RESEARCH LETTER

Phytophthora cinnamomi and other fine root pathogens in north temperate pine forests

Didier Chavarriaga[1], William J.A. Bodles[1], Carlo Leifert[2], Lassaad Belbahri[3] & Steve Woodward[1]

[1]Department of Plant and Soil Science, School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland, UK; [2]Nafferton Ecological Farming Group, University of Newcastle, Newcastle, UK; and [3]Department of Applied Genetics, University of Applied Sciences Lullier, Jussy, Switzerland

Correspondence: Steve Woodward, Department of Plant and Soil Science, School of Biological Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen AB24 3UU, Scotland, UK. Tel.: +44 1224 272669; fax: +44 1224 273731; e-mail: s.woodward@abdn.ac.uk

Present addresses: Didier Chavarriaga, Instituto Colombiano Agropecuaria ICA, 51764 Medellin, Colombia.

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Abstract

A number of fine root pathogens, including *Phytophthora cinnamomi*, *Pythium ultimum* var. *ultimum*, *Pythium undulatum*, *Pythium violae*, *Fusarium* sp., and two incompletely identified *Verticillium* species, were isolated from soils taken from under Scots pine trees at five sites in north Scotland, including semi-natural forests and plantations. At least two root pathogens were recovered from each forest. Morphological and molecular data supported the identification of *Phytophthora cinnamomi* from three of the sites investigated. Isolates of *Phytophthora cinnamomi*, *Pythium ultimum* var. *ultimum* and an incompletely identified *Fusarium* sp. caused growth reductions of Scots pine seedlings, as determined by dry weight; the most virulent species were *Phytophthora cinnamomi* and *Fusarium* sp. The most severe disease symptoms were caused by a mixed inoculum containing *Phytophthora cinnamomi*, *Pythium ultimum* var. *ultimum* and *Fusarium* sp., or by the *Fusarium* isolate alone. These nonspecific pathogens may persist on the roots of understorey and herbaceous plants in the pine forests.

Introduction

Despite serious problems caused in forest ecosystems worldwide by fine root pathogens, their role in ecosystem dynamics is poorly understood, except when causing large-scale epidemics. Devastating diseases are caused by *Phytophthora cinnamomi*, typified by jarrah dieback in western Australia (Shearer & Tippet, 1989) and little leaf disease of pines (Tainter, 1997). Dieback of *Chamaecyparis lawsoniana* in the Pacific north-west, caused by *Phytophthora lateralis*, also threatens the natural forest ecosystem stability (Hansen, 2000). In the last 15 years, 26 previously unrecognized *Phytophthora* species causing significant emerging diseases on tree hosts have been characterized, including three subspecies of *Phytophthora alni* attacking *Alnus* species (Brasier *et al.*, 1999), *Phytophthora quercina* on *Quercus* species (Jung *et al.*, 1999), *Phytophthora ramorum* causing sudden oak death in the Pacific north-west (Goheen *et al.*, 2002) and *Phytophthora pseudosyringae* causing a collar rot on oaks and beech (Jung *et al.*, 2003). Complexes of *Phytophthora* species have also been reported in semi-natural and plantation forest ecosystems (Vettraino *et al.*, 2002), although the ecological significance of these disease complexes is unclear.

Other genera of fine root pathogens are also known to affect tree seedlings in forest nurseries, but their possible effects in forest ecosystems have received little attention. For example, *Pythium dimorphum, Pythium ultimum* and *Pythium sylvaticum* are frequent causal agents of damping-off and seedling decline and dieback in tree nurseries in Norway and Finland (Lilja, 1994). Recently, a newly described species, *Pythium montanum*, has been associated with fine root necroses and death in stands of *Picea abies* in Bavaria, Germany (Nechwatal & Oßwald, 2003). *Pythium undulatum* may also be a component in oak and beech decline (Jung *et al.*, 1996, 2000).

