# Phytophthora and Oak Decline - Impact on Seedlings and Mature Trees in Forest Soils

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# *Phytophthora* and Oak Decline

## – Impact on Seedlings and Mature Trees in Forest Soils

# *Phytophthora* and Oak Decline
# – Impact on Seedlings and Mature Trees in Forest Soils

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*Till Mamma och Pappa*

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# *Phytophthora* and Oak Decline – Impact on Seedlings and Mature Trees in Forest Soils

## Abstract

This thesis investigated the occurrence of soilborne species of the plant pathogenic genus *Phytophthora* in southern Swedish oak forests and their possible involvement in southern Swedish oak decline. The emphasis was on the impact of *P. quercina* on *Quercus robur* in acid forest soils. Several different methods, including screening for *Phytophthora* in oak forest soils, greenhouse experiments with seedlings grown in soils inoculated with *Phytophthora*, and field studies of mature oaks growing in *Phytophthora*-infested stands, were used. The results showed that *Phytophthora* species occur in southern Swedish oak forests. The most frequently recovered species was *P. quercina*, which was found in 10 of 32 investigated oak stands. In addition, *P. cactorum* and *P. cambivora* (mating type A2) were recovered from one stand each. The soil conditions at the sites from which *Phytophthoras* were recovered ranged from mesic sediments to moraines, with clayey to silty textures and with pH($BaCl_2$) in the rhizosphere soil between 3.5 and 5.0.

In greenhouse studies, *P. quercina* and *P. cactoum* were found to infect and cause substantial damage to roots of *Q. robur* seedlings grown in acid forest soils under a mesic water regime. The presence of the natural soil microflora did not hinder the pathogens from infecting and damaging the roots. In addition, significant differences in live fine-root length were found between healthy mature oaks and declining mature oaks growing in stands infested with *P. quercina*. No such difference was found between trees growing in non-infested stands. The impact of the pathogen on mature oaks seemed to depend on tree vitality, site and climatic conditions. Despite the significant reductions in live fine-root length of both seedlings and mature oaks, few differences in aboveground growth (measured only in seedlings) and leaf nutrient concentrations were detected. However, it seems likely that continuous root infections, with subsequent replacement of roots, will deplete the carbohydrate stores in the plant and cause reductions in the production of new root and shoot tissue as well as in the production of secondary metabolites. This may result in an increased susceptibility of the tree to further pathogen infections as well as to other stress factors. A weak association was found between the occurrence of *P. quercina* and the vitality of oak stands (determined from estimates of crown defoliation).

The impact of soil type on carbon allocation patterns in plants and its consequences for the extent of root damage caused by *P. quercina* is discussed, as well as the influence of abiotic and biotic factors on the aggressiveness of *Phytophthora*, susceptibility of oak and subsequent disease development.

This thesis is based on the following papers, which are referred to by their Roman numerals.

I Jönsson U, Lundberg L, Sonesson K and Jung T. 2003. First records of soilborne *Phytophthora* species in Swedish oak forests. – Forest Pathology 33: 175–179. 47

II Jönsson U, Jung T, Rosengren U, Nihlgård B and Sonesson K. 2003. Pathogenicity of Swedish isolates of *Phytophthora quercina* to *Quercus robur* in two different soils. – New Phytologist 158: 355–364. 55

III Jönsson U. 2004. *Phytophthora* species and oak decline – can a weak competitor cause significant root damage in a nonsterilized acidic forest soil. – New Phytologist 162: 211–222. 67

IV Jönsson U and Rosengren U. Can *Phytophthora quercina* have a negative impact on mature penduncluate oaks under field conditions? – Manuscript. 81

V Jönsson U, Jung T, Sonesson K and Rosengren U. Relationships between *Q. robur* health, occurrence of *Phytophthora* species and site conditions in southern Sweden. – Submitted manuscript. 105

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# Contents

# *Phytophthora* and Oak Decline – Impact on Seedlings and Mature Trees in Forest Soils

## Preface

Today, most scientists consider European oak decline to be a complex phenomenon in which different site-specific factors interact with a succession of abiotic and biotic factors causing a multi-faceted tree decline. My research has focused on one part of this complex of factors, namely the plant pathogens *Phytophthora*, and the primary aim of the work was to evaluate the involvement of soilborne *Phytophthora* species, particularly *P. quercina*, in southern Swedish oak decline. As a plant ecologist, I have tried to approach the subject of plant pathology from an ecological point of view, evaluating the impact of *P. quercina* on its host, *Quercus robur*, under more natural conditions. This approach was chosen since studies where maximum infection and disease development are induced are abundant in the literature, while attempts to simulate more natural scenarios are largely lacking.

I once read in the proceedings from a pathology conference on *Phytophthora* (*Phytophthora* in Forests and Natural Ecosystems, 2nd International IUFRO Working Party Meeting in Albany, W. Australia, 2001) that the subject of Plant Ecology is "The quantification of the obvious". However, I hope I have managed to bring some ecological knowledge to the involvement of *Phytophthora* species in European oak decline and especially, to their involvement in southern Swedish oak decline.

## 1. Background

### 1.1 Oak decline – symptoms and causal agents

Several studies performed during the past two decades have shown extensive decline of *Quercus* species throughout Europe and North America, indicating episodes of severe stress followed by high mortality (Tainter *et al.*, 1983; Oleksyn & Pryzbyl, 1987; Hartmann *et al.*, 1989; Ragazzi *et al.*, 1989; Anonymous, 2000; Oszako, 2000; Rizzo *et al.*, 2002). In Europe, pedunculate oak (*Quercus robur*) is the species which has shown the most marked crown defoliation (Anonymous, 2000). According to the UN/ECE Federal Research Center for Forestry and Forest Products, 35% of the pedunculate oaks in Europe had a crown defoliation that exceeded 25% in 1999 (i.e. could be considered damaged; Anonymous, 2000). Primarily two different types of decline have been identified; a rapid mortality where apparently healthy trees die within one or two growing seasons, and, a slower chronic decline, where trees gradually weaken before they die, or, alternatively, recover. The two types of decline are characterized by a variety of symptoms exhibited by the oak trees, including thinning of the crown, formation of leaf clusters, die-back of branches, discoloration or yellowing of the leaves, formation of epicormic shoots, exudations from the bark of the lower trunks, and bark lesions, many of which are nonspecific and may be due to several different causal agents. Despite the low specificity of the symptoms and the different patterns of disease development, the search for a common cause behind oak decline, especially across Europe, has been intense, and many different factors have been suggested to trigger the outbreak of the damage (Führer, 1998; Oszako, 2000; Thomas *et al.*, 2002).

### *1.1.1 Biotic factors*

Among the biotic factors, defoliation by insect larvae during several consecutive years seems to be the most accepted explanation of the onset of oak decline. All over Europe (for example, in France, Germany, Hungary, Poland, Romania and Russia), various investigations have provided evidence that defoliation by larvae of phyllophagous insects plays a dominant role in the outbreak of oak decline. The most important defoliating insects seem to be *Operophthera brumata* L., *Tortrix viridana* L. and, in warmer regions, *Lymantria dispar* L. (Thomas *et al.*, 2002). Bark beetles, borers and pathogenic microorganisms, on the other hand, have mostly been regarded as secondary agents, killing only already severely weakened trees (Manion, 1991; Wargo, 1996). However, the extensive necrotic areas and the increased tylosis of stems and roots of declining oak trees, indicating infection by biotic agents (Luisi *et al.*, 1993; Blaschke, 1994; Kaus *et al.*, 1996; Jung, 1998), directed some interest to the involvement of pathogenic microorganisms in European oak decline. In the literature, it has been suggested that several different pathogens, for example, *Armillaria* species, *Collybia fusipes*, *Fusarium eumartii*, *Microsphaera alphitoides*, mycoplasma-like organisms, bacteria and viruses, are involved in the decline process (Nienhaus, 1987; Ragazzi *et al.*, 1993; Ahrens & Seemüller, 1994; Schlag, 1995; Wargo, 1996; Marcais *et al.*, 1999; Piou *et al.*, 2002: Biosca *et al.*, 2003; Camy *et al.*, 2003). Most attention has been directed towards a group of Oomycota, *Phytophthora* species, almost all of which are aggressive primary plant pathogens (Brasier *et al.*, 1993; Jung & Blaschke, 1996; Jung *et al.*, 1996; 1999; 2000; Robin *et al.*, 1998; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003a,b; see also Chapter 4). If attacks are repeated, borers, especially *Agrilus biguttatus* Fabr. (Buprestidae), may also cause girdling and death of initially healthy trees (Hartmann, 1996).

### *1.1.2. Abiotic factors*

Of the abiotic factors, three main groups have been considered to contribute to oak decline: climatic extremes, site conditions and air pollutants. Climatic extremes and site conditions that are unfavourable for the growth of *Quercus* have been widely recognized to significantly affect the health of the trees. Of the climatic factors, changes in precipitation patterns and summer droughts seem to be frequently associated with aggravated decline (Oosterbaan & Nabuurs, 1991; Gibbs & Greig, 1997; Siwecki & Ulfnarski, 1998; Thomas & Hartmann, 1998), but exceptionally cold winters have also been found to have major damaging effects on oak trees, with large areas of bark being killed (Hartmann *et al.*, 1989; Hartmann & Blank, 1992; Barklund & Wahlström, 1998; Barklund, 2002). Spring frost is believed to occasionally contribute to oak decline (Hartmann & Blank, 1998). With regard to site conditions, close correlations were found between soil water conditions and tree health. In several central European countries, oak decline was found to be increased at hydromorphic sites with fluctuating water tables (Thomas *et al.*, 2002). Air pollutants, on the other hand, are generally not believed to contribute to oak decline. Thomas *et al.* (2002) concluded in their review of oak decline in central Europe that there is no evidence of substantial contributions by any gaseous air pollutant ($SO_2$, ozone, gaseous N compounds) to recent outbreaks of oak decline, and the studies performed by Berger & Glatzel (1994), Thomas & Kiehne (1995), Simon & Wild (1998) and Thomas & Büttner (1998) on leaf and soil nutrient concentrations in oak stands support the suggestion that air-pollutant-induced changes in the nutritional status of the trees is not a causal factor behind oak decline, at least not in Austria or Germany. Although few direct correlations between nutrient status and oak health have been found, chemical changes in soil and trees resulting from a high input of N and acidifying compounds may destabilize oak ecosystems and thereby reduce the stress tolerance of trees. Excess N may, under certain circumstances, reduce the frost hardiness of oaks (Thomas & Blank, 1996; Thomas & Ahlers, 1999), increase the susceptibility of trees to parasite attack (Flückiger & Braun, 1998) or, in combination with drought, possibly lead to reduced concentrations of allelochemicals in the leaves of *Q. robur* (Thomas & Schafellner, 1999; Thomas *et al.*, 2002).

### *1.1.3 Interactions between biotic and abiotic factors*

Although many and diverse, the various hypotheses presented above have usually explained only regional and short-term incidents of oak decline. No general long-term, large-scale factor has yet been found. These findings, together with the low specificity of the symptoms of decline, have led many scientists to believe that the decline of oak is a complex phenomenon where several different abiotic and biotic factors interact according to the spiral theory for tree decline suggested by Manion (1991). Manion divided the causal factors into three groups: predisposing, inciting and contributing (Figure 1). The predisposing factors decrease the vitality of the trees over a long period of time, thereby increasing their sensitivity to the inciting and the contributing factors.

