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Inoculation and field testing of Sitka spruce and Douglas fir with ectomycorrhizal fungi in the United Kingdom

Abstract *Picea sitchensis* and *Pseudotsuga menziesii* seedlings were grown in containers, inoculated with ectomycorrhizal fungi, and planted in British forestry sites. Root samples taken during the year after planting were assessed for mycorrhiza formation. Survival and shoot height were assessed at the end of each year. Observations were made each autumn on the occurrence of sporophores of ectomycorrhizal fungi. Pot experiments were used to assess the colonization potential of soils from the experimental locations. Assessment of mycorrhiza formation by the inoculant fungi both before planting and the following year showed much variation among the fungi used. Similar variation was found among field sites. Inoculation with *Laccaria* isolates was most successful. Height measurements are reported for the first 2 years after planting, at which time there were few significant effects on growth of *Picea sitchensis* or *Pseudotsuga menziesii* seedlings. Experimental assessment of colonization potential was of little value in this work for predicting events in the forest.

Key words Ectomycorrhiza · Inoculation · Field testing · *Laccaria* spp · *Thelephora terrestris*

Introduction

There have been few published descriptions of field experiments on inoculation of conifers with ectomycorrhizal fungi in the United Kingdom (Le Tacon et al. 1993; Thomas and Jackson 1983; Wilson et al. 1986), but numerous reports from other countries (e.g., Lamb and Richards 1971; Le Tacon et al. 1988; Marx et al. 1984, 1988, 1989b; Mikola 1989; Shaw et al. 1987), including several from continental Europe (Kropácek et al. 1989; Moser 1958; Stenström and Unestam 1986). Various forms of inoculum were used in these experiments, including basidiospores, peat-vermiculite (PV) based cultures, mycelial suspensions and alginate entrapped mycelium (Kropácek et al. 1989; Marx et al. 1989; Montier et al. 1989). Inoculation success, measured by short root colonization, was variable (Last et al. 1989). Positive growth responses to inoculation feature in much of the published work and lack of response or negative responses have been infrequently reported (Thomas and Jackson 1983).

The concepts of 'infective potential' and 'receptiveness' of soils with respect to ectomycorrhizal fungi have been developed and the latter studied by Perrin et al. (1988). Because the word 'infection' should properly be confined to the description of pathogens, we have chosen to replace it with 'colonization'. Thus, a term such as 'infective potential' becomes 'colonization potential'.

This work is part of a series of long-term experiments to assess inoculation of seedlings with ectomycorrhizal fungi to produce economically important improvements in survival and growth of conifers in British forests. The field experiments are designed to be assessed fully after 5 years; interim results for only the first 2 years are presented here.

Materials and methods

Plants

Seed was obtained commercially from the Forestry Commission (FC) Seed Supply Branch. The Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco; identity number 85(797)403] was of Darrington, Ore., origin and provenance. The Sitka spruce [*Picea sitchensis* (Bong.) Carr.; identity number 83(1012)N] was of Queen Charlotte Islands origin with a provenance of northeast England (Kielder Forest). All seed was pre-chilled (24 h in water at 2° C), followed by 7 days at 2° C in moist towelling (Aldhous 1972). Sowing substrates were irradiated (2.5 Mrad gamma irradiation) before use to eliminate naturally occurring mycorrhizal fungi.

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Two seeds per cell were sown in either Japanese Paper Pots (JPPs) or Sherwood Rootrainers (Ronash Ltd) and seedlings were thinned to one after germination. Undercuts were produced in FC nurseries following normal practice (Aldhous 1972).

Fungi

In all, eleven isolates from among six species of ectomycorrhizal fungi were used as inoculants. Five of these were different strains of *Thelephora terrestris* (Ehr.) Fr. (isolates R34, R38, R67, S62, and S63). Two different species of *Laccaria* [*L. proxima* (Boud.) Pat. (isolate S64) and *L. bicolor* (R. Mre.) Orton (isolate S238)], a strain of *Paxillus involutus* (Batsch) Fr. (isolate S59), and three species of *Hebeloma* [*H. subsaponaceum* Karst. (isolate S68), *H. crustuliniforme* (Bull) Quél (isolate S66), and *H. cylindrosporum* Romagn. (isolate S20)] were used. The isolate number refers to the collection of mycorrhizal fungi held at the University of Surrey, except for isolate number S238 which came from the United States Forest Service Forestry Sciences Laboratory, Corvallis, Ore.