Excluding *Phytophthora* spp., reports of fine root pathogens causing problems in the forest rather than the nursery are rare. Severe root rot and mortality in *Pinus patula* plantations on old agricultural land in the north-eastern Cape Province of South Africa were attributed to infection by *Pythium irregulare* (Linde *et al.*, 1994). Packer & Clay (2000) demonstrated that the presence of a *Pythium* spp. in the rooting zones of mature *Prunus serotina* in forests reduced the survival of *Prunus serotina* seedlings, compared with seedlings growing at a distance from the mature trees.

It is clear that diseases of fine roots, particularly those caused by *Phytophthora* spp., pose significant threats to the health of dominant species in forest ecosystems world-wide. In other situations, these pathogens may regulate forest succession. Extrapolating from models for climate change, however, suggests that the activities of pathogens, which may currently be constrained by temperature limitations, could increase markedly in north temperate regions, posing greater threats to forest ecosystem stability (Bergot *et al.*, 2004).

The aims of the work reported here were to (1) establish the diversity in fine root-infecting fungi inhabiting semi-natural and plantation *Pinus sylvestris* forests in northern Scotland, and (2) determine the potential effects of isolated pathogens on the growth of Scots pine seedlings.

Materials and methods

Soil collection

Five forest sites where Scots pine (*Pinus sylvestris*) grows naturally or in plantations were sampled: (1) Glen Strathfarrar National Nature Reserve (57:24:41N, 4:46:24W), an uneven-aged remnant of the Caledonian forest, with individual trees up to 400 years old; (2) Darnaway forest, Alves, Morayshire (57:37:58N, 3:29:50W), an agricultural soil within 5 m of the boundary with a Scots pine forest; (3) Culbin forest (57:38:10N, 3:40:59W), an uneven-aged plantation of predominantly Scots pine; (4) Glen Dye (56:59:46N, 2:35:11W), a two-storey Scots pine plantation *c.* 60 and 20 years in age; and (5) Royal Balmoral Estate (57:00:02N, 3:18:15W), a 120 year old plantation.

At each site, soil was collected under five Scots pine trees, between June and September in 1998 and in 2001. The tools used were surface sterilized by 70% ethanol between each sample. Five 200 g samples of soil were removed from the upper 5–15 cm within the rooting zones of the trees and thoroughly mixed after collection.

Baiting methods

Method 1

Unblemished apples (varieties Golden Delicious and Royal Gala) were used for fruit baiting (Jung *et al.*, 1996). Baited soil samples (500 g) were maintained under ambient conditions with *c.* 12 h daylight and 12 h dark. After 10 days, when rot appeared on the fruit surface, fruits were withdrawn from the soil and rinsed thoroughly with sterile water. Three pieces of peel with flesh, *c.* 10 mm^3, were excised from lesion margins, immersed in 70% ethanol for 1 min, 35% ethanol for 1 min, 17.5% ethanol for 1 min, rinsed three times in sterile distilled water (SDW) and blotted dry on a sterile filter paper. Tissues were trimmed and three pieces were placed in Petri dishes containing potato dextrose agar (PDA; Oxoid) containing 400 mg L^{-1} oxytetracycline, or benomyl-amended PDA [PDAB; PDA containing 100 mg L^{-1} benomyl (DuPont Agricultural Chemicals)]. Duplicate dishes of both media were prepared for each apple. Cultures were incubated at 25 °C in the dark, checked for fungal growth at 12, 24, 48 and 72 h and emerging hyphae were subcultured repeatedly to fresh PDA medium until pure cultures were obtained.

Method 2

The *Quercus robur* leaf method of Jung *et al.* (1996) was used. Six young leaves, three with the axial and three with the abaxial surfaces upwards, were floated on the surface of 600 mL SDW over 200 g of mixed soil in sterilized clear plastic trays (24 cm × 21.5 cm, 3 L capacity). A tray lacking soil but with distilled water and the same arrangement of leaves was used as a control. Trays were incubated under ambient conditions, and observed for necrotic lesions at 24-h intervals over 20 days. Necrotic lesions on leaf margins were excised aseptically and placed on PDA or PDAB, using the methods described above.