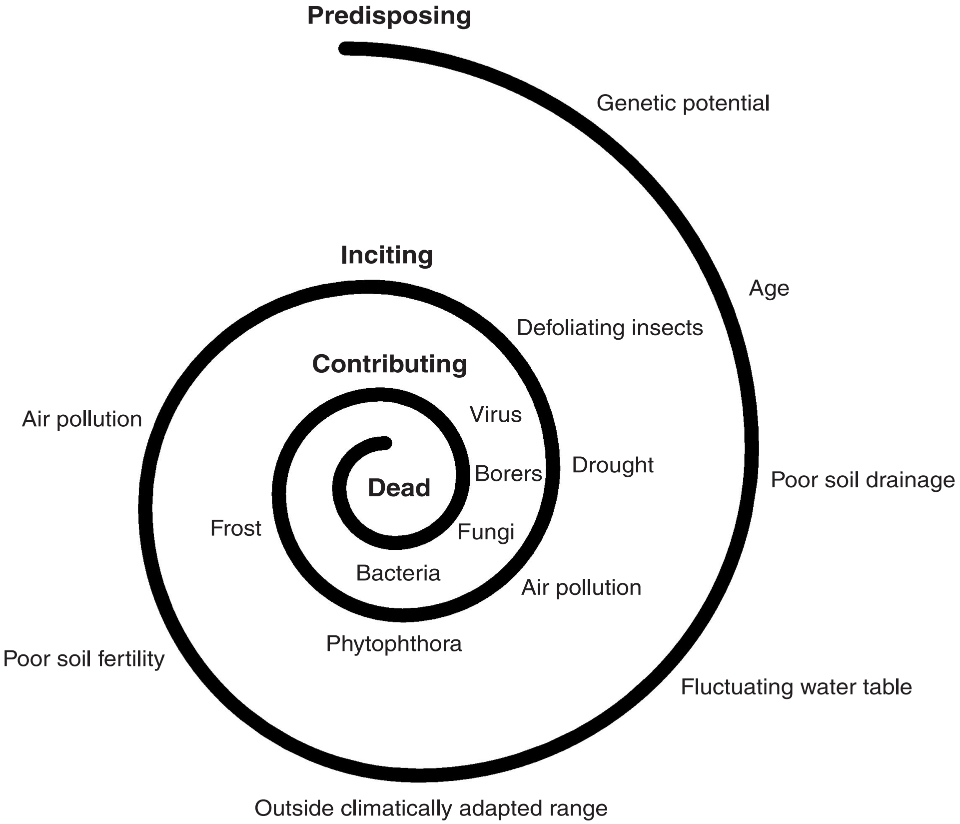

**Figure 1.** Disease spiral for tree decline showing predisposing, inciting and contributing factors suggested to contribute to oak decline (modified after Manion, 1991). The predisposing factors decrease the vitality of the tree over a long period of time, thereby increasing its susceptibility to inciting and contributing factors. The inciting factors act over a shorter time and cause direct damage to tree tissue, while the contributing factors act only on severely weakened trees.

The inciting factors act on a shorter time scale and cause direct damage to the tree tissue. The contributing factors act on the tree when it is already severely weakened and usually have considerable effects on the mortality level of the stand. The contributing factors are often of biotic origin. With regard to oak decline, the factors suggested to be involved fall into all three of these groups (Figure 1). In addition to the factors already mentioned, forest management practices attempting to achieve high production on soils not suitable for oak production are likely to influence disease severity. Which of the factors in Figure 1 has the greatest impact on the decline of oaks, and how these different factors interact with each other, subsequently causing a complex course of decline, is still unclear and requires further investigation. However, Thomas *et al.* (2002) suggested a conceptual model for northern Germany involving three of these factors: insect defoliation, summer drought and winter/spring frosts. Normally, at least two of these factors (although always including defoliation) have to occur simultaneously to trigger decline. These three factors are regarded as decisive causal factors for past, as well as present, occurrences of oak decline. However, single factors are likely to be responsible for degradation and tree die-back in specific stands.

### 1.2 Oak decline in southern Sweden

Similar to the situation in the rest of Europe, oaks in southern Sweden (primarily *Q. robur*), have shown a dramatic deterioration in health during recent decades. In 1999, the proportion of damaged trees (crown defoliation > 25%) in the southern part of the country reached a level of 59%, compared with 9% in 1988 (Sonesson & Anderson, 2001). Barklund & Wahlström (1998) suggested that the initial cause of the decline was severe bark necrosis, due to frost damage caused by the unusually cold winters at the end of the 1980s. However, very little research has been done in Sweden to elucidate other possible causes of the decline. A study was therefore initiated to investigate whether *Phytophthora* species, which had previously been suggested to be involved in central European oak decline, were present in southern Swedish oak forests and, if they were, whether they might be involved in oak decline in southern Sweden.

## 2. Aims and Hypotheses

The primary aim of this work was to evaluate whether soilborne *Phytophthora* species were present in southern Swedish oak forests, and if these pathogens might be involved in southern Swedish oak decline (Papers I–V). In addition, I wanted to evaluate at what kind of sites *Phytophthora* species occur (Paper V), and how different abiotic and biotic environments affect the development of disease in the oaks (Papers II, III & IV). In particular, I was interested in studying the influence of soil type (Paper II) and microbial competition (Paper III) on the pathogenicity of *P. quercina* to *Q. robur*. Based on this, the following hypotheses were tested.

(i) *Phytophthora* species are present in southern Swedish oak stands. Soils in which these pathogens occur are less acidic and more nutrient-rich than soils without the pathogen (Papers I & V)

(ii) Swedish isolates of *Phytophthora* can infect roots and cause damage to root systems of seedlings grown in acid forest soils in the greenhouse. However, the effects of the pathogens will be less severe in an acid forest soil than in a nutrient-rich soil with a higher pH (Papers II & III).

(iii) *P. quercina* can infect, and cause damage, to root systems of mature oaks under field conditions (Paper IV).

(iv) Above-ground growth and nutrient concentrations in leaves are negatively affected by the presence of *P. quercina* in the soil (Papers II, III & IV).

(v) *Phytophthora* species are more likely to occur in declining than in healthy stands (Paper V).

In addition, the importance of soil chemistry and climate for oak decline at 32 sites in southern Sweden was briefly evaluated (Paper V). A schematic presentation of the factors that have been studied in this work are shown in Figure 2.

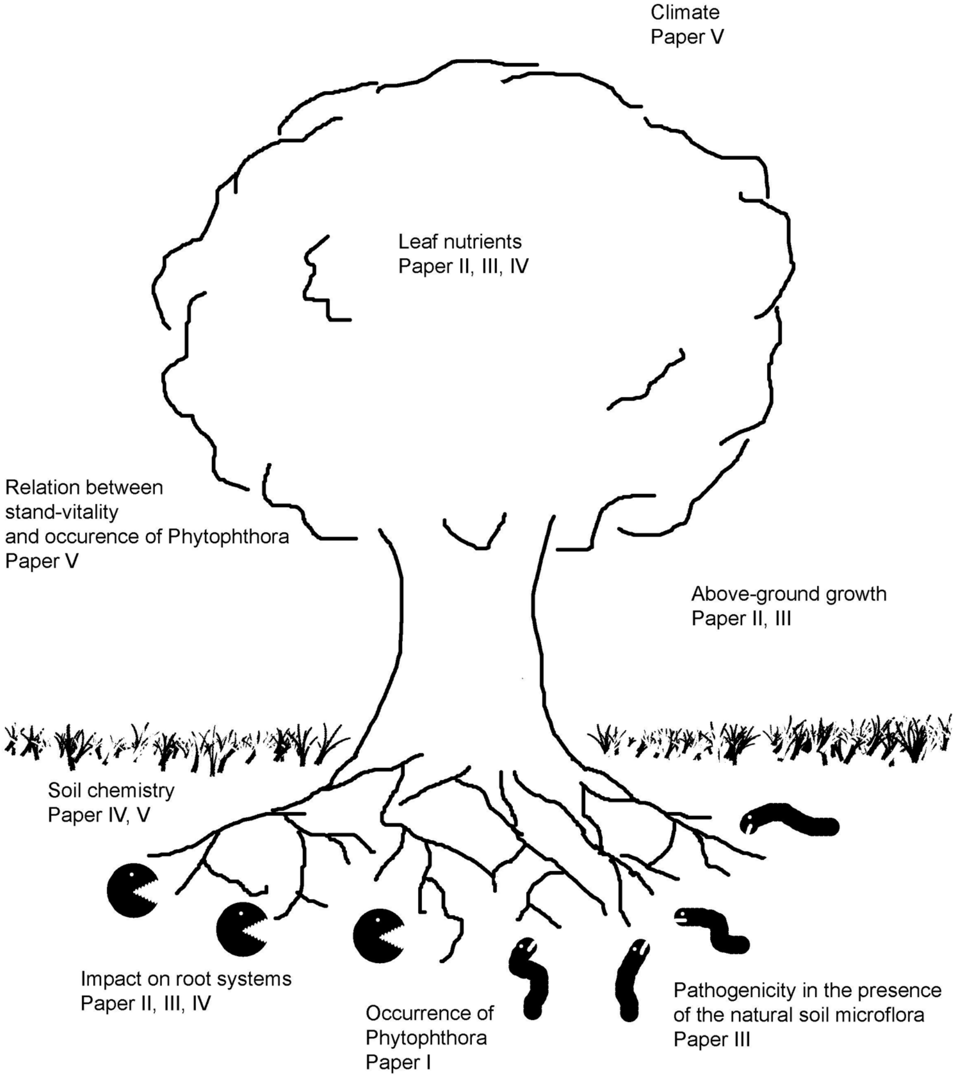

**Figure 2.** A schematic presentation of the factors studied within the scope of this thesis.

## 3. Methods

Several different approaches were used to investigate the involvement of *Phytophthora* species in southern Swedish oak decline. In the first study (Paper I), rhizosphere soil from 32 oak stands was sampled in order to determine whether *Phytophthora* species were present at all in southern Swedish oak stands. A combination of two greenhouse experiments, where oak seedlings (*Q. robur* L.) were grown under controlled conditions (Papers II & III), and a field experiment using mature oaks (Paper IV), was then used to evaluate the aggressiveness of *Phytophthora* to the root systems of *Q. robur*. In addition, the association between the presence of *Phytophthora* and the state of decline of the stand was determined (Paper V). All the methods used are described in Papers I–V. Some additional comments on the methods used and features of specific interest are given below.

### 3.1 Detection of *Phytophthora* species

The soil samples used for detection of *Phytophthora* species were analysed using the soil baiting method described by Jung *et al.* (1996; 2000; Figure 3). This method was proven to be the most efficient for the detection of *Phytophthora* species that occur in oak forest soils compared with several other diagnostic methods (Jung *et al.*, 1996; Jung, 1998). Since *Phytophthora* species are slow growing, their presence is often masked on agar plates by faster growing *Pythium* species, which are commonly found in southern Swedish oak forest soils (Paper I). To minimize contamination by *Pythium* species, soil was dried at room temperature (17–20°C) before baiting (Jung *et al.*, 1996), and slight alterations in the composition of the selective PARPNH agar described in Paper I was made. Soil drying for one week or more proved most useful in reducing the growth of *Pythium* spp. and other fast-growing fungi.

### 3.2 Inoculation method for pot experiments with seedlings

In both greenhouse experiments, the inocula and soil were mixed thoroughly before the seedlings were transplanted (Papers II & III). This ensured a more uniform distribution of *Phytophthora* propagules in the soil. This approach seemed most appropriate considering that a restricted flooding regime was applied and that the experiments were run for a relatively short time. If extensive flooding had been applied, or if water availability had been unlimited, the ability of *Phytophthora* zoospores to disperse in the entire soil volume by themselves would have been greater, and the method of inoculation would have been less important for the development of disease (Robin *et al.*, 2001). The amount of primary inoculum used in the pathogenicity tests has been used previously in a number of studies (Jung *et al.*, 1996; 1999; 2003a,b). The amount of inoculum is likely to be less important for the extent of damage caused by the pathogens, due to the multicyclic nature of *Phytophthora* diseases (Erwin & Ribeiro, 1996), implying that inoculum begets inoculum within a relatively short period of time (hours) and continues to do so as long as environmental conditions favour rapid reproduction and dissemination of sporangia and zoospores.

### 3.3 Excavation of roots in the field

Excavation of monoliths was chosen as the most appropriate method of collecting roots in the field (Paper IV). Soil coring is difficult in these soils, since there are many stones and rocks. Monoliths also give a more coherent root system than cores. However, the disintegration of the root system when collecting, washing and scanning the roots was still found to be too extensive for reliable measurements of the number of fine roots and the number of fine-root tips per unit length of mother root. Further investigations are therefore needed to determine possible differences in the branching pattern between infected and non-infected mature trees (and seedlings).

### 3.4 Determining the effects of *Phytophthora* species on the root systems

Visible signs of infection on the roots, recovery of *Phytophthora* from necrotic tissue, and measurements of root length and biomass were used to determine the impact of *Phytophthora* species on oak root systems (Papers II, III & IV). Signs of infection were determined by comparing the symptoms in oak seedlings and mature trees grown in infested soil with seedlings and trees grown in soils without *Phytophthora* and with symptoms described in the literature (Blaschke, 1994; Jung, 1998; Jung *et al.*, 1996; 1999).

