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Published in:
New Phytologist

DOI:
10.1111/j.1469-8137.2004.01016.x

2004

New Phytologist

Research

Phytophthora species and oak decline – can a weak competitor cause significant root damage in a nonsterilized acidic forest soil?

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Received: *20 September 2003*
Accepted: *28 November 2003*

doi: 10.1111/j.1469-8137.2004.01016.x

Summary

• *Phytophthora* species in general, and *P. quercina* in particular, have been suggested in several studies to be a contributing factor to the problem of oak decline in Europe. Although *Phytophthora* species are generally regarded as weak competitors, few studies of the pathogenicity of species causing root rot on oaks have hitherto been performed in natural, nonsterilized forest soils. This study describes the effects of seven southern Swedish isolates of *P. quercina* and one isolate of *P. cactorum* on root vitality of *Quercus robur* seedlings grown in a natural, nonsterilized, acidic forest soil.

• The pathogenicity of *P. quercina* and *P. cactorum* were tested using a soil infestation test. The climatic conditions applied were an attempt to simulate summer conditions in southern Sweden.

• Both species of *Phytophthora* caused a significant dieback of fine roots, and necrotic lesions on coarser roots, of *Q. robur* seedlings. Total and live root lengths were significantly lower in infected seedlings than in controls. No significant effects of *Phytophthora* on above-ground growth or leaf nutrient concentration were found.

• The results demonstrate that *P. quercina* and *P. cactorum* can cause substantial root dieback of seedlings of *Q. robur* in natural, acidic forest soils in competition with the inhabiting soil microflora under a mesic water regime.

Key words: *Phytophthora* spp., *Quercus robur*, soil infestation, nonsterilized forest soil, acidity, mesic water regime.

Introduction

During the past decade, several studies have demonstrated the involvement of soilborne species of the well known plant pathogenic genus *Phytophthora* in European oak decline (Brasier *et al.*, 1993; Jung *et al.*, 1996, 1999, 2000; Robin *et al.*, 1998; Gallego *et al.*, 1999; Hansen & Delatour, 1999; Sanchez *et al.*, 2002; Vettraino *et al.*, 2002). In central, western and southern Europe a diverse *Phytophthora* population consisting of, among others, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. europea*, *P. syringae* and *P. cactorum*, is usually present in oak forests (Brasier *et al.*, 1993; Jung *et al.*, 1996, 1999, 2000; Robin *et al.*, 1998; Hansen & Delatour, 1999; Bianco *et al.*, 2000; Vettraino *et al.*, 2002). However in these areas, as well as in northern Europe, the most widespread and most frequently isolated species is *P. quercina*, an oak-specific, fine-root pathogen (Jung *et al.*, 1999, 2000; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003; Jönsson *et al.*, 2003a). *Phytophthora quercina* has proved to be very aggressive towards root systems of *Quercus robur* seedlings in several soil infestation tests (Jung, 1998; Jung *et al.*, 1996, 1999, 2002, 2003a, 2003b; Jönsson *et al.*, 2003b). Apart from dieback of nonsuberized and suberized roots, the pathogen has been shown to cause abnormal root branching (Jung *et al.*, 1996) and to produce elicitins: proteins that may cause necrosis of the leaves and induce yellowing and wilting of the infected host plant (Heiser *et al.*, 1999; Brummer *et al.*, 2002). In addition to *P. quercina*, other *Phytophthora* species have been demonstrated to have pathogenic effects on the

roots of *Q. robur*. These include *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. europea*, *P. gonapodyides*, *P. megasperma*, *P. pseudosyringae* and *P. uliginosa* (Jung *et al.*, 1996, 1999, 2002; 2003a, 2003b). Although many studies of pathogenicity have been performed, information about the impact of root damage on above-ground growth and nutrient status of the seedlings is scarce.

In addition, the soil infestation tests previously used to examine the pathogenicity to oak of *P. quercina* and other *Phytophthora* species have usually been performed on oak seedlings grown in sterile mixtures of peat, vermiculite and sand with pH values of 6.5–7.0 and, in many cases, under favourable environmental conditions for the pathogen (Jung & Blaschke, 1996; Jung *et al.*, 1996, 1999, 2002, 2003a, 2003b; Robin & Desprez-Loustau, 1998; Robin *et al.*, 1998, 2001; Luque *et al.*, 2000; Sanchez *et al.*, 2002). Few studies have focused on the effects of the pathogens under more natural conditions, using forest soils, which have an active soil microflora and also usually have a higher acidity, lower amounts of nutrients and a lower water-holding capacity than the peat–vermiculite–sand mixtures. An exception is the pathogenicity of *P. cinnamomi* to *Quercus rubra* and *Quercus ilex*, where forest soils have been used to a certain extent when assessing the interaction between pathogen and host (Marcais *et al.*, 1996; Gallego *et al.*, 1999). In a first attempt to determine the ability of *P. quercina* to cause root damage in forest soils, Jönsson *et al.* (2003b) compared the pathogenicity of two southern Swedish isolates of *P. quercina* to seedlings of *Q. robur* in two different soils – an acidic, N-rich but otherwise nutrient-poor forest soil; and a nutrient-rich peat–sand mixture with a higher pH. By contrast to what might have been expected, the results showed that the physical and chemical environment of the forest soil did not inhibit *P. quercina*, and that the pathogen can have detrimental effects on oak fine-root systems in acidic, nutrient-poor forest soils under a mesic water regime. However, both soils were initially sterile, and the results gave no indication of the pathogenic abilities of *P. quercina* when in competition with the natural soil microflora.

The next logical step is therefore to determine the interaction between *Phytophthora* spp. and *Q. robur* in a natural, nonsterilized forest soil. The influence of other microorganisms on the pathogenicity of *Phytophthora* species is important in defining the role of this pathogen in European oak decline, as *Phytophthora* species are usually regarded as weak competitors (Tsao, 1990; Erwin & Ribeiro, 1996), and the previous use of sterile soils may have influenced the survival and root infection of *P. quercina* and other *Phytophthora* species as well as the susceptibility of the host. In particular, mycorrhizal infection has been suggested to be an efficient barrier protecting the roots against *Phytophthora* infection (Zak, 1964; Marx, 1973; Barham *et al.*, 1974) but also other types of microbial competition and antagonism have been suggested to be of importance for the activity of Phytophthora species (Weste & Vithanage, 1977; Keast & Tonkin, 1983).