Identification methods

The growth patterns and morphological features of isolates were compared with known isolates obtained from Dr D.E.L. Cooke, Scottish Crop Research Institute, Dundee, and with published descriptions (Domsch *et al.*, 1980; Nelson *et al.*, 1983; Waterhouse *et al.*, 1983; Ellis & Ellis, 1985; Barnett & Hunter, 1998). Colony morphologies and growth rates were recorded for three replicate cultures per isolate on PDA following incubation at 20 °C in the dark.

Sporangia production in candidate *Phytophthora* was induced by subculturing isolates to modified kidney bean agar [KBA; Cooke *et al.*, 2000; supplemented with 10 mg L^{-1} pimaricin (Sigma), 10 mg L^{-1} rifampicin (Sigma), 200 mg L^{-1} ampicillin (Sigma) and 50 mg L^{-1} hymexazol]. Six 10 mm squares cut from the margins of 10-day-old KBA cultures, grown at 25 °C in the dark, were arranged in a 9 cm Petri dish and flooded with 25 mL of soil extract water (Chee & Newhook, 1965). Cultures were incubated at 25 °C in the dark for 24 h and transferred to 8 °C for 3–4 days. Sporangia were mounted on glass slides, stained in methylene blue and

observed using a Wild M20 microscope. Shapes were noted and sizes were recorded for 50 sporangia per isolate.

Identifications of Oomycota and true fungi were confirmed using PCR and sequencing. Isolates were grown in 25 mL V8 juice broth (Vettraino *et al.*, 2002) for 10 days at 20 °C and DNA was extracted from the mycelium using the DNeasy Plant Mini Kit (Qiagen Ltd, Crawley, England). Ribosomal DNA internal transcribed spacer (ITS) fragments were amplified using standard primer sequences ITS4 and ITS6 (White *et al.*, 1990; Bonants *et al.*, 1997) and standard methods. All primers were synthesized by TAG Newcastle Ltd; PCR reactions were carried out with reagents from Promega UK Ltd.

Purified PCR product (5 μL) concentrations were quantified following horizontal agarose gel electrophoresis at 80 V for 2 h on a 1.5% agarose gel (Sigma) and comparison with a λDNA ladder (Promega). Sequencing was carried out using a BIG DYE-terminator sequencer (Applied Biosystems, ABI prism 377 × l DNA sequencer). Forward and reverse primers were supplied at 2.5 μM concentrations; PCR product concentrations ranged from 10 to 40 ng μL^{-1}. Forward and reverse sequences were aligned to give a 5′–3′ sequence of between 850 and 1000 bp. Sequences were compared against the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov) database. In addition, the CLUSTAL W nucleotide alignment computer package (Thompson *et al.*, 1994) was used to compare sequences from unknown with known isolates. Sequences from *Pythium* isolates were compared against the database held at the Pacific Agrifood Research Center (Dr André Lévesque, Agriculture and Agricultural Food Canada).

Pathogenicity to scots pine seedlings

Preparation of pathogen inoculum

All isolates used were from the Alves site. Bottles (200 mL) containing rye grain medium (50 g rye grain, 0.8 g sucrose in 80 mL distilled water) were inoculated from 10 day old cultures of *Fusarium* sp., *Pythium ultimum* var. *ultimum* or *Phytophthora cinnamomi* on PDA or KBA medium by transferring a 4 mm diameter agar plug to each bottle. Bottles were incubated at 25 °C for 22 days. Following incubation, 10 mL of 1% sterile aqueous Tween® was added to each bottle and the culture was thoroughly stirred before transferring to a 600 mm × 400 mm plastic bag containing 2 kg of compost [two parts loam, two parts acid-washed sand, one part commercial sphagnum moss peat (Midland Iris Moss Peat Ltd, Ireland)] previously autoclaved three times at 105 kPa for 20 min. Three bottles of each pathogen were blended with 2 kg compost. The mixture of pathogens comprised a 200 mL clinical bottle of each pathogen culture blended with 2 kg compost.

