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# Pathogenicity of Swedish isolates of *Phytophthora quercina* to *Quercus robur* in two different soils

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## Summary

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- Several studies have demonstrated the involvement of soil-borne *Phytophthora* species, especially *Phytophthora quercina*, in European oak decline. However, knowledge about the pathogenicity of *P. quercina* in natural forest soils is limited.
- The short-term effects of two south-Swedish isolates of *P. quercina* on root vitality of *Quercus robur* seedlings grown in two different soils, one high pH, nutrient-rich peat–sand mixture and one acid, nitrogen-rich but otherwise nutrient-poor forest soil are described. Pathogenicity of *P. quercina* was tested using a soil infestation method under a restricted mesic water regime without prolonged flooding of the seedlings.
- There was a significant difference in dead fine-root length between control seedlings and seedlings grown in soil infested with *P. quercina*. Trends were similar for both soil types and isolates, but there was a higher percentage of fine-root die-back and more severe damage on coarse roots in the acid forest soil. No effects on above-ground growth or leaf nutrient concentration between control seedlings and infected seedlings were found.
- The results confirm the pathogenicity of south-Swedish isolates of *P. quercina* in acid forest soils under restricted water availability. Stress-induced susceptibility of the seedlings and/or increased aggressiveness of the pathogen in the forest soil are discussed as key factors to explain the difference in root die-back between soil types.

**Key words:** *Phytophthora quercina*, *Quercus robur*, soil infestation, acidity, nitrogen, mesic water regime.

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## Introduction

Since the early 1980s, extensive decline of several oak species has occurred throughout Europe (Oleksyn & Przybyl, 1987; Hartmann *et al.*, 1989; Ragazzi *et al.*, 1989; Luisi *et al.*, 1993; Anonymous, 2000; Sonesson & Anderson, 2001). Various possible causes of the decline have been discussed, many of them focusing on the susceptibility of different oak species to excess water and drought (Oosterbaan & Naabuurs, 1991; Hartmann, 1996; Gibbs & Greig, 1997; Siwecki & Ulfarski, 1998; Thomas & Hartmann, 1998), or to exceptional frosts or late frosts during winter and spring (Hartmann *et al.*, 1989; Hartmann & Blank, 1992). Pathogenic organisms, such as *Armillaria* spp., have mostly been regarded as secondary organisms, killing only already weakened trees (Wargo, 1993,

1996). However, during the past decade, several studies have demonstrated the involvement of soil-borne species of the well-known plant pathogenic genus *Phytophthora* in European oak decline. Brasier *et al.* (1993) suggested that *Phytophthora cinnamomi* was a contributory factor in the decline of *Quercus ilex* and *Quercus suber* in Iberia. Blaschke (1994) demonstrated a progressive deterioration of fine roots and mycorrhizal systems on mature, declining *Quercus robur* trees, and suggested that the damage was caused by *Phytophthora* species. Since then, *Phytophthora* species have been recovered from declining oak stands growing on a wide range of site conditions all over Europe (Jung & Blaschke, 1996; Jung *et al.*, 1996, 2000; Robin *et al.*, 1998; Gallego *et al.*, 1999; Hansen & Delatour, 1999; Vettrano *et al.*, 2002). In Central, Western and parts of southern Europe, the most widespread

and most frequently isolated species was *Phytophthora quercina*, an oak-specific, newly described, fine-root pathogen (Jung *et al.*, 1999). In several soil infestation tests, *P. quercina* proved to be very aggressive towards root systems of *Q. robur* seedlings (Jung *et al.*, 1996, 1999, 2002a, 2002b). In addition to die-back of nonsuberized and suberized roots, the pathogen caused abnormal root branching (Jung *et al.*, 1996) and produced proteins causing necrosis of the leaves and inducing yellowing and wilting of the plants (Heiser *et al.*, 1999). Jung *et al.* (2000) and Vettriano *et al.* (2002) also found significant correlations between the presence of *P. quercina* in the rhizosphere, the condition of the fine roots (investigated only in Germany) and crown symptoms of mature oaks in Germany and Italy, respectively.

Recently, *P. quercina* was recovered from 10 out of 27 declining stands in the southernmost part of Sweden, indicating that this pathogen might also be of importance in the decline of oak in Sweden (Jönsson *et al.* 2003a). This is of particular interest because of the high acidity of the oak forest soils in that region. The majority (85.7%) of 258 beech and oak forest soils tested had pH(BaCl<sub>2</sub>) values below 4.2 at a depth of 20–30 cm in the mineral soil in 1999 (Sonesson & Anderson, 2001), and almost all of the South-Swedish sites from which *Phytophthora* species were recovered had pH(BaCl<sub>2</sub>) values below 4.0 (Jönsson *et al.* 2003a). Previously, the pathogenicity of soil-borne *Phytophthora* species was usually tested on oak seedlings grown in sterile mixtures of peat, vermiculite and sand with pH-values of 6.5–7.0 and under favourable environmental conditions for the pathogen (Jung *et al.*, 1996, 1999, 2002a). Knowledge of the pathogenicity in natural forest soils is therefore limited (Jung, 1998). Environmental constraints, such as the chemical characteristics of the soil, may influence the pathogen directly or, by increasing the susceptibility of the host to the pathogen, indirectly promote disease development. Among other soil characteristics, soil pH has been shown to influence the aggressiveness of *Phytophthora* species, and Ribeiro (1983) showed that sporangia cannot be formed at pH(H<sub>2</sub>O) values below 4.0. The latter finding was supported by Jung *et al.* (2000), who demonstrated that *P. quercina*, and most other *Phytophthora* species occurring in oak stands, did not produce any sporangia in nonsterile soil filtrate when the pH(H<sub>2</sub>O) was below 4.0. In addition, Jung *et al.* (2000) showed an increase in the production of sporangia with increasing pH. Soil pH may therefore have a strong impact on disease incidence. In general, *Phytophthora* diseases are considered to be more severe at higher pH values (Schmitthenner & Canaday, 1983).