In order to evaluate the effects of microbial presence on the pathogenicity of *Phytophthora* species, a soil infestation test using two different *Phytophthora* species, *P. quercina* and *P. cactorum*, was performed. *Phytophthora quercina* was chosen as a test species as it is the most frequently occurring *Phytophthora* species in many European oak stands (Jung *et al.*, 1999, 2000; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003; Jönsson *et al.*, 2003a), and it has often been shown to be the most aggressive *Phytophthora* species towards root systems of *Q. robur* (Jung *et al.*, 1999, 2002; 2003a, 2003b). Several different isolates, obtained from different sites in southern Sweden, but with similar soil conditions, were used to verify the impact of *P. quercina* on the seedlings. *Phytophthora cactorum* is found worldwide on a wide variety of hosts (Erwin & Ribeiro, 1996), and is relatively common in European oak stands (Jung *et al.*, 1996, 2000; Bianco *et al.*, 2000; Vettraino *et al.*, 2002). Only one isolate of *P. cactorum* was available for the pathogenicity test.

The following hypotheses were tested:

- *P. quercina* and *P. cactorum* can infect and induce root damage and root dieback of fine and coarser roots of *Q. robur* seedlings grown in a nonsterilized, acidic forest soil under a mesic water regime.
- There is no significant variation in pathogenicity between the different isolates of *P. quercina*.
- *P. quercina* is more aggressive than *P. cactorum* on roots of *Q. robur*.
- Above-ground growth and nutrient concentration in leaves of *Q. robur* are adversely affected by the presence of *P. quercina* and *P. cactorum* in the soil.

The climatic conditions applied were an attempt to simulate natural growth conditions for *Q. robur* during the summer season in southern Sweden. A mesic water regime was applied, where the soil was saturated with water for only a short period each time, a condition which is assumed to be less favourable for *Phytophthora* infection than long periods of high or unlimited water availability (Erwin & Ribeiro, 1996). This approach was chosen because studies where maximum infection and disease development are induced are abundant in the literature (Jung & Blaschke, 1996; Jung *et al.*, 1996, 1999, 2002, 2003a, 2003b; Robin *et al.*, 1998; Luque *et al.*, 2000; Sanchez *et al.*, 2002), while attempts to simulate more natural scenarios are largely lacking. Root vitality, as well as above-ground growth and leaf nutrient concentrations, were used as measures to evaluate the direct and indirect effects of the pathogen.

Materials and Methods

Soil infestation test

Seven different isolates of *P. quercina* Jung. Sp. nov. and one isolate of *P. cactorum* (Lebert & Cohn) Schroeter, recovered from seven declining oak stands in southern Sweden, were tested for their pathogenicity towards seedlings of pedunculate

Table 1 Species and origin of *Phytophthora* isolates tested for pathogenicity towards seedlings of *Quercus robur*, and some soil properties for sites from which isolates were recovered

Isolate	Species	Isolation site* (*X*/*Y*)	Geological substrate	Soil texture	pH ($BaCl_2$)†
1	*P. quercina*	6162490/1372500	moraine	loam	3.74
2	*P. quercina*	6231290/1406230	moraine	silt	3.90
3	*P. quercina*	6231250/1398750	sediment	clay	3.53
4	*P. quercina*	6213790/1346220	moraine	loam	3.96
5	*P. quercina*	6284800/1547500	moraine	silt	4.97
6	*P. quercina*	6273300/1500200	moraine	silt	3.55
7	*P. quercina*	6233770/1458760	sediment	loam	3.87
8	*P. cactorum*	6273300/1500200	moraine	silt	3.55

*Swedish National Map Projections RT90. †In mineral soil at 10–30 cm depth.

oak (*Q. robur* L.). The seven oak stands are located between 55.3° and 56.4° latitude, on the border between the northern nemoral vegetation zone and the southern boreal zone. The mean annual temperature and mean annual precipitation in this area ranged from 6.6 to 9.0°C and 451–728 mm, respectively, between 1991 and 2001 (SMHI, 1991–2001). For the summer period (June–August), the mean temperature and precipitation ranged from 14.6 to 17.9°C and 40–73 mm (SMHI, 1991–2001). The pH ($BaCl_2$) of the rhizosphere soil at a depth of 10–30 cm in the seven oak stands from which samples were collected varied between 3.53 and 4.97. Further information on the eight isolates of *Phytophthora* is given in Table 1. The pathogenicity of the *Phytophthora* isolates was determined by a soil infestation test, modified after Matheron & Mircetich (1985) and Jung *et al.* (1996, 1999).

A natural forest soil, sampled from a pedunculate oak stand in southern Sweden, was used in the soil infestation test. This forest soil is acidic and silty, with a chemistry representative of southern Swedish oak stands (based on data from the National Board of Forestry). The soil was sampled at a depth of 10–30 cm in the mineral soil and sieved through a 4 mm sieve to exclude roots and large particles. Apart from low pH, this soil is rich in aluminium (Al) and nitrogen (N), but low in base cations (Table 2). Subsamples of the soil were tested, at several occasions, for the presence of *Phytophthora* species by an oak leaf-baiting method, performed according to Jung *et al.* (1996, 1999). No *Phytophthora* or *Pythium* species were found in the soil.

The inocula consisted of 6-wk-old cultures of individual isolates of *P. quercina* and *P. cactorum*. The isolates were grown at 20°C in the dark on an autoclaved mixture consisting of 250 cm^3 vermiculite and 20 cm^3 whole oat grains, thoroughly moistened with 175 ml multivitamin juice broth (consisting of 200 ml l^{-1} vegetable juice, 800 ml l^{-1} demineralized water and 3 g l^{-1} $CaCO_3$). The inoculum of each isolate was rinsed with demineralized water to remove excess nutrients, then mixed with the forest soil at a concentration of 20 cm^3 inoculum per 1000 cm^3 soil (Matheron & Mircetich, 1985; Jung *et al.*, 1996). Controls received only a rinsed, uninfested mixture of vermiculite, oat grains and multivitamin juice broth at the same concentration.

Table 2 Nutrient concentration of forest soil used in pathogenicity test

Element	Concentration*
Ca	27.0 μg g^{-1}
K	18.6 μg g^{-1}
Mg	5.0 μg g^{-1}
Na	4.0 μg g^{-1}
Al	116.8 μg g^{-1}
Mn	10.6 μg g^{-1}
Fe	1.0 μg g^{-1}
B	0.0 μg g^{-1}
N	1.3 mg g^{-1}
C	14.7 mg g^{-1}
Total exchangeable acidity	1.4 $cmol_c$ kg^{-1}
Cation-exchange capacity	1.6 $cmol_c$ kg^{-1}
Base saturation	14.6%
pH ($BaCl_2$)	4.2

*Concentrations of base cations Al, Mn, Fe and B were determined by inductively coupled plasma spectroscopy (Perkin Elmer, CT, USA) after extraction of 20 g soil in 100 ml 0.1 M $BaCl_2$ for 2 h. The total concentration of C was determined using an automatic LECO instrument, and total N was analysed using the Kjeldahl technique (ICP-Forest, 1998). Analyses based on five subsamples.

