

D. Flynn · A. C. Newton · K. Ingleby

## Ectomycorrhizal colonisation of Sitka spruce [*Picea sitchensis* (Bong.) Carr] seedlings in a Scottish plantation forest

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**Abstract** The ectomycorrhizal (ECM) colonisation of seedling Sitka spruce (*Picea sitchensis*) was examined in an uneven-aged plantation forest in southern Scotland. The extent of ECM colonisation of individual seedlings was 43.8–97.2%, with an overall mean of  $80.3 \pm 1.1\%$ . A total of 13 ECM morphotypes were differentiated, with 1–4 ECM types colonising an individual seedling. ECM colonisation was dominated by a single species, *Tylospora fibrillosa*, which accounted for 72.4–97.7% of the ECM colonisation recorded, on a plot mean basis. Other ECM types appeared to be distributed very patchily, only two types (*Lactarius* sp. and *Mycelium radicis atrovirens* Melin) exceeding a mean of 10% colonisation in any one plot. No significant correlations were recorded between ECM colonisation and seedling growth, or between ECM colonisation and soil pH, loss-on-ignition, or water content.

**Key words** Ectomycorrhiza · Diversity · Sitka spruce · Regeneration

### Introduction

The importance of ectomycorrhizal (ECM) colonisation for the survival and growth of tree seedlings has been widely acknowledged (Harley and Smith 1983; Allen 1991). However, the processes influencing the colonisation of tree seedlings by ECM fungi have rarely been investigated in the field (Newton 1991; Visser 1995 and references therein). The extent of ECM colonisation may be influenced by a variety of factors,

such as soil characteristics, host physiology, availability of inoculum, etc. (Perry et al. 1987). It has also been suggested that the composition of the ECM mycota influences seedling growth and survival (Perry et al. 1987). The processes determining ECM diversity are poorly understood, but may involve competitive interactions between different ECM species and the availability of different sources of inoculum (Bruns 1995). The aim of this investigation was to evaluate variation in the extent and composition of ECM colonisation of naturally regenerating seedlings of Sitka spruce [*Picea sitchensis* (Bong.) Carr.], in relation to variation in surrounding host trees and soil conditions. This was achieved by assessment of ECM colonisation of seedlings regenerating in open areas in an uneven-aged plantation forest in Scotland.

### Materials and methods

The experimental field site is situated within an area of 117 ha of Glentress Forest, Peeblesshire (Forestry Commission), located on a southwesterly, moderately exposed upland site at an altitude of 240–560 m and with an annual precipitation of 1000–1500 mm. The soils are classified as leached brown forest soils and grade from well-drained brown earths on the lower slopes to podsolised peaty iron-pan soils on the ridgetops (Blyth and Malcolm 1988). The main tree species in the experimental site are Japanese larch (*Larix kaempferi*), Scots pine (*Pinus sylvestris*), Norway and Sitka spruce (*Picea abies* and *P. sitchensis*). Since the early 1950s, the area has been transformed into a mixed forest of uneven-aged structure (Blyth and Malcolm 1988), divided into six management blocks (A–F) and worked in a 6-year cycle, treating each block in turn. An area of 2 ha is regenerated annually by group felling and restocking, either by natural regeneration or planting (Blyth and Malcolm 1988).

Three circular plots of 40 m diameter, with natural regeneration of Sitka spruce, were randomly located in separate groups in three management blocks (B, D and E), which differed in the composition of the mature trees surrounding each plot (Table 1). Within each plot, 10 seedlings of < 20 cm height were sampled at a minimal distance of 1 m apart (to avoid root interference) and excavated by cutting a 2-cm<sup>2</sup> block of soil to a depth of 15 cm. Each sample was stored in a sealed polythene bag at 4°C prior to analysis. The age of each seedling was determined by counting