To increase the probability of inoculation success, fungal cultures were produced in more than one way. Most frequently this was by growing cultures in Petri dishes on modified Melin Norkrans (MMN) agar (Marx 1969) for 2–3 weeks at 25° C. The contents of one Petri dish were introduced aseptically into a plastic bag and disrupted for approximately 5–10 s in a Stomacher 80 (A. J. Seward Ltd, Colworth, UK), a device that gently breaks up the mycelium by peristaltic action, thus minimizing damage to the fungus. The resultant fragments were used to inoculate MMN broth either in 500-ml flasks containing 200 ml of medium or in simple fermenters constructed from 2-l screw-capped borosilicate bottles (Duran; Schott, Mainz, Germany). The flasks were incubated, without shaking, for 2–3 weeks at 25° C. To provide aeration and agitation, air was bubbled into the fermenters from aquarium aerator pumps, through sintered glass spargers. Sterility was maintained by positioning hydrophobic filters of pore size <2 μm (Gelman, Ann Arbor, Mich.) in the inlet and exit lines. Good mycelial growth with little pelleting occurred after 1–2 weeks at 25° C. Cultures macerated by stomaching were also used to inoculate PV cultures (Marx and Kenney 1982). PV cultures of *Paxillus involutus* and *L. bicolor* prepared by Somycel, a French company specializing in the production of mushroom spawn, were also used.

Inoculation

Mycelium from one or two fermenters or several flask cultures was combined and washed thoroughly on a 250-μm sieve under a stream of tapwater to remove most of the residual nutrients. It was then resuspended in approximately 300 ml of tapwater and homogenized for a few seconds in a domestic food blender to break up the mycelium and increase the numbers of potential colonizing units. This homogenate was then added to sufficient tapwater to allow 10 ml to be injected into the root zone of each seedling. Seedlings were inoculated by injecting with a peristaltic pump (a commercial agar dispenser). Two pulses of 5 ml of the suspension were introduced into the root zone of each seedling through a hollow metal lance, providing approximately 2 μg dry wt. equivalent of mycelium to the root zone of each seedling. When PV cultures were used as inoculum, they were rinsed with tap water to remove excess nutrient and mixed with the compost immediately before sowing. With PV cultures, it was impossible to ascertain the dry weight equivalent of fungal material.

Field sites

Four field sites were used in forests belonging to the FC, representing a range of soils, topography, history and climate typical of British forestry conditions (Table 1).

Experimental design

The experiments at any one site were planted with either Sitka spruce or Douglas fir. Six experiments were set up, although two of these failed to establish successfully. Each experiment com-

Table 1 Details of the field sites used for planting cell-grown Sitka spruce or Douglas fir seedlings inoculated with different mycorrhizal fungi

Experiment name, location of field site, tree species used, and planting year	Site description
1. Experiment Bedgebury 40 Flimwell, Bedgebury Forest District, Kent, south east England; one Douglas fir experiment; planted 1990	A new planting site on a silty clay/loam soil (Tunbridge Wells sand-Wadhurst Clay) disturbed by cultivation. Used as a pasture for the 27 years before establishment of this experiment. Altitude 250 m amsl; slope southerly, 15–20°; exposure moderate; annual precipitation 838 mm
2, 3. Experiments Kielder 123 and Kielder 127 Clintburn, Kielder Forest District, Northumberland, north England; two Sitka spruce experiments; planted 1988 and 1989	A second-rotation site on a peaty gley over Tournaisean and Visean (Carboniferous limestone series) lithology, previously planted with Sitka spruce felled 2 years before use. No vegetation after felling; gradually invaded by *Holcus* spp., *Molinia caerulea* and *Agrostis canina*. Altitude 240–260 m amsl; slope north-easterly, 7°; exposure moderate; annual precipitation 1150 mm
4, 5. Experiments Shin 125 and Shin 127 Loch Borralan Research Reserve, Dornoch Forest District, Ross and Cromarty, north-west Scotland; two Sitka spruce experiments; planted 1988 and 1989	This is a new planting site on deep peat (>2 m) on type 12 intrusive igneous lithology. Formerly grazed by sheep and deer. Mixed upland vegetation dominated by *Scirpus caespitosa*, *Juncus squarrosus*, *Sphagnum* spp., *Calluna vulgaris* lichens. Altitude 165 m amsl; slope north-easterly 3–5°; very exposed; annual precipitation 1700 mm
6. Experiment North York Moors 43 Broxa, North York Moors Forest District, Yorkshire, north England; one Douglas fir experiment; planted 1989	A second rotation site on an iron pan soil over lower calcareous grit (Jurassic), previously planted (1949) with Japanese larch, felled 2 years before use. Vegetation *Deschampsia flexuosa*, *Holcus lanatus* and *H. mollis*. Altitude 198 m amsl; slope southerly, very slight ($<1°$); exposure moderate; annual precipitation 864 mm

Table 2 The locations, tree species and fungal species of the field trials of mycorrhizal fungi. *P88* and *P89* mean planted in 1988 and 1989, respectively. Tree species: *SS* Sitka spruce, *DF* Douglas fir; fungal treatments: *Cont* tapwater control, *Hc Hebeloma crustuliniforme*, *Hcy Hebeloma cylindrosporum*, *Hs H. subsaponaceum*, *Lb Laccaria bicolor*, *Lp Laccaria proxima*, *Pi Paxillus involutus*, *Rv Rhizopogon vinicolor*, *Tt Thelephora terrestris*, *U'cut* undercut seedling control. See text for explanation of identity numbers of fungal species