To avoid uncertainties due to individual differences in the handling of roots, the same person washed all roots included in an experiment. Separation of roots into living and dead was al-

**Figure 3.** The soil baiting method used for detection of *Phytophthora* species from soil samples (Photo T. Jung).

ways done by me. Since the surface area and the volume of roots were highly correlated with the root length, and the results of the statistical analysis were similar, only root length is discussed in the papers. In addition to the measurements of length, area and volume of roots, their dry weight was determined. For fine roots (0–2 mm), root length was found to be a more sensitive parameter than biomass.

To investigate the occurrence of *Phytophthora* species in southern Swedish oak stands, to determine if these isolates are pathogenic to oak root systems in acid forest soils, and to evaluate whether these pathogens are involved in southern Swedish oak decline, the methods described in Papers I–V seem appropriate and complement each other. However, more thorough investigations in the laboratory, employing methods developed to answer only one or a few specific questions, are required to determine the specific role of *Phytophthora* species in southern Swedish oak decline, and the interactions between environmental factors, aggressiveness of *P. quercina*, host susceptibility, and subsequent disease expression.

# 4. *Phytophthora* and Oak Decline

## 4.1 An introduction to the genus *Phytophthora*

*Phytophthora* species are a genus of fungus-like microorganisms that belong to the phylum Oomycota in the kingdom Chromista (Figure 4). Species of *Phytophthora* cause a variety of diseases in many different types of plants, ranging from seedlings of annual crops to mature forest trees. Most species cause root rot, damping off of seedlings, and rot of lower stems and tubers (mainly soilborne species). Others cause rot or blight of buds, fruit or foliage (mainly airborne species; Erwin & Ribeiro, 1996; Agrios, 1997). Soilborne *Phytophthoras* occur worldwide, and cause damage to their hosts in nearly every part of the world where the soil becomes too wet for good growth and the temperature remains fairly low (Erwin & Ribeiro, 1996; Agrios, 1997), since these conditions favour the reproduction of the pathogens as well as increase the susceptibility of the host. Unlike most other soilborne fungal pathogens, *Phytophthoras* attack only fresh healthy plant tissue or newly exposed wounds, but do not invade tissue previously colonized by other microorganisms. *Phytophthora*, therefore, is almost never a secondary pathogen (Tsao, 1990). In all hosts affected by

**Figure 4.** Sporangia and zoospores of *P. quercina* (Photo T. Jung).

*Phytophthora* root rot, many of the small and fine roots are dead and necrotic brown lesions are often present on the larger roots. Some *Phytophthora* species attack the stem, where they cause water soaked and dark lesions in the bark. The infected bark area usually spreads and may, under certain circumstances, encircle the entire stem, or if it is restricted to one side of the stem, become a depressed canker below the level of surrounding healthy bark. The cankers may spread up into the trunk, and sometimes the branches, or down into the root system. As the cankers spread and enlarge, they may girdle the trunk or roots (Agrios, 1997). *Phytophthora* is a cortical invader in the majority of cases, and generally does not extend deep into the wood or attack the xylem (Tsao, 1990).

Plants infected with root- and bark-rotting *Phytophthora* species first show symptoms of drought stress and starvation, and then become weakened and susceptible to attack by other pathogens or abiotic factors. Annual plants and young seedlings of trees may be killed within a few weeks or months if environmental conditions are favourable for the pathogen (Agrios, 1997). In older trees, the die-back of the root system may be slower, and affected trees usually do not die until several years after initial root infection by *Phytophthora* (Tsao, 1990). Meanwhile, above-ground symptoms such as sparse foliage, shorter, cupped and yellow leaves and die-back of twigs and branches may occur (Tsao, 1990; Agrios, 1997). In most cases when a large tree dies as a result of root rot, almost all the fine roots will have succumbed to *Phytophthora* infection, and additional stress, such as adverse climatic conditions, is often present and aggravates the course of events (Tsao, 1990). Among the *Phytophthora* species causing severe diseases in forest ecosystems, *P. cinnamomi* in jarrah ecosystems (*Eucalyptus marginata*) in Australia, *P. lateralis* on Port-Orford cedar in the USA and *P. alni* sp. nov. on *Alnus* spp. in Europe are probably the most well-known (Shearer & Tippett, 1989; Brasier *et al.*, 1995; Hansen, 2003). Recently, the newly described airborne *P. ramorum* has caused widespread mortality of coast live oak (*Q. agrifolia*) and tan oak (*Lithocarpus densiflorus*) along the west coast of USA (Rizzo *et al.*, 2002; Davidson *et al.*, 2003).

### 4.2 Occurrence of *Phytophthora* species in European oak ecosystems and their involvement in European oak decline

During the past decade, it has been suggested that several different *Phytophthora* species are involved in the decline of oak in Europe. Brasier *et al.* (1993) suggested that *P. cinnamomi* contributed to the decline of *Q. ilex* and *Q. suber* in Iberia. Blaschke (1994) observed progressive deterioration of fine roots and mycorrhizal systems in mature, declining *Q. robur* trees, and suggested that the damage was caused by *Phytophthora* species. Since then, *Phytophthora* species have been recovered from oak stands growing in a wide variety of soil conditions across Europe (Jung & Blaschke, 1996; Jung *et al.*, 1996; 2000; Robin *et al.*, 1998; Gallego *et al.*, 1999; Hansen & Delatour, 1999; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003a). In central, western and southern Europe, a diverse *Phytophthora* population is usually present in oak forests. Among the 13 *Phytophthora* species recorded in Europe, *P. quercina*, *P. citricola* and *P. cambivora* are widespread, while others seem to be restricted to sites with a warmer climate (*P. cinnamomi*, *P. cryptogea*), wet sites (*P. gonapodyides*, *P. europea*, *P. uliginosa*) or acid sites (*P. pseudosyringae*). Additionally, *P. cactorum*, *P. megasperma*, *P. psychrophila* and *P. syringae* are occasionally isolated (Brasier *et al.*, 1993; Jung *et al.*, 1996; 2000; 2002; 2003b; Robin *et al.*, 1998; Hansen & Delatour, 1999; Bianco *et al.*, 2000; Hartmann & Blank, 2002; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003a). Hitherto, *Phytophthora* species have been recovered from rhizosphere soil and roots of *Q. cerris*, *Q. frainetto*, *Q. ilex*, *Q. palustris*, *Q. petraea*, *Q. pubescens*, *Q. robur*, *Q. rubra* and *Q. suber*.

The mere presence of *Phytophthora* species at a site does not necessarily mean that they are involved in oak decline. However, several studies have demonstrated a significant negative impact of these pathogens on oak fine-root systems under controlled conditions (Jung *et al.*, 1996; 1999; 2002; 2003a,b; Robin & Desprez-Loustau, 1998; Robin *et al.* 1998; Gallego *et al.*, 1999; Sanchez *et al.*, 2002; Papers II & III). Complementary field studies have led to the suggestion that *P. cinnamomi* is involved in the decline of *Q. ilex* and *Q. suber* in Spain, Portugal and France (Brasier *et al.*, 1993; Robin *et al.*, 1998; Sanchez *et al.*, 2002), that several different *Phytophthora* species are involved in central European oak decline and oak decline in Turkey (Jung *et al.*, 2000; Balci & Halmschlager, 2003a,b), and that *P. quercina* is involved in oak decline in Italy (Vettraino *et al.*, 2002). Under certain circumstances, *Phytophthora* species may act as the single factor causing disease of oaks (Jung *et al.*, 2000; Brasier *et al.*, 1995), but the disease process is usually influenced by various abiotic factors, often extreme climatic conditions (Brasier *et al.*, 1993; Erwin & Ribeiro, 1996). Few studies have failed to find associations between the presence of these pathogens and crown condition, but in France, no correlations could be detected between health of trees and the presence of *Phytophthora* (Robin *et al.*, 1998; Hansen & Delatour, 1999; Camy *et al.*, 2003). However, Hansen & Delatour (1999) stated that their sampling method was inadequate for supporting firm conclusions about correlations between crown status and the *Phytophthora* population in the soil. Furthermore, *Q. rubra* has been shown to be relatively resistant to infection by *Phytophththora* (Marcais *et al.*, 1996; Robin *et al.*, 2001).

## 4.3 Occurrence of *Phytophthora* species in oak forests in southern Sweden

In contrast to the diverse population of *Phytophthora* species found in oak ecosystems in the rest of Europe, only three *Phytophthora* species were found in soils of southern Swedish oak stands (*Q. robur* L.; Paper I). The oak-specific fine-root pathogen *P. quercina* was the most frequently recovered species. *P. quercina* seemed to be geographically widespread in southern Sweden and was recovered from 10 out of 32 sampled oak stands, ranging from the southernmost part of Skåne to the northern part of the province of Kalmar. The findings of *P. quercina* in southern Sweden extend the northern limit of this species and complement recent records, which extended the geographical range of this species into Turkey in the south-east and the UK in the west (Balci & Halmschlager, 2003b; Brasier & Jung, 2003). Besides *P. quercina*, *P. cactorum* and *P. cambivora* (mating type A2) were recovered from rhizosphere soil in one stand each. The limited number of *Phytophthora* species found in the oak forests suggest that the climatic conditions or the predominantly acid soils of southern Sweden may not be suitable for more than a few *Phytophthora* species. Another explanation may be that other *Phytophthora* species have not yet been introduced into Sweden. However, since additional sampling (Paper IV) increased the frequency of isolation of *P. quercina* in the stands where it was present (compared with the findings presented in Paper I), repeated sampling during different seasons and investigations of a larger number of sites are needed before any firm conclusions can be drawn about the general presence or not of other *Phytophthora* species in Swedish oak forests.

The soil conditions in the stands from which *Phytophthora* species were recovered ranged from mesic sediments to moraines, with clayey, loamy and silty textures, and soil pH($BaCl_2$) in the rhizosphere between 3.5 and 5.0 (Paper I). Oak stands with *Phytophthora* had a significantly higher pH($BaCl_2$) in the rhizosphere soil than stands without *Phytophthora* (Paper V). The most frequently occurring *Phytophthora* species in southern Sweden, *P. quercina*, seemed to occur more frequently in clayey and loamy soils than in silty and sandy soils. In general, *Phytophthora* species were found at more nutrient-rich sites, primarily with regard to Ca, and where the proportion of base cations in relation to acid ions was high (Paper V). These results are consistent with findings in Germany (Jung *et al.*, 2000; Hartmann & Blank, 2002) and to some extent with findings in Austria (Balci & Halmschlager, 2003a). Although the pathogens occur mainly at relatively nutrient-rich sites with a somewhat higher pH, it was shown by both Jung *et al.* (2000) and in Paper I that *Phytophthora* species may occur in all but the most acidic soils, i.e. soils with pH($BaCl_2$/$CaCl_2$) <3.5.

## 4.4 Infection and symptoms in oak roots

*Phytophthora* species may attack the leaves (*P. ramorum*), stems and roots of oaks. Most commonly, fine roots and stems are infected, with subsequent die-back of the fine-root system and necroses and lesion development on the stems and collars. The first description of symptoms in roots of declining oaks (*Q. ilex* and *Q. suber*) resembling those caused by *Phytophthora* infection came from Spain and Portugal (Brasier *et al.*, 1993). Brasier *et al.* (1993) described dead and dying structural roots up to 20 cm in diameter with substantial necrotic inner-bark lesions. Isolation attempts gave positive results for *P. cinnamomi*. In 1994, Blaschke found similar symptoms in roots of *Q. robur*. He described the infection process as starting with limited necrosis and maceration of the primary tissues of fibrous roots. In advanced stages, the deterioration of non-woody roots progressed towards the suberized mother roots. Having spread from shallow necrotic lesions in the peripheral phellem, some hyphal branches, when not impeded in their growth by the formation of lignitubers in the innermost phellem cells, were capable of colonizing secondary cortex tissue, and induced discoloration of the adjacent phloem. In advanced stages of necrosis, penetrating hyphae could also be found in phloem and xylem elements. In some cases, root rot extended from non-woody fine roots into mother roots. Blaschke (1994) also observed tylose formation in the xylem of the oaks. Coralloid hyphae and thick-walled oospores, typical of *Phytophthora*, were present in the necrotic tissue (Jung, 1998).

