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Pathogen profile

Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. *s.l.*

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SUMMARY

The root and butt rot caused by *Heterobasidon annosum* is one of the most destructive diseases of conifers in the northern temperate regions of the world, particularly in Europe. Economic losses attributable to *Heterobasidion* infection in Europe are estimated at 800 million euros annually. The fungus has been classified into three separate European intersterile species P (*H. annosum*), S (*H. parviporum*) and F (*H. abietinum*) based on their main host preferences: pine, spruce and fir, respectively. In North America, two intersterile groups are present, P and S/F, but these have not been given scientific names. The ecology of the disease spread has been intensively studied but the genetics, biochemistry and molecular aspects of pathogen virulence have been relatively little examined. Recent advances in transcript profiling, molecular characterization of pathogenicity factors and establishment of DNA-transformation systems have paved the way for future advances in our understanding of this pathosystem.

Taxonomy: *Heterobasidion annosum* (Fr.) Bref., *H. parviporum* Niemelä & Korhonen and *H. abietinum* Niemelä & Korhonen; kingdom Fungi; class Basidiomycotina; order Aphyllophorales; family Bondarzewiaceae; genus *Heterobasidion*.

Identification: presence of the fungus fruit bodies, basidiocarps whitish in the margins, upper surface is tan to dark brown, usually irregular shaped, 3.5 (–7) cm thick and up to 40 cm in diameter; pores 5–19, 7–22 and 13–26 mm^2 for the P, F and S groups, respectively. Small brownish non-sporulating postules develop on the outside of infected roots. Asexual spores (conidiospores) are 3.8–6.6 × 2.8–5.0 μm in size. Mating tests are necessary for identification of intersterility groups.

Host range: The fungus attacks many coniferous tree species. In Europe, particularly trees of the genera *Pinus* and *Juniperus* (P), *Picea* (S), *Abies* (F) and in North America *Pinus* (P) and *Picea*, *Tsuga* and *Abies* (S/F). To a lesser extent it causes root rot on some decidous trees (*Betula* and *Quercus*).

Disease symptoms: symptoms (e.g. exhudation of resin, crown deterioration) due to *Heterobasidion* root rot in living trees are not particularly characteristic and in most cases cannot be distinguished from those caused by other root pathogens. *Heterobasidion annosum s.l.* is a white rot fungus. Initial growth in wood causes a stain that varies in colour depending on host tree species. Incipient decay is normally pale yellow and it develops into a light brown decay to become a white pocket rot with black flecks in its advanced stage.

Control: silvicultural methods (e.g. stump removal), chemicals (urea, borates) and biological control agent (*Phlebiopsis gigantea*, marketed as PG Suspension® in the UK, PG IBL® in Poland and Rotstop® in Fennoscandia) are commonly used approaches for minimizing the disease spread.

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INTRODUCTION

Conifer trees colonize the earth to a greater extent than any other plant group and include some of the most important tree species in the northern hemisphere. Timber from conifers forms the basis for one of the largest industries in Europe, contributing *c.* 100 billion euros net export income yearly and the value on a global perspective is estimated at 370 billion dollars (http://english.forestindustries.fi). One of the most destructive conifer diseases is root and butt rot caused mainly by basidiomycete decay fungi (Allen *et al.*, 1996) including *Heterobasidion annosum*, *Armillaria* spp. (Shaw and Kile, 1991) and *Phellinus* spp. (Hansen and Goheen, 2000). However, this review is primarily focused on annosum root rot. Most conifer trees are susceptible to infection by the basidiomycete *H. annosum*, causative agent of a root and butt rot disease (Fig. 1a–d), and widely regarded as the most economically important forest pathogen in temperate forests of the northern hemisphere. Early descriptions of the fungus were published by Fries (1821) who gave the fruit body the name *Polyporus annosum*; no connection to any tree disease was made. The first reports characterizing and linking

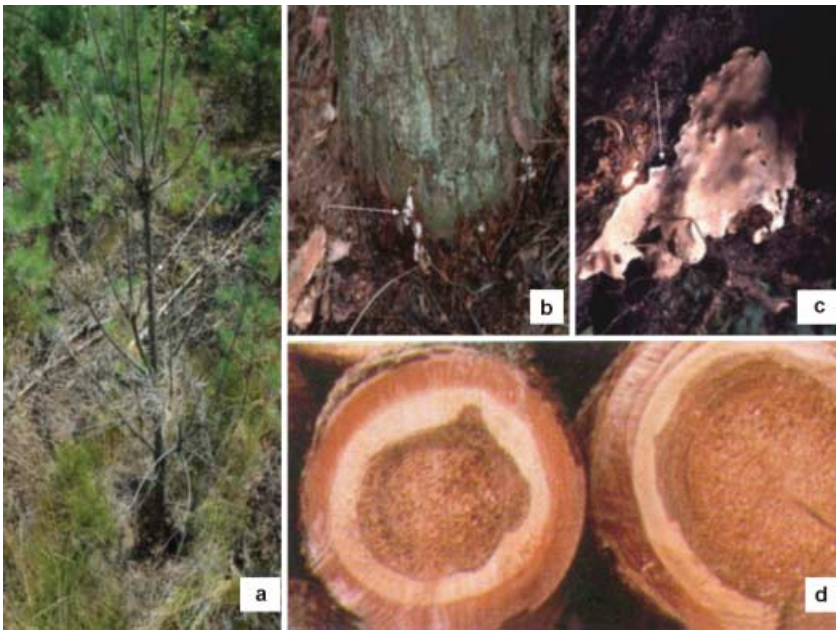

Fig. 1 (a) A dead pine tree killed by *Heterobasidion annosum*. (b) Fruit body of *H. annosum* at the base of the trunk. (c) Enlarged fruit body of *H. annosum*. (d) Decayed tree trunk due to *H. annosum* infection.

H. annosum to root and butt rot disease of conifers were published by Hartig (1874). Before the end of the 19th century, investigators gave various names to the root and butt rot fungus, including *Fomes annosus* by H. Karsten. Brefeld (1888) created a new genus named *Heterobasidion* but still retained the original species name (*annosum*) as given by Fries. Despite the new taxonomic descriptions, *Fomes annosus* remained the dominant name for the fungus for most of the twentieth century (Hodges, 1969; Hüttermann and Woodward, 1998). Currently, eight distinct taxonomic species have been described within the genus *Heterobasidion*: *H. annosum*, *H. parviporum*, *H. abietinum*, *H. araucariae*, *H. insulare*, *H. pahangense*, *H. perplexum* and *H. rutilantiforme* (Niemelä and Korhonen, 1998).

Taxonomy, phylogeny and geographical distribution of *H. annosum* complex

The *H. annosum* complex has a wide geographical distribution (Fig. 2) particularly in many parts of Europe, North America, China and Japan (Dai and Korhonen, 1999; Dai *et al.*, 2003). Besides *H. annosum*, the other species are known from East Asia, Australia and adjacent areas (Niemelä and Korhonen, 1998). *Heterobasidion araucariae* Buchanan has been reported from eastern Australia, New Zealand, New Guinea and the Fiji Islands (Fig. 2) where it lives as a saprotroph on dead wood of *Araucaria*, *Cunninghamia* and *Pinus* (Niemelä and Korhonen, 1998). *Heterobasidion insulare* (Murr.) Ryvarden has been reported from eastern and southern Asia: Russian far east and Japan in the north, Philippines, Borneo and New Guinea in the south, and Nepal and India in the west (Fig. 2) (Niemelä and Korhonen, 1998).

The fungus was long considered to represent a single species until mating experiments revealed host specialized intersterile groups (IGs) within *H. annosum* (Capretti *et al.*, 1990; Chase and Ullrich, 1988; Korhonen, 1978). The intersterility groups were classified as P, S and F-types based on their host preferences. In Europe, P-type shows preference for Scots pine (*Pinus sylvestris*), but attacks many other conifers and broad-leaved trees as well. It occurs all over the continent except on sites north of 64°N (66°N in Norway) as well as the driest areas in the south (Capretti *et al.*, 1990; Korhonen and Stenlid, 1998; Korhonen, 1978; La Porta *et al.*, 1997; Stenlid, 1987). The S-type shows relatively strict specialization for Norway spruce (*Picea abies*) and occurs primarily in areas where Norway spruce grows naturally. The major host of the F-type is silver fir (*Abies alba*) although it can infect other hosts as well and it occurs where *Abies* species grow naturally in southern and central Europe. Interestingly, in eastern Europe, the S-type rather than the F-type infects *Abies sibirica* (Korhonen *et al.*, 1997). In China, two separate populations of the S-type exist in the northern and southern conifer regions (Korhonen *et al.*, 2001). In North America, the P-type attacks *Pinus* species both in eastern and in western forests, but is less common in the central parts of the continent. It occurs in the west from British Columbia to Mexico and in the east from Quebec to Florida (Korhonen *et al.*, 1998a). The S-type has been found only in western North America, from Alaska in the north to California in the south, where it attacks mainly *Abies*, *Tsuga*, *Picea*, *Pseudotsuga* and *Sequoiadendron* (Harrington *et al.*, 1989; Korhonen and Stenlid, 1998; Korhonen *et al.*, 1998a; Otrosina *et al.*, 1993). The phylogeny within the *H. annosum* complex has recently been studied using isozymes (Karlsson and Stenlid, 1991), polymorphism in ITS

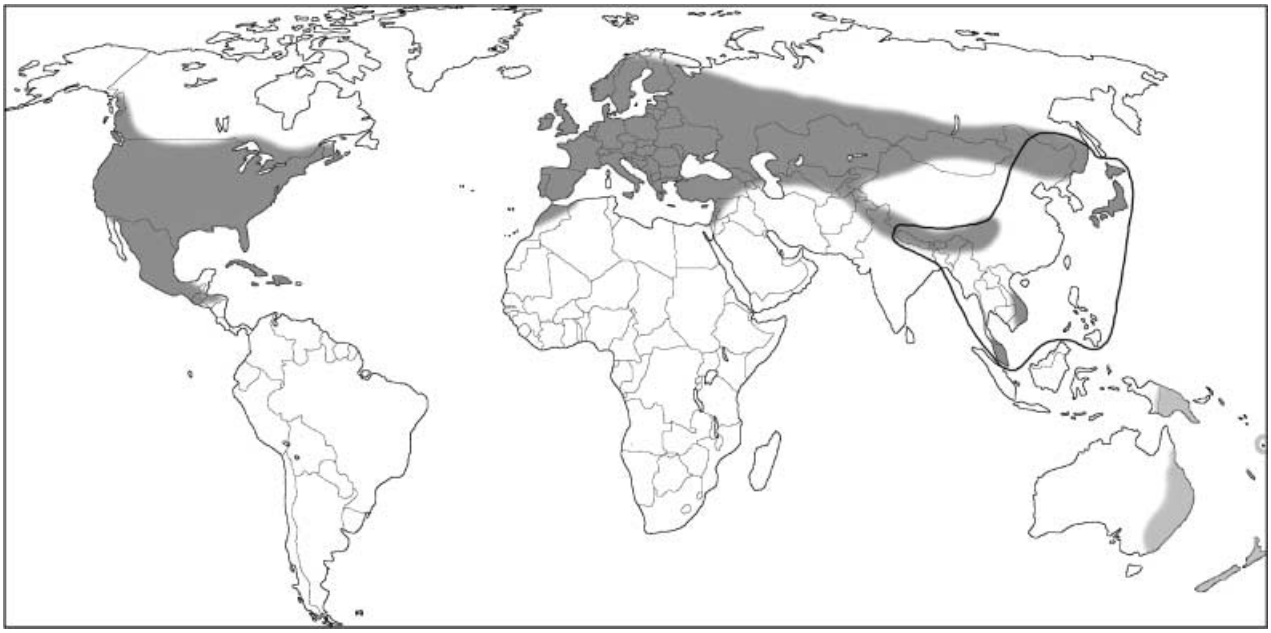

Fig. 2 Global distribution of *Heterobasidion annosum* complex (dark shaded areas). The distribution area of *H. araucariae* (light shaded) and *H. insulare* (line drawing) is also shown. This figure was kindly provided by Kari Korhonen.