D. Flynn · A.C. Newton (✉)  
The Institute of Ecology and Resource Management, University of Edinburgh, Darwin Building, Mayfield Road, Edinburgh, EH9 3JU, UK  
Fax: +44-131-662-0478; e-mail: a.newton@ed.ac.uk

K. Ingleby  
Institute of Terrestrial Ecology (ITE), Bush Estate, Penicuik, Midlothian, EH26 0QB, Scotland, UK

**Table 1** Details of experimental plots from which seedling Sitka spruce were sampled in an uneven-aged forest in southern Scotland. Values for pH, water content and loss-on-ignition are means

Block	Plot	Species present as mature trees surrounding plot	Year of planting	pH	Moisture content (%)	Loss-on-ignition (%)
B	6	<i>Picea sitchensis</i> / <i>Pinus sylvestris</i> / <i>Larix kaempferi</i>	1926	4.06 c	51.7 bc	35.5 cd
	7	<i>Picea sitchensis</i> / <i>Pinus sylvestris</i> / <i>Larix kaempferi</i>	1926	4.34 c	40.9 e	22.3 e
	9	<i>Picea sitchensis</i> / <i>Pinus sylvestris</i> / <i>Larix kaempferi</i>	1926	3.98 c	67.4 a	59.2 a
D	4	<i>Picea sitchensis</i>	1949	4.01 c	50.7 bcd	35.6 cd
	6	<i>Picea sitchensis</i>	1949	4.19 b	48.0 cd	30.9 cde
	7	<i>Picea sitchensis</i>	1949	4.25 ab	44.0 de	28.9 de
E	5	<i>Picea abies</i>	1945	4.06 c	51.0 bcd	37.8 bcd
	13	<i>Picea abies</i>	1928	3.97 c	50.7 bcd	42.8 bc
	22	<i>Picea abies</i>	1927	3.98 c	57.0 b	48.8 ab

( $n = 10$ ). Means grouped by the same letter are not significantly different at  $P < 0.05$

the number of whorls on the main stem, a standard practice for this species, and root collar diameter was measured using calipers. Stem and leaf dry mass of each seedling were determined on a precision balance after drying in an oven for 24 h at 80 °C.

Soil samples to a depth of 10 cm were taken from sites immediately adjacent to each seedling, using a soil corer of 2 cm diameter, and stored in sealed polythene bags at 4 °C prior to analysis. Soil pH, moisture content and loss-on-ignition (l.o.i.) were measured following the techniques described by Moore and Chapman (1986).

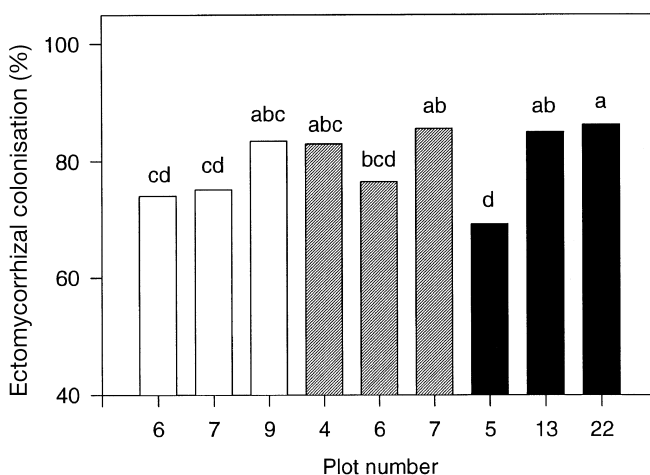
Roots were carefully washed free of soil using a fine jet of water and all of the fine roots of each seedling were manually dissected out from the organic material under a  $\times 8$ –40 zoom objective prior to assessment of ECM colonisation. Infected and non-infected root tips were then counted on each root system under the  $\times 40$  objective; broken tips and incomplete root fragments were not recorded. ECM colonisation was indicated by the presence of a fungal mantle surrounding the root tip. Mycorrhizal morphotypes were counted separately, initial separation being made on macroscopic characters (colour, morphology, branching pattern etc.), and subsequently confirmed by examination of slide preparations viewed under a compound microscope with a total magnification of  $\times 400$ –1000 (Ingleby et al. 1990). Where possible, identification was by comparison with published descriptions (Agerer 1987; Taylor and Alexander 1990; Ingleby et al. 1990).