Preparation of seedlings

Pinus sylvestris seeds (UK Forestry Commission, provenance 20) were soaked in 30% H_2O_2 for 1 h and germinated aseptically in distilled water agar (DWA) in 140 mm diameter Petri dishes. Germinated seeds free from microbial contamination were transferred to 200 mm × 150 mm × 70 mm plastic seed trays (Ward, UK) containing autoclaved (105 kPa, 20 min) 0.71–0.25 mm acid-washed white sand (Garside Sands, UK) and covered with a thin layer of sand. Trays were watered regularly with SDW. Seedlings were grown for 30 days in a Phytotron (FISONS 600) at 20 °C, ± 70% relative humidity, with a 12 h light (fluorescent lamps providing 118 $\mu E\, m^{-} s^{-1}$), 12 h dark photoperiod. Following harvest, roots were rinsed thoroughly in DW to remove sand and seedlings were transferred to pots (130 mm × 130 mm × 125 mm) containing inoculated compost. Two seedlings were planted in each pot.

Six pots were inoculated with individual pathogens, or with a mixture of the pathogens, giving a total of 12 replicate plants per treatment. Control plants were potted into compost containing uninoculated rye grain medium that had been incubated as described above. The number of fungal propagules in each treatment was estimated in serial dilutions from 2 g inoculated compost and plating 1 mL aliquots from the 10^{-3}–10^{-5} dilutions onto PDA supplemented with 400 mg L^{-1} oxytetracycline. Dishes were incubated inverted at 20 °C for 24–72 h and separated colonies were counted.

Pots were arranged on the glasshouse bench in a completely randomized manner and watered to field capacity with distilled water as required at 48-h intervals. Symptoms were recorded 4, 8, 15, 30 and 60 days after inoculation, assessed on a categorical scale, where 0 = healthy plants, and 5 = damage > 75% (cotyledons and hypocotyl highly necrotic). Disease index scores were calculated as the means of 12 plants per treatment at each assessment date. Pathogens were reisolated from the root collar or roots of inoculated plants by plating onto PDA amended with 400 mg L^{-1} oxytetracycline. Immediately after recording symptoms on day 60, seedlings were harvested and dried to a constant weight at 60 °C.

Statistical analyses

Differences between inoculation treatments were assessed using *t*-tests. Duncan's multiple-range test was used to determine differences between categorical data. Regression analyses were used to test the significance of disease progress, based on disease index scores.

Results

Identification of isolates

At least two pathogen species were isolated from each forest area sampled (Table 1). Isolates were identified conclusively

Table 1. Identification of pathogen isolates from soils in five pine forests of north Scotland

Location	Bait*	Closest species to morphology match†	Closest species to ITS sequence match‡	Number of isolates	NCBI Accession Number§	Number of nucleotides aligned
Glen Strathfarrar	O	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*	1	EF055300	894
	A	*Pythium ultimum*	*Pythium ultimum* var. *ultimum*	2	EF055298	889
	A+O	*Pythium undulatum*	*Pythium undulatum*	2	EF055297	796
	A	*Verticillium* sp.	N/A	2		
Alves	O	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*	1	EF055303	883
	O	*Pythium ultimum*	*Pythium ultimum* var. *ultimum*	2	EF055301	871
	O	*Pythium* sp.	*Pythium violae*	1	EF060053	476
	A	*Fusarium* sp.	*Fusarium* sp.	2	EF055302	583
Culbin forest	A	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*	1	EF055293	882
	A	*Pythium ultimum*	*Pythium ultimum* var. *ultimum*	1		
	A	*Verticillium* sp. 1	N/A	1	EF055299	878
	A	*Verticillium* sp. 2	N/A	2		
Glen Dye	A	*Pythium undulatum*	*Pythium undulatum*	1	EF055295	795
	A	*Verticillium* sp. 1	N/A	2		
Royal Balmoral estate	A	*Pythium undulatum*	*Pythium undulatum*	2	EF055296	792
	A	*Verticillium* sp. 1	N/A	2		

Except where indicated, identifications were based on macro- and micro-morphology, plus ITS4 and ITS6 sequences.
*Bait used to obtain isolate. A, apple; O, oak leaf.
†Closest match by morphological characteristics.
‡Closest match after BLAST comparison of ITS sequences.
§Accession number in NCBI database.

as *Phytophthora cinnamomi*, *Fusarium* sp., *Pythium ultimum* var. *ultimum*, *Pythium undulatum* and *Pythium violae*. Two different species of *Verticillium* were identified to the genus level.