regions (Chase *et al.*, 1991; Harrington *et al.*, 1989), RFLP analyses of interribosomal gene regions, RAPD and DNA sequence analyses of ITS and IGS regions (DeScenzo and Harrington, 1994; Kasuga and Mitchelson, 1994; Kasuga *et al.*, 1993; Stenlid *et al.*, 1994), house keeping genes (Johanesson and Stenlid, 2003), manganese peroxidase (Maijala *et al.*, 2003) and laccases (Asiegbu *et al.*, 2004). The results indicate that the three European (P, S, F) and the North American (P, S) groups form separate clades. New scientific names have now been proposed for the three European intersterility groups P, S and F: *H. annosum*, *H. parviporum* and *H. abietinum*, respectively (Niemelä and Korhonen, 1998). Although separated on different continents for a long period of time (Johanesson and Stenlid, 2003), the North American and European P groups are morphologically indistinguishable (Korhonen and Stenlid, 1998) and fully interfertile (Karlsson and Stenlid, 1991). Furthermore, they also share similar broad host preferences and are thus probably best regarded as two subpopulations of the same species. An interesting observation of intercontinental introduction of the American P group into Italy was recently reported (Gonthier *et al.*, 2004). Based on distinctive mitochondrial markers, the authors concluded that the fungus was probably introduced with woody material to a military camp during the Second World War, thereby creating an opportunity for gene flow between the two P group populations. The taxonomic status of the North American S group is less clear; it is partly interfertile with both the S and the F groups from Europe, but has a distinct evolutionary history and in contrast to its European relatives it has a broad host range.

The intersterility in *H. annosum s.l.* is controlled by a genetic system consisting of at least five loci: P, S, V1, V2 and V3 (Chase and Ullrich, 1990). Similar + alleles at any of the loci allow for mating between two homokaryotic strains. This system opens up possibilities for hybridization between the intersterility groups (Garbelotto *et al.*, 2001; Olson and Stenlid, 2001, 2002). Hybrid mycelia have been detected in the field (Garbelotto *et al.*, 2001) and laboratory tests show that heterokaryons carrying nuclei of the American P and S types express the pathogenicity representative of the parent cytoplasm (Olson and Stenlid, 2001). Although the genetic background for interfertility between species in Europe has not been formally sorted out, an interesting study on higher degree of intersterility was reported between the S and F group populations growing in sympatry in northern Italy as compared with Italian F populations and Finnish S populations (Capretti *et al.*, 1990; Korhonen *et al.*, 1997). It would be of interest to study whether selection against hybrids has driven the alpine *H. parviporum* and *H. abietinum* into more distinctive intersterility gene genotypes as compared with the allopatric northern European *H. parviporum* vs. *H. abietinum*. In addition to fascinating possibilities for reticulate evolution, the hybridization also allows for genetic analysis of pathogenicity traits. The first steps have been taken for QTL analysis of pathogenicity by analysing progeny of such hybrids (M. Lind *et al.*, in preparation).

Life cycle and aetiology

The fungus produces both conidiospores and basidiospores but the primary infection is mediated by basidiospores that infect fresh stump surfaces or wounds on the roots or stem (Redfern and Stenlid, 1998; Rishbeth, 1959). Although conidiospores have

been reported to be present in air (Hsiang *et al.*, 1989), their role in the spread of the fungus in nature is unclear (Korhonen and Stenlid, 1998) and they are most likely to be important for short-distance transmission in substrates or vectored by root-feeding insects (Kadlec *et al.*, 1992). In temperate regions, the basidiospores are mainly released during summer when tree stumps are most susceptible to infection (Redfern and Stenlid, 1998). The fungus establishes much less frequently when the temperature drops below 5 °C, either because of lack of basidiospores, inability to grow at low temperature or a combination of both (Meredith, 1959). Similarly, the fungus does not infect during periods when the substrate temperature exceeds +35 °C (Ross, 1973). In Europe, homokaryotic mycelia developing from basidiospores may live on stumps or logs for many years without causing disease in a living tree (Stenlid, 1994). But virulent homokaryons have been demonstrated in the natural forest habitat in North America (Garbelotto *et al.*, 1997a) and in pure culture (Olson and Stenlid, 2001). Following stump colonization, the fungus has been reported to spread via root contacts from infected to healthy trees (Fig. 3). The spores are not known to infect healthy uninjured roots, although they may be able to penetrate bark to a limited extent (Peek *et al.*, 1972). However, supported from a nutrient base in already colonized root material, the vegetative mycelium can infect healthy uninjured trees by growth through root contacts or grafts (Fig. 3). In nature, the infection of fine roots has been reported to be rare (Moller, 1939; Schönhar, 1992; Siepmann, 1981) but has been demonstrated in pure culture (Asiegbu *et al.*, 1993, 1994) and non-suberized lateral roots (Heneen *et al.*, 1994), which suggests that the pathogen may be capable of infecting conifer roots of all ages. *Heterobasidion annosum* grows necrotrophically within the sapwood of living trees but with time will expand readily in the heartwood in most species except for pines where the heartwood extractives are fungistatic (Korhonen and Stenlid, 1998). The rate of spread varies depending on vitality of the trees and moisture content of the wood and can reach 2 m yr^{-1} in roots (Rishbeth, 1962) and 1 m yr^{-1} in stems (Huse and Venn, 1994).

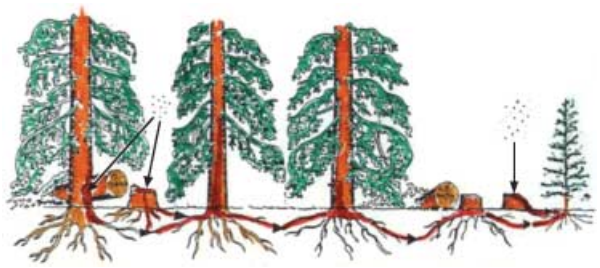

Fig. 3 A schematic illustration of the infection biology of *Heterobasidion annosum* under natural conifer forest habitat. Spores fall on freshly cut stumps (arrows), germinate, form infective hyphae (red colour) and invade the stumps, spreading to neighbouring healthy trees by root to root contact.

In many coniferous forests the rate of spread depends largely on stand type and history, forest composition and soil properties including pH (Korhonen and Stenlid, 1998). In the northern temperate forests, average growth rates of 20–50 cm yr^{-1} have been documented (Gibbs, 1968; Morrison and Redfern, 1994; Roll-Hansen and Roll-Hansen, 1981; Swedjemark and Stenlid, 1993). Based on the estimated annual growth rate, the vegetative spread of the fungus via the root systems within forest stands could produce > 100-year-old expanding disease centres with one individual *H. annosum* genet (clone) capable of occupying areas about 50 m in diameter (Garbelotto *et al.*, 1994; Lygis *et al.*, 2004a,b; Piri *et al.*, 1990; Stenlid, 1985). However, it is not known how long the fungus can stay alive on a given site although it can survive and still remain infectious in stumps for up to 62 years after felling (Greig and Pratt, 1976; Piri *et al.*, 1990; Piri, 1996). Stenlid (1987) and Lygis *et al.* (2004a) also reported that *H. annosum* can persist in root systems of diseased trees for decades and efficiently spread from one forest generation to the next. The survival of *H. annosum* in stumps following clear cutting is of the utmost importance in determining the period that a site remains infectious (Lygis *et al.*, 2004a; Stenlid and Redfern, 1998). Stenlid and Redfern (1998) reported that two major sources of infection are those originating from trees that were infected before felling and those that became infected by spores at the time of felling. Extracting stumps and sieving out fine roots ensures removal of all inoculum and a virtually disease-free site in the next generation (Stenlid, 1987).

Ecological impact of *H. annosum* root rot

Heterobasidion annosum, like all wood decayers, influences species composition, ecosystem diversity, stand structure, stand density, and direction and rate of forest succession (Goheen and Otrosina, 1998). In addition, as a saprotroph, the fungus is thought to contribute substantially to nutrient recycling by returning vital nutrients locked up within wood tissues back to the soil and providing habitat for a wide variety of animals (Filip and Morrison, 1998). In North America, *H. annosum* root rot has been reported to affect successive patterns of forest development by selectively killing certain tree species (Filip and Morrison, 1998). The authors also stated that in coastal areas of North America, the wood decayed due to *annosum* root rot provides a niche for many species of insects and other wildlife (Filip and Morrison, 1998; Hansen and Goheen, 2000). Infected trees are often attacked by bark beetles, except during droughts; for instance activity of fir engraver beetles (*Scolytus ventralis* LeConte) is closely associated with occurrence of root disease in firs (Schmitt *et al.*, 2000). Mortality caused by the root rot and bark beetles is important in creating gaps in the forest canopy (Filip and Morrison, 1998). Forest gaps change the light, moisture and temperature in the forest and thus change the habitat for a diverse range of plants and animals.