Soil aluminum (Al) and calcium (Ca) concentrations are often inversely and intimately linked with soil pH and many forest soils in southern Sweden have high Al concentrations and low Ca concentrations owing to acidification-related chemical changes in the soil. As a result of high atmospheric deposition, most of them are also rich in nitrogen (N) (Jönsson *et al.* 2003b, K. Sonesson, pers. com.). High concentra-

tions of soluble Al have been shown to be detrimental to *Phytophthora* species (Erwin & Ribeiro, 1996), and although there are examples of high Ca concentrations suppressing root rot caused by *Phytophthora* species, the diseases are generally considered to be less severe at low Ca concentrations (Schmitthenner & Canaday, 1983). The influence of N on the disease seems to depend on the host-pathogen combination and the form of soil N (Klotz *et al.*, 1958; Newhook & Podger, 1972; Schmitthenner & Canaday, 1983; Utkhede & Smith, 1995; Erwin & Ribeiro, 1996). Jung *et al.* (2002b) demonstrated a stimulation of *in vitro* production of sporangia by several *Phytophthora* species, including *P. quercina*, with increasing concentrations of nitrate in the soil leachate. This stimulation was probably a major cause of the increasing difference in fine-root length and the number of fine root tips between uninfected and infected *Q. robur* seedlings with increasing nitrate concentration.

Considering the environmental constraints discussed above, and the chemical characteristics of south-Swedish oak forest soils, we tested the following hypotheses:

- 1 South-Swedish isolates of *P. quercina* can infect and induce die-back of fine roots of *Q. robur* seedlings.
- 2 The effects of *P. quercina* on fine roots of *Q. robur* seedlings are more severe in a high pH, nutrient-rich peat–sand mixture than in an acid, N-rich but otherwise nutrient-poor, forest soil.
- 3 Above-ground growth and nutrient concentrations in leaves are negatively affected by *P. quercina*.

The hypotheses were tested using a soil infestation method. 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. Usually, pathogenicity of *P. quercina* has been tested under prolonged flooding of the plants. However, as the pathogen has been recovered from oak stands experiencing variable conditions of soil moisture (Hansen & Delatour, 1999; Jung *et al.*, 2000; Jönsson *et al.* 2003a), indicating an ability to survive and infect fine roots also at drier sites, this paper describes the pathogenicity of *P. quercina* under a restricted, mesic water regime (without extreme flooding of the oak seedlings).

## Materials and Methods

### Soil infestation test

The pathogenicity of *P. quercina* isolated from the rhizosphere soil of two south-Swedish oak stands was determined by a soil infestation test, modified after Matheron & Mircetich (1985) and Jung *et al.* (1999). Isolate 1 was recovered from a declining *Q. robur* stand in Blekinge (Swedish National Map Projections RT 90 623377/145876), in the south-eastern part of Sweden, where the average pH(BaCl<sub>2</sub>) of the rhizosphere soil at a depth of 10–30 cm was 3.87. Isolate 2 was recovered from a declining *Q. robur* stand in Scania (Swedish National Map Projections RT 90 616249/137250), in the

**Table 1** Nutrient concentration<sup>1</sup> in the peat–sand mixture and in the forest soil after sterilization at 122°C (1.1 bar) for 1 h

Element	Peat–sand mixture	Forest soil
Aluminium ( $\mu\text{g g}^{-1}$ )	0.4	98
Boron ( $\mu\text{g g}^{-1}$ )	0.1	0.3
Calcium ( $\mu\text{g g}^{-1}$ )	1228	31
Carbon (C) ( $\text{mg g}^{-1}$ )	40	14
Iron ( $\mu\text{g g}^{-1}$ )	0.9	17
Magnesium ( $\mu\text{g g}^{-1}$ )	113	6
Manganese ( $\mu\text{g g}^{-1}$ )	9.3	113
Nitrogen (N) ( $\text{mg g}^{-1}$ )	0.9	1.4
Potassium ( $\mu\text{g g}^{-1}$ )	109	20
Sodium ( $\mu\text{g g}^{-1}$ )	17	7
Total exchangeable acidity ( $\text{cmolc kg}^{-1}$ )	0.05	1.6
Cation exchange capacity ( $\text{cmolc kg}^{-1}$ )	7.5	1.9
Base saturation (%)	99	14
pH(BaCl <sub>2</sub> )	4.7	4.1

<sup>1</sup>Concentrations of base cations, aluminium, manganese, iron and boron were determined by inductively coupled plasma spectroscopy (Perkin Elmer, CT, USA) after extraction of 20 g of soil in 100 ml 0.1 M BaCl<sub>2</sub> for 2 h. The total concentration of carbon was determined using a Carbon Determinator CR12 (LECO Corporation, Michigan, USA) and total nitrogen was analysed using the Kjeldahl technique. (ICP-Forest, 1998) The analyses were based on five subsamples for each soil type.

southernmost part of Sweden, where the average pH(BaCl<sub>2</sub>) of the mineral soil was 3.74