Quercus robur seedlings, 14–18 wk old, grown from surface-sterilized acorns with a weight of 1.6–2.6 g, were classified into four different size categories based on above-ground size. The control and the eight different isolates (= nine treatments) included an equal number of seedlings from each size category. The seedlings were transplanted into individual pots with 2.5 l soil and 50.0 cm^3 inoculum. In total, 20 seedlings (five from each size category) were used for each treatment, giving a total of 180 seedlings for the whole experimental setup. All seedlings were kept in a glasshouse at approx. 20–25°C and a relative humidity (RH) of 60%

during daytime, and 10–15°C and 40% RH during the night. The choice of temperature was based on calculations of summer temperature (June–August) in southern Sweden (latitude 55.3–56.4°) between 1991 and 2001 (SMHI, 1991–2001). The photoperiod was approximately 15 h. A mesic water regime was applied: seedlings were flooded with deionized water for 15 min once a day, on three consecutive days, every third week, and received 200 ml deionized water once between each flooding cycle (10 d after the third day of flooding). In total, eight flooding cycles were applied. The seedlings never reached their wilting point before watering or flooding was repeated.

After 25 wk, when the seedlings were 10–11 months old, they were harvested. Based on the yellowing and wilting of seedlings and the presence of necrotic leaf spots, the aboveground condition of each seedling was estimated on a scale from 0–3 (0 = healthy; 3 = dead). The number of leaves and extensions (number of internodes on the stem) were counted and stem length was measured. Roots were separated from the rest of the plant at the point where the dicotelydon had been attached, and the soil was sieved through a 2 mm sieve to collect remaining pieces of the root system. The roots (apart from the small fragments of fine roots used for reisolation tests) were stored in sealed plastic bags at −18°C until further processing. Leaves and stems were dried at 40°C and weighed. Reisolation from the soil of each pot and from a small number of diseased fine-root fragments of each plant was performed to confirm survival and infection of the pathogen. Reisolation from the soil was performed using the oak leaf-baiting method (Jung *et al.*, 1996, 1999), and from the diseased fine-root fragments by direct plating onto selective PARPNH agar (Jung *et al.*, 1996, 100 ml l^{-1} vegetable juice and 20 g l^{-1} agar amended with 3 g l^{-1} $CaCO_3$, 10 mg l^{-1} pimaricin, 200 mg l^{-1} ampicillin, 10 mg l^{-1} rifampicin, 25 mg l^{-1} pentachloronitrobenzene, 62 mg l^{-1} nystatin and 50 mg l^{-1} hymexazol).

Root analysis

The evening before washing, the roots were removed from the freezer and incubated in a cold room (5°C) to thaw. After washing, roots were separated into dead or living based on general visible criteria, resilience, brittleness, bark integrity and colour of the stele. Live roots had an intact stele and cortex, were slightly elastic and were often white or brown in colour. Dead roots often had fragmented bark, were inelastic and brittle, and were often very dark in colour. The roots were scanned, and root length, surface area and volume were measured for different root diameter classes (0–1, 1–2, 2–5, >5 mm) using the software WinRhizo Pro 5.0 (Regent Instruments, Québec, Canada). Roots were then sorted into three different diameter classes (0–2, 2–5, >5 mm), dried at 40°C and weighed. In addition, the number of seedlings subjected to each kind of treatment with a visually detectable mycorrhizal infection in any of the root tips (a well developed mantle visible in the stereomicroscope) was noted. In the following, roots with a diameter of 0–1 mm are referred to as the finest roots, roots with a diameter of 1–2 mm as finer roots, and both diameter classes together as fine roots.

Chemical analysis of leaves

The 20 seedlings subjected to treatment with a particular isolate were sorted randomly into groups of four and the leaves bulked, giving five composite samples per treatment. The leaves were then ground and subsamples digested in concentrated HNO_3. The concentrations of Ca, K, Mg, Na, B, Al, Fe, Mn, Cu, Zn, S and P were determined using an inductively coupled plasma analyser (Perkin Elmer, CT, USA). The concentration of N was determined by Kjeldahl distillation. The ratios of Ca, K, Mg and P to N by weight were calculated.

Statistical analysis

The data were analysed using parametric tests, and nonparametric data were log-transformed to achieve normal distribution and homogenous variances before statistical analyses. A one-way ANOVA was used to test for significant differences between the means for each kind of treatment. The Tukey test was used as *post hoc* test for multiple comparisons between all pairs of means in the case of significant differences using one-way ANOVA. All statistical calculations were performed using the software SPSS 10 for Macintosh (SPSS Inc., IL, USA).

Results

Reisolation

Phytophthora quercina and *P. cactorum* were reisolated from all soil samples and from most diseased, fine-root fragments of oak seedlings that had been growing in the infested soils for 25 wk. This confirms the survival and root infection of both *P. quercina* and *P. cactorum* in the acidic forest soil. None of the pathogens was recovered from the soil samples or the fine-root fragments of the control seedlings.

Root growth

There was a visible difference in the dieback of the roots, as well as in the size of the root systems, of many of the infected seedlings and the control seedlings (Fig. 1). Both *Phytophthora* species caused fine-root decay, with dieback of nonsuberized as well as suberized fine roots. Necroses were observed mainly on fine roots, although some necroses and dieback of coarser roots (diameter 2–5 mm) was also observed. The necroses on the coarser roots usually developed via infection of nonsuberized lateral roots. In many of the infected seedlings the tap roots were dead, and dieback of mother roots was common. A few seedlings showed an abnormal branching pattern, with