Experiment	Tree	Fungal treatment												
Bedgebury 40 P90	DF	Cont				LbS238	LpS64	PiS59	TtR34	TtR38	TtR67	TtS62	TtS63	U'cut
Kielder 123 P88	SS	Cont	HcyS20	HcS66	HsS68		LpS64	PiS59	TtR34	TtR38	TtR67			U'cut
Kielder 127 P89	SS	Cont				LbS238	LpS64	PiS59	TtR34	TtR38	TtR67	TtS62	TtS63	U'cut
NYM 43 P89	DF	Cont				LbS238	LpS64	PiS59	TtR34	TtR38	TtR67	TtS62	TtS63	U'cut
Shin 125 P88	SS	Cont	HcyS20	HcS66	HsS68		LpS64	PiS59	TtR34	TtR38	TtR67			U'cut
Shin 127 P89	SS	Cont				LbS238	LpS64	PiS59	TtR34	TtR38	TtR67	TtS62	TtS63	U'cut

prised a randomized block matrix of 10 treatments in 10 blocks. Nine of the treatments consisted of inoculated containerized trees, the 10th being undercut seedlings. The undercuts served as a reference to indicate the likely performance of the type of planting material normally used for planting. Each experimental unit was a 5 × 5 matrix of plants. The fungal treatments in any one experiment usually differed according to tree species and time of planting (Table 2).

The experimental results were analysed by analysis of variance (ANOVA) ($P \leq 0.001$, 0.01, or 0.05 as indicated in results for individual trials), followed by Tukey's Studentized range test to locate any significant differences ($P \leq 0.05$).

Samples and assessments

The mycorrhizal status of seedlings was assessed twice for each experimental planting, once before planting to determine the success or otherwise of inoculation and again in the following season to assess survival of inoculants and colonization by other mycorrhizal fungi. Samples for each treatment usually comprised a minimum of 25 root tips from each of 10 plants. For these assessments, root tips were excised under a stereoscopic microscope and squash preparations made in lactophenol with 0.05% trypan blue. The squashes were examined under a compound microscope (× 500) to assess mycorrhizal colonization. Mycorrhizas in most samples were classified as being formed by *T. terrestris* or by other unidentified fungi. Field assessments of survival and shoot height were made annually. These measurements will be continued for 5 years when root collar diameters will also be measured.

Colonization potential experiments

Soil was collected from the upper 30 cm at 10 random positions on each site and aggregated to give one composite sample. The samples were then passed through a 6-mm mesh sieve after partial air-drying when necessary. The sieved soil was subsequently distributed into 18-cm diameter plastic pots. Bedgebury soil, which had a high clay content, was mixed with medium grade steamed vermiculite (2:1 soil:vermiculite v/v) to improve its physical properties. Seeds of Sitka spruce and Douglas fir (seed identity number as for the field experiments) were sown in pasteurized peat in a greenhouse and subsequently transplanted to the pots. Five seedlings were planted in each pot to provide 20 seedlings for each destructive sampling. Seedling samples were treated as follows: the roots were gently washed in water to remove adhering soil; they were then examined under a low-power stereoscopic microscope and 25 short roots were excised from each seedling; these were mounted on microscope slides in lactophenol–trypan blue and gently squashed under cover slips for examination by transmitted light microscopy; roots were classified as mycorrhizal or nonmycorrhizal, but a complete analysis of mycorrhizal types was not attempted.

Table 3 Mycorrhizal status of Sitka spruce seedling short roots inoculated for planting in field experiments at Clintburn, near Kielder, Northumberland and Loch Borralan, near Shin, Sutherland in 1989 (*PV* peat-vermiculite)

Inoculant	Percentage short roots colonized by	
	Thelephora terrestris	Other
Thelephora terrestris S63	62.4	2.4
Thelephora terrestris R38	28.8	7.7
Thelephora terrestris R67	87.2	5.6
Thelephora terrestris R34	48.4	4.0
Thelephora terrestris S62	65.6	7.2
Paxillus involutus S59 Somycel PV inoculum	19.2	0.8
Laccaria bicolor S238 Somycel PV inoculum	6.4	4.8
Laccaria proxima S64	18.4	53.6 (*Laccaria*)
None (water control)	93.6	0
None (undercut control)	0	96.0

Results

Seedling inoculation

The success of seedling inoculations was variable. Many of the seedlings raised for the first series of experiments in JPPs were either uncolonized, or were only weakly colonized, probably because of the tendency of JPPs to become waterlogged. Reducing watering to a level below normal practice did not solve this problem, and it was therefore necessary to change the type of container for future experiments.

