years of field growth (Fig. 7B). At the end of the 5-year period, inoculated plants were taller and diameters and stem volumes greater than non-inoculated plants (Table 1).

Five years after field planting, *Rhizopogon*-type mycorrhizas were consistently observed in root samples taken from both control plants and those initially inoculated with *Rhizopogon* in the nursery. These types of mycorrhizas were also present in the plants inoculated in the nursery with *M. ambiguus*. *M. ambiguus* mycorrhizas were observed in the roots of 25% of the non-inoculated plants. At the same time, sporocarps of *Rhizopogon* spp. (section *villosuli*) were collected in plots planted with *R. colossus*- and *R. subareolatus*-inoculated plants.

Discussion

In general, the effect of ectomycorrhizal inoculation on plant survival after outplanting was very limited. The experimental plots were established in forest productive areas with edafoclimatic conditions suitable for Douglas-fir growth where a significant seedling mortality was not expected. Differences in plant survival were only significant at the Guipuzkoa site, where nursery plants inoculated with *L. bicolor* S238 survived better than non-inoculated plants grown in non-fumigated soil. Soil fumigation may alter microbial populations and reduce or eliminate pathogenic microorganisms. As well as a reduction in disease, a frequent response to fumigation is enhanced crop growth. This effect results from the elimination of organisms that cause inconspicuous damage to the fine feeder roots but nevertheless impede uptake of nutrients and water (Altman 1970). Thus, plants cultivated in fumigated nursery

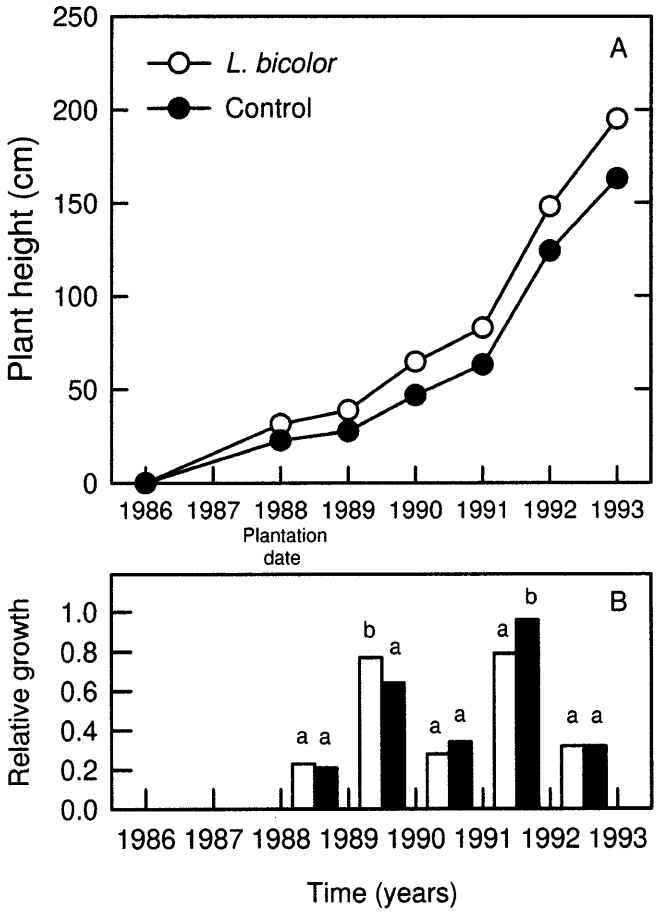

Fig. 2 Growth curves (**A**) and relative annual growth (**B**) of *L. bicolor* S238 inoculated and non-inoculated plants after 5 years of field outplanting at Girona. For each year, bars with the same letter are not significantly different ($P=0.05$)