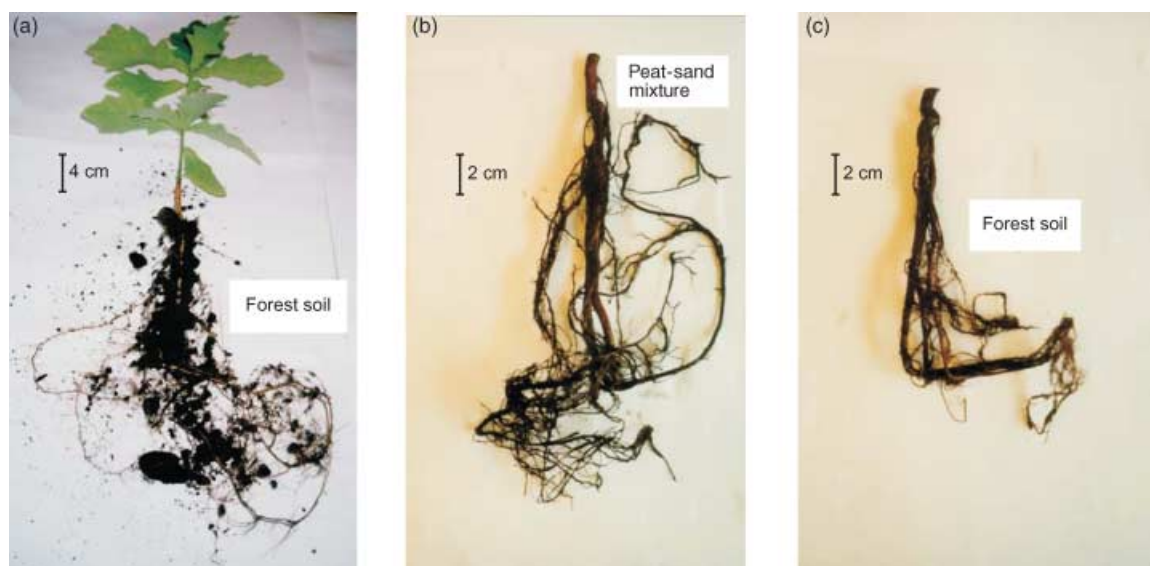
Two different soil types were used for the soil infestation test. Soil type 1 (F) was an acid, silty forest soil (Swedish National Map Projections RT 90 6197460/1357500) with a chemistry representative of south-Swedish oak stands (K. Sonesson, pers. com.). The soil was sampled from 10 to 30 cm depth (i.e. excluding the organic layer) and sieved through a 4 mm mesh to exclude roots and large particles. In addition to low pH, this soil is rich in Al and N, but low in base cations (Table 1). Soil type 2 (P) was a commercial compost (95% peat), which was mixed with sand in a ratio of 2 : 1. This peat–sand mixture is rich in nutrients, has a good water-holding capacity and a relatively high pH (Table 1). The peat–sand mixture resembles (in chemical and physical characters) the mixtures of peat, sand and vermiculite previously used in pathogenicity tests with *P. quercina* (Jung *et al.*, 1996, 1999, 2002a). Both soils were autoclaved at 122°C (1.1 bar) for 1 h before infestation with *P. quercina*.

The inocula consisted of 4- to 6-wk-old cultures of individual isolates of *P. quercina*. The isolates had been grown in the dark at 20°C on an autoclaved mixture consisting of 250 cm<sup>3</sup> vermiculite and 20 cm<sup>3</sup> of whole oat grains, thoroughly moistened with 175 ml of multivitamin juice broth (consisting of 200 ml l<sup>-1</sup> vegetable juice, 800 ml l<sup>-1</sup> demineralized water and 3 g l<sup>-1</sup> CaCO<sub>3</sub>). The inoculum of each isolate was rinsed with demineralized water to remove excess nutrients and then mixed with autoclaved soil to a concentration of 20 cm<sup>3</sup> of inoculum per 1000 cm<sup>3</sup> of soil. Controls received only rinsed, uninfested, vermiculite–oat grain–multivitamin juice broth mixture at the same concentration (Jung *et al.*, 1996, 1999).

Eight- to 12-wk-old *Q. robur* seedlings, grown from surface-sterilized acorns with a weight of 2.7–2.9 g, were classified

into five different size categories based on above-ground size. Each of the two soil types and control, isolate 1 and isolate 2, respectively (six treatments), had an equal number of seedlings from each size category in order to avoid any biasing effects of size on the results of the pathogenicity test. The seedlings were transplanted into individual pots with 2.36 l of soil and 47.2 cm<sup>3</sup> inoculum. In total, 20 seedlings (four seedlings from each size category) were used for each treatment, giving a total of 120 seedlings for the whole experimental setup. All seedlings were kept in a green house at approximately 20°C and a relative humidity of 60% during the day, and 10–15°C and 40% relative humidity during the night, with the exception of 2 wk in July (in the middle of the experiment) when day temperatures exceeded 30°C. The photoperiod was 15 h. Seedlings were flooded every third week with deionized water for 5 min once a day, on three consecutive days, and received 200 ml of deionized water once in between the floodings (10 days after the third day of flooding).

After 3 months, when the seedlings were 5–6 months old, they were harvested. Figure 1 gives an indication of the test system and the above- and below-ground size of the seedlings at the end of the experiment. Based on the yellowing and wilting of the plants, and the presence of necrotic leaf spots, the above-ground condition of each seedling was estimated on a scale from 0 to 3 (0 = healthy and 3 = dead). The number of leaves and the number of internodes on the stem were counted and stem length measured. Roots were separated from the rest of the plant at the point where the cotyledons had been attached, and the soil was sieved through a 2 mm mesh to collect remaining pieces of the root system. The roots (except for the fine roots used for re-isolation tests) were stored in sealed plastic bags at –18°C until further processing. Leaves and stems were dried at 40°C and weighed. Re-isolations