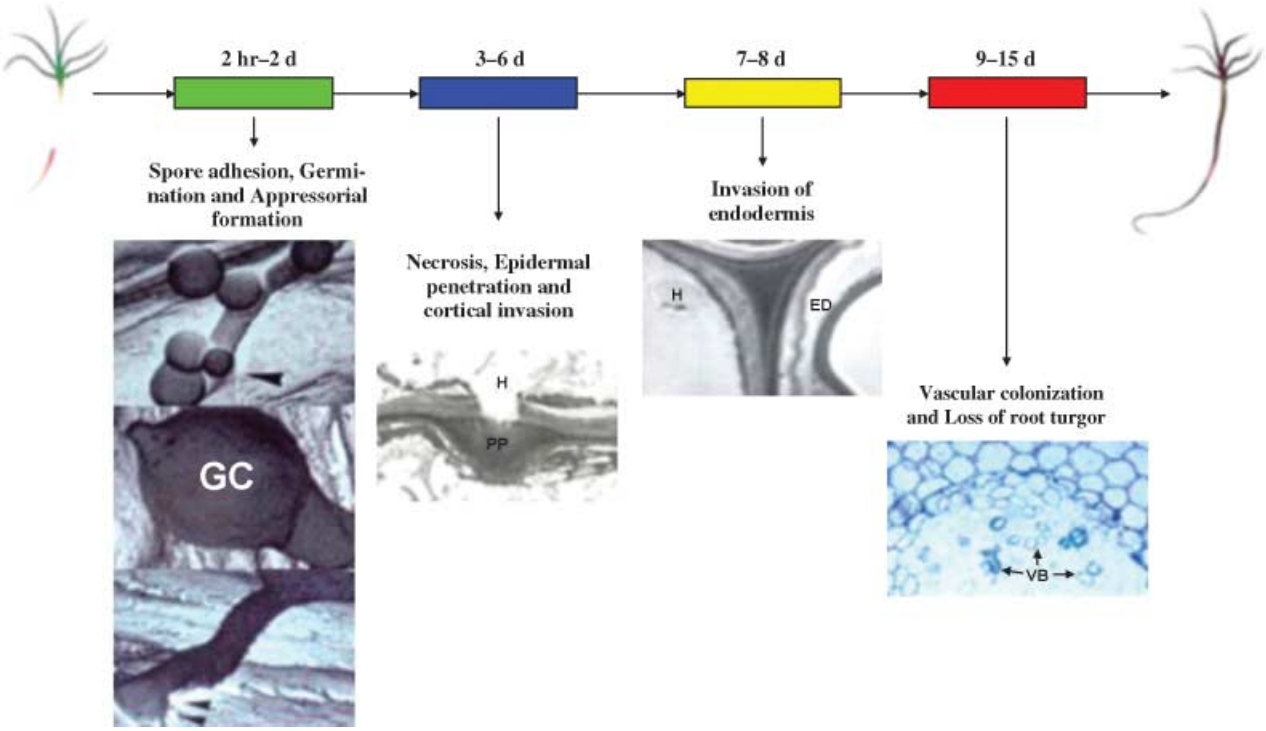

Fig. 4 An experimental model illustrating stages in the development of conidiospores of *Heterobasidion annosum* on seedling roots of conifer trees. Within 2 h to 2 days spores adhere (arrowhead), and develop germ tube and appressorium (double arrowheads). By 3–6 days invasive hyphae have penetrated into the cortical region, reaching the endodermis by 7–8 days and the vascular region by 9–15 days. (GC, germinating conidiospore; H, hyphae; PP, papillae; ED, endodermis; VB, vascular tissue.)

BIOCHEMICAL, MOLECULAR AND GENOMIC STUDIES ON *H. ANNOSUM*–CONIFER PATHOSYSTEMS

Plant–pathogen interactions have been studied extensively in agricultural and horticultural crops but relatively little work has been done on tree pathosystems (Asiegbu *et al.*, 1998). Crop studies have led to the selection of disease resistant cultivars and varieties and have improved our understanding of gene regulation in the pathogenic interaction (Dangl and Jone, 2001; Keen, 1990). There are several reasons for the slow pace of biochemical, molecular and genomic studies in conifer pathosystems. Mature trees do not readily lend themselves to laboratory-based studies on account of their large size and long life span. There are also no known avirulent strains of the conifer pathogen and no host genotype in Pinaceae with total resistance. Recent advances in transcript profiling, genetic mapping and development of a transformation system makes *H. annosum*–conifer interactions a candidate system for such studies. Knowledge of the molecular and genomic aspects of host–parasite interactions in a conifer–root rot pathosystem is critical for understanding variation in natural resistance and may also serve as a basis for increasing resistance through breeding or genetic engineering.

Pathogenesis and virulence

Adhesion, recognition and development

Earlier authors noted that the survival of a pathogen is partly dependent upon successful attachment of dispersive propagules to the host substratum (Jones, 1994). In angiosperm systems, the expression of virulence by a pathogen has been reported to be initiated at the point of attachment whereupon host–parasite recognition is concomitant with the onset of defence reactions and often presumed to be a determinant of host plant specificity (Albersheim and Anderson-Prouty, 1975; Jones, 1994; Manocha, 1984). Despite the importance of adhesion in survival and virulence of fungal pathogens, studies on the mechanisms and factors influencing the establishment of fungal pathogens to their host are still in their infancy in conifer pathosystems. Using non-suberized roots as an experimental model (Fig. 4), spore adhesion has been documented within 2 h following inoculation of primary roots of juvenile conifer seedlings with conidiospores of *H. annosum* (Asiegbu *et al.*, 1994; Asiegbu, 2000a). Adhesion occurred mainly on the mucilaginous regions of the root but rarely on non-slimy regions and was significantly reduced by treatment of spores with potassium hydroxide, di-ethyl ether,

pronase E or periodic acid (Asiegbu, 2000a). The effect of periodic acid and KOH suggests that the adhesive component and part of the nutrient source for the spores was a sugar or carbohydrate. Several sugars (pinitol, dulcitol, xylitol, mannitol, D-glucose, mannose, fructose) have been detected on both wood discs and fine root surfaces of Scots pine and Norway spruce (Asiegbu, 2000a).

Germination of adhered spores was enhanced in the presence of glucose, mannose or fructose whereas xylose and arabinose were poorly utilized. The effect of diethyl ether also indicates that the adhesive component on spore or host surface was either a lipid or was bound to a lipid. Evidence that proteins on root and spore surfaces and mucilage influenced adhesion and spore germination was obtained by treatment with proteases. Significant differences documented on viability of adherant spores on root surfaces were attributed to possible variations in accumulation and metabolism of storage sugars (trehalose and mannitol) on spore mucilage (Asiegbu, 2000a). The influence of abiotic factors on spore viability and survival in nature has not been properly investigated but their impact on surface constituents of spores may also affect the viability. For example, with fresh conifer stumps, variations in the period of susceptibility ranging from 1 to 4 weeks have been documented (Redfern and Stenlid, 1998). Very little is known of the reasons for the observed variations during stump infection. Photochemical oxidation with a direct effect on essential nutrients (fructose, glucose, mannose) required for initial spore development could be a potential contributory factor.

Penetration and disease development

On germling non-suberized roots of *P. sylvestris* and *P. abies*, development of infection structures such as germ tubes and appressoria by *H. annosum* occurred within 24 h of spore adhesion. Direct penetration through natural openings on root surfaces as well as by direct enzymatic degradation of waxes on root surfaces have been reported (Asiegbu *et al.*, 1993). Spore germination and appressoria formation were often followed within 72 h by internal colonization of the cortical tissues with the fungus reaching the endodermal region 3–7 days after infection (Fig. 4). Colonization and disintegration of the vascular region occurred within 9–15 days. During the process of colonization, the pathogen encounters various types of root tissues with differing levels of susceptibility or resistance which are partly dependent on the defence properties and characteristics displayed by the particular tissues. Initial studies revealed that the meristem and vascular region were the most susceptible regions of the non-suberized roots whereas root cap and endodermis were the most resistant to attack (Asiegbu *et al.*, 1994).

Cell-wall-degrading enzymes during pathogenesis

Plant polysaccharides and lignified tissues, which are the main structural framework of conifer woods, constitute the main obstacles to invasive growth of the pathogen within host tissues. To access and obtain nutrients locked up in these tissues, *H. annosum* as an aggressive necrotrophic wood decayer secretes a wide range of extracellular enzymes which can degrade and detoxify structural and soluble host constituents such as sugars, starch, pectin, cellulose, lignin and various phenolic compounds (Asiegbu *et al.*, 1998; Nord and Hata, 1969). The digestion of plant cell-wall polymers provides nutrients and aids the penetration of cells, allowing survival and spread through woody tissues. However, very few of the enzymes (amylase, catalase, cellulase, esterase, glucosidase, hemicellulase, manganese peroxidase, laccase, pectinase, phosphatase, proteases) secreted by *H. annosum* have been thoroughly studied (Asiegbu *et al.*, 2004; Hüttermann, 1980; Johansson, 1988; Karlsson and Stenlid, 1991; Korhonen and Stenlid, 1998; Maijala *et al.*, 1995, 2003) and almost nothing is known about their role in pathogenesis. One of the enzymes that has received much more attention is laccase, a copper-containing enzyme that presumably contributes substantially to lignin degradation as well as detoxification of host chemical and structural defences (Have and Teunissen, 2001). The correlation between laccase secretion and wood decay capability was highlighted by a study which reported that P-types of *H. annosum* which possess significantly greater wood-degrading ability than S-types secrete 5–6 times more laccase than the S-type (Daniel *et al.*, 1998). Recent molecular data revealed the existence of several copies of laccase (Asiegbu *et al.*, 2004) and manganese peroxidase (Maijala *et al.*, 2003) genes in the *H. annosum* genome. Analysis of mutation frequencies in the P, S and F groups indicates that *H. annosum* laccase genes may be under positive selection, suggesting a potential role for the gene in host adaptation (Asiegbu *et al.*, 2004).

Low-molecular-weight compounds (toxins and oxalic acid)

Among low-molecular-weight compounds secreted by *H. annosum*, several toxins including fommanoxin, fommanosin and fommanoxin acid, oosponol and oospoglycol have been isolated in cultures of the pathogen (Basset *et al.*, 1967; Donelly *et al.*, 1988; Holdenrieder, 1982; Sonnenbichler *et al.*, 1989). Although earlier studies reported that application of fommanosin to stem wounds provoked a systemic response leading to accumulation of pinosylvin (Basset *et al.*, 1967), substantive evidence as to the role of *H. annosum* toxins in the natural pathogenesis of conifer trees has yet to be established. Another low-molecular-weight compound produced in large amounts by several white rot fungi including *H. annosum* is oxalate (Hüttermann *et al.*, 1980; Volger *et al.*, 1982). Oxalate appears to have versatile roles in fungi. It has been proposed to have an important connection with manganese peroxidase (MnP) and manganese in the oxidation of aromatics (Lequart *et al.*, 1998; Moreira *et al.*, 1998; Shimada *et al.*, 1994). Oxalate can also function in lignin peroxidase (LiP) systems (Popp *et al.*, 1990). Low-molecular-weight iron-binding chelators as well as oxalate have also been implicated as potential compounds

with vital roles in wood degradation (Goodell *et al*., 1997). Oxalate might also chelate calcium, thereby allowing for a better pectin access for pectinases (Johansson, 1988). However, the importance of oxalate in the *H. annosum* conifer pathosystem remains unclear.

Mitochondrial and extrachromosomal viral nucleic acids elements: implications in pathogenicity

A number of pathogenicity factors have been described for fungal pathogens of agricultural crop plants (Idnurm and Howlett, 2001). However, less is known about pathogenicity determinants in phytopathogenic fungi of forest trees such as *H. annosum*. Olson and Stenlid (2001) demonstrated the role of inheritance of mitochondrial DNA in the control of fungal hybrid virulence in *H. annosum*. Using artificially created S–P hybrids from S and P types of North American isolates of *H. annosum*, they observed significant correlations between the mitochondrial type acquired by hybrids and their virulence. Although the exact reason for the correlation between mitochondrial origin and virulence was not indicated, it could be either that the mitochondrial genome encodes factors that determine the virulence of hybrids or alternatively that the lower virulence was caused by a mismatch between interspecific combinations of nuclei and cytoplasm. By contrast, Irhmark *et al*. (2002, 2004) investigated the role of double stranded RNA (dsRNA) mycoviruses as virulence determinants in *H. annosum*. Their studies showed that there was little difference in virulence between dsRNA-infected and dsRNA-free *H. annosum* isolates of the same genotype. The virulence of the fungus was also not affected when dsRNA was transferred from isolates of the S-intersterility group to a P-isolate.