Five days after subculture to fresh PDA, *Phytophthora cinnamomi* isolates produced typical white petaloid cottony mycelial mats, 4–4.5 cm in diameter. Hyphal diameter was 4–8.9 μm. Other features typical of *Phytophthora cinnamomi* included coralloid branching mycelium, numerous hyphal swellings, vesicles and sessile, terminal or lateral protuberances 6–8 μm in diameter, proliferation of nonpapillate sporangia 22–(44)–61 μm (length) × 21.5–(35)–43 μm (width) and clusters and chains of globose chlamydospores 25–(31.34)–47 μm (length) × 18–(30.42)–35 μm (width).

Pythium ultimum var. *ultimum* isolates were initially identified by the rapid growth and hyaline appearance on culture media. *Pythium ultimum* var. *ultimum* produced hyphal swellings and mainly terminal, globose sporangia 15–28 μm in diameter. Luxuriant mycelial growth occurred on PDA and abundant smooth-walled oogonia, 16–(18.3)–22 μm diameter, sporangia and monoclinous short-stalked hypogynous antheridia were induced in soil extract agar.

Cultures of the Alves *Fusarium* isolate were rose in colour, becoming blood red; some areas of mycelium remained white floccose. The colony diameter after 4 days on PDA was 4.2 cm. Final identification of the *Fusarium* was inconclusive using the methods described here. Distinct curved macroconidia, four-septate, 27.8–(43.1)–54 μm (length), 2.3–(4.7)–7 μm (width), and simple lemon-shaped microconidia, with 0–1 septa, 10.1–(11.9)–13.4 μm (length), 6.1–(8.2)–9.3 μm (width), were observed.

Verticillium isolates produced white mycelium and aerial, verticilliate conidiophores. With ageing, cultures developed limited melanization.

Molecular identifications

Identifications of the Oomycota isolates were confirmed by sequencing. *Phytophthora cinnamomi* isolates showed high homologies with other Genebank accessions of this species (Fig. 1). Sequences derived from the *Phytophthora cinnamomi* isolates (EF055300, EF055303 and EF055293) matched with 99% homology those of an American isolate collected from infected *Vaccinium macrocarpon* and a Japanese isolate recovered from *Larix kaempferi*. The *Fusarium*-derived sequence (EF05332) matched with 95–98% identity sequences of *Fusarium* spp. derived either from *Fusarium* sp. inhabiting soil or forests (data not shown. The same homologies were also observed for *Verticillium* sequences.

Pathogenicity to Scots pine seedlings

Inoculated soil contained 1.5×10^5 infective propagules of *Phytophthora cinnamomi*, 1.5×10^6 of *Pythium ultimum* var. *ultimum* and 1.7×10^6 of the *Fusarium* sp.

All tested pathogens showed virulence to Scots pine based on disease index scores (Fig. 2) and the impact of infection on seedling dry weights 60 days after inoculation (Fig. 3). Inoculations with *Phytophthora cinnamomi* or *Fusarium*

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EF055300        CCACACCTAAAAAACTTTCCACGTGAACCGTATCAACCCAATTAGTTGGGGGCCTGCTCT
AF087478        CCACACCTAAAAAACTTTCCACGTGAACCGTATCAACCCAATTAGTTGGGGGCCTGCTCT
AY964101        CCACACCTAAAAAACTTTCCACGTGAACCGTATCAACCCAATTAGTTGGGGGCCTGCTCT
EF055303        --CCACCTAAAAAACTTTCC-CGTGAACCGTATCAACCCAATTAGTTGGGGGCCTGCTCT
EF055293        -CCCACCTAAAAAACTTTCC-CGTGAACCGTATCAACCCAATTAGTTGGGGGCCTGCTCT
                   ***************** ****************************************