**Fig. 1** Examples of a 6-month-old *Quercus robur* seedling included in the pathogenicity test (a) and the root system of a seedling grown in the peat-sand mixture (b) and in the forest soil (c), respectively. The photographs give an indication of the test system and the above- and below-ground size of the seedlings at the end of the soil infestation test.

from the soil of each pot and from a small number of diseased, fine-root fragments of each plant were made to confirm survival and infection of the pathogen. The re-isolations from the soil were performed using the oak leaf baiting method (Jung *et al.*, 1996, 1999, 2000), and from the diseased fine-root fragments by direct plating onto selective PARPNH agar (Jung *et al.*, 2000; 100 ml l<sup>-1</sup> vegetable juice and 20 g l<sup>-1</sup> agar amended with 3 g l<sup>-1</sup> CaCO<sub>3</sub>, 10 mg l<sup>-1</sup> pimaricin, 200 mg l<sup>-1</sup> ampicillin, 10 mg l<sup>-1</sup> rifampicin, 25 mg l<sup>-1</sup> pentachloronitrobenzene, 62 mg l<sup>-1</sup> nystatin and 50 mg l<sup>-1</sup> hymexazol).

### Root analysis

The evening before washing, the roots were taken from the freezer and incubated in a cold room (5°C) to thaw. After washing, the roots were separated into dead or living, based on general visible criteria, resilience, brittleness, and colour of the stele. The roots were then scanned, and root length, surface area, and volume were measured for different root diameter classes (0–1, 1–2, 2–5, and > 5 mm) using the software WINRHIZO PRO 5.0 (Regent Instruments, Québec, Canada). Roots were then sorted into three different diameter classes (0–2 mm, 2–5 mm and > 5 mm), dried at 40°C and weighed. In this text, roots with a diameter of 0–1 mm are referred to as 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

Leaves from each seedling within a treatment were randomly grouped in fours, giving five composite samples per treatment.

The leaves were crumbled and subsamples were digested in concentrated HNO<sub>3</sub>. The concentrations of Ca, potassium (K), magnesium (Mg), sodium (Na), boron (B), Al, iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and sulphur (S) were determined using an inductively coupled plasma analyser (Perkin Elmer, Norwalk, CT, USA). The concentration of N was determined by Kjeldahl distillation.

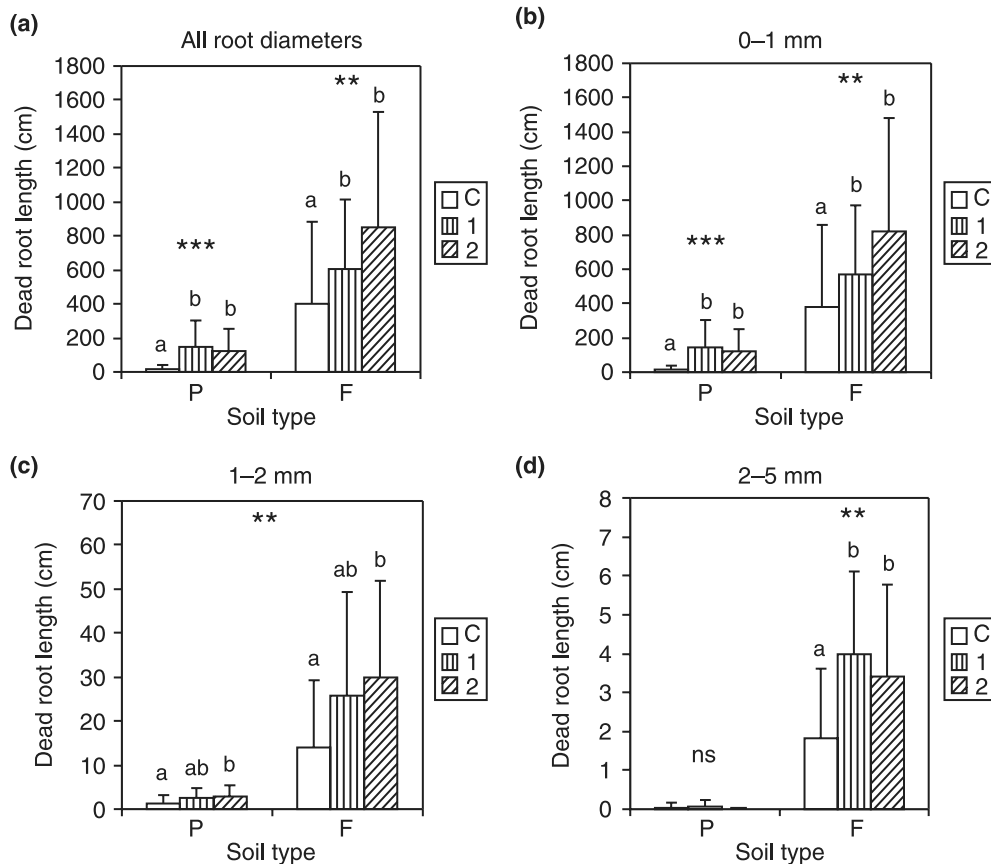
### Statistical analysis

A two-way ANOVA was used to test for significant differences between the means for isolate and soil type. For parameters that showed a significant interaction when applying a two-way ANOVA, a one-way ANOVA was calculated for each soil type separately, as the effects of the isolates (including control) were considered as the most interesting. The Tukey test was used for multiple comparisons between all pairs of means, in the case of significant differences using one-way or two-way ANOVA, respectively. All statistical calculations were performed using the software SPSS 10 for Macintosh (SPSS Inc., Illinois, USA).

## Results

### Root growth

*Phytophthora quercina* was re-isolated from all soil samples and from most diseased, fine-root fragments of oak seedlings that had been growing in the infested soils for 3 months. The recovery proves the survival and root infection of both isolates of the pathogen, in the peat-sand mixture as well as in the acid forest soil. The pathogen could not be recovered from the soil samples or the fine root fragments of the control plants.



