Heart Rot of Spruce and Alder in Forests of Latvia

Impact and Possibilities for Silvicultural Control

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Abstract
Heart rot causes great decrease in timber quality throughout the world. In the conifer forests of Northern Hemisphere the most significant losses are caused by fungi from the Heterobasidion annosum species complex. The spread of the disease is favored by forest management, as the fungus can use freshly cut stumps as an infection court, and so extend through root contacts to infect neighboring trees.

The country-scale inventory revealed that about 22% of Latvian spruce trees contained heart rot, which extended on average 6.6 m within the tree stem; the most commonly associated fungus being H. parviporum. These facts highlight the need to take actions to reduce the level of inoculum in already infested stands. Stump removal and the planting of resistant tree species are two options for that.

Long-term trials carried out in Scandinavia showed that stump removal could significantly decrease the proportion of infected trees in regenerated stands, although the effectiveness of this procedure may eventually decrease with time. In addition, this method is quite drastic and the adverse influence of continuous stump removal on stand biodiversity and productivity should be taken into consideration. The trail conducted in Finland showed that stump removal had a positive impact on seedlings growth, but influenced negatively mycorrhization and species richness.

The other management method which could be employed is the planting of resistant tree species, especially broadleaved trees. In the work undertaken for this thesis heart rot incidence, associated fungi and decay caused yield losses were studied in Latvian grey (Alnus incana) and black alder (A. glutinosa) stands. Despite the high decay incidence and the number of associated fungal species, no single H. annosum s.l. was found. Also, our data showed that the proportion and size of the heart rot column increased with tree age. These data suggest that a short rotation of fast growing broadleaved trees (not only Alnus, but also species of Populus, Betula, and Salix) could be used for clearing up the Heterobasidion inoculum.

Keywords: Decay incidence, Heterobasidion, Picea abies, Alnus incana, Alnus glutinosa, decay causing fungi, stump removal, mycorrhiza.

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Dedication

This work is dedicated to my mother, Maria, for all the love and support what you have given to me.
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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:


Papers I-V are reproduced with the permission of the publishers.
The contribution of Natalija Arhipova to the papers included in this thesis was as follows:

I  Part of the field work and most of the laboratory work. Analysis of data and writing up the paper in collaboration with the co-authors.
II Field work done together with supervisors. All laboratory work, analysis of data and writing up the Brynge and Ramsåsa part of manuscript.
III Conducted part of field work.
IV Field work done together with supervisors. All laboratory work. Analysis of data and writing up the paper in collaboration with the co-authors.
V All laboratory work. Analysis of data and writing up the paper in collaboration with the co-authors.
<table>
<thead>
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<tr>
<td>BLAST</td>
<td>Basic Local Alignment Search Tool</td>
</tr>
<tr>
<td>CIAP</td>
<td>Calf Intestinal Alkaline Phosphatase</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyl trimethyl ammonium bromide</td>
</tr>
<tr>
<td>DBH</td>
<td>Diameter at breast height</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>ECM</td>
<td>Ectomycorrhizae</td>
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<td>Exo I</td>
<td>Escherichia coli exonuclease I</td>
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<td>ITS</td>
<td>Internal transcribed spacer</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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1 Introduction

1.1 Heart rot

Heart rot in living trees causes serious economical losses due to mortality, growth reduction and decrease of timber quality throughout the world. The term “heart rot” is used to describe decay caused by fungi in the central core of the tree (Berry, 1973). Only in temperate regions of the Northern Hemisphere heart rots caused by different decay fungi reduce timber quality by several million cubic meters annually (Hinds, 1977; Woodward et al., 1998). For example, inventories of spruce (Picea abies (L.) Karst.) stands in Finland demonstrated that direct losses in sawn timber volume due to stem rot varied on average from 8.5% to 30% (Kallio, 1972; Kallio & Tamminen, 1974; Tamminen, 1985). In broadleaved stands losses caused by heart rot can be even higher. For instance, inventories conducted in Populus tremuloides Michx. stands in different regions of the United States showed that on average 42-80% of aspen trees contained stem decay (Davidson et al., 1959; Jones & Ostry, 1998; Warall & Fairweather, 2009).

Many fungal species, mainly basidiomycetes, are capable of causing considerable amount of stem decay in forest trees (Figure 1). Entry occurs through different infection courts, such as fire scars, dead branches, roots, parent stumps, or mechanical injuries (Berry, 1973). Heart rot in stems and roots could be considered as economically the most important cause of decrease of timber quality in managed forests, where cut stumps and wounds on living stems are abundant. For example, the highly destructive fungal pathogens from the genus Heterobasidion, which cause considerable heart rot in P. abies, are promoted by the forest management (Woodward et al., 1998, Berglund et al., 2012). As the disease is very common, in some studies heart rot of P. abies was associated with Heterobasidion based only on visual stump assessment (Enerstvedt & Venn, 1979; Nilsen, 1983; Vasiliauskas, 1989; Huse et al.,
However, many other decay fungi can cause heart rot in living *P. abies* trees (Pechmann & Aufsess, 1971; Pechmann *et al*., 1973; Kallio & Tamminen, 1974; Norokorpi, 1979; Hallaksela, 1984; Stenlid & Wästerlund, 1986; Piri *et al*., 1990; Vasiliauskas *et al*., 1996; Vasiliauskas & Stenlid, 1998; Vasiliauskas, 1998, 1999; Mäkinen *et al*., 2007). Furthermore, communities of heart rot pathogens of *P. abies* may vary considerably depending on geographical region, stand age, site characteristics and management history, as reported from Germany (Pechmann *et al*., 1973) and Finland (Kallio & Tamminen, 1974; Norokorpi, 1979; Hallaksela, 1984).

Figure 1. Heart rot in spruce tree. (Photo: T. Gaitnieks).