Several investigations on oak seedlings, performed in artificial soil mixtures as well as in natural forest soils under controlled conditions, have verified the symptoms found by Brasier *et al.* (1993) and Blaschke (1994). For *Q. robur*, substantial die-back of fine roots in oak seedlings infected with several different *Phytophthora* species, including southern Swedish isolates of *P. quercina*, have been demonstrated in a number of studies (Jung *et al.*, 1996; 1999; 2002; 2003a,b; Jung, 1998; Papers II & III), and similar symptoms have also been observed in *Q. ilex*, *Q. rubra* and *Q. suber* infected with *P. cinnamomi* (Marcais *et al.*, 1996; Robin & Desprez-Loustau, 1998; Robin *et al.*, 1998; Gallego *et al.*, 1999; Robin *et al.*, 2001; Rodriguez-Molina *et al.*, 2002). Apart from root rot of fine roots, *P. quercina* and the other *Phytophthora* species may cause orange-brown discoloration at the site of invasion, necrotic lesions of differing sizes and induce formation of abnormal branching (Jung *et al.*, 1996; Marcais *et al.*, 1996; Robin *et al.*, 1998; Rodriguez-Molina *et al.*, 2002, Papers II & III). Furthermore, *P. quercina* has been shown to infect suberized coarse roots of *Q. robur* (Jung *et al.*, 1996; Papers II & III). Necrosis usually developed via infection of non-suberized, lateral roots. In some infected oak seedlings, *Phytophthora* species may cause death of the entire tap rot, and when environmental conditions are favourable for the pathogen, the whole plant may succumb to the infection and die (Jung *et al.*, 1996; 1999; 2002; 2003b; Marcais *et al.*, 1996; Gallego *et al.*, 1999; Rodriguez-Molina *et al.*, 2002; Papers II & III). In stem inoculation tests, the pathogens were shown to induce oval to fusiform, sometimes girdling, necrotic lesions which, in some cases, were associated with dark exudates (Jung *et al.*, 1996; Luque *et al.*, 2000; Brasier & Kirk, 2001). However, *P. quercina* was only weakly pathogenic, or not pathogenic at all, in these tests (Jung *et al.*, 1996; Balci & Halmschlager, 2003b; Bianco *et al.*, 2003). *P. quercina* therefore seems to be aggressive only to root systems of *Quercus* species. The symptoms of *Phytophthora* infection described by Brasier *et al.* (1993) and Blaschke (1994), and the symptoms found in seedlings grown under controlled conditions, have also been observed in other field studies (Jung & Blaschke, 1996; Marcais *et al.*, 1996; Jung, 1998; Robin *et al.*, 1998; Jung *et al.*, 2000; Paper IV).

## 4.5 Quantitative effects of *Phytophthora* on oaks in natural forest soils

The aggressiveness of *Phytophthora* species is strongly influenced by both environmental conditions and the activity of other soil microorganisms (Tsao, 1990; Erwin & Ribeiro, 1996). Despite this, the soil infestation tests used to examine the pathogenicity of *P. quercina* and other *Phytophthora* species have usually been performed on oak seedlings grown in sterile mixtures of peat, vermiculite and sand, with relatively high pH values and high concentrations of mineral nutrients (Jung *et al.*, 1996; 1999; 2002; 2003a,b; Robin & Desprez-Loustau, 1998; Robin *et al.*, 1998; 2001; Luque *et al.*, 2000; Sanchez *et al.*, 2002). Temperature and water regimes applied in these pathogenicity tests are often designed to favour the pathogen, in an attempt to maximize disease development. In these studies, the root rot is usually severe (often exceeding 50% of the root system when aggressive pathogen isolates are used) and large lesions on roots may be formed, occasionally resulting in high mortality of the seedlings (Jung *et al.*, 1996; 1999; 2002; 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 higher acidity, lower amounts of nutrients, a lower water-holding capacity and are more compacted than artificial peat-vermiculite-sand mixtures. Exceptions are some studies on the pathogenicity of *P. cinnamomi* to *Q. rubra* and *Q. ilex*, where forest soils have been used to a certain extent when assessing the interaction between the pathogen and the host (Marcais *et al.*, 1996; Gallego *et al.*, 1999), and stem inoculation tests with *Phytophthora* isolates from Turkey on *Q. petraea* and *Q. cerris* grown in forest soils (Balci & Halmschlager, 2003b). Furthermore, few field studies, in which the quantitative effects of these pathogens on mature trees in forests have been estimated, can be found in the literature (i.e. Jung *et al.*, 2000). Considering southern Swedish oak forests, it is particularly interesting to determine the effects of *Phytophthora* species under more natural conditions, since the majority of our oak forest soils have pH($BaCl_2$) values below 4.2, and it is common that the base saturation is lower than 20% at 20–30 cm depth in the mineral soil (Sonesson & Anderson, 2001). These are conditions known to be unfavourable to *Phytophthora* species (Schmitthenner & Canaday, 1983; Erwin & Ribeiro, 1996; Jung *et al.*, 2000).

### *4.5.1 Effects on root systems of seedlings grown in forest soil under controlled conditions*

In a first attempt to determine the ability of *P. quercina* to cause root damage in forest soils, the pathogenicity of two southern Swedish isolates of *P. quercina* to seedlings of *Q. robur* in an acidic, N-rich but otherwise nutrient-poor forest soil was investigated (Paper II). In this experiment, the forest soil was autoclaved, to kill the natural soil microflora. The experiment was therefore limited to studying the pathogenicity of *P. quercina* in the chemical and physical environment of a forest soil. A restricted watering regime and relatively high temperature, attempting to simulate southern Swedish summer conditions, were applied. In contrast to what was expected based on previous investigations of the influence of soil chemical factors on *Phytophthora* diseases (Schmitthenner & Canaday, 1983; Erwin & Ribeiro, 1996), the soil infestation test showed that the physical and chemical environment of the forest soil did not inhibit *P. quercina*. The pathogen was recovered from root fragments of all seedlings grown in soil infested with the pathogen, and was shown to cause significant root rot. Infected seedlings had on average 34 to 35% of dead fine-root length compared with 15% in the control seedlings. In addition, the dead root length of coarser roots (2–5 mm in diameter) was significantly higher in infected seedlings, verifying the ability of *P. quercina* to induce damage in suberized tissue. Results from this first experiment demonstrated that *P. quercina* can cause substantial damage to the root systems of oak seedlings under, for the pathogen, adverse chemical and physical conditions in the soil: low pH, high concentration of Al, low concentration of Ca, a compact soil structure and restricted periods of high water availability.

In the second experiment (Paper III), the same forest soil, but not autoclaved, was used. The aim of this experiment was to investigate the effects of *P. quercina* and *P. cactorum* in a forest soil when in competition with the natural soil microflora. This is necessary in defining the role of *Phytophthora* species in European oak decline, since these pathogens are usually regarded as weak competitors, and have been suggested to only infect plant tissue under conditions of reduced competition from rhizosphere microorganisms due to the presence of water (Tsao, 1990, Erwin & Ribeiro, 1996). In this experiment, the seedlings were older (14–18 weeks old, compared with the 8–12-weeks old seedlings in the first experiment), and the duration of the experiment was longer (six months instead of three months). The watering regime and temperature were similar to those in the first experiment. The results showed that both *P. quercina* and *P. cactorum* could cause fine-root decay, with die-back of non-suberized as well as suberized fine roots, despite the presence of the natural soil microflora. Live fine-root length was significantly higher in control seedlings than in seedlings infected with *P. cactorum* and all isolates of *P. quercina*, except one (seven isolates of *P. quercina* were tested). The reduction in the live fine-root length due to *Phytophthora* infection varied between 31 and 64% depending on the isolate. The proportion of dead root length in relation to total root length was on average 26%, while control seedlings had only 7% dead root length. However, the length of dead roots was influenced by the presence of saprophytic microorganisms, and thereby depended not only on the activity of *Phytophthora,* but also on the decomposition rate. In accordance with the first experiment, suberized coarser roots (diameter 2–5 mm) were also infected and the dead root length of these was significantly higher in infected seedlings than in control seedlings. Obviously, *P. quercina* and *P. cactorum* can cause significant damage to root systems of *Q. robur* seedlings also when the natural soil microflora is present in the forest soil, in the absence of extended periods of flooding.

In contrast to previous studies, which have often attempted to maximize disease development by extended periods of waterlogging, the aim of these two greenhouse experiments was to examine the pathogenicity of *P. quercina* (and to a lesser extent *P. cactorum*) to seedlings grown under conditions designed to mimic a southern Swedish summer. The lower proportion of root rot in these two studies, compared with several previous studies examining the pathogenicity of *P. quercina* and other *Phytophthora* species to root systems of oak seedlings, is therefore not surprising, since the infection of fine roots by *Phytophthora* species and symptom expression is generally believed to be favoured by a high availability of water (Erwin & Ribeiro, 1996). However, it may also be due to chemical and physical constraints of the soil on the pathogen. For further discussion of the effects of abiotic factors on the relationship between *Phytophthora* and oak, see Section 5.1.

#### *4.5.2 Effects on root systems of mature trees*

Qualitative studies, determining the presence of *Phytophthora* species at a site, their infection of tree tissue and their association with declining oaks, are common in the literature (Brasier *et al.*, 1993; Jung *et al.*, 1996; 1999; 2000; Robin & Desprez Loustau, 1998; Robin *et al.*, 1998; Gallego *et al.*, 1999; Hansen & Delatour, 1999; Balci & Halmschlager, 2003a,b). However, little is still known about the quantitative effects on mature trees in oak forests (i.e. Jung *et al.*, 2000). In southern Sweden, healthy, moderately declining and severely declining trees in ten stands, five of which were infested with *P. quercina* and five which were not, were sampled to evaluate the impact of this pathogen on root systems of mature *Q. robur* trees (Paper IV). Each stand where the pathogen was present, was paired with another stand where the pathogen was not present, in order to verify the impact of *P. quercina*. The matching of stands into pairs was primarily based on soil texture, soil chemistry and geographical location of the stands, but geological substrate, stand type and stand age were also taken into consideration. Results showed that live fine-root length per unit soil volume was significantly higher for healthy than for declining trees in infested stands. In non-infested stands, on the other hand, there were no significant differences in live fine-root length between trees. The impact of the pathogen was dependent on the season of sampling, being most severe after an unusually dry summer (August 2002), and least pronounced at the beginning of the growing season (June 2002). This suggests that there may be an interaction between drought and *Phytophthora* attack, supporting previous investigations on oak seedlings, where Jung *et al.* (2003a) demonstrated that *P. quercina* caused higher amounts of root damage to *Q. robur* under conditions where drought and flooding were alternated than when moist soil conditions prevailed between flooding cycles.

The data presented in Paper IV support the results of a German study on the impact of *Phytophthora* species on mature trees of *Q. robur,* which also revealed significant differences in the root systems between healthy and declining trees (Jung *et al.*, 2000). In the German study, declining trees showed a higher amount of visible root damage and significantly lower number of fine roots per unit length mother root, lower specific root tip density and lower fine-root length per unit mother root length than healthy trees in 19 stands infested with *Phytophthora* (Jung *et al.*, 2000). Similar to the Swedish study, there were few differences in root parameters between healthy and declining trees in the 16 stands where the pathogen was not present. These results indicate that *Phytophthora* species have a significant impact on the live fine-root length, on the proportion of fine roots and fine root tips and on the branching patterns of the root systems of mature oaks under field conditions. In contrast to the Swedish study, almost all root parameters in the stands without *Phytophthora* in Germany were superior to those of trees in stands with the pathogens. However, Jung *et al.* (2000) stated that these differences might be due to differences in site conditions rather than to fine-root damage caused by *Phytophthora*. The influence of soil type on the extent of damage caused by *P. quercina* on oak root systems is discussed in Chapter 6.

### *4.5.3 Effects on crown vitality, above-ground growth and mineral nutrition*

Significant associations between the presence of *Phytophthora*, particularly *P. quercina*, in the rhizosphere soil, fine-root damage (investigated only in Germany) and crown vitality of individual oak trees have been found in several European countries (Jung *et al.*, 2000; Vettraino *et al.*, 2002; Balci & Halmschlager, 2003a,b). In southern Sweden, declining oaks growing in stands infested with *P. quercina* were found to have significantly lower live fine-root length than healthy trees (see Section 4.5.2; Paper IV). Furthermore, a weak association (p=0.088) was found between the occurrence of *P. quercina* in soil and the vitality of oak stands (determined from estimates of crown defoliation), indicating that oak stands were more likely to be declining if *P. quercina* was present in the soil (Paper V).