H. annosum *transcriptome during spore development and pathogenesis*

The successful initiation of infection in *H. annosum* as in other plant pathosystems requires production of spores, and formation and development of germ tubes, appressoria or infection hyphae. The resulting interaction between *H. annosum* and conifer trees is complex and it is hard to predict how it will develop in nature. This is quite unlike the well-defined and established hypothesis of biotrophic crop pathosystems responding on a gene-for-gene basis (Keen, 1990), in which the outcome of the interaction can be either compatible or incompatible. The ability of a pathogen to develop specific cell types necessary for successful establishment on its host and overcome host defences implies a complex dynamic communication involving the up and down regulation of many genes (Birch and Whisson, 2001). To obtain an overall view of all the processes that occur during fungal growth and invasion, it is necessary to identify as many as possible pathogen genes that are expressed during the infection process. The use of high-thoroughput DNA sequencing has facilitated genomic and molecular studies of phytopathogenic fungi. Such large-scale sequencing of cDNAs (expressed sequenced tags, ESTs) has led to the identification and characterization of a diverse collection of genes expressed during developmental stages or fungal pathogenicity (Abu *et al*., 2004; Asiegbu *et al*., 2005; Karlsson *et al*., 2003; Keon *et al*., 2000; Rauyaree *et al*., 2001; Thomas *et al*., 2001). EST analysis is a robust approach for detecting novel genes as well as for obtaining information about previously uncharacterized genes. The technique was recently applied for the identification of *H. annosum* genes induced during spore and hyphal development (Abu *et al*., 2004) and during pathogenesis (Abu, 2004; Karlsson *et al*., 2003). About 2000 genes from both conditions are currently deposited in the NCBI Genebank database. Analysis of over 1200 ESTs from germinated spores of *H. annosum* revealed a significant number of genes without any similarity to sequences in the international databases (Abu *et al*., 2004). A selected number of genes with roles in signal transduction (MAP kinase), oxidative phosphorylation (NADH dehydrogenase), fatty and amino acid metabolism (Acyl-CoA, glutamine synthase), carbohydrate metabolism (phosphoglucomutase), cell growth and development (beta tubulin, Gbeta-like protein) were identified to be expressed at initial stages of spore germination and hyphal development (Abu *et al*., 2004; G. Li and F. O. Asiegbu, unpublished observations). Interestingly, very few genes (transcript antisense to ribosomal RNA, cytochrome P450 mono-oxygenase) were found to be up-regulated both during spore development as well as in invasive growth within host tissues (Abu, 2004; Asiegbu *et al*., 2005). A particular gene encoding a cytochrome P450 mono-oxygenase was found to have increased transcript levels during all stages of spore development (ungerminated spore, germ tube stage, mycelia stage, pathogenesis stage), suggesting the gene may have a significant biological function. In the early infection stage, genes involved in handling oxidative stress [superoxide dismutase (SOD), glutathione-S-transferase], secondary metabolite synthesis (farnesyl-pyrophosphate synthetase, a cytochrome P450 mono-oxygenase) and polysaccharide degradation (arabinase) as well as hydrophobins have been shown to be significantly up-regulated (Karlsson *et al*., 2003, and in preparation)

Resistance and defence mechanisms

To achieve successful host resistance against microbial attack early recognition of invading phytopathogens is vital. Several factors, including genes with putative functions in recognition and signal transduction, have been documented in biotrophic crop–pathogen systems responding in a gene-for-gene specific manner (Dangl and Jone, 2001; Keen, 1990). Although gene-for-gene interactions have not been observed for the *H. annosum*–conifer pathosystem, a number of proteins (*P. nigra* chitin binding lectin, PNL) and genes [*PsACRE*, leucine rich repeat (*LRR*)] with roles in recognition and signal transduction have recently been described (Asiegbu *et al*., 2005; Li and Asiegbu, 2004; Nahalkova *et al*.,

2001). In several pathosystems (biotrophs, necrotrophs), following the activation of such R gene-mediated recognition, the hypersensitive reaction (HR) is triggered, leading to production of reactive oxygen species and phytoalexins, induction of defence-related genes, and finally host cell browning and death (Keen, 1990; Mayer *et al.*, 2001). Although the HR barrier prevents spread of biotrophic fungi (Lamb and Dixon, 1997), it does not deter the growth of necrotrophic fungi such as *H. annosum* (Asiegbu *et al.*, 1994, 1998).

Morphological and chemical basis for resistance

At the late stages of infection, a number of chemical and morphological responses are activated. A diverse array of phenolic compounds, including phenylpropanoids, stilbenes, flavonoids and lignans, are accumulated after fungal attack (Asiegbu *et al.*, 1998; Johansson *et al.*, 2004; Lindberg *et al.*, 1992; Nagy *et al.*, 2004). Although phenolic monomers may inactivate fungal membranes, lignification prevents penetration and diffusion of enzymes and toxins from the pathogen. In addition to lignification, suberization (Asiegbu *et al.*, 1998; Solla *et al.*, 2002) and papillae formation (Asiegbu *et al.*, 1998) have been implicated as substantial barriers to penetration. In response to infection, conifers also secrete oleoresin consisting of a mixture of volatile and non-volatile terpenes. Resin acids act as mechanical barriers, whereas the volatile compounds (monoterpens) are fungitoxic (Cobb *et al.*, 1968; Shain, 1967). Krekling *et al.* (2004) observed anatomical responses of Norway spruce to infection such as accumulation of phenolic inclusions in ray parenchyma cells, activation of phloem parenchyma cells and formation of traumatic resin ducts in the xylem.

Pathogenesis-related proteins

Pathogenesis-related (PR) proteins produced in conifer tissues in response to *H. annosum* infection include a large number of enzymes (chitinases, glucanases, peroxidases). Chitinases and glucanases are implicated in the *in vivo* degradation of fungal cell walls (Asiegbu *et al.*, 1995; Hietala *et al.*, 2004). Peroxidases have been studied extensively (Asiegbu *et al.*, 1994; Johansson *et al.*, 2004; Nagy *et al.*, 2004). As polymerizing enzymes, they play a role in lignin and suberin synthesis, and may be involved in defence by cross-linking phenolic compounds into papillae containing callose and enhance defence reactions by production of toxic radicals. Production of phenolics depends also on two other enzymes: phenylalanine lyase (PAL) and chalcone synthetase, catalysing the first steps of phenyl propanoid synthesis (Karjalainen *et al.*, 1998; Nagy *et al.*, 2004).

Transcript profiling of host defences

Only few papers have been published relating to the application of EST technology to identify genes involved in host–pathogen interactions (Rauyaree *et al.*, 2001). Application of this technology will advance our knowledge of host–pathogen interactions that will have direct relevance to conifers. Recent studies on *H. annosum*–conifer interactions have employed a genomic approach. Analysis of a subtractive cDNA library of Scots pine roots led to the isolation of numerous genes differentially expressed during infection with *H. annosum.* The identified transcripts encode proteins with diverse functions, including those involved in cell rescue and defence such as peroxidase, antimicrobial peptide (*SpAMP*), thaumatin, metallothionein-like protein and R gene homologue (Asiegbu *et al.*, 2003, 2005). Similarly, increased transcript levels of chitinases class II and IV (Hietala *et al.*, 2004) and chalcone synthase (Nagy *et al.*, 2004) have also recently been reported in Norway spruce following infection with *H. annosum*, further substantiating observations on the recognition response. A microarray study of early infection events showed a shift in transcription towards genes involved in metabolism, cell rescue and defence as well as genes involved in energy generation (F. O. Asiegbu *et al.*, unpublished observations).

Influence of abiotic factors in host resistance

Resistance against the necrotroph *H. annosum* may be affected by the age of the tree (Johansson *et al.*, 2004), time of the year and abiotic factors. A high partial pressure of oxygen within the tree, soil type (Asiegbu *et al.*, 1998), nutrients (Piri, 1998), high pH (Gibbs *et al.*, 2002) and stress (Lindberg and Johansson, 1992; Swedjemark and Stenlid, 1997) can increase susceptibility to infection by *H. annosum.* Seasonal variations in resistance may be partially caused by changes in concentration of stilbenes (Lindberg *et al.*, 1992) and starch (from which phenolic compounds are produced) (Johansson and Stenlid, 1985).

DISEASE MANAGEMENT

Theoretically, a root rot pathogen can be suppressed during all stages of its life cycle, starting from adhesion, early establishment and infection, through spreading inside the host to formation of its propagules and transferring between trees. Curative measures against the annosum root rot are not feasible because a decay inside the tree cannot really be healed, although spread can be reduced in the attacked root system in order to minimize economic losses. Prophylactic protection measures against *H. annosum* have been focused on preventing basidiospore deposition, germination and growth of the fungus. Current strategies for the control of annosum root rot include silvicultural, chemical and biological methods.

Silvicultural control measures

Differential degrees of resistance against *H. annosum* among conifer species and the fact that broad-leaved trees are relatively less susceptible (Delatour *et al.*, 1998) can be exploited in forest

management. Planting a species with low susceptibility can diminish the root rot problem and has the potential to free an infested site from inoculum (Korhonen *et al.*, 1998b; Lygis *et al.*, 2004a,b). A more radical measure to clean a site from inoculum is by the practice of stump removal. This has to be carried out rigorously in order to be 100% effective as the fungus can survive and carry over the disease to the subsequent stand even in 1-cm-thick roots (Greig, 1984; Korhonen *et al.*, 1998b; Stenlid, 1987). Losses caused by the disease in mixed stands are reported to be lower than in pure stands (Linden and Vollbrecht, 2002; Piri *et al.*, 1990). In addition, choosing proper planting (wide spacing preferable) and mixture schemes (avoiding monocultures of susceptible species) allows thinning to be delayed and to obtain a better yield than in pure plantations (Lygis *et al.*, 2004a). An alternative to delaying thinning is performing it in periods outside spore dispersal and establishment (see above). Ferilization is another silvicultural approach that may be used for protection against *H. annosum*. Although the effects of nutrients on annosum root rot incidence seem to be variable (Piri, 1998), studies have shown that application of compound fertilizers (NPK) increases the resistance of Scots pine to *H. annosum* (Fedorov, 1994; Piri, 2000).

Chemical methods

Chemical and biological control encompass prophylactic stump treatment immediately after felling in an attempt to prevent infection. Over the last 50 years a large number of commercially produced compounds have been tested as stump protectants (Pratt *et al.*, 1998a). Of these, urea and borates have been shown to be the most effective against *H. annosum* and are used commercially (Brandtberg *et al.*, 1996; Johansson *et al.*, 2002; Pratt and Lloyd, 1996). Both compounds are cheap, widely available and easy to handle. Lloyd (1997) concluded that the primary mode of action of borates is on the metabolism of basidiomycetes. With urea, stump protection is achieved by hydrolysis of the compound by urease in the living wood tissues resulting in formation of ammonia and a rise in pH to a level at which spores of *H. annosum* are unable to germinate and mycelia are unable to survive (Johansson *et al.*, 2002). This mechanism explains why urea sometimes fails in wetter climates where the heartwood rather than the sapwood of spruce normally is the target for *H. annosum* (Pratt *et al.*, 1998a) as no host urease activity is available in the dead heartwood of the stumps. In spite of the effectiveness of these two commercially produced compounds, there have been increased objections against their use, for practical and environmental reasons. Westlund and Nohrsted (2000) showed that both borate and urea solutions caused severe damage to common ground-vegetation species. The treatments also resulted in transient changes in soil chemistry. Moreover, urea treatment had a major influence on fungal community structure in freshly cut spruce stumps (Vasiliauskas *et al.*, 2004). It strongly promoted colonization of stumps by ascomycetes and deuteromycetes, led to a significant decrease of zygomycetes and almost completely eliminated basidiomycetes.