Forests cover about 50% of the total land area of Latvia, and are the most important renewable natural resource in the country. Wood is one of the most essential forest products and makes up a significant component of all Latvian exports (Latvian Forest Industry Federation, 2008). Because of their significance Latvia’s forests are intensively managed and, furthermore, the proportion of forest lands is likely to increase in the future due to a country scale policy of afforestation of abandoned farmlands. Despite this, little data are available about the distribution and importance of heart rot in spruce *P. abies* and alders, *Alnus incana* (L.) Moench. and *A. glutinosa* (L.) Gaertn. in Latvian forests. Early studies have shown that the proportion of decayed stumps in spruce clear-cuts varies from 18-68%, and in some stands – even reaches 87% (Eglitis, 1938; Jurēvičs, 1939; Lange, 1960; Mangalis, 1975; Gaitnieks *et al*., 2004). However, fungi were not isolated in the cited studies, thus the causal agents of the disease remain unidentified. Studies on heart rot incidence in alders were never been previously performed in Latvia. The aim of the current work (Papers I-V) was to investigate frequency of infection by
stem decay fungi, the extent of decay they cause, the associated yield losses and management possibilities for reducing decay incidence in Latvian forests. The identification of stem rot causing fungi (Papers I, IV and V) also assist future silvicultural management decision-making and in helping in planning the most suitable stand regeneration strategies.

1.2 Heterobasidion root rot

Heterobasidion (or annosum) root rot is considered to be one of the most destructive conifer diseases in the north temperate regions of the world (Woodward et al., 1998). In addition to root rot, *H. annosum* s.l. is able to cause extensive heart rot in a number of tree species, including *P. abies*. The disease usually occurs in managed conifer stands where the pathogen may cause direct tree mortality, growth reduction and root and stem decay (Risbeth, 1951c; Hodges, 1969; Bradford et al., 1978; Bendz-Hellgren & Stenlid, 1997; Gibbs et al., 2002). The *H. annosum* s.l. species complex consists of five species that have a wide geographical distribution, particularly, in many parts of Europe, Asia and North America (Dalman et al., 2010). These fungi were once considered to belong to a single species under the name *Fomes annosus* (Fr.) Karst, or more recently, *Heterobasidion annosum* (Fr.) Bref., until mating experiments conducted by Korhonen (1978) revealed there were a number of host specialized intersterility groups. These intersterility groups were initially classified as the P, S (Korhonen, 1978), and F-type (Capretti et al., 1990) based on their host preferences, namely pine, spruce and fir. However, as a result of subsequent phylogenetic studies, polymorphism in the DNA regions proved that three European (P, S and F) and two North American (P and S) intersterility groups belong to different clades, and that the *H. annosum* complex is in fact composed of five species (Johannesson & Stenlid, 1998; Otrosina & Garbelotto, 2010; Dalman et al., 2010). As a result, new scientific names were proposed: *H. annosum* (Fr.) Bref. s.s. (former European P type), *H. parviporum* Niemelä & Korhonen (former European S type) (Figure 2), *H. abietinum* Niemelä & Korhonen (former European F type), *H. irregulare* Otrosina & Garbelotto (former American P type) and *H. occidentale* Otrosina & Garbelotto (former American S type).
These *Heterobasidion* species have two ways of dispersing: one - by airborne basidiospores, which invade through the surface of freshly cut stumps or fresh wounds, especially where these are close to the tree base and on roots; and another - by mycelia, which infect roots of neighboring trees via root contacts (Risbeth, 1951a, 1951b, 1951c; Meredith, 1959; Hodges, 1969; Roll-Hansen & Roll-Hansen, 1980; Vollbrecht & Stenlid, 1999). The disease symptoms in living trees, e.g. resin exudation, crown deterioration, butt swelling are not very characteristic and cannot be distinguished from symptoms, caused by other root pathogens (Woodward et al., 1998). *H. annosum* species grow necrotrophically within the sapwood of the living trees, but with the exception of pine infections, extends to the heartwood, causing intensive heart rot, usually without any external symptoms (Gibbs, 1968; Woodward et al., 1998). The decay caused by *H. annosum* is described as a white pocket rot. The color of the stain depends on host species. The incipient decay in most trees is pale-yellow or straw colored, usually surrounded by a darker stained zone, eventually becoming soft, fibrous and light-brown in the advanced stage, sometimes leading to hollowing in the center of stem (Woodward et al., 1998). The spread rate within the individual stem depends on the tree vitality and wood moisture content and can reach rates of 2 m per year in roots and 1 m per year in stems (Hodges, 1969). The spread rate of disease foci through the stand depends on stand type, management history, tree species composition, soil properties and other factors (Risbeth, 1951b; Greig, 1962; Pratt & Greig, 1988; Lindén & Vollbrecht, 2002; Puddu et al., 2003). In the northern temperate forests spread rate may reach on average 20-50 cm per year and the largest detected area occupied by a single genet was about 50 m at diameter (Stenlid, 1985; Piri et al., 1990; Piri, 1996; Garbelotto et al., 1997). The fungus can
survive in the wood residues (for example, stumps) for up to 60 years and still retain the ability to infect the next forest generation (Morrison & Johnson, 1978).

1.3 Disease management

About 46% of Latvian forests are composed of conifer stands, mainly of Scots pine (*Pinus sylvestris* (L.)) and Norway spruce (*P. abies*) (Central Statistical Bureau of Latvia, 2011). Both of these tree species are highly susceptible to infection by *H. annosum* s.l. and disease management is of high importance. As effective control of *H. annosum* s.l. is difficult, all practical methods that help to reduce the infection frequency would be of great value. There are three generally accepted ways to minimize damage in the future: stump removal, freshly-cut stump treatment with chemical or biological preparations and planting of resistant tree species in the succeeding stand generation (Hodges, 1969; Hallaksela & Nevalainen, 1981, Greig, 1984, Woodward *et al.*, 1998, Thor & Stenlid, 2005, Oliva *et al.*, 2008, Vasaitis *et al.*, 2008). Other possible control methods which have been suggested by different authors to reduce economic losses created by root rot are: planting mixed stands with a high proportion of deciduous trees, prescribed burning, seedling planting with wider spacing, reduced or delayed thinning in first rotation stands, site fertilization, and reducing the rotation time in diseased stands (Greig, 1984; Woodward *et al.*, 1998; Piri, 2000; Piri, 2003, Lygis *et al.*, 2004b, 2010).