EF055300        GGGCGGCGGCTGTCGATGTCAAAGTCGACGGCTGCTGCTGCGTGGCGGGCCCTATCACTG
AF087478        GGGCGGCGGCTGTCGATGTCAAAGTCGACGGCTGCTGCTGCGTGGCGGGCCCTATCACTG
AY964101        GGGCGGCGGCTGTCGATGTCAAAGTCGACGGCTGCTGCTGCGTGGCGGGCCCTATCACTG
EF055303        GGGCGGCGGCTGTCGATGTCAAAGTCGACGGCTGCTGCTGCGTGGCGGGCCCTATCACTG
EF055293        GGGCGGCGGCTGTCGATGTCAAAGTCGACGGCTGCTGCTGCGTGGCGGGCCCTATCACTG
                ************************************************************

EF055300        GCAAGCGTTTGGGTCCCTCTCGGGGGAACTGAGCTAGTAGCCTCTCTTTTAAACCCATTC
AF087478        GCGAGCGTTTGGGTCCCTCTCGGGGGAACTGAGCTAGTAGCCTCTCTTTTAAACCCATTC
AY964101        GCGAGCGTTTGGGTCCCTCTCGGGGGAACTGAGCTAGTAGCCTCTCTTTTAAACCCATTC
EF055303        GCGAGCGTTTGGGTCCCTCTCGGGGGAACTGAGCTAGTAGCCTCTCTTTTAAACCCATTC
EF055293        GCGAGCGTTTGGGTCCCTCTCGGGGGAACTGAGCTAGTAGCCTCTCTTTTAAACCCATTC
                ** ********************************************************

EF055300        TGTAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAG
AF087478        TGTAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTGGATAG
AY964101        TGTAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAG
EF055303        TGTAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAG
EF055293        TGTAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATG-
                ******************************************* *** 
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Fig. 1. Alignment of the ITS1 region of *Phytophthora cinnamomi* isolates EF055300 (Glen Strathfarrar), EF055303 (Alves) and EF055293 (Culbin forest) with the closely matching *Phytophthora cinnamomi* isolates AY964101 (*Vaccinium macrocarpon*, NJ) and AF087478 (*Larix kaempferi*, Japan).

caused the greatest decreases in plant biomass, although the pathogen mixture resulted in the greatest development of disease symptoms. *Pythium ultimum* var. *ultimum* had little impact on plant dry weight. Pathogens were reisolated from inoculated plants showing disease symptoms and identities confirmed by comparison with stock cultures.

Discussion

This is the first report demonstrating the presence of a range of fine root pathogens in Scots pine forests in northern Scotland. Although eight different fine root pathogens were isolated in this work, further root pathogens may be present on the sites, and more extensive sampling over different seasons would determine the full range of species in these forests (Hansen & Delatour, 1999; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003). For example, no isolates of *Cylindrosporium* or *Rhizoctonia*, pathogens known to cause serious problems in forest nurseries (Lilja, 1994), were obtained. The baiting techniques used here encouraged recovery of Oomycota, and may not be efficient enough to enable isolation of true fungi present in low abundance.

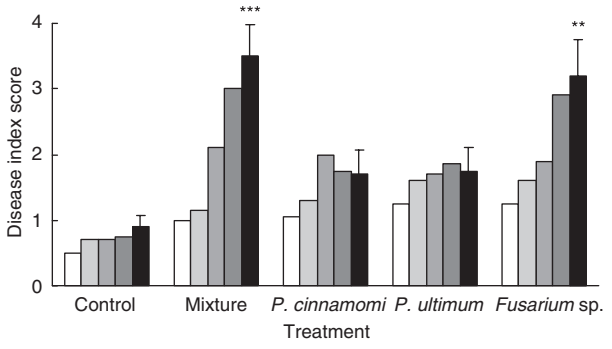

Fig. 2. Disease index scores on *Pinus sylvestris* seedlings four (□), nine (▫), 15 (▩), 30 (■) and 60 (■) days after inoculation with *Phytophthora cinnamomi, Pythium ultimum* var. *ultimum*, *Fusarium* sp. or a mixture of these pathogens. Treatments with asterisks are significantly different from controls (independent *t*-test, equal variances not assumed). $^{**}P < 0.01$; $^{***}P < 0.001$. Scale bars represent SE of the mean. Means of 12 replicates per treatment. Regression analysis showed that disease increased significantly with time for all treatments ($P > 0.05$), except *Pythium ultimum* and *Phytophthora cinnamomi*.