The variation in colonisation between different plots was examined by nested ANOVA using SAS (1980). Percentage data were arcsin transformed prior to analysis. Differences between treatment means were analysed using Duncans Multiple Range Test in SAS. The relationships between mycorrhizal colonisation, seedling growth measurements and soil characteristics were examined by regression using SIGMAPLOT (Jandel Scientific GmbH, Erkrath, Germany).

## Results

A mean of 213 ( $\pm 36$ ) root tips was assessed per individual seedling. The extent of ECM colonisation was 43.8–97.2%, with an overall mean of  $80.3 \pm 1.1\%$ . When results were analysed by ANOVA following arcsin transformation, the extent of colonisation was significantly higher ( $P = 0.006$ ) in plots E22, E13 and D7 than in plots B6, B7 and E5 (Fig. 1). The effect of block was not significant ( $P > 0.05$ ).

A total of 13 ECM morphotypes was recorded during this analysis, with one type remaining unidentified (Table 2). The numbers of ECM types recorded on an individual seedling was 1–4, with an overall mean of



**Fig. 1** Variation in the extent of ectomycorrhizal colonisation of seedling Sitka spruce, sampled from different experimental plots in an uneven-aged forest in southern Scotland. *Open bars* block B; *hatched bars* block D; *filled bars* block E (for details of blocks see Table 1). Values are treatment means ( $n = 10$ ). Means grouped by the same letter are not significantly different at  $P < 0.05$  (ANOVA, carried out on arcsin-transformed values)

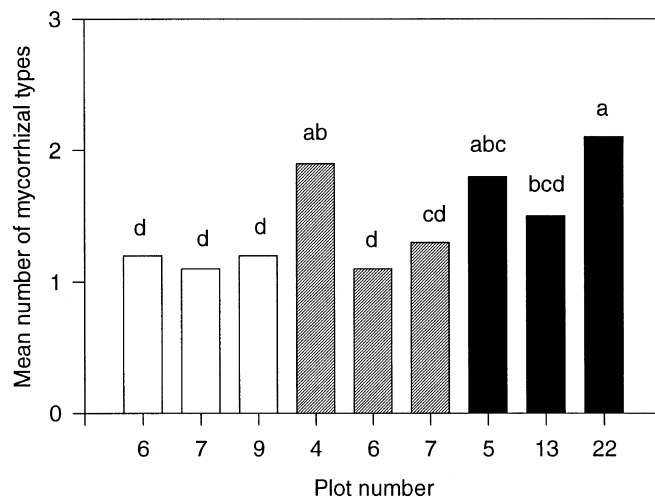
$1.47 \pm 0.07$ . The mean number of ECM types colonising an individual seedling was significantly higher in block E than either blocks B and D ( $P < 0.001$ ; treatment means of 1.8, 1.2 and 1.4 respectively) (Fig. 2).

*Tylospora fibrillosa* dominated the ECM flora in all plots, the plot mean values of relative proportion being 72.4–97.7% (Table 2). A higher mean relative proportion of *T. fibrillosa* was recorded from block B than E (96.3 versus 83.6%). The other ECM morphotypes were recorded at relatively low proportions, with only two (attributable to *Lactarius* sp. and *Mycelium atrovirens* Melin) exceeding a mean of 10% colonisation in any one plot (Table 2).