1.3.1 Biological and chemical stump treatment as a control method

Treatment of freshly cut stumps with biological and chemical preparations is widely used to prevent infection by airborne basidiospores. Today, the most widespread method of *Heterobasidion* control is stump treatment with a *Phlebiopsis gigantea* (Fr.) Jülich spore suspension. The treatment was first suggested by Risbeth (1950, 1951b, 1952, 1961, 1963) and was found to be an effective way of preventing spore infection of freshly cut stumps, especially on different pine species (Greig, 1976; Annesi *et al.*, 2005; Nicolotti & Gonthier, 2005). Nowadays, a number of preparations based on *P. gigantea* have been produced in Europe: “PG Suspension” (UK), “PgIBL” (Poland), “Rotex” (Germany) and “Rotstop” (Finland). Stump treatment with *P. gigantea* preparations is widely used in many European countries (Pratt *et al.*, 2000) for reducing new infections by air-borne basidiospores, but has no effect, if disease is already present in stumps (Pettersson *et al.*, 2003).
Amongst chemical agents used for stump treatment, urea is the most commonly employed. The method works by changing the pH of the stump surface thus making it unsuitable for colonization of *Heterobasidion* airborne basidiospores (Johansson *et al*., 2002). This method does have a negative impact on the stump inhabiting basidiomycete community (Vasiliauskas *et al*., 2004) and also on plant species population within the stand (Westlund & Nohrstedt, 2000). However, it has been shown to be quite effective for controlling *Heterobasidion*, especially at urea concentrations of 20-30% (Brantberg *et al*., 1996; Nicolotti & Gonthier, 2005; Lehtijärvi *et al*., 2011; Wang *et al*., 2012) and the toxicity of urea is low. As *P. gigantea* preparations are not as effective in spruce as in pine wood (Berglund & Rönnberg, 2004), stump treatment with urea could be an alternative method for this species (Oliva *et al*., 2008).

### 1.3.2 Stump removal as a control method

Stump removal (Figure 3) to reduce the probability of root disease transmission from stumps of the previous generation to newly planted trees was first suggested for controlling *H. annosum* by Hartig in 1878. Numerous experiments have shown that stump removal is an effective method for controlling *H. annosum* in heavily infested forest areas (Greig, 1980, 1984, Stenlid, 1987, Greig *et al*., 2001, Vasaitis *et al*., 2008).

![Figure 3. Stump removal (Photo: T. Gaitnieks).](image-url)
Stump removal has also been recommended for reducing the inoculum potential of other root pathogens, e.g. *Armillaria solidipes* Peck. and *Phellinus sulphurascens* Pilát. in British Columbia (B.C.), Canada (Cleary *et al*., 2008, 2011; Strurrock *et al*., 2006). Although this operation is expensive, there has been renewed interest in this procedure during the last few years because of the possibility of using stumps as an energy source (Björheden, 2006, Eriksson & Gustavsson, 2008; Laitila *et al*., 2008; Walmsley & Godbold, 2010). Moreover, Saarinen (2006) showed that slash and stump removal will increase the work productivity and quality of mechanized forest regeneration. However, little is known about the long term impact of stump removal on root rot incidence. In paper II results from long-term experiments carried out in Sweden, Denmark and Canada for the control of the different root rot diseases: heterobasidion, armillaria and laminated root rot were compared. Data about the possible ecological impact of stump removal are scarce, especially from Europe (Walmsley & Godbold, 2010.). In paper III influence of stump and slash removal on the growth and mycorrhization of *P. abies* seedlings was studied.

1.3.3 Planting of resistant tree species as a control method

The relative resistance of tree species to pathogens is of special interest for reducing losses created by forest diseases. Planting of resistant species on the heavily infested sites has been suggested by number of authors (Stambaugh, 1989; Woodward *et al*., 1998; Greig *et al*., 2001). Broadleaved trees are considered to be more resistant to infection than conifers, as *H. annosum* s.l. does not occur or is very rare in pure deciduous tree stands (Domański, 1983; Lygis *et al*., 2004a). Susceptibility to *Heterobasidion* infection varies greatly not only between different tree species, but also between individuals within one species (Swedjemark & Stenlid, 1995, 1997). Little information about planting alders on diseased sites is yet available, although several reports have been published dealing with the occurrence of *H. annosum* s.l. on *A. incana* (Rishbeth, 1957; Domański & Kowalski, 1987; Kraj & Kowalski, 2010). *Alnus glutinosa* is listed among the host species for *H. annosum* s.l (Woodward *et al*., 1998). However, an inoculation experiment conducted in Sweden showed that both *H. annosum* s.s. and *H. parviporum* developed poorly in the wood of *A. glutinosa* (Swedjemark & Stenlid, 1995). In papers IV and V, the disease situation in natural *A. incana* and *A. glutinosa* stands was studied. Data on decay incidence and causal agents are very scarce and improved information would help in the planning of the best-practice management strategies in the future. Although broadleaved species are not generally as valuable
economically as conifers, in recent years interest about the short-term energy plantations has increased considerably, which gives a new aspect to this question. The work published in papers IV and V included the results of studies on the frequency of infection by stem decay fungi, extent of decay, and the associated yield losses in Latvian alder stands.
2 Objectives of the thesis

The overall aim was to study the incidence of heart-rot in *P. abies* and *Alnus* spp. stands, the associated fungi, their impact on forest yield and possible silvicultural measures to control *Heterobasidion* spp. with a special emphasis on stump removal.