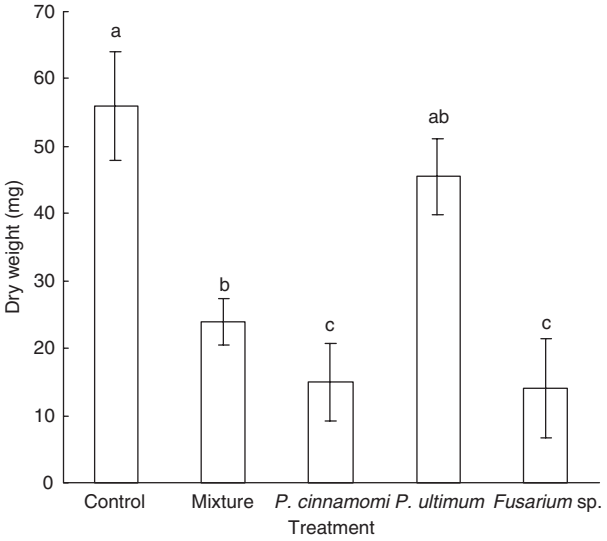

Fig. 3. Dry weights of *Pinus sylvestris* seedlings 60 days after inoculation with *Phytophthora cinnamomi, Pythium ultimum* var. *ultimum*, *Fusarium* sp. or a mixture of these pathogens. Treatments with different letters are significantly different (Duncan test, $n = 3$, $P < 0.05$).

Until recently, most work on the pathogens isolated here focused on forest nurseries, where severe problems with *Phytophthora* and *Pythium* spp. arise under wet soil conditions, and drier conditions promote *Fusarium* infections (Garrett, 1970). In forests, the presence of *Phytophthora* species causing severe disease has been noted for many years, but their role in forest decline syndromes was recognized only in the 1990s (Brasier, 2000). Work on decline (e.g. Brasier *et al.*, 1993; Jung *et al.*, 1996; Hansen & Delatour, 1999; Sanchez *et al.*, 2002; Vettraino *et al.*, 2002; Hansen *et al.*, 2003) demonstrated the presence of many previously unrecognized species of *Phytophthora* in different forest ecotypes, suggesting widespread occurrence of this genus in the wild.

Pythium spp. are reported from forests, sometimes causing serious damage (Hamm *et al.*, 1988; Linde *et al.*, 1994; Jung & Blaschke, 1996; Jung *et al.*, 1996). Certainly, species of *Pythium*, including *Pythium ultimum* var. *ultimum* and *Pythium undulatum*, are frequent causal agents of damping-off disease in forest nurseries under wet conditions. *Pythium undulatum* was found in soils from declining oak sites in Germany and Turkey (Jung *et al.*, 1996; Balci & Halmschlager, 2003) and is known to be pathogenic to oak (Jung & Blaschke, 1996). *Pythium violae* is known to cause cavity spot on carrot (Groom & Perry, 1985).

Fusarium spp. have rarely been reported as root pathogens in temperate forests, although both anamorphs and teleomorphs in *Gibberella* and *Nectria* are known to cause canker diseases of trees (Thomas & Hart, 1986). *Fusarium subglutinans* f.sp. *pini*, the cause of pine pitch canker for example, is currently causing concern in several areas of the world (Correll *et al.*, 1991).

The most common species of *Verticillium* known to attack trees are *Verticillium albo-atrum* and *Verticillium dahliae*, which are associated with wilt diseases of a wide range of hosts in ornamental plantings and in nurseries (Pegg, 1984). *Verticillium rexianum* was recently reported in soils near Scots pine forests in Scotland (Anderson *et al.*, 2003).