**Fig. 2** Mean and SD for length of dead roots of *Quercus robur* seedlings for (a) all root diameter classes together and (b–d) for three different root diameter classes separately. Note that statistics given are for isolate for two-way ANOVA in (c). In (a) (b) and (d), statistics given are for isolate for one-way ANOVA because of a significant interaction when applying the two-way ANOVA. \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; ns, not significant. Where there were significant differences when applying ANOVA, lower case letters denote statistical results of the *post hoc* test (Tukey); different letters indicate significant differences. Open columns, control; striped columns, *P. quercina* isolate 1; hatched columns, *P. quercina* isolate 2; P, peat–sand mixture; F, forest soil.

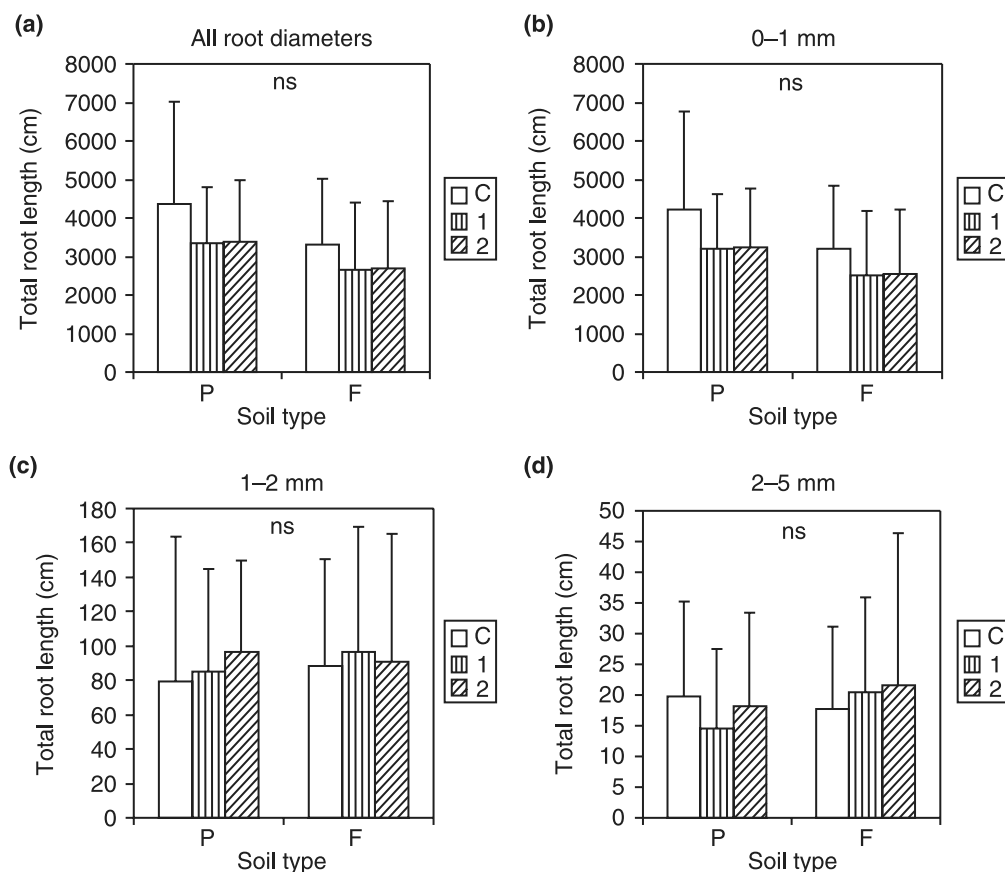
**Table 2** Effects of *Phytophthora quercina* and soil type on dead roots as a percentage of total root length of *Quercus robur* seedlings

Root diameter	Soil type <sup>1</sup>	Isolate		
		Control	<i>P. quercina</i> isolate	<i>P. quercina</i> isolate 2
All root diameters	P	0.7 ± 1.5	8.9 ± 19.9	6.0 ± 8.1
	F	15.0 ± 20.6	34.1 ± 29.4	34.1 ± 20.3
0–1 mm	P	0.7 ± 1.5	9.5 ± 22.0	6.2 ± 8.4
	F	14.9 ± 20.9	34.3 ± 30.2	34.2 ± 20.7
1–2 mm	P	3.8 ± 11.6	4.5 ± 6.2	4.2 ± 5.3
	F	22.6 ± 25.5	37.5 ± 27.7	38.5 ± 28.8
0–2 mm	P	0.7 ± 1.5	9.4 ± 21.9	6.1 ± 8.3
	F	15.0 ± 21.0	34.6 ± 29.9	34.4 ± 20.8

Values are mean ± SD,  $n = 20$ . <sup>1</sup>P, peat–sand mixture; F, forest soil.

Both isolates of *P. quercina* caused fine-root decay, with die-back of nonsuberized as well as suberized fine roots, and necrosis of fine and coarse roots (diameter 2–5 mm). Necroses on suberized, coarse roots usually developed via infection of nonsuberized, lateral roots. In some seedlings, the tap roots were dead. No signs of pathogen infection could be seen on the roots of the control plants. Die-back of fine roots occurred

in some of the control plants, but not in the same proportion as in infected plants. Consequently, the length of dead roots differed significantly between control plants and infected plants (Fig. 2). In the peat–sand mixture, on average 1% of the root length of the control plants was dead, while 9% and 6% of the root length of the plants infected with isolate 1 and 2, respectively, were dead (Table 2). For the forest soil, the



**Fig. 3** Mean and SD for total root length of *Quercus robur* seedlings for (a) all root diameters together and (b–d) for three different root diameter classes separately. Statistics given are for isolate for two-way ANOVA. \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; ns, not significant. Open columns, control; striped columns, *P. quercina* isolate 1; hatched columns, *P. quercina* isolate 2; P, peat-sand mixture; F, forest soil.

corresponding values were 15% for the control plants and 34% for the plants infected with *Phytophthora* (Table 2). However, the variation in the percentage of dead root length was high among plants in both soils. Both isolates of *P. quercina* were equally aggressive to the oak seedlings (Fig. 2, Table 2).

