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Succession of mycorrhizal fungi on birch: infection of seedlings planted around mature trees

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Summary Non-mycorrhizal seedlings of *Betula pendula* were planted around an 11-year old tree of *B. pubescens* in an experimental plot at Bush Estate, south of Edinburgh. Half (23) of the seedlings were in untreated planting positions and half (24) in positions that had been cored to sever connections of the roots with the parent tree. After 17 weeks, seedlings in the non-cored positions bore mycorrhizas mainly of *Lactarius pubescens* whereas mycorrhizas of this species were uncommon on seedlings in cored positions. Much smaller and usually non-significant differences were found with respect to other mycorrhizal types in cored and non-cored positions. It is concluded that *L. pubescens* infected seedling roots by means of mycelial strands which must remain attached to the parent tree (food base) in order to infect. The significance of the results for the concept of early-stage and late-stage mycorrhizal fungi is discussed.

Introduction

A succession of mycorrhizal fungi has been reported on roots of birch (*Betula pendula* Roth. and *B. pubescens* Ehrl.)[2,6,7]. In this respect a distinction has been made between early-stage and late-stage fungi, because the former can infect seedlings grown in cores of soil taken beneath their fruitbodies whereas the latter cannot do so[2,6]. A common feature of the reported late-stage fungi, like *Lactarius pubescens* Fr. and *Leccinum* spp., is that they form conspicuous mycelial strands. Most work on such mycelial strands has concentrated on their abilities to absorb and translocate materials from the external environment to the plant root (*e.g.* reference 3). But Garrett[4] considered that the major role of mycelial strands and rhizomorphs in root-infecting fungi as a whole was as agents of infection. The possibility that strands serve this role in mycorrhizal fungi has been recognized by several workers (*e.g.* reference 1) but never conclusively demonstrated.

Because the infectivity of mycelial strands and rhizomorphs depends on nutrients supplied by a food base[5] it was thought possible that the failure of late-stage mycorrhizal fungi to infect seedlings in soil cores was a result of the severence of inoculum in these cores from the food base provided by the parent tree. An experiment was done to investigate this.

Experimental

An 11-year old tree of *B. pubescens* was selected in an experimental plot at Bush Estate, south of Edinburgh, where the distributions of mycorrhizas and fruitbodies had been recorded in previous years[7]. The tree had mycorrhizas of *L. pubescens* more or less evenly distributed around it to a distance of at least 1 metre from the trunk. The ground beneath the tree was covered with black polyethylene sheeting from December 1980 until May 1981, to suppress growth by herbaceous plants. Meanwhile, non-mycorrhizal seedlings of *B. pendula* were grown in vermiculite-peat in laboratory conditions[8], hardened-off in a cold-frame and prepared for transplanting into the field when they were 55 days old. They were then 20.1 ± 1.75 (S.E.) mm tall, weighed 3.6 ± 0.51 mg (oven-dry) and bore 49.3 ± 5.26 uninfected root tips.

Forty-eight planting positions were chosen, in four annuli around the tree, at 20, 35, 50 and 75 cm radii from the trunk. The planting positions were evenly spaced within the annuli, so there were progressively more seedlings further out from the tree. Alternate positions were untreated and received one seedling each – 'non-isolated'. At other positions a core of soil 8 cm deep and of 10 cm diameter was taken with a corer and then replaced with minimal disturbance, knowing that roots within the core had been severed from the parent tree. Seedlings were then planted – 'isolated'. All plantings were done on 3 June 1981 by removing a narrow core of soil with a cork-borer (1 cm diameter) and inserting into the hole a root system of a seedling grown in a 1 cm diameter plastic sleeve (the sleeve being removed at planting)[8].

The seedlings were watered as required in the summer of 1981, and a 2 cm deep mulch of sphagnum peat was applied to the site to help conserve moisture. At intervals the cores containing the 'isolated' seedlings were carefully lifted and replaced immediately, to prevent re-invasion by roots or mycelial strands. Light intensities at seedling level, and temperatures and rainfall were recorded, and the positions of fungal fruitbodies were recorded as they appeared.

Seedlings were sampled 17 weeks after they had been planted into the field, when the isolated seedlings were both taller (39.1 ± 1.42 mm) and heavier (37.6 ± 3.93 mg dry wt.) than the non-isolated ones (34.1 ± 1.43 mm and 27.5 ± 3.53 mg). The root systems were washed and assessed for mycorrhizal types, distinguished on the basis of colour, gross morphology and microscopical features, using as standards mycorrhizas formed in bixenic culture or consistently associated with fruitbodies in the field (Table 1).

Results

There were no significant differences in (a) total numbers of root tips and (b) numbers of uninfected root tips between isolated and non isolated seedlings (Table 1). Four predominant mycorrhizal types were seen on the seedling root systems. Unidentified types '4' and '5' were found on similar numbers of isolated and non-isolated seedlings, and the numbers on these seedlings did not differ significantly (Table 1). By contrast, Lactarius-type mycorrhizas were present on significantly more non-isolated than isolated seedlings (Table 1) and they were frequently the dominant type on non-isolated seedlings (in 11 out of 23 instances) but were never dominant on isolated seedlings. The difference in the case of Lactarius-type mycorrhizas can be illustrated in another way: a total of 73 mycorrhizas of this type was recorded on the 24 isolated seedlings, compared with 1469 on the 23 non-isolated seedlings (one of the original 24 seedlings in this category died).