Despite significant associations between the presence of *Phytophthora* species, loss of fine roots and unhealthy appearance of the crown of seedlings and trees, there are often none, or very few, effects on the above-ground growth and nutrient status of seedlings (Papers II & III; Wilcox *et al.*, 1993; Jung *et al.*, 1999; Sanchez *et al.*, 2002) or on the nutrient status of mature trees infected with *Phytophthora* (Paper IV). The small effects on seedlings suggest that there is a significant timelag between root loss and above-ground effects, or that the nutrient uptake efficiency of the remaining roots increases when roots are lost due, for example, to pathogen attack. Alternatively, plants may have an excess of roots, which may be a trait that has been of evolutionary advantage during periods of multiple stress. Mature trees that are suffering from fine-root losses usually have a reduced crown and the fine-root system may thus be able to take up enough nutrients for the remaining crown. Nutrient deficiencies may therefore be obvious only in severely declining trees. Results from both the greenhouse experiments (Papers II & III) and the field experiment (Paper IV) are in good agreement with those of previous studies, suggesting that *Phytophthora* may infect roots of woody plants several 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 plants (Tsao, 1990; Maurel *et al.*, 2001).

## 5. Influence of Abiotic and Biotic Factors on the Host–Pathogen Relationship

The development of diseases caused by *Phytophthora* is strongly influenced by a large number of abiotic and biotic factors and the literature on the influence of these factors on *Phytophthora* epidemiology is too voluminous for complete review here. I have therefore emphasized some factors that I believe are important for the development of *Phytophthora* diseases on oak in acid forest soils in a temperate climate.

## 5.1 Abiotic factors

### *5.1.1 Water availability*

*Phytophthora* diseases are multicyclic, which means that inoculum may amplify rapidly when the environmental conditions, the most important of which is the presence of free water, are favourable (Erwin & Ribeiro, 1996). The increase in inoculum from low, often undetectable levels to high levels within a few days or weeks, is caused by the rapid production of sporangia and zoospores at the surface of infected plant tissues. The short regeneration time and great reproductive capacity imply that *Phytophthora* diseases may develop epidemically when the soil remains excessively wet for prolonged periods and temperatures remain fairly low (Erwin & Ribeiro, 1996). However, considering the disease development of oak, drought or variations in water availability, especially fluctuations between drought and flooding, also seem to be detrimental to the fine-root systems (Brasier *et al.*, 1993; Jung *et al.*, 2003a). In particular *P. quercina* seems to be less dependent on soil moisture for its survival under natural conditions (Hansen & Delatour, 1999; Jung *et al.*, 2000; Balci & Halmschlager, 2003a), which may be due to its thick-walled oospores, making it well adapted to survive in dry soil conditions. In greenhouse experiments with *P. quercina* and *Q. robur*, the pathogen was shown to cause substantial damage to root systems of oak seedlings under restricted mesic water regimes (attempting to simulate summer conditions in southern Sweden; Papers II & III), demonstrating that *P. quercina* can cause substantial damage not only when weather or site conditions involve periods of high or unlimited water availability. Similar results were also found for disease development in *Q. ilex* and *Q. suber* infected with *P. cinnamomi* (Robin *et al.*, 2001; Sanchez *et al.*, 2002). Furthermore, Jung *et al.* (2003a) demonstrated the ability of *P. quercina* to survive during periods of drought, and to cause higher amounts of root damage to seedlings under conditions where drought and flooding were alternated than when moist soil conditions prevailed between flooding cycles. Drought also seemed to be a critical factor for the degree of root damage caused by *P. quercina* on root systems of mature trees in oak forests in southern Sweden (Paper IV). Live root length per unit soil volume was significantly lower for both moderately and severely declining oaks compared with healthy oaks after an unusually dry summer (August 2002), while no difference could be detected between healthy and moderately declining trees on the other sampling occasions (June 2002 and March 2003). The high amount of damage when *Phytophthora* and drought interact may be due to the simultaneous increase in the production of sporangia upon rewetting and the production of new, unsuberized fine roots, which are highly susceptible to infection. Extended periods of drought may also increase root exudation, thereby facilitating the initial establishment of soilborne pathogens in the roots (Duniway, 1977), and may, in the event of an increase in soil moisture, favour *Phytophthora* over other soil microorganisms, since *Phytophthoras* respond very rapidly to changes in soil moisture (as can be seen when drying the soil before soil baiting). Restricted water availability, or drought, may also critically reduce the tolerance of the host to the pathogen through its influence on the defence mechanisms of the plants. Horner (1990) suggested that severe drought may lead to a reduction in stomatal conductance and, thus, carbon gain. These reductions can result in the preferential allocation of C to primary metabolites and a decrease in the formation of secondary compounds, such as allelochemicals (Bryant *et al.*, 1983). Severe drought may therefore increase the risk of pathogen attack as well as defoliation. Performance of phyllophagous insects has been shown to increase with reduced concentrations of foliar allelochemicals (Joseph *et al.*, 1993; Schafellner *et al.*, 1994; Hättenschwiler & Schafellner, 1999). A mild water deficit, on the other hand, may lead to growth reduction prior to distinct reduction of stomatal conductance, thereby inducing an increase in C flow to secondary metabolites and increased concentrations of secondary compounds (Kramer, 1983; Horner, 1990; Lambers *et al.*, 1998).

### *5.1.2 Soil chemical properties*

Many different chemical properties of the soil have been shown to influence the aggressiveness of *Phytophthora* species. In general, *Phytophthora* diseases are considered to be more severe at higher pH values (Schmitthenner & Canaday, 1983). For *Phytophthora* species occurring in oak stands, Jung *et al.* (2000) showed that sporangia can not be formed at pH($H_2O$) values below 4.0. In addition, the production of sporangia was found to increase with increasing pH. Concentrations of Al and Ca in the soil are often inversely and intimately related to soil pH, and may also affect the aggressiveness of *Phytophthora*. High concentrations of Al have been shown to inhibit mycelial growth, as well as sporangial formation and germination in *P. capsici* and *P. infestans* (Muchovej *et al.*, 1980; Andrivon, 1995; Erwin & Ribeiro, 1996). High concentrations of Ca may suppress diseases caused by *Phytophthora* species (Von Broembsen & Deacon, 1996; 1997), but generally, the diseases are considered to be more severe at high Ca levels (Schmitthenner & Canaday, 1983; Erwin & Ribeiro, 1996). Minimum levels of Ca are necessary for zoospore production as well as cyst germination (Halsall & Forrester, 1977; Von Broembsen & Deacon, 1996; 1997; Xu & Morris, 1998), and Ca levels also affects *Phytophthora* infection of roots through its effect on zoospore taxis and adhesion to solid surfaces (Gubler *et al.*, 1989; Deacon & Donaldson, 1993). Furthermore, external Ca stimulates oospore germination (Ribeiro, 1983). As for Ca, high concentrations of Mg usually increase the severity of disease caused by *Phytophthora* (Schmitthenner & Canaday, 1983). High concentrations of K and P, on the other hand, are generally thought to decrease disease severity, or not affect it at all (Schmitthenner & Canaday, 1983; Graham, 1988; Rehm & Stienstra, 1993). The influence of cations and P may depend on the concentration of N and the ratio of the cations and P to N, since application of complete fertilizer has been shown to decrease disease severity (Schmitthenner & Canaday, 1983; Pacumbaba *et al.*, 1997).

The effect of N on disease development is variable and seems to depend on both the host–pathogen combination and the type of soil N (Klotz *et al.*, 1958; Newhook & Podger, 1972; Schmitthenner & Canaday, 1983; Utkhede & Smith, 1995; Erwin & Ribeiro, 1996). For *P. quercina* and *Q. robur*, Jung *et al.* (2003a) demonstrated a stimulation of *in vitro* production of sporangia with increasing concentrations of nitrate in the soil leachate, and an increasing difference in fine-root length and the number of fine-root tips between uninfected and infected seedlings with increasing nitrate concentration in the soil. In contrast to this, the study described in Paper IV showed higher concentrations of total N in the organic and uppermost mineral soil layers around healthy and moderately declining mature trees in *Phytophthora*-infested stands, and these trees also had higher N concentrations in the leaves than severely declining trees. It seems likely that under conditions of N limitation, the trees are favoured by good tissue N status, since it is likely to improve the trees' ability to sustain root production and replace roots destroyed by the pathogen (see Chapter 6). It may also affect the ability of the plants to produce defensive compounds (Agrios, 1997; Crawley, 1997; Lambers *et al.*, 1998). Studies of "*little leaf disease*" of shortleaf pine (*Pinus echinata*), caused by *P. cinnamomi* on degraded soils in the south-eastern USA, have shown that fertilization with N may prevent symptom development of healthy trees and improve the condition of little leaf trees in early stages of disease (Tainter & Baker, 1996). In situations of excess N, on the other hand, *P. quercina* is likely to have an advantage, since smaller amounts of carbohydrates may be available in the tree for the production of defensive compounds, and nutrient imbalances may render tissues more susceptible to pathogen infection (Marschner, 2003). A decrease in the protein precipitating capacity of trees and in the foliar concentrations of phenolics and tannins in response to increased N has commonly been observed in both gymnosperms and deciduous trees (Balsberg-Påhlsson, 1992; Muzika & Pregitzer, 1992; Joseph *et al.*, 1993; Schafellener *et al.*, 1994; Hättenschwiler & Schafellner, 1999), and Thomas & Schafellner (1999) demonstrated that the combination of excess N and drought led to a decline in foliar tannin concentration and protein precipitation capacity of pedunculate oak seedlings.

## 5.2 Biotic factors

A number of studies have shown that various biotic populations in the soil influence the severity of disease caused by *Phytophthora* species. Some organisms, such as nematodes, facilitate the infection of *Phytophthora* by creating wounds on the roots (Barham *et al.*, 1974). Others accelerate the damage already caused by *Phytophthora* through secondary infections. A third group reduces the amount of damage caused by *Phytophthora* species through parasitism on the *Phytophthora* propagules in the soil (Erwin & Ribeiro, 1996), or by exerting competitive pressure on the *Phytophthora* species, thereby excluding them from the root surface (Weste & Vithanage, 1977; Marx, 1972; Keast & Tonkin, 1983; Malajczuk, 1983). In particular, mycorrhizal colonization has been suggested to protect roots against *Phytophthora* infection (Zak, 1964; Barham *et al.*, 1974; Marx, 1972).

Zak (1964) suggested several different mechanisms by which ectomycorrhiza may protect plants from diseases caused by root pathogens. (i) The additional physical barrier formed by the mycorrhizal mantle may hinder zoospores and mycelia from penetrating roots. (ii) The utilization of surplus carbohydrates by the mycorrhizal fungi may decrease the chemotaxis of the pathogen to the root. (iii) The secretion of antibiotics by the fungus may reduce the pathogen inoculum potential. (iv) The ectomycorrhizal fungi may, together with the root, support a protective microbial rhizosphere population. Furthermore, Marx (1969) suggested that the symbiosis between the plant roots and the fungi might initiate changes in the host cells that inhibit infection and spread of the pathogen. Mycorrhizal symbiosis may also enhance plant nutrition, thereby reducing the host susceptibility to pathogen infection (Linderman, 1994). Several of these mechanisms probably act synergistically, and their relative importance probably depends on the plant species involved, the specific ectomycorrhizal fungi and the prevailing environmental conditions.

The majority of studies aimed at investigating the interactions between ectomycorrhizal fungi and *Phytophthora* species on woody plants have been performed by Marx and colleagues (Marx, 1969; 1970; 1973; Marx & Davey, 1969a, b). Using agar plates and liquid cultures, Marx (1969) demonstrated an inhibitory effect of several ectomycorrhizal species on the growth of mycelia, as well as on the motility and germination of zoospores of *P. cinnamomi*. Furthermore, the presence of fully developed mycorrhiza with a fungal mantle and a Hartig net on individual roots of shortleaf pine seedlings was seen to provide resistance to zoospore and mycelial infection, and to have a protective influence on adjacent non-mycorrhizal roots (Marx & Davey, 1969a,b; Marx, 1970). The protective effect of the mycorrhizal symbiosis was evident throughout at the whole root system of seedlings (Marx, 1973). In contrast, non-mycorrhizal roots and roots with incomplete fungal mantles were infected by the pathogen (Marx & Davey, 1969a,b; Marx, 1970; Barham *et al.*, 1974). Similar results were found by Branzanti *et al.* (1999) when investigating the importance of ectomycorrhizal fungi in the reduction of chestnut ink disease. Mycorrhizal chestnut seedlings infected with *P. cinnamomi* or *P. cambivora* showed no signs of pathogen infection and the growth of seedlings was not reduced by the presence of the pathogens in the soil. Non-mycorrhizal seedlings, on the other hand, were severely affected by the infection of *Phytophthora*.