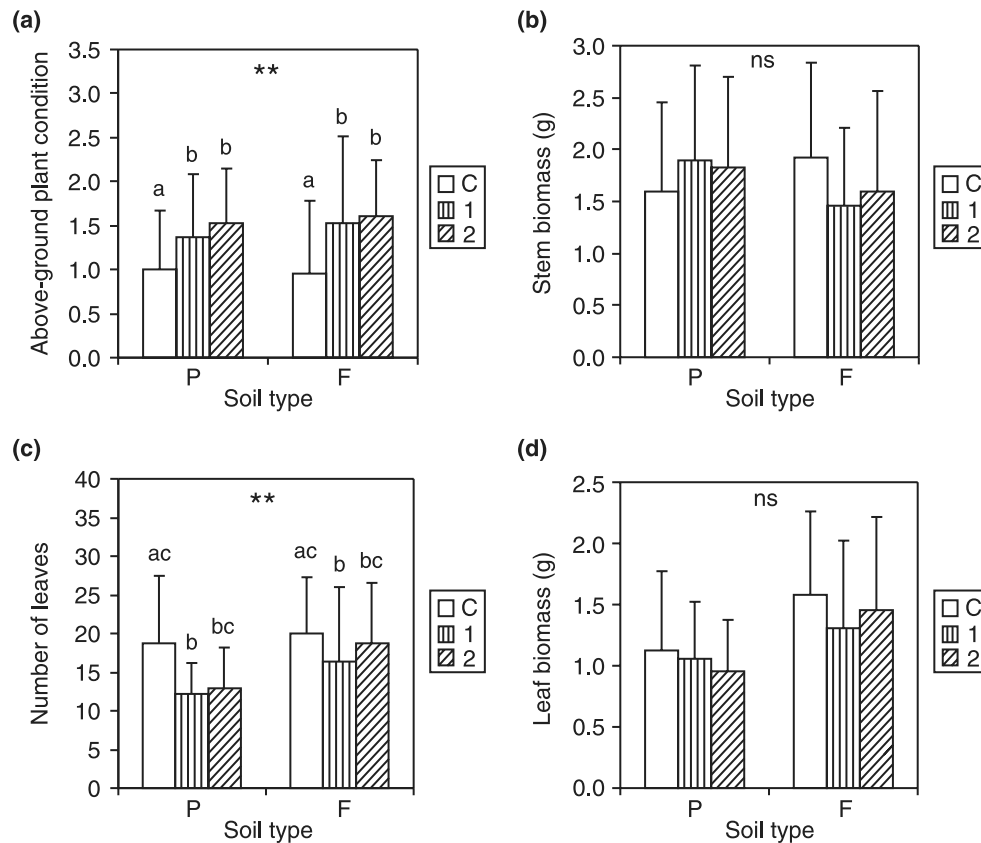
The difference in dead root length comprised mainly the difference in the length of dead fine roots. For finest roots and finer roots, dead root length differed significantly between the control and the infected plants (Fig. 2). However, in the peat-sand mixture, no significant difference was found in dead root length of the finer roots when the soil character was statistically separated (forest soil: one-way ANOVA  $P = *$ , *post hoc* C vs isolate 2  $P = *$ ) The control plants in the peat-sand mixture had, on average, 1% dead fine-root length, while the infected plants had 9% and 6% dead fine-root length, respectively (Table 2). In the forest soil, on average 15% of the fine root length of the control plants was dead, while the corresponding numbers for seedlings infected with isolate 1 and 2 were 35% and 34%, respectively (Table 2). For suberized coarse roots (2–5 mm), significant differences in dead root length were found only in the forest soil. No roots with a diameter greater than 5 mm were dead, and there was no difference between

the control and the infected seedlings in the length of these roots. The surface area and volume of the roots followed approximately the same pattern as root length. Root biomass was a less sensitive parameter, and differences were significant only between the control seedlings and those infected with isolate 2 for dead fine-roots in the forest soil (one-way ANOVA  $P = **$ , *post hoc* C vs isolate 2  $P = ***$ ).

Despite the substantial amount of dead root length in the infected seedlings, there was no significant difference in total root length (= living root + dead root) or total fine-root length between the control seedlings and the infected ones (Fig. 3). However, there was a tendency for the infected plants to have shorter total root and finest root length than the control plants in both soils (Fig. 3). As expected, overall root production was considerably higher in the nutrient-rich peat-sand mixture than in the acid forest soil (Fig. 3).

#### Above-ground condition and growth

The control plants appeared healthier than the plants infected with *Phytophthora*. Many of the infected plants were yellowish, with necrotic leaf spots, and in the forest soil, two of the



**Fig. 4** Mean and SD for (a) for the estimated above-ground condition of the seedlings (0–3) (b) stem biomass (c) the number of leaves, and (d) leaf biomass at the end of the soil infestation test. Statistics given are for isolate for two-way ANOVA. \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; ns, not significant. Where there were significant differences when applying ANOVA, lower case letters denote statistical results of the *post hoc* test (Tukey); different letters indicate significant differences. Open columns, control; striped columns, *P. quercina* isolate 1; hatched columns, *P. quercina* isolate 2; P, peat–sand mixture; F, forest soil.

seedlings infected with isolate 1 died during the experiment. Several of the seedlings with severe root rot showed wilting of leaves and interveinal chlorosis. Accordingly, the estimated above-ground condition (based on yellowing, wilting and leaf necrotic spots) of the control plants and the infected plants differed significantly (Fig. 4).