The pathogens were present in areas without clear symptoms attributable to root disease on the trees or the understorey vegetation. This effect was previously observed in native forests of New Zealand, where potentially susceptible *Agathis australis* and *Nothofagus* sp. showed no obvious above-ground symptoms of disease, despite the presence of *Phytophthora cinnamomi* (Podger & Newhook, 1971). Under suitable conditions, however, these pathogens may cause serious damage to forest ecosystems (Shearer & Tippet, 1989; Sanchez *et al.*, 2002; Jönsson *et al.*, 2005). In horticulture, many infections have rather subtle effects and may go undetected (Tsao, 1990); a similar situation is likely in forest ecosystems. Perturbations to the forest caused by human interventions may change conditions sufficiently for pathogens to become more active.

Sequencing proved unequivocally the identities of *Phytophthora cinnamomi*, and the *Pythium* spp. isolated, a factor of particular importance in confirming the presence of *Phytophthora cinnamomi* in Scots pine forests at such northerly latitudes. Based on published temperature thresholds, this pathogen would not be expected to survive in northerly latitudes (Benson, 1982) and, although oospores may survive at $-20\,^{\circ}C$ under laboratory conditions (Erwin & Ribeiro, 1996), the pathogen exists in mycelial forms within host tissues or as chlamydospores, which probably display differing temperature thresholds for survival. In northern Scotland, the soil temperature rarely exceeds $16\,^{\circ}C$, the cardinal growth temperature for *Phytophthora cinnamomi* (Erwin & Ribeiro, 1996); during summer 2004, soil temperatures higher than $16\,^{\circ}C$ occurred only in the daytime during July and August (UK Meteorological Office, 2005, pers. commun.). The times at which *Phytophthora cinnamomi* could become active in northerly latitudes, therefore, are limited.

The ecological role of these fine root pathogens in temperate forest ecosystems is uncertain. Clearly, each pathogen tested was capable of causing some damage, in terms of growth reductions, on the Scots pine seedlings used in the present work. The greatest reductions in growth, in terms of biomass production, were found with *Phytophthora cinnamomi* and the *Fusarium* sp. The mixture of pathogens also caused some damage, and was clearly the most virulent inoculum tested in terms of the appearance of visible above-ground symptoms. Differences in the apparent virulence of the pathogens in single or mixed inoculations, and in relation to symptomology vs. impact on biomass, may arise from interspecific competition in the rhizosphere in combination with the absence of edaphic conditions suitable for high rates of infection.

In Scots pine-dominated ecosystems, pathogens may have multiple effects, causing plant death and reduced vigour and fecundity, leading to changes in the composition of the plant community (Gilbert, 2002). Jarrah dieback provides a dramatic example of the impact of a root pathogen on a particular ecosystem (Shearer & Tippet, 1989), and the work of Packer & Clay (2000) clearly demonstrated the modulating effect of a *Pythium* species on the regeneration of *Prunus serotina* in forests. Scots pine is reported as a host for *Phytophthora cinnamomi* (Zentmeyer, 1980), but there is no indication to date that serious problems, such as Littleleaf disease (Tainter, 1997; Jung & Dobler, 2002), occur in this interaction.

Climate change will undoubtedly alter host–pathogen interactions in the future (Coakley *et al.*, 1999; Bergot *et al.*, 2004). Changes in response to Arctic sea ice anomalies for the near future (Alexander *et al.*, 2003) may alter conditions in northern Scotland sufficiently to allow *Phytophthora cinnamomi* to become a problem in the forests, provided

that both temperature and soil water increase. Brasier & Scott (1994) suggested that temperature increases of *c.* +3 °C would be sufficient to result in *Phytophthora cinnamomi* activity at the latitudes of northern Scotland.

Future work will further examine the range and distribution of potential fine root pathogens present in forest ecosystems and determine the relative virulence of the different pathogen species obtained to the woody plants characteristic of Scots pine forests in Scotland.

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