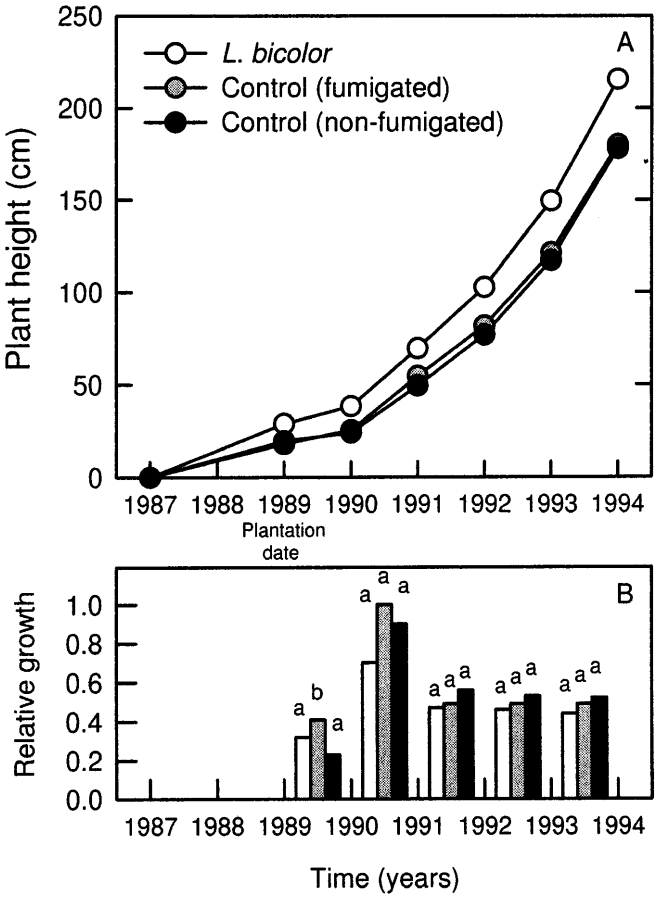

Fig. 3 Growth curves (**A**) and relative annual growth (**B**) of *L. bicolor* S238 inoculated and non-inoculated plants (grown in fumigated soil and in non-fumigated soil) after 5 years of field outplanting at Guipuzkoa. For each year, bars with the same letter are not significantly different ($P=0.05$)

soil, whether inoculated or not, may obtain more nutrients than plants cultivated in non-fumigated soil. Such differences in nutrient status or rhizospheric microflora, at the time of field establishment, may explain slight differences in survival.

Field experiments with trees are conducted over a long time-scale and several years of growth may be required before treatment differences are detectable. At the same time, field experiments are affected by variation in climatic, edafic and microbiological factors which cannot be controlled and that tend to influence, and often confound, responses to ectomycorrhizal treatments. Variation in the extent of ectomycorrhizal colonisation of the test seedlings and in initial seedling size must also be considered.

Fluctuation in ectomycorrhizal development of test seedlings was minimised by selecting only plants mycorrhizal with the inoculated fungus and with an average degree of root infection higher than 30% of all short roots. Therefore, non-mycorrhizal plants or mycorrhizal with a low percentage of root colonisation were rejected for field outplanting. Douglas-fir plants inoculated with *L. bicolor* S238 were produced in bareroot nurseries for 2 years, whereas seedlings inoculated with hypogeous fungi were grown in containers for 1 year. The number and diversity of native ectomycorrhizal contaminants was clearly different. Plants inoculated with *L. bicolor*, as well as their respective non-inoculated controls, showed random but consistent presence of ectomycorrhizal contaminants colonising their root systems, mainly *Thelephora terrestris* and an unidentified *Rhizopogon*. No contaminants were detected in the root systems of container-grown plants and the root systems of uninoculated container-grown seedlings were free of any mycorrhiza.

The seedlings inoculated in the bareroot nurseries were larger than the non-inoculated controls, as were seedlings inoculated with *L. bicolor* S238. If initial size at outplanting is not taken into account, this tends to confuse interpretation of subsequent field results, since large seedlings usually grow faster than smaller ones. Grading of seedlings to a particular size eliminates the bias of size differences attributable to treatment in the nursery but may introduce a new bias. Selection of