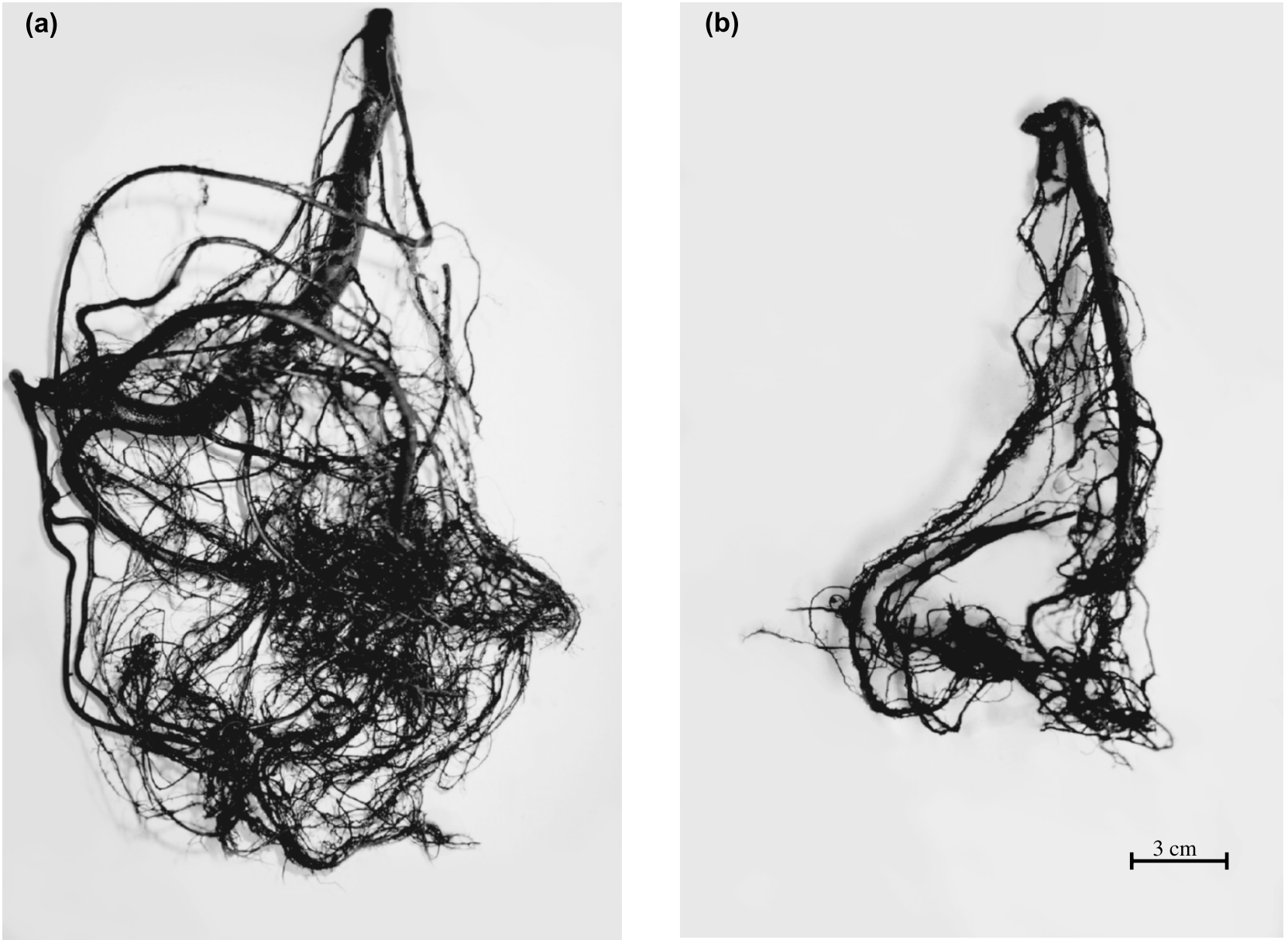

Fig. 1 Example of a representative *Quercus robur* root system of (a) a control seedling, (b) an infected seedling. Photographs indicate the below-ground size of seedlings at the end of the soil infestation test.

extensive sprouting of fine roots where dieback of the tap root had occurred. On the coarser roots (diameter 2–5 mm and >5 mm), wounds in the bark were apparent. The lesions varied in size, but were usually around 2–5 mm in width and 5–10 mm in length. However, some of the lesions reached a length of several cm, with the longest lesion being 55 mm in length. Many, but not all, of the wounds were callusing. Lesions were also present on some of the surviving tap roots. No signs of pathogen infection could be seen on the roots of the control seedlings. Some dieback of fine roots occurred in the control seedlings, but not to the same extent as in infected seedlings. Only a few of the control seedlings showed dieback of tap roots. Both control seedlings and infected seedlings had mycorrhizal root tips, but visible infections were sparse (≤ 10% of the root tips). No significant difference in the number of seedlings with mycorrhizal root tips could be detected between treatments. The mean number of seedlings with visually detectable mycorrhizal infection in any of the root tips was 16.1 (SD ± 0.8, $n = 180$).

Live root length was significantly higher in control seedlings than in seedlings infected with *P. cactorum* and all isolates of *P. quercina* except for isolate 1 (Fig. 2). The difference in live root length consisted mainly of the difference in the length of live fine roots. The differences were most pronounced for the finest roots, where seedlings infected with any isolate of *P. quercina* and *P. cactorum* differed significantly from the control seedlings (Fig. 2b). For coarser roots there were no differences between control and infected seedlings.

Dead root length showed a less obvious pattern. The total dead root length differed significantly only between the control seedlings and some of the isolates. Infection with *P. cactorum* and *P. quercina* isolates 3, 5 and 7 caused a significantly higher amount of dead root length than was observed in the control seedlings, while the effects of other isolates did not differ from the control (Fig. 3). The differences in dead root length were most pronounced with regard to finer roots and roots with a diameter of 2–5 mm, where *P. cactorum* and all isolates of *P. quercina*, except for isolate 6, showed significant differences compared with the control (Fig. 3c,d).

On average, less than 7% of the total root length of the control seedlings was dead, while for seedlings infected with *P. quercina* the percentage of dead root length varied between 18 and 32% of total root length (Table 3). Seedlings infected with *P. cactorum* had, on average, 25% dead root length (Table 3). The fine roots showed similar values, with 7% dead root length in the control seedlings and between 18 and 33% dead root length for the infected seedlings. However, the variation was high within each type of treatment, and dead root length varied between 0 and 50% for infected seedlings. The percentage of dead root length in relation to total root length differed significantly for all isolates of *P. quercina* and *P. cactorum* compared with control seedlings for all roots, as well as for fine roots (Table 3). There were also significant differences between the isolates of *P. quercina*. None of the roots with a diameter above 10 mm was dead.

The total length of roots was significantly higher in control seedlings than in seedlings infected with *P. quercina* (Table 3). The exceptions are *P. cactorum* and *P. quercina* isolate 1, where total root length did not differ from that of control seedlings. The differences in total root length were mainly caused by differences in the length of fine roots (Table 3). The surface area and volume of roots followed approximately the same pattern as root length. Root biomass was found to be a less sensitive parameter (data not shown).