Resources permitted us to assess preplanting mycorrhizal status of inoculated seedlings only for the second experiments at Kielder and Shin and the North York Moors experiment (Tables 3, 4). For each treatment, 125 roots were examined. These assessments showed that all inoculated Sitka spruce, including the water control, had short root colonization by *T. terres-*

Table 4 Mycorrhizal status of Douglas fir seedling short roots inoculated for planting in field experiments at North York Moors, near Wykeham, Yorkshire in 1989

Inoculant	Percentage short roots colonized by	
	Thelephora terrestris	Other
Thelephora terrestris S63	7.2	0
Thelephora terrestris R38	22.4	0
Thelephora terrestris R67	74.0	0
Thelephora terrestris R34	0	0
Thelephora terrestris S62	0	0
Paxillus involutus S59 Somycel PV inoculum	0	0
Laccaria bicolor S238 Somycel PV inoculum	0	0
Laccaria proxima S64	0	0
None (water control)	0	0
None (undercut control)	Not assessed	Not assessed

Table 5 Mycorrhizal status of Sitka spruce seedlings planted at Loch Borralan, near Shin, Sutherland in 1989 and sampled in September 1990

Inoculant	Percentage short roots colonized by	
	Thelephora terrestris	Other
Thelephora terrestris S63	80.8	6.3
Thelephora terrestris R38	86.4	4.8
Thelephora terrestris R67	92.0	3.2
Thelephora terrestris R34	82.4	11.2
Thelephora terrestris S62	86.4	8.8
Paxillus involutus S59 Somycel PV inoculum	60.0	19.2
Laccaria bicolor S238 Somycel PV inoculum	31.2	65.6 (partly *Laccaria*)
Laccaria proxima S64	17.6	76.0 (*Laccaria*)
None (water control)	84.0	12.8
None (undercut control)	60.8	37.6

tris ranging from 6.4 to 93.6% . Least colonization by *T. terrestris* occurred in the seedlings inoculated with *L. bicolor* (Somycel PV inoculum). Inoculation with *L. proxima* S64 was successful, the majority of the short roots being colonized by this fungus. From the large numbers of roots colonized by *Thelephora*, including those of water controls, it can be deduced that spores of this fungus were dominant aerial contaminants in the environment in which the seedlings were produced. It was impossible to estimate success of inoculation with specific *Thelephora* isolates because of the high level of naturally occurring contamination.

Colonization assessments of the Douglas fir used at North York Moors contrasted with those for Sitka spruce. Only the three strains of *T. terrestris* (S63, R38 and R67) resulted in root colonization by *Thelephora*. Samples from all other treatments, including the water control and other *T. terrestris* isolates, lacked any short root colonization. While it appears that Douglas fir was much less susceptible than Sitka spruce to colonization by the contaminating *Thelephora*, it is again impossible to distinguish between the inoculant and contaminant fungi. Inoculation of Douglas fir with *L. proxima*, *L. bicolor* or *Paxillus involutus* all failed to produce colonization.

Assessment of roots from field samples

Root samples from three of the field experiments (Shin, Kielder and North York Moors) were assessed for short root colonization approximately 18 months

Table 6 Mycorrhizal status of Sitka spruce seedlings planted at Clintburn, Kielder, Northumberland in 1989 and sampled in September 1990

Inoculant	Percentage short roots colonized by	
	Thelephora terrestris	Other
Thelephora terrestris S63	40.0	54.4
Thelephora terrestris R38	53.6	44.0
Thelephora terrestris R67	52.8	44.0
Thelephora terrestris R34	42.4	53.6
Thelephora terrestris S62	20.0	75.2
Paxillus involutus S59 Somycel PV inoculum	46.4	48.0
Laccaria bicolor S238 Somycel PV inoculum	8.0	82.0 (partly *Laccaria*)
Laccaria proxima S64	0	96.8 (*Laccaria*)
None (water control)	32.0	61.6
None (undercut control)	10.4	86.4

Table 7 Mycorrhizal status of Douglas fir seedlings planted at Flimwell, near Bedgebury, Kent in 1988 and sampled in September 1989

Inoculant	Percentage short roots mycorrhizal with	
	Ascomycetous fungus	Other
Thelephora terrestris S63	9.1	0.9
Thelephora terrestris R38	76.0	8.0
Thelephora terrestris R34	13.6	9.6
Paxillus involutus S59 liquid inoculum	27.2	18.4 (including 2.4 *T. terrestris*)
Laccaria proxima S64	64.8	16.8 (all *T. terrestris*)
Hebeloma subsaponaceum S68	12.8	52.8
Hebeloma crustuliniforme S60	6.6	60.0
Hebeloma cylindrosporum S20	27.7	12.6
None (water control)	20.8	8.0
None (undercut control)	37.0	7.0

Table 8 Mycorrhizal status of Douglas fir seedlings planted at North York Moors, near Wykeham, Yorkshire in 1989 and sampled in September 1990