Hebeloma-type mycorrhizas were present on many non-isolated seedlings but

Table 1. Occurrence of mycorrhizal types on birch seedlings grown for 17 weeks in isolated (cored) * and non-isolated positions around a mature birch tree

Mycorrhizal type	No. seedlings bearing each mycorrhizal type (no. with > 20% root tips of that type in parentheses)		Mean no. root tips of each mycorrhizal type (means of log x + 1 in parentheses)		
	Isolated (Max. 24)	Non-isolated (Max. 23)	Isolated	Non-isolated	LSD (P = 0.05)
Hebeloma	6 (3) **	19 (4)	16 (0.35)	18 (0.89)	(0.39)
Lactarius	3 (2) ***	22 (17)	3 (0.15)	64 (1.66)	(0.32)
Type 4	17 (16)	11 (11)	70 (1.33)	34 (0.87)	(0.50)
Type 5	19 (13)	17 (9)	54 (1.28)	47 (1.12)	(0.49)
Total no. mycorrhizal root tips/seedling			143	166	
Total no. uninfected root tips/seedling			11	7	
Total no. root tips/seedling			154	173	

* For explanation see text

** Difference significant at $P = 0.01$

*** Difference significant at $P = 0.001$

not on the isolated seedlings. In most cases, however, they were present in very low numbers and if the value of 20% of total root tips represented by Hebeloma-type mycorrhizas is taken as an indication of good establishment on a seedling (even this is much less than would be true of a dominant mycorrhizal type) then Table 1 shows that few seedlings in either category were heavily colonized by it. In fact, there was no difference between treatments in the number of Hebeloma-type mycorrhizas: 389 were recorded on the 24 isolated seedlings, compared with 408 on the 23 non-isolated seedlings.

The distribution of mycorrhizal types on the parent tree root system was examined early in November 1981 by taking small cores of soil (8 × 2 cm diameter) at 25 cm distance intervals on ten radiating transects from the tree trunk. Lactarius-type mycorrhizas represented only 12% of the total number of mycorrhizal root tips, the highest recorded percentage being 31.0 for the ten cores at 100 cm from the trunk. Type '5' was the dominant type, representing 48.8% of root tips overall. Hebeloma-type represented 26.1% type '4' 9.9% and other types 3.2%. Thus, comparing the results for seedlings and the parent tree root system, Lactarius was markedly over-represented on the non-isolated seedlings and under-represented on the isolated seedlings, Hebeloma and type '5' were under-represented on the seedling roots in general, and type '4' was over-represented on the seedling roots in general.

Most fruitbodies that appeared around the parent tree in autumn 1981 were of *L. pubescens*, *Hebeloma velutipes* Bruchet and *Cortinarius* sp. Three species of *Russula* were also seen (*R. versicolor* J. Sch., *R. betularum* Hora and *R. grisea* (Secr.) Gill) but neither they nor the *Cortinarius* sp. seemed to represent the unidentified mycorrhizal types on the roots.

Discussion

L. pubescens was the only one of the mycorrhizal types observed that formed mycelial strands; indeed, it was the only late-stage type (in the sense defined in reference 2) that occurred on the seedlings, *Russula* and *Cortinarius* spp. being excluded from consideration because they apparently did not infect seedling roots.

The results show clearly that Lactarius can establish mycorrhizas on seedlings in soil, provided that the fungus is not separated from the parent tree; furthermore, it can colonize the seedling roots to a greater degree than its level of establishment on the parent tree root system would suggest. This is consistent with the view that Lactarius colonized the seedling roots by means of mycelial strands or, at least, by means of hyphae that need to remain attached to the food base provided by the parent tree. In this respect the results parallel those previously obtained for root-infecting pathogens, like *Armillaria mellea* (Vahl ex Fr.) Kummer[5].

Supporting evidence for the view that mycelial strands are important in colonization of seedling roots by late-stage mycorrhizal fungi has recently been obtained. Naturally occuring birch seedlings were collected in a chestnut (*Castanea sativa* Mill.) coppice with oak standards near Rochester, Kent, late in July 1981. The seedlings were less than one year old but 73% of them bore a late-stage mycorrhizal type associated with thick yellow mycelial strands and resembling some mycorrhizas formed by the Boletaceae. Soil in which these seedlings were growing was taken to a glasshouse and sown to birch, but only the usual range of early-stage mycorrhizal types occurred on the seedling roots. This could be interpreted as evidence that seedlings developing around parent trees or their living coppice stools in the field are infected by late-stage mycorrhizal fungi but that isolation of these fungi from the parent tree leads to failure to infect seedlings.

The results of this study raise interesting questions about the respective roles of early- and late-stage mycorrhizal fungi. It is unclear at present if early-stage mycorrhizal fungi have a significant role in the establishment of naturally regenerating tree seedlings in the woodland environment.

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