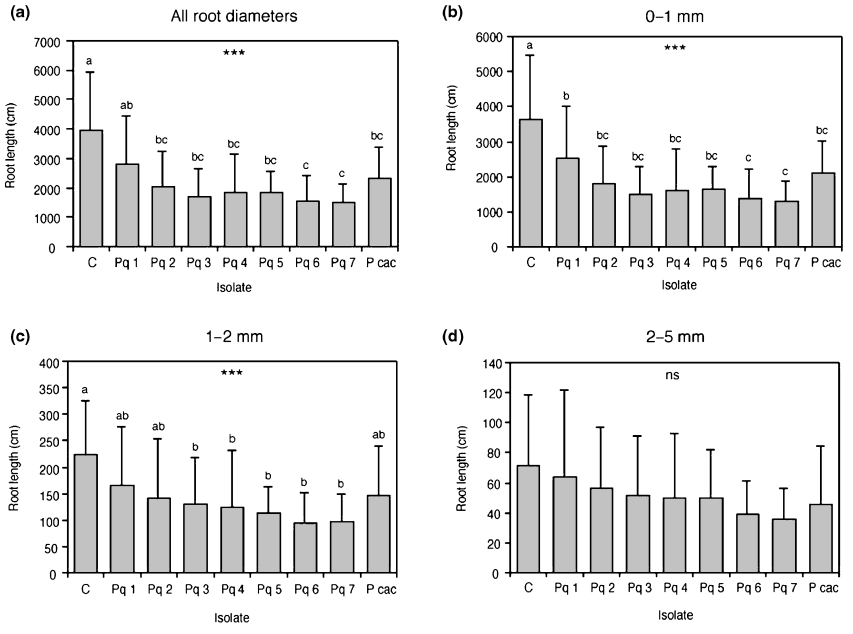

Fig. 2 Length of live roots (mean ± SD) of *Quercus robur* seedlings for (a) all root diameters together and (b–d) three different root diameter classes separately. Statistics are for one-way ANOVA. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; ns, not significant. In the case of significant differences when applying the one-way ANOVA, small letters denote the statistical results of the *post hoc* test (Tukey). Different letters indicate significant differences. C, control; Pq 1–7, *P. quercina* isolates 1–7; P cac, *P. cactorum*.

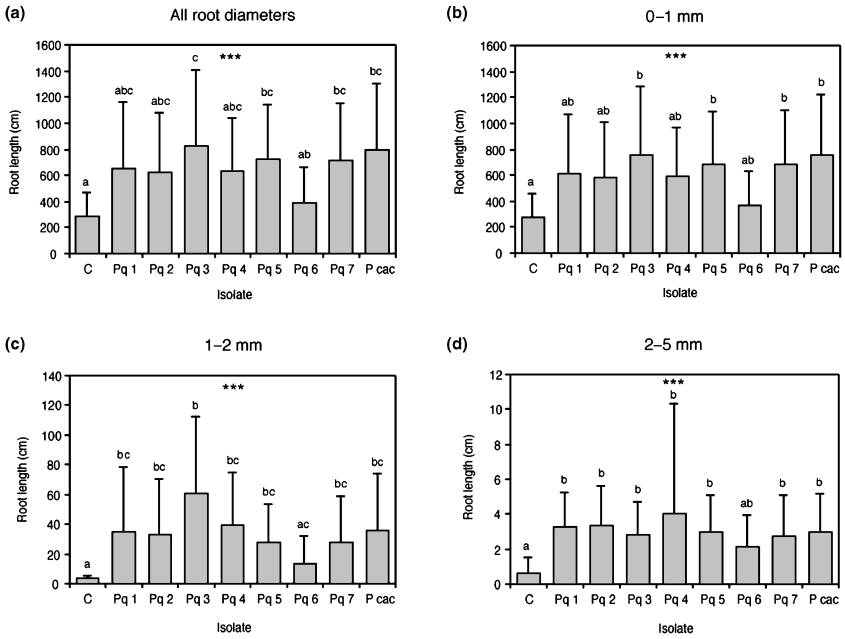

Fig. 3 Length of dead roots (mean ± SD) of *Quercus robur* seedlings for (a) all root diameters together and (b–d) three different root diameter classes separately. Statistics are for one-way ANOVA. $*P \le 0.05$; $**P \le 0.01$; $***P \le 0.001$; ns, not significant. In the case of significant differences when applying the one-way ANOVA, small letters denote the statistical results of the *post hoc* test (Tukey). Different letters indicate significant differences. C, control; Pq 1–7, *P. quercina* isolates 1–7; P cac, *P. cactorum*.

Table 3 Total root length and percentage of dead root length in relation to total root length for all root diameters together and for fine roots of *Quercus robur* seedlings

	Total root length (cm)‡		Dead root length (%)‡	
Isolate†	All root diameters	0–2 mm	All root diameters	0–2 mm
C	4258 ± 2042 a	4144 ± 2008 a	6.7 ± 3.5 a	6.8 ± 3.6 a
Pq 1	3437 ± 2077 ab	3325 ± 2014 ab	19.1 ± 6.5 cd	19.5 ± 6.7 cd
Pq 2	2659 ± 1616 b	2561 ± 1575 b	21.8 ± 9.5 cd	22.5 ± 10.2 cd
Pq 3	2533 ± 1452 b	2443 ± 1414 b	30.9 ± 8.2 b	31.8 ± 8.6 b
Pq 4	2467 ± 1620 b	2376 ± 1573 b	26.2 ± 11.0 bcd	27.0 ± 11.3 bcd
Pq 5	2565 ± 995 b	2477 ± 972 b	27.5 ± 10.2 bc	28.3 ± 10.7 bc
Pq 6	1935 ± 1107 b	1864 ± 1090 b	18.0 ± 9.5 d	18.3 ± 10.1 d
Pq 7	2196 ± 938 b	2122 ± 917 b	31.7 ± 10.6 b	32.6 ± 11.2 b
P cac	3129 ± 1478 ab	3040 ± 1442 ab	24.7 ± 7.9 bcd	25.4 ± 8.2 bcd
One-way ANOVA	***	***	***	***

†C, control; Pq 1–7, *Phytophthora quercina* isolates 1–7; P cac, *Phytophthora cactorum*. ‡Values are mean ± SD ($n = 20$). Statistics are for one-way ANOVA. $*P \le 0.05$; $**P \le 0.01$; $***P \le 0.001$; ns, not significant. In the case of significant differences when applying one-way ANOVA, small letters denote statistical results of *post hoc* test (Tukey). Different letters indicate significant differences.