These results suggest that the presence of other microorganisms in the rhizosphere soil may render oak trees less susceptible to infection by *Phytophthora* species, although root infection is most likely not completely inhibited (Paper III). In particular, ectomycorrhizal oak roots may be less affected by *Phytophthora* infection than non-mycorrhizal ones, provided that the symbiotic relationship is well established. However, the influence of the soil microflora on *Phytophthora* species is likely to vary substantially between sites and seasons, depending on the composition of the microbial community, the microbial activity and the growth rate of the new fine roots. Furthermore, low vitality of oak trees (Causin *et al.*, 1996; Kovacs *et al.*, 2000) and a high input of N into ecosystems (Brunner, 2001; Nilsson, 2004), have been found to influence the ectomycorrhizal colonization of roots negatively. Epidemiological studies under controlled conditions and field conditions are thus required before the significance of ectomycorrhizal colonization of roots for disease resistance of oak can be determined.

## 6. Soil Type, Carbon Allocation and the Extent of Root Damage

The negative influence of low pH and high concentrations of Al on sporangial production and mycelial growth and the necessity of minimum levels of Ca for zoospore production, cyst germination and the infection process (see Section 5.1.2), make it reasonable to assume that *Phytophthora* species would cause less severe damage to root systems of oaks growing in acid forest soils than in the artificial soil mixtures which are commonly used in pathogenicity tests. Contrary to this, the study presented in Paper II showed that seedlings of *Q. robur* were more severely affected by *P. quercina* when grown in an acid, N-rich forest soil, than when grown in a peat–sand mixture. This was expressed as a greater reduction in live fine-root length, higher proportion of dead root length and infection of coarser suberized roots (Paper II). One possible explanation of these results may be N-induced stimulation of sporangial production in the forest soil, promoting the infection of greater numbers of fine roots, thereby deteriorating the fine-root system, as was shown by Jung *et al.* (2003a) when enriching an artificial soil with nitrate. However, since the growth of seedlings in the forest soil was reduced compared with the growth of seedlings in the peat–sand mixture (Figure 6a), I hypothesize that the results are due to the influence of soil type on the C fixation and C allocation patterns in the seedlings, thereby affecting the capacity of seedlings to regenerate roots and produce defensive compounds. It is well known that mineral nutrients may affect root growth, morphology and distribution of root systems in the soil (Lambers *et al.*, 1998; Marschner, 2003). The effect is particularly pronounced with N, but also P, S, K, Mg and Mn can affect the C allocation patterns in trees, thereby influencing the relation between root and shoot growth (Ericsson *et al.*, 1996; Lambers *et al.*, 1998, Marschner, 2003). Shortage of N, P and/or S usually results in an increased allocation of C to the roots, thereby favouring root growth over shoot growth, while a shortage of K, Mg or Mn results in a decrease in the allocation of assimilates to the roots (Ericsson *et al.*, 1996; Marschner, 2003). To visualize a possible mechanism by which nutrient availability may influence the C fixation and allocation patterns within the tree, a causal loop diagram was constructed (Figure 5). The diagram is based on the theory for C allocation in trees suggested by Ericsson *et al.* (1996). The purpose of the causal loop diagram is to look at the balance between root- and shoot-growth and allocation to secondary metabolites in the oak seedlings. The model thus only describes these three pools of carbohydrates in the tree and the flow of carbohydrates between them. It does not consider various groups of compounds or individual compounds, and it does not explain the physiological processes. In addition, I assume that the seedlings are not limited by water, light or $CO_2$. The diagram is described below.

For seedlings grown in the acid N-rich forest soil, K (or possibly Mg) is likely to limit growth, since the concentration of K in the soil and in the leaves is low, especially when compared with N (Tables 1 & 2). Shortage of K, Mg, or Mn, elements that are important in photosynthesis (denoted photosynthesis nutrients in Figure 5), will result in a reduced rate of photosynthesis and subsequently, in reduced C fixation (Ericsson *et al.*, 1996). This will impede the replenishment of the pool of non-structural carbohydrates, and lower amounts of carbohydrates will then be available for growth and the production of secondary metabolites. Since the carbohydrates are formed in the photosynthetically active part of the seedlings, the available carbohydrates will probably be metabolized for growth in the above-ground part first (Ericsson *et al.*, 1996), leading to low translocation of C to the roots and, consequently, low root growth compared with shoot growth. If root growth is decreased, the uptake of base cations is likely to be further impaired. In addition, the high availability of N (or P, S) which is important to metabolize carbohydrates for growth (denoted growth nutrients in Figure 5; Ericsson *et al.*, 1996; Marschner, 2003), may reduce the amount of carbohydrates allocated to secondary

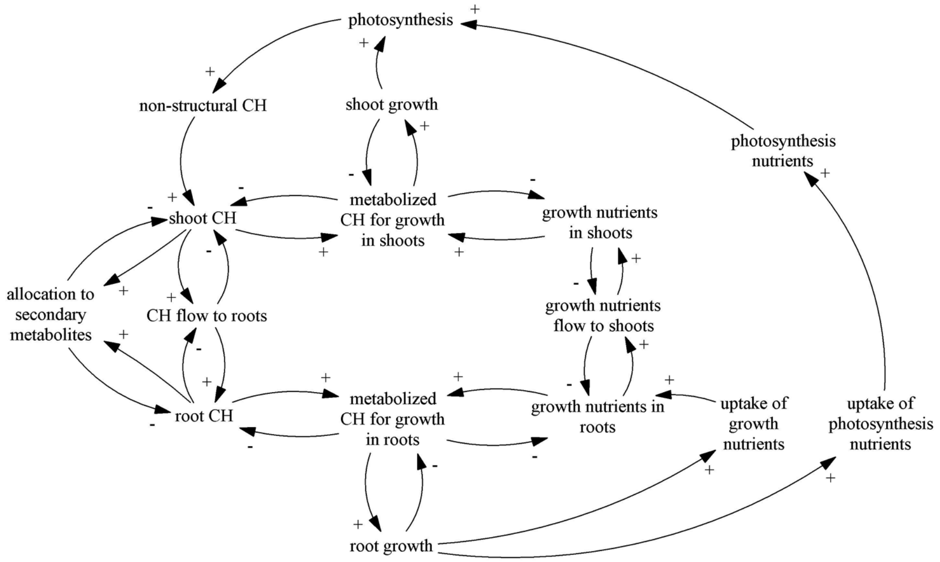

**Figure 5.** A causal loop diagram describing how soil type may influence the C allocation patterns in plants. CH = carbohydrates; photosynthesis nutrients = K, Mg, Mn; growth nutrients = N, P, S. Note that arrows only indicate the direction of the causality. An arrow with a positive causality sign implies that a change in the affecting factor drives a change in the affected factor in the same direction. Arrows with negative causality signs imply a change in the opposite direction. For further information and a description of the diagram, see Chapter 6.

**Table 1.** Concentration of chemical elements[1] in the artificial peat–sand mixture and in the acid forest soil after sterilization at 122°C (1.1 bar) for one hour.

| Element | Peat–sand mixture | Forest soil |
|---|---|---|
| Ca (μg $g^{-1}$) | 1228 | 31 |
| K (μg $g^{-1}$) | 109 | 20 |
| Mg (μg $g^{-1}$) | 113 | 6 |
| Mn (μg $g^{-1}$) | 9.3 | 113 |
| N (mg $g^{-1}$) | 0.9 | 1.4 |
| P (μg $g^{-1}$) | 33 | 39 |

[1] Concentrations of base cations and Mn were determined by an inductively coupled plasma analyzer (Perkin Elmer, Norwalk, USA) after extraction of 20 g of soil in 100 ml 0.1 M $BaCl_2$ for two hours, while P was extracted according to the Bray method. Total N was analyzed using the Kjeldahl technique (Anonymous, 1998). The analyses were based on five samples for each soil type.

**Table 2.** Leaf nutrient concentrations[1] of seedlings grown in an artificial peat–sand mixture and of seedlings grown in an acid forest soil. Values given are average values ± SD (mg $g^{-1}$) for control seedlings (n = 5).

| Element | Peat–sand mixture | Forest soil |
|---|---|---|
| Ca | 20.3 ± 3.2 | 12.5 ± 3.5 |
| K | 13.6 ± 1.8 | 8.9 ± 1.7 |
| Mg | 3.5 ± 0.5 | 3.5 ± 0.1 |
| Mn | 1.8 ± 0.6 | 8.5 ± 2.1 |
| N | 20.0 ± 2.6 | 24.0 ± 0.6 |
| P | 2.6 ± 0.5 | 1.9 ± 0.7 |
| S | 3.7 ± 0.4 | 2.9 ± 0.3 |
| Ca/N | 102.6 ± 20.8 | 52.5 ± 15.5 |
| K/N | 17.7 ± 4.2 | 14.6 ± 0.8 |
| Mg/N | 68.2 ± 6.9 | 37.1 ± 6.4 |
| P/N | 13.2 ± 2.2 | 8.1 ± 2.9 |

[1] Crumbled leaf subsamples were digested in concentrated $HNO_3$. The concentrations of the elements were then determined using an inductively coupled plasma analyzer (Perkin Elmer, Norwalk, USA).

metabolites, since a high uptake of these nutrients will result in an increased metabolization for growth, thus depleting the carbohydrate pools. Roots may therefore have less protection against pathogen infection and subsequent damage.

In the peat–sand mixture, on the other hand, N is likely to be limiting, since the soil and leaf concentrations are relatively low compared with other nutrients (Tables 1 & 2). If a growth nutrient is limiting, the available mineral is likely to be metabolized in the roots first due to the close proximity between the sites of uptake and mineral assimilation (Ericsson *et al.*, 1996). Consequently, less carbohydrates will be metabolized in the shoot and shoot growth will be reduced. A larger fraction of carbohydrates will be transported to the roots and thus be available for metabolization in the root, and subsequently root growth. Furthermore, the relatively high availability of non-structural carbohydrates is likely to lead to an increased flow through pathways that result in the synthesis of carbon-rich secondary metabolites (Lambers *et al.*, 1998), some of which may be used to restrict root infection by *Phytophthora*. The seedlings grown in the peat–sand mixture may therefore have a better protection of their roots and they may also be able to maintain a high root production, replacing the roots that are lost due to infection by *P. quercina*. The greater total biomass (Figure 6a) and the significantly higher root:shoot ratios (Figure 6b) of seedlings grown in the peat–sand mixture (control as well as infected) compared with seedlings grown in the forest soil support this hypothesis. Measuring the concentrations of some defence compounds may confirm the hypothesis.

Differences in carbon allocation patterns in trees, as a consequence of soil chemistry, is also a likely reason for the lack of difference in live fine-root length between mature oaks in stands infested with *P. quercina* and those growing in a corresponding stand where the pathogen was not present (Paper IV). The concentrations of P in the organic layer and in the mineral soil were lower in several of the stands where *P. quercina* was present than in the corresponding stand where the pathogen was not present. In accordance with the suggested mechanism in Figure 5, low availability of P is likely to lead to a high allocation of carbohydrates to root growth. Subsequently, the trees may replace the roots that are lost due to *Phytophthora* infection and may also have a high allocation of carbohydrates to defence. This is supported by the significant correlation between the concentration of P in

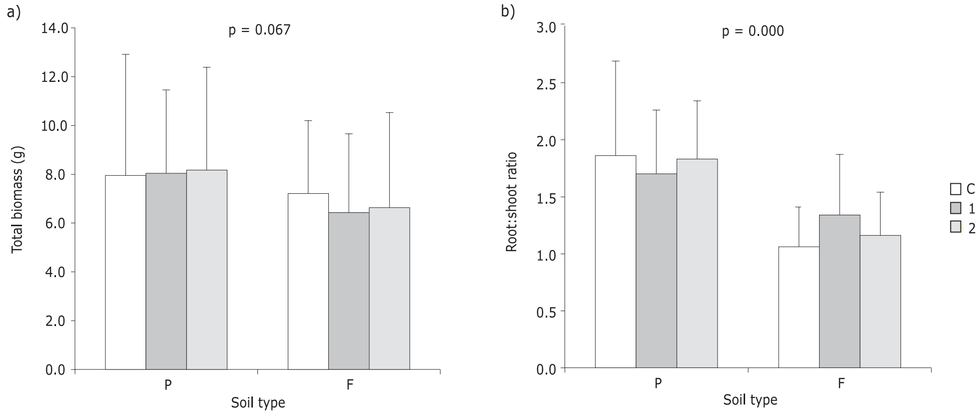

**Figure 6.** a) Total biomass and b) root:shoot ratios of control seedlings and *Phytophthora*-infected seedlings grown in an artificial nutrient-rich peat–sand mixture and in an acidic, N-rich forest soil with low concentrations of base cations. Statistics given are for soil type for two-way ANOVA. There were no significant differences between control and infected seedlings. C = control seedlings; 1 = seedlings infected with *P. quercina* isolate 1; 2 = seedlings infected with *P. quercina* isolate 2; P = peat–sand mixture; F = forest soil.

the leaves and the live fine-root length (Pearson correlation: rho = –0.77, p = 0.025, n = 8). In the severely declining trees, the continuous production of new roots have probably depleted the carbohydrate stores, and the production of new carbohydrates is obviously too low to compensate for the loss of roots, resulting in an imbalance between root death and root replacement, thereby enhancing tree decline (see also Chapter 7; Figure 7).