Inoculant	Percentage short roots colonized by	
	Thelephora terrestris	Other
Thelephora terrestris S63	32.8	24.0
Thelephora terrestris R38	19.2	25.6
Thelephora terrestris R67	26.4	22.4
Thelephora terrestris R34	41.6	23.2
Thelephora terrestris S62	31.2	16.0
Paxillus involutus S59 Somycel PV inoculum	0.8	29.6
Laccaria bicolor S238 Somycel PV inoculum	0	30.4 (partly *Laccaria*)
Laccaria proxima S64	0	22.4 (*Laccaria*)
None (water control)	14.4	18.0
None (undercut control)	3.2	15.2

after planting (Tables 5–7). *T. terrestris* remained the dominant fungus colonizing the short roots of Sitka spruce seedlings in all but one of the treatments at Shin but was rather less dominant at Kielder. Most short roots of these seedlings had mycorrhizas similar to those formed by *Laccaria* spp. (Ingleby et al. 1990). Seedlings inoculated with *L. bicolor* from Shin and Kielder had respectively 65.6 and 82% of short roots colonized by fungi other than *Thelephora*. Although most of these mycorrhizas were probably caused by *Laccaria*, it was impossible to identify them with certainty. The undercut controls from Kielder had only 10.4% of their roots colonized by *Thelephora* and 86.4% had mycorrhizas typical of those formed by *Hebeloma* spp. Root samples of Douglas fir from North York Moors (Table 8) showed a similar pattern of short root colonization to those of the spruce seedlings, but with generally lower levels of *Thelephora*. Only the samples from seedlings inoculated with *L. proxima* or *L. bicolor* lacked *Thelephora* mycorrhizas, but the *Paxillus* treatment and the undercuts had little colonization. Assessment of short root colonization in samples from Bedgebury are available only from an earlier experiment, planted in 1988, that was terminated following severe drought damage. The striking feature of this assessment, confirmed by other samples and the colonization potential experiments, is the predominance of one mycorrhizal type. This is characterized by a thin sheath and well-developed Hartig net composed of rather large cells, differing markedly from the mycorrhizas of *Thelephora* or any of the other inoculant fungi. Further study of these has shown them to be due to a fungus with ascomycetous characteristics. Roots from only one treatment, the inoculation with *L. proxima*, showed significant colonization by *Thelephora*.

Field assessment

Shin 125

Height measurements of the outplanted Sitka spruce are shown in Fig. 1A. At the time of planting, there were no statistically significant height differences among treatments. Good establishment was achieved by the end of the planting season with some height increment. At the end of the first year, significant differences were shown by the ANOVA ($P \leq 0.05$). Tukey's test showed that plants treated with *T. terrestris* S63 were significantly smaller ($P \leq 0.05$) than the undercuts, but by the end of the second year, this difference had disappeared.

No fungal fruiting bodies were found in the plots during the first year. In the second year basidiocarps of *L. proxima* were found in four plots, but only two were of seedlings inoculated with that fungus, the other two had been inoculated with *Thelephora* and *Hebeloma*. By the end of the third year, *L. proxima* was fruiting in a *Paxillus* plot and two control plots (though on only one tree in each plot of the controls). The *Paxillus* plot was heavily contaminated, 12 plants possessing attached basidiocarps of *Laccaria*. This plot is next to a heavily fruiting *Laccaria* plot in an adjacent block, and it must be presumed that contamination has taken place from this, either by air-borne basidiospores, or accidental contamination. The control plots which contained *Laccaria* fructifications were also adjacent to *L. proxima* treatments. *Hebeloma* basidiocarps were found in three of the *H. cylindrosporum* plots, one of the *H. subsaponaceum* plots, and two *T. terrestris* plots.

Shin 127

Height measurements of the outplanted Sitka spruce are shown in Fig. 1B. At all three sampling dates, the ANOVA was significant ($P \leq 0.001$). Tukey's test indicated that at the time of planting the undercuts were significantly taller than all other treatments, and *T. terrestris* R34 and S62, *L. proxima* S64, and the water control treatments produced significantly taller plants than those inoculated with *L. bicolor* S238. Good establishment was achieved by the end of the planting season. By this time, *L. bicolor* S238 and the undercuts were significantly smaller than the *T. terrestris* treatments R34, S62, R67 and water control. The undercuts had changed ranking from first to ninth in total height. At the end of the second season, no statistically significant height differences among fungal treatments were found, but the undercuts were significantly smaller than any of the other treatments (including the water control).

In the autumn of the year of planting, *Laccaria* basidiocarps were present in six of the 10 *Laccaria* plots. They were also found in three of the undercut plots,

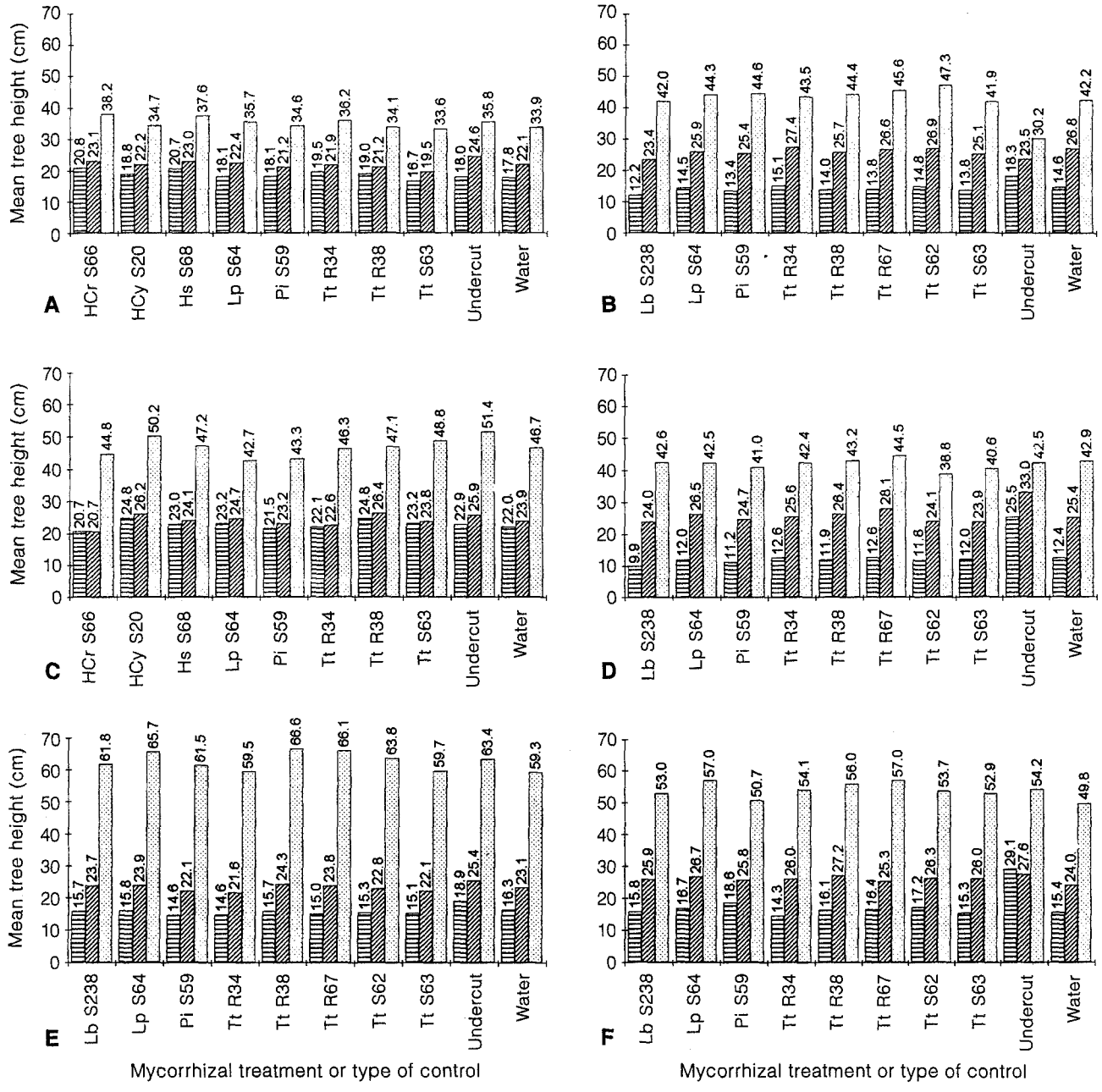

Fig. 1A–F Mean heights (cm) of seedlings inoculated with mycorrhizal fungi or with water as a control, planted in forest sites. A normal forest undercut transplant is also included as a 'forestry control'. For details of the individual sites and the fungal treatments, see Tables 1 and 2. Minimum Significant Difference (MSD) ($P \leq 0.05$) values (to 1 decimal place) from Tukey's test are as follows (listed in order for initial heights (▤), end of year 1 (▨), and end of year 2 (▦)): **A** Shin 125. Sitka spruce. Planted 1988: 5.4, 4.2, 6.4; **B** Shin 127. Sitka spruce. Planted 1989: 2.0, 2.9, 5.5; **C** Kielder 123. Sitka spruce. Planted 1988: 3.2, 4.0, 9.1; **D** Kielder 127. Sitka spruce. Planted 1989: 2.2, 4.4, 10.5; **E** North York Moors 43. Douglas fir. Planted 1989: 2.4, 3.0, 8.7; and **F** Bedgebury 40. Douglas fir. Planted 1990: 3.2, 7.3, 15.3

one of the *L. bicolor* plots and six others. It is likely that *Laccaria* of nursery origin was present on some of the undercuts. All but one of the remaining plots in which it fruited were adjacent to either an undercut or a *Laccaria* plot.

Kielder 123

Mean plant height measurements are shown in Fig. 1C. The ANOVA showed that significant differences were present among treatment means ($P \leq 0.001$) at planting and after the first year's growth. Tukey's test showed that the initial mean heights of *H. crustuliniforme* (S66) and *Paxillus involutus* (S59) treatments were significantly less than those treated with *H. cylindrosporum* (S20) and *T. terrestris* (R38). By the end of the first year, treatment S66 was significantly smaller than R38, S20 and undercuts. However, by the end of the second

year, significant differences among treatments had disappeared.