Above-ground growth showed no response to the loss of fine roots, except for the number of leaves at the end of the experiment. This parameter differed significantly only between the control seedlings and the seedlings infected with isolate 1 (Fig. 4). Leaf biomass, stem biomass, stem length and the number of internodes did not differ between the control and the infected seedlings (Fig. 4).

#### Leaf nutrient concentration

There were no consistent differences in leaf nutrient concentrations between the control and the infected seedlings. However, the infected oak seedlings grown in the peat–sand mixture had higher concentrations of P than the control plants (significant only between control and isolate 2; one-

way ANOVA  $P = *$ , *post hoc* C vs isolate 2  $P = **$ ) and the plants infected with isolate 2 had a significantly lower concentration of N than the control plants or the plants inoculated with isolate 1 (two-way ANOVA  $P = **$ , *post hoc* C vs isolate 2  $P = **$ , isolate 1 vs isolate 2  $P = *$ ). This effect was most pronounced in the forest soil.

The soil type had more influence on the nutrient status of the oak seedlings than the root damage caused by *P. quercina*. Compared with the seedlings in the peat–sand mixture, the oaks grown in the forest soil had consistently lower concentrations of Ca, K, Na, B and P and higher concentrations of N and acid ions such as Al, Fe and Mn.

#### Discussion

This study demonstrates the ability of south-Swedish isolates of *P. quercina* to induce fine-root die-back of *Q. robur* seedlings grown in an acid, N-rich but otherwise nutrient-poor forest soil, as well as in a high pH, nutrient-rich soil, under a restricted mesic water regime. The recovery of *P. quercina* from the acid forest soil proves the ability of the



pathogen to survive at the low pH(BaCl<sub>2</sub>) average value of 4.1, and the high Al and the low Ca concentrations. The re-isolations from the fine root fragments demonstrate the ability of *P. quercina* not only to survive, but also to infect and induce die-back of fine roots at high soil acidity. Both *P. quercina* isolates tested were equally, and highly, aggressive to roots of *Q. robur* (Fig. 2, Table 2). Their aggressiveness, together with the high infection rate (all seedlings infected), demonstrates the efficiency and potential of *P. quercina* as a pathogen in acid forest soils. As found by Jung *et al.* (1996, 1999, 2002b), fine roots were most susceptible to infection. In the peat-sand mixture, *P. quercina* induced a 9- to 14-fold increase in root damage of the finest roots (0–1 mm) compared with the control plants, while there was no significant increase in root damage of the finer roots (1–2 mm). In the forest soil, the increase was only twofold for both finest and finer roots, but the extent of root damage of fine roots by *P. quercina* was markedly greater than in the peat-sand mixture (35% and 34% compared to 9% and 6%, respectively). Moreover, in the forest soil, some coarser roots (2–5 mm) were also affected, primarily via infection of nonsuberized, lateral fine roots. In contradiction to our second hypothesis, the substantial amount of fine-root die-back in the forest soil, together with the infection of coarser roots, a more severe effect of *P. quercina* in the acid, N-rich forest soil than in the high pH, nutrient-rich peat-sand mixture.

In field studies, pH as well as nitrate and Ca concentrations were significantly higher in soils infested with *Phytophthora* than in uninfested soils (Jung *et al.*, 2000). However, *P. quercina* has also been recovered from heavy forest soils with pH(BaCl<sub>2</sub>) values as low as 3.5 (Jung *et al.*, 2000; Jönsson *et al.* 2003a). Apparently, the pH value and the Ca and Al levels in the forest soil used in this study were within the tolerance range of *P. quercina*, confirming the known high plasticity of this pathogen (Jung *et al.*, 2000) and adaptation to acidic conditions. Despite these characteristics of the pathogen, one would have expected more severe root damage in the peat-sand mixture, considering the higher pH, the higher root density, and the better water-holding capacity (due to the large amount of organic matter) in this less compacted soil. However, the contradictory result may be explained by the same soil properties that are apparently advantageous for *Phytophthora* species, but which may also stimulate plant production, thereby compensating the negative effects of the pathogen by enhanced replacement of infected fine-root tips. In the forest soil, the low pH, the high N concentration, the low amount of other nutrients and the more compacted soil structure apparently restricted plant production, compared with the peat-sand mixture, as indicated by the inferior root growth and the substantially greater root die-back of the control plants. In the infested soil, the ratio between rootlet death caused by *P. quercina*, and the rootlet replacement by the seedlings, may have got out of balance, thus further weakening the plants and enhancing the ability of the fine-root pathogen to

invade even suberized, coarse roots. The influence of soil type and soil condition on symptom expression and disease incidence is well known from littleleaf disease of shortleaf pine (*Pinus echinata*), caused by *P. cinnamomi* in the south-eastern USA. Intense investigations revealed that both root damage of shortleaf pine and isolation frequency of *P. cinnamomi* are the greatest on degraded and nutrient-poor soils (Oak & Tainter, 1988; Tainter & Baker, 1996). Environmental stress as a possible factor predisposing trees to pathogens was previously proposed by Manion (1981). An abnormal root morphology, reduced fine-root production and partial root death of *Q. robur* seedlings, as a consequence of high soil acidity, was also suggested by Sonesson (1994). A complementary hypothesis may be N-induced stimulation of sporangia production in the forest soil, promoting the infection of greater numbers of fine roots and the progressive deterioration of the fine-root system, as shown by Jung *et al.* (2002b).