These results suggest that when oak growth is limited by N or P, more carbohydrates will be available for root regeneration and the production of defence compounds unless the deficiency is severe. If trees are limited by K, Mg or Mn, the amount of carbohydrates available for defence and root production will be relatively low, and rootlet death caused by *Phytophthora* may exceed rootlet replacement by the trees, thus weakening the plants and enhancing the ability of the pathogen to invade the roots. Acidification-induced reductions in soil-available K and Mg, and a high N deposition (see also Chapter 7), may therefore accelerate the damage caused by *P. quercina* on root systems of oak in southern Sweden. The influence of soil type and soil condition on symptom expression and disease incidence is well-known in *little leaf disease* of shortleaf pine (*Pinus echinata*), caused by *P. cinnamomi* in the south-eastern USA (Oak & Tainter, 1988; Tainter & Baker, 1996) and in New Zealand (Newhook & Podger, 1972).

## 7. A Conceptual Model for the Disease Development in Oak

Based on the literature presented in Chapter 5, describing how different abiotic and biotic factors affect *Phytophthora* species and the inter-

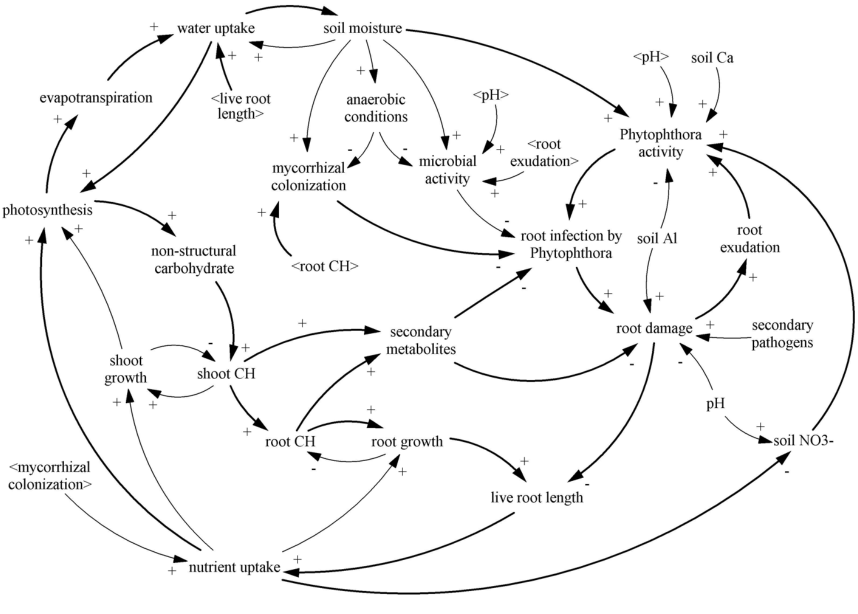

**Figure** 7. A conceptual model of how different abiotic and biotic factors influence the aggressiveness of *P. quercina*, the susceptibility of *Q. robur* and the subsequent disease development. The variable "*Phytophthora* activity" includes processes that affect the capacity of the pathogen to infect roots (such as zoospore production, dispersion, taxis, adhesion and cyst germination); CH = carbohydrates. Note that arrows only indicate the direction of the causality. An arrow with a positive causality sign implies that a change in the affecting factor drives a change in the affected factor in the same direction. Arrows with negative causality signs imply a change in the opposite direction. Thick arrows indicate major feedback loops in the system. For further information and a description of the causal loop diagram, see Chapter 7.

action between the pathogens and their hosts, a conceptual model was constructed (Figure 7). The aim of this simplified model was to give an overview of how the different abiotic and biotic factors act simultaneously on the pathogen–host system, sometimes accelerating disease development, and sometimes hindering it. The model is applicable for the interaction between *Phytophthora* species and *Quercus* species growing in acid forest soils in a temperate climate (similar to southern Swedish conditions). Only effects of *Phytophthora* on the fine-root system have been considered. For a more thorough description of the C allocation in trees, see Chapter 6 (Figure 5). Note that the variable "microbial activity" does not include pathogens, since these are considered separately, and that the arrows in the causal loop diagrams show only the direction of the causality. An arrow with a positive causality sign implies that a change in the affecting factor drives a change in the affected factor in the same direction (i.e. an increase leads to and increase, and a decrease leads to a decrease). Arrows with negative causality signs imply a change in the opposite direction (i.e. an increase leads to a decrease, and a decrease leads to an increase). Temperature has not been included in the figure, since it is likely to influence *Phytophthora* activity, microbial activity and physiological processes in the tree in a similar direction. Note that increased soil moisture does not necessarily imply increased uptake of water. However, the link has been included since a low availability of water will lead to a reduced water uptake.

The causal loop diagrams in Figure 7 can be explained as follows. High soil moisture, as a result of heavy rain or prolonged periods with rain, will increase the production and dispersion of *Phytophthora* zoospores (the infecting unit), thereby increasing the probability of root infection and consequently also root damage. Root damage may lead to an increased exudation of carbohydrates from the roots, thereby facilitating zoospore taxis of *Phytophthora* (Carlile, 1983). When the zoospores can find the roots easily, this is likely to result in a higher degree of infection and thus an increase in the amount of damage. A high amount of root damage results in a reduction in the amount of live fine-root length, unless high production replaces the roots that are lost. If the live fine-root length is decreased, the uptake of water and nutrients will be impaired. Shortage of nutrients that are important in photosynthesis will lead to reduced C fixation, and subsequently reduced tree vitality (see Chapter 6; Figure 5). However, increased root exudation may also enhance the microbial activity. This increase in microbial activity may reduce the root infection by *Phytophthora*, since *Phytophthora* species usually are weak competitors (Tsao, 1990). Flooding of soils for a long period of time may result in anaerobic conditions in the soil, which may affect the activity of the soil microbial community (including mycorrhizal fungi) negatively, thereby increasing the probability of *Phytophthora* infection of roots. Furthermore, the lack of $O_2$ may cause direct damage to the roots, although *Q. robur* is believed to be relatively tolerant to flooding (Colin-Belgrand *et al.*, 1991).

Low soil moisture, or drought, has the reverse effect on sporangial production and the dispersion of zoospores, and may thus decrease the degree of root infection. However, drought also influences the soil microbial community negatively, thereby increasing the potential for *Phytophthora* to infect roots in the event of a subsequent increase in soil moisture, since these pathogens respond extremely quickly to such an increase. Furthermore, severe drought may decrease the rate of photosynthesis (Horner, 1990; see also Section 5.1.1), and subsequently impede the replenishment of the carbohydrate pools in the plant. If the pools of carbohydrates decrease, the allocation of C to the production of defence compounds decreases, and this may increase the ability of *Phytophthora* to infect roots in the event of an increase in soil moisture, and render tissue more susceptible to damage once infection has taken place.

Accumulation of N in the soil may influence the development of the disease through the positive effect of nitrate on zoospore production. In addition, high uptake of N is likely to increase the allocation of C to growth, particularly shoot growth (Marschner, 2003), and subsequently decrease the relative amount of C available for root growth and the production of secondary compounds (see also Chapter 6; Figure 5). Root tissue may thereby become more susceptible to root infection and damage. Low C allocation to root growth may also lead to a reduced ectomycorrhization and growth of external mycelia (Brunner, 2001), thereby increasing the potential for *Phytophthora* to infect roots. Other soil chemical characteristics may also influence disease development (see Section 5.1.2). Low concentrations of Ca, high concentrations of Al and low pH all influence zoospore production negatively, thereby decreasing the probability of root infection. Low concentrations of Ca may also influence zoospore adhesion to solid surfaces, zoospore taxis and oospore germination negatively. However, low concentrations of Ca may also increase the susceptibility of the plant to root infection, due to the necessity of Ca for structural growth in cell walls and plasma membranes (Marschner, 2003). In addition to influencing zoospore production negatively, low pH and high concentrations of Al are known to have negative effects on root elongation and cell division (Marschner, 2003; Puhe, 2003) and to influence the microbial activity negatively. Regarding the influence of other nutrients (K, Mg, P), which have no effect, or variable effects, on *Phytophthora*, but may influence the C allocation patterns in plants, see Chapter 6 (Figure 5).

Secondary pathogens often follow infection of roots by *Phytophthoras*, and may exacerbate the root damage, thus increasing the carbohydrate exudation from the roots, and, may thereby influence the activity of *Phytophthora* as well as other microbes. Decreased tree vitality (defined as increased crown defoliation) will result in a reduction in photosynthesis, thereby reducing the pool of available carbohydrates. Subsequently, less carbohydrates will be available for growth and defence.

In conclusion, *P. quercina* has the ability to reduce the live fine-root length of oak substantially, but as long as the tree can sustain new root production to replace the lost roots, nutrient and water uptake will not be affected. However, continuous root infection may gradually deplete the carbohydrate stores, and less carbohydrates will then be available for root and shoot growth and defence. The susceptibility of the tree to pathogen infection as well as to other types of stress will thus increase. If environmental conditions are favourable for the pathogen, the live fine-root length is likely to eventually decrease. A reduced fine-root length, together with the infection of coarser roots, will result in a lower capacity for uptake and transport of water and nutrients. This may result in a reduction in the rate of photosynthesis, and smaller amounts of carbohydrates will thus be available to regenerate the root system, as well as for the production of secondary metabolites. Subsequently, tree vitality will decrease, as disease development is accelerated through negative feedback loops. The range of field responses to *Phytophthora*, from slow decline to sudden death of trees, is likely to reflect differences in the interaction between the pathogen and the host, as a consequence of their response to the different abiotic and biotic factors presented in Figure 7.

## 8. Oak Decline in Southern Sweden – A Hypothesis

The ability of *P. quercina* to infect and cause substantial root damage to both seedlings and mature trees in forest soils under varying environmental conditions suggests that this pathogen contributes to oak decline in southern Sweden. Since both pathogen aggressiveness and host susceptibility are strongly influenced by various abiotic and biotic factors, a combination of events is probably necessary for decline to occur as a consequence of *Phytophthora* infection. Severe flooding, drought, low availability of K and Mg in the soil or high availability of N may all enhance the negative effects of *P. quercina* (see Chapter 5 & 7). However, many of the sites from which *Phytophthora* species were not recovered also show severe symptoms of crown damage. This suggests that the decline of oaks in southern Sweden cannot be attributed to this single factor.

During the past decades, an extensive acidification of southern Swedish forest soils has taken place, leading to a diminishing nutrient capital, particularly of base cations, in the soil (Falkengren-Grerup, 1987; Falkengren-Grerup *et al.*, 1987; Tamm & Hallbäcken, 1988). A low availability of base cations, or other nutrients, might at elevated concentrations of N, result in nutrient imbalances in the tree (Nihlgård, 1985; Thelin, 2000). As a consequence, tree vitality may decrease. In the oak stands investigated in this work, no significant differences in soil acidity, the concentration of base cations or the concentration of N in the soil were found between healthy and declining trees, or between healthy and declining stands (Papers IV & V). Instead, leaf chemical analysis and the patterns of root growth indicated that trees were N or P limited (Paper IV). However, a large-scale survey of 109 oak stands in southern Sweden showed that stands growing in soils with very low base saturation values (<10%) had significantly higher crown defoliation than stands growing in soils with normal base saturation values (>20%; Sonesson & Anderson, 2001). Oak vitality therefore seems to be related in some way to nutrient availability in the soil, at least in certain regions of southern Sweden. Possibly, the low availability of base cations have led to a decrease in the C fixation of the trees (see Chapter 6; Figure 5), which may have caused a reduction in the production of secondary metabolites in the trees, thereby predisposing them to factors such as pathogens or frost. Considering the low concentrations of base cations in the oak forest soils in southern Sweden, continued deterioration of this cation pool seems likely to further destabilize the oak ecosystems.