The age of seedlings sampled was 1–6 years, with a mean at  $2.6 \pm 0.1$  years, and the mean height recorded was  $69.7 \pm 3.7$  mm (the range was 15–200 mm). Seedling height differed significantly between the plots ( $P < 0.001$ , ANOVA), with significantly higher values recorded in plots E5 and E13 (125.6 and 93.9 mm, re-

**Table 2** Relative proportion of different ectomycorrhizal types infecting naturally regenerating seedling Sitka spruce in an uneven-aged forest in southern Scotland. Values are means ( $n=10$ )  $\pm$  sem. For details of ECM types see Materials and methods (*Indet* unidentified type)

Plot	Ectomycorrhizal type												
	<i>Tylospora fibrillosa</i>	<i>Boletus</i> sp.	<i>Laccaria</i> sp.	<i>Russula ochroleuca</i>	Indet	<i>Lactarius</i> sp.	<i>Hebeloma</i> sp.	<i>Mycelium radicans atroirens</i>	<i>Cortinarius</i> sp.	ITE 2	<i>Suillus</i> sp.	<i>Cenococcum geophilum</i>	<i>Elaphomyces granulatus</i>
B6	96.4 $\pm$ 2.6	0	0	3.6 $\pm$ 2.6	0	0	0	0	0	0	0	0	0
B7	97.1 $\pm$ 2.9	2.9 $\pm$ 2.9	0	0	0	0	0	0	0	0	0	0	0
B9	95.4 $\pm$ 3.6	0	4.6 $\pm$ 3.6	0	0	0	0	0	0	0	0	0	0
D4	68.5 $\pm$ 12.8	0	0	5.6 $\pm$ 3.1	8.5 $\pm$ 8.5	14.0 $\pm$ 10.1	1.4 $\pm$ 1.4	2.0 $\pm$ 2.0	0	0	0	0	0
D6	97.7 $\pm$ 2.3	0	0	0	0	2.3 $\pm$ 2.3	0	0	0	0	0	0	0
D7	88.6 $\pm$ 6.6	0	0	1.6 $\pm$ 1.6	0	0	0	5.6 $\pm$ 5.6	4.3 $\pm$ 4.3	0	0	0	0
E5	85.0 $\pm$ 8.0	1.5 $\pm$ 1.5	0	0	0	1.8 $\pm$ 1.8	0	10.4 $\pm$ 8.0	0	1.3 $\pm$ 1.3	0	0	0
E13	93.5 $\pm$ 5.3	0	0	0	0	1.1 $\pm$ 1.1	0	5.1 $\pm$ 5.1	0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0	0
E22	72.4 $\pm$ 10.5	0	0	0	0	3.0 $\pm$ 2.2	5.8 $\pm$ 2.8	18.1 $\pm$ 9.7	0	0	0	0	0.8 $\pm$ 0.8



**Fig. 2** The number of ectomycorrhizal morphotypes colonising individual Sitka spruce seedlings from different experimental plots in an uneven-aged forest in southern Scotland. *Open bars* block B; *hatched bars* block D; *filled bars* block E (for details of blocks see Table 1). Values are treatment means ( $n = 10$ ). Means grouped by the same letter are not significantly different at  $P < 0.05$  (ANOVA)

spectively) than any of the others; mean height growth in plot B9 (48.0 mm) was significantly lower than in the other plots. Soil pH, moisture content and l.o.i. also differed significantly between plots ( $P < 0.001$ , ANOVA, Table 1).

No significant relationships were observed ( $P > 0.05$ ) between either the extent of ECM colonisation or the number of ECM morphotypes and seedling height, root collar diameter or seedling age, either when individual seedlings or plot mean values were assessed. Similarly, no significant relationships were recorded between extent of ECM colonisation or number of ECM types, on either an individual seedling or plot mean basis, with any of the soil characteristics measured (pH, l.o.i. or water content).