The restricted flooding regime applied in the present study was an attempt to supplement earlier investigations, which have usually demonstrated the pathogenicity of *P. quercina* under repeated, prolonged flooding (Jung *et al.*, 1996, 1999, 2002a). In this study, flooding is limited to what may be considered as natural rainfalls (i.e. saturation of the soil for a short period and then a return to field capacity). In general, infection of fine roots by *Phytophthora* species and expression of symptoms in the host plants are considered to be favoured by waterlogged conditions (Duniway, 1977, 1983; Erwin & Ribeiro, 1996). Conversely, restricted water availability, or drought, may critically reduce the tolerance of the host to the pathogen, and large numbers of both susceptible, fresh, fine roots and sporangia that release zoospores may be produced concurrently after rewetting. For *P. quercina* and *Q. robur*, Jung *et al.* (2002b) demonstrated the ability of the pathogen to survive during droughts, and also to cause root damage during re-flooding in close conjunction with droughts. The results of the present study add to previous knowledge by demonstrating that *P. quercina* has a substantial potential to cause root damage at restricted, mesic water regimes, and not only when weather or site conditions are extremely wet. In a similar study, Sanchez *et al.* (2002) found that *P. cinnamomi* was able to infect and produce root damage on *Q. ilex* and *Q. suber* plants when waterlogging was absent or occurred only during short periods.

Despite the significant root rot caused by *P. quercina*, no effects on the above-ground growth (except for the number of leaves) or on the leaf nutrient status of the seedlings could be detected (Fig. 4). An explanation may be that despite a significant root loss, especially in the forest soil, the oak seedlings still had enough roots remaining for sufficient water and nutrient uptake. Tsao (1990) stated that trees can withstand a substantial loss of fine roots without showing visible above-ground symptoms, provided that they receive adequate water and nutrients from the remaining fine roots. This is supported by Wilcox *et al.* (1993), who detected no effects on the aerial

parts of raspberry, despite up to 20% root rot, and by Sanchez *et al.* (2002), who found low levels of foliar symptoms on *Q. ilex* although root symptoms were severe. The unhealthy appearance (yellowing and interveinal chlorosis of the leaves) of the infected seedlings in the present study might instead be caused by the leaf necrotic proteins (elicitors) produced by *P. quercina* (Heiser *et al.*, 1999). Another likely reason for the absence of effects on above-ground growth is the short duration of the experiment. Plants were harvested 3 months after the infestation of the soil with *P. quercina*. A late infection of the pathogen may have caused substantial root rots, but probably gave no time for the above-ground parts of the seedlings to respond to the root die-back.

Although there were significant differences in dead root length, the total root length did not differ between the control and the infected plants (Fig. 3). However, this was to be expected, considering the short duration of the experiment and the use of autoclaved soils. Using autoclaved soils implies a lack of saprophytic soil organisms at the beginning of the experiment, and the recolonization of the soil takes time and is likely to produce an altered microbial community, perhaps affecting the decomposition rate in the soil. In addition, it seems likely that the seedlings can withstand the pathogen for a while, consistently producing new roots as other fine roots die off. Marçais *et al.* (1996) suggested three different disease phases for *P. cinnamomi* attack on *Q. rubra*. The first is root infection by the pathogen, though without apparent seedling reaction. The infection is followed by reduced root growth, and a reduced rate of pathogen advance, which then gradually ceases. In the third phase, seedling root growth resume, although in some plants *P. cinnamomi* advance is not halted and the oaks die. Depending on pathogen aggressiveness and host susceptibility, a longer duration of the present pathogenicity test might have led to further deterioration or recovery of fine-root production.

With respect to the use of a natural forest soil and the restricted, more natural flooding regime, the results of this experiment can be extrapolated to a certain extent to the field. However, *Phytophthora* species have been suggested to be weak competitors, and the use of autoclaved soils might have favoured the survival and root infection of *P. quercina*. Mycorrhiza, for example, has been suggested to be an effective mechanical and biochemical barrier against *Phytophthora* infection (Zak, 1964; Barham *et al.*, 1974), and its presence may influence the extent of root damage in unautoclaved soils. However, during the past decade, several studies have shown a decrease in mycorrhizal infection in declining oak trees (van Driessche & Pierart, 1995; Causin *et al.*, 1996; Kovacs *et al.*, 2000), which may favour the infection of fine roots by *Phytophthora* spp. in the field.

In conclusion, this study demonstrates that the two south-Swedish isolates of *P. quercina* tested were pathogenic to *Q. robur* seedlings, causing die-back of fine roots in both soils. Root damage in the acid, nitrogen-rich, but otherwise nutrient-

poor, forest soil was substantial, despite the high acidity and Al concentration, and was more severe than in the high-pH, nutrient-rich peat-sand mixture. This might be due to a stress-induced susceptibility of the seedlings, or an increased aggressiveness of the pathogen in the forest soil. The extensive root damage was attained without prolonged flooding of the seedlings, demonstrating the pathogenic potential of *P. quercina* under mesic, natural site and weather conditions. Hitherto, a number of studies performed under controlled conditions and in the field, have demonstrated the high ecological plasticity of *P. quercina* and the ability of this pathogen to cause root damage on seedlings and mature oak trees. However, more studies of the plant-pathogen interaction under various environmental constraints, using nonautoclaved, natural forest soils with mycorrhizal oak plants of different ages and provenances, are necessary before the interaction between pathogenicity of *P. quercina*, host susceptibility and subsequent disease expression is fully understood.

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