Biocontrol

Biological control has been proposed as an alternative to chemical control, and a number of fungal species (*Phlebiopsis gigantea*, *Bjerkandera adusta*, *Fomitopsis pinicola*, *Resinicium bicolor*, *Hypholoma* spp., *Trichoderma* spp., *Scytalidium* spp.) have been tested on stumps as competitors and antagonists against *H. annosum* (Holdenrieder and Greig, 1998; Holmer and Stenlid, 1994, 1997; Kallio and Hallaksella, 1979; Nicolotti and Varese, 1996; Nicolotti *et al.*, 1999). Among these, only *Phlebiopsis gigantea* (Fr.) Jül is currently being used with good results (Berglund *et al.*, 2005; Holdenrieder and Greig, 1998; Thor and Stenlid, 2005). Three distinct products based on this saprotrophic fungus have been developed: PG Suspension® in the UK, PG IBL® in Poland and Rotstop® in Fennoscandia (Pratt *et al.*, 2000).

However, very little is known about the mode of action of *P. gigantea*. Presently, there is no evidence of either antibiotics or toxins being secreted (Holdenrieder and Greig, 1998). Ikediugwu *et al.* (1970) and Ikediugwu (1976) found that *P. gigantea* hyphae changed the structure of adjacent *H. annosum* hyphae: penetration, granulation and vacuolation of the cytoplasm and loss of opacity were observed. The hypothesis of resource competition as the most probable mechanism for the biocontrol action (Holdenrieder and Greig, 1998) was recently verified (A. Adomas and F. O. Asiegbu, unpublished observations). Under varying nutrient sources *P. gigantea* had a higher growth rate than *H. annosum* and it also possessed the ability to secret significant levels of wood-degrading enzymes (A. Adomas and F. O. Asiegbu, unpublished observations). At the molecular level, a number of genes encoding proteins involved in carbohydrate and nitrogen metabolism (fructose-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, endo-galacturonase, glutamine synthase) were identified to be up-regulated in the interaction zone formed by the biocontrol agent and the pathogen, which may partly explain the competitive advantage of *P. gigantea* (A. Adomas and F. O. Asiegbu, unpublished observations).

When the effect of *P. gigantea* on ground vegetation was tested, no noticeable effect was recorded (Westlund and Nohrstedt, 2000). Even though species richness of fungi inhabiting freshly cut spruce stumps was negatively affected, the stumps were colonized mainly by the same species that occurred in untreated stumps (Vainio *et al.*, 2001; Vasiliauskas *et al.*, 2004).

Management of annosus root rot in North America

In North America, stump infection by *H. annosum* can be largely prevented by treating freshly cut stump surfaces with a light

coating of granular sodium tetraborate decahydrate or disodium octaborate tetrahydrate. Currently, the only borate products registered for annosum control are Sporax® and Tim-Bor® (Schmitt *et al.*, 2000). Borate stump treatment is not effective on stumps of trees that are already infected. In stands with numerous infected stumps, silvicultural treatments should be employed to deal with the disease (Filip and Morrison, 1998; Garbelotto *et al.*, 1997b; Schmitt *et al.*, 2000). Where possible, stands should be managed by conversion to full stocking of site-adapted and less susceptible species. Reducing rotation length have been found to limit losses where other root diseases and stem decays are not a concern. When thinning and harvesting are completed, care needs to be taken to minimize both wounding of residuals and site disturbance.

Molecular breeding

Significant variation in fungal growth among host species (Redfern and MacAskill, 2003; Swedjemark and Stenlid, 1995) and genotypes (Hietala *et al.*, 2004; Swedjemark and Karlsson, 2004) has been identified. About 35% of variations in fungal growth is attributed to the genetic differences in the susceptibility of clonal materials (Swedjemark *et al.*, 1997). However, as stated earlier, there is virtually no host genotype or conifer species with total resistance against the pathogen, which apparently justifies the need for molecular methods. It should be noted that forest trees have long generation cycles with extended vegetative phases ranging from one to several decades, and this may pose a limitation in breeding programmes (Ahuja, 2001). Molecular breeding offers the possibility to shorten the breeding cycle as well as to facilitate the introduction of desired resistance traits to the plant and mass-propagate selected clones. Högberg *et al.* (1998) demonstrated that somatic embryogenesis can be integrated into Norway spruce breeding programmes. Elfstrand *et al.* (2001) produced transgenic Norway spruce cell cultures overexpressing defensin *sp1*. The regenerated plants were less susceptible to infection with *H. annosum.* Another approach is to screen naturally occurring or conventionally bred genotypes for high levels of molecular markers associated with resistance.

Disease modelling

A major objective for developing a disease model is to incorporate knowledge of root rot problems into forest planning (Pukkala *et al.*, 2005). The use of control methods may be acceptable when the treatment can be justified by its efficacy, cost effectiveness and limited impact on the environment (Pratt *et al.*, 1998b). Stand-alone applications can also be useful research tools, identifying areas requiring further investigations. Construction of a model requires simulating complex processes within the disease cycle: primary infection, root contact, secondary spread of infection and development of decay. Perhaps the most comprehensive attempt to model dynamics and impact of root rot was made in North America, where through a series of meetings of biologists and foresters a Western root disease (WRD) model was created (Frankel *et al.*, 1994). In Europe, models have been developed for *H. annosum* on Sitka spruce in the UK (Pratt *et al.*, 1989), and on Norway spruce in Finland (Moykkynen *et al.*, 1998, 2000) and Sweden (Thor *et al.*, 2005; Vollbrecht and Agestam, 1995). Pukkala *et al.* (2005) simulated the infection and spread of *H. annosum* and *H. parviporum* in Fennoscandia stands of Norway spruce and Scots pine; in a European research programme (MOHIEF) this work has been extended to be used in several hosts over the entire continent.

PROSPECTS AND CONCLUDING REMARKS

Although earlier authors have reported that mature woody tissues are the primary target of *H. annosum* infection, most basic biochemical and molecular work has been performed on non-suberized seedling roots. During development the conifer root undergoes several morphological and physiological transformations. The non-suberized seedling roots, mainly containing epidermis, cortex, endodermis, meristem and stele, are successively suberized. The cortex disappears and the secondary xylem in the vascular region (stele) becomes dominant. Later the epidermis, cortex and endodermis are replaced by the rhyitidome, phelloderm and phloem layers. The living parts of the xylem consist mainly of cambium, rays and resin parenchyma cells. The successive morphological development occurs concomitantly with changes in constitutive and inducible defence mechanisms. Several recent studies have shown that *H. annosum* is one of the very few fungal pathogens able to infect conifer roots of all ages. The differences in defence mechanisms between suberized and non-suberized roots would most likely hinge on the timing and spatial pattern of their regulation. We believe that a lesson could equally be learnt by studies performed on seedling roots that may be of value in understanding defence reactions occurring in living parts of suberized woody tissues

Recently, progress has been made on QTL mapping of the pathogenicity factors in *H. annosum* using a hybrid between North American P and S homokaryons (M. Lind, A. Olson and J. Stenlid, unpublished observations). Based on AFLP markers, a genetic linkage map was established that allowed for mapping QTLs for pathogenic growth towards seedling roots and pine inner bark (M. Lind *et al.*, unpublished observations). The next step is underway to verify the identity of candidate genes located within the established region of the genome. Future functional analysis of both QTL- and EST-derived candidate genes should be aided by the fact that the fungus is now shown to be amenable to molecular genetic analysis through a recently established biolisitc, protoplast and *Agrobacterium*-mediated DNA-transformation

system (Asiegbu, 2000b; N. Samils *et al.* unpublished observations). Equally, QTL mapping of host resistance could aid in the identification of resistance factors relevant for future molecular breeding. Finally, future genetic and functional work on this severe pathogen would be very much helped by more complete sequence information from the genome.

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REFERENCES

Abu, S. (2004) Application of genomics for the study of the root rot fungus. *Heterobasidion* spp. Licentiate (M.Phil) Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Abu, S., Li, G. and Asiegbu, F.O. (2004) Identification of *Heterobasidion annosum* (S-type) genes expressed during initial stages of conidiospore germination and under varying culture conditions. *FEMS Microbiol. Lett.* **233**, 205–213.

Ahuja, M.R. (2001) Recent advances in molecular genetics of forest trees. *Euphytica*, **121**, 173–195.

Albersheim, P. and Anderson-Prouty, A.J. (1975) Carbohydrates, proteins, cell surfaces and the biochemistry of pathogenesis. *Annu. Rev. Plant Physiol.* **26**, 31–52.

Allen, E., Morrison, D. and Wallis, G. (1996) *Common Tree Diseases of British Coloumbia Canadian*. Victoria, Canada: Forest Service (cat no. Fo42–257/1996E).

Asiegbu, F.O. (2000a) Adhesion and development of the root rot fungus (*Heterobasidion annosum*) on conifer tissues: effects of spore and host surface constituents. *FEMS Microbiol. Ecol.* **33**, 101–110.

Asiegbu, F.O. (2000b) Genetic transformation of the root rot fungus *Heterobasidion annosum* by particle bombardment. *Metsanduslikud Uurimused (Forry Studies)*, **XXXIV**, 9–16.

Asiegbu, F.O., Abu, S., Stenlid, J. and Johansson, M. (2004) Sequence polymorphism and molecular characterisation of laccase genes of the conifer pathogen *Heterobasidion annosum*. *Mycol. Res.* **108**, 136–148.

Asiegbu, F.O., Choi, W.B., Li, G.S., Nahalkova, J. and Dean, R.A. (2003) Isolation of a novel antimicrobial peptide gene (Sp-AMP) homologue from *Pinus sylvestris* (Scots pine) following infection with the root rot fungus *Heterobasidion annosum*. *FEMS Microbiol. Lett.* **228**, 27–31.

Asiegbu, F.O., Daniel, G. and Johansson, M. (1993) Studies on the infection of Norway spruce roots by *Heterobasidion annosum*. *Can. J. Bot.* **71**, 1552–1561.

Asiegbu, F.O., Daniel, G. and Johansson, M. (1994) Defence related reactions of seedling roots of Norway spruce to infection by *Heterobasidion annosum*. *Physiol. Mol. Plant Pathol.* **45**, 1–19.

Asiegbu, F.O., Denekamp, M., Daniel, G. and Johansson, M. (1995) Immunocytochemical localization of pathogenesis-related proteins in roots of Norway spruce infected with *Heterobasidion annosum*. *Eur. J. For. Pathol.* **25**, 169–178.

Asiegbu, F.O., Johansson, M., Woodward, S. and Hütterman, A. (1998) Biochemistry of the host–parasite interaction. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 167–195. London: CAB International.

Asiegbu, F.O., Nahalkova, J. and Li, G. (2005) Pathogen inducible cDNA's from the interaction of the root rot fungus *Heterobasidion annosum* with Scots pine. *Plant Sci.* **168**, 365–372.

Basset, C., Sherwood, R.T., Kepler, J.A. and Hamilton, P.B. (1967) Production and biological activity of fommannosin, a toxic sesquiterpene metabolite of *Fomes annosus*. *Phytopathology*, **57**, 1046–1052.