More specifically the objectives were to evaluate:
- butt rot frequency in Latvian *P. abies* stands, associated fungi and the economical impact on a country scale
- the long-term effect of stump removal on root rot incidence
- the influence of stump and slash removal on the growth and mycorrhization of *P. abies* seedlings
- stem decay frequency in Latvian *A. incana* stands, associated fungi and related yield losses
- heart-rot in Latvian *A. glutinosa* stands, associated fungi and possible yield losses
3 Materials and methods

3.1 Study sites and field work

3.1.1 Butt rot incidence, causal fungi and related losses in P. abies stands of Latvia (Paper I)

The study was conducted during years 2005-2006 in 322 thinned and clear-cut P. abies-dominated stands distributed throughout Latvia. The sites represented Oxalidosa (102 sites), Hylocomiosa (86 sites), Myrtillosa (67 sites), Mercurialiosa (37 sites) and Carico-phragmitosa, Oxalidosa turf. mel. and Myrtillosa turf. mel. (together, 27 sites) forest site types according to the Latvian forest classification system (Bušs, 1997). The age of surveyed stands ranged from 27 to 164 years and their area from 0.2 to 8.7 ha. In total, 24 745 spruce stumps were examined using randomly oriented transects (Bloomberg et al., 1980). Depending on the stand area, 15 to 240 stumps per site were measured and the presence or absence of decay in each was recorded. If present, the diameter of the decay zone was measured and the stage of decay recorded. In addition, cores were collected aseptically using an increment borer from a random selection of 1-10 decayed stumps at each site for subsequent isolation of decay-causing fungi (Section 3.2).
To estimate the extent of heart rot in living stems 114 decayed mature *P. abies* trees were selected in four stands. All trees were cut, and height, diameter at stump level and DBH of trees, as well as diameter at stump level and total length of decay were measured. Discs were cut from these trees at the stem base (0.2 – 0.3 m above ground level), at breast height (1.3 m), at a height of 2.3 m from the stump, and then continuing at 3 m intervals until the final length of decay column was determined. Discs from the tree base, the middle and top of the decay column were brought to the laboratory and used for isolation of decay causing fungi (Section 3.2).

### 3.1.2 Stump removal to control *Heterobasidion* root rot (Paper II)

The study was conducted in 2008 in a stump removal trial established earlier at two localities: one at Brynge in northern Sweden and the other at Ramsåsa in southern Sweden. The trial was previously described by Hyppel (1978) and Stenlid (1987). Briefly, in two clear-cut spruce stands, heavily infected by *H. annosum* s.l., six sample plots were established: three at Brynge and three at Ramsåsa. In Brynge in one sample plot stumps were removed using a caterpillar tractor (B1), in other stumps were left intact (B2) and in the remaining plot stumps were treated with 15 g of sodium fluoride (NaF) through a bole hole in the middle of each stump (B3). All plots were planted with 2-year-old *P. abies* seedlings. In Ramsåsa, in the first sample plot stumps were removed using caterpillar tractor, soil was plowed and sieved, leaving no roots larger than 5 mm and two-year-old *P. abies* seedlings were planted at 1.5 m spacing (R1); at the second sample plot stumps left intact and *P. abies* seedlings planted at 1.2 m spacing (R2); and at the third stumps were also not removed and *A. incana* were planted (R3) (Hyppel, 1978). Tree condition and
the frequency of *Heterobasidion* infection were assessed twice before the present study: during 1975/78 by Hyppel (1978) and during 1984/85 by Stenlid (1987). In addition, all trees and stumps from the previous spruce generation and from the thinning (carried out in 1977 at Ramsåsa and in 1978 at Brynge) were mapped.

In 2008, both sites were reassessed. Due to wind storm, plots R2 and R3 at Ramsåsa were destroyed, but plot R1 though severely damaged was still functional. At this and all the Brynge plots (R1, B1, B2 and B3) tree DBH was measured and wood samples were taken using an increment borer. All trees were sampled twice at the stem base, but if the one of the wood samples indicated soft rot, a third sample was taken at breast height. Dead trees were also recorded and wood samples taken only from trees dead 1-3 years previously. All wood cores were used for subsequent fungal isolation.

### 3.1.3 Effect of stump and slash removal on growth and mycorrhization of *P. abies* seedlings outplanted on a forest clear-cut (Paper III)

This study was conducted in Hyytiälä Forestry Field Station of the University of Helsinki in Central Finland. The experiment was established in May 2005. On a clear-felled area four plots were established using different treatments: 1 – mounding (M); 2 – stump removal (K), 3 – mounding and slash removal (MH); 4 – stump and slash removal (HK). All plots were planted with 1-year-old containerized *P. abies* seedlings. In each treatment, the growth of all *P. abies* seedlings was measured twice: in September 2007 and in May 2009. In May 2009 also root systems from 12 randomly selected plants from each treatment were collected.

### 3.1.4 Decay, yield loss and associated fungi in stands of *A. incana* in Latvia (Paper IV)

This study was conducted in 46 recently cut clear-felled *A. incana* stands in different regions of Latvia. *Alnus incana* was dominant in all surveyed stands. The ages of the felled stands ranged from 25 to 65 years and represented mostly the *Oxalis* forest site type (Bušs, 1997). In total, 4434 *A. incana* stumps were examined for the presence of decay (52 -100 stumps per site, depending on site size), using randomly oriented transects (Bloomberg *et al.*, 1980). For all stumps diameter of the cut surface, and diameter of the decay zone, if present, were measured using a ruler. The presence of spongy rot (decomposed wood squeezable with fingers) and hollowed stumps were also recorded. On each site, from 1 to 12 decayed stumps increment cores were collected for
subsequent isolation of decay causing fungi. In total, 227 stumps were sampled.

