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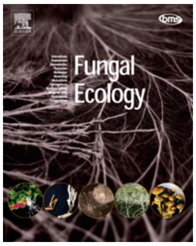

Short Communication

A few exotic mycorrhizal fungi dominate eucalypts planted in England

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ABSTRACT

Eucalypts have been planted widely outside of their native habitat for the purposes of commercial forestry. Mycorrhizal fungi are known to have spread with these trees around the world but little is known of the degree to which pre-existing mutualistic associations are maintained, or new associations are formed with *Eucalyptus* spp. outside of their native range. Molecular methods were used in this study to assess ectomycorrhizal communities colonising eucalypts in England. Our analyses revealed that only a dozen, mainly exotic, fungi were associated with eucalypts and that these mycorrhizal communities were dominated by a *Laccaria* sp.

Introduction

Eucalypts form symbioses with mycorrhizal fungi both in their native habitat and in plantations. For instance, one study of sporocarps under *Eucalyptus globulus* in both plantation and forest settings reported 44 putative ectomycorrhizal (ECM) species; 30 of which occurred in plantations (Lu *et al.* 1999). The identification of mycorrhizal fungi associated with eucalypts within the context of large-scale planting will be necessary if these trees are to be grown long-term in a sustainable and productive manner, and to understand and predict their invasive potential and that of their fungi (Giachini *et al.* 2000; Diez 2005; Chen *et al.* 2007). The transportation of plants with intact root systems, for example tree seedlings, can facilitate the spread of their mycorrhizal partners (Mikola 1969). The use of pre-grown *Eucalyptus* seedlings has been shown to be the primary cause of exotic mycorrhizal fungal introductions (Tedersoo *et al.* 2007). Little information is available, however, on the interaction of indigenous ECM fungi with introduced tree species, or regarding which species of ECM fungi form symbioses with *Eucalyptus* in Britain (Brundrett *et al.* 1996; Giachini *et al.* 2004; Chen *et al.* 2007).

In 2005, eucalypt plantations were established in Rogate (Hampshire), and in the Daneshill Energy Forest (Nottinghamshire) using seedlings imported from Navia, Spain (M. Maynard & D. Maynard pers. comm.; T. Wooddisse pers.

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Comm.). The goal of these plantings was to establish the viability and appropriateness of short-rotation forestry as a renewable energy source. A total of 55,500 *Eucalyptus gunnii* and 7,400 *Eucalyptus nitens* were planted at Daneshill, and *ca.* 2,000 *E. nitens* at Rogate. The present study aims to address a knowledge gap in the ecology of British eucalypt plantations through the identification of mycorrhizal fungi growing in association with *E. gunnii* and *E. nitens*, by revealing the abundance, diversity and origin of the fungi.

Methods

The fine roots of *E. nitens* and *E. gunnii* trees were sampled for ECM at three sites in England. These were: (1) a *Eucalyptus* plantation in Rogate, Hampshire, where soil cores were collected among *E. nitens* trees, established in 2005; (2) a *Eucalyptus* plantation in Daneshill, Nottinghamshire, where soil cores were collected among separate stands of *E. nitens* and *E. gunnii* trees, also established in 2005; and (3) individual *E. gunnii* trees planted as ornamentals in Kew Gardens, Surrey. The Rogate plantation was established on the site of a former conifer plantation. The total area of each plantation was estimated using aerial photographs. The area occupied by the eucalypts at Rogate was approximately 2.2 ha; and samples were collected from an area no larger than *ca.* 0.5 ha due to the presence of other tree species. The Daneshill site is separated into six plots of *E. gunnii* with a total area of *ca.* 21.6 ha; and three plots of *E. nitens* with a total area of *ca.* 2.6 ha. The Daneshill site was previously occupied by a factory and used as a landfill site. The Daneshill plots were separated from each other, and from other tree species, by borders of greater than 10 m.