Berglund, M., Rönnberg, J., Holmer, L. and Stenlid, L. (2005) Comparison of the effectiveness of five strains of *Phlebiopsis gigantea* and two *Trichoderma* formulations against natural *Heterobasidion* spore infections on Norway spruce stumps. *Scand. J. For. Res.* **20**, 12–17.

Birch, P.R.J. and Whisson, S.C. (2001) *Phytophthora infestans* enters the genomics era. *Mol. Plant Pathol.* **2**, 257–263.

Brandtberg, P.O., Johansson, M. and Seeger, P. (1996) Effects of season and urea treatment on infection of stumps of *Picea abies* in stands on former arable land. *Scand. J. For. Res.* **11**, 261–268.

Brefeld, O. (1888) *Untersuchungen aus dem Gesamtgebiet der mykologie. Basidiomyceten III. Autobasidiomyceten und die Begrundung des naturlichen Systems der Pilze*. Heft 8, 305pp. Leipzig (1888–1889).

Capretti, P., Korhonen, K., Mugnai, L. and Romgnoli, C. (1990) An intersterility group of *Heterobasidion annosum* specialized to *Abies alba*. *Eur. J. For. Pathol.* **20**, 231–240.

Chase, T.E., Otrosina, W.J., Spieth, P.T. and Cobb, F.W. (1991) Use of PCR to distinguish biological species within the *Heterobasidion annosum* complex. *Phytopathology*, **81**, 1190 [abstract].

Chase, T.E. and Ullrich, R.C. (1988) *Heterobasidion annosum* root and butt rot of trees. *Adv. Plant Pathol.* **6**, 501–510.

Chase, T.E. and Ullrich, R.C. (1990) Five genes determining intersterility in *Heterobasidion annosum*. *Mycologia*, **82**, 73–81 .

Cobb, F.W., Krstic, M., Zavarin, E. and Barber, H.W. (1968) Inhibitory effects of volatile oleoresin components on *Fomes annosus* and four *Ceratocystis* spp. *Phytopathology*, **58**, 1327–1335.

Dai, Y.C. and Korhonen, K. (1999) *Heterobasidion annosum* group S identified in north-eastern China. *Eur. J. For. Pathol.* **29**, 273–279.

Dai, Y.C., Vainio, E.J., Hantula, J., Niemela, T. and Korhonen, K. (2003) Investigations on *Heterobasidion annosum* s. lat. in central and eastern Asia with the aid of mating tests and DNA fingerprinting. *For. Pathol.* **33**, 269–286.

Dangl, J.L. and Jone, J.D.G. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, **411**, 326–333.

Daniel, G., Asiegbu, F.O. and Johansson, M. (1998) The saprotrophic wood degrading abilities of *Heterobasidion annosum* intersterility groups P and S. *Mycol. Res.* **102**, 991–997.

Delatour, C., von Weissenberg, K. and Dimitri, L. (1998) Host resistance. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 143–167. London: CAB International.

DeScenzo, R.A. and Harrington, T.C. (1994) Use of (CAT) 5, as a fingerprinting probe for fungi. *Phytopathology*, **84**, 534–540.

Donelly, D.M.X., Martin, M. and Polonsky, J. (1988) High performance liquid chromatographic determination of fommanoxin in European strains of *Heterobasidion annsoum*. *J. Chromatogr.* **440**, 65–69.

Elfstrand, M., Fossdal, C.G., Swedjemark, G., Clapham, D., Olsson, O., Sitbon, F., Sharma, P., Lonneborg, A. and von Arnold, S. (2001)

Identification of candidate genes for use in molecular breeding—A case study with the Norway spruce defensin-like gene, Spi 1. *Silvae Genet.* **50**, 75–81.

Fedorov, N. (1994) Root rots of pine plantations in Belarus. In: *Proceedings of Eighth IUFRO Conference on Root and Butt Rots, Sweden/Finland, August 1993* (Johansson, M. and Stenlid, J., eds), pp. 572–576. Uppsala: Swedish University of Agricultural Sciences.

Filip, G.M. and Morrison, D.J. (1998) Impact, control and management of *Heterobasidion annosum* root and butt rot in North America. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hüttermann, A., eds), pp. 405–429. London: CAB International.

Frankel, S., Eav, B., Kleijunas, J. and Shaw Iii, C. (1994) Modelling the dynamics and impact of annosus root disease in western North America. In: *Proceedings of the Eighth IUFRO Conference on Root and Butt Rots, Sweden/Finland, August 1993* (Johansson, M. and Stenlid, J., eds), pp. 762–770. Uppsala: Swedish University of Agricultural Sciences.

Fries, E. (1821) *Systems Mycologicum, Sistens Fungorum Ordines, Genera et Species, Huc Usque Cognitas Quas Ad Norman Methodi Naturalisdeterminavit, Disposuit Atque Descripsit*, 1. Sumptibus Ernesti Mauritii, Gryphiswaldie.

Garbelotto, M., Cobb, F., Bruns, T., Otroina, W., Slaughter, G. and Popenuck, T. (1994) Preliminary results on the genetic structure of *Heterobasidion annosum* in white fir (*Abies concolor*) root decay centres. In: *Proceedings of the 8th IUFRO Conference on Root and Butt Rots. Sweden/Finland, August 1993*. (Johansson, M. and Stenlid, J., eds), pp. 227–232. Uppsala: Swedish University of Agricultural Sciences.

Garbelotto, M., Lee, H.K., Slaughter, G., Popenuck, T., Cobb, F. and Bruns, T. (1997a) Heterokaryosis is not required for virulence of *Heterobasidion annosum*. *Mycologia*, **89**, 92–102.

Garbelotto, M.M., Otrosina, W.J., Chapela, I.H. and Gonthier, P. (2001) Studies on the ecology and genetics of hybridization in *Heterobasidion*. In: *10th International Conference on Root and Butt Rots of Forest Trees* (Laflamme, G., Berube, J.A. and Bussieres, G., eds), pp. 238–244. Quebec, Canada: Natural Resources Canada.

Garbelotto, M., Otrosina, W., Cobb, F. and Bruns, T. (1997b) Population biology of the forest pathogen *Heterobasidion annosum*: implications for management. In: *Proceedings of 46th Annual Meeting*, pp. 24–35. California Forest Pest Council, USA.

Gibbs, J.N. (1968) Resin and the resistance of conifers to *Fomes annosus*. *Ann. Bot.* **32**, 649–665.

Gibbs, J.N., Greig, B.J.W. and Pratt, J.E. (2002) Fomes root rot in Thetford Forest, East Anglia: past, present and future. *Forestry*, **75**, 191–202.

Goheen, D. and Otrosina, W. (1998) Characteristics and consequences of root diseases in forets of Western North America. *User's Guide to the Western Root Disease Model*. U.S. Department of Agriculture, Forest Service.

Gonthier, P., Warner, R., Nicolotti, G., Mazzaglia, A. and Garbelotto, M.M. (2004) Pathogen introduction as a collateral effect of military activity. *Mycol. Res.* **108**, 468–470.

Goodell, B., Jellison, J., Liu, J., Daniel, G., Paszcyfiski, A., Fekete, F., Krishnamurthy, S., Jun, L. and Xu, G. (1997) Low molecular weight chelators and phenolic compounds isolated from wood decay fungi and their role in the fungal biodegradation of wood. *J. Biotechnol.* **53**, 133–162.

Greig, B.J.W. (1984) Management of East England pine plantations affected by *Heterobasidion annosum* root rot. *Eur. J. For. Pathol.* **14**, 392–397.

Greig, B.J.W. and Pratt, J.E. (1976) Some observations on the longevity of *Fomes annosus* in conifer stumps. *Eur. J. For. Pathol.* **6**, 250–253.

Hansen, E.M. and Goheen, E.M. (2000) *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in western North America. *Annu. Rev. Phytopathol.* **38**, 515–539.

Harrington, T.C., Worrall, J.J. and Rizzo, D.M. (1989) Compatibility among host specialized isolates of *Heterobasidion annosum* from Western North America. *Phytopathology*, **79**, 290–296.

Hartig, R. (1874) *Wichtige Krankheiten der Waldbäume: Beitrage zur mycologie und Phytopathologie fur Botaniker und Forstmänner*. Berlin: Julius Springer [Translation: Phytopathological Classics (1975) no. 12. American Phytopathological Society, St. Paul, Minnesota.]

Have, R.T. and Teunissen, P.J.M. (2001) Oxidative mechanisms involved in lignin degradation by white rot fungi. *Chem. Rev.* **101**, 3397–3413.

Heneen, W.K., Gustafsson, G., Brismar, K. and Karlsson, G. (1994) Interaction between Norway spruce and *Heterobasidion annosum*. I. Infection of non-suberized and young suberized roots. *Can J. Bot.* **72**, 872–883.

Hietala, A.M., Kvaalen, H., Schmidt, A., Johnk, N., Solheim, H. and Fossdal, C.G. (2004) Temporal and spatial profiles of chitinase expression by Norway spruce in response to bark colonization by *Heterobasidion annosum*. *Appl. Environ. Microbiol.* **70**, 3948–3953.

Hodges, C.S. (1969) Modes of infection and spread of *Fomes annosus*. *Annu. Rev. Phytopathol.* **7**, 247–266.

Högberg, K.A., Ekberg, I., Norell, L. and von Arnold, S. (1998) Integration of somatic embryogenesis in a tree breeding programme: a case study with *Picea abies*. *Can. J. For. Res.* **28**, 1536–1545.

Holdenrieder, O. (1982) Kristallbildung bei *Heterobasidion annosum* (Fr.) Bref. (*Fomes annosus* P. Karst.) und anderen holzbewohnenden Pilzen. [Production of crystals by *Heterobasidion annosum* (Fr.) Bref. (*Fomes annosus* P. Karst.) and some other wood-inhabiting fungi]. *Eur. J. For. Pathol.* **12**, 41–58.

Holdenrieder, O. and Greig, B.J.W. (1998) Biological methods of control. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hüttermann, A., eds), pp. 235–259. London: CAB International.

Holmer, L. and Stenlid, J. (1994) Diffuse competition for heterogenous substrate in soil among six species of wood decomposing basidiomycetes. *Oecologia*, **106**, 531–538.

Holmer, L. and Stenlid, J. (1997) Resinicium bicolor; *a potential biological control agent for* Heterobasidion annosum. *Eur. J. For. Pathol.* **27**, 159–172.

Hsiang, T., Edmonds, R.L. and Driver, C.H. (1989) Conidia of *Heterobasidion annosum* from *Tsuga heterophylla* forests in western Washington. *Can. J. Bot.* **67**, 1262–1266.

Huse, K.J. and Venn, K. (1994) Vertical spread of *Heterobasidion annosum* in stems of Norway spruce. In: *Proceedings of the 8th IUFRO Conference on Root and Butt Rots. Sweden/Finland, August 1993*. (Johansson, M. and Stenlid, J., eds), pp. 208–212. Uppsala: Swedish University of Agricultural Sciences.

Hüttermann, A., Kuhl, W.E. and Chet, I. (1980) The effect of 1-threonine on oxalic acid synthesis by *Fomes annosus*. *Eur. J. For. Pathol.* **10**, 339–344.