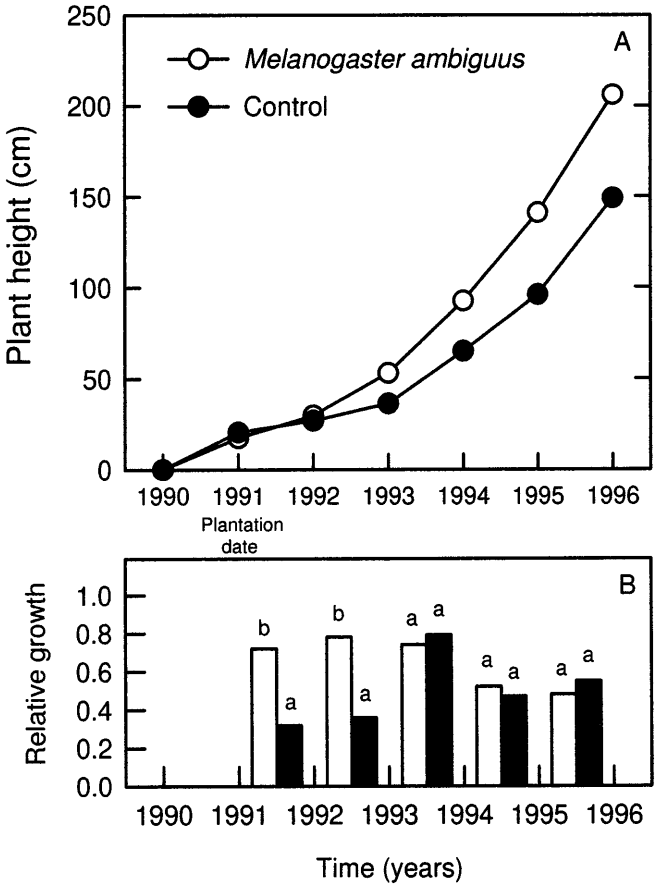

Fig. 4 Growth curves (**A**) and relative annual growth (**B**) of Douglas-fir plants inoculated with *Melanogaster ambiguus* and non-inoculated controls after 5 years of field outplanting at Álava. For each year, bars with the same letter are not significantly different ($P=0.05$)

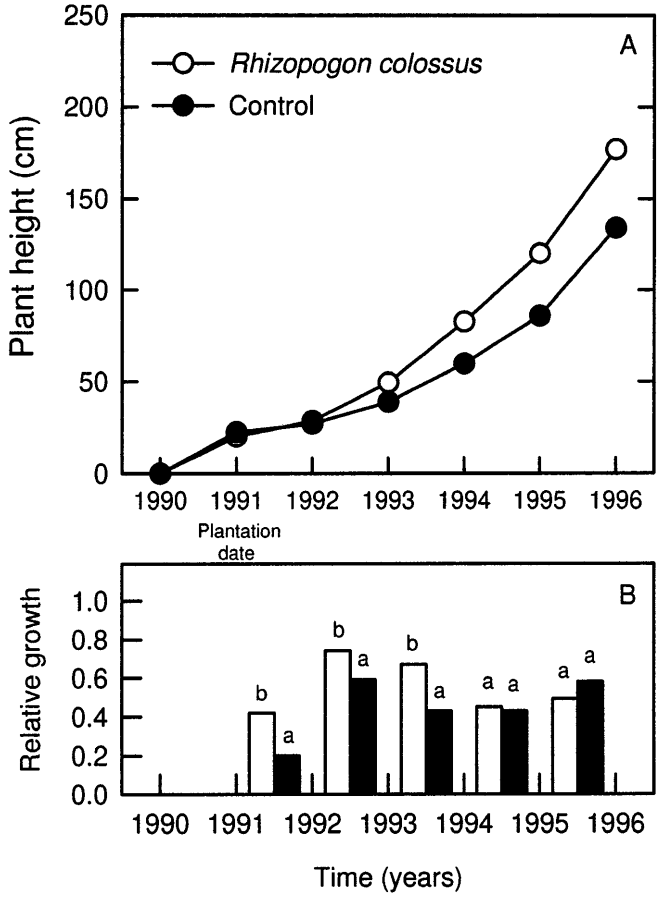

Fig. 5 Growth curves (**A**) and relative annual growth (**B**) of Douglas-fir plants inoculated with *Rhizopogon colossus* and non-inoculated controls after 5 years of field outplanting at Álava. For each year, bars with the same letter are not significantly different ($P=0.05$)

larger control seedlings and the smallest inoculated seedlings may tend to select different genotypes from the initial mixed population or create differences in initial seedling nutritional status. To minimize (or eliminate) the effect of initial differences in height, relative growth ($(h_f–h_i)/h_i$; h_f = height at the end of the growing season, h_i = height at the beginning of the growing season) was used to compare annual gains in height (stem length), and initial height has been introduced as a covariant in the statistical analysis of plant height at the end of the 5-year assessment period.