In addition, 431 living *A. incana* trees were sampled using an increment borer close to the stem base for evaluation of the presence or absence of heart rot in seven between 25- and 65-year-old stands (40-100 trees per stand). All collected wood cores were used for subsequent fungal isolation. In order to estimate the correlations between stem and decay parameters, 175 decayed stems from among those that were sampled, were felled, dissected and the following parameters recorded: age of tree, stump diameter, tree DBH, tree height, decay diameter at stump level and length of decay column. The presence of spongy heart rot in each stem was also recorded and its diameter at stump level and length measured.

3.1.5 Heart-rot and associated fungi in *A. glutinosa* stands in Latvia (Paper V)

This study was conducted in four between 51- and 84- year-old *A. glutinosa* stands located in the Kalsnava forest district in central-eastern Latvia. A total of 450 trees (100 – 150 trees per site) were sampled using an increment borer for evaluation of the presence or absence of heart rot. Each tree was sampled once close to the stem base and wood cores were collected for subsequent fungal isolation. In addition, 34 stems which showed the presence of heart rot were felled, dissected and the followed parameters recorded: tree age, stem diameter at stump level, tree DBH, tree height, decay and spongy rot diameter at stump level and length of the decay and spongy rot column.

3.2 Identification of fungi

All collected samples were transported to the laboratory and held in a refrigerator at +4 °C. Isolations from wood samples (Papers I, III, IV, V) were performed 1-2 days after field sampling. Each wood sample was flame-sterilized, placed on malt or Hagem agar media and incubated in the dark at 19 – 20 °C. To obtain pure cultures, the emergent mycelia were subcultured on Hagem agar media in separate Petri dishes. After 1-3 weeks of incubation, all pure cultures were examined under the light microscope (Leica DM400B) and grouped into cultural morphotypes according to macro- and microscopic morphological features of the fungal mycelia (Figure 5). For identification of fungal species and genera, morphological and molecular techniques were used. For identification of *Heterobasidion* species, intersterility tests were performed (Korhonen, 1978) using homokaryotic test cultures 96119 and 98036 (*H.
parviporum); 03013 and 03015 (H. annosum s.s.). Clones of Heterobasidion were identified using somatic incompatibility pairing test (Stenlid, 1987).

Spruce roots (Paper III) were washed in tap water, and 100 single root tips from each plant were randomly selected from different parts of root systems using forceps. ECM roots were identified using dissection microscope and morphotyped according to their morphological features.

One to three representatives from mycelial morphotypes (with some exceptions) and one to three randomly selected representatives of each distinct ECM morphotype from each root system were subjected to molecular identification following modified procedures from previous studies (Vasiliauskas et al. 2004, 2005). For DNA extraction, fungal mycelia were collected from Petri dishes using a flame-sterilized scalpel and placed into impact-resistant tubes with 800 μl 2% CTAB solution and 3 glass beads. After that, mycelia were ground using FastPrep (MP Biomedicals) at speed 6000 and tubes placed for 1 hour in a heater (DB2P, Techne Dri-block®, Labasco) at 65 °C. After centrifugation (Biofuge Pico, Heraeus) 750 μl of supernatant were placed into a new Eppendorf tube and under a fume hood 750 μl of chloroform were added. After centrifugation 450 μl of supernatant were very carefully removed from the upper part of the solution and placed in a new Eppendorf tube. The DNA was precipitated using 2-propanol in the proportion 1:2. After centrifugation 2-propanol was removed, and the DNA pellet was centrifugated with 200 ml of 70% ethanol. Then ethanol was carefully removed and DNA dissolved in 50 μl of purified water (Milli-Q). The DNA concentration was determined using spectrophotometer (ND-1000, NanoDrop®).

For PCR amplification, primers ITS1F (CTTG GTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) with an annealing temperature 55
°C were used (Kären et al., 1997). The thermal cycling was applied in a thermal cycler (Thermocycler GeneAmp®, PCR System 2700, Applied Biosystems). The ready PCR products were purified using CIAP and Exo I. After purification, PCR products were Sanger sequenced (Sanger et al., 1977) by Macrogen (Seoul, Republic of Korea) using the primer ITS4 for every specimen. Sequencing was performed in one direction. All sequences were manually edited using the Lasergene software package SeqMen (version 5.07, DNASTAR, Madison, Wisconsin and BioEdit version 7.0.5.2. (Hall, 1999). BLAST searches were performed using three reference sequence databases: one at GenBank (http://www.ncbi.nlm.nih.gov/blast), one at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences and at UNITE (Kõljalg et al., 2005). The ITS sequence homology was set at 98%-100% for delimiting fungal taxon (presumed species) and 94%-97% for delimiting at the genus level. ITS sequences of each sequenced morphotype (from pure fungal cultures and ECM) were deposited in GenBank.

3.3 Volume calculations and statistical analysis

Stand characteristics (species composition, age and site type) of Latvian trial plots were obtained from the stand inventory database in the Forest State Register (Mr. Indulis Brauners, Latvian State Forest Inc., personal communications). Calculations of decayed log volume were based on actual lengths of the columns of decay (Papers I, IV and V) and spongy rot (Paper IV and V) in the felled trees, according to Ozoliņš (2002). Calculations of decay volume in the stand were based on the volume of decayed log and the number of stems containing butt rot. Data were applied in the context of regional forest yield models and tables (Kuliesis, 1993). The calculations were accomplished following the procedure described in details in Papers I and IV. The correlation analyses were done using Microsoft Excel and significance levels were evaluated according to Liepa (1974). The analyses of proportions (chi-squared tests) and t-tests were conducted using Minitab® 15 software and their significance evaluated according to Fowler et al. (2001). In cases when the same dataset was subjected to repeated comparisons, confidence limits were reduced as required by the Bonferroni correction (Sokal & Rohlf, 1995). Analysis of similarity between fungal communities (Papers I, III, IV and V) was performed by calculation of qualitative Sorensen similarity indices (Magurran, 1988).
4 Results and discussion