Another factor that is probably involved in oak decline is climate (Barklund & Wahlström, 1998; Paper V). Severe bark necrosis, due to frost damage caused by the unusually cold winters at the end of the 1980s was suggested to be the initial cause of oak decline in southern Sweden (Barklund & Wahlström, 1998; Barklund, 2002). In Paper V, significant positive correlations are reported between the average crown defoliation as well as the proportion of damaged trees and average summer precipitation and average annual precipitation for the years 1984 to 1999 in 21 oak stands in the southernmost part of the country. The interpretation of the data is complicated, since many different factors may be correlated with precipitation, and the measurements of precipitation were only rough estimates, but, together with the study by Barklund & Wahlström (1998), the results indicate that extreme weather events during the 1980s and 1990s, with fluctuations between years with exceptionally high precipitation, years with hot, dry summers and years with low winter temperatures, may have affected the trees negatively and predisposed them to the action of other abiotic and biotic stress factors.

Several studies carried out in central Europe have emphasized the necessity of defoliators for oak decline to occur (e.g. Thomas *et al.*, 2002). The most important defoliators of oak in Sweden are *Tortrix viridana*, *Erannis defoliaria* and *Operophthora brumata* (Barklund, 2002). Repeated defoliation by these insects is likely to result in the depletion of carbohydrate stores, followed by reduced growth and reduced production of secondary metabolites. Thus, the susceptibility of trees to other stress factors will increase. Attack by the ascomycete *Microsphaera alphitoides* on the second flush of leaves may further increase the susceptibility of the tree. In later stages of decline, when the tree is already severely weakened, several different secondary pathogens may infect the tree, occasionally causing the death of the tree. Apart from the factors already mentioned, forest management is likely to be involved in the decline process of oaks through attempts to achieve high production at sites that are not especially suitable for oak, or by neglecting to perform measures necessary to support good growth.

Based on the research hitherto performed in southern Sweden, it appears that several different factors may be involved in oak decline. In some cases, one single factor may be responsible for the decline at a particular site. However, the nonspecific symptoms of disease in trees, and the inconclusive results when attempting to find causal factors, suggest that at most sites, several of these factors interact with each other, causing a complex development of disease. Climatic fluctuations, low availability of base cations and forest management probably act as predisposing factors, increasing the susceptibility of the tree to the actions of inciting factors such as *Phytophthora* species and defoliating insects. After the tree has become substantially weakened, secondary pathogens may infect the trees, thereby contributing to the decline, and occasionally causing death of the tree.

A conceptual model describing how the factors discussed above (infection by *P. quercina*, low availability of base cations in the soil, climatic extremes, defoliators and secondary pathogens) may affect oak vitality and how they may interact with each other in causing decline of oaks in southern Sweden is shown in Figure 8. I would like to emphasize that the model describes a possible chain of events and is only intended as a starting point for further discussion. More thorough investigations are needed to determine the importance of the factors included, and to evaluate whether there are other causal factors that are not yet described. For visibility, only the most prominent links between factors are included. For more information regarding *Phytophthora* activity, tree susceptibility and C allocation in trees, see Chapters 5, 6 and 7. Note that increased soil moisture or availability of base cations will not necessarily result in an increased uptake of water and cations. However, the links have been included since low availability of water or base cations will lead to reduced uptake. The model can be explained as follows. If defoliating insects attack a tree, the amount of foliage will decrease, with a subsequent decrease in the ability of the tree to photosynthesize. New foliage will therefore have to be produced using stored carbohydrates. If defoliation is repeated, the carbohydrate stores are likely to become depleted. Together with the reduction in leaf area, this will reduce the amount of carbohydrates available for the production of

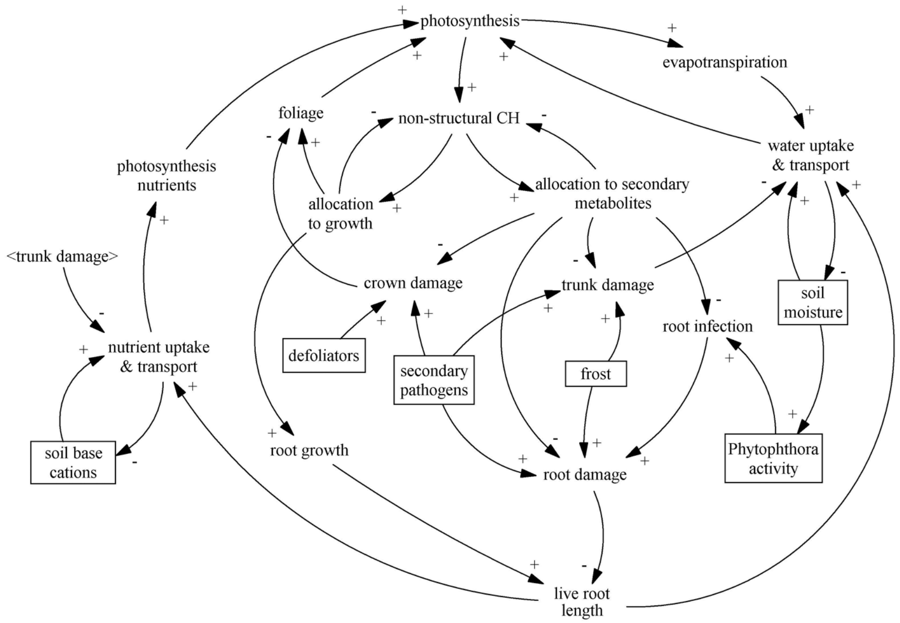

**Figure 8.** A conceptual model of how the different factors hypothesized to be involved in southern Swedish oak decline may interact with each other and affect tree vitality. The suggested causal factors are indicated with a frame. The variable "*Phytophthora* activity" includes processes that affect the capacity of the pathogen to infect roots (such as zoospore production, dispersion, taxis, adhesion and cyst germination); CH = carbohydrates; photosynthesis nutrients = K, Mg, Mn. Note that arrows only indicate the direction of the causality. An arrow with a positive causality sign implies that a change in the affecting factor drives a change in the affected factor in the same direction. Arrows with negative causality signs imply a change in the opposite direction. For further information and a description of the causal loop diagram, see Chapter 8.

secondary metabolites, thus increasing the susceptibility of the tree to other stress factors, such as pathogens or frost. Severe drought may also reduce the rate of C fixation through its negative influence on the stomatal conductance of leaves (Kramer, 1983; Horner, 1990). Less carbohydrates will then be available for growth and defence. Water fluctuations in the soil, such as alternating periods of flooding and drought, may enhance the effects of a prospective drought on the tree by causing the oaks to develop a more shallow root system (Oosterbaan & Nabuurs, 1991). If *Phytophthoras* are present in the soil, the production of zoospores will increase rapidly when soil moisture increases after a drought. The ability of *Phytophthora* to infect root tissue will then be high, at the same time as the ability of the tree to defend itself and replace roots lost due to pathogen attack will be low. A reduction in the amount of live fine roots will impede the ability of the tree to take up water and nutrients, and may thereby affect the C fixation negatively. Apart from defoliators and soil moisture, shortage of certain base cations may also affect photosynthesis negatively, since K and Mg are important for stomata regulation, capture of light energy and functioning of enzymes central to carbon fixation (Ericsson *et al.*, 1996; Marschner, 2003; Figure 5). Frost may cause necrosis of both trunks and roots, thereby possibly reducing the water and nutrient uptake, and also providing a gateway for secondary pathogens.

In conclusion, all the factors suggested to be of importance in southern Swedish oak decline are likely to eventually influence the production of carbohydrates, and thereby the ability of the tree to replace lost tissue and to produce secondary metabolites. The susceptibility of the tree to the other stress factors will thus increase, and tree vitality will decrease. The rate of tree decline will vary depending on how many of these factors are acting on the tree simultaneously, and on how they interact with other abiotic and biotic factors present at a site. With regard to *P. quercina*, low availability of certain base cations in the soil, periods of drought or excessive flooding, and defoliation by insects, are likely to accelerate the development of disease.

## 9. Conclusions

Based on the results presented in this thesis, I have drawn the following conclusions.

(i) *Phytophthora* species are present in southern Swedish oak stands. The most frequently recovered species is the oak-specific *P. quercina*. The oak forest soils in which *Phytophthora* species occurred had a somewhat higher rhizosphere pH and a higher base saturation than the forest soils in which the pathogens were not present.

(ii) The southern Swedish isolates of *P. quecina* can infect roots and cause damage to fine roots as well as coarser roots of *Q. robur* seedlings in acidic forest soils under a mesic water regime. Substantial damage was caused both in the presence and in the absence of the inherent soil microflora. In contrast to what was expected, the pathogen caused more damage to the root system of seedlings grown in an acid forest soil than to seedlings grown in a nutrient-rich artificial mixture of peat and sand with a higher pH. It is hypothesized that the more severe damage in the forest soil is due to low availability of certain base cations (K, Mg) in relation to N in this type of soil. A low availability of these cations may reduce the rate of photosynthesis of seedlings and may also affect the allocation patterns in the plant, thereby reducing the seedlings' ability to produce defence compounds and to replace roots that are lost due to pathogen infection.

(iii) *P. quercina* can infect and cause significant damage to root systems of mature trees. The effect of the pathogen seemed to depend on tree vitality and climatic conditions. In stands without *P. quercina*, the length of the fine-root system did not differ between trees of differing vitality, demonstrating that fine-root decay does not necessarily occur prior to noticeable above-ground symptoms in oaks.

(iv) Although a significant reduction in live fine-root length of seedlings infected with *P. quercina* was observed, no significant effects on above-ground growth or on leaf nutrient concentration could be detected after the short-term greenhouse experiments. There were also few differences in leaf nutrient concentration between mature oaks of differing vitality. This is consistent with previous studies, suggesting that root rot must usually be severe before above-ground effects in trees can be detected.

(v) A weak association was found between vitality of oak stands and occurrence of *P. quercina*, indicating that oak stands were more likely to be declining if *P. quercina* was present in the soil.

The observation that *P. quercina* causes substantial root damage to both seedlings and mature trees in forest soils suggests that this pathogen is involved in oak decline in southern Sweden. The continuous replacement of roots lost due to pathogen infection is likely to gradually deplete the carbohydrate reserves of the tree, and may subsequently result in a reduction in root and shoot growth, as well as in an increased susceptibility of the tree to further infections and other types of stress. Since the effects of *P. quercina* on root systems of oak are highly dependent on the prevailing environmental conditions, the development of the disease is likely to vary substantially depending on geographical location, season and soil type. However, under severe environmental conditions, epidemic development with die-back of oaks seems possible. In some southern Swedish oak stands, *P. quercina* may be the factor of greatest importance in the decline of the stand. In most stands, tree decline is more likely to be due to a combination of abiotic and biotic factors, which gradually decreases the vitality of the tree, occasionally resulting in its death.

## 10. Future Perspectives

Despite a substantial improvement in our knowledge concerning the involvement of *Phytophthora* species in oak decline and in our general understanding of the interactions between these pathogens and their hosts during the past decades, there are still significant gaps that need to be filled regarding the response of pathogen aggressiveness, host susceptibility and subsequent disease severity to various abiotic and biotic factors. A possible reduction of pathogen infection as a consequence of extensive mycorrhizal colonization of roots or high microbial activity in the soil remains to be confirmed for *Phytophthora* species and oaks. This is highly interesting when considering oak decline, since some studies have indicated reduced mycorrhizal colonization of declining oaks (Causin *et al.*, 1996; Kovacs *et al.*, 2000), thereby possibly enhancing their susceptibility to *Phytophthora* infection. Since the response to different abiotic factors may vary depending on the specific species combination, supplementary studies on how increasing concentrations of soil N and Al and decreasing soil concentrations of base cations affect the aggressiveness of *P. quercina* and, equally important, the susceptibility of *Q. robur*, are needed. In addition, it would be interesting to evaluate the effects of soil amelioration, such as liming, on disease development, since this kind of treatment may be necessary in the future to improve soil quality in the areas most exposed to soil acidification.

Regarding oak decline in general, more studies are required to evaluate the interactions between several different abiotic and biotic factors. Today, few studies exist in which three or more factors included in a hypothetical model have been tested together. Greenhouse experiments on defoliation followed by drought and infestation by *Phytophthora*, or a high input of N, followed by defoliation and pathogen infestation, would be very interesting in order to evaluate whether symptoms similar to those observed in the field can be artificially induced.

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