Field results reported in the literature for Douglas-fir inoculated with *Laccaria* species are controversial. Usually inoculation had no effect in North America (Bledsoe et al. 1982; Berch and Hunt 1988; Castellano 1996), whereas in Europe, where Douglas-fir is planted as an exotic, it ranged from non-effective (Jackson et al. 1995) to stimulatory (Le Tacon and Bouchard 1988; Le Tacon et al. 1988; Villeneuve et al. 1991; Généré 1995).

In our experience, non-significant differences in height, root collar diameter and stem volume occurred between *L. bicolor* S238-inoculated plants and non-inoculated controls produced in the Morvan nursery. Plants of both treatments performed similarly in terms of relative annual growth. Plants produced at Peyrat-le-Château and inoculated with *L. bicolor* S238 had higher diameters and stem volumes 5 years after outplanting in the field sites of Pontevedra, Girona and Guipuzkoa. The inoculated plants were significantly taller than their respective non-inoculated controls at the time of planting (30 cm versus 20 cm). As pointed out by other authors (Le Tacon et al. 1985; Le Tacon and Bouchard 1986), inoculation with *L. bicolor* S238 increased plant development during the period of growth in the nursery. In all cases, differences in height were maintained and, at the end of the 5 years growth in the field, inoculated plants were 15–25% taller than their respective controls. However, these differences were only significant at the Guipuzkoa site. In general, non-inoculated plants and plants inoculated with *L. bicolor* had equal relative annual increments in height throughout the assessment period. Differences in plant development at the end of 5 years of field growth may

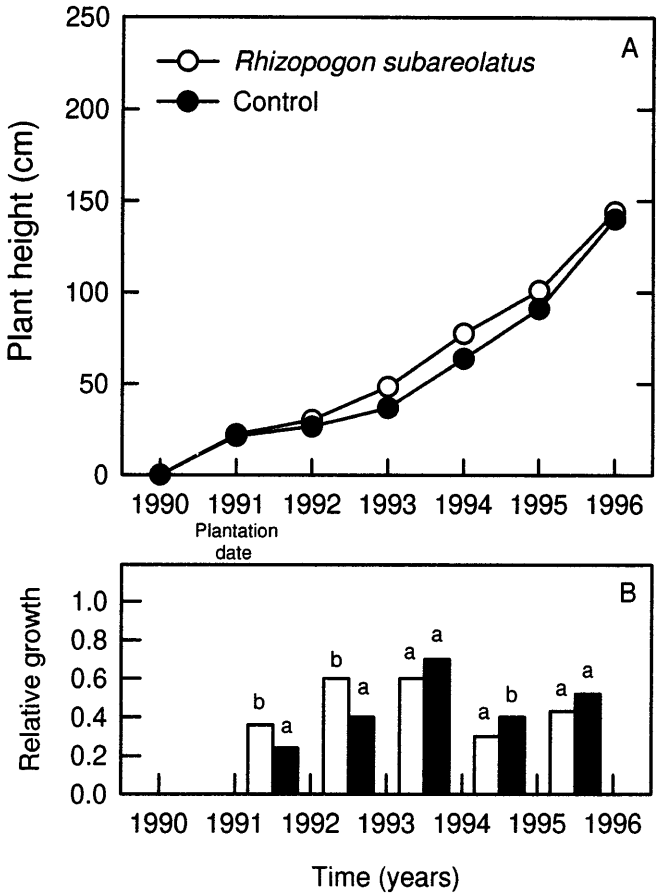

Fig. 6 Growth curves (**A**) and relative annual growth (**B**) of Douglas-fir plants inoculated with *Rhizopogon subareolatus* and non-inoculated controls after 5 years of field outplanting in the experimental plot established in 1991 at Álava. For each year, bars with the same letter are not significantly different ($P=0.05$)