4.1 Heart rot: incidence, causal fungi and related losses

4.1.1 Heart rot of spruce (Paper I)

Heart rot was detected in about one fifth (22%) of Latvian *P. abies* trees, a value which is compatible with the 10-30% rot frequency detected during previous studies done in Nordic and Baltic countries (Enerstvedt & Venn, 1979; Norokorpi, 1979; Nilsen, 1983; Vasiliauskas, 1989; Huse et al., 1994; Vasiliauskas et al., 2002). Significant relationships were found between both stand age and forest site type and rot incidence and intensity (the possibility of finding heart rot was higher in larger stumps), which generally agree with reports by other authors (Enerstvedt & Venn, 1979; Norokorpi, 1979; Nilsen, 1983; Mangalis, 1975). However, despite the reports of several authors that butt rot frequency in *P. abies* stands tends to decrease with an increasing proportion of deciduous and Scots pine trees (Enerstvedt & Venn, 1979; Huse et al., 1994; Linden & Vollbrecht, 2002, Vasiliauskas et al., 2002; Gaitnieks et al., 2008), a positive correlation between an admixture of other tree species and rot frequency was not found in present study. This, as well as the generally high variation of rot incidence (from 0 to 83% in individual stands), may be due the different history of the surveyed stands: the previous tree rotation, the level of stem damages by big game or during forest operations, forest management operations during growing season and in previous generations, and others factors, all of which can have a significant influence on rot frequency (Isomäki & Kallio, 1974; Piri et al., 1990; Swedjemark & Stenlid, 1993; Rönnberg, 2000; Piri & Korhonen, 2001; Vasiliauskas, 2001; Piri, 2003; Mäkinen et al., 2007; Piri & Korhonen, 2008).
The length of the heart rot columns in the felled trees was 6.6 m on average, which means that approximately 30% of tree stem of harvested trees, including the valuable butt log component, was spoiled by decay. In Paper I the relationship between the average volume of decayed logs and various stem parameters were presented in order to calculate actual timber losses due to heart rot in Latvian *P. abies* stands. The results indicated that 6-16% of the total standing volume in Latvian spruce stands could be damaged by decay, which generally agrees with the 8-30% of yield loss reported in Finnish studies (Kallio, 1972; Kallio & Tamminen, 1974; Tamminen, 1985). Yield losses increased with time. They were quite low in the relatively young spruce stands (40-50 years), two times higher in mature spruce stands (60-100) and three times higher in over-mature spruce stands (120-150 years).

*Heterobasidion parviporum*, following by *Stereum sanguinolentum* (Alb. & Schwein.) Fr. were identified as the main causes of heart rot in Latvian *P. abies* stands. Both of these fungi are known as common butt and stem rot agents in living *P. abies* trees (Pechmann & Aufsess, 1971; Pechmann et al., 1973; Isomäki & Kallio, 1974; Hallaksela, 1984; Woodward et al., 1998; Vasiliauskas, 1999, 2001, and references therein). In agreement with earlier reports, other decay-causing agents in living *P. abies*, namely *Amylostereum areolatum* (Chaillet ex Fr.) Boidin, *A. chailletii* (Pers.) Boidin, *Armillaria cepistipes* Velen., *Climacocystis borealis* (Fr.) Kotl. & Pouzar and *Porodaedalea chrysoloma* (Fr.) Fiasson & Niemelä were found only incidentally (Pechmann & Aufsess, 1971; Pechmann et al., 1973; Norokorpi, 1979; Hallaksela, 1984). An unusual observation in the present study (Paper I) was the occurrence of some fungi, known to behave as dead wood decomposers or saprophytes, in the active decay columns of study trees. However, some of these species (namely *Bjerkandera adusta* (Willd.) Karst., *Gloeophyllum sepiarium* (Wulfen) Karst. and *Polyporus brumalis* (Pers.) Fr.) have been reported as occasional butt rot agents in southern Germany and northern Finland (Pechmann & Aufsess, 1971; Pechman et al., 1973: Norokorpi, 1979).

The incidence of *Heterobasidion* - infected trees in Latvian spruce forests confirms that active management measures should be taken in order to reduce the extent of the yield losses caused by this disease. In 2007 the commercial Rotstop preparation was registered in Latvia and stump treatment now is a common procedure in thinned stands (Kenigsvalde et al., 2011). However, as this stump treatment is only able to prevent the formation of new disease centers, other methods which could reduce the inoculum in already infected
stands should be taken into consideration. Two options that should be considered in a programme to reduce disease levels are the removal of decayed stumps and the planting of resistant tree species on heavily infected areas.

4.2 Heart rot of grey and black alders

The heart rot incidence found in this study in *A. incana* and *A. glutinosa* stands was generally high (1-54% in *A. incana* stands and 53-98% in *A. glutinosa* stands). The larger proportion of decay in *A. glutinosa* stands could be related to the older age of the surveyed stands, as well as to the fact, that a much smaller number of *A. glutinosa* stands (4) was investigated in comparison with the larger number of *A. incana* stands (46). The mean length of the heart rot column was generally similar for both species: 7.1 m for *A. incana* and 7.7 m for *A. glutinosa*. However, the mean length of spongy rot within the heart rot column was larger in *A. glutinosa* stems (4.2 m) than in *A. incana* stems (2.8 m). The spongy rot incidence was also higher in the *A. glutinosa* stands. The length of the heart rot zone correlated with tree age and tree size, indicating that the extent of rot tended to increase with time. However, in both cases the variation within the data was high, a result also found by other authors (Kärki, 1999; Kärki *et al*., 2000).

Despite the fact that *A. incana* and *A. glutinosa* belong to the same genus, the most significant heart rot causing fungi were different in each species. In *A. incana* the most commonly isolated fungus was *Chondrostereum purpureum* (Pers.) Pouzar, whereas in *A. glutinosa* it was *Inonotus radiatus* (Sowerby) Karst. This may explain the lower incidence of spongy rot in the *A. incana* stands, compared with those of *A. glutinosa*, as well as the smaller extent of spongy rot within the decay column in *A. incana*, since *C. purpureum* is a less powerful decay agent. The biology of these two fungi is quite different; *I. radiatus* being a true heart rot fungus, while *C. purpureum* is a typical wound pathogen.

Yield losses caused by decay in alders tend to increase with time and may reach 20% of the standing volume by age 60-65 years for *A. incana* and over 50% of the standing volume at the age 80 years for *A. glutinosa*. The mean volume of wood degraded by the particularly serious spongy heart rot, which significantly reduces wood quality, was determined as 32.7 m³ha⁻¹ in fully stocked 60-65 years old *A. incana* stands and 94.4 m³ha⁻¹ in fully stocked 80 years old *A. glutinosa* stands. Since the harvesting age for *A. glutinosa* stands is prescribed at 71 years under Latvian law (corresponding clause nr. 9, Forest
Law of Latvia), high yield losses can be anticipated for diseased stands of this age. However, most of *A. glutinosa*-dominated stands currently have protected status in Latvia as key biotopes. For *A. incana*, the situation is better, as the harvesting age is not determined by law, and stands may be cut at any time. This should enable forest managers to avoid significant yield losses if stands are felled before heart rot increases to unacceptable levels. Also, a lower decay incidence was demonstrated in mixed *A. glutinosa* stands, and this question is worth further investigation.

4.3 Heart rot: possibilities for silvicultural control

4.3.1 Stump removal as method to control root rot (Papers II and III)

Stump removal cannot completely eradicate *H. annosum* s.l. from the stand, but it may help to reduce the inoculum potential in the next rotation stand and so reduce future timber losses. Numerous studies done in North America and Europe show that in most cases stump removal reduces root rot incidence in the next tree generation and increases stand productivity (Vasaitis *et al.*, 2008). Results of the long-term trials described in Paper II also show that stump removal significantly reduced damage caused by root rot fungi in the regenerated stands. Although the frequency of heterobasidion root rot increased with stand age, it was nevertheless 20-72% lower than in the control areas. Recent interest in the harvesting of tree stumps for fuel may give additional benefits and partially cover stump removal costs (Björheden, 2006; Laitila *et al.*, 2008). Historically, although stumps have previously been used as a fuel source, a subsequent decline in the demand for fuel wood had reduced its use for this purpose by the first part of 20th century (Walmsley & Godbold, 2010). Although interest in stump harvesting for energy production increased during the 1970s in Scandinavia, this source was eventually found to be too expensive (Hakkila, 2004). However, since the demands for forest fuels continue to grow, a re-evaluation of the possibility of stump harvesting had recommenced (Erikson & Gustavsson, 2008; Lazdiņš *et al.*, 2009; EUSTAFOR, 2010).

Data on the environmental impact of stump removal are scarce and more research is needed under European conditions. Some American trials showed a decrease in the amounts of C and N present in the soil after stump removal, especially on poor sites (Zabowski *et al.*, 2008; Butnor *et al.*, 2012). However, a comparative analysis done by Vasaitis *et al.* (2008) showed mostly a positive influence of stump removal on seedling establishment, tree growth and stand...
productivity. Our results (Paper III) also revealed that stump removal improved seedling growth, which mainly agrees with reports by other authors (Vasaitis et al., 2008; and references therein), but had a negative impact on seedling mycorrhization and diversity of mycorrhizal fungi. To date, a number of authors have recorded possible negative effects of stump removal on stand biodiversity, as many species of wood decay fungi, invertebrates, lichens and bryophytes use the stumps as their habitat (Jonsell, 2007; Rudolphi, 2007; Caruso, 2008; Hjältén et al., 2010). However, cut stumps do not occur in natural forests and are attributed solemnly to the human activity in the recent hundreds years. Large quantities of stumps created each year in forest stands provide a favourable substrate for many pests and pathogens, such as the large pine weevil, *Hylobius abietis* L., and the root rot fungi belonging to *H. annosum* species complex. However, no evidence so far exists that any of the rare or protected species is restricted solely to a stump habitat. Moreover, not all stands are suitable for stump removal. Stump removal should not be attempted, for instance, on sites of slopes greater than 20º or with sandy or peat soils (the peat depth exceeding 45 cm) or on riparian buffer zones (EUSTAFOR, 2010). Large numbers of stumps are also created during stand thinning. The inventory conducted in Latvia (Paper I) showed that rather similar communities of wood inhabiting fungi were detected in *P. abies* stumps created during clear felling and thinning operations (Sorensen similarity index 0.68). However more species were found in stumps on the clear cut sites, a question which needs further investigation. In fact, *H. parviporum* was the most abundant fungus in both clear-felled and thinned stumps. The ability of this fungus to infect healthy trees through root contacts as well as survive in cut stumps for decades means that inoculum is able to accumulate in forest stand. Moreover the fruitbodies of *H. annosum* s.l. can be quite abundant on colonised stumps (Vasiliauskas et al., 2002; Müller et al., 2007) as well as on cull pieces left in the forest (Schütt et al., 1979; Müller et al., 2007; Stivriņa et al., 2010), providing an additional risk of infection not only to neighboring trees, but also to adjacent stands.

Results from trials reported in Paper II indicate that stump removal has a long-term impact and significantly decreases *Heterobasidion* infection 50 years after planting. Furthermore, although the disease incidence increased with time, it still remained on an acceptable level. Combining stump removal with other root rot control methods could achieve even better results. Treatment of the thinned stumps of the subsequent generation with *P. gigantea* spore suspensions, thinning during the cold season, when the probability of spore infection is low (Yde-Andersen, 1962) and avoiding damage to living trees
from heavy machinery (Vasiliauskas, 2001) will prevent the development of new infection centers and help to reduce losses. Modern methods of stumping have improved the quality of stump lifting by extracting the whole root system with minimal soil damage. However, more research is needed for the evaluation of the possible ecological effects of removing such a great amount of dead wood within stands, especially if stump removal will become a standard forest management procedure. The long-term consequences of stump removal on stand productivity, soil quality, biodiversity and tree growth in different geographical regions of Europe also needs further investigation.

4.3.2 Planting resistant tree species

As stump removal should be avoided on some sites because of the soil type, slope or other factors, the planting of Heterobasidion-resistant trees, especially broadleaved species, could be considered as one of the disease management options (Woodward et al., 1998; Piri, 2003; Pavlov et al., 2008). For example, Silver birch *Betula pendula* Roth was found to be resistant to *H. parviporum* infection (Piri, 1996), although *H. annosum* s.s. can quite heavily infect this tree species (Lygis et al., 2004a). Concerning the two alder species, *A. incana* and *A. glutinosa*, little is known about the susceptibility of pure stands of these hosts to infection by *H. annosum* s.l. Although the decay incidence in the evaluated alder stands was high (Papers IV and V) and large number of different fungal species was detected, no species from the *H. annosum* species complex was found among them. Frequency of decay in these studies was mostly attributed to the stand age and timber losses caused by heart rot significantly increased in stands older than 50 years. This finding indicates that the 10- to 20-year-rotation age proposed for energy plantations in Scandinavia and Baltic countries (Rytter, 1995, 1996; Lohmus et al., 1996; Daugavietis et al., 2009) may be suitable not only for biomass production, but also to improve forest health. The additional beneficial effect of planting *Alnus* spp. on heavily infested sites could result from the antagonistic effect of alder rhizosphere microflora to *H. annosum* s.l. (Johansson & Marklund, 1980; Arhipova et al., 2008), as well as the ability of alders to fix atmospheric nitrogen, which could improve soil productivity (Huss-Danell & Lundmark, 1988; Uri et al., 2002; 2003; Kuznetsova et al., 2010). Even if the tested species is not fully resistant to *H. annosum* s.l. infection, a short rotation time is likely to reduce the build-up of new inoculum. Stands of coppice origin, especially on sites with a high decay incidence, are better avoided, as the newly emerging sprouts could be infected by decay fungi from the parent stump. This could reduce sprouting incidence, decrease growth and survival, and could lead to stem decay at a very
young age (Roth & Sleeth, 1936; Berry & Lombard, 1978; Kärki et al., 2000; Wolken et al., 2009; Bakys et al., 2011). For example, the most common heart rot causal agent in Latvian *A. incana* stands, *C. purpureum*, is known as a sprout killer and this species were proposed by some authors for forest vegetation management (Harper et al., 1999; Pitt et al., 1999). Also, the ability of *Heterobasidion* sp. to infect stumps of short rotation tree species is unknown. Therefore, it would be useful to establish trials in short term rotation stands of fast-growing native *Alnus*, *Populus*, *Betula*, *Salix* and other broadleaved species on sites, heavily infected by *H. annosum* s.l., in order to study their resistance potential in more detail under field conditions. Such studies might give promising results, especially taking into account the recommended short rotation age (10 – 20 years), so that the planted species might escape serious damage by *Heterobasidion*, even if the host trees are not completely resistant.

### 4.4 Conclusions

Studies conducted in this thesis show that *H. parviporum* is the main cause of heart rot in *P. abies* stands of Latvia and that the associated yield losses are significant. The ability of this pathogen to spread within stands through root contacts and to survive in plant residues for decades indicates that such forest sites are permanently infested, and that the disease will persist over future forest generations. Stump treatment could prevent, or at least, reduce infections of healthy stands from airborne basidiospores. However, as the disease is already present in a great number of Latvian *P. abies* stands, other methods for reducing the future yield losses should be attempted, especially on heavily infected sites. The most promising methods, both from a forest health and an economic point of view, are likely to be stump removal and the planting of fast-growing broadleaved tree species, such as native species of birch, aspen and alder, on heavily infected sites. However, as the long term impact of this type of forest management is still unknown, more research is needed concerning its potential ecological impact. Stump and slash removal, as well as the introduction of short rotation energy plantation could lead to a dramatic decrease in nutrient concentrations, at least in some types of forest soils with the removal of large amounts of biomass for energy production. The long-term consequences of this procedure to future forests productivity are still unclear. However, taking ecological and silvicultural consideration into account, optimally healthy and productive forest stands could be achieved.
5 Future prospects

New stump removal trials should be set up in different geographical regions of Europe to evaluate the long term effects of this treatment on site productivity, biodiversity and other related factors. A number of associated questions also deserve to be answered. In particular, what is the size limit of the *Heterobasidion* colonised wood remnants left on the site after stump removal which will still make the infection of new tree generation possible? There is little interest in the use of small dimension stumps (less than 10 cm in diameter) as an energy resource due to their small size and yet stumps of these dimensions are not usually treated with *P. gigantea* preparations. The question therefore arises as to whether and to what extent they may serve as a substrate for colonisation by *H. annosum* s.l. basidiospores and act as a centre for the establishment of new disease foci in the next generation. Also, could fruitbodies produced on colonised stumps and other wood residues left in forest increase the possibility of infection of neighbouring healthy stands by *Heterobasidion* spores?

Experimental energy plantations of native fast growing broadleaved species should be planted on infected sites and their potential resistance to *heterobasidion* root rot evaluated under the field conditions. Also, the economic aspects should be taken into consideration when designing such trials.
References


Lange, V. (1960) [Silvicultural characteristics of Norway spruce with different branching]. In *Proceedings of the Latvia University of Agriculture*, 9, Riga, p. 537-542. [In Latvian.]


Mangalis, I. (1975) [Heterobasidion annosum in spruce plantations and spruce forests]. In: [Spruce and spruce forests in Latvia]. Zinātne, p. 35-42. (in Latvian).


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