In the first year, only three *Laccaria* plots had basidiocarps of that fungus, and in one of these one fruiting body was clearly associated with a self-sown birch seedling. Some of the lodgepole pine buffer trees also had *Laccaria* fruiting bodies, a possible source of future contamination by basidiospores.

Kielder 127

Mean plant height measurements are shown in Fig. 1D. The overall ANOVA was significant for planting and the end of year one ($P \leq 0.001$), but not for the end of the second year. Tukey's test showed that the undercuts were significantly taller at planting than all other treatments, whereas the plants inoculated with *L. bicolor* (S238) were smaller than those inoculated with *T. terrestris* R67 and R34, and water control. At the end of the first year, the undercuts remained taller than all other treatments, and the plants inoculated with *L. bicolor* were no longer smaller than other plants. By the end of the second year, there were no significant differences among any of the treatments. *L. bicolor* – inoculated plants changed their relative rank in height during this time. At planting they ranked 10th, at the end of the first year they were ninth, and by the end of the second year, they had outperformed the undercuts and ranked fourth.

In the first year, *Laccaria* basidiocarps were found in all 10 *Laccaria*-treated plots, and in only two others (both controls). In the second year, nine of the 10 *Laccaria* plots had basidiocarps of *Laccaria*, as had also two of the *Thelephora* plots and one of the *Paxillus* plots.

North York Moors 43

Mean plant height measurements are shown in Fig. 1E. This experiment was established during an extremely dry spell; most plants survived but growth during the first year was very poor. Significant differences were found among treatments by ANOVA for initial heights ($P \leq 0.001$), end of first year heights ($P \leq 0.01$) and end of second year heights ($P \leq 0.05$). The only significant difference located by Tukey's test at establishment was that the undercuts were significantly taller than all other plants, but by the end of the first year's growth, all except the *T. terrestris* (S63 and R34) and *P. involutus* S59 treatments had caught them up. By the end of the second year, the treatments could not be separated by Tukey's test.

During the first year, probably as a result of the dry weather, no basidiocarps were observed on the *Laccaria* plots. During the second year, when weather conditions were more favourable, *Laccaria* fruiting bodies were observed in seven of the 10 plots inoculated with *L. proxima*, but only three of the 10 plots inoculated with *L. bicolor*. Contamination of seedlings, or natural inoculation after planting, had occurred in some plots of all treatments. Basidiocarps of *Laccaria* species were found in five control plots, four plots of *Thelephora* R38, S62 and S63, two plots of *Thelephora* R34 and R67 and undercuts, and one of *Paxillus involutus*.

Bedgebury 40

This experiment was planted in autumn to reduce the risk of droughting, a problem experienced previously at this location when seedlings were planted in the spring. The ANOVA showed significant differences among treatments at planting ($P \leq 0.001$), but not at the end of the first or second growing seasons. Initially, the height measurements (Fig. 1F), when analysed by Tukey's test, showed the undercuts to be significantly taller than all other treatments. Also, *Paxillus involutus* S59-treated plants were significantly taller than those given two of the *T. terrestris* treatments, S63 and R34. Heights at the end of the two seasons following planting showed no significant differences among the treatments, the undercuts having lost their earlier advantage.

Planting was too late in the season for any fruit body production in the first year. A survey of fruit body production in the autumn following planting showed *Laccaria* fruit bodies to be present in many plots. Nine of the 10 plots inoculated with *L. bicolor* had *Laccaria* fruit bodies, but they were found in only two of the plots inoculated with *L. proxima*. Two plots inoculated with *Paxillus involutus* produced *Laccaria* fruit bodies but only one of these was adjacent to another *Laccaria*-treated plot. *Laccaria* fruit bodies were produced in five plots inoculated with *Thelephora*, but these were all adjacent to other plots which produced *Laccaria* fruit bodies. One water control plot, adjacent to a *L. bicolor* plot, had *Laccaria* fruit bodies. Unexpectedly, eight of the 10 undercut plots had between them a total of 32 fruit bodies of *Laccaria*. It is probable that these plants had become colonized naturally in the nursery. Because of the extreme variability of *Laccaria* fruit bodies, it was not possible to ascribe them to species or isolate with absolute confidence, though the majority found in the *L. bicolor*-treated plots had a slightly different appearance from those found amongst other treatments.