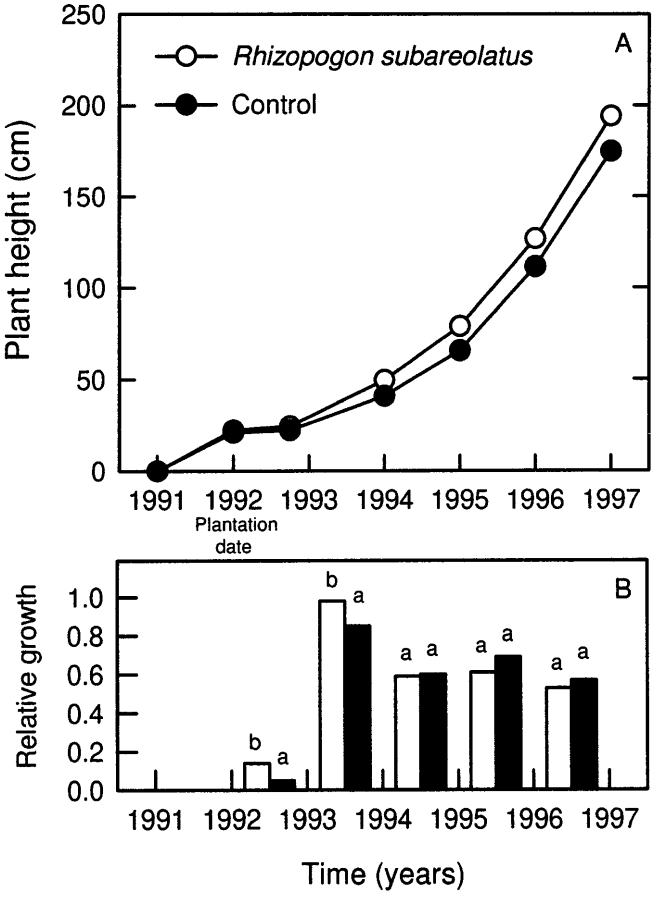

Fig. 7 Growth curves (**A**) and relative annual growth (**B**) of Douglas-fir plants inoculated with *Rhizopogon subareolatus* and non-inoculated controls after 5 years of field outplanting in the experimental plot established in 1992 at Álava. For each year, bars with the same letter are not significantly different ($P=0.05$)

result from the initial differences at the time of field outplanting.

Because of the difficulties of monitoring the fungal strain in field samples without using molecular techniques (Di Battista et al. 1996), no data on the persistence of *L. bicolor* S238 in the root systems of outplanted seedlings are available. Sporocarps of *L. bicolor* were only collected at the Guipuzkoa site during the first 2 years of field monitoring.

Douglas-fir has been reported to be stimulated when inoculated with host-specific fungi such as *Rhizopogon parksii* A. H. Smith and *R. vinicolor* in North America (Castellano and Trappe 1985; Owston and Castellano 1988; Castellano 1996). In our experiments, plants inoculated with *M. ambiguus* and the host-specific fungi *R. colossus* and *R. subareolatus* (experimental plantation established in 1992), showed significant differences in height, root collar diameter and stem volume 5 years after field outplanting. Relative annual growth of inoculated plants was significantly higher during the first 2 and 3 years of field growth, reflecting a positive effect of the inoculation treatment after outplanting that would be useful for a forester in a practical situation.

Five years after outplanting, *M. ambiguus* and *Rhizopogon* sp. ectomycorrhizas were observed in the root systems of the inoculated plants, and had spread to the root systems of non-inoculated plants. The spread of both fungi may explain the similarity in the relative annual growth of control and inoculated plants after 2 and 3 years of field outplanting. *Rhizopogon* sporocarps were collected in the plots planted with inoculated plants.

The results obtained indicate that nursery inoculations with *M. ambiguus*, *R. colossus* and *R. subareolatus* can significantly improve Douglas-fir growth following outplanting. *L. bicolor* S238 had a significant effect on seedling growth during the period of bareroot nursery production, and this difference in growth was carried over to the field. Inoculation with these ectomycorrhizal fungi could be considered for improving the quality of nursery-grown plants used in reforestation with Douglas-fir in northern Spain.

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