Phytophthora quercina and *P. cactorum* were equally aggressive to the oak seedlings (Figs 2, 3; Table 3). However, seedlings infected with *P. quercina* isolate 1 and with *P. cactorum* tended to have a somewhat greater live and total root length (although not significantly different from seedlings infected with the other isolates: Fig. 2; Table 3).

Above-ground condition and growth

None of the seedlings died during the experiment. The control seedlings appeared healthier than those infected with *P. quercina*, as many of the infected seedlings had yellowish leaves with green veins and scattered necrotic leaf spots or coherent brown areas. Most of the infected seedlings with severe root rot showed yellowing and wilting of leaves and interveinal chlorosis. However, the estimated above-ground condition (based on yellowing, wilting and necrotic spots on leaves) of the infected seedlings differed significantly from the control only for seedlings infected with *P. quercina* isolates 2, 4 and 7 (Fig. 4a). The seedlings infected with *P. cactorum* appeared healthier than those infected with *P. quercina*, and could not be distinguished from the control seedlings based on above-ground appearance.

Above-ground growth, as expressed by leaf biomass and stem length, showed no significant response to the loss of fine roots and the damage of coarser roots (Fig. 4c,d). Consequently there was no difference in total above-ground biomass between

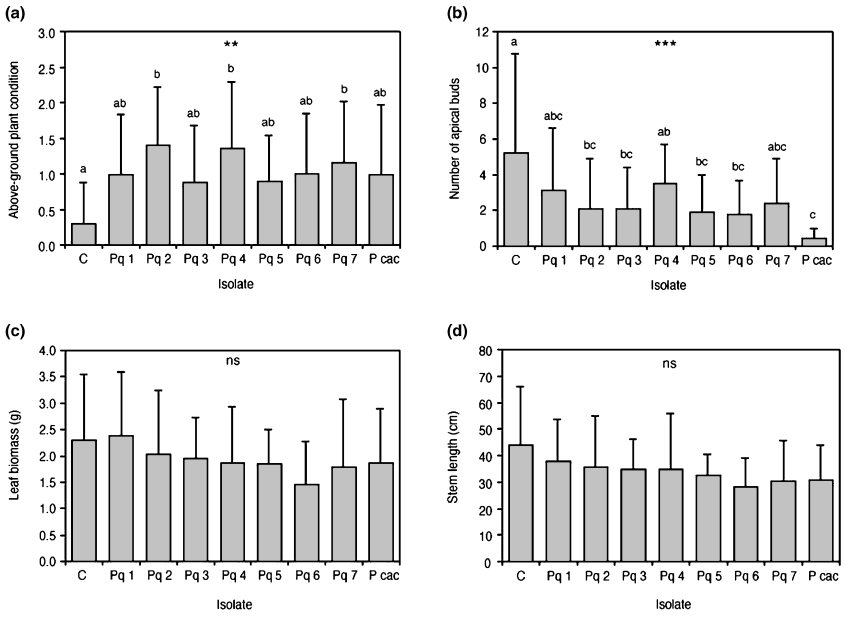

Fig. 4 Mean and SD of (a) estimated above-ground condition of *Quercus robur* seedlings (0–3); (b) number of apical buds; (c) leaf biomass; (d) stem length at harvest. Statistics are for one-way ANOVA. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; ns, not significant. In the case of significant differences when applying the one-way ANOVA, small letters denote the statistical results of the *post hoc* test (Tukey). Different letters indicate significant differences. C, control; Pq 1–7, *P. quercina* isolates 1–7; P cac, *P. cactorum*.

Table 4 Nutrient concentrations and nutrient ratios to N (by weight) in leaves of *Quercus robur* seedlings at the end of the soil infestation test

Element†	One-way ANOVA	Isolate‡ C	Pq 1	Pq 2	Pq 3	Pq 4	Pq 5	Pq 6	Pq 7	P cac
N	ns	19.2 ± 1.3	17.2 ± 1.6	16.5 ± 1.1	19.5 ± 2.6	17.7 ± 0.8	17.6 ± 1.6	18.1 ± 2.6	16.4 ± 1.5	17.8 ± 2.8
P	ns	3.0 ± 0.5	2.3 ± 0.3	2.6 ± 0.9	2.6 ± 0.6	2.9 ± 0.7	2.6 ± 0.5	3.0 ± 0.4	2.6 ± 0.3	3.1 ± 0.6
K	ns	9.5 ± 0.5	9.2 ± 0.6	9.1 ± 0.7	9.9 ± 0.7	10.1 ± 1.6	9.5 ± 0.8	9.5 ± 0.9	8.9 ± 1.7	9.4 ± 1.2
Ca	ns	11.2 ± 1.3	10.0 ± 0.6	12.2 ± 1.8	12.4 ± 2.7	12.8 ± 0.9	11.2 ± 2.0	12.3 ± 2.7	11.3 ± 2.7	13.0 ± 2.6
Mg	**	2.5 ± 0.4	2.1 ± 0.2	2.5 ± 0.2	2.8 ± 0.2	2.4 ± 0.4	2.6 ± 0.2	2.1 ± 0.2	2.3 ± 0.2	2.4 ± 0.3
Mn	ns	3.2 ± 0.7	4.0 ± 0.9	3.6 ± 0.9	5.9 ± 2.4	4.2 ± 0.4	3.7 ± 0.7	4.3 ± 1.2	4.0 ± 1.3	5.0 ± 1.9
Fe	*	0.20 ± 0.04	0.20 ± 0.04	0.21 ± 0.05	0.35 ± 0.12	0.29 ± 0.09	0.19 ± 0.08	0.29 ± 0.06	0.29 ± 0.12	0.28 ± 0.07
B	ns	47.3 ± 12.2	39.3 ± 7.8	42.1 ± 10.4	47.8 ± 16.9	48.0 ± 8.5	40.3 ± 10.0	54.7 ± 21.6	47.4 ± 14.0	58.8 ± 20.9
Cu	ns	5.8 ± 2.0	4.4 ± 0.8	4.6 ± 0.9	6.4 ± 2.8	8.7 ± 3.6	6.3 ± 3.1	5.8 ± 0.8	6.6 ± 1.5	5.0 ± 0.8
Zn	*	37.6 ± 3.9	39.1 ± 4.8	46.0 ± 5.5	39.7 ± 4.2	49.6 ± 10.1	41.2 ± 4.1	37.1 ± 4.6	41.0 ± 6.1	40.8 ± 6.1
S	ns	2.4 ± 0.4	2.1 ± 0.2	2.0 ± 0.2	2.3 ± 0.2	2.4 ± 0.3	2.1 ± 0.2	2.3 ± 0.3	2.2 ± 0.3	2.2 ± 0.3
Ca/N	ns	58.7 ± 9.3	58.4 ± 5.8	74.7 ± 16.4	65.9 ± 22.4	72.6 ± 4.9	63.8 ± 11.6	70.6 ± 24.0	69.4 ± 17.5	75.4 ± 22.4
K/N	ns	49.7 ± 3.0	53.9 ± 3.2	55.5 ± 7.4	51.7 ± 8.0	57.4 ± 9.7	53.9 ± 5.6	53.4 ± 10.5	54.2 ± 11.7	53.8 ± 10.5
Mg/N	ns	13.2 ± 2.8	12.5 ± 1.2	15.3 ± 1.9	14.6 ± 3.2	13.8 ± 2.4	14.7 ± 1.9	12.0 ± 1.5	13.9 ± 1.0	14.0 ± 3.3
P/N	ns	15.7 ± 2.7	13.5 ± 1.7	16.0 ± 6.3	13.6 ± 3.6	16.1 ± 4.0	14.9 ± 2.6	16.9 ± 4.1	15.9 ± 2.9	17.9 ± 4.6