Hüttermann, A. (1980) Biochemical studies on *Fomes annosus*. In: *Proceedings of the Fifth IUFRO Conference on Problems of Root and Butt Rot in Conifers* (Dimitri, L., ed.), pp. 109–123. Hann. Munden: Hessische Forstliche Versuchsanstalt.

Hüttermann, A. and Woodward, S. (1998) Historical aspects. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S.,

Stenlid, J., Karjalainen, R. and Hüttermann, A., eds), pp. 1–26. London: CAB International.

Idnurm, A. and Howlett, B.J. (2001) Pathogenicity genes of phytopathogenic fungi. *Mol. Plant Pathol.* **2**, 241–255.

Ikediugwu, F.E.O. (1976) The interface in hyphal interference by *Peniophora gigantea* against *Heterobasidion annosum*. *Trans. Br. Mycol. Soc.* **66**, 291–296.

Ikediugwu, F.E.O., Dennis, C. and Webster, J. (1970) Hyphal interference by *Peniophora gigantea* against *Heterobasidion annosum*. *Trans. Br. Mycol. Soc.* **54**, 307–309.

Irhmark, K., Johannesson, H., Stenström, E. and Stenlid, J. (2002) Transmission of double stranded RNA in *Heterobasidion annosum*. *Fungal Gen. Biol.* **36**, 147–154.

Irhmark, K., Stenström, E. and Stenlid, J. (2004) Double stranded RNA transmission through basidiospores of *Heterobasidion annosum*. *Mycol. Res.* **108**, 149–153.

Johanesson, H. and Stenlid, J. (2003) Molecular markers reveal genetic isolation and phylogeography of the S and P intersterility groups of the wood decay fungus *Heterobasidion annosum*. *Mol. Phylog. Evol.* **29**, 94–101.

Johansson, M. (1988) Pectic enzyme activity of spruce (S) and pine (P) strains of *Heterobasidion annosum*. *Physiol. Mol. Plant Pathol.* **33**, 333–349.

Johansson, S.M., Lundgren, L.N. and Asiegbu, F.O. (2004) Initial reactions in sapwood of Norway spruce and Scots pine after wounding and infection by *Heterobasidion parviporum* and *H. annosum*. *For. Pathol.* **34**, 197–210.

Johansson, S.M., Pratt, J.E. and Asiegbu, F.O. (2002) Treatment of Norway spruce and Scots pine stumps with urea against the root and butt rot fungus *Heterobasidion annosum*—possible modes of action. *For. Ecol. Manag.* **157**, 87–100.

Johannsson, M. and Stenlid, J. (1985) Infection of roots of Norway spruce (*Picea abies*) by *Heterobasidion annosum*. *Eur. J. For. Pathol.* **15**, 32–45.

Jones, E.B.G. (1994) Fungal adhesion. *Mycol. Res.* **98**, 961–981.

Kadlec, Z., Stary, P. and Zumr, V. (1992) Field evidence for the large pine weevil, *Hylobius abietis* as a vector of *Heterobasidion annosum*. *Eur. J. For. Pathol.* **22**, 316–318.

Kallio, T. and Hallaksela, A.M. (1979) Biological control of *Heterobasision annosum* (Fr.) bref. (*Fommes annosus*) in Finland. *Eur. J. For. Pathol.* **9**, 298–308.

Karjalainen, R., Ernst, D. and Woodward, S. (1998) Molecular biology of host defence. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control*. (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 195–213. London: CAB International.

Karlsson, J.-O. and Stenlid, J. (1991) Pectic isozyme profiles of the intersterility groups in *Heterobasidion annosum*. *Mycol. Res.* **95**, 531–536.

Karlsson, J.-O., Olson, Å. and Stenlid, J. (2003) Expressed sequences from the basidiomycetous tree pathogen *Heterobasidion annosum* during early infection of Scots pine. *Fungal Genet. Biol.* **39**, 51–59.

Kasuga, T. and Mitchelson, K. (1994) The rapid identification of *Heterobasidion annosum* intersterility groups by ribosomal RNA gene polymorphism and by minisatellite allele polymorphism. In: *Molecular Detection Assays for Plant Pathogenic Fungi* (Schots, A., Dewey, F.M. and Oliver, R., eds), pp. 117–126. Wallingford, UK: CAB International.

Kasuga, T., Woods, C., Woodward, S. and Mitchelson, K.R. (1993) *Heterobasidion annosum* 5.8S ribosomal DNA and internal transcribed spacer sequence: rapid identification of European intersterility groups by ribosomal DNA restriction polymorphism. *Curr. Genet.* **24**, 433–436.

Keen, N.T. (1990) Gene for gene complimentarity in plant pathogen interactions. *Annu. Rev. Genet.* **24**, 447–463.

Keon, J., Bailey, A. and Hargraves, J. (2000) A group of expressed cDNA sequences from the wheat fungal leaf blotch pathogen, *Mycosphaerella graminicola* (*Septoria tritici*). *Fungal Genet. Biol.* **29**, 118–133.

Korhonen, K. (1978) Intersterility groups of *Heterobasidion annosum*. *Commes Instituti Foralis Fenniae*, **94**, 25 pp.

Korhonen, K., Capretti, P., Karjalainen, R. and Stenlid, J. (1998a) Distribution of *Heterobasidion annosum* intersterility groups in Europe. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control*. (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 93–105. London: CAB International.

Korhonen, K., Dai, Y.-C., Hantula, J. and Vainio, E. (2001) Investigations on *Heterobasidion* in central and eastern Asia. In: *10th International Conference on Root and Butt Rots of Forest Trees* (Laflamme, G., Berube, J.A. and Bussieres, G., eds), pp. 27–32. Natural Resources Canada.

Korhonen, K., Delatour, C., Greig, B.J.W. and Schönhar, S. (1998b) Silvicultural control. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 283–315. London: CAB International.

Korhonen, K., Fedorov, N.I., La Porta, N. and Kovbasa, N.P. (1997) Abies sibirica *in the Ural region is attacked by the S type of* Heterobasidion annosum. *Eur. J. For. Pathol.* **27**, 273–281.

Korhonen, K. and Stenlid, J. (1998) Biology of *Heterobasidion annosum*. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 43–71. London: CAB International.

Krekling, T., Franceschi, V.R., Krokene, P. and Solheim, H. (2004) Differential anatomical response of Norway spruce stem tissues to sterile and fungus infected inoculations. *Trees-Structure Function*, **18**, 1–9.

La Porta, N., Capretti, P., Korhonnen, K., Kammiovirta, K. and Karjalainen, R. (1997) The relatedness of the Italian intersterility group of *Heterobasidion annosum* with the S group as revelead by RAPD assay. *Mycol. Res.* **101**, 1065–1072.

Lamb, C. and Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 251–275.

Lequart, C., Kurek, B., Debeire, P. and Monties, B. (1998) MnO_2 and oxalate: an abiotic route for the oxidation of aromatic components in wheat straw. *J. Agric. Food Chem.* **46**, 3868–3874.

Li, G. and Asiegbu, F.O. (2004) Induction of *Pinus sylvestris PsACRE*, a homology of *Avr9/Cf-9* rapidly elicited defence-related gene following infection with root rot fungus *Heterobasidion annosum*. *Plant Sci.* **167**, 535–540.

Lindberg, M. and Johansson, M. (1992) Resistance of *Picea abies* seedlings to infection by *Heterobasidion annosum* in relation to drought stress. *Eur. J. For. Pathol.* **22**, 115–124.

Lindberg, M., Lundgren, L., Gref, R. and Johansson, M. (1992) Stilbenes and resin acids in relation to the penetration of *Heterobasidion annosum* through the bark of *Picea abies*. *Eur. J. For. Pathol.* **22**, 95–106.

Linden, M. and Vollbrecht, G. (2002) Sensitivity of *Picea abies* to butt rot in pure stands and in mixed stands with *Pinus sylvestris* in Southern Sweden. *Silva Fennica*, **36**, 767–778.

Lloyd, J. (1997) Borates and their biological applications. In: *Proceedings of the Second International Conference on Wood Protection with Diffusible Preservative and Pesticides* (Stamm, S., ed.), pp. 45–54. Madison, WI: Forest Products Society.

Lygis, V., Vasiliauskas, R. and Stenlid, J. (2004a) Planting *Betula pendula* on pine sites infested by *Heterobasidion annosum*: disease transfer,

silvicultural evaluation and community of wood inhabiting fungi. *Can. J. For. Res.* **34**, 120–130.

Lygis, V., Vasiliauskas, R., Stenlid, J. and Vasiliauskas, A. (2004b) Silvicultural and pathological evaluation of Scots pine afforestations mixed with deciduous trees to reduce the infections by *Heterobasidion annosum*. *For. Ecol. Manag.* **201**, 275–285.

Maijala, P., Harrington, T.C. and Raudaskoski, M. (2003) A peroxidase gene family and gene trees in *Heterobasidion* and related genera. *Mycologia*, **95**, 209–221.

Maijala, P., Raudaskoski, M. and Viikari, L. (1995) Hemicellulolytic enzymes in P- and S-strains of *Heterobasidion annosum*. *Microbiology*, **141**, 743–750.

Manocha, M.S. (1984) Cell surface characteristics of *Mortierella* and their interaction with a mycoparasite. *Can. J. Microbiol.* **30**, 290–298.

Mayer, A.M., Staples, R.C. and Gil-ad, N.L. (2001) Mechanisms of survival of necrotrophic fungal plant pathogens in hosts expressing the hypersensitive response. *Phytochemistry*, **58**, 33–41.

Meredith, D.S. (1959) The infection of pine stumps by *Fomes annosus* and other fungi. *Ann. Bot., New Series*, **23**, 455–476.

Moller, C.M. (1939) Nye Danske undersogelser over rodfordaerveren. *Dansk Skovforeningens Tidskrift*, **1939**, 433–454.

Moreira, M.T., Feijoo, G., Mester, T., Mayorga, P., Sierra-Alvarez, R. and Field, J.A. (1998) Role of organic acids in the manganese independent biobleaching system of *Bjerkandera* sp. Strain BOS55. *Appl. Environ. Microbiol.* **64**, 2409–2417.

Morrison, D.J. and Redfern, D.B. (1994) Long term development of *Heterobasidion annosum* in basidiospores-infected Sitka spruce stumps. *Plant Pathol.* **43**, 897–906.

Moykkynen, T., Miina, J. and Pukkala, T. (2000) Optimizing the management of a *Picea abies* stand under risk of butt rot. *For. Pathol.* **30**, 65–76.

Moykkynen, T., Miina, J., Pukkala, T. and von Weissenberg, K. (1998) Modelling the spread of butt rot in a *Picea abies* stand in Finland to evaluate the profitability of stump protection against *Heterobasidion annosum*. *For. Ecol. Manag.* **106**, 247–257.

Nagy, N.E., Fossdal, C.G., Krokene, P., Krekling, T., Lonneborg, A. and Solheim, H. (2004) Induced responses to pathogen infection in Norway spruce phloem: changes in polyphenolic parenchyma cells, chalcone synthase transcript levels and peroxidase activity. *Tree Physiol.* **24**, 505–515.

Nahalkova, J., Asiegbu, F.O., Daniel, G., Hrib, J., Vookova, B., Pribulova, B. and Gemeiner, P. (2001) Isolation and immunolocalization of a *Pinus nigra* lectin (PNL) during interaction with the necrotrophs—*Heterobasidion annosum and Fusarium avenaceum*. *Physiol. Mol. Plant Pathol.* **59**, 153–163.