Colonization potential experiments

The colonization potential experiments with Kielder and Bedgebury soils were established and sampled at the same times and are therefore reported together (Table 9). Mycorrhizal colonization of short roots of both Douglas fir and Sitka spruce in the Bedgebury soil occurred at very much the same rate, with maximal col-

Table 9 Percentage of short roots mycorrhizal in samples from colonization potential experiments with Douglas fir and Sitka spruce (soils from Clintburn, near Kielder, Northumberland and Flimwell, near Bedgebury, Kent). All ascomycetous at Bedgebury, all unidentified basidiomycetous at Kielder

Plant species, soil origin	Time from planting (weeks)		
	8	16	24
Douglas fir, Bedgebury soil	39.6	92.1	92.1
Douglas fir, Kielder soil	0	0	0
Sitka spruce, Bedgebury soil	35.9	98.4	91.4
Sitka spruce, Kielder soil	8.6	29.8	34.2

onization (92.1 and 98.4%, respectively) being reached by 16 weeks. The mycorrhizas in Bedgebury soil were all of the ascomycetous type, having a thin sheath and well-developed Hartig net. Results for the Kielder and Bedgebury soils contrast markedly. Douglas fir had no mycorrhizas at 24 weeks, but had 50% short root colonization at 45 weeks. Colonization of Sitka spruce short roots increased steadily from 8.6% at 8 weeks to 58.4% at 45 weeks. In the colonization potential experiment with Shin soil, there was no mycorrhizal colonization of Douglas fir seedling roots up to the end of the experiment (48 weeks). Sitka spruce also showed no colonization in the 30-week sample and only 2.0% at 48 weeks. Douglas fir had 54.8% of short roots mycorrhizal at 20 weeks and 74.7% at 32 weeks. Sitka spruce had 72.2% colonization at 20 weeks and 84% at 32 weeks.

Discussion

Inoculation of Sitka spruce and Douglas fir seedlings in containers with mycelium of several fungi had variable and conflicting success, but in no case did the statistically significant differences reflect growth effects that would be useful to a forester in a practical situation. The water control usually performed as well as other treatments, and in some instances the fungal treatments depressed growth a little. The experiments will, however, be maintained for a further 3 years, at which time it is proposed to carry out regression analyses to examine growth curves.

Several different isolates of *T. terrestris* were included in our work for three reasons: previous trials had shown some positive responses to inoculation with this fungus (Holden et al. 1983; Walker 1986); it grows vigorously in culture; and it is generally regarded as having high colonization potential. A major disadvantage of using this fungus experimentally is that inoculant strains cannot usually be distinguished from contaminants of the same species without the use of sophisticated techniques. Such contamination can result from spore rain or from mycelium already existing in the substrate. It is clear from the subsequent occurrence of fruit bodies associated with seedlings in the field plots, and from microscopical examination of roots, that inoculation with either *L. proxima* (S64) or with *L. bicolor* (S238) was successful on several occasions. Examination of inoculated seedlings before planting showed that Sitka spruce seedlings are generally more susceptible to colonization by *T. terrestris* than those of Douglas fir. The variable success of inoculation in this work emphasizes the need to improve both inoculum quality, in terms of colonization potential, and our understanding of the factors affecting the colonization of seedling short roots by mycorrhizal fungi.

In this work, it was not possible to make direct comparisons of the development of mycorrhizas of Sitka spruce and Douglas fir in the field, because only one tree species was used at each site. Some comparisons can, however, be made between mycorrhizal development of the two trees in the pot colonization potential experiments, although here only the total proportion of short root colonization and the incidence of *T. terrestris* were assessed. In Bedgebury soil, colonization of the two trees progressed at very similar rates. However, the great majority of mycorrhizas in this soil were in association with an ascomycetous fungus, the identity and significance of which are being investigated. It was remarkable that in the Kielder soil Douglas fir seedlings had acquired no mycorrhizas by the end of the experiment after 24 weeks. Sitka spruce seedlings grown in the same soil under the same conditions produced mycorrhizas with *T. terrestris*, as did Douglas fir in other soils, providing evidence that a suitable fungus was available in the soil. These results lead us to suggest that colonization potential trials made in a glasshouse are of limited value because of the difficulty of reflecting natural conditions. The failure of *Paxillus involutus* to colonize the roots should not necessarily be ascribed to competition from other mycorrhizal fungi. The inoculum of this fungus was commercially produced and was found to be contaminated by *Aspergillus* spp. and *Penicillium* spp. Even if some viable *Paxillus involutus* remained in the inoculum, its ability to colonize roots is likely to have been much reduced. Only in the case of Bedgebury were initial heights (Fig. 1F) of *Paxillus involutus*-treated plants significantly greater than those of other fungal treatments. Since this difference was small, and colonization with this fungus was not found, this is probably nothing more than a type I error, where significance was indicated that did not exist.

There was little evidence that any of our treatments induced significant growth effects in the field with Sitka spruce or Douglas fir, and various factors may account for this. Examination of trees planted at both Kielder and Shin showed a large proportion of short roots had become naturally mycorrhizal, even in the absence of fungal inoculation, and thus any benefits gained by inoculation may have been swamped by equally efficient naturally available fungi. This is likely also to have been true at the other locations. Tree species differ in their degree of dependence on mycorrhizas and may

also differ in their growth response to colonization by different fungi.

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