†N, P, K, Ca, Mg, Mn, Fe, S (mg g^{-1}); B, Cu, Zn (μg g^{-1}); Ca/N, K/N, Mg/N, P/N (%). ‡C, control; Pq 1–7, *Phytophthora quercina* isolates 1–7; P cac, *Phytophthora cactorum*. Values are mean ± SD (n = 20). Statistics are for one-way ANOVA. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; ns, not significant.

seedlings (data not shown). However, the number of apical buds was significantly higher in control seedlings than in those infected with *P. cactorum* and *P. quercina* isolates 2, 3, 5 and 6 (Fig. 4b). As root biomass is a less sensitive parameter than root length, the total biomass of seedlings (above- and below-ground) did not differ either (data not shown).

Leaf nutrient concentration

There were no consistent differences in leaf nutrient concentrations or nutrient ratios to N between the control seedlings and those infected with *P. quercina* and *P. cactorum* (Table 4). However, the N concentration tended to be somewhat higher in control than in infected seedlings (except for those infected with *P. quercina* isolate 3), and some seemingly random differences in concentration of a few elements appeared between the isolates of *P. quercina* (Table 4).

Discussion

This study investigated the aggressiveness of seven southern Swedish isolates of *P. quercina* and one southern Swedish

isolate of *P. cactorum* to seedlings of *Q. robur* in a natural, nonsterilized, acidic oak forest soil under a mesic water regime. The average fine root damage of about 26%, together with the reisolation of all seven isolates of *P. quercina* and the one isolate of *P. cactorum*, both from forest soil and from necrotic fine-root fragments, demonstrates the ability of the pathogens to survive, infect and induce dieback of fine and coarse roots of oaks in the presence of other soil microorganisms, and under adverse environmental conditions in the soil (acidic soils and limited water availability). This indicates that both *Phytophthora* species can compete well with other microbes in the forest soil. Previous studies on the pathogenicity of *Phytophthora* species to *Q. robur* have been performed in sterilized soils, and the initial lack of microbes in these soil infestation tests might have favoured the survival and root infection of the pathogen, as well as altering the susceptibility of the host, and thereby biasing the results of the tests (Jung *et al.*, 1996, 1999, 2002, 2003a, 2003b; Jönsson *et al.*, 2003b). However, performing the pathogenicity test in a natural, nonsterilized forest soil, harbouring a natural community of microorganisms, makes the results obtained here valuable for the interpretation of the pathogenicity of *P. quercina*, and to a certain extent also *P. cactorum*, in the field.

In particular, mycorrhizal infection of plants has been suggested to be important in protecting the roots mechanically and biochemically against *Phytophthora* infection (Zak, 1964; Marx, 1973; Barham *et al.*, 1974). In the present study there was no difference between treatments at the end of the experiment in the number of seedlings with visually detectable ectomycorrhizal infection. However, the measure used is very coarse, and does not quantify the mycorrhizal infection of the seedlings. A more thorough estimate would have been to count the number of mycorrhizal root tips, or preferably to quantify the mycorrhizal infection of fine roots by ergosterol analysis (Nylund & Wallander, 1992).

The high infection rate and the considerable dieback of fine roots, together with necrosis and lesions on coarser roots in the nonsterilized forest soil, demonstrate the aggressiveness towards the host of both *P. quercina* and *P. cactorum*. In accordance with Jung *et al.* (1996, 1999) and Jönsson *et al.* (2003b), fine roots were most susceptible to infection (Fig. 3b,c; Table 3). The effects of the pathogens on the fine roots of *Q. robur* seedlings were expressed as a significant difference in live root length and total root length, but to a lesser degree in the amount of dead root length (Figs 2, 3; Table 3). This is likely to be caused by the presence of saprophytic soil microorganisms from the beginning of the experiment, implying that decomposition proceeded continuously. Hence the amount of dead root length in the soil at harvest is a consequence not only of dieback of roots, but also of decomposition rate. The influence of decomposition rate is most pronounced for the finest roots, as indicated by the significant difference in dead root length for all isolates compared with control seedlings for finer and coarser roots, while there are only a few significant differences for the finest roots (Fig. 3). This is in accordance with several studies on decomposition of roots of different tree species, which show an initially lower decomposition rate as a function of a larger root diameter (Berg, 1984; Fahey *et al.*, 1988; King *et al.*, 1997; Usman *et al.*, 2000; Silver & Miya, 2001).

In addition to the decomposition of dead roots, the rate of root production is likely to contribute to differences in total root length and in live root length between control and infected seedlings. The infected seedlings are exposed to a stress factor that destroys an essential part of the plant, and in an advanced stage of pathogen infection their growth rate is likely to become lower than that of the control seedlings. The ratio between root death and root replacement by seedlings may easily become unbalanced, thereby reducing the amount of living roots. In addition, the adverse chemical conditions in forest soil – low pH, high Al concentration and low amounts of base cations (Table 2) – as well as competition for resources with soil microorganisms, may add to the stress caused by the pathogens. Soil chemical stress was suggested to be a possible reason for the differences in damage caused by *P. quercina* on root systems of seedlings grown in an acidic forest soil and a nutrient-rich peat–sand mixture (Jönsson *et al.*, 2003b).