At the Rogate site, soil samples were collected across a 20 m × 6 m section of the plantation comprising four rows of nine trees. At the Daneshill site, samples were collected across areas of approximately 20 m × 40 m of the plantation comprising 10 rows of 20 trees. For the plantation sites, a random number generator was used to choose a pair of trees to sample. Wherever a dead tree was present in the plantation stands, samples were taken from between the next pair of live trees in that row. Two replicate soil cores (2 cm diameter, 9–19 cm depth) were removed 10 cm to the right and 10 cm to the left of the midpoint between each pair of live *Eucalyptus* trees in each row. Fewer samples were collected at Rogate than at Daneshill due to the smaller size of the stand, and due to the presence of other tree species in the surrounding area. One large larch (*Larix* sp.) tree and a number of silver birch (*Betula pendula*) seedlings were present growing amongst the eucalypts. Where possible, sampling near to other tree species was avoided. The fine roots of the silver birch and *Eucalyptus* could be distinguished morphologically. At Kew Gardens, soil cores were obtained from the base of two *E. gunnii* trees. The samples were removed from within 15 cm of the trunk of the tree.

Soil cores were stored at 4 °C and processed within 5 d of collection to minimise degradation of mycorrhizas. Fine roots were washed in tap water on a 500 μm sieve. Roots were extracted from each sample within an 8 min period using a dissecting microscope. A second selection was then completed to remove dead and non-mycorrhizal roots. Roots were ranked according to length, and one live ectomycorrhiza sampled from each root with forceps, starting with the longest root until eight (Rogate, Kew), or four (Daneshill) ectomycorrhizas had been obtained. This selection method was used to minimise sampling bias and to maximise sample independence.

Genomic DNA was extracted from individual ectomycorrhizas using 5 μl Extract-N-Amp (Sigma, Dorset, UK) following the manufacturer's instructions. PCR amplification was performed using 0.5 μl of extracted DNA; 5 μl 2X Extract-N-Amp PCR ready Mix; 4.4 μl dH$_2$0; and the fungal specific primer ITS1F with the eukaryotic-specific primer ITS4 (White *et al.* 1990) in a 10 μl reaction. Samples were incubated at 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 53 °C for 35 s, 72 °C for 45 s; followed by final extension at 72 °C for 5 min in the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). ExoSAP-IT (USB, Cleveland, OH, USA) was used to purify successfully amplified products as per manufacturer's instructions. The eukaryotic primer ITS1 was used to replace ITS1F, and nested PCR carried out using a 1:100 dilution for samples which had not initially been successfully amplified.

Following optimisation, 1 ml 5X buffer; 2 ml H$_2$O, 0.5 ml BigDye v3.1 (Applied Biosystems, Carlsbad, California) and 1 ml 1.25 mM primer were added to each 0.5 ml DNA sample, thermo-cycled and precipitated following the manufacturer's protocols, analysed using an ABI Prism 3730 Genetic Analyser (Applied Biosystems) and edited using Sequence Navigator v1.01 (Applied Biosystems). The DNA sequences generated were used to perform a BLASTn search on GenBank (www.ncbi.nlm.nih.gov/blast). The sequences were then checked against UNITE (unite.ut.ee), and where possible a taxonomic name was applied. Species level identification was considered correct if the identity was ≥97 %. The genus only was used for DNA sequences with an identity <97 %. Samples which were of an insufficient quality to perform a BLASTn search were excluded from further analysis, as were non-ECM species. Statistical analyses were performed using 'R' version 2.11.1 (R Development Core Team 2009).

Results and discussion

A literature search was performed to infer the origin of the ECM species identified in this study (Table 1), based on

Table 1 – Ectomycorrhizal fungi identified in association with *Eucalyptus* spp. by site. Eucalypt specific fungi are indicated with *, and non-native or imported fungi with +

Site	Species identified
Kew Gardens	*Laccaria lateritia**; *Descomyces albus**+; *Scleroderma citrinum* and *Xerocomus badius*.
Rogate	*L. lateritia**; *S. citrinum*; *X. badius*; *Lactarius* sp; *Tomentella* sp and *Helotiales* sp.
Daneshill *E. gunnii*	*L. lateritia**; *Scleroderma 'aurantium'*; *Thelephora terrestris*; *Setchelliogaster* sp*+; *Hebeloma* sp*; *Pezizales* sp; and *Helotiales* sp.
Daneshill *E. nitens*	*L. lateritia**; *S. 'aurantium'*; *Setchelliogaster* sp*+; *Hebeloma* sp*; and *Helotiales* sp.