Nicolotti, G., Gonthier, P. and Varese, G.C. (1999) Efectiveness of some biocontrol and chemical treatments against *Heterobasidion annosum* on Norway spruce stumps. *Eur. J. For. Pathol.* **29**, 339–346.

Nicolotti, G. and Varese, G.C. (1996) Screening of antagonstic fungi against air-borne infection by *Heterobasidion annosum* on Norway spruce. *For. Ecol. Manag.* **88**, 249–257.

Niemelä, T. and Korhonen, K. (1998) Taxonomy of the genus *Heterobasidion*. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 27–35. London: CAB International.

Nord, F.F. and Hata, K. (1969) Fungal degradation of pine bark lignin. In: *Current Aspects of Biochemical Energetics* (Kaplan, N.O. and Kennedy, E.P., eds), pp. 315–329. New York: Academic Press.

Olson, Å. and Stenlid, J. (2001) Mitochondrial control of fungal hybrid virulence. *Nature*, **411**, 438.

Olson, A. and Stenlid, J. (2002) Pathogenic fungal species hybrids infecting plants. *Microb. Infect.* **4**, 1353–1359.

Otrosina, W.J., Chase, T.E., Cobb, F.W. and Korhonen, K. (1993) Population structure of *Heterobasidion annosum* from North America and Europe. *Can. J. Bot.* **71**, 1064–1071.

Peek, R.D., Liese, W. and Parameswaran, N. (1972) Infection and degradation of *Picea abies* root wood by *Fomes annosus*. *Eur. J. For. Pathol.* **2**, 237–248.

Piri, T. (1996) The spreading of the S-type of *Heterobasidion annosum* from Norway spruce stumps to the subsequent tree stand. *Eur. J. For. Pathol.* **26**, 193–204.

Piri, T. (1998) Effects of vitality fertilization on the growth of *Heterobasidion annosum* in Norway spruce roots. *Eur. J. For. Pathol.* **28**, 391–397.

Piri, T. (2000) Response of compensatory-fertilized *Pinus sylvestris* to infection by *Heterobasidion annosum*. *Scand. J. For. Res.* **15**, 218–224.

Piri, T., Korhonen, K. and Sairanen, A. (1990) Occurence of *Heterobasidion annosum* in pure and mixed stands in Southern Finland. *Scand. J. For. Res.* **5**, 113–125.

Popp, J.L., Kalyanaraman, B. and Kirk, T.K. (1990) Lignin peroxidase oxidation of Mn^{2+} in the presence of veratryl alcohol, malonic or oxalic acid and oxygen. *Biochemistry*, **29**, 10475–10480.

Pratt, J.E., Johansson, M. and Hütterman, A. (1998a) Chemical control of *Heterobasidion annosum*. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 259–283. London: CAB International.

Pratt, J.E. and Lloyd, J.D. (1996) *The Use of Disodium Octaborate Tetrahydrate to Control Conifer Butt Rot Caused by Heterobasidion annosum*. Dundee: Crop Protection in Northern Britain, Scottish Crops Research Institute, pp. 207–212.

Pratt, J.E., Niemi, M. and Sierota, Z.H. (2000) Comparison of three products based on *Phlebiopsis gigantea* for the control of *Heterobasidion annosum*. Europe. *Biocont. Sci. Technol.* **10**, 467–477.

Pratt, J.E., Redfern, D.B. and Burnand, A. (1989) Modelling the spread of *Heterobasidion annosum* in Sitka spruce plantations in Britain. In: *Proceedings of the Seventh International Conference on Root and Butt Rots* (Morisson, D.J., ed.), pp 308–319. Victoria, British Columbia: Forestry Canada.

Pratt, J.E., Shaw Iii, C. and Vollbrecht, G. (1998b) Modelling disease development in forest stands. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 213–235. London: CAB International.

Pukkala, T., Moykkynen, T., Thor, M., Ronnberg, J. and Stenlid, J. (2005) Modeling infection and spread of *Heterobasidion annosum* in even-aged Fennoscandian conifer stands. *Can. J. For. Res.* **35**, 1–11.

Rauyaree, P., Choi, W., Fang, E., Blackmon, B. and Dean, R.A. (2001) Genes expressed during early stages of rice infection with the rice blast fungus *Magnaporthe grisea*. *Mol. Plant Pathol.* **2**, 347–354.

Redfern, D.B. and Macaskill, G.A. (2003) Susceptibility of Sitka spruce and grand fir trees to decay by *Heterobasidion annosum*. *For. Pathol.* **33**, 39–52.

Redfern, D.B. and Stenlid, J. (1998) Spore dispersal and infection. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 105–125. London: CAB International.

Rishbeth, J. (1959) Dispersal of *Fomes annosus* and *Peniophora gigantea*. *Trans. Br. Mycol. Soc.* **42**, 243–260.

Rishbeth, J. (1962) Rate of spread of *Fomes annosus*. *Trans. Br. Mycol. Soc.* **45**, 280.

Roll-Hansen, F. and Roll-Hansen, H. (1981) Root wound infection of *Picea abies* at three localities in Southern Norway. *Meddelser Fra Norsk Inst for Skogforskning*, **36**, 1–18.

Ross, E.W. (1973) *Fomes annosus* in the southeastern United States: relation of environmental and biotic factors to stump colonization losses in the residual stand. *For. Service, Techn. Bull.* **1459**, 26 pp.

Schmitt, C., Parmeter, J. and Kliejunas, J. (2000) Annosus root rot disease of western conifers. In: *Forest Insect and Disease Leaflet*. U.S. Department of Agriculture, Forest Service, 172.

Schönhar, S. (1992) Feinwurzelschäden und Pilzbefall in Fichtenbeständen. *Allgemeine Forstzeitschrift*, **47**, 384–385.

Shain, L. (1967) Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. *Phytopathology*, **57**, 1034–1045.

Shaw, C.G. and Kile, G.A. (1991) *Armillaria Root Disease*. *Agricultural Handbook No. 691*. Washington, DC: Forest Service: United States department of Agriculture.

Shimada, M., Ma, D.B., Akamatsu, Y. and Hattori, T. (1994) A proposed role of oxalic acid in wood decay systems of wood rotting basidiomycetes. *FEMS Microbiol. Rev.* **13**, 285–296.

Siepmann, R. (1981) *A Contribution to the Regeneration and Infection of Roots of Picea abies and Pseudostuga menziesii by Fungi Present in the Soil. Root and Butt Rots in Scots Pine Stand*, pp. 138–141. European Regional Meeting, IUFRO Working Party S2.06.01, Poznan, Poland.

Solla, A., Tomlinson, F. and Woodward, S. (2002) Penetration of *Picea sitchensis* root bark by *Armillaria mellea*, *Armillaria ostoyae* and *Heterobasidion annosum*. *For. Pathol.* **32**, 55–70.

Sonnenbichler, J., Bliestle, I.M., Peipp, H. and Holdenrieder, O. (1989) Secondary fungal metabolites and their biological activities. I. Isolation of antibiotic compounds from cultures of *Heterobasidion annosum* synthesized in the presence of antagonisitic fungi or host plant cells. *Biol. Chem. Hoppe-Seyler*, **370**, 1295–1303.

Stenlid, J. (1985) Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility and isozyme patterns. *Can. J. Bot.* **63**, 2268–2273.

Stenlid, J. (1987) Controlling and predicting the spread of *Heterobasidion annosum* from infected stumps and trees of *Picea abies*. *Scan. J. For. Res.* **2**, 187–198.

Stenlid, J. (1994) Homokaryotic *Heterobasidion annosum* mycelia in stumps of Norway spruce. In: *Procedings of the 8th IUFRO Conference on Root and Butt Rots, Sweden/Finland, August 1993* (Johansson, M. and Stenlid, J., eds), pp. 243–248. Uppsala: Swedish University of Agricultural Sciences.

Stenlid, J., Karlsson, J.-O. and Högber, N. (1994) Interspecific genetic variation in *Heterobasidion annosum* revealed by amplification of minisatellite DNA. *Mycol. Res.* **98**, 57–63.

Stenlid, J. and Redfern, D.B. (1998) Spread within the tree and stand. In: *Heterobasidion annosum: Biology, Ecology, Impact and Control* (Woodward, S., Stenlid, J., Karjalainen, R. and Hütterman, A., eds), pp. 125–143. London: CAB International.

Swedjemark, G. and Karlsson, B. (2004) Genotypic variation in susceptibility following artificial *Heterobasidion annosum* inoculation of *Picea abies* clones in a 17-year-old field test. *Scand. J. For. Res.* **19**, 103–111.

Swedjemark, G. and Stenlid, J. (1993) Population dynamics of the root rot fungus *Heterobasidion annosum* following thinning of *Picea abies*. *Oikos*, **66**, 247–254.

Swedjemark, G. and Stenlid, J. (1995) Susceptibility of conifer and broad leaf seedlings to Swedish S and P strains of *Heterobasidion annosum*. *Plant Pathol.* **44**, 73–79.

Swedjemark, G. and Stenlid, J. (1997) Between-tree and between-isolate variation for growth of S-group *Heterobasidion annosum* in sapwood of *Picea abies* cuttings. *Can. J. For. Res.* **27**, 711–715.

Swedjemark, G., Stenlid, J. and Karlsson, B. (1997) Genetic variation among clones of *Picea abies* in resistance to growth of *Heterobasidion annosum*. *Silvae Genetica*, **46**, 369–374.

Thomas, W.S., Rasmussen, W.S., Glaring, A.M., Rouster, A.J., Christiansen, K.S. and Oliver, P.R. (2001) Gene identification in the obligate fungal pathogen *Blumeria graminis* by expressed sequence tag analysis. *Fungal Genet. Biol.* **33**, 195–211.

Thor, M., Ståhl, G. and Stenlid, J. (2005) Root rot incidence, correlation with environmental conditions and potential for predicting stand decay levels. *Scand. J. For. Res.* **20**, 165–176.

Thor, M. and Stenlid, J. (2005) *Heterobasidion annosum* infection following mechanised first thinning and stump treatment in *Picea abies*. *Scand. J. For. Res.* **20**, 154–164.

Vainio, E.J., Lipponen, K. and Hantula, J. (2001) Persistence of a biocontrol strain of *Phlebiopsis gigantea* in conifer stumps and its effects on within-species genetic diversity. *For. Pathol.* **31**, 285–295.

Vasiliauskas, R., Lygis, V., Thor, M. and Stenlid, J. (2004) Impact of biological (Rotstop) and chemical (urea) treatments on fungal community structure in freshly cut *Picea abies* stumps. *Biol. Control.* **31**, 405–413.

Volger, C., Hesse, C. and Vogt, A. (1982) On the occurrence of calcium oxalate crystals in *Heterobasidion annosum* (Fr.) Bref. *Eur. J. For. Pathol.* **7**, 262–282.

Vollbrecht, G. and Agestam, E. (1995) Modeling incidence of root-rot in *Picea abies* plantations in southern Sweden. *Scand. J. For. Res.* **10**, 74–81.

Westlund, A. and Nohrstedt, H.-Ö. (2000) Effects of stump treatment substances for root-rot control on ground vegetation and soil properties in a *Picea abies* forest in Sweden. *Scand. J. For. Res.* **15**, 550–560.