The quantitatively homogeneous effects of the isolates of *P. quercina* (Figs 2, 3; Table 3) may be due to a small genetic variation. All *Phytophthora* isolates were recovered from sites with similar soil conditions (loamy to clayey soil texture, mesic soil moisture and pH ($BaCl_2$) between 3.5 and 4.0, with the exception of isolate 5 where the pH was 5.0) and they were therefore probably adapted to acidic mesic soil conditions. The tendency (although not significant) for seedlings infected with *P. quercina* isolate 1 and *P. cactorum* to have a somewhat greater live and total root length than seedlings infected with other isolates may be caused by a lower aggressiveness or competitive ability of these isolates, or possibly a slower rate of infection and disease development. For *P. quercina* isolate 1, a lower competitive ability or a decrease in aggressiveness during storage is likely as isolates 1 and 7 were equally aggressive in the study of Jönsson *et al.* (2003b). In many cases, adaptive changes or mutations may occur after prolonged culturing (Erwin & Ribeiro, 1996) and, for *P. infestans* and *P. parasitica* var. *nicotianae*, several studies have shown a loss of virulence when the pathogen was grown in culture or was continuously subcultured (Apple, 1957; Jeffrey *et al.*, 1962; Pietkiewicz, 1979; Goth, 1981). Evaluation of the pathogenicity of *P. cactorum* is complicated by the use of only one isolate.

The climatic conditions applied in this study were an attempt to evaluate the impact of *Phytophthora* on oak seedlings under conditions less favourable for pathogen infection and disease development, rather than estimating the maximum potential pathogenicity of *Phytophthora*. The results confirm the previous findings by Jönsson *et al.* (2003b) that *P. quercina*, and also *P. cactorum*, can cause substantial root

damage under mesic water regimes, and not only when weather or site conditions involve periods of high or unlimited water availability. This is in accordance with studies of the interaction between *P. cinnamomi* and *Q. ilex* and *Q. suber*, which have demonstrated that flooding did not enhance the root rot caused by *P. cinnamomi* (Robin *et al.*, 2001), and that this *Phytophthora* species is able to infect and induce damage even when waterlogging is absent or occurs only during short periods (Sanchez *et al.*, 2002). Periods of drought may reduce the tolerance of host to pathogen, thereby enhancing the ability of *Phytophthora* species to cause root damage on rewetting. The ability of *P. quercina* to survive droughts, and to cause higher amounts of root damage to *Q. robur* after re-flooding than in treatment with moist soil conditions between flooding cycles has been shown by Jung *et al.* (2003a). For *P. cinnamomi*, on the other hand, drought stress did not increase the amount of damage caused on roots of *Q. ilex*, *Q. suber* and *Q. rubra* (Robin *et al.*, 2001).

The nonsignificant effects on above-ground growth (Fig. 4) and the few random differences in leaf nutrient concentration (Table 4) appear to agree well with previous studies, suggesting that *Phytophthora* can infect the roots of woody plants months to years before foliage symptoms are detected, and that root rot must usually be severe before significant effects can be seen on the aerial parts of the plants (Tsao, 1990; Erwin & Ribeiro, 1996). However, an early indication of a possible influence of the fine-root damage on above-ground growth is the significantly lower number of apical buds in the infected seedlings (Fig. 4). The significant amount of root rot in infected seedlings (19–33% of dead fine-root length) apparently did not influence leaf nutrient concentration during the period of this experiment. Both infected and control seedlings had leaf nutrient concentrations within the range of what is considered sufficient according to Bergmann (1988) and Linder (1995), indicating that infected seedlings must have had enough roots remaining for adequate nutrient uptake. However, the concentrations of K, Cu and N in the leaves were in the lower range of what is considered as optimum levels for oak according to Bergmann (1988), but the lack of significant differences between control and infected seedlings suggests that the low concentrations are more probably due to low availability of the elements in the soil, or strong competition for the them, rather than to the size and activity of the root system. The ratios of each of the elements to N exceeded the target values suggested by Bergmann (1988) and Linder (1995), indicating that the seedlings are N-limited. The yellowing and interveinal chlorosis of the leaves of the infected seedlings is likely to have been caused by leaf necrotic proteins (elicitins), which can be produced by *P. quercina* (Heiser *et al.*, 1999; Brummer *et al.*, 2002).

Although not significant, the N concentration in the leaves showed a tendency to be somewhat lower in infected than in control seedlings. A similar tendency was found by Jönsson *et al.* (2003b). It is possible that this may be an early indication of reduced nutrient uptake, but it may also be caused by a translocation of N in the infected seedlings, from the leaves to the roots, in order to sustain new root production and/or to produce defensive compounds to counteract the *Phytophthora* attack. Alkaloids, for example, are efficiently used as defensive agents in plants, and may be moved around within the plant to parts that need greater protection during growth and development (Crawley, 1997). Other antimicrobial substances that may be produced in attacked root cells, and which may influence the distribution of N within the plant, are pathogenesis-related proteins and possibly also N-containing enzymes necessary for the production of phytoalexins (Agrios, 1997; Lambers *et al.*, 1998).

In conclusion, this study demonstrates that both *P. quercina* and *P. cactorum* can cause substantial root dieback of fine roots, as well as necrotic lesions on coarse roots of *Q. robur* in a natural acidic forest soil in the presence of the inherent soil microflora and under a mesic water regime. The stress caused by the two *Phytophthora* species led to a low replacement of dead roots in infected seedlings. Although severe dieback of fine roots was observed, no significant effects on above-ground growth or on leaf nutrient concentration could be detected after 6 months. These results improve our knowledge about the pathogenicity of *P. quercina* and *P. cactorum* to *Q. robur*, which previously has been limited to effects in sterilized soil. As a complement to the findings in this study, a thorough investigation of how mycorrhizal infections affect the pathogenicity of *Phytophthora* species, host susceptibility and subsequent disease severity would be desirable in order to understand the pathogenicity of *Phytophthora* to seedlings of *Q. robur* in natural forest soils.

Acknowledgements

This project was generously funded by CF Lundströms Fund, Erik and Ellen Sökjers Petersens Fund, The Consolidated Funds of the Royal Swedish Academy of Agriculture and Forestry, and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning. I am very grateful to Thomas Jung and Ulrika Rosengren for constructive discussions and valuable comments. I am also grateful to Håkan Wallander for reading and commenting on the manuscript. Anita Balogh and Maj-Britt Larsson helped in starting up the experiment and in harvesting the plants. Ragnhild Ohlin washed the roots, and performed the chemical analyses together with Maj-Lis Gernersson. Helen Sheppard corrected the language.

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