## Discussion

At this study site, ECM colonisation of Sitka spruce seedlings was found to be dominated by a single fungal species, *Tylospora fibrillosa*. Similar results have been obtained at another site in Scotland, where nearly 70% of the ECM colonisation of a 37-year-old Sitka spruce stand was attributable to this same species (Taylor and Alexander 1989, 1990). These values compare with the 21% of mycorrhizas attributable to this fungus in a 45-year-old *Picea abies* stand in Sweden (Erland 1995). In the UK, Sitka spruce is considered to have a relatively depauperate ECM flora: Alexander and Watling (1987) list 84 putative ECM species recorded in association with Sitka spruce in Scotland, based on the occurrence of sporocarps. This partly reflects the fact that Sitka spruce is not native to the UK; many of the ECM asso-

ciates recorded occur naturally with either birch or pine (Alexander and Watling 1987). However, both Alexander and Watling (1987) and Walker (1987) failed to mention *T. fibrillosa* as an ECM associate of Sitka spruce, perhaps because its sporocarps are inconspicuous and are rarely encountered in the field (Breitenbach and Kränzlin 1986). The present results support previous observations on the limitations of surveys based solely on sporocarp abundance for assessment of ECM diversity (Deacon and Fleming 1992; Visser 1995).

The ability of certain ECM fungi to dominate the root systems of host tree species appears to be a widespread phenomenon (Harvey et al. 1976; Pilz and Perry 1984; Mason et al. 1987; Newton 1991; Lee and Alexander 1996), and may be attributable to competitive interactions between ECM species (Newton 1991, 1992). Such interactions may account for the relatively low number of ECM types recorded with individual seedlings, compared with the total number encountered in the survey. Similar results have been obtained in other studies. For example, Newton (1991) recorded 6–7 ECM types on individual seedlings of oak (*Quercus robur*) and birch (*Betula pendula*) established on soil from two field sites, out of a total of 41 ECM types encountered in the entire investigation. Similarly, although Lee and Alexander (1996) recorded 23 ECM types associated with *Shorea leprosula* on three Malaysian sites, individual seedlings were never infected by more than five types, and usually by only one or two. However, interpretation of the current results must necessarily be tentative, as ECM identifications based solely on morphological criteria may be subject to error (Gardes and Bruns 1993).

Apart from *T. fibrillosa*, which was dominant throughout all the plots, most species occurred sporadically across the plots at relatively low intensities of colonisation. The only two ECM types to occur at relative proportions of above 10%, *Lactarius* sp. and *Mycelium radialis atrovirens*, were both absent from block B, but were largely present throughout blocks D and E. Six of the 13 ECM types encountered were recorded from a single plot only. This suggests that inoculum of many of the ECM species present was distributed very patchily. Many of the ECM genera recorded in this investigation (such as *Boletus*, *Lactarius*, *Russula* and *Cortinarius*) are generally associated with well-established or mature forests (Visser 1995) and depend largely on mycelial inoculum for colonisation. Pioneer species able to colonise readily from spore inocula (Newton 1992), such as *Laccaria* spp. and *Hebeloma* spp., were recorded at low frequencies in only one and two plots, respectively. The processes influencing ECM colonisation by different groups of ECM fungi are poorly understood. Disturbances to forest stands such as windthrow, fire or logging activities may fragment mycelia and provide opportunities for ECM colonisation from spore inocula (Dahlberg and Stenlid 1995). Analysis of the genetic structure of ECM populations would be re-

quired to evaluate more precisely the impacts of such disturbances, including those associated with forest management, on ECM colonisation at this site (Dahlberg and Stenlid 1995).

The lack of any relationship between the extent or diversity of ECM colonisation and seedling height or diameter growth is consistent with a number of other field investigations (Newton 1991 and references therein). Although it has been suggested that ECM diversity is important for seedling survival and growth, as a result of the contrasting influence of different ECM species on the host (Perry et al. 1987) or variation in the symbiotic efficiency of different associations, this has rarely been evaluated in the field. Further experimental investigations are clearly required to examine whether ECM diversity is functionally important in field situations or whether some degree of functional redundancy exists within ECM communities (Bruns 1995).

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