METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 880, 2002
FINNISH FOREST RESEARCH INSTITUTE, RESEARCH PAPERS 880, 2002

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PUNKAHARJUN TUTKIMUSASEMA – PUNKAHARJU RESEARCH STATION

METLA

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Akateeminen väitöskirja metsänjalostuksen alalta

Esitetään Helsingin yliopiston maatalous-metsätieteellisen tiedekunnan luvalla julkisesti tarkastettavaksi Suomen metsämuseo ja metsätietokeskus Luston auditoriossa, 23. päivänä tammikuuta 2003 kello 12.

Academic dissertation in forest tree breeding

To be presented with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in the auditorium of Lusto, at Punkaharju, on the 23rd of January 2003, at 12 noon.

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Venäläinen, Martti 2002. Decay resistance of heartwood timber as a quality characteristic in Scots pine breeding. Seloste: Sydänpuutavaran lahonkestävyys laatuominaisuutena männynjalostuksessa. Metsäntutkimuslaitoksen tiedonantoja 880. Finnish Forest Research Institute, Research Papers 880. Academic dissertation. 53 + 69 p. ISBN 951-40-1866-4. ISSN 0358-4283.

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Julkaisija Publisher	Metsäntutkimuslaitos, Punkaharjun tutkimusasema Finnish Forest Research Institute, Punkaharju Research Station Accepted to be published by **Eeva Korpilahti**, editor-in-chief, 2.12.2002

Abstract

The aim of this study was to evaluate the biological possibility of improving the decay resistance of Scots pine (*Pinus sylvestris* L.) heartwood by means of forest tree breeding. The two fundamental prerequisites for successfully carrying out this task were: a method suitable for testing the decay resistance of standing trees, and tree material suitable for analysis of the genetic parameters of heartwood characteristics. A satisfactory method for investigating the natural decay resistance of the wood of standing trees proved to be the in vitro accelerated malt agar plate decay test, which uses increment core sections as samples. The decay tests, carried out with two relatively young, 32-34 year-old, Scots pine progeny test materials, showed that there was phenotypic variation in the decay resistance of juvenile heartwood among the individual trees. In the two progeny tests, the coefficient of additive genetic variation was estimated to be $CV_A = 10.6$ % and $CV_A = 28.5$ %, and the proportion of the additive genetic variation out of the total phenotypic variation, i.e. the individual heritability, was estimated to be $h^2 = 0.02$ and $h^2 = 0.37$. It was concluded that the additive genetic variation was adequate for a considerable response to phenotypic selection in the decay resistance of juvenile heartwood. However, the decay tests with mature, 170-year-old, Scots pines showed the most durable part to be the outer heartwood. In practical tree breeding direct selection for the durability of outer heartwood would prolong the generation interval unrealistically.

In order to elucidate the possibilities of indirect selection, the relationships between decay resistance and other chemical or physical wood characteristics were studied in the progeny test material as well as in a mature natural stand. The most promising characteristic for indirect selection appeared to be the concentration of two secondary phenolic compounds, pinosylvin (PS) and pinosylvin monomethyl ether (PSM), both of which are stilbenes. The resin acids, which are important for the active defence of living trees, seemed to play such a weak role in passive defence that their concentration does not provide any useful information for indirect durability selection. The stilbenes, which are normally synthesised in heartwood formation when the last living sapwood cells die, but which evidently can be induced by artificial stress factors to form already in young seedlings, could even provide a tool for an early indirect selection. However, before any indirect selection will be performed, it is advisable to carry out a thorough investigation of the genetic correlations between the characteristics used in indirect selection and the target characteristic as well as the growth and quality characteristics already included in the Scots pine breeding programme.

Keywords: *Pinus sylvestris*, juvenile heartwood, genetic parameters, early indirect selection, *Coniophora puteana,* accelerated decay test, resin acids, stilbenes, pinosylvin, water sorption

Seloste

Sydänpuutavaran lahonkestävyys laatuominaisuutena männynjalostuksessa

Puuaineen luontainen lahonkestävyys johtuu ensisijaisesti sydänpuuhun muodostuvista lajikohtaisista uuteaineista, jotka eri tavoin haittaavat lahottajasienten hajotustoimintaa. Lahonkestävyys vaihtelee sekä puulajien että rungon eri osien välillä. Ihmiset ovat vuosituhansien myötä oppineet käyttämään tätä vaihtelua hyväkseen ja valitsemaan puutavaran käyttöolosuhteiden mukaisesti. Nykyinen kiinnostus luontaiseen lahonkestävyyteen on virinnyt sitä mukaa kuin kyllästettyä puutavaraa koskevat viranomaismääräykset ovat tiukentuneet ja kuluttajien asenteet kyllästyskemikaaleja kohtaan ovat muuttuneet varovaisemmiksi. Meillä Suomessa männyn sydänpuu voisi olla kuluttajien arvostama kotimainen ja ekologisesti hyväksyttävä puutavaralaji sellaisiin kosteudelle alttiisiin rakennuskohteisiin, joissa ei edellytetä ehdotonta lahonkestävyyttä, mutta joissa toivotaan kohtuullisen pitkää käyttöikää. Tällaisia ovat esimerkiksi ne piharakenteet ja laiturien osat, jotka eivät ole jatkuvassa maa- tai vesikosketuksessa. Tämän väitöskirjatyön tarkoituksena on ollut selvittää ja pohtia biologiselta kannalta, voidaanko männyn sydänpuun lahonkestävyyttä pitää metsänjalostuksessa varteenotettavana laatuominaisuutena.

Väitöskirjatyön empiiriset havainnot on julkaistu kuudessa tutkimusartikkelissa. Artikkelissa **I** kuvataan kasvukairalla pystypuusta otettuun sydänpuunäytteeseen perustuva pikalahotusmenetelmä. Tällainen tarvittiin, koska puutavaran lahonkestävyyden arvioimiseen kehitetyt standardimenetelmät, jotka vaativat puun kaatamista, eivät sovellu metsänjalostustutkimuksiin. Tutkimuksissa **II** ja **III** tarkasteltiin kahdesta (32- ja 34-vuotiaasta) männyn jälkeläiskokeesta peräisin olevan, sydänpuuksi muuttuneen nuorpuun ominaisuuksien vaihtelua. Tarkastelu kohdistui erityisesti lahonkestävyydessä havaitun additiivisen geneettisen vaihtelun määrään suhteessa lahonkestävyyden keskiarvoon ja fenotyyppisen vaihtelun kokonaismäärään. Tutkimuksissa **IV** ja **V** tarkasteltiin, poikkeavatko hitaasti ja nopeasti lahoava sydänpuu toisistaan hartsihappojen tai fenoliyhdisteiden pitoisuuksien suhteen. Nämä molemmat uuteaineryhmät ovat tyypillisiä männyn sydänpuulle. Tutkimuksessa **VI** tarkasteltiin lahoamisnopeuden ja puuaineen kemiallisten ja fysikaalisten ominaisuuksien välisiä riippuvuuksia 170-vuotiaissa luonnonmetsän puissa.

Kairanlastunäytteestä tehty pikalahotuskoe antoi tarkkuudessaan tyydyttävän tuloksen pystypuun lahonkestävyydestä, joten jalostusaineistoon kuuluvien, elävinä säilytettävien puiden testaaminen todettiin mahdolliseksi (**I**). Nuorten mäntyjen sydänpuun lahonkestävyyden vaihtelu havaittiin varsin laajaksi, mikä puolestaan tekee fenotyyppisen valinnan mahdolliseksi. Lahonkestävyyden additiivisen geneettisen vaihtelun

määrä suhteessa keskiarvoon havaittiin kummassakin jälkeläiskokeessa siinä määrin korkeaksi, CV_A = 10.6 % (**II**) ja CV_A = 28.5 % (**III**), että vanhempien valinnalla voidaan olettaa saavutettavan merkittävää vastetta jälkeläisten keskiarvossa. Additiivisen geneettisen vaihtelun ja fenotyyppisen kokonaisvaihtelun suhteesta eli heritabiliteetista saatiin kaksi erisuuruista estimaattia. Korpilahden jälkeläiskokeessa havaittu korkea heritabiliteetti, h^2 = 0.37 (**III**), viittasi siihen, että yksilöiden fenotyyppinen arvo korreloi merkittävässä määrin jalostusarvon kanssa. Kerimäen jälkeläiskokeessa heritabiliteetti oli alhainen (h^2 = 0.02, **II**). Tuloksista pääteltiin, että lahonkestävyyden additiivinen geneettinen vaihtelu on jalostuksellista valintaa ajatellen riittävä, ja että Korpilahden jälkeläiskoe edustaa yksilövalintaan sopivaa kohdetta.

Lahonkestävyyden suora varhaisvalinta manto- eli pintapuun perusteella todettiin hyödyttömäksi (**II**). Suora valinta on mahdollista vasta sitten, kun mänty alkaa ilmentää lahonkestävyysominaisuuttaan. Tämä tapahtuu noin 30-vuotiaana, kun sydänpuun muodostuminen rungon keskellä olevassa nuorpuussa on päässyt vauhtiin (**II, III**). Vanhoista luonnonmännyistä todettiin, että sydänpuun ulompi osa oli merkittävästi kestävämpää kuin sisäosan nuorsydänpuu (**VI**). Mikäli jalostuksellisessa valinnassa jäätäisiin odottamaan kypsän mantopuun muuttumista sydänpuuksi, mikä merkinnee vähintään 50 vuotta, sukupolvien väli venyisi jalostuksen etenemisen kannalta epäedullisen pitkäksi.

Lahonkestävyyden epäsuoran mittaamisen ja valinnan mahdollisuuksien kannalta lupaavimmaksi ominaisuudeksi osoittautui fenoliyhdisteisiin kuuluvien stilbeenien (pinosylviini ja pinosylviinimonometyylieetteri) pitoisuus (**V, VI**). Elävän puun aktiivisen puolustautumisen kannalta tärkeiden hartsihappojen määrän vaikutus passiiviseen lahonkestävyyteen todettiin vähäiseksi (**IV, VI**). Stilbeenien tiedetään normaalisti syntyvän sydänpuuksi muuttumassa olevan mantopuun ydinsäteiden tylppysolujen, ts. viimeisten elävien solujen, kuollessa hitaasti. Kirjallisuuden perusteella todettiin, että stressitekijöiden (esim. UV-säteilyn tai mekaanisen vaurioittamisen) avulla stilbeenien synteesi saadaan tapahtumaan jo pienissä taimissa, mikä saattaa mahdollistaa lahonkestävyyden epäsuoran varhaisvalinnan. Epäsuoran valinnan mahdollisuuksien selvittäminen vaatii perusteellisia jatkotutkimuksia erityisesti valinnan kohteena olevien ominaisuuksien, lahonkestävyyden ja männyn jalostusohjelmassa jo mukana olevien kasvu- ja laatuominaisuuksien geneettisistä korrelaatioista.

Väitöskirjan loppupäätelmissä ehdotetaan männyn jalostuksen painopisteen siirtämistä kasvunopeudesta rungon ulkoisten ja puuaineen sisäisten laatuominaisuuksien suuntaan.

Avainsanat: mänty, kellarisieni, nuorpuu, sydänpuu, lahotustesti, epäsuora valinta, periytyvyys, pihka, hartsihappo, fenoliyhdiste, pinosylviini

Alkusanat

Tämä väitöskirja on metsänjalostuksen näkökulmasta tehty yhteenveto niistä tutkimuksista, jotka tehtiin vuosina 1998-2002 Metsäntutkimuslaitoksen tutkimushankkeen 3220, "Suomalaisten puulajien luontainen käyttökelpoisuus rakentamisessa", osahankkeen 01, "Männyn ja lehtikuusen perimän vaikutus puun lahonkestävyyteen" sekä Wood Wisdom metsäalan tutkimusohjelmaan sisältyneen Suomen Akatemian rahoittaman tutkimushankkeen 43140, "Männyn ja siperianlehtikuusen puuaineen lahonkestävyyden geneettinen vaihtelu" saumattomana yhteistyönä. Kuten alkuperäisten julkaisujen luettelosta käy hyvin ilmi, tutkimukset ovat olleet samalla usean eri tieteenalan, usean tutkimuslaitoksen ja ennen kaikkea usean tutkijan yhteistyötä. Olen kiitollinen teille Anni Harju, Teijo Nikkanen, Leena Paajanen, Pirkko Velling, Hannu Viitanen, Egbert Beuker, Pirjo Kainulainen, Markku Tiitta, Pekka Saranpää ja Hanna Nikulainen kanssakirjoittajina sopuisasta ja tunnollisesta uurastuksesta niin havaintoaineistojen hankkimisessa ja käsittelyssä kuin raporttien valmistelussakin. Kaikkiaan olen kiitollinen sille monien vaiheiden ketjulle, joka on vähitellen avannut minulle kiehtovan ikkunan puun ja sen uuteaineiden ominaisuuksiin ja lahottajasienten maailmaan.

Opinnäytetyötäni ovat ohjanneet Anni Harju ja Hannu Viitanen. Hannulta perin sen hankeratsun ohjakset, joka vei urakelkkani jalostusohjelmista ja puun ulkoisen laadun tutkimuksesta keskelle lahonkestävyyden tutkimisen hetteikköä. Anni tuli "lahohankkeeseen" pätevöitymään metsägenetiikan dosentiksi ja mitä geneettisten parametrien estimoimisen opettamiseen tulee, sai harjoitusmateriaalikseen kyllin haastavan oppilaan. Kiitän sydämellisesti teitä kumpaakin omasta kontribuutiostanne työn aikana ja käsikirjoituksen korjailussa.

Oheiset tutkimukset eivät olisi valmistuneet koskaan ilman poikkeuksellisen mittavaa avustajakaartia. Esko Oksa, Pentti Manninen, Reijo Rauniomaa, Hannu Partti, Tapani Relander, Outi Kylliäinen, Markku Tiainen, Liisa Seppänen, Heikki Paajanen, Heikki Kinnunen, Teemu Moilanen, Eija Matikainen, Tore Ericsson, Matti Haapanen, Seppo Ruotsalainen, Tarja Salminen, Jussi Tiainen, Arto Haatainen, Terhi Vuorinen, Tapio Laakso, Veikko Kitunen, Ari Haapasaari, Pentti Konttinen, Hannu Heinonen, Sari Lignell, Katriina Lipponen, Anna-Maija Hallaksela, Kerttu Rainio, Auvo Silvennoinen, Hannele Makkonen ja Seija Vatanen, osuuttanne on kuvattu suppeasti alkuperäisten julkaisujen yhteydessä. Kaikille teille ja niille nimeltä mainitsematta jääneille Punkaharjun, Vantaan ja Joensuun yksikköjen henkilöille, jotka ovat tutkimuksiamme

eteenpäin vieneet, lausun vilpittömät kiitokseni. Ulotan kiitokseni koskemaan myös huolto- ja toimistohenkilökuntaa, sekä yksiköiden päällikköjä, tutkimuksen asiantuntijaryhmää että Metlan koko esikuntaa.

Two qualified scientists, nominated as official reviewers by Helsinki university, Björn Hannrup from Sweden and John H. Russell from Canada worked thoroughly but rapidly in evaluating the thesis. I extend cordial thanks for you for your constructive contribution!

Käsikirjoituksen ovat lukeneet Matti Haapanen, Pirjo Kainulainen ja Pirkko Velling. Kiitän teitä arvokkaista kommenteistanne ja siitä, toimititte ne minulle eikä vastaväittäjälle. Oman erityiskiitoksensa ansaitsee kielieditori John Derome, joka on sinnikkäästi korjannut samoja heikkouksia kaikista osajulkaisuista ja lopulta yhteenvedosta. Eija Matikaiselle kiitos kirjan taittamisesta.

Loppulauseeni osoitan maailman parhaille tyttärille Sirjelle, Pinjalle, Pihlalle ja Milkalle. Moni asia, jota olette kärsivällisesti pyytäneet, on jäänyt viime aikoina tekemättä. Toivon vähitellen voivani korvata takaisin sen poissaolon, jonka tämän väitöskirjan kirjoittaminen on aiheuttanut.

Punkaharjulla, joulukuun 12. päivänä 2002

Martti Venäläinen

Original publications

The thesis is based on the following publications, which are referred to in the text by Roman numerals. All the publications are reprinted with the permission of the copyright holders.

I Venäläinen, M., Harju, A., Nikkanen, T., Paajanen, L., Velling, P., & Viitanen, H. 2001. Genetic variation in the decay resistance of Siberian larch (Larix sibirica Ledeb.) wood. Holzforschung 55(1): 1–6.

II Harju, A.M., Venäläinen, M., Beuker, E., Velling, P., & Viitanen, H. 2001. Genetic variation in the decay resistance of Scots pine wood against brown rot fungus. Can. J. For. Res. 31(7):1244-1249.

III Harju, A.M. & Venäläinen, M. 2002. Genetic parameters regarding the resistance of *Pinus sylvestris* heartwood to decay caused by *Coniophora puteana*. Scand. J. For. Res. 17:199-205.

IV Harju, A.M., Kainulainen, P., Venäläinen, M., Tiitta, M., & Viitanen, H. 2002. Differences in resin acid concentration between brown-rot resistant and susceptible Scots pine heartwood. Holzforschung 56(5):479-486.

V Venäläinen, M., Harju, A.M., Saranpää, P., Kainulainen, P., Tiitta, M., and Velling, P. 2003. The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood. Wood Sci. Technol. In press.

VI Venäläinen, M., Harju, A.M., Kainulainen, P., Viitanen, H. and Nikulainen, H. 2003. Variation in the decay resistance and its relationship with other wood characteristics in old Scots pines. Ann. For. Sci. In press.

The contribution of Martti Venäläinen in the preparation of the original articles:

II, VI	main responsibility for planning the experiment, collecting the material and handling the samples
I, V, VI	main responsibility for data analysis and writing the manuscripts
III, IV, V	joint responsibility for planning the experiment, collecting the material and handling the samples
II, III, IV	joint responsibility for data analysis and writing the manuscripts

Contents

1. Introduction

1.1 Scots pine breeding guided by the ideotype

Tree breeding is an essential part of intensive forestry, which is based on the regeneration of stands through cultivation and on the active management of the stands during their growth. In addition to the natural conditions, such as soil, climate, and the risk of damage, the demands of the wood markets direct the forest owners to decide how the stands should be regenerated and managed in order to maximise their economic return. Although the quantity of wood produced will always be an important target of forestry, the quality of wood seems to become an ever more important factor in the wood markets (Paavilainen 2002). The tree breeders have to be far ahead of the forest owners when searching for signs of what kind of wood material will be considered valuable in the future. The improved products of the tree breeders, i.e. cultivars, have to survive and grow vigorously for decades and produce a large amount of high quality timber. In addition, it would also be advantageous for the cultivars to have a positive interaction with the management practices, i.e. that the stands would 'pay back with a high rate of interest' the investments made in intensive management aimed at enhancing either volume production or a specific quality property of the wood.

In order to integrate all the requirements set on improved trees, the concept of an ideotype, which Donald (1968) has introduced to crop plant breeding, has been adopted and developed to tree breeding (Dickmann 1985, Dickmann et al. 1994). Today the ideotype of the cultivated tree can be seen as an ideal model of a tree that meets several, if not all, of the requirements set on the cultivar as regards the yield and behaviour in a specific environment. Thus the adoption of the ideal model as a breeding goal provides an important practical tool for breeding strategy planning. For Scots pine breeding in Finland the ideotype has been delineated by Kärki and Tigerstedt (1985), Velling (1988) and Pöykkö (1993). High volume growth on an areal basis, combined with good external quality for the sawing industry, have been the key components of the Scots pine ideotype. Good external quality is associated with thin branches in relation to the diameter of the stem, and thus the ideotype also includes the target of high harvest index, i.e. a high yield of harvested timber compared to the total biomass of the stem (Donald 1962, Tigerstedt and Velling 1986). In actual fact, the ideotype was intuitively applied already in the phenotypic selection of plus trees (Oskarsson 1995), although the concept itself was not familiar to the tree breeders at that time.

The requirements set on a cultivar should not represent a biological contradiction, e.g. simultaneous extractive-rich and extractive-free heartwood. Otherwise more than one ideotype is needed. A comprehensive physiological and genetic investigation is always needed within each ideotype, in order to decide whether the breeding efforts can be directed simultaneously at all the components of the ideotype.

In the implementation of the Finnish breeding programme for Scots pine, recurrent selection of the plus trees has so far been mainly based on the height growth of the progenies (Venäläinen and Ruotsalainen 2002). In terms of Hannrup (1999), tree height has been used as "an operational selection criterion" for the target trait, "the total dry matter production", as has also been the case in Sweden. This is partly due to the fact that the quality characteristics are manifested at a later age in the progeny tests, and the costs of quality measurements limit the amount of data that can be collected. In order to simplify the quality measurements and analysis of the data, visual assessment of young trees has been proposed (Venäläinen et al. 1996). On the other hand, Haapanen et al. (1997) recently stated that the total height has been a lucky choice as a selection criterion because it appears to have a favourable genetic correlation with the external quality characteristics.

1.2 Need to estimate genetic parameters

The genetic parameters of a quantitative characteristic in a certain population include the additive genetic variance, the heritability, and the genetic correlation between pairs of characteristics. It would be beneficial to have estimates for these parameters in the populations with which the tree breeders operate: natural stands in the early phases of selection, progeny tests at a later stage and perhaps the virtual breeding populations in the advanced phases of breeding. In practice, in the initial phase of tree breeding programmes, plus tree selection is started with no information about the genetic parameters if the phenotypic variation is considered to be sufficient. However, the existence of additive genetic variation is the prerequisite for the selection response in the next generation. On the other hand, the statistical requirement for estimation the genetic variance components is the relatedness of the individuals used in the analysis (Falconer 1981). As a result, estimation is not possible before the progeny tests have been established. This is the rationale on which the traditional tree breeding procedure is based.

The additive genetic variance is, as such, a relatively abstract parameter. Analogous to the coefficient of phenotypic variation (CV %), defined as the ratio of the phenotypic standard deviation and the phenotypic

mean, is the coefficient of additive genetic variation, CV_A %, which is defined as the ratio of the additive genetic standard deviation, σ_A, and the phenotypic mean (Houle 1992, Cornelius 1994). Thus the estimate of CV_A helps us to evaluate the absolute scale of the gain, i.e. the magnitude of the selection response compared to the current average of the characteristic in the population.

The individual heritability, h^2, i.e. the ratio between the additive genetic variance and the phenotypic variance, expresses how precisely the genotypic value of the individual tree can be predicted from its phenotype in the analysed population (Falconer 1981). If the estimate of the heritability is low, then the phenotype mainly reflects environmental effects. Thus in the tree breeding context, the heritability of a characteristic, estimated in a specific progeny test, tells us how good the progeny test is as a selection environment for the characteristic in question (White and Hodge 1989). The average level of several heritability estimates is interpreted to depict the "strength of genetic control" and thus "the possible gains through selection and breeding" for the characteristic in question, e.g wood density (Zobell and Jett 1995).

Estimates of the genetic correlation between characteristics are necessary before the recurrent selection can be started. If indirect selection is to be applied, the genetic correlations enable the breeder to predict the response of indirect selection in the target characteristic. In the case of index selection, the genetic correlations are essential elements when constructing a selection index (Cotterill and Dean 1990). A genetic correlation can also reveal an 'indirect response' of selection in a characteristic that is not supposed to change.

The heritability and the CV_A together provide prospects of improving the average value of a characteristic through selection. Both estimates are also needed to determine which is the most efficient breeding strategy for each characteristic (Hannrup 1999). In the planning of a breeding programme it might be advisable to set the different components of the ideotype into a 2 x 2 table (Table 1) as soon as the necessary parameters are available.

Table 1. Prospects of improving a characteristic by breeding, predicted on the basis of the scale of the heritability and the coefficient of the additive genetic variation. Modified from Hannrup (1999).

	High CV_A	Low CV_A
High h^2	easy breeding	progress only by intensive selection
Low h^2	progress only through intensive testing	difficult breeding

1.3 The dilemmas of simultaneous selection for several characteristics and early selection

Even a fairly simple ideotype leads to a breeding task in which the gain in the next generation should be achieved in several characteristics simultaneously. The solution currently suggested for multiple characteristic selection is index selection, the other possibilities being tandem selection and independent culling. Building up a successful selection index demands economic weighting of the characteristics included in the index, and reliable estimates of the heritabilities and genetic correlations between the characteristics (Cotterill and Dean 1990). The selection for two characteristics with a strong unfavourable genetic correlation cannot be effective for the both characteristics at the same time. Thus including a new trait in the selection programme demands, not only an investigation of the additive genetic variation of the characteristic itself, but also a comprehensive study of the relationships with the characteristics that have already been included.

Wood quality characteristics are often not manifested in young seedings. In the case of such characteristics, investigation of the age-age type genetic correlations is not as relevant as with the early selection of production characteristics. However, in order to keep the interval between breeding generations as short as possible, there is a need for early selection also in the quality characteristics. A successful early indirect selection requires the breeder to find, either by carefully investigating the genetics and physiological background of the target characteristic or merely by screening several characteristics, a high genetic correlation between the target and any other characteristic which is manifested at an early age of the tree with a high heritability. In the case of heartwood properties, the question about early indirect selection is not only important, but also exciting, because the wood tissues of the future heartwood already exist in fairly young stems, but still possess the properties of sapwood (Fig. 1)!

1.4 The current criteria of Scots pine wood quality

The difficulty of determining wood quality has sometimes been overemphasised. Nevertheless, 'high quality' wood is a fairly simple concept: it means a good suitability for the purpose the wood is to be used for (Zobel and van Buijtenen 1989, Kellomäki et al. 1992) or, in economic terms, it is something the customers are willing to pay extra for (Venäläinen et al. 1996). However, an important consequence of the definition

is that different, sometimes even contradictory, properties are deemed to mean high quality, depending on the purpose the wood is used for. The classical example of this are the studies on the chemical properties of Scots pine heartwood which led to the discovering of the phenolic extractives playing a role in decay resistance. The studies were initiated because heartwood was found to be detrimental for the sulphite pulping process in the paper industry (Erdtman 1939b). Since then, Nordic pulp engineers have turned their main attention to the fibres of other tree species, such as spruce, birch and aspen (Paavilainen 2002), and thus the mechanical industry, together with its customers, has alone set the quality requirements for Scots pine wood.

The Nordic grading rules for sawn timber, dating from the 1930's and updated in the 1980's, set upper limits first of all for the size and number of knots (Pohjoismainen ... 1994). The occurrence of actual defects (such as scars and reaction wood) is also limited, but there is no strict upper limit for the growth rate as such. In practice, the high phenotypic correlation between rapid growth and thick branches has led to a rule of thumb: the inner knot quality of a log is predicted by the width of the annual rings visible at the end of the log (Heiskanen 1965, Halinen 1985). Rapid growth as such, leading to wide annual rings, low late wood proportion and low basic density, may decrease the quality of timber only in those usages where high strength is required for beams. Overall, it can be inferred that rapid growth and high timber quality are not logically contradictory properties to be included in the same ideotype. Neither does there seem to be any evidence that a Scots pine tree, growing rapidly and producing wood which fulfils the current quality criteria, would be a biological impossibility.

1.5 Natural durability

1.5.1 The 'rehabilitation' of natural durability

In the early days, before the invention of artificial wood preservatives, the people who used wood were fully aware of the differences in the natural durability between tree species and between different parts of the stem, as well as the use of natural wood extractives as preservatives (Plinius ~A.D.77, Richardson 1978, Hillis 1987). Nowadays, when the reputation of coal-tar distillate 'creosote', patented in 1838, and the range of metallic salt preservatives, such as copper-chromium-arsenic (CCA) products, that have been developed during the 19th and 20th centuries (Richardson 1978, Zabel and Morrell 1992), have been questioned, there has been a recurrence of interest in the natural durability of wood. In Finland the rules

concerning the production, sale, use (decisions of Finnish Environment Institute, Suomen ympäristökeskus) and especially the disposal of impregnated wood were considerably tightened in 2002 (Suomen säädöskokoelma 1128/2001, 1129/2001). On the other hand, consumers have begun to appreciate the advantages of ecologically benign over foreverlasting wood, for example in garden fittings and playground structures (Life Cycle Assessment). In massive buildings such as houses, a long service life for untreated wooden elements can best be guaranteed by keeping the wood relatively dry, i.e. by maintaining the moisture content below 20 % (Viitanen 1996). In general, it will be difficult to avoid the use of impregnated wood in places with the highest decay risk (e.g. railway sleepers and electricity transmission poles in contact with the soil), as well as in constructions that are difficult and therefore expensive to repair, or the weakening of which may cause a safety hazard (e.g. guard rails of a bridge) (Richardson 1978). The variation in the durability of impregnated wood is smaller than that of natural durability, which increases the value of impregnated wood as a standardised building material.

Among the tree species growing in Finland the trees considered to have wood of the highest natural durability are yew (*Taxus baccata* L.) and oak (*Quercus robur* L.) (Adopted European ... 1994b). However, they have no use on a practical scale because the wood production of them in our climate is relatively limited. The medium level of durability is represented by the heartwood of larch (*Larix sp*) and Scots pine. According to the five-class scale of the European standard EN 350-2, Scots pine heartwood is classified as 3-4, i.e. moderately to slightly durable against wood-destroying fungi, while Siberian larch (*L. sibirica* Ledeb.) is not included in the list of species (Adopted European ... 1994b). Global trade makes it possible to utilise non-domestic durable softwoods, such as western red cedar (*Thuja plicata* Donn) from North America, or the tropical, very durable hardwoods, in the Nordic countries. However, the easy accessibility of raw material favours the use and price of domestic species. Moreover, if the wood, even though less durable, is produced near to the site where it will be used, it gains some additional value as regards ecological indicators (Material Flow Analysis).

1.5.2 Factors affecting natural durability

The wide variation in the durability among and within species has led to a number of scientific reports, starting in the 1920's (see the early references given by Zabel and Morrel, 1992), as well as speculation about the factors that may play role in natural durability. These factors are mainly associated with the wood extractives, "the principal source of decay resis-

tance", that inhibit the primary metabolism or degradation processes of the fungi, or with the permeability of the wood for water, air and fungal hyphae (Scheffer and Cowling 1966). Approximately the same factors are involved in the formation of heartwood (Rudman 1966). Other factors, which affect the suitability of wood as a living environment for fungi and the variation of which could thus contribute to the durability variation, are lignification of the wood cell walls, degree of crystallinity of the cellulose, nitrogen content of the wood, and the depletion of reserve food materials (Scheffer and Cowling 1966, Gref et al. 2000).

The wood degradation mechanisms of brown-rot fungi have been gradually elucidated during the past two decades (Highley and Dashek 1998). Both oxidative free radicals (Ritschkoff 1996) and cellulolytic enzymes (Eriksson et al. 1990, Maijala 2000) are involved in the processes, as soon as there is sufficient water within the wood cells for the functioning of fungal hyphae (Zabel and Morrell 1992, Viitanen 1996). The understanding of the oxidative degradation mechanisms especially has revealed new potential sources of durability variation, such as the functioning of iron ions (Fekete et al. 1989, Goodell et al. 1997, Hyde and Wood 1997, Goodell and Jellison 1998, Xu and Goodell 2001), or the role of extractives as antioxidants (Schultz et al. 1997, Schultz and Nicholas 2000).

According to Zabel and Morrell (1992), there are four major groups of heartwood extractives that include compounds known or believed to contribute to the natural durability: phenolics including stilbenes and flavonoids, tannins, terpenoids and tropolones. A more comprehensive grouping of heartwood extractives is presented by Hillis (1987), and the biosynthesis and biological activity of them by Seigler (1998). In the case of Scots pine the relevant groups of extractives are stilbenes and terpenoids.

The compound called pinosylvin (PS), named after *Pinus sylvestris* by Erdman (1939a), and its derivates pinosylvin monomethylether (PSM) and pinosylvin dimethylether (PSD), represent stilbenes and are typical for the heartwood of *Pinus* species. Very low concentrations of free forms of PS and PSM have shown to be toxic for fungi (Rennerfelt 1943, 1945). Based on his investigation on the metabolism of microorganisms, Lyr (1961) concluded that PSM acts as an uncoupling toxin that inhibits the oxidative phosphorylation, and assumed that PS acts in an equal manner. However, the role of stilbenes in situ, as integrated in the wood substrate, has been a puzzling and even argued conundrum (Erdtman and Rennerfelt 1944, Rennerfelt 1947, Rudman 1963, Loman 1970a,b, Hart and Shrimpton 1979, Hart 1981, Celimene et al. 1999, Schultz et al. 1997, Schultz and Nicholas 2000).

The oleoresin of Scots pine contains two types of terpenoids: volatile monoterpenoids, such as α-pinene and 3-carene, and non-volatile diterpe-

noids, resin acids (Sjöström 1993). The fungitoxic monoterpenoids in oleoresin makes it important for the active defence of pines (Flodin and Fries 1978, Yamada 1992 and references therein). It has also been suggested that the resin acids, that remain in timber during the drying processes, play a role in decay resistance (Hart et al. 1975 and references therein). As regards the mechanism of inhibition, the resin acids are supposed to make the wood hydrophobic (Verrall 1938, Eberhardt et al. 1994) or act as weak fungicides (Hartman et al. 1981, Micales et al. 1994).

In any case, the decay resistance of sawn timber can be based only on passive defence mechanisms (see the definition in Merrill 1992) possessed by tissues that have no living cells. Induced processes that are important for the active defence of the living tissues of standing trees can no longer occur.

1.5.3 In vitro methods for studying natural durability

The accelerated testing of preservatives against wood destroying fungi has a long tradition, and several testing procedures have been developed (see Zabel and Morrell 1992). In today's Europe the testing of wood preservatives against Basidiomycetes has been standardised by the norm EN 113 (European standard EN 113, 1996). The accelerated testing procedure for the natural durability of wood, based on EN 113, has been standardised by the norm EN 350-1 (Adopted European ... 1994a). In the standardised test, the size of the wood block is 50 x 25 x 15 mm and the blocks are incubated for 16 weeks in contact with pure cultures of three different fungus species (which in the case of softwood are *Gloeophyllum trabeum* (Persoon ex Fries) Murrill, *Serpula lacrymans* (Schumacher ex Fries) S.F Gray, *Poria placenta* (Fries) Cooke sensu J.Eriksson). According to Hannu Viitanen from VTT (pers. comm. 11.1.2002), the use of *Coniophora puteana* (Schumacher ex Fries) Karsten, which is an obligatory brown-rot species in the EN 113 test, is today also accepted in the testing of natural durability. Reference specimens from *Pinus sylvestris* sapwood are obligatory when softwoods are tested. The mass loss of the samples is used as an inverse measure of the decay resistance. The results are expressed as the ratio of the loss in mass and the oven dry (103°C) initial mass of the sample, i.e. as mass loss percentage (%).

The first obstacle in using the standardised testing method in estimating the genetic parameters of decay resistance, and in testing breeding material, is the destructive sampling. In the breeding procedures the trees are needed, after the testing, to produce the next generation (i.e. in pollen and seed production) or to be used in vegetative propagation. Thus they

have to remain alive and vigorous for several years after sampling. The other disadvantage of the standardised method are the experimental costs. The number of samples needed to meet the requirements of statistical precision in parameter estimation is high, several hundred at the minimum. Thus testing would occupy a lot of laboratory space for a long period, especially if parallel samples are tested against several fungus species.

1.6 Aim of the study

The aim of this doctoral thesis study was to evaluate the biological possibility of improving the decay resistance of Scots pine heartwood timber by means of forest tree breeding. In order to be able to draw conclusions on this topic, the following questions had to be addressed. The Roman numerals refer to the original articles, including empirical results, on which the answers and discussion were based on.

Fundamental questions:

a) Is it possible to assess the decay resistance of the timber, i.e. sawn and dried wood product, of a standing tree ? (I, II, III, VI)
b) What is the magnitude of the natural phenotypic variation in the decay resistance of Scots pine heartwood timber? (II, III, VI)
c) How large is the additive genetic component of variance? (II, III)

Further questions provided additive genetic variation exists:

d) In the implementation of the Scots pine breeding programme, is it possible to select directly for the target characteristic, i.e. the decay resistance of timber? (II, III, VI)
e) Which characteristics can be used in the indirect selection and in the early indirect selection ? (IV, V, VI)
f) What are the genetic correlations between decay resistance and those used in the indirect selection? What are the genetic correlations between decay resistance and those already included in the Finnish Scots pine breeding programme? (discussion based mainly on the literature)
g) What is the role of the environment in the recurrent selection of Scots pine breeding material and in the management of the stands established with the improved regeneration material. (II, III)

2. Material and methods

2.1 Wood material

The planning of sampling in studies such as the present one is troublesome if the organisation of different types of woody tissue within a standing tree is not fully understood. Figures 3 and 4 in the Appendix provide information about the age of the tissues and the internal variation of the stem. The sampling in young trees especially is fundamentally associated with the concept of juvenile wood (see e.g. Zobel and van Buijtenen 1989, Thörnquist 1993). The most accepted explanation for the formation of juvenile wood is the juvenile stage of the cambium; ageing of the cambium gradually leads to the formation of mature wood. The main differences between juvenile and mature wood lie in the dimensions of the tracheids, the basic density and the microfibril angle. The duration of the juvenile stage in Scots pine is poorly known, but it can be assumed to continue for 20-30 years (Thörnquist 1993). Thus the core of the stem, i.e. approximately the first 20-30 annual rings around the pith, at each relative height of the tree consists of juvenile wood. Thus, when the formation of heartwood begins, the first 20-30 annual rings around the pith will be *juvenile heartwood.*

The material used to develop the accelerated decay test for standing trees was obtained from a Siberian larch clonal seed orchard at Jämsänkoski (**I**). The samples were taken with a 5 mm diameter, increment core borer. A 40 mm-long piece of the outer heartwood section of the core was separated for the decay test. In studies **II** and **III** an equal-sized increment core borer was used to take samples from standing Scots pines. In the planning of the sampling, the main emphasis was put on the precise estimation of the genetic parameters. The progeny tests established with half-sib families are well suited for this purpose. However, a compromise was needed in order to adjust the number of families, and trees per family to be sampled (Xie and Mosjidis 1997), as well as the number of samples per tree, with the total number of samples that could be included in the decay test at same time (about 1000). On the other hand, in order to obtain as mature wood as possible the stand had to be selected from among the oldest progeny tests, the designs of which set their own limitations on the sampling. At Kerimäki, 10 trees from 25 families (totalling 250 trees) were sampled from a 32-year-old progeny test (**II**), and at Korpilahti 413 trees from a total of 26 families in a 34-year-old progeny test were included in the statistical analysis (**III**). In study **II**, four sapwood samples and one heartwood sample per tree were studied. In study **III**, only one heartwood sample per tree was subjected to the decay fungus. For estima-

tion of the offspring-parent regression, 20 clones representing the mother trees of the half-sib families of the Korpilahti progeny test were sampled in the clonal archive in Punkaharju (**III**).

In order to study the hypotheses concerning the relationships between decay resistance and other wood characteristics, derived from the framework presented in Figure 2 (Chapter 3.6.2), a new set of sample trees was selected from among the trees included in studies **II** and **III**. Two groups of trees with extreme rates of juvenile heartwood decay were obtained by culling the tails of the in vitro mass-loss distributions. The group of trees with slowly decaying heartwood, referred to later on as resistant, comprised 10 trees from Kerimäki and 10 trees from Korpilahti. The 20-tree-group with rapidly decaying heartwood, referred to later on as susceptible, were also derived from the same locations (Table 2 in **IV**). Sampling was carried out on an individual tree basis, but only one tree from a single half-sib family was accepted in order to avoid a kin structure among the sampled trees. This time the sampling was carried out with an 8 mm increment core borer as described in study **IV**.

The sampling procedure limited the use of parametric statistical methods in the data analysis in studies **IV** and **V**. In contrast, the sampling for the study **VI** was random as regards the decay resistance and the distribution of the samples was continuous, thus allowing better use of parametric statistical methods. Two additional elements were included in the material for study **VI**: variation in an old natural stand, and the radial variation in large-sized mature trees. Destructive sampling was applied by taking disks from twenty felled trees and by re-sampling the disks (figure 1 in **VI**).

2.2 Decay tests

In order to avoid the problems associated with the use of standardised decay test EN 113 in studies like the present one, VTT *Building and transport* developed a simplified testing method. The main advantage of this malt agar plate method, described in detail in article **I**, is the possibility to study standing trees. This was enabled by the use of an increment core borer in the sampling. The small size of the specimen permitted a short incubation time, 6-8 weeks, and only one brown rot fungus, *C. puteana* (strain BAM Ebw. 15), was used instead of the three fungus species. The 5 mm x 35 - 40 mm increment core section was used in studies **II** and **III**. The destructive sampling in study **VI** made it possible to use a small (5x15x30 mm) block as a specimen, but otherwise the decay testing procedure was similar in studies **I, II, III** and **VI**.

According to the norm EN 113, the result of the decay test is expressed as the relative mass loss (loss in mass/initial dry mass x 100 %). However, because of the large natural variation of basic density, the relative mass loss alone does not provide sufficient information in the durability analysis. In the present study the mass loss was expressed as an absolute measure per fresh wood volume (mg/cm^3), which better describes the degrading activity of the fungus. Only in study **III** the genetic parameters were also estimated for the relative mass loss. Irrespective of whether the variable is either absolute or relative, the information about the basic density of the samples should always be included in the reporting.

2.3 Quantification of extractives

For the quantification of resin acids (**IV, VI**), wood powder was extracted with petroleum ether-diethyl ether following the procedures of Gref and Ericsson (1985). The extracts were analysed by gas chromatography - mass spectrometry (GC-MS) as described by Manninen et al. (2002). For quantification of individual resin acids, calibrations were made using known amounts of pure resin acids and the response factors were determined for each substance relative to known amounts of the internal standard (heptadecanoic acid).

For the analysis of the total concentration of all phenolic secondary compounds, wood powder was extracted with 80 % (v/v) acetone. The phenolics were determined by the Folin-Ciocalteu technique using tannic acid as a standard (Julkunen-Tiitto 1985, Turtola et al. 2002). The concentrations of soluble phenolics other than the stilbenes PS and PSM are low in Scots pine heartwood, and thus the result of the Folin-Ciocalteu determination is assumed to mainly depict the concentration of stilbenes.

For the quantification of the individual stilbenes, pinosylvin (PS), pinosylvin monomethyl ether (PSM), and pinosylvin dimethyl ether (PSD), two different techniques were used. In study **V** wood powder was extracted with acetone in a mini-Soxhlet apparatus for 6 hrs. An internal standard, diethylstilbestrol, was added and the extracts were evaporated to dryness and stored under nitrogen. Trimethylsilyl esters were prepared, after which PS, PSM, and PSD were determined by gas liquid chromatography - mass spectrometry (GLC-MS). In study **VI,** wood powder was extracted with 80 % (v/v) methanol. The extraction was carried out in tubes with mixing for 30 minutes. Vanillin was used as an internal standard. Samples were centrifuged and the supernatants were analysed by high performance liquid cromatography (HPLC). The analysis was performed as described by Lieutier et al. (1996). Peak areas were used to quantify the individual

substances PS and PSM, and the results (mg/g dry wt) were calculated relative to known amounts of internal standard. However, in study **VI**, the final results of all the chemical analyses were expressed, correspondingly to the absolute mass loss, as concentration per fresh volume of wood.

2.4 Water sorption capacity

Water bound to hygroscopic cell wall constituents and into voids of wood of radius less than 1.5 µm is called *ad*sorbed water. This critical point of sorption is called the fibre saturation point. It represents a water potential of –0.1 MPa and, in theory, a relative humidity of 99.93 % (Griffin 1977). The water present in the cell lumens and intercellular space is called free or *ab*sorbed water (Walker 1993).

The adsorption capacity of the Scots pine heartwood was first (**IV**) studied using increment core samples taken from the Korpilahti progeny test in January 2001 from the same 10 resistant and 10 susceptible trees on which the analysis of extractives had been performed. After drying at 60°C for 48 hours and weighing, the samples were conditioned at a relative humidity (RH) of 100 % at 24°C. After two weeks of conditioning the samples were weighed, oven dried at 103°C for 24 hours, and reweighed. The quantity of water in the equilibrium stage was expressed as mg/cm^3, and the equilibrium moisture content, MC %, as the mass of water at RH 100 %, divided by the oven dry mass of wood at 103°C. The moisture content can be regarded as the quantity of water standardised by the wood density.

The water sorption capacity was then studied in two different kinds of experiment using 5 x 15 x 30 mm-sized, wood blocks (**VI**). The adsorption was first determined in a tightly closed steel tank that was half-filled with tap water (+25°C). The wood blocks (dried at 60°C for 48 hours) were placed on steel racks immediately above the water surface. The blocks were weighed at increasing intervals from 4 hours up until 14 days after the start of the test, and then dried at 103°C to obtain the dry mass. The results were expressed as the moisture content values, MC %. The same blocks were then immersed in water. The mass of the wet blocks was measured at increasing intervals from 1 hour to 7 days after the start of the test. The quantity of sorbed water was expressed as the gross mass of water (i.e. adsorbed and absorbed) per fresh volume of wood (mg/cm^3).

2.5 Statistical analysis

In the studies **II** and **III**, in which variance components were estimated the models of analysis were based on individual tree observations, assum-

ing that all effects associated with the observations were incorrelated. In contiguous plot designs, there is the possibility of spatial auto-correlation. If several trees per plot are sampled and the assumption of independence is wrong, the additive genetic component of variance is overestimated. In the case of wood characteristics the danger of this bias was not considered to be serious.

The measurable phenotypic value (P) an individual tree is assumed to be a sum of an additive genetic effect (A), and an environmental effect (E) which here also includes the possible non-additive genetic effects (P=A+E). Correspondingly, the observed phenotypic variance splits into additive genetic and environmental components: $\sigma_P^2 = \sigma_A^2 + \sigma_E^2$. The heritability in a narrow sense is calculated as $h^2 = \sigma_A^2/\sigma_P^2$ (Zobel and Talbert 1984).

In the data of study **II,** the estimation of the variance components was based on a random model

$$[1] \quad y_{bfi} = \mu + a_b + b_f + c_{bf} + e_{bfi}$$

where y_{bfi} is an observation for a tree, and μ is a fixed general mean. The terms a_b and b_f are the random effects of the block b and the family f, c_{bf} is the random block × family interaction effect, and e_{bfi} is the residual random effect of tree i in plot bf. The individual tree heritability was estimated, assuming true half-sibs and unrelated parents, as

$$[2] \quad \hat{h}^2 = \frac{4\hat{\sigma}_f^2}{\hat{\sigma}_b^2 + \hat{\sigma}_f^2 + \hat{\sigma}_{bf}^2 + \hat{\sigma}_e^2}.$$

This may be an overestimate due to the possible existence of full-sibs (Squillace 1974, Hill et al. 1998). According to Cotterill (1987), this model and heritability estimate are useful when no corrections for block effects are made prior to selection.

The MIXED procedure of SAS® provided approximate standard deviations for the estimated variance components (SAS Institute Inc. 1996) . The standard deviation for the heritability estimate was calculated using 'Dickerson's approximation'(e.g. Dieters *et al.* 1995), and for the additive genetic component as $\sigma_{\sigma_A^2} = 4\sigma_{\sigma_f^2}$.

The coefficient of additive genetic variation (CV_A) was calculated by dividing σ_A by the overall mean value of the trait.

In the data of study **III,** estimation of the variance components was based on a mixed model without interaction (a simplified genetic model, Jacquard 1983, Ericsson 1997):

[3] $$y_{bfi} = \mu + \alpha_b + b_f + e_{bfi}.$$

The block effect (α_b) was regarded as fixed (corrections for block effects should be applied prior to selection), and the family effect (b_f) as random. This model is constructed assuming that the interaction between the design and treatment factors within a single site does not describe the true genotype-environment interaction.

In order to estimate additive genetic, environmental and phenotypic correlation (r_A, r_E, r_P), the covariance between properties was obtained using corresponding variance estimates of each pair of traits and of their sum (e.g. Williams & Matheson 1994). Standard deviation for the genetic correlation was estimated according to Falconer (1981).

In study **III** the heritability of the heartwood properties was also estimated by utilising the measurements from 20 parents, together with the measurements from their offspring (progeny). First, the degree of resemblance between the relatives was expressed as the regression (b) of offspring (O) on parents (P) (Falconer 1981, Nyquist 1991). Heritability was estimated as $h^2_{OP}=2b_{OP}$. Second, heritability was estimated by a correlation approach using the coefficient of genetic prediction between offspring and parents as $h^2 = \mathrm{CGP}_{\mathrm{OP}}$ (Baradat 1976).

In order to test whether the heartwood of decay resistant and susceptible trees was equal as regards the studied characteristics, the Mann-Whitney U-test using Wilcoxon scores (ranks of data) was employed (**IV**, **V**). The test was carried out by the SAS procedure NPAR1WAY, which provides the exact p values for the rank statistics (SAS Institute Inc. 1996). A one-sided test was applied for resin acids and phenolics, since the alternative hypothesis for the null hypothesis was that the concentration of these compounds is higher in the heartwood of decay resistant trees. The one-sided alternative hypothesis was justified since, according to earlier reports, these compounds reduce fungal growth in laboratory tests.

In study **VI**, one-way ANOVA using tree-wise means was applied to test whether the sapwood and the outer and the inner heartwood differed from each other in the studied wood characteristics. The pair-wise comparisons between the stem sections were performed by Tukey's test. A simple regression model ($y= \beta_0 + \beta_1 x + \varepsilon$, were y= response variable and x= independent variable) was applied to study whether the mass loss was dependent on the chemical or physical wood characteristics. The relationships between the independent characteristics were studied with correlation analysis.

3. Results and discussion

3.1 Measuring the decay resistance of timber from standing trees

In article **I** there is an introduction to an accelerated decay testing method in which samples, taken from standing trees with a 5 mm increment core borer, were incubated on malt agar plates containing a pure culture of *C. puteana*. In spite of its simplicity compared to the standardised EN 113 test, the method appeared to be effective enough to reveal the clonal repeatability (**I**) and the additive genetic variance component (**III**) with a statistical significance. The small size of the wood specimens enabled the testing of a sufficiently large number of samples. Especially when genetic parameters are estimated or breeding materials tested, large numbers of individuals need to be studied. The accelerated method also performed satisfactorily as a screening test for standing trees when the material of studies **II** and **III** was selected for studies **IV** and **V**. Increment core sampling from standing trees for decay resistance testing has been used earlier by Wazny and Krajewski (1984).

The introduced method also has some shortcomings. In addition to the intra-tree variation described in the Appendix (figures 3 and 4), trees typically contain spots of random internal variation. A small piece of wood taken from such a spot will poorly represent the average properties of the tree. The use of several replicate samples per tree would lessen this problem. The small sample may also be sensitive to random variation during the in vitro test. The accuracy could be partly increased by using an 8 mm-diameter sample. At least the proportion of intact tracheids would then be higher, which may smooth out the random variation in the adsorption of water.

The finding in study **VI** that the variation in the sapwood durability of the mature pines could not be explained by any independent variable, suggests that the accelerated and simplified in vitro decay test has some nuisance factors as a considerable source of error variation. This doubt was raised already by the results of study **II**: was it the growing environment of the trees, together with the reaction wood, that caused the differences in the durability between the trees in the Kerimäki progeny test, or was the observed variation a result of the variation in the activity of the degrading fungus? The introduced method, as a complicated biological test, is obviously a relatively imprecise tool for measuring decay resistance. This problem could be avoided through replication and randomisation, i.e. by using several parallel samples, distributed on different plates.

The extra variation caused by the nuisance factors has two kinds of implication. The results overestimate the true phenotypic variation of the decay resistance. On the other hand, the genetic parameters and the R^2 values of the regression models are underestimates as regards the pure natural variation.

3.2 Phenotypic variation in the decay resistance of Scots pine

Tree breeders have typically been interested in the visible differences between individual trees because this phenotypic variation indicates possibilities for phenotypic selection. Large phenotypic variation in the decay resistance of Scots pine wood against one brown-rot fungus, *C. puteana*, was found in all three in vitro decay tests. In study **II**, the average absolute mass loss (mg/cm^3) of juvenile heartwood was about 70 % compared to that of sapwood, and the coefficient of variation, CV, among individuals was 76 % for heartwood and 18 % for sapwood (unpublished). In study **III,** the CV was 47 % for the juvenile heartwood of the progeny test trees, and 67 % for the heartwood of grafts, which was on the average more durable. In study **VI,** the mass loss of the sapwood was the highest; the mass loss of the inner heartwood reached 77 %, and that of the outer heartwood 40 % of the sapwood mass loss. The CV values were: 14 % in the sapwood, 51 % in the outer heartwood, and 21 % in the inner heartwood. Because the testing time and the size and form of the wood specimens varied from test to test, comparison of the absolute mass loss means between the materials in the different tests is not appropriate, while comparisons within the tests are meaningful. The most marked feature in the phenotypic variation was that the variation was especially large where the average mass loss was low.

CV provides a parameter for comparing the scale of phenotypic variation in decay resistance and in the other wood characteristics. In the juvenile heartwood of young pines (**II, III**) and in the outer heartwood of mature pines (**VI**), the CV of mass loss was 6-8 fold compared to the CV of basic density. According to Zobel and van Buijtenen (1989), the amount of tree-to-tree variation in basic density promises "very successful" breeding in most, if not all, of the tree species studied. Even if our CV estimates for mass loss are obviously overestimates, they show that the lack of phenotypic variation does not represent an obstacle for the breeding efforts.

3.3 Additive genetic variation of decay resistance in sapwood and juvenile heartwood

The additive genetic variance, σ_A^2, was estimated through the variance of half-sib family effects, σ_f^2. The estimate of σ_A^2 was then used to calculate the heritability in a narrow sense, h^2, and the coefficient of additive genetic variation, CV_A. These parameters were estimated for the mass loss of the sapwood (**II**) and for the mass loss of the juvenile heartwood of the young pines (**II, III**). In the sapwood data (**II**), there was no significant additive genetic component in the observed mass loss variation ($h^2 = 0.03$, SD=0.11; $CV_A = 1.9$ %). Thus the variation in sapwood mass loss was deemed to be caused by the environmental factors, and hence to be a futile source of variation for breeder's selection. In this context the term 'environmental' is used in a broad sense, i.e. it includes all the non-genetic sources of variation from the growing environment of the individual tree up to the possible nuisance factors of the malt agar plate test, as well as all the non-additive genetic effects.

The results obtained for the heartwood decay resistance promised a fairly good selection response in the advanced tree generations. For the heartwood mass loss in study **III**, the estimate of CV_A was high, 28.5 %, and the estimate of h^2 was also high, 0.37 (SD=0.17). (According to Cotterill and Dean (1990), individual heritabilities of between $h^2 = 0.10$ and 0.30 are considered intermediate.) For the heartwood mass loss in study **II**, the estimate of CV_A was also fairly high (10.6 %) indicating the existence of additive genetic variation. However, the estimate of h^2 remained low (h^2=0.02, SD=0.14) because of the relatively large residual variation. The difference in the estimate of h^2 between the two progeny test gives a warning: there are populations in which the correlation between the phenotypic and genetic value is low, and which are therefore not suitable populations for the phenotypic selection. On the other hand, a high single-site heritability can be inflated by a family-by-test site interaction (Haapanen 2002) and in such a case the selection gain will be overestimated.

In study **III,** h^2 was also estimated from the regression of the offspring means on their mother clones, and using the coefficient of genetic prediction, CGP. The regression and the correlation approach gave lower heritability estimates for each of the characteristics, probably due to the exceptional growing environment of the grafts. What was remarkable however, was that the estimates of mass loss heritability were of the same magnitude as those for basic density. According to the review of Zobel and Jett (1995), the heritability values of wood density for the hard pines, including Scots pine, are very high. Thus the results support the conclusion concerning the possibilities to improve heartwood durability through tree breeding.

Table 2. Summary of the results of studies **II, III** and **VI** on the mass loss of Scots pine wood caused by *C. puteana* in an in vitro decay test. The testing time was 6 weeks in studies **II** and **VI**, and 8 weeks in the study **III**. The estimates of mean and standard deviation (SD), narrow-sense individual heritability (h^2), and the coefficient of genetic variation (CV_A) are given.

	sample	mean (SD) (mg/cm^3)	h^2(SD)	CV_A (%)
Kerimäki (**II**)	[1]s	114 (20)	0.03 (0.11)	1.9
	[2]h	80 (61)	0.02 (0.14)	10.6
Korpilahti (**III**)	h	123 (58)	0.37 (0.17)	28.5
Punkaharju (**III**)	h	80 (54)	[3]0.29 (0.34)	
Punkaharju (**VI**)	s	141 (19)		
Punkaharju (**VI**)	h(outer)	57 (29)		
Punkaharju (**VI**)	h(inner)	108 (23)		

[1] s = sapwood

[2] h = heartwood

[3] heritability based on offspring parent regression

So far, only a few studies have been carried out on the genetic variation associated with the decay resistance of the wood of any tree species. Broad-sense heritability was estimated in this study (**I**) using clonal material of Siberian larch, resulting in a moderate value of 0.39. The study of Schmidtling and Amburgey (1982) on *Pinus taeda* L. showed a broad-sense heritability of 0.22. Studies on Scots pine have obviously been lacking before studies **II** and **III**.

3.4 Quantity of heartwood

The clear difference in the durability between the sapwood and heartwood in several tree species, known for thousands of years, demonstrated in studies **II** and **VI**, is as such not very interesting from the tree breeders' point of view. However, this difference well demonstrates the potential of natural wood-preservation mechanisms, and provides motivation for studying the factors behind natural durability. Furthermore, it raises the question about the genetic variation in the volume of heartwood, and the

possibilities to enhance the quantitative production of heartwood through tree breeding.

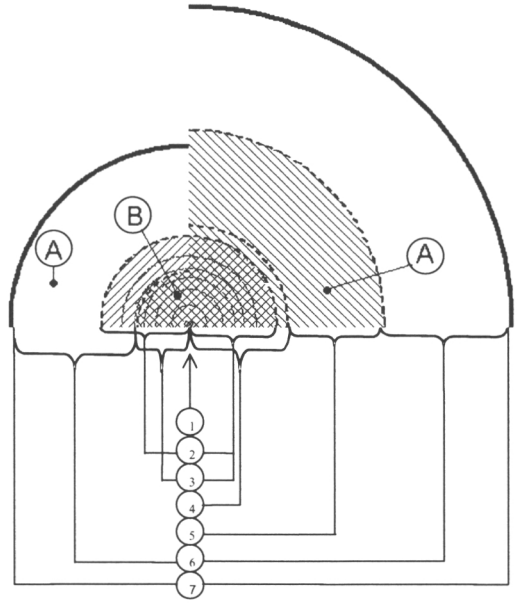
Two characteristics, measurable in an increment core, were used to describe the amount of heartwood in a stem: the number of heartwood annual rings, and the radius of heartwood. The sometimes proposed variable, sapwood:heartwood ratio in the cross-section area, was not used in the statistical analyses because of its non-linear nature (see the discussion in Fries and Ericsson (1998)). In study **II,** h^2 was estimated to be low (h^2=0.06, SD=0.16) for the radius of heartwood, and high (h^2=0.39, SD=0.25) for the number of heartwood annual rings. The respective estimates for the CV_A were moderate (6.2 %) and high (12.6 %). In study **III,** the estimate of h^2 was exceptionally high for the radius of heartwood (h^2=0.59, SD=0.20), and high (h^2=0.43, SD=0.17) for the number of heartwood annual rings. The estimate of h^2 for the radius of heartwood was equal to that of basic density (h^2=0.61, SD=0.20). What is interesting from the tree breeders' point of view, however, is that the estimate of CV_A for the radius of heartwood (18.8 %) was three-fold compared to that of basic density (6.3 %). Fries and Ericsson (1998) studied the heartwood diameter in a 25-year-old Scots pine full-sib progeny test and reported a heritability of 0.30 and CV_A of 20 %. In a 44-year-old progeny test, Ericsson and Fries (1999) estimated 0.54 for the heritability and 17 % for the CV_A in the heartwood diameter. Together these results indicate that also the quantity of heartwood would be a favourable target for selection in the tree breeding programme.

3.5 Prospects of direct selection

The outline of early selection and indirect selection in the context of selection for heartwood properties is presented in Figure 1. The simplest option, direct early selection utilising the differences in durability of the mature sapwood (point A in the figure) of some 20 – 30-year-old-trees to select for the durability of the future mature heartwood, appeared to be unsuitable (**II**). Since the observed mass loss variation in sapwood seemed not to be additive, there will be no response to the selection. The next option of direct durability selection is to wait until heartwood formation in the juvenile wood begins, i.e. until the age of over 30 years (point B in the figure). Studies **II** and **III** showed additive genetic variation in the durability of juvenile heartwood. Study **V** showed that the stilbenes, which according to the results of **VI** are the most important factor determining durability, were present in the juvenile heartwood. Thus this option seems more promising. However, these studies do not provide any estimates for the genetic correlation between the decay resistance of the

30-year-old tree now (23 annual rings at breast height)

70-year-old tree in the future (23+40 annual rings at breast height)



1 = parenchymic pith; 2 = juvenile wood; 3 = juvenile heartwood; 4 = inner heartwood; 5 = outer heartwood; 6 = sapwood; 7 = cambium, phloem and bark

selection 20 years earlier than now, in the juvenile sapwood (point B)

- direct early selection of decay resistance: no success in sapwood (**II**)
- indirect selection: no reliable characteristics known

selection now in point A

- direct early selection of decay resistance: no success in sapwood (**II**)
- indirect selection: no reliable characteristics known although the wood cells of the future heartwood already exist

selection now in point B

- direct selection of decay resistance is possible (**II, III**), but
- the differences between the juvenile and mature wood make the selection indirect as regards the outer heartwood in the future (**VI**)

selection when point A forms into heartwood

- wait for at least 20 years more

Figure 1. Aspects associated with the early selection of heartwood characteristics. The target is to improve the quality of the outer heartwood that will be formed at breast height at the tree age of 50 years and onwards.

juvenile and the mature heartwood. According to the data from 16 mature trees (**VI**), the phenotypic correlation between the decay resistance of the inner and outer heartwood was not significant. Within the limitations of accelerated tests with increment core samples, the direct selection for decay resistance is possible when the mature wood (point A) is converted to heartwood, which is not expected to occur earlier than the age of 50 years. However, waiting over 50 years for the selection is definitely too impractical for tree breeding.

3.6 Prospects of indirect selection: phenotypic relationships between decay resistance and other wood characteristics

3.6.1 Phenotypic versus genetic correlation

The possibilities of early direct selection (point A in Fig. 1) were found to be unpromising (**II**), and the suitable time for direct selection (in point A) is considered to be too far in the future for any efficient breeding effort. This makes the question of the possibilities of indirect selection highly relevant and, as a result, attention was paid to the phenotypic and genetic correlations between decay resistance and other wood characteristics. If our task would be to *screen* decay resistant timber among the existing trees, we could utilise phenotypic correlations for the indirect screening. Phenotypic correlations may also give useful hints about true cause and effect relationships between the characteristics. When we are concerned about the response of indirect selection in the next tree generation, we need to know the genetic correlations. It would also be wise to estimate genetic correlations in the breeding programme in order to predict the indirect responses to selection of those important characters which should not be deteriorated.

3.6.2 Resistance gatework

The ample variation found in the decay resistance of juvenile heartwood (**II, III**) encouraged to consider which characteristics, assessable in the wood, are associated with the dynamics of fungus colonisation and degradation processes, and are thus correlated with the durability. More fundamentally, the question is about the basic physiological factors causing the differences in the natural decay resistance. The constitutive factors which could interfere with the degradation processes in the form of passive defence are inadequately known. Several specific toxic secondary

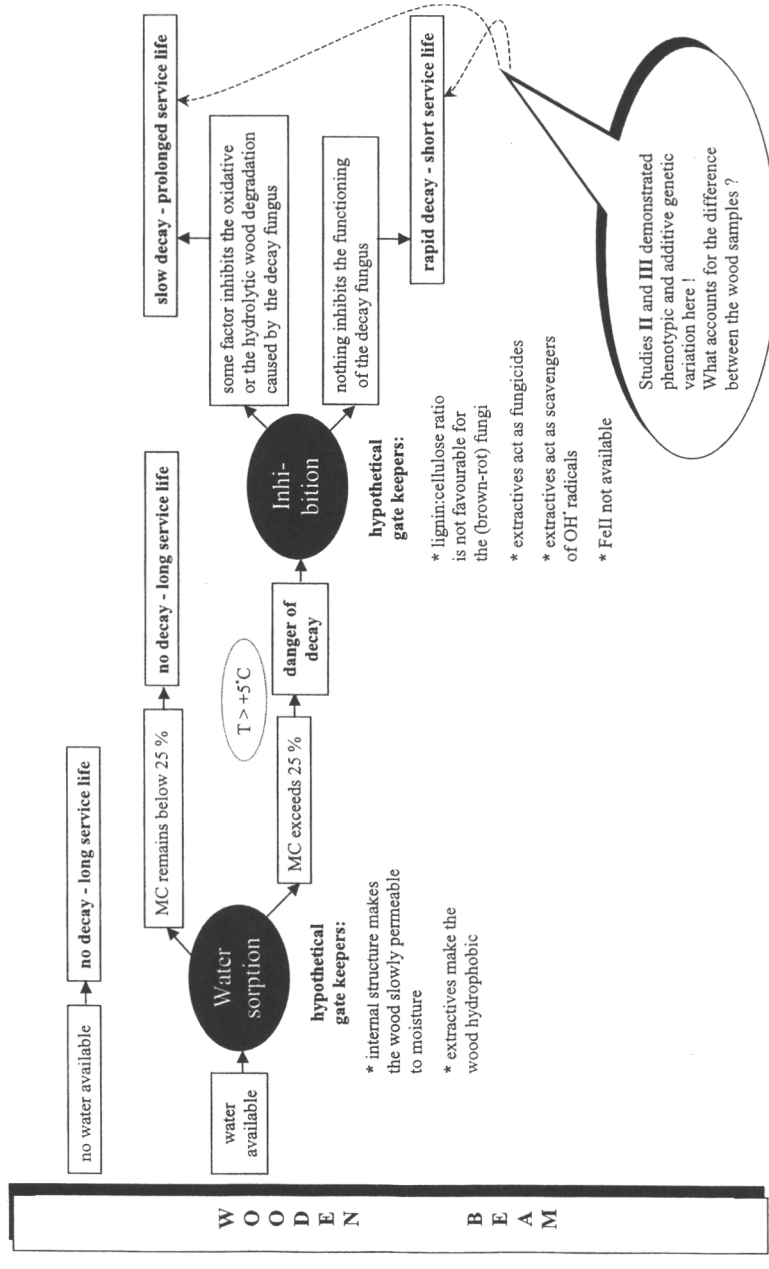


Figure 2. Resistance gatework. A simplified diagramme describing the possible reasons for the phenotypic differences in the decay rate of Scots pine increment core samples in II and III.

metabolites have been identified (Lyr 1961, Rudman 1963), but several additional hypotheses have also been presented (Chapter 1.5.2). In order to formulate hypotheses that could be tested in order to find characteristics related to decay resistance and useful in indirect selection, a simplified framework, called 'Resistance gatework' (Fig. 2), was devised. The first gate in the frame is 'the gate of water sorption'. As long as the moisture content of the wood remains clearly below the fibre saturation point, there is no danger of decay because readily available water is necessary for several of the metabolic functions of the fungus. If the moisture content remains high for extended periods, then the risk of fungus invasion is high (Viitanen 1996). If desiccation does not take place after colonisation, only the constitutional substances of the wood can maintain 'the gate of inhibition', i.e. interfere with the enzymatic or oxidative reactions caused by the fungus and thus decrease the rate of degradation.

Four possible factors affecting the durability differences were investigated: water repellency (**IV, V, VI**), the concentration of resin acids (**IV, VI**), the concentration of stilbenes (**V, VI**), and the proportions of main wood constituents, i.e. lignin, cellulose and hemicelluloce (Harju et al. 2003).

3.6.3 Water repellency

The equilibrium moisture content (MC) of the increment core sections obtained from decay resistant and susceptible heartwood in Korpilahti (**IV**) was determined. The adsorption capacity of the decay susceptible heartwood was slightly higher, the difference between the two extreme groups being close to statistical significance ($p = 0.089$). The results with block samples (**VI**) provide more evidence about the weak relationship between decay resistance and MC: the difference in MC between the sapwood and the outer and inner heartwood was close to the statistical significance ($p = 0.075$), while the differences in the durability were highly significant between all the stem sections. Furthermore, according to the regression analysis, the MC significantly explained the mass loss differences within the inner heartwood ($p = 0.006$) and nearly significantly that within the outer heartwood ($p = 0.077$). The amount of water retained by the wood at the end of the immersion experiment was also studied (**VI**): in the sapwood the amount was significantly higher than that in the heartwood, but the significance of the amount of water as a regressor for the mass loss differences within the heartwood sections was only indicative ($p = 0.091$-0.111). We can infer that the differences in water repellency may contribute to the differences in the decay rate, at least

in the beginning of the process, but they are unlikely to provide a tool for indirect selection for decay resistance.

3.6.4 Resin acids

The difference in the concentration of total resin acids between the decay resistant and susceptible heartwood was significant in the Korpilahti progeny test ($p = 0.004$), and nearly significant in the Kerimäki progeny test ($p = 0.072$) (**IV**). According to the results of **VI**, the concentration of resin acids was significantly lower ($p < 0.001$) in the sapwood than in the outer and inner heartwood, while between the outer and inner heartwood the difference was not significant. According to the regression analysis, the concentration of total resin acids explained poorly the mass loss differences within the inner heartwood ($p = 0.123$), and not at all within the outer heartwood ($p = 0.724$).

The results in studies **IV** or **VI** do not support the hypothesis about the strong negative correlation between the concentration of resin acids and the water sorption capacity of natural wood substrate. Obviously the relatively weak role of resin acids in the decay resistance of sound heartwood timber is not associated with water repellency. The overall conclusion is that the concentration of any individual resin acid or the total amount of resin acids is unlikely to serve as a tool for indirect durability selection.

3.6.5 Stilbenes

The juvenile heartwood of relatively young Scots pines contained considerable amounts of heartwood phenolics (**V**). In both progeny tests the concentration of total phenolics, determined by the Folin-Ciocalteu method, was significantly higher in the decay resistant than in the decay susceptible juvenile heartwood (at Korpilahti, $p < 0.001$; at Kerimäki, $p = 0.002$). The difference in the concentration of the most abundant individual stilbenes, PS and PSM, was also significant in both progeny tests (p values varying from < 0.001 to 0.045). Study **VI** showed a significant difference in the average concentration of total phenolics, as well as in the concentration of the individual stilbenes, PS and PSM, between the sapwood, the outer heartwood, and the inner heartwood. The average level of heartwood phenolics and the average mass loss were in inverse relation. The dependence between the mass loss and the stilbene concentration was significant within the inner heartwood (for PS, $p < 0.001$; for PSM, $p = 0.011$), while in the outer heartwood the dependence was less clear (for PS+PSM, $p = 0.058$). Overall these results show that the concentration of

stilbenes is the most important single factor determining the natural durability of Scots pine heartwood. This conclusion is in accordance with the earlier conclusions made by Rennerfelt (1943, 1945, 1947), Erdtmann and Rennerfelt (1944), and Rennerfelt and Nacht (1955) on the basis of studies carried out using several methods and several fungi.

In addition to the main results concerning the contribution of stilbenes to the reduction of the decay rate, studies **V** and **VI** provide two interesting side results.

First, as pointed out earlier by Loman (1970a), *C. puteana* was able to degrade wood containing stilbenes in concentrations at which free stilbenes would have been lethal (Rennerfelt 1945). Thus the stilbenes integrated in the wood substrate are either not toxic in the way reported by Lyr (1961), or the enzymes of the fungi are able to detoxify them (Lyr 1962a, 1962b, Loman 1970b).

Second, the concentration of stilbenes and the water sorption capacity seemed to be negatively correlated. The negative correlation in the immersion experiment (**VI**) especially was significant (p values varying from 0.018 to 0.005). Erdtman (1958) concluded, after failing to restore the longitudinal water permeability of heartwood by extraction with several solvents, that the extractives, including the phenolics, are not the reason for the low permeability of the heartwood. The result obtained by Vologdin et al. (1979) was the opposite: the *radial* permeability to water (and preservative liquids) was restored by extracting out the phenolic compounds. The apparent contradiction of the results can be understood from the results of Erdtman and Rennerfelt (1944) and Jorgensen (1961): the stilbenes are formed in the parenchyma cells of the radial-orientated rays and obviously also accumulate in the vicinity of these cells. Recently, Celimene et al. (1999) treated wood specimens with stilbenes extracted from cones, then carried out decay tests, and concluded that the stilbenes protect the wood through their water-repellent properties and dehydrating effects. The results in studies **V** and **VI** are in accordance with the conclusions of Celimene et al. (1999), but they do not prove the existence of a true causal relationship.

The relationship between the Folin-Ciocalteu results and the mass loss (**V**,**VI**) suggest that the Folin-Ciocalteu technique could be used in predicting the decay resistance of wood, and thus in the screening of durable timber. The significant correlation between the Folin-Ciocalteu results and the stilbene concentration (**VI**) suggests that this relatively simple method could also be used in phenotypic selection for the stilbene concentration. Another method for assessing the stilbene concentration in solid wood seems to be Fourier transform Raman and infrared spectroscopy (Holmgren et al. 1999). DeBell et al. (1997) have earlier suggested

the use of tropolone content as an indicator of decay resistance of western redcedar stems.

Although the stilbenes are not the only factor contributing to heartwood durability, and although the mechanism through which PS and PSM slow down the degradation processes is still questionable, the contribution of the stilbenes provides, so far, the most exciting clue to the indirect selection and early selection of decay resistance. Normally the stilbenes are not formed until the last living cells of the sapwood, the ray parenchyma cells, slowly die due to desiccation and the sapwood tissue is converted into heartwood (Erdtman and Rennerfelt 1944, Jorgensen 1961). However, the findings with *Pinus resinosa* show that the synthesis of stilbenes can be induced in sapwood by wounding the cambium (Jorgensen 1961, Rudloff and Jorgenssen 1963), as well as in callus tissue cultures (Jorgensen and Balsillie 1969). Stilbenes were detected in the reaction zone formed as a response to infection by fungi in the sapwood of *Pinus taeda* L. (Shain 1967). In the sapwood of *Pinus contorta* the stilbene synthesis was a result of a bark beetle attack (Shrimpton 1973). Stilbene induction also took place in young Scots pine seedlings exposed to UV radiation (Schöppner and Kindl 1979, Gehlert et al. 1990) or when stimulated with a fungus (Gehlert et al. 1990). Inoculation with fungi associated with bark-beetles induced phenolic metabolites in the phloem of Scots pine (Lieutier et al. 1991, Lieutier et al. 1996, Bois and Lieutier 1997). The debarking of mature Scots pines induced the formation of stilbene-containing lightwood (Gustafsson 2001). These results suggest that the differences in the hereditary tendency to synthesise stilbenes could be tested before the onset of natural heartwood formation. In this kind of testing the assistance of advanced molecular biology and biotechnology will be indispensable (Raemdonck et al. 2001).

3.6.6 Other factors

The relationship between the mass loss and the amount of main cell wall constituents, i.e. cellulose, hemicellulose and lignin, was studied by Harju et al. (2003) using the same trees as those included in **IV** and **V**. The variation in the amount of the main constituents was minor, and there was no significant difference between the decay resistant and susceptible heartwood either. Thus the study of Harju et al. (2003) leaves it open what kind of difference there would be in the decay rate if there would be remarkable differences for example in the content of lignin. Vance et al. (1980) have emphasised the role of lignin in protecting cellulose against brown-rot fungi.

Tiitta et al. (unpublished manuscript) have studied the electrical properties of wood using the same trees as those included in **IV** and **V.** Electrical impedance spectroscopy (EIS) revealed significant differences between the decay resistant and susceptible heartwood: the samples from the resistant trees had e.g. increased electrical resistance and decreased capacitance. Furthermore, the concentration of extractives affected on certain electrical properties. These results encourage us to continue the development of non-destructive methods for testing wood durability.

3.7 Genetic correlations

Estimates of additive genetic variance were produced in **II** for the mass loss and for several wood and tree characteristics, and it would have been of great interest to calculate the genetic correlations between the mass loss and the other characteristics. However, the genetic correlation estimates would be very unstable when the additive variation estimate is very unstable (or 0), as was the one for mass loss in study **II**. Thus the genetic correlations were ignored in study **II**. In study **III** the estimate of the genetic correlation ($\hat{r}_A$) between the mass loss and heartwood density was 0.36 (SD=0.26), while the phenotypic correlation ($\hat{r}_P$) was –0.11 and the environmental correlation ($\hat{r}_E$) –0.56. According to this result, the selection for low mass loss would reduce the wood density in the next generation. The phenotypic correlation did not reveal this unfavourable implication because the strong negative environmental correlation masked the genetic effect. The estimates for the relative mass loss and the heartwood density (not published) were ($\hat{r}_A$) = 0.17, ($\hat{r}_P$) = –0.26, and ($\hat{r}_E$) = –0.65. A similar phenomenon was pointed out for Siberian larch (**I**): the absolute mass loss increased along with an increase in wood density, while the relative mass loss decreased. Thus the higher density prolonged the service life of the timber.

Two recent studies have been carried out in Sweden on the genetic correlations between Scots pine heartwood characteristics. Fries and Ericsson (1998) analysed a 25-year-old full-sib progeny test and reported a significant positive genetic correlation between the heartwood diameter and the crown limit, and non-significant positive genetic correlations between the heartwood diameter and height. The estimate between the diameter and heartwood diameter was low, although the phenotypic correlation was positive and high. Ericsson et al. (2001) analysed two progeny tests (25 and 44 years old) and reported about "some evidence of positive correlations" between the heartwood diameter and the concentrations of resin acids. The genetic correlation between the concentrations of resin acids and stilbenes was strongly positive.

The first inference concerning the genetic correlations between heartwood characteristics is that they are poorly known. More studies are needed to make reliable predictions about the consequences of selection but, as has been clearly shown, the studies have to be carefully planned in order to obtain statistical significance for the results. The second inference concerns the genetic correlations between heartwood characteristics and the characteristics now included in the ideotype. As regards the quantity of heartwood, the conclusion can be quoted directly from Fries and Ericsson (1998): " Since no correlations with production traits were unfavorable, we conclude that including heartwood formation capacity in a breeding programme may be done without drawbacks and with good prospects for success ". As regards the quality, i.e. the true durability of heartwood, no results are available. For the third inference concerning the genetic correlations between the decay resistance and the characteristics that are intended to be used in the indirect selection, results are so far totally lacking.

3.8 The role of environment

3.8.1 Selection environment

The two progeny tests provided valuable information about the role of the environment (*sensu lato*) in the recurrent selection of tree breeding material (**II, III**). They showed that progeny tests growing in different environments can differ considerably as regards the precision at which the phenotypic values reflect the genetic values, i.e. breeding values. This kind of efficiency of a progeny test is estimated on the basis of the heritability. The environmental variation was defined in a broad sense in this study, including the variation due to all effects other than the additive genetic effects. They presumably also include real differences in the soil and other growing conditions (including the variation among years), which more or less disturb the manifestation of genetic differences. Furthermore, there is the possibility of a real genotype by environment interaction, which means that certain types of growing environment systematically favour or disfavour certain genotypes.

According to Haapanen (2002), however, an unpredictable family-by-test site interaction seems to be a considerable source of variation in a single progeny test, at least in case of the growth characteristics. Thus the single-site estimate of additive genetic variation, such as that presented in **III**, can be biased upwards and the selection gain will be overestimated as regards other growing sites.

The estimate of genetic correlation between the heartwood density and mass loss, together with the other component of phenotypic correlation, the environmental correlation, also gave us an important lesson about the role of environment (**III**): the phenotypic correlation may reflect more the relationship due to the environmental effects, which may be contradictory to the direction of the genetic relationship (see discussion in King et al. 1997, Fries and Ericsson 1998). This again emphasises the importance of careful investigations on the genetic correlations between the characteristics included in the breeding programme, and also the importance of interpreting the environmental component of the equation.

3.8.2 The role of environment in the growing of improved Scots pines

During the past four decades the role of the growing environment has been crucial for the reputation, and consequently for the volume, of the artificial regeneration of Scots pine carried out by planting, and thus for the justification of the expensive breeding efforts. The numerous studies which clearly established that vigorous growth, for any reason, also leads to vigorous growth of the branches (Heiskanen 1966 and the 13 references therein) had been overlooked, and massive plantations of Scots pine were also planted on fertile sites. High site fertility, combined with an inadequate planting density or low survival of the planted seedlings (Turkia and Kellomäki 1987, Kellomäki et al. 1992, Varmola 1996), led to the deterioration of timber quality (Uusvaara 1981, Kärkkäinen and Uusvaara 1982), which is the important component of the Scots pine ideotype. The consequences were excessive favouring of natural regeneration over cultivation, direct forest sowing over planting, and regeneration with other tree species, Norway spruce mainly, over Scots pine. Thus the whole breeding programme of Scots pine was soon faced with the question: to be needed or not to be needed.

At the same time as the 'justification crisis' of Scots pine breeding, the emphasis on traditional quality characteristics, mainly knot size, and the need to add new characteristics to the quality component of the ideotype, such as the decay resistance of heartwood, are confounding the tree breeders with the puzzle of having to select too many characteristics simultaneously.

My suggestion to help solve the puzzle is to more fully utilise the power of environmental effects to raise Scots pines resembling the ideotype. Since the production characteristics have somewhat lower heritabilities and less additive genetic variation on the average than the quality characteristics (Cornelius 1994, Haapanen et al. 1997), and since there is

abundantly sites where Scots pine grows fast, the gain in volume production could be obtained, instead of intensive selection, by planting Scots pine on sites which are 'slightly too fertile'. In this way, selection potential would be released for quality characteristics that have higher heritabilities and thus will give a better response of selection. A high heritability may also reflect relatively strict genetic control over the environmental effects in the development of the quality characteristic. Thus the branch characteristics of improved seedlings may be less sensitive for the undesired effects of fast growth caused by the fertile environment. On the other hand, since height growth has, up until the present day, been the main criterion in the progeny testing of the Southern Finnish breeding zones (Venäläinen and Ruotsalainen 2002), dropping the volume growth away from the selection index in the forthcoming selection cycles would correspond with the idea of tandem selection.

4. Conclusions

The estimates of additive genetic variance derived from the data on half-sib families in the progeny tests showed that the phenotypic variation in decay resistance of Scots pine juvenile heartwood was partly genetic, i.e. not due entirely to environmental factors. The coefficients of additive genetic variation calculated in both progeny tests, $CV_A = 28.5$ % and $CV_A = 10.6$ %, predicted that the amount of additive genetic variation is sufficient for successful tree breeding by the means of selection and sexual propagation. One high estimate for heritability, $h^2 = 0.37$, showed that there exist progeny tests in which the correlation between phenotypic values and breeding values is high, and which are thus suitable for the phenotypic selection of timber decay resistance. The low estimate for heritability, $h^2 = 0.02$, in another case showed that some progeny tests are not suitable for selection because of abundant environmental variation.

According to the first conclusion, which concerns the amount of additive genetic variation and the heritability, the possibility to improve the decay resistance of Scots pine heartwood timber seems to be at least as good as the possibility of improving the production and quality characteristics included in the present tree breeding programme. Our knowledge of genetic correlations between the decay resistance and the characteristics included in the current ideotype is deficient, but thus far no results show these characteristics to be too much in conflict to be improved simultaneously.

Secondly, one indisputable obstacle for adoption of heartwood decay resistance into the multiple generation tree breeding programme is the late age at which this characteristic naturally manifests itself. The most durable heartwood develops outside the juvenile core of the stem, and direct assessment of its decay resistance cannot be performed until the age of approximately 50 years – too late for an efficient selection cycle. The current oldest progeny tests, planted about 30 years ago, enable restricted selection for the decay resistance of juvenile heartwood as a complement to the first cycle of recurrent selection. This unique option of direct selection will not be available in the advanced selection cycles.

Thirdly, therefore, the possibilities of indirect early selection require investigation. Such an investigation should include the physiological factors that affect natural decay resistance and, furthermore, assessable characteristics that correlate with the future decay resistance. The most promising factor found in this study was the concentration of the phenolic

compounds, pinosylvin (PS) and pinosylvin monomethyl ether (PSM), which belong to the stilbene group. Woody tissues synthesise stilbenes naturally when sapwood changes into heartwood, but different types of stress factor can stimulate stilbene production even in young seedlings. This finding could facilitate the development of an early testing method.

Furthermore, the possibilities of modern biotechnology should be integrated in the traditional testing and selection procedures. Identifying the genes responsible for heartwood formation and decay resistance, such as the genes encoding stilbene biosynthesis, may considerably enhance breeding for heartwood quality.

Future studies should also concentrate on obtaining reliable estimates of the genetic correlations between heartwood decay resistance, its early predictors, and characteristics already included in the Finnish Scots pine breeding programme. These estimates are needed to predict the gain in the multi-characteristic selection, as well as to avoid defective indirect selection responses. The role of environmental factors as a source of decay resistance variation should also be studied in more detail before the tree breeders start their selection operations, and before the forest owners begin the intensive growing of decay-resistant wood.

If the decay resistance of heartwood or any other new quality characteristics is to be included in the breeding programme, the number of characteristics to be selected simultaneously will rise to an unpractically high level. In such a situation the tree breeders should consider omitting volume growth characteristics from the intensive recurrent selection. This suggestion is justified by the fact that it is relatively simple to increase the volume growth of Scots pine through environmental factors.

The methods of direct decay resistance testing are not of prior interest for tree breeders because having to wait for mature heartwood would excessively prolong the generation interval. However, the durability screening of the existing Scots pine heartwood resources would be of high interest for the forest owners and the sawing industry. Therefore the further development of economic and reliable in vitro methods for testing decay resistance is a relevant task. Furthermore, chemical or physical methods for detecting a high stilbene concentration in wood at the moment of stem-cut would be necessary for the large-scale screening of timber.

Because this study provides no data for a proper comparison of Siberian larch and Scots pine wood as a source of naturally decay-resistant timber, the frequently asked question, which is more decay resistant, cannot be answered directly. Among all the tree species, the durability of

Siberian larch and Scots pine heartwood has been classified as approximately equal. The most important advantage of larch is its high relative proportion of heartwood and the readily visible colour difference between heartwood and sapwood. These two properties make it easy to saw and to sort pure pieces of larch heartwood timber. The most important advantage of Scots pine is the domestic timber resources. As regards the systematic growing of decay-resistant timber in Finland, these two species are not competitors but are complementary choices, because they are cultivated on different types of soil.

5. References

Adopted European standard EN 350-1. 1994a. Durability of wood and wood-based products - Natural durability of solid wood - Part 1: Guide to the principles of testing and classification of the natural durability of wood. European Committee for Standardization. Brussels. 20 p.

Adopted European standard. EN 350-2. 1994b. Durability of wood and wood-based products - Natural durability of solid wood - Part 2: Guide to Natural durability and treatability of selected wood species of importance in Europe. European Committee for Standardization. Brussels. 42 p.

Baradat, P.H. 1976. Use of juvenile–mature relationships and information from relatives in combined multitrait selection. Advanced generation breeding. Proc. IUFRO Joint Meeting. Bordeaux, June 14–18, 1976, pp. 121–138.

Bois, E. & Lieutier, F. 1997. Phenolic response of Scots pine clones to inoculation with Leptographium wingfieldii, a fungus associated with Tomicus piniperda. Plant Physiol. Biochem. 35(10): 819-825.

Celimene, C.C., Micales, J.A., Ferge, L. & Young, R.A. 1999. Efficacy of pino-sylvins against white-rot and brown-rot fungi. Holzforschung 53: 491-497.

Cornelius, J. 1994. Heritabilities and additive genetic coefficients of variation in forest trees. Can. J. For. Res. 24:372-379.

Cotterill, P.P. 1987. On estimating heritability according to practical applications. Silvae Genet. 36:46–48.

Cotterill, P.P. & Dean, C.A. 1990. Succesfull tree breeding with index selection. CSIRO Australia. 80 p.

DeBell, J.D., Morrell, J.J. & Gartner, B.L. 1997. Tropolone content of increment cores as an indicator of decay resistance in western redcedar. Wood Fiber Sci. 29(4): 364-369.

Dickmann, D.I. 1985. The ideotype concept applied to forst trees. In: Attributes of trees as crop plants. Eds. Cannell, M.G.R. & Jackson, J.E.. Inst. Terr. Ecol. Monks wood, Abbots Ripton, Hunts, UK. pp. 89-101.

Dickmann, D. I., Gold, M. A., & Flore, J. A., 1994: The ideotype concept and the genetic improvement of tree crops. Plant Breeding Reviews 12: 163-193.

Dieters, M.J., White, T.L., Littell, R.C., & Hodge, G.R. 1995. Application of approximate variances of variance components and their ratios in genetic tests. Theor. Appl. Genet. 91:15–24.

Donald, C.M. 1962. In search of yield. Journal of the Australian Institute of Agricultural Science 28:171-178.

Donald, C.M. 1968. The breeding of crop ideotypes. Euphytica 17: 385-403.

Eberhardt, T.L., Han, J.S., Micales, J.A. & Young, R.A.. 1994. Decay resistance in conifer seed cones: role of resin acids as inhibitors of decomposition by white-rot fungi. Holzforschung 48: 278–284.

Erdtman, V. H. 1939a. Die phenolischen Inhaltsstoffe des Kiefernkernholzes, ihre physiologische Bedeutung und hemmende Einwirkung auf die nor-

male Aufschließbarkeit des Kiefernkernholzes nach dem Sulfitverfahren. Justus Liebigs Annalen der Chemie 539: 116-127.

Erdtman, H., 1939b. Tallvedkärnans extraktivämnen och deras inverkan på uppslutningen enligt sulfitmetoden. Svensk Papperstidning 42: 344-349.

Erdtman, V.H. & Rennerfelt, E. 1944. Der Gehalt des Kiefernkernholzes an Pinosylvin-Phenolen. Ihre quantitative Bestimmung und ihre hemmende Wirkung gegen Angriff verschiedener Fäulpilze. Svensk Papperstidning 47: 45-56.

Erdtman, H., 1958. Kärnved och kärnvedskemi. Svensk Papperstidning 61: 625-632.

Ericsson, T. 1997. Enhanced heritabilities and best linear unbiased predictors through approriate blocking of progeny trial. Can. J. For. Res. 27: 2097–2101.

Ericsson, T., Fries, A. & Gref, R. 2001. Genetic correlations of heartwood extractives in Pinus sylvestris progeny test. Forest Genetics 8: 73–80.

Ericsson, T. & Fries, A. 1999. High heritability for heartwood in north Swedish Scots pine. Theor. Appl. Genet. 98: 732-735.

Eriksson, K.-E.L., Blanchette, R.A. & Ander, P. 1990. Microbial and enzymatic degradation of wood and wood components. Springer-Verlag. Berlin. 407 p.

European standard EN 113. 1996. Wood preservatives – Test method for determining the protective effectiveness against wood destroying basidiomycetes – Determination of the toxic values. European Committee for Standardization. Brussels. 32 p.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. 2 ed. Longman Group Ltd, New York. 340 p.

Fekete, F. A., Chandhoke, V., and Jellison, J., 1989: Iron-binding compounds produced by wood-decaying basidiomycetes. Appl. Environ. Microbiol., 55: 2720-2722.

Flodin, K. & Fries, N. 1978. Studies on volatile compounds from Pinus silvestris and their effect on wood-decomposing fungi. II. Effects of some volatile compounds on fungal growth. Eur. J. For. Path. 8:300-310.

Fries, A. & Ericsson, T. 1998. Genetic parameters in diallel-crossed Scots pine favor heartwood formation breeding objectives. Can. J. For. Res. 28: 937-941.

Gehlert, R., Schöppner, A. & Kindl, H. 1990. Stilbene synthase from seedlings of Pinus sylvestris: Purification and induction in response to fungal infection. Mol. Plant. Microbe Interaction 3(6): 444-449.

Goodell, B. & Jellison, J. 1998. The role of biological metal chelators in wood degradation and in xenobiotic degradation. In: Forest Products Biotechnology. Eds. A. Bruce and J.W. Palfreyman. Taylor & Francis Ltd, London. pp. 235–249.

Goodell, B., Jellison, J., Liu, J., Daniel, G., Paszczynski, 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.

Gref, R. & Ericsson, A. 1985. Wound-induced changes of resin acid concentrations in living bark of Scots pine seedlings. Can. J. For. Res. 15: 92–96.

Gref, R., Håkansson, C., Henningsson, B., & Hemming, J., 2000. Influence of wood extractives on brown and white rot decay in Scots pine heart-, light- and sapwood. Material und Organismen, 33: 119-128.

Griffin, D.M. 1977. Water potential and wood-decay fungi. Ann. Rev. Phytopathol. 15:19–29.

Gustafsson, G. 2001. Heartwood and lightwood formation in scots pine – A physiological approach. Doctoral thesis. Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Umeå. Silvestria 193: 38 p.

Haapanen, M. 2002. Evaluation of options for use in efficient genetic field testing of *Pinus sylvestris* (L.). Academic dissertation. Metsäntutkimuslaitoksen tiedonantoja 826. 67 p. + app.

Haapanen, M., Velling, P. & Annala, M.-L. 1997. Progeny trial estimates of genetic parameters for growth and quality traits in Scots Pine. Silva Fennica 31(1): 3-12.

Halinen, M. 1985. Männyn nuoruusvaiheen kasvunopeuden vaikutus sahatavaran laatuun. Summary: The effect of the growth rate of young pine on the quality of sawn goods. Silva Fennica 19(4): 377-385.

Hannrup, B. 1999. Genetic parameters of wood properties in *Pinus sylvestris* (L.). Acta Universitatis Agriculturae Sueciae. Silvestria 94. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, 36 + 58 p.

Harju, A., Venäläinen, M., Anttonen, S., Viitanen, H., Kainulainen, P., Saranpää, P. & Vapaavuori, E. 2003. Chemical factors affecting the brown-rot decay resistance of Scots pine heartwood. Trees (in press).

Hart, J.H. 1981. Role of phytostilbenes in decay and disease resistance. Ann. Rev. Phytopathol. 19: 437–458.

Hart, J.H. & Shrimpton, D.M. 1979. Role of stilbenes in resistance of wood to decay. Phytopathology 69: 1138-1143.

Hart, J.H., Wardell J.F. & Hemingway, R.W. 1975. Formation of oleoresin and lignans in sapwood of white spruce in response to wounding. Phytopathology 65: 412–417.

Hartmann, E., Renz, B. & Jung J.A. 1981. Untersuchungen über Bakterien- und Pilzhemmstoffe in höheren Pflanzen. Isolierung, Identifizierung und Wirkungsspectrum von zwei Harzsäuren aus Fictenrinden. Phytopath. Z. 101:31-42.

Heiskanen, V. 1966. Puiden paksuuden ja nuoruuden kehityksen sekä oksaisuuden ja sahapuulaadun välisistä suhteista männiköissä. Summary: On the relations between the development of the early age and thickness of trees and their branchiness in pine stands. Acta For. Fenn. 80(2):1-62.

Highley, T.L. & Dashek, W.V. 1998. Biotechnology in the study of brown- and white-rot decay. In: Forest products biotechnology. Eds. Bruce, A. and Palfreyman, J.W.. Taylor & Francis, Padstow. pp. 15-36

Hill, J., Becker, H.C. & Tigerstedt, P.M.A. 1998. Quantitative and Ecological Aspects of Plant Breeding. Chapman and Hall, London. 275 p.

Hillis, W.E. 1987. Heartwood and Tree Exudates. Springer Series in Wood Science. Springer-Verlag, Berlin. 268 p.

Holmgren, A., Bergström, B., Gref, R., & Ericsson, A. 1999. Detection of pinosylvins in solid wood of Scots pine using fourier transform raman and infrared spectroscopy. Journal of Wood Chemistry and Technology 19:139-150.

Houle, D. 1992. Comparing Evolvability and Variability of Quantitative Traits. Genetics 130:195-204.

Hyde, S. M. & Wood, P. M. 1997. A mechanism for production of hydroxyl radicals by the brown-rot fungus Coniophora puteana: Fe(III) reduction by cellobiose dehydrogenase and Fe(II) oxidation at a distance from the hyphae. Microbiology 143: 259-266.

Jacquard, A. 1983. Heritability: One word, three concepts. Biometrics 39: 465–477.

Jorgensen, E., 1961. The formation of pinosylvin and its monomethyl ether in the sapwood of Pinus resinosa Ait. Can. J. Bot. 39: 1765-1772.

Jorgensen, E. & Balsillie, D. 1969. Formation of heartwood phenols in callus tissue cultures of red pine (Pinus resinosa). Can. J. Bot. 47:1015-1016.

Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. Journal of Agriculture and Food Chemistry 33: 213-217.

Kellomäki, S., Lämsä, P., Oker-Blom, P. & Uusvaara, O. 1992. Männyn laatukasvatus. Summary: Management of Scots pine for high quality timber. Silva Carelica 23. 133 p.

King, J. N., Yanchuk, A. D., Kiss, G. K. & Alfaro, R. I. 1997. Genetic and phenotypic relationships between weevil (Pissodes strobi) resistance and height growth in spruce populations of British Columbia. Can. J. For. Res. 27: 732–739.

Kärki, L., & Tigerstedt, P. M. A., 1985. Definition and exploitation of forest tree ideotypes in Finland. In: Attributes of trees as crop plants. Eds. Cannell, M.G.R. & Jackson, J.E.. Inst. Terr. Ecol. Monks wood, Abbots Ripton, Hunts, UK. pp. 102-109.

Kärkkäinen, M. & Uusvaara, O. 1982. Nuorten mäntyjen laatuun vaikuttavia tekijöitä. Abstract: Factors affecting the quality of young pines. Folia Forestalia 515. 28 p.

Lieutier, F., Sauvard, D., Brignolas, F., Picron, V., Yart, A., Bastien, C. & Jay-Allemand, C.H. 1996. Changes in phenolic metabolites of Scots-pine phloem induced by Ophiostoma brunneo-ciliatum, a bark-beetle-associated fungus. Eur. J. For. Path. 26: 145-158.

Lieutier, F., Yart, A., Jay-Allemand, C.H. & Delorme, L. 1991. Preliminary investigations on phenolics as a response of Scots pine phloem to attacks by bark beetles and associated fungi. Eur. J. For. Path. 21: 354-364.

Loman, A. 1970a. Bioassays of fungi isolated from Pinus contorta var. latifolia with pinosylvin, pinosylvinmonomethyl ether, pinobanksin, and pinocembrin. Can. J. Bot. 48:1303-1308.

Loman, A. 1970b. The effect of heartwood fungi of Pinus contorta var. latifolia on pinosylvin, pinosylvinmonomethyl ether, pinobanksin, and pinocembrin. Can. J. Bot. 48: 737-747.

Lyr, H. 1961. Die wirkungsweise toxischer kernholz-inhaltsstoffe (thujaplicine und pinosylvine) auf den stoffwechsel von mikroorganismen. Flora 150: 227-242.

Lyr, H. 1962a. Enzymatische detoxifikation der kernholztoxine. Flora 152: 570-579.

Lyr, H. 1962b. Detoxification of heartwood toxins and chlorophenols by higher fungi. Nature 195(4838): 289-290.

Maijala, P. 2000. Heterobasidiom annosum and wood decay: Enzymology of cellulose, hemicellulose, and lignin degradation. University of Helsinki. Department of Biosciences. Academic dissertation. (PDF- publication 90 p.)

Manninen, A.-M., Tarhanen, S., Vuorinen, M. & Kainulainen, P. 2002. Comparing the variation of needle and wood terpenoids in Scots pine provenances. J. Chem. Ecol. 28: 211-228.

Merrill, W. 1992. Mechanism of resistance to fungi in woody plants: a historical perspective. In: Defense Mechanisms of Woody Plants against Fungi. Eds. R.A. Blanchette and A.R. Biggs. Springer Verlag, New York, pp. 1-12.

Micales, J.A., J.S. Han, J.L. Davis and R.A. Young. 1994. Chemical composition and fungitoxic activities of pine cone extractives. In: Biodeterioration Research 4. Mycotoxins, Wood Decay, Plant Stress, Biocorrosion, and General Biodeterioration. Eds. G.C. Llewellyn, W.V. Dashek and C.E. O'Rear. Plenum Press, New York. 317-332 p.

Nyquist, W. E. 1991. Estimation of heritability and prediction of selection response in plant populations. Critical Rev. In Plant Sci. 10(3): 235-322.

Oskarsson, O. 1995. Silmällä tehty savotta. Pluspuiden valinnan historia ja arki. Metsäntutkimuslaitoksen tiedonantoja 579. 68 p.

Paavilainen, L. (ed.) 2002. Finnish forest cluster research programme Wood Wisdom (1998-2001). Final report. Tampere. 441 p.

Plinius Secundus, G. ~A.D.77. Historia Naturalis. Liber XVI. Silvestrium arborum naturae.

Pohjoismainen sahatavara. 1994. Mänty- ja kuusisahatavaran lajitteluohjeet. Suomen Sahateollisuusmiesten yhdistys. Gummerus Kirjapaino Oy. Jyväskylä. 64 s. + 16 liites.

Pöykkö, T. 1993. Selection criteria in Scots pine breeding with special reference to ideotype. Männyn jalostuksen valintaperusteet erityisesti viljelypuun ihannemallia soveltaen. Academic dissertation. Metsänjalostussäätiön tiedonantoja 6: 1-66.

van Raemdonck, D., Jaziri, M., Boerjan, W. & Baucher, M. 2001. Advances in the improvement of forest trees through biotechnology. Belg. Journ. Bot. 134(1): 64-78.

Rennerfelt, E. 1943. Die Toxizität der phenolischen Inhaltsstoffe des Kiefernkernholzes gegenüber einigen Fäulnispilzen. Svensk Bot. Tidskr. 37: 83-93.

Rennerfelt, E. 1945. The influence of the phenolic compounds in the heartwood of Scots pine (Pinus silvestris L.) on the growth of some decay fungi in nutrient solution. Svensk Bot. Tidskr. 39: 311-318.

Rennerfelt, E. 1947. Några undersökningar över olika rötsvampars förmåga att angripa splint- och kärnved hos tall. Summary: Some investigations over the capacity of some decay fungi to attack sapwood and heartwood of Scots pine. Meddel. Från Statens skogforskningsinstitut 36(9): 1–24.

Rennerfelt, E. & Nacht, G. 1955. The fungicidal activity of some constituents from heartwood of conifers. Svensk Bot. Tidskr. 49: 419–432.

Richardson, B.A. 1978. Wood preservation. Spon Press. London. 226 p.

Ritschkoff, A.-C. 1996. Decay mechanisms of brown-rot fungi. Academic dissertation. Technical Research Centre of Finland, Espoo. VTT Publications 268: 67 p.

von Rudloff, E. & Jorgensen, E. 1963. The biosynthesis of pinosylvin in the sapwood of Pinus resinosa Ait. Phytochemistry 2: 297-304.

Rudman, P. 1963. The causes of natural durability in timber. Part XI. Some tests on the fungi toxicity of wood extractives and related compounds. Holzforschung 17(2):54-57.

Rudman, P. 1966. Heartwood formation in trees. Nature 210: 608-610.

SAS Institute Inc. 1996. SAS/STAT® Software: Changes and Enhancements through Release 6.11. SAS Institute Inc. Cary, N.C. 1104 p.

Scheffer, T.C. & Cowling, E.B. 1966. Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4: 147-170.

Schultz, T.P. & Nicholas, D.D. 2000. Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry 54: 47-52.

Schultz, T.P., Nicholas, D.D. & Fisher, T.H. 1997. Quantitative structure-activity relationships of stilbenes and related derivatives against wood-destroying fungi. Recent Research Development in Agricultural and Food Chemistry 1: 289-299.

Schmidtling, R. C. & Amburgey, T. L. 1982. Genetic variation in decay susceptibility and its relationship to growth and specific gravity in loblolly pine. Holzforschung 36: 159–161.

Schöppner, A. & Kindl, H. 1979. Stilbene synthase (pinosylvine synthase) and its induction by ultraviolet light. FEBS letters 108(2): 349-352.

Seigler, D.S. 1998. Plant secondary metabolism. Kluwer Academic. Boston. 759 p.

Shain, L. 1967. Resistance of sapwood in stems of Loblolly pine to infection by Fomes annosus. Phytopathology 57:1034-1045.

Shrimpton, D.M. 1973. Extractives associated with wound response of lodgepole pine attacked by the mountain pine beetle and associated microorganisms. Can. J. bot. 51: 527-534.

Sjöström, E. 1993. Wood chemistry. Fundamentals and applications. 2nd ed. Academic Press. San Diego. 293 p.

Squillace, A. E. 1974. Average genetic correlations among offspring from open-pollinated forest trees. Silvae Genet. 23: 149–156.

Suomen säädöskokoelma 1128/2001. Valtioneuvoston asetus jäteasetuksen liitteen 4 muuttamisesta.

Suomen säädöskokoelma 1129/2001. Ympäristöministeriön asetus yleisimpien jätteiden sekä ongelmajätteiden luettelosta.

Thörnquist, T. 1993. Juvenile wood in coniferous trees. Byggforskningsrådet. Swedish Council for Building Research. Stockholm. D13:1993. 110 p.

Tigerstedt, P. & Velling, P. 1986. The genetic anatomy of harvest index in Scots pine and some suggestions for applications in breeding and silviculture. In: Fujinori, T. & Whitehead, D. (eds.). Proceedings Crown and Canopy Structure in Relation to Productivity. Forestry and Forest Products Research Institute, Ibaraki, Japan. pp. 49-69.

Tiitta, M., Kainulainen, P., Harju, A.M., Venäläinen, M., Manninen, A-M., Vuorinen, M,. & Viitanen, H. Comparing the effect of chemical and physical properties on complex electrical impedance of Scots pine wood. Submitted manuscript.

Turkia, K. & Kellomäki, S. 1987. Kasvupaikan viljavuuden ja puuston tiheyden vaikutus nuorten mäntyjen oksien läpimittaan. Abstract: Influence of the site fertility and stand density on the diameter of branches in young Scots pine stands. Folia Forestalia 705. 16 p.

Turtola, S., Manninen, A.-M., Holopainen, J.K., Levula, T., Raitio, H. & Kainulainen, P. 2002. Secondary metabolite concentrations and terpene emissions of Scots pine xylem after long-term forest fertilization. J. Env. Qual. 31:1694-1701.

Uusvaara, O. 1981. Viljelymänniköiden puun tekninen laatu ja arvo. Akateeminen väitöskirja. Metsäntutkimuslaitoksen tiedonantoja 28. 47 p.

Vance, C. P., Kirk, T. K., & Sherwood, R. T., 1980. Lignification as a mechanism of disease mechanism of disease resistance. Annu. Rev. Phytopathol. 18:259-288.

Varmola, M. 1996. Nuorten viljelymänniköiden tuotos ja laatu. Akateeminen väitöskirja. Metsäntutkimuslaitoksen tiedonantoja 585. 70 p. + app.

Velling, P. 1988. The relationships between yield components in the breeding of Scots pine. Academic dissertation. University of Helsinki, Department of Plant Breeding. 59 p.

Venäläinen, M., Hahl, J. & Pöykkö, T. 1996. Assessing the quality of young stems in predicting the total monetary yield of Scots pine progenies Can. J. For. Res. 26(12): 2227-2231.

Venäläinen, M. & Ruotsalainen, S. 2002. Procedure for managing large-scale progeny test data: a case study of Scots pine in Finland. Silva Fennica 36(2): 475-487.

Verrall, A.F. 1938. The probable mechanism of the protective action of resin in fire wounds on red pine. J. Forest. 36:1231-1233.

Viitanen, H. 1996. Factors affecting the development of mould and brown rot decay in wooden material and wooden structures. Effect of humidity, temperature and exposure time. The Swedish University of Agricultural Sciences, Department of Forest Products. Dissertation. Uppsala. 58 p.

Vologdin, A.I., Razumova, A.F. & Charuk, E.V. 1979. Die Bedeutung der Extraktstoffe für die Permeabilität von Kiefern- und Fichtenholz. Holztechnologie 20(2): 67-69.

Walker, J.C.F. 1993. Primary Wood Processing: principles and practice. Chapman, Hall, London. 595 p.

Wazny, J. & Krajewski, K.J. 1984. Jahreszeitliche Änderungen der Dauerhaftigkeit von Kiefernholz gegenüber holzzerstörenden Pilzen. Holz als Roh- und Werkstoff 42:55-58.

White, T.L. & Hodge, G.R. (eds.) 1989. Predicting Breeding Values with Applications in Forest Tree Improvement. Forestry Sciences vol. 33. Kluwer Academic Publishers. 367 p.

Williams, E. & Matheson, A. 1994. Experimental design and analysis for use in tree improvement. CSIRO, Australia. 174 p.

Xie, C. & Mosjidis, J.A. 1997. Influence of sample size on precision of heritability and expected selection response in red clover. Plant Breed. 116:83-88.

Xu, G., & Goodell, B., 2001. Mechanisms of wood degradation by brown-rot fungi: chelator-mediated cellulose degradation and binding of iron by cellulose. J. Biotechnol. 87: 43-57.

Yamada, T. 1992. Biochemistry of gymnosperm xylem responses to fungal invasion. *In*: Defense Mechanisms of Woody Plants against Fungi. Eds. R.A. Blanchette and A.R. Biggs. Springer Verlag, New York, pp. 147–164.

Zabel, R.A. & Morrell, J.J. 1992. Wood microbiology. Decay and its prevention. Academic Press, Inc, California. 476 p.

Zobel, B.J., & van Buijtenen, J.P. 1989. Wood variation, its causes and control. Springer-Verlag, Berlin, Heidelberg. 363 p.

Zobel, B.J. & Jett, J.B. 1995. Genetics of wood production. Springer-Verlag. Berlin Heidelberg. 337 p.

Zobel, B. & Talbert, J. 1984. Applied forest tree improvement. John Wiley & Sons. 505 p.

Appendix

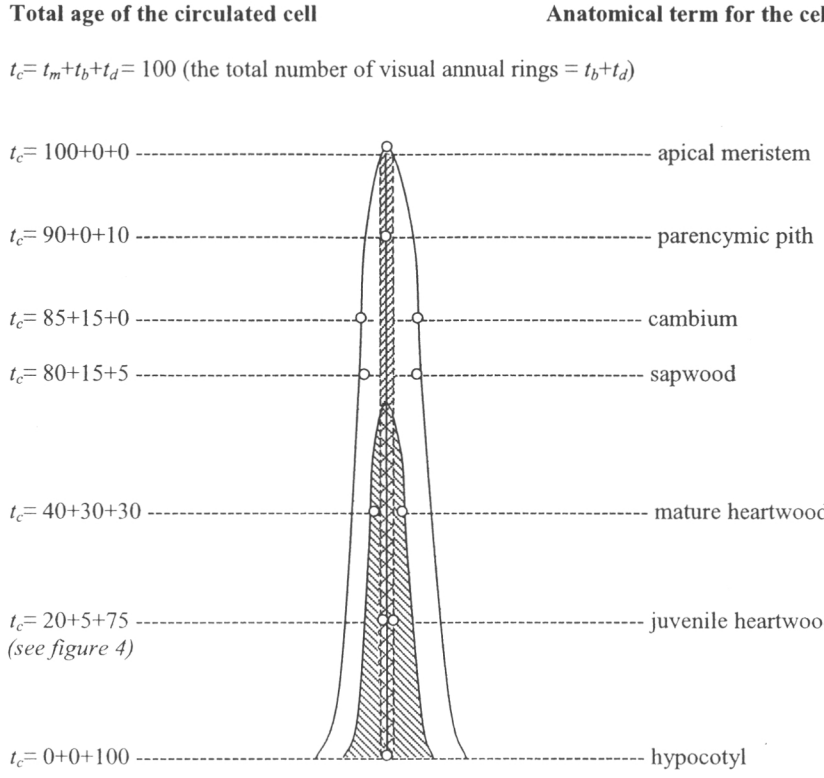


Figure 3. The age of the wood cells of a 100-year-old living tree (inspired by Zobel and Jett 1995). The total age of each cell (t_c) can be postulated to be 100 years, provided that the total age is divided into 3 components: the age of the apical meristem (t_m), the age of the cambium (t_b), and the age of the differentiated cell (t_d). Thus $t_c = t_m + t_b + t_d = 100$ years. The comprehension of wood age is essential for successful sampling of wood specimens. The importance of the age of the apical meristem is obviously not crucial for the wood characteristics, in contrast to that of the cambium, which contributes to certain of the differences between juvenile and mature wood, or that of differentiated cells which contribute e.g. to the difference between sapwood and heartwood.

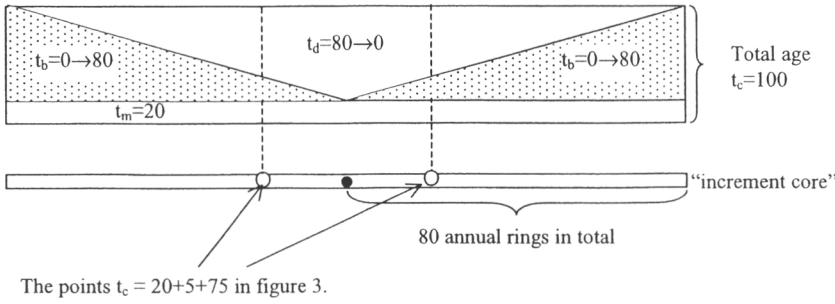


Figure 4. The age profile of a line of cells (compare with an increment core), running from the cambium through the parenchymic pith to the opposite cambium, taken from a height of 1.3 m ("breast height") from the 100-year-old tree presented in Figure 3.

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Holzforschung
55 (2001) 1–6

Genetic Variation in the Decay Resistance of Siberian Larch (*Larix sibirica* Ledeb.) Wood

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Keywords

Larix sibirica
Coniophora puteana
Heartwood
Wood decay resistance
Clonal repeatability
Heritability

Summary

The aim of the study was to estimate the degree of genetic determination in the decay resistance of Siberian larch (*Larix sibirica* Ledeb.) wood and its correlation to other wood traits. The wood samples were taken from 25-year-old grafted seed orchard clones with an increment core borer, dried, weighed, and subjected to a laboratory decay test using a modified method based on the standardised EN 113 method. One brown rot fungus, *Coniophora puteana* (Schum. ex Fr.) Karst., was used as the decaying organism. The advantages of the method were the savings in time, the possibility to study standing trees, and the potential for screening large numbers of samples at reasonable costs. The clonal repeatability was used to estimate the degree of genetic determination. The genetic determination appeared to be stronger for decay resistance than for growth characteristics or heartwood formation, but weaker than for wood density or latewood formation. Decay resistance and the growth characteristics did not correlate.

Introduction

Siberian larch (*Larix sibirica* Ledeb.) is one of the most promising and the most widely used (15 000 ha) exotic tree species in Finland. The oldest cultivated stands are about 150 years old. The natural range of Siberian larch covers western Siberia and north-western Russia, the westernmost stands growing only about 300 kilometres to the east of Finland (Fig. 1). Larch has a good reputation among the users of wood as a fairly decay resistant species, although in some cases also the larch wood has been noticed to degrade quite soon. This controversy has been repeatedly mentioned in the literature dealing with studies on the durability of larch wood against decay (e.g. Björkman 1944; Kiellander 1966; Ueckermann and Lülfing 1974; Viitanen *et al.* 1997). However, the results of these studies are in agreement and show that the durability of larch heartwood is comparable with that of Scots pine (*Pinus sylvestris* L.) heartwood.

One obvious reason for the traditional controversy must have been the large inter-species variation in durability within the genus of larch and, furthermore, between stands and individual trees, as well as within individual stems (Zabel and Morrell 1992). Most of the larch studies carried out in Europe deal with European larch (*Larix decidua* Mill.). In the European standard EN 350-2 concerning the natural durability of wood species of importance in Europe, natural durability of European larch heartwood is equal to class 3 or 4 (moderately or slightly durable) and comparable with the durability of Scots pine heartwood (European standard 1994). Siberian larch is not even mentioned in this standard. Viitanen *et al.* (1997) tested the heartwood durability of several larch species and hybrids according to the standard EN 113 (European standard 1996) and EN 350-1 (European standard 1994). The durability of Siberian larch was equal to class 3 or 4, but large variation was found in the decay resistance.

The proportion of heartwood in Siberian larch stems is higher than that of Scots pine. This difference may have confused some of the comparisons made between the

Fig. 1. The introduction of Siberian larch (*Larix sibirica*) from the original Arkhangelsk region *via* Raivola stand (▲) to Finland. The dark shared area shows the natural range of Siberian larch in north-western Russia. ● = planted stand from which plus trees have been selected, ■ = seed orchard no. 309.

decaying rates of these two species. In old Siberian larches the proportion of heartwood can be as high as 80 % of the volume (Lappi-Seppälä 1927). According to Hakkila and Winter (1973), the proportion of heartwood at a height of about 5 m in 25 m Siberian larches is nearly 70 % while, according to Kellomäki (1981), it is about 35 % of the volume in Scots pine butt logs. Thus it has often been suggested in Finland that larch wood could replace the use of ecologically suspect impregnated pinewood. In fact only the sapwood reacts to preservatives, the heartwood being difficult to impregnate (e.g. Löyttyniemi 1986).

The natural decay resistance of wood is dependent on the amount and quality of primary metabolites, storage compounds and the extractives that are deposited in the heartwood (Zabel and Morrel 1992). In Scots pine heartwood the role of stilbenes is considered to be important for the decay resistance (Rennerfelt 1943; Hart and Shrimpton 1979). In Siberian larch heartwood the concentration of water-soluble extracts, mainly arabinogalactan, is high, reaching up to 16 % in old trees (Hakkila and Winter 1973 and references therein). The concentration increases with the age of the trees (Viitanen *et al.* 1997). The concentrations of resin acids, free fatty acids and triacylglycerols are found to be low (Viitanen *et al.* 1997). The role played by different chemical compounds for the durability of larch heartwood has not yet been elucidated.

In Scots pine, the variation in the amount of heartwood may be influenced by the genotype of individual tree, but also environmental factors have affect (Ericsson and Fries 1999; Fries and Ericsson 1998). The effect of the genotype and the environment on the durability of wood has not been studied in the tree species grown in Finland.

In this study we analysed the variation in the decay resistance of Siberian larch heartwood, and estimated the genetic and environmental components of this variation. In order to find characteristics that could be used to predict the wood durability indirectly, we studied whether the weight loss of increment core samples in a decay test correlated with ring width, earlywood/latewood proportion, heartwood proportion, or wood density.

Materials and Methods

Sampling and preliminary analyses of wood material

The Siberian larch wood used in the decay test was mainly obtained from seed orchard grafts. Reference material for the grafts was obtained from mature plus trees growing in stands. The seed orchard (number 309) is located at Jämsänkoski, central Finland (Fig. 1), and owned by the Finnish Forest and Park Service. The seed orchard was established in 1974. The grafts were planted in the orchard at a spacing of 3.5 × 7 m (400 grafts/ha). The orchard consists of 54 clones, one of which was omitted from the sampling because of the bad quality of grafts. At the time of sampling the age of the grafts was about 25 years. The plus trees, i.e. the ortets of the clones, had been selected from a few cultivated stands in northern Finland. Eleven of the still standing plus trees were sampled as reference material. Their age, calculated at breast height, varied from 56 to 88 years. The origin of the plus trees is the Raivola stand, which is a famous plantation established between the years 1738–1821 (Fig. 1).

Wood samples were taken from the standing seed orchard grafts during October-November 1997 using an increment borer (diam. 5 mm). Five good quality grafts in each clone were bored at 1.3 m height from four different directions of the stem: south, west, north and east. The sampling design gave 265 grafts and 1060 increment cores. The core samples were stored in sealed plastic tubes at a temperature of − 22 °C.

The plus trees were bored in May 1998. Increment cores were taken from four directions at 0.65 m and 1.3 m heights on each tree, giving eight cores per tree and a total of 88 cores. This material was investigated in a separate decay test series from the graft material.

The bored grafts were felled in April 1998 when the seed orchard was thinned. Cross-sectional samples were taken from 15 clones (75 grafts). These disks were used to determine the basic density of the wood using the water displacement method described by Olesen (1971).

The increment core samples were first analysed under a microscope connected to a data-recording ruler. The width of the earlywood and latewood in each annual growth ring was measured. The transition point between the sapwood and heartwood was determined on the basis of the colour difference, thus making it possible to calculate the number of sapwood and heartwood rings. A 40 mm section of the outermost heartwood of each increment core was then removed for the decay test (Fig. 2). Two 40 mm sections were taken from the increment cores of the plus trees, one from the inner and one from the outer part of the heartwood.

Decay test in the laboratory

The core samples were dried at 60 °C for 48 hours, cooled in desiccator for 3–6 hours and weighed to an accuracy of 1 mg. After weighing the samples were packed into paper bags and sterilised at VTT Chemical Technology using a radiation dose of between 25 kGy and 50 kGy of radioisotope ^{60}Co source.

Decay resistance was studied using a malt agar medium decay test, which had been developed previously at VTT Building Technology on the basis of the standardised EN 113 test (European standard 1996; Viitanen *et al.* 1997). The wood samples were not leached prior to the test. The organism used in the test was a pure culture of the brown rot fungus *Coniophora puteana* (Schum. ex Fr.) Karst. (strain BAM Ebw. 15). The cylindrical wood samples were exposed to the fungal hyphae growing on agar in petri dishes. The samples were placed on a glass rack so that they were not in contact with the agar (Fig. 2). The incubation time was 6 weeks, after which the samples were dried and reweighed.

Data analysis

The calculations and statistical analyses of the variables were performed using the graft-wise means as observations. The graft-wise means were first obtained as the mean of the four increment cores

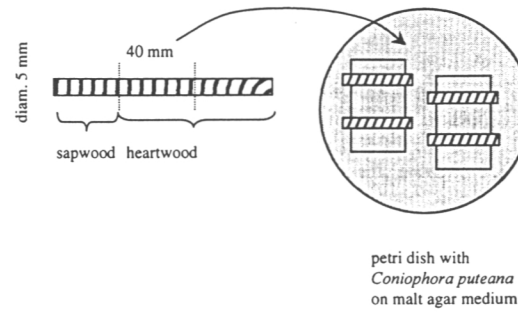

Fig. 2. The wood sample removed from the increment core and its exposure to the decay test. The four samples in each petri dish come from different increment cores.

taken from each stem. This smoothed out the possible intra-tree variation which was not a point of interest in this study. Furthermore, this meant that the use of complicated statistical models with repeated observations could be avoided. In cases where the clone-wise means were needed they were obtained as the mean of the five grafts. The values for the plus trees were calculated as the mean of all the 8 increment cores or the 16 samples per tree, depending on the variable.

For the even-aged grafts the mean width of the annual ring, i.e. the mean annual increment, was calculated as the mean of the whole radius from the cambium to the pith. For the uneven-aged plus trees the mean annual increment was calculated for the 25-year-period, 1947–1972. Latewood percentage was calculated for the same years as the annual increment. Heartwood percentage was calculated as the ratio between the cross-sectional areas of sapwood and heartwood, although this may cause, according to Fries and Ericsson (1998), an overestimation of the genetic parameters. The wood density was obtained using the dry weight of the sample and its calculated fresh volume (a cylinder with a diameter of 5 mm and length of 40 mm).

The resistance of the wood samples against the test fungus was expressed as the weight loss (mg), calculated as the difference between the dry weight before and after the decay exposure. This absolute weight loss can also be related to the sample volume (mg cm^{-3}). The relative weight loss (%) was calculated as the ratio between the difference and the dry weight before the decay exposure. However, the relative weight loss is seldom useful in statistical analyses because it is partially dependent on the wood density. After the decay test there was a total of 1028 core-wise weight loss measurements available. Because the attack by the fungus did not start normally in some of the petri dishes, the distribution of the measurements was skewed to the left. The skewness was corrected by rejecting the measurements less than 50 mg cm^{-3} (69 in number) before calculating the graft-wise means.

The degree of genetic determination of a quantitative trait such as 'the weight loss in the decay test' can be estimated since the trees included in the experiment have been propagated vegetatively and thus form clones. The phenotypic variance observed (V_P) can be partitioned into genotypic variance (V_G) and environmental variance (V_E), $V_P = V_G + V_E$. The grafts within a clone have an equal genetic constitution, and thus the within-clone component of the variance is due to the environment or to the error in sampling and measuring. The between-clone component of the variance is an estimate of the total genotypic variance V_G. This total genotypic variance consists of the additive component V_A and the non-additive component V_{NA} (van Buijtenen 1992). However, these variance components cannot be separated by using clonal testing material. The V_G:V_P ratio expresses the degree of genetic determination of the trait. In genetics, this ratio of the variance components, is called 'heritability in the broad sense' and more specifically, when estimated using clones, 'clonal repeatability' (Falconer 1981; Hill *et al.* 1998). This is equivalent to the intraclass correlation in statistical terms.

The statistical analysis was carried out according to the model equation $y_{ij} = \mu + a_i + e_{ij}$, where y_{ij} is an observation made on a graft and μ is a fixed general mean. The term a_i is the random effect of a clone i, and e_{ij} is the residual random effect where j varies from 1 to 5 as the number of graft in the clone i. Estimation of the variance components was performed using the VARCOMP procedure, and calculation of the correlation coefficients using the CORR procedure of SAS/STAT® statistical software (SAS Institute Inc., Cary, NC, 1989).

Results

Analysis of the variance components showed that the clonal repeatability for the absolute weight loss (mg cm^{-3}) during the decay exposure was 0.39. This figure estimates the degree of genetic determination of wood decay resistance. The estimates of clonal repeatability for all the studied characteristics are listed in Table 1. The genetic control over decay resistance appeared to be stronger than that over the growth characteristics (0.22–0.26), but weaker than that over the wood density (0.57). The range of absolute weight loss was 133–195 mg cm^{-3}, with a mean of 160 mg cm^{-3}. The coefficient of variation was 10 %. On the average, the relative weight loss was 39 %.

The absolute weight loss did not correlate with the rate in growth of graft diameter (Table 2). The connection between the weight loss and wood density was more complicated: the slight positive correlation between the absolute weight loss and wood density indicated that the decay activity of the fungus was higher in heavy wood than in light wood. The negative correlation between the relative weight loss and wood density showed, however, that increasing wood density provided greater durability against the decay i.e. the remaining wood mass after the decay exposure was higher. The coefficient between the heartwood percentage of the cross-sectional area and weight loss was negative, indicating less decay in trees with more heartwood, even though the decay samples consisted totally of heartwood (Fig. 2).

Moreover, the correlation analyses showed that the annual ring width was negatively correlated with latewood percentage and wood density and, remarkably, positively with heartwood percentage. The positive correlation

Table 1. Variation in the wood characteristics of 53 Siberian larch clones. Weight loss refers to the 6 weeks' treatment with brown-rot fungus *Coniophora puteana*. The characteristics are ranked according to the degree of genetic determination, as estimated by the clonal repeatability

Characteristic	mean	SE	range	clonal repeatability	
				estimate	SE
wood density, mg cm^{-3}	419	4	353–485	0.57	0.062
latewood, %	24	0.4	20–33	0.51	0.065
weight loss, %	39	0.5	30–46	0.41	0.068
weight loss, mg cm^{-3}	160	2	133–195	0.39	0.069
heartwood, %	53	1	39 – 69	0.30	0.076
width of annual ring, mm	4.7	0.1	3.4–6.0	0.26	0.066
diameter of graft, cm	20	0.3	15–25	0.26	0.066
height of graft, m	12	1	10–14	0.22	0.064
distance to living crown, m	1.7	0.5	1.0–2.5	0.18	0.062

Table 2. The phenotypic Pearson correlation coefficients and their p-values (in italics) between the wood characteristics of 53 Siberian larch clones

	diameter	height	distance to living crown	wood density	weight loss mg cm^{-3}	weight loss %	width of annual ring	latewood %
height	0.663							
	0.000							
distance to living crown	0.153	0.101						
	0.274	*0.471*						
wood density	− 0.282	− 0.067	− 0.176					
	0.041	*0.632*	*0.209*					
weight loss, mg cm^{-3}	0.007	− 0.043	− 0.032	0.258				
	0.959	*0.761*	*0.821*	*0.062*				
weight loss, %	0.201	0.005	0.095	− 0.382	0.790			
	0.150	*0.974*	*0.500*	*0.005*	*0.000*			
width of annual ring	0.854	0.560	0.169	− 0.327	− 0.090	0.131		
	0.000	*0.000*	*0.227*	*0.017*	*0.524*	*0.348*		
latewood, %	− 0.361	− 0.132	− 0.190	0.629	0.193	− 0.222	− 0.355	
	0.008	*0.347*	*0.173*	*0.000*	*0.166*	*0.111*	*0.009*	
heartwood, %	0.439	0.438	0.323	0.067	− 0.250	− 0.288	0.393	− 0.152
	0.001	*0.001*	*0.020*	*0.640*	*0.074*	*0.038*	*0.004*	*0.281*

Table 3. Comparison of wood characteristics between 11 mature plus tree ortets growing in northern Finnish stands and their grafted clones growing in a central Finnish seed orchard

	mean of ortets	mean of grafts	grafts/ortet ratio	correlation coefficient	correlation p-value
wood density, mg cm-3	504	410	0.81	0.73	0.01
latewood, %	35	25	0.71	0.36	0.28
weight loss, %	25	39	1.56	0.52	0.10
weight loss, mg cm^{-3}	125	161	1.29	0.27	0.43
heartwood, %	78	53	0.68	0.48	0.16
width of annual ring, mm	2.3	4.7	2.04	− 0.23	0.49

between latewood percentage and wood density was strong. The heartwood percentage had a slight positive correlation with the height to the lowest living branch (Table 2).

The wood density figures presented in Tables 1, 2 and 3 have been measured from the increment core samples. The verifying measurements made on the cross-section disks were similar, the correlation on the clonal level being 0.90 ($p < 0.0001$, $n = 16$).

Comparison of the 11 old plus trees and their young grafts showed that the graft wood had a greater absolute weight loss. The correlation coefficient between the plus trees and their grafts was slightly positive but not statistically significant. The correlation for the wood density was significant ($r = 0.726$, $p = 0.011$) although the diameter growth rate of the grafts was doubled (Table 3). The density of the grafts was lower, as also the latewood and heartwood percentages. For the heartwood percentage the correlation was positive and almost significant. The coefficient for the latewood percentage was also slightly positive but not significant.

Discussion

The results of this study showed that the durability of dried Siberian larch wood against degradation by the brown rot fungus, *C. puteana*, was to a moderate extent under genetic control. The degree of genetic determination for decay resistance was stronger than that for heartwood formation or the growth characteristics, but weaker than that for wood density or latewood formation.

Our conclusion was based on the clonal repeatability, i.e. the between-clone component of the variance compared to the total phenotypic variance observed in the laboratory test. The vegetatively propagated grafts within each clone resemble each other mainly because they have the same genetic constitution. A minor reason for the resemblance could be that the grafts reflect, for example, the physiological condition of the plus tree. In any case, the clonal repeatability, as the heritability in the broad sense always does, exaggerates the genetic determination of the characteristic compared to the estimates of the 'heritability in the narrow sense'. The latter is the parameter calculated on the basis of sexually reproduced groups of related individuals (e.g. half-sib or full-sib families). The reason for the difference is that the heritability in the broad sense includes, in addition to the additive component of genetic variation, also the non-additive component. Thus, the use of the broad sense heritability is appropriate, particularly when the gain obtained by vegetative propagation is predicted (van Buijtenen 1992).

Although there are a large number of reports on the proportion of genetic variation for a range of wood character-

istics, few of them deal with the genus of larch or decay resistance (Zobel and Jett 1995 and references therein). Most of the reports deal with wood density, and they show that the heritability of wood density is moderate to strong in almost all tree species. The results of our study were in agreement with this. The highest estimate of clonal repeatability was found for wood density. Moreover, the high correlation in wood density between the plus trees and their grafts, growing in totally different environments, indicated strong genetic control over the wood density of Siberian larch.

The estimates of the strength of genetic control, such as heritability or clonal repeatability, are valid only in the context of the studied material, in our case the clones in the seed orchard no. 309. Thus generalising any single heritability estimate to other populations must be made with care. In our case especially, the genetic structure of the studied population is probably far from the natural one owing to the selection and introduction to new environments during several successive generations.

Our results were in agreement with the earlier results reported by Viitanen *et al.* (1997) concerning the decay resistance of different larch species. Viitanen *et al.* (1997) obtained their results using the EN 113 standardised testing method, which includes 16 weeks' incubation of $15 \times 25 \times 50$ mm samples with three different fungus species (European standard 1996). In their test *C. puteana* and *Poria placenta* (Fries) Cooke sensu J. Eriksson appeared to be aggressive to degrade Siberian larch wood. On the average, the weight loss for 25-year-old Siberian larches was 150 mg cm^{-3} (35 % on a weight loss/weight basis), and 136 mg cm^{-3} (22 %) for 70-year-old trees.

In the present study, the absolute weight loss did not correlate with any other wood or stem characteristic in a way suggesting that indirect selection for decay resistance could be possible. Our finding that the proportion of heartwood was high when the growth of the graft has been rapid, is in accordance with the conclusion of Hakkila and Winter (1973): "heartwood seemed to be more abundant the faster the tree grew". The recent findings of Björklund (1999) with Scots pine suggest that the heartwood percentage has a slight positive correlation with the growth rate of early annual rings.

The statistically non-significant correlation between the grafts and the plus trees in decay resistance suggests that the transfer to a totally different kind of environment may interact with the genetic determination of this characteristic. Another reason could be the large difference in the cambial age and the biological age of the tissues compared. Before decay resistance can be used as a selection criterion in a vegetative propagation programme or in a breeding programme of Siberian larch, the components of the genotype x environment interaction and the additive genetic variation should be studied more closely with another type of field trial material.

The test fungus *C. puteana* is a typical brown rot fungus that causes decay in wooden material at wet points in buildings. It is not very sensitive to individual wood species, but is normally more common in softwoods than in hardwoods (Käärik 1981). The strain BAM Ebw. 15 was originally isolated in Germany (Bundesanstalt für Materialforschung und -prüfung, Berlin) and has been used successfully in laboratory tests for many years. According to VanAcker *et al.* (1999), the number of test fungi in the EN 113 test can be limited to *C. puteana* for all wood species and the durability classification can be derived directly from the percentage mass loss using the criteria mentioned in the standard EN 350-1. However, some variation has been found in the results between different test series, as well as between different strains of the fungus (Wazny and Greaves 1984). There may be several reasons for this variation. In the present study, attack by the fungus did not start normally in some of the petri dishes, leading to the rejection of a small part of the data.

The laboratory method applied in this study performed satisfactorily as a screening test. The main advantages compared to the standardised EN 113 method are the savings in time, from 16 to only 6 weeks, and the benefits offered by the use of increment core samples taken from standing trees. Furthermore, a small piece of wood saves space in the test facilities and thus large numbers of samples can be screened at reasonable costs. Especially when genetic parameters are estimated or breeding materials tested, large numbers of individuals need to be screened. On the other hand, the use of small pieces of wood as samples is associated with the large intra-tree variation. The weight loss figures obtained with core samples and cube samples are not directly comparable due to the different shape and size. However, they should give a similar ranking for the materials tested.

Acknowledgements

This study is a part of the consortium study "Genetic variation of decay resistance in Scots pine and Siberian larch wood" financed by the Academy of Finland. Esko Oksa, Pentti Manninen and Reijo Rauniomaa of the Finnish Forest Research Institute, and Hannu Partti and Tapani Relander of the Forest and Park Service, collected the increment core samples. Outi Kylliäinen and Markku Tiainen at the Finnish Forest Research Institute analyzed the samples in the laboratory. Liisa Seppänen at VTT Building Technology performed the decay tests. The authors are grateful to the above mentioned and to all participants for their contribution.

References

Björklund, L. 1999. Identifying heartwood-rich stands or stems of *Pinus sylvestris* by using inventory data. Silva Fennica *33* (2), 119–129.

Björkman, E. 1944. Om röthärdigheten hos lärkvirke. Norrlands Skogsvårdsförbunds Tidskrift 1944 (1), 18–45.

Ericsson, T. and A. Fries. 1999. High heritability for heartwood in north Swedish Scots pine. Theor. Appl. Genet. *98*, 732–735.

European standard. EN 113. 1996. Wood preservatives – Test method for determining the protective effectiveness against wood destroying basidiomycetes – Determination of the toxic values. European Committee for Standardization. Brussels. 31 p.

European standard. EN 350-1. 1994. Durability of wood and wood-based products - Natural durability of solid wood - Part 1: Guide to the principles of testing and classification of the natural durability of wood. European Committee for Standardization. Brussels. 19 p.

European standard. EN 350-2. 1994. Durability of wood and wood-based products - Natural durability of solid wood - Part 2: Guide to Natural durability and treatability of selected wood species of importance in Europe. European Committee for Standardization. Brussels. 37 p.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. 2 ed. Longman Group Ltd, New York.

Fries, A. and T. Ericsson. 1998. Genetic parameters in diallel-crossed Scots pine favor heartwood formation breeding objectives. Can. J. For. Res. *28*, 937–941.

Hakkila, P. and A. Winter. 1973. On the properties of larch wood in Finland. Commun. Inst. For. Fenn. *79* (7), 45.

Hart, J.H. and D.M. Shrimpton. 1979. Role of stilbenes in resistance of wood to decay. Phytopathology *69*, 1138–1143.

Hill, J., H.C. Becker and P.M.A. Tigerstedt. 1998. Quantitative and Ecological Aspects of Plant Breeding. Chapman and Hall, London.

Kellomäki, S. 1981. Mäntysahapuun laadun ja sydänpuuosuuden yhteys tukin ulkoisiin tunnuksiin. Summary: Quality of pine logs and proportion of heartwood as related to properties of the logs. Folia Forestalia *489*, 1–13.

Kiellander, C.L. 1966. Om lärkträdens egenskaper och användning med särskild hänsyn till europeisk och japansk lärk. Summary: On the properties and use of larch with special reference to European and Japanese larch. Föreningen Skogsträdsförädling. Årsbok 1965. pp. 65–106.

Käärik, A. 1981. *Coniophora puteana* (Schum. ex. Fr.) Karst. *In*: Some wood-destroying Basidiomycetes. Ed. Crockroft. Internat. Res. Group on Wood Pres.. *1*, 11–25. Stockholm.

Lappi-Seppälä, M. 1927. Tutkimuksia siperialaisen lehtikuusen kasvusta Suomessa. Referat: Untersuchungen über den Zuwachs der sibirischen Lärche in Finnland. Metsätieteellisen koelaitoksen julkaisuja. Commun. Inst. For. Fen. *12*, 1–72.

Löyttyniemi, K. 1986. Männyn sydänpuu – luonnon kestopuuta. Männyn sydänpuun luontaisen lahon- ja hyönteistuhonkestävyyden hyväksikäytöstä. Summary: On natural durability of pine heartwood. Metsäntutkimuslaitoksen tiedonantoja *231*, 1–50.

Olesen, P.O. 1971. The water displacement method: A fast and accurate method of determining the green volume of wood samples. Arboretet Hørsholm. Forest Tree Improvement *3*, 3-23.

Rennerfelt, E. 1943. Die Toxizität der phenolischen Inhaltsstoffe des Kiefernkernholzes gegenüber einigen Fäulnispilzen. Svensk Bot. Tidskr. *37*, 83–93.

Ueckermann, E. and D. Lülfing. 1974. Zur Dauerhaftigkeit von Zaunpfählen der Baumarten Kiefer *(Pinus silvestris)*, Lärche *(Larix europaea)* und Douglasie *(Pseudotsuga douglasii)* für Gehege- und Kulturzaunbau. Summary: A study of the durability of posts made from pine (*Pinus silvestris*), larch (*Larix europaen*) and Douglas fir (*Pseudotsuga douglasii*) used in the fencing of enclosures and tree-cultivation plots. Zeitschrift für Jagdwissenschaft *20* (3), 150–156.

van Buijtenen, J. P. 1992. Fundamental Genetic Principles. *In*: Handbook of Quantitative Forest Genetics. Eds. L. Fins *et al.* Kluwer Academic Publishers. Netherlands. pp. 29–68.

VanAcker, J., J. Carey, R. Sierra-Alvarez, I. LeBayon, M. Grinda, H. Militz and R-D. Peek. 1999. Laboratory testing of natural durability including Basidiomycetes and soil inhabiting microorganisms. The final conference of Cost action 2: Wood Durability. WG 1. Natural protection mechanisms. St Gallen, January 25–27, 1999.

Viitanen, H., L. Paajanen, P. Saranpää and P. Viitaniemi. 1997. Durability of larch (*Larix* spp.). wood against brown-rot fungi. Internat. Res. Group on Wood Pres.. IRG Doc. No. IRG/WP 97–10228. Stockholm.

Wazny, J. and H. Greaves. 1984. A comparison of fungal strains used in the bioassay of wood preservatives. Internat. Res. Group on Wood Pres.. Doc. No. 2220. Stockholm.

Zabel, R.A. and J.J. Morrell. 1992. Wood microbiology. Decay and its prevention. Academic Press, Inc, California.

Zobel, B.J. and J.B. Jett. 1995. Genetics of wood production. Springer-Verlag. Berlin Heidelberg.

Received December 2nd 1999

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II

Genetic variation in the decay resistance of Scots pine wood against brown rot fungus

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Abstract: The role of genotype in the durability of Scots pine (*Pinus sylvestris* L.) wood against decay by brown rot fungus (*Coniophora puteana* (Schum. ex Fr.) Karst. (strain Bam EBW 15)) was studied in a laboratory test. The wood material was obtained from 32-year-old half-sib progenies of Scots pine. The increment core samples of sapwood and juvenile heartwood were decayed using a modification of the standardized EN 113 method. The mean densities of the sapwood and heartwood samples were 391 and 337 mg·cm^{-3}, respectively, and the mean mass losses were 114 and 80 mg·cm^{-3}, respectively. The additive genetic components were small compared with the total phenotypic variance, which resulted in small narrow-sense heritabilities in mass loss. The most marked feature was the wide phenotypic variation in mass loss observed in heartwood (range 199 mg·cm^{-3}) compared with sapwood (range 72 mg·cm^{-3}) samples. Low heritability, together with the relatively high coefficient of additive genetic variation (CV_A) in heartwood mass loss, suggests that advances in breeding can only be made through intensive testing in the environments which the studied experiment represents.

Résumé : Le rôle du génotype dans la résistance du bois du pin sylvestre (*Pinus sylvestris* L.) à la carie causée par le champignon de carie brune (*Coniophora puteana* (Schum. ex Fr.) Karst. (race Bam EBW 15)) a été étudié en laboratoire. Le matériel ligneux provenait de descendants uniparentaux de pin sylvestre âgés de 32 ans. Des carottes de bois d'aubier et de bois de cœur juvénile ont été soumises à un test de décomposition en utilisant une modification de la méthode standard EN 113. La densité moyenne des échantillons de bois d'aubier et de bois de cœur était respectivement de 391 et 337 mg·cm^{-3}. En moyenne, la perte de poids des échantillons de bois d'aubier était de 114 mg·cm^{-3} et celle des échantillons de bois de cœur de 80 mg·cm^{-3}. Les composantes génétiques additives étaient faibles comparativement à la variance phénotypique totale, ce qui correspond à de faibles héritabilités au sens strict pour la perte de poids. La caractéristique la plus marquante est la grande variation phénotypique dans la perte de poids observée dans les échantillons de bois de cœur (écart de 199 mg·cm^{-3}) comparativement aux échantillons de bois d'aubier (écart de 72 mg·cm^{-3}). La faible héritabilité, accompagnée d'un coefficient de variation génétique additive (CV_A) relativement élevé pour la perte de poids du bois de cœur, indiquent que seuls des tests intensifs dans les milieux représentatifs de l'étude permettraient d'obtenir des résultats significatifs grâce à l'amélioration génétique.

[Traduit par la Rédaction]

Introduction

Durability against decay is needed when wood is used in structures exposed to moisture and fungal attack. Such structures should be protected by using construction techniques that ensure that the wood remains as dry as possible. In situations with an increased risk of decay, chemical treatment and impregnating the wood with preservatives should be used. However, synthetic wood preservatives contain chemicals that may have harmful effects on the environment and human health during their utilization. Thus, it would be both an environmentally and ecologically sound option to utilize wood which is naturally durable against decay. In Finland, the heartwood of old-growth Scots pine (*Pinus sylvestris* L.) has been a traditional material in structures exposed to a risk of decay (e.g., Löyttyniemi 1986).

Two explanations have been proposed for the traditional experience that heartwood is naturally more durable against fungal attack than sapwood. Firstly, the concentration of extractives in heartwood is higher than that in sapwood. These extractives have a toxic effect on fungal growth in bioassays (e.g., Zabel and Morrell 1992, p. 408; Bruce 1998, p. 255 and references therein). Secondly, structural and chemical properties of heartwood result in a lower permeability to water (Panshin and de Zeeuw 1980, p. 352). When sapwood is converted into heartwood, pit aspiration in gymnosperms increases (e.g., Dadswell 1958; Kramer and Kozlowski 1979, pp. 602, 605–606), tylosoids fill the resin canals, and resin accumulates into the cells. All these factors prevent water movement in the tissues. Resin acids are suggested to provide resistance against decomposition as a result of their hydrophobic properties rather than their general toxicity (Eberhardt

Received July 6, 2000. Accepted February 25, 2001. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on June 26, 2001.

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 DOI: 10.1139/cjfr-31-7-1244

et al. 1994). The relative importance of these defence mechanisms may differ among tree species.

To increase the production and quality of Scots pine heartwood for use as a construction material, it is essential to know the variation in the rate of formation and properties of heartwood between different stands and between the trees within the same stand. Not only the range of variation but also the reasons for the variation are important. Such variation, which is mainly caused by the growing environment, could be controlled by silvicultural measures such as regulating the growing density. If the variation is primarily of genetic origin, this could be utilized in tree breeding.

Several studies have reported large variation in heartwood percentage among individual Scots pine stems and among stands (Tamminen 1962; Uusvaara 1974; Kärkkäinen 1972, 1976; Kellomäki 1981; Björklund and Walfridson 1993; Björklund 1999). Apart from the correlation with tree size, which reflects tree age and the growing environment, the correlations between the amount of heartwood and other stand and tree variables have been poor.

Studies on *Pinus radiata* D. Don (Hillis and Ditchburne 1974; Wilkes 1991) and *Pinus canariensis* Chr. Sm. ex DC (Climent et al. 1993) report that the amount of heartwood formed is strongly dependent on the rate of diameter growth in the part of the stem that is becoming heartwood. In absolute terms, more heartwood is produced in the parts where the annual rings are wider. Björklund (1999) has reported the same positive correlation, although weak, for Scots pine. He also concluded that the rate of heartwood formation (at a specific stem height), expressed as the number of new heartwood rings per year, increases with increasing age of the cambium.

Relatively few studies have been carried out on the genetics of Scots pine heartwood traits. The narrow-sense heritability for heartwood diameter in 25- and 44-year-old Scots pine, full-sib progeny tests was 0.30 and 0.54, respectively (Fries and Ericsson 1998; Ericsson and Fries 1999). The coefficient of additive genetic variation was about 0.20 in both tests. These values suggest that it would be possible to breed for or against heartwood formation.

The number of studies carried out on the genetic parameters associated with the resistance of the wood of any tree species against fungi is low. Two studies report broad-sense heritabilities estimated using clonal material. The studies of Schmidtling and Amburgey (1982) on *Pinus taeda* L., and Venäläinen et al. (2001) on *Larix sibirica* Ledeb. showed moderate broad-sense heritabilities (0.22 and 0.39, respectively) in durability against brown rot decay fungus in a laboratory test. Similar studies on Scots pine are lacking.

The aim of the present study was to test in vitro the durability of Scots pine wood against a common brown rot fungus, *Coniophora puteana* (Schum. ex Fr.) Karst. We studied whether the decay activity of one strain of a brown rot fungus causes measurable genetic variation in mass loss in the sapwood and heartwood of Scots pine. Wood samples were collected from relatively young trees. The heartwood samples represented juvenile wood, while the sapwood samples originated from mature wood, which will eventually be the most durable outer part of the heartwood. Although it is generally known that sapwood is not resistant against decay, we expected to find genetic variation in the decay resistance of sapwood. Early selection of heartwood durability would be possible if there was genetic variation in the durability of sapwood. High genetic correlation between heartwood and sapwood durability, or any easily measurable growth or wood trait with high heritability, would enable indirect selection.

Materials and methods

Sampling and preliminary analysis of the increment cores

The Scots pine wood samples used in our study were 5 mm diameter increment cores obtained from a progeny trial consisting of 38 open-pollinated half-sib families. The mother trees of the families were plus trees originating from several stands in southern Finland. The trial (no. 307/1 in the Forest Genetic Register of the Finnish Forest Research Institute) is located in eastern Finland (Kerimäki, Mäkrä, 61°50′N, 29°23′E, 90 m a.s.l.) and is owned by the Finnish Forest Research Institute. The trial was planted in 1968 using 2-year-old bare-root seedlings. The original trial design was six randomized complete blocks with square plots of 16 seedlings at a spacing of 2 × 2 m. The trial was thinned in winter 1992–1993 to a density of eight trees per plot. The site is relatively fertile with respect to the nutrient requirements of Scots pine. All the boles in the progeny test were not straight, most probably because of the planting method and site effects. Our sample included both straight and somewhat curved trees.

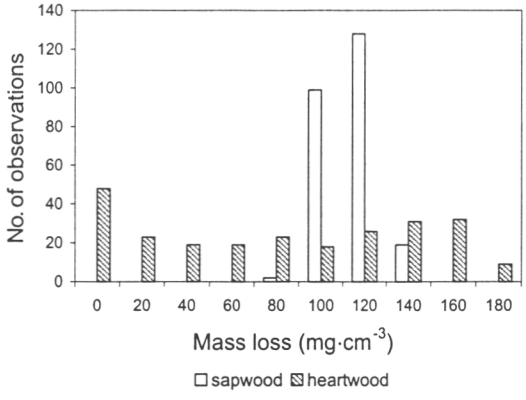
Owing to the limitations set by the laboratory facilities, only 25 of the 38 families from 5 of the 6 blocks were sampled for our study. To ensure the presence of heartwood, two trees that were among the thickest on each plot were tagged on the five blocks. These 10 trees per family represent trees that will be present in the final harvesting of the stand about 50 years from now. Tree height, diameter at breast height over bark, and the distance to the living crown were measured before the increment core sampling. The straightness of the stems was also assessed visually. The increment core samples were bored during a 1-week period at the beginning of May 1998, about 4 weeks before the initiation of annual height growth. The increment cores were taken from four directions (north, east, south, and west) approximately at breast height (1.3 m). The cores were bored through the pith for a distance of a few centimetres on the opposite side. Thus 25 families, 2 trees per plot, 5 blocks, and 4 samples per tree gave a total of 1000 increment cores. The samples were placed in sealed plastic tubes and stored at a temperature of –22°C.

The increment core samples were examined under a microscope connected to a data-recording measuring device. The width of the earlywood and the latewood in each annual growth ring was measured. The transition point between the sapwood and heartwood was recorded according to visually observed moisture and colour differences. It was thus possible to count the number of sapwood and heartwood rings.

Laboratory experiment

A 35-mm section was cut from the outermost part of the sapwood in each of the 1000 increment cores. A 35-mm heartwood section, centred on the pith, was cut from one core per tree (the northern or the southern core) making 250 heartwood samples. Because the study was primarily concerned with the possibilities of utilizing early selection, the number of sapwood samples was greater than that of heartwood. Some sapwood sections were only 30 mm long, because pure sapwood samples were also required from slender stems with a narrow sapwood area. The sections were dried at 60°C for 48 h, cooled in a desiccator, and weighed to an accuracy of 1 mg. The dry–fresh wood density was obtained on the basis of the dry mass of the sample and its calculated fresh volume (a cylinder with a diameter of 5 mm and length 35 or 30 mm). The

Fig. 1. The distribution of sapwood and heartwood mass loss in two hundred and forty-nine 32-year-old Scots pines. The samples were incubated with a pure culture of the brown rot fungus *Coniophora puteana* for 6 weeks.



weighed samples were packed in paper bags and sterilized at VTT Chemical Technology using a radiation dose of between 25 kGy and 50 kGy of radioisotope ^{60}Co source.

The decay resistance was studied at VTT Building and Transport using a malt agar plate decay test, which is a modification of the standardized EN 113 decay test described by Viitanen et al. (1998) and Venäläinen et al. (2001). The increment core sections, from which the wood extractives had not been removed, were placed on a pure culture of a brown rot fungus, *C. puteana*. The incubation time was 6 weeks, after which the samples were dried at 60°C for 48 h and reweighed.

Statistical analysis

For the sapwood traits, the mean of the four increment cores per tree was used in the statistical analysis to even out the possible effects of the sampling direction. The number of heartwood annual rings, the heartwood radius, and latewood percentage were measured from two directions (north, south), and their average was used in the statistical analysis. Heartwood density and mass loss was obtained from only one increment core, which included both the southern and northern sides of the heartwood.

We constructed a model and estimated the heritabilities on an individual tree basis, assuming that all random effects were pairwise independent in the studied traits. Because of the spatial autocorrelation introduced by the plot design, however, this assumption may overestimate the additive genetic component of the variance. This violation of the independence rule is not regarded as serious in the case of wood quality traits. The distribution of the observations appeared normal or close to normal for all the traits except for the heartwood mass loss (Fig. 1). To evaluate the possibilities of selecting a breeding population producing durable heartwood, the phenotypic variance of the wood durability has to be partitioned into environmental and genetic components. On the individual tree level, a measured phenotypic value (P) of a trait is assumed to be the sum of the additive genetic effect (A) and of the independent environmental effect (E). The environmental effect also includes the remaining genetic effects that are independent of A. Thus, $P = A + E$. Furthermore, the phenotypic variance is assumed to be composed of genetic and environmental components, $\sigma_P^2 = \sigma_A^2 + \sigma_E^2$. The narrow-sense heritability is calculated as $h^2 = \sigma_A^2/\sigma_P^2$.

Variance components were estimated with the REML technique utilizing the MIXED procedure of the SAS system (SAS Institute Inc. 1992). The random model equation was

$$[1] \quad y_{bfi} = \mu + a_b + b_f + c_{bf} + e_{bfi}$$

where y_{bfi} is an observation for a tree; μ is a fixed general mean; a_b and b_f are the random effects of the block b and the family f, which vary from 1 to 5 and from 1 to 25, respectively; c_{bf} is the random block × family interaction effect; and e_{bfi} is the residual random effect of tree i in plot bf. All the effects are assumed to be independent. The random effects were assumed to be normally distributed with the expectation zero and the variance σ_b^2, σ_f^2, σ_{bf}^2, and σ_{bfi}^2 [N(0, σ_b^2), etc.]. The individual-tree heritability for half-sib progenies was estimated as

$$[2] \quad \hat{h}^2 = \frac{4\hat{\sigma}_f^2}{\hat{\sigma}_b^2 + \hat{\sigma}_f^2 + \hat{\sigma}_{bf}^2 + \hat{\sigma}_e^2}$$

This estimate of the heritability is used when no corrections for block effects are applied prior to selection (Cotterill 1987), and when the purpose is to describe the effects of the sources of variation in a particular experiment.

The MIXED procedure of the SAS system also provided approximate standard deviations for the estimated variance components. The standard deviation for the heritability estimate was calculated using "Dickerson's approximation" (e.g., Dieters et al. 1995), and for the additive genetic component $\hat{\sigma}_{\hat{\sigma}_A^2} = 4\hat{\sigma}_{\hat{\sigma}_f^2}$. The coefficient of additive genetic variation (CV_A) was calculated by dividing $\hat{\sigma}_A$ by the overall mean value of the trait.

Results

There was minor genetic variation in the durability of Scots pine sapwood against a single strain of the brown rot fungus *C. puteana*. The heritability for the sapwood mass loss and CV_A were among the lowest in the range of traits studied (Table 1). The heritability estimates for the heartwood mass loss were of the same low order of magnitude as for the sapwood. However, CV_A for the heartwood mass loss was high, 10.6% (Table 1).

The mean mass loss differed considerably between the sapwood and heartwood, the heartwood losing less mass than the sapwood (Table 1). The difference between the average mass losses was 33.6 mg·cm^{-3} during the 6-week decay test. The range for the heartwood mass loss was 198.8 mg·cm^{-3} compared with 72.4 mg·cm^{-3} for sapwood (Fig. 1). There was unimportant phenotypic correlation between the sapwood and heartwood mass loss at both the individual tree (r = –0.062) and the family mean (r = 0.047, SD = 0.507) level. Because of the low number of families in the study and low heritability of the sapwood and heartwood mass loss, it was not reasonable to estimate the genetic correlation between these parameters. At least in this progeny test, indirect selection for the heartwood mass loss on the basis of sapwood mass loss would not be effective.

A number of wood and growth traits, including the distance to the crown limit, had heritabilities ranging from moderate to high. However, the stem diameter at breast height, sapwood width, and the heartwood radius, had low heritabilities (Table 1). Among the wood traits, the number of annual rings in sapwood and heartwood had heritabilities of 0.47 and 0.39, respectively, and the number of heartwood annual rings also had a high CV_A (Table 1). The density of the heartwood reached the highest heritability among all traits ($\hat{h}^2$ = 0.57).

Table 1. Estimates of genetic parameters of wood and growth traits.

Trait	*N*	Mean	$\hat{\sigma}^2_P$	$\hat{\sigma}^{2\,a}_A$	$\hat{h}^{2\,b}$	CV_A, %
Sapwood						
Mass loss, mg·cm^{-3}	248	113.6	144.79	4.74±16.00 (0.77)	0.03±0.11	1.9
Wood density, mg·cm^{-3}	248	391.0	607.37	204.78±140.78 (0.14)	0.34 ±0.23	3.7
No. of annual rings	238	17.4	1.79	0.85±0.49 (0.08)	0.47±0.27	5.3
Width, cm	245	5.3	0.61	0.03±0.10 (0.75)	0.05±0.16	3.3
Latewood, %[c]	246	39.2	22.26	12.61±4.43 (0.19)	0.26±0.20	6.1
Heartwood						
Mass loss, mg·cm^{-3}	248	80.0	3677.03	71.87±534.36 (0.89)	0.02±0.14	10.6
Wood density, mg·cm^{-3}	248	337.1	1014.98	580.90±286.29 (0.04)	0.57±0.28	7.1
No. of annual rings	241	6.4	1.70	0.66±0.43 (0.13)	0.39±0.25	12.6
Radius, cm	242	3.0	0.61	0.04±0.10 (0.72)	0.06±0.16	6.2
Latewood, %	242	13.2	6.10	2.73±1.47 (0.06)	0.45±0.24	5.4
Height, m	249	15.3	1.58	0.38±0.33 (0.25)	0.24±0.21	4.0
Crown limit, m	249	7.6	1.54	0.58±0.37 (0.11)	0.38±0.24	10.1
Diameter (1.3 m), cm	249	18.5	4.54	0.00±0.00 (—)	0.00±0.00	0.0

Note: Sample size (*N*) and mean values for the traits studied and the estimates for the phenotypic and additive genetic variances ($\hat{\sigma}^2_P$, $\hat{\sigma}^2_A$), for the narrow-sense heritability ($h^2 = \hat{\sigma}^2_A/\hat{\sigma}^2_P$), and the additive genetic component of variation (CV_A, ×100).
[a]Values are estimates ± SD; the *p* value for the approximate test about whether the additive component of variance is zero is given in parentheses.
[b]Values are estimates ± SD.
[c]Latewood percentage is for 10 outermost annual rings.

Discussion

We obtained low narrow-sense heritabilities for mass loss in both sapwood and heartwood, which means that, in this progeny test, the effects of environmental factors on the mass loss were much more important than the genetic determination. Genetic variation of these traits may actually be low or some of the environmental factors, which effected the wood durability, were unexpectedly heterogeneous in this progeny test. Nuisance factors connected to the laboratory experiment might have been one reason for the high proportion of unexplained residual variation. A suitable covariate describing the fungal activity in each agar plate would have probably decreased the unexplained variation. However, a similar laboratory experiment with Siberian larch gave a broad-sense heritability of 0.39 (Venäläinen et al. 2001), and therefore, we do not expect the experimental inaccuracy to be the main reason for low heritabilities in the mass loss.

Both the phenotypic variation and CV_A for sapwood mass loss were low, indicating minor variation in the passive defence system of sapwood against fungal attack. In the sapwood, the defence mechanisms involved in the wounding of a standing tree are based on active processes, i.e., when the sapwood is wounded the injured area will be compartmentalized by changes in wood anatomy and chemistry. The poor passive resistance of the sapwood does not necessarily indicate poor active or induced resistance of the standing trees against plant pathogens.

Our results revealed considerable phenotypic variation and a high CV_A in the decay resistance of heartwood. A large fraction of the cores did not lose mass at all. As a similar distribution was not found for the sapwood samples, the cause of this is most probably due to the wood material rather than the experimental conditions during the decay test. In western redcedar (*Thuja plicata* Donn.), the decay resistance of the wood near the pith was extremely variable (DeBell et al. 1999). In western redcedar the decay resistance has been connected to the presence of toxic extractives, tropolones. In the study of DeBell et al. (1999), the highest and the most variable mass losses were associated with samples containing very low tropolone levels, while the samples with more tropolones had a lower, less variable degree of mass loss. Eberhardt et al. (1994) has reported that resin acids extracted from *Pinus ponderosa* Dougl. ex Laws. and *Pinus banksiana* Lamb. seed cones provided decay resistance against white rot fungi as a result of their hydrophobic properties rather than their general toxicity when applied to test blocks of sweetgum (*Liquidambar styraciflua* L.) wood. In Scots pine, a phenolic compound, pinosylvin, has been shown to cause the decay resistance of heartwood (e.g., Rennerfelt 1956; Rennerfelt and Nacht 1955). However, Hart and Shrimpton (1979) emphasize caution in such interpretations because of the complicated nature of the in situ interaction between decay fungi and stilbenes to which pinosylvin belongs. However, the reason for the great phenotypic variation in heartwood mass loss in our material remains unclear for the present. One reason may be that the sample included both straight and curved trees, which may have different anatomical structure and chemical composition and thus a varying effect on fungal activity. It also could be hypothesized that young Scots pine trees in the early stages of heartwood formation differ with respect to the timing of events connected to decay resistance. The relatively high CV_A, accompanied by a low heritability for heartwood mass loss, necessitates selection with intensive testing of the genotypes.

We did not estimate genetic correlation between sapwood and heartwood mass loss, because the family components of the variance were low, with standard deviations being three- to seven-fold larger than the component itself, respectively. Unimportant phenotypic correlation was found at the individual and the family mean level. Thus, at least outside the growing season, it is not possible to perform indirect selection on the basis of measurements of sapwood mass loss.

Heartwood formation is evidently an age-dependent process. Lappi-Seppälä (1952) studied naturally regenerated, 50- to 150-year-old Scots pines growing on relatively infertile sites. He concluded that heartwood formation had started at the age of 30–40 years. However, Fries and Ericsson (1998) found heartwood diameters ranging between 5.5 and 53.5 mm at a height of 80 cm above the ground in 25-year-old saplings in a progeny test. Moreover, the study of Björklund (1999) suggests that heartwood formation begins in the pith when the cambium is about 15 years old. In our material, the average numbers of annual rings in heartwood and sapwood at breast height were 6 and 17, respectively. Heartwood diameter has been reported to have a high heritability (Fries and Ericsson 1998; Ericsson and Fries 1999). In our study, the somewhat curved growth habit of the trees may have caused the low narrow-sense heritability for the heartwood radius.

In practice, the highest heritabilities are obtained in progeny tests where the environment is homogeneous, and to a great extent the individual differences are due to additive genetic effects. We suggest that, in this study, one environmentally induced factor which probably affected the mass loss through anatomical structure and chemical composition of the wood, was the fact that all the sampled boles were not straight. There were marked differences in the mean width of the annual rings on different sides of the stem, indicating the existence of reaction wood. It has also been reported that compression wood is more common in juvenile wood than in mature wood (e.g., Dadswell 1958). Thus, it is apparent that the wood samples used in our study contained reaction wood.

Blanchette et al. (1994) reported that, compared with normal wood, the compression wood of *Abies balsamea* (L.) Mill., *Picea mariana* (Mill.) BSP, and *Pinus strobus* L. was more resistant to decay caused by white and brown rot fungi, but the reason for this remained unclear. It has also been reported that compression wood contains higher concentrations of lignin than normal wood (Panshin and de Zeeuw 1980, p. 308, and references therein; Zobel and van Buijtenen 1989, p. 145, and references therein; Blanchette et al. 1994). On the other hand, the opposite wood may have higher cellulose and lower lignin content than normal wood (Zobel and van Buijtenen 1989, p. 144, and references therein; Haygreen and Bowyer 1996, p. 112). Thus, the curved growth form of the stems in our study may have resulted in changed lignin and cellulose concentrations compared with those of normal wood, thus affecting the amount of substrate available for cellulose decomposing, brown rot fungus. Moreover, our sample was most probably a mixture of increment cores from normal, compression, and opposite wood. As a result of the uncontrolled and irregular occurrence of the different types of the wood, the residual variance for heartwood mass loss became very high compared with the additive genetic variance, resulting in low narrow-sense heritability. In our study we did not find any connection between the visual observations of the stem form and decay resistance against *C. puteana*. However, because we did not determine the presence of reaction wood directly from the wood samples, the relationship between decay and reaction wood could not be studied.

Decay resistance as such is a complicated combination of traits that are poorly known. The traits and the progeny test we studied did not promise good possibilities for increasing the decay resistance of Scots pine wood against brown rot fungus through tree improvement. Caution is needed when generalizing about genetic parameters estimated from a single progeny test (see e.g., Haapanen et al. 1997; Jacquard 1983).

Acknowledgements

The progeny test was planned by the late Risto Sarvas and the late Reino Saarnio. Heikki Paajanen, Heikki Kinnunen, and Teemu Moilanen took the increment cores. Outi Kylliäinen and Markku Tiainen carried out the microscopic study at Joensuu Research Station. The decay resistance tests were performed by Liisa Seppänen at VTT Building and Transport. Eija Matikainen assisted with the data files, and John Derome revised the language. We thank all the above-mentioned collaborators. We are grateful to Tore Ericsson and Matti Haapanen for discussions concerning the statistical models and their applications. We thank Seppo Ruotsalainen for reading the manuscript with care and making many constructive comments. Two anonymous referees made valuable comments on the manuscript. The Academy of Finland (Resource Council for the Environment and Natural Resources, project No. 43140) supported this study, which belongs to the Finnish Forest Cluster Research Programme Wood Wisdom (1998–2001).

References

Björklund, L. 1999. Identifying heartwood-rich stands or stems of *Pinus sylvestris* by using inventory data. Silva Fenn. **33**(2): 119–129.

Björklund, L., and Walfridsson, E. 1993. Properties of Scots pine wood in Sweden—basic density, heartwood, moisture and bark content. [In Swedish with English summary.] Department of Forest Products, Swedish University of Agricultural Sciences, Uppsala. Rep. 234.

Blanchette, R.A., Obst, J.R., and Timell, T.E. 1994. Biodegradation of compression wood and tension wood by white and brown rot fungi. Holzforschung, **48**: 34–42.

Bruce, A. 1998. Biological control of wood decay. *In* Forest products biotechnology. *Edited by* A. Bruce and J.W. Palfreyman. Taylor & Francis Ltd., London. pp. 251–266.

Climent, J., Gil, L., and Pardos, J. 1993. Heartwood and sapwood development and its relationship to growth and environment in *Pinus canariensis* Chr. Sm ex DC. For. Ecol. Manage. **59**: 165–174.

Cotterill, P.P. 1987. On estimating heritability according to practical applications. Silvae Genet. **36**: 46–48.

Dadswell, H.E. 1958. Wood structure variations occuring during tree growth and their influence on properties. J. Inst. Wood Sci. **1**: 11–24.

DeBell, J.D., Morrell, J.J., and Gartner, B.L. 1999. Within-stem variation in tropolone content and decay resistance of second-growth western redcedar. For. Sci. **45**(2): 101–107.

Dieters, M.J., White, T.L., Littell, R.C., and Hodge, G.R. 1995. Application of approximate variances of variance components and their ratios in genetic tests. Theor. Appl. Genet. **91**: 15–24.

Eberhardt, T.L., Han, J.S., Micales, J.A., and Young, R.A. 1994. Decay resistance in conifer seed cones: role of resin acids as inhibitors of decomposition by white-rot fungi. Holzforschung, **48**: 278–284.

Ericsson, T., and Fries, A. 1999. High heritability for heartwood in north Swedish Scots pine. Theor. Appl. Genet. **98**: 732–735.

Fries, A., and Ericsson, T. 1998. Genetic parameters in diallel-crossed Scots pine favour heartwood formation breeding objectives. Can. J. For. Res. **28**: 937–941.

Haapanen, M., Velling, P., and Annala, M.-L. 1997. Progeny trial estimates of genetic parameters for growth and quality traits in Scots pine. Silva Fenn. **31**(1): 3–12.

Hart, J.H., and Shrimpton, D.M. 1979. Role of stilbenes in resistance of wood decay. Phytopathology, **69**: 1138–1143.

Haygreen, J.G., and Bowyer, J.L. 1996. Forest products and wood science: an introduction. 3rd ed. Iowa University Press, Ames.

Hillis, W.E., and Ditchburne, N. 1974. The prediction of heartwood diameter in radiata pine trees. Can. J. For. Res. **4**: 524–529.

Jacquard, A. 1983. Heritability: one word, three concepts. Biometrics, **39**: 465–477.

Kellomäki, S. 1981. Quality of pine logs and proportion of heartwood as related to properties of the logs. [In Finnish with English summary.] Folia For. 489. pp. 1–13.

Kramer, P.J., and Kozlowski, T.T. 1979. Physiology of woody plants. Academic Press, Inc., Orlando, Fla.

Kärkkäinen, M. 1972. On the proportion of heartwood in Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.). [In Finnish with English summary.] Silva Fenn. **6**(3): 193–208.

Kärkkäinen, M. 1976. Wood science prerequisites for the weight measurement of pine and spruce logs. [In Finnish with English summary.] Commun. Inst. For. Fenn. 89(1). pp. 1–58.

Lappi-Seppälä, M. 1952. Über Verkernung und Stammform der Kiefer. [In Finnish with German summary.] Comm. Inst. For. Fenn. 40(25). pp. 1–26.

Löyttyniemi, K. 1986. On natural durability of pine heartwood. [In Finnish with English summary.] Metsäteknologian tutkimusosasto, Puuntutkimussuunta, Finland. Metsäntutkimuslaitoksen tiedonantoja 231.

Panshin, A.J., and de Zeeuw, C. 1980. Textbook of wood technology. Structure, identification, properties, and uses of the commercial woods of the United States and Canada. 4th ed. McGraw-Hill, Inc., New York.

Rennerfelt, E. 1956. The natural resistance to decay of certain conifers. Friesia, **3**(5): 361–365.

Rennerfelt, E., and Nacht, G. 1955. The fungicidal activity of some constituents from heartwood of conifers. Sven. Bot. Tidskr. **49**: 419–432.

SAS Institute Inc. 1992. SAS© technical report P-229, SAS/STAT© software: changes and enhancements, release 6.07 ed. SAS Institute Inc., Cary, N.C.

Schmidtling, R.C., and Amburgey, T.L. 1982. Genetic variation in decay susceptibility and its relationship to growth and specific gravity in loblolly pine. Holzforschung, **36**(3): 159–161.

Tamminen, Z. 1962. Moisture content, density and other wood properties of wood and bark of Scots pine. [In Swedish with English summary.] Department of Forest Products, Royal College of Forestry, Stockholm, Sweden. Rep. 41.

Uusvaara, O. 1974. Wood quality in plantation-grown Scots pine. Commun. Inst. For. Fenn. 80(2). pp. 1–105.

Venäläinen, M., Harju, A., Nikkanen, T., Paajanen, L., Velling, P., and Viitanen, H. 2001. Genetic variation in the decay resistance of Siberian larch (*Larix sibirica* Ledeb.) wood. Holzforschung, **55**(1): 1–6.

Viitanen, H., Paajanen, L., Nikkanen, T., and Velling, P. 1998. Decay resistance of Siberian larch wood against brown rot fungi. Part 2. The effect of genetic variation. International Research Group on Wood Preservation, Stockholm. Doc. IRG/WP 98-10287.

Wilkes, J. 1991. Heartwood development and its relationship to growth in *Pinus radiata*. Wood Sci. Technol. **25**: 85–90.

Zabel, R.A., and Morrell, J.J. 1992. Wood microbiology: decay and its prevention. Academic Press Inc., San Diego, Calif.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood variation, its causes and control. Springer-Verlag, Berlin, Heidelberg.

III

Scand. J. For. Res. 17: 199–205, 2002

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Genetic Parameters Regarding the Resistance of *Pinus sylvestris* Heartwood to Decay Caused by *Coniophora puteana*

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Scandinavian Journal of Forest Research

Harju, A. M. and Venäläinen, M. (Finnish Forest Research Institute, Punkaharju Research Station, FI-58450 Punkaharju, Finland). *Genetic parameters regarding the resistance of Pinus sylvestris heartwood to decay caused by Coniophora puteana.* Received June 8, 2001. Accepted October 15, 2001. Scand. J. For. Res. 17: 199–205, 2002.

Genetic variation in the durability of *Pinus sylvestris* L. heartwood to a brown rot fungus, *Coniophora puteana*, was studied using an *in vitro* decay test. Juvenile heartwood was sampled from 33-yr-old half-sib families growing in a progeny test and from their mothers in a clonal archive. The narrow-sense heritability for the heartwood weight loss was 0.37, and the coefficient of additive genetic variation was 28%. Heritability estimated by the regression of the offspring on mothers was 0.29, and the coefficient of genetic prediction was 0.24. These results indicated that it would be possible to improve the decay resistance of *P. sylvestris* heartwood by direct selection. According to the genetic correlation ($r_A = 0.36$), selecting for heartwood density would result in an unfavourable response in weight loss caused by *C. puteana*. However, it appears that unknown environmental factors, which increase heartwood density, also decrease the heartwood weight loss ($r_E = -0.56$). This result emphasizes the need for better understanding of the relationships among wood density, decay fungi, and environmental factors. *Key words: basic density, brown rot, clonal archive, genetic correlation, heritability, offspring–parent regression, progeny test, Scots pine, wooden structures.*

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INTRODUCTION

The best way to protect wooden structures against fungal attack is to use construction techniques which ensure that the wood remains dry. If there is a risk of wood being exposed to moisture for longer periods, it would be both an environmentally and ecologically sound option to use wood that is naturally durable against decay. In Finland, the heartwood of old-growth *Pinus sylvestris* L. and an extract made from this material (pine tar) have been traditionally used in structures exposed to the risk of decay (e.g. Löyttyniemi 1986).

To increase the production and quality of *P. sylvestris* heartwood, it is essential to know the variation in the rate of formation and the properties of heartwood between different stands and between the trees within the stands. If the variation is to a great extent of genetic origin, it could be used by means of tree breeding, e.g. recurrent selection.

Several studies on *P. sylvestris* suggest that it would be possible to improve the rate of heartwood formation and its chemical properties by breeding (Fries & Ericsson 1998, Ericsson & Fries 1999, Fries et al. 2000, Ericsson et al. 2001). Studies by Schmidtling & Amburgey (1982) on *Pinus taeda* L. and Venäläinen et al. (2001) on *Larix sibirica* Ledeb. also showed moderate broad-sense heritabilities (0.22 and 0.39, respectively) in durability against brown-rot decay fungus in a laboratory test.

This group previously carried out a study on *P. sylvestris*, and found very low narrow-sense heritabilities for both sapwood and heartwood durability (0.04 and 0.07, respectively), although the coefficient of additive genetic variation for the heartwood durability was relatively high (19%) (Harju et al. 2001). In that progeny test several of the studied trees were slightly curved. The curved growth habit was induced by an unspecified and experimentally uncontrolled factor which occurred irregularly in the experiment. It was speculated that the reaction wood present in the curved stems degraded at a different rate to the "normal" wood. As a result of the uncontrolled and irregular occurrence of the reaction wood, the residual variance for heartwood durability became very high compared with the additive genetic variance, resulting in low narrow-sense heritability.

The aim of the present study was to evaluate further the possibility of improving the heartwood decay resistance of *P. sylvestris* by tree breeding. To eliminate the disturbing effects of the curved growth habit of the stems, the wood material for the present study was obtained from a *P. sylvestris* progeny trial with straight stems. The necessary genetic parameters, narrow-sense

 ISSN 0282-7581

heritability (h^2) and the coefficient of genetic variation (CV_A), were estimated for heartwood weight loss, which is inversely related to decay resistance. Heartwood weight loss data were obtained from a laboratory test in which heartwood samples were exposed to a primarily cellulose-degrading brown rot fungus, *Coniophora puteana* (Schum. ex Fr) Karst., which causes decay in wooden constructions exposed to moisture and water (Viitanen & Ritschkoff 1991). Heritability was estimated as parent–offspring regression and by the coefficient of genetic prediction. The genetic parameters for heartwood weight loss were compared with the parameters for the wood density, which were well known, and to the parameters for the amount of heartwood.

MATERIALS AND METHODS

Study populations, sampling and preliminary analysis of increment cores

The primary set of *P. sylvestris* heartwood samples, consisting of 5-mm diameter increment cores, was obtained from a progeny trial comprising half-sib families originating from open pollination of selected plus trees. The trial (no. 310/1 according to the Finnish Forest Genetic Register) is located in Korpilahti in central Finland (62°11′ N, 25°23′ E, on 135 m elevation) and is owned by the Finnish Forest and Park Service. The trial was planted in 1968 in eight randomized complete blocks with square plots of 16 2-yr-old, bare-rooted seedlings at a spacing of 2 × 2 m. The trial was thinned in winter 1989–1990 to a density of eight trees per plot.

Twenty-six families of similar east Finnish origin were used to estimate the narrow-sense heritabilities. Core samples were taken from two dominant straight-stemmed trees per plot from each block, in total 16 trees per family.

The increment core samples were drilled during a 1 week period at the end of August 1999. One increment core was sampled from a random direction at the midpoint between two whorls on each tree. As the diameter of the heartwood was required to be at least 40 mm for the decay test, the sampling height varied to meet this criterion. The average sampling height was 91 cm and it ranged from 60 to 128 cm above ground level. As the samples were not taken from a specific height (e.g. breast height) this resulted in an approximately equal number of annual rings in the increment cores sampled from individual trees. The mean number of annual rings was 28 (SD = 0.9). The cores were drilled through the pith for a distance of a few centimetres on the opposite side of the pith. The transition point between the sapwood and heartwood was marked according to visually observed moisture differences immediately after the increment core was sampled. This made it possible to count the number of sapwood and heartwood rings later in the laboratory. The samples were stored in sealed plastic tubes at a temperature of −5°C. The total number of increment cores was 413 (samples from three trees were missing).

A second set of increment cores, which was used to estimate offspring–parent regression, was obtained from a clonal archive located in Punkaharju in southeastern Finland (61°48′ N, 29°20′ E, on 85 m elevation, established in 1968–1971). Increment cores were taken from two grafts of each of 20 clones during a 3 day period at the beginning of September 1999. The clones were grafted from the mother trees of the 20 half-sib families in the progeny test described above. Because the grafts were slightly curved, two increment cores were bored at right angles to each other and at different heights. This was done to make the clone-wise measurements more precise. The lower sampling height was 98 ± 11.4 cm (mean ± SD) and the upper one was 133 ± 8.2 cm. Cores were sampled and stored in the same way as those used for the progeny test.

Laboratory experiment

A 40 mm section was cut from the heartwood part of each increment core. However, in a few cases only a 30 mm section was cut, because the aim was to obtain pure heartwood. The sections were dried at 60°C for 48 h, after which they were cooled in a desiccator and weighed to an accuracy of 1 mg. The basic density of the wood specimen (mg cm^{-3}) was calculated from the dry weight of the sample and its fresh volume (a cylinder with a diameter of 5 mm and length of 40 or 30 mm). The weighed samples were packed into paper bags and sterilized at VTT Chemical Technology using a radiation dose of between 25 and 50 kGy from a radioisotope ^{60}Co source.

The decay resistance was studied at VTT Building Technology using a malt agar plate decay test, which is a modification of the standardized EN 113 (European Standard 1996) as described by Viitanen et al. (1998) and Venäläinen et al. (2001). The cylindrical increment core sections, from which the wood extractives had not been removed, were placed on a pure culture of a brown rot fungus, *C. puteana* (Schum. ex

Fr.) Karst. (strain BAM Ebw. 15), growing on agar in Petri dishes. The samples were placed on a glass rack so that they were not in contact with the agar. A random set of four wood specimens was placed in each Petri dish. The incubation time was 8 weeks, after which the samples were dried at 60°C for 48 h and reweighed. The weight loss during the experiment was expressed in absolute terms as mg cm^{-3}, and in relative terms [(weight loss/start weight) × 100%].

Statistical analysis

On the individual tree level, a measured phenotypic value (P) of a trait is assumed to be the sum of the additive genetic effect (A) and the independent environmental effect (E). E also includes the remaining genetic effects that are independent of A. Thus, $P = A + E$. Furthermore, the phenotypic variance at the population level is assumed to be composed of genetic and environmental components, $\sigma_P^2 = \sigma_A^2 + \sigma_E^2$. The narrow-sense heritability is estimated using $h^2 = \sigma_A^2/\sigma_P^2$.

For the progeny test, the model was constructed and the narrow-sense heritabilities were estimated on an individual tree basis, assuming that all random effects in the studied properties were pairwise independent. Because of the spatial autocorrelation introduced by the plot design, however, this assumption may overestimate the additive genetic component of the variance. The violation against the independence rule is not regarded as serious for the properties describing wood quality.

Variance components were estimated with the REML technique using the MIXED procedure of the SAS system (Anon. 1992). Interaction between block and family effect was not significant. Thus, a mixed model equation without interaction was used (a simplified genetic model; Jacquard 1983, Ericsson 1997):

$$y_{bfi} = \alpha_b + b_f + e_{bfi}.$$

The block effect (α_b) was regarded as fixed (corrections for block effects should be applied before selection) and the family effect (b_f) as random. Application of this model assumes that the interaction between the design and treatment factors within a single site does not describe the true genotype–environment interaction, and is thus not regarded as useful for ordinary breeding purposes, when the goal is to perform effective selection.

The random effects were assumed to be normally distributed with the expectation zero and the variance σ_f^2 and σ_{bfi}^2 [$N(0, \sigma_f^2)$, etc.]. The individual-tree heritability for the half-sib progenies was estimated using the formula $h^2 = 4\hat{\sigma}_f^2/(\hat{\sigma}_f^2 + \hat{\sigma}_\epsilon^2)$, assuming true half-sibs and unrelated parents. This may be an overestimate owing to the possible existence of full-sibs (see Squillace 1974).

The MIXED procedure of the SAS system also provided approximate standard deviations for the estimated variance components. The standard deviation for the heritability estimate was calculated using Dickerson's approximation (e.g. Dieters et al. 1995). The coefficient of additive genetic variation (CV_A) was calculated by dividing $\hat{\sigma}_A$ by the overall mean value of the trait.

To estimate additive genetic, environmental and phenotypic correlation (r_A, r_E, r_P, respectively) at the progeny test, the covariance between properties was obtained using corresponding variance estimates of each pair of traits and of their sum (e.g. Williams & Matheson 1994, p. 112). The correlations were estimated between the heartwood weight loss and the heartwood density. Standard deviation for the genetic correlation was estimated according to Falconer (1981, p. 285).

Heritability of the heartwood properties was estimated also by using the measurements from 20 parents together with the measurements from their offspring (progeny). First, the degree of resemblance between the relatives was expressed as the regression (b) of offspring (O) on parents (P) (Falconer 1981, pp. 134–147, Nyquist 1991, pp. 280–281). Heritability was estimated as $h_{OP}^2 = 2b_{OP}$ and its sampling variance was estimated according to Lynch & Walsh (1998, p. 543). Secondly, heritability was estimated by a correlation approach using the coefficient of genetic prediction between offspring and parents ($h^2 = CGP_{OP}$) (Baradat 1976).

The coefficient of genetic prediction (CGP) can also be used in calculating the correlated response in trait b by selecting for a trait a (e.g. van Buijtenen 1992, p. 64).

RESULTS

The narrow-sense heritabilities (h^2) for the measured heartwood traits are presented in Table 1. The heritability estimate for the absolute weight loss was high, but it did not reach the level of the estimates for the basic density and the radius of the heartwood. Taking the sampling height into account as a covariate in the analysis of variance did not change the estimates for the narrow-sense heritability. Except for the basic

Table 1. *Estimates of the genetic parameters of the heartwood properties of 26 families of Pinus sylvestris (n = 413)*

Heartwood trait	Mean	$\hat{\sigma}_P^2$	$\hat{\sigma}_A^2$ (SD, p)	h^2 (SD)	CV_A (%)
Weight loss (mg cm^{-3})	123.1	3313.3	1227.3 (562.36, 0.029)	0.37 (0.17)	28.5
(%)	33.1	250.6	79.1 (38.92, 0.042)	0.32 (0.15)	26.9
Basic density (mg cm^{-3})	375.7	936.0	569.1 (217.70, 0.010)	0.61 (0.20)	6.3
Radius (mm)	28.0	47.5	27.8 (10.76, 0.010)	0.59 (0.20)	18.8
Annual rings (n)	6.7	2.1	0.9 (0.40, 0.020)	0.43 (0.17)	18.6

Weight loss was due to *Coniophora puteana* degradation. Mean values of the properties, and the estimates of the phenotypic and additive genetic variances ($\hat{\sigma}_P^2$, $\hat{\sigma}_A^2$) and for the narrow-sense heritability (h^2) p-values for additive genetic variation, as well as the additive genetic component of variation (CV_A) are presented.

density of heartwood, the coefficients of additive genetic variation (CV_A) were high for all the properties studied, especially for the absolute heartwood weight loss (Table 1).

The heritabilities of the wood properties estimated by the regression of the offspring on the mother clone, and by the coefficient of genetic prediction (Table 2), were substantially lower than the estimates obtained from the half-sib progeny test. The mean heartwood weight loss and density among the mother clones are presented in Table 3, which also includes the corresponding information for the progeny test.

The heartwood weight loss had large variation. In the progeny test the coefficient of variation (CV, which is not presented in the tables) was 46.7%, and among the mother clones 67.4%. The figures for the heartwood density were 8.2% and 12.6%, respectively.

The genetic correlation ($\hat{r}_A$) between heartwood density and weight loss was 0.36 ± 0.26, while the phenotypic correlation ($\hat{r}_P$) was -0.11. The environmental correlation ($\hat{r}_E$) between heartwood weight loss and density was -0.56. The CGP was 0.17.

Table 2. *Heritability of the heartwood properties of Pinus sylvestris estimated from the regression of the offspring means on their mother clones* h_{OP}^2

Heartwood trait	h_{OP}^2 (SD)	CGP_{OP}
Weight loss (mg cm^{-3})	0.29 (0.34)	0.24
Basic density (mg cm^{-3})	0.20 (0.18)	0.30
Radius (mm)	0.02 (0.13)	0.04
Annual rings (n)	−0.08 (0.26)	−0.09

Weight loss was due to degradation by a brown rot fungus, *Coniophora puteana*. The coefficient of genetic prediction (CGP_{OP}) gives an estimate for the heritability using the correlation approach. Number of paired observations = 20.

DISCUSSION

The additive genetic variation accounted for a relatively large proportion of the phenotypic variation found in the weight loss of the juvenile heartwood of *P. sylvestris* exposed to degradation by the brown rot fungus *C. puteana* in a laboratory test. The estimates for the genetic parameters (Table 1) predict a good selection response in environments similar to that of this progeny trial, and suggest that there are possibilities to improve the heartwood decay resistance by tree breeding.

The narrow-sense heritability for basic density (Table 1) fits well with the knowledge on other tree species (e.g. Zobel & van Buijtenen 1989, Hannrup 1999), and thus provides a meaningful comparison level for the heritability of the other properties studied. The narrow-sense heritability for heartwood weight loss is lower than that for basic density. However, assuming breeding populations of identical size, the improvement of heartwood durability or radius will probably be a more successful effort than the improvement of heartwood density, because the low coefficient of additive genetic variation (Table 1) would restrict progress in breeding for heartwood density (see e.g. Hannrup 1999).

The heritabilities for the heartwood weight loss and heartwood density estimated from the regression of the offspring on the parent support the finding that there is additive genetic variation in those traits. Since the mother clones and the progenies were grown in different environments, it was expected that the regression (or correlation) would be strongly affected by the environmental differences. The amount of heartwood, in particular, appeared to be strongly affected by this fact (h_{OP}^2 and CGP_{OP} in Table 2). Another important difference between the mother clones and the offspring, which could have had an

Table 3. *Comparison of heartwood weight loss due to brown rot fungus, Coniophora puteana, and basic density between progeny test and a clone archive of Pinus sylvestris*

	Weight loss (mg cm^{-3})			Basic density (mg cm^{-3})		
Sample	Mean	CV (%)	Range	Mean	CV (%)	Range
Korpilahti, progeny test						
Individual trees ($n = 413$)	123.1	47	−6 to 243	375.7	8	297 to 540
Family means ($n = 26$)		18	61 to 165		4	352 to 405
Punkaharju, clone archive						
Individual grafts ($n = 40$)	80.0	67	−8 to 182	389.5	13	326 to 525
Clone means ($n = 20$)		49	16 to 155		11	331 to 482

CV, coefficient of variation.

effect on the regression of the offspring on the parent, was the ontogenetic age of the heartwood tissues. The mother clones, which were about 30 yrs old, had been propagated vegetatively from scions of plus-trees whose physiological age had been more than 100 yrs. In drawing conclusions in this study, the main weight has to be put on the result obtained in the progeny test material.

The results of the present study differed from those of an earlier study carried out with material from another progeny test in Kerimäki (Harju et al. 2001). In the previous study the narrow-sense heritability was practically zero compared with 0.37 in the present study. However, the wide range of the distribution and the high coefficient of additive genetic variation of the heartwood weight loss observations were similar between the progeny tests. It is impossible to conclude whether the difference between the progeny tests was caused by the sampling effects, experimental conditions during the laboratory test, bias in the measuring process or by the properties of the wood, which may have been strongly affected by several unknown environmental factors.

The obvious external difference between the two progeny tests was that the stems at Kerimäki were slightly curved and those at Korpilahti were straight. Thus, the present study indirectly supports the authors' speculation (Harju et al. 2001) concerning the effect of the curved growth habit on the heartwood decay resistance of *P. sylvestris*. Stem-form defects are known to induce the formation of reaction wood, which has specific anatomical and chemical properties (e.g. Zobel & van Buijtenen 1989 and references therein, Blanchette et al. 1994). These properties may have played an important role during the fungal degradation processes in the laboratory test. The divergence in the estimates for the narrow-sense heritability could be explained by the absence of a disturbing environmental factor that affected the decay resistance in the present study.

Negative phenotypic correlation between heartwood density and heartwood weight loss has been reported in slash pine (specific gravity in Schmidtling & Amburgey 1977). No relationship between the density and decay resistance has been found in other studies (e.g. Southam & Ehrlich 1943, Rennerfelt 1947, 1956, Richards 1950). Suolahti (1948) also concluded that it was equally difficult for a rot fungus to degrade dense as light *P. sylvestris* wood. The phenotypic correlation in the present study was negative. However, there was an unfavourable positive genetic correlation between the heartwood density and weight loss. According to the present results, selection for higher heartwood density would increase the expected weight loss in the next generation. The increased heartwood density may have enhanced the availability of cellulose for the degrading fungus. However, the negative environmental correlation suggests that certain environmental factors have had a reverse effect on the heartwood weight loss and on the density. Environmental factors that increased the density may also have induced the production of fungicidal compounds, or enhanced lignification at the expense of the proportion of cellulose (e.g. Zobel & van Buijtenen 1989 and references therein, Blanchette et al. 1994). The combination of correlations indicates that the environmental effects, which make the denser heartwood more resistant to the degradation by brown rot fungus, are independent of the fact that families with denser wood represent a more favourable substrate for the brown rot fungus (see discussion in King et al. 1997).

In conclusion, this study showed that there is enough additive genetic variation in the decay resistance of *P. sylvestris* heartwood to consider genetic improvement of this trait by selection. However, to carry out the phenotypic selection successfully, and especially to manage the stands to produce durable wood, the role of environmental factors in the mechanisms associated with the passive decay resistance of heartwood should be elucidated. Moreover, the results concerning the phenotypic distribution of, for example, the weight loss among trees, strongly stress the importance of including an adequate number of trees in the studies of wood quality traits.

ACKNOWLEDGEMENTS

We are grateful to Heikki Kinnunen, Eija Matikainen, Heikki Paajanen, Tarja Salminen and Jussi Tiainen for sampling and measuring the increment cores. Liisa Seppänen carried out the decay test in the laboratory of VTT Building Technology. We thank Pirkko Velling for the valuable comments on the manuscript, and Hannu Viitanen, VTT, for co-operation during the study. We also thank John Derome for checking the language. The Academy of Finland (Resource Council for the Environment and Natural Resources, project no. 43140) supported this study, which is part of the Finnish Forest Cluster Research Programme WOOD WISDOM (1998–2001).

REFERENCES

Anon. 1992. SAS® Technical Report P-229, SAS/STAT® Software: Changes and Enhancements, Release 6.07, 620 pp. SAS Institute, Cary, NC. ISBN 1-55544-473-3.

Baradat, Ph. 1976. Use of juvenil–mature relationships and information from relatives in combined multitrait selection. Advanced generation breeding. Proc. IUFRO Joint Meeting. Bordeaux, 14–18 June 1976, pp. 121–138.

Blanchette, R. A., Obst, J. R. & Timell, T. E. 1994. Biodegradation of compression wood and tension wood by white and brown rot fungi. Holzforschung 48: 34–42.

Buijtenen, J. P. van. 1992. Fundamental Genetic Principles. *In* Fins, L., Friedman, S. T. & Brotschol, J. V. (eds). Handbook of Quantitative Forest Genetics. pp. 29–68. Kluwer Academic, Dordrecht. ISBN 0-7923-1568-5.

Dieters, M. J., White, T. L., Littell, R. C. & Hodge, G. R. 1995. Application of approximate variances of variance components and their ratios in genetic tests. Theor. Appl. Genet. 91: 15–24.

Ericsson, T. 1997. Enhanced heritabilities and best linear unbiased predictors through approriate blocking of progeny trial. Can. J. For. Res. 27: 2097–2101.

Ericsson, T. & Fries, A. 1999. High heritability for heartwood in north Swedish Scots pine. Theor. Appl. Genet. 98: 732–735.

Ericsson, T., Fries, A. & Gref, R. 2001. Genetic correlations of heartwood extractives in *Pinus Sylvestris* progeny test. For. Genet. 8: 73–78.

European Standard. EN 113. 1996. Wood preservatives–Test method for determining the protective effectiveness against wood destroying basidiomycetes–Determination of the toxic values, 32 pp. European Committee for Standardization, Brussels.

Falconer, D. S. 1981. Introduction to Quantitative Genetics. 2nd ed., 340 pp. Longman, London. ISBN 0-582-44195-1.

Fries, A. & Ericsson, T. 1998. Genetic parameters in diallel-crossed Scots pine favour heartwood formation breeding objectives. Can. J. For. Res. 28: 937–941.

Fries, A., Ericsson, T. & Gref, R. 2000. High heritability of wood extractives in *Pinus sylvestris* progeny test. Can. J. For. Res. 30: 1–7.

Hannrup, B. 1999. Genetic parameters of wood properties in *Pinus sylvestris* (L.). Acta Universitatis Agriculturae Sueciae. Silvestria 94, 36 + 58 pp. Doctoral Thesis, Swedish Uni. of Agric. Sci., Uppsala, ISBN 91-576-5628-2.

Harju, A. M., Venäläinen, M., Beuker, E., Velling, P. & Viitanen, H. 2001. Genetic variation in the decay resistance of Scots pine wood against brown rot fungus. Can. J. For. Res. 31: 1244–1249.

Jacquard, A. 1983. Heritability: one word, three concepts. Biometrics 39: 465–477.

King, J. N., Yanchuk, A. D., Kiss, G. K. & Alfaro, R. I. 1997. Genetic and phenotypic relationships between weevil (*Pissodes strobi*) resistance and height growth in spruce populations of British Columbia. Can. J. For. Res. 27: 732–739.

Löyttyniemi, K. 1986. On natural durability of pine heartwood. Metsäntutkimuslaitoksen tiedonantoja 231: 50. ISBN 951-40-0877-4. (In Finnish with English summary.)

Lynch, M. & Walsh, B. 1998. Genetics and Analysis of Quantitative Traits, 980 pp. Sinauer Associates, Sunderland, MA. ISBN 0-87893-481-2.

Nyquist, W. E. 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10 (3): 235–322.

Rennerfelt, E. 1947. Some investigations over the capacity of some decay fungi to attack sapwood and heartwood of Scots pine. Meddel. från Statens skogforskningsinstitut 36(9): 1–24. (In Swedish with English summary.)

Rennerfelt, E. 1956. The natural resistance to decay of certain conifers. Friesia 3: 361–365.

Richards, D. B. 1950. Decay resistance and physical properties of wood. J. For. 48: 420–422.

Schmidtling, R. C. & Amburgey, T. L. 1977. Growth and wood quality of slash pines after early cultivation and fertilization. Wood Sci. 9: 154–159.

Schmidtling, R. C. & Amburgey, T. L. 1982. Genetic variation in decay susceptibility and its relationship to growth and specific gravity in loblolly pine. Holzforschung 36: 159–161.

Southam, C. M. & Ehrlich, J. 1943. Decay resistance and physical characteristics of wood. J. For. 41: 666–673.

Squillace, A. E. 1974. Average genetic correlations among offspring from open-pollinated forest trees. Silvae Genet. 23: 149–156.

Suolahti, O. 1948. Dependence of the resistance to decay on the quality of Scots pine. Finn. Paper Timber J. 30(23): 421–425. (In Swedish with English summary.)

Venäläinen, M., Harju, A., Nikkanen, T., Paajanen, L., Velling, P. & Viitanen, H. 2001. Genetic variation in the decay resistance of Siberian larch (*Larix sibirica* Ledeb.) wood. Holzforschung 55: 1–6.

Viitanen, H. & Ritschkoff, A.-C. 1991. Brown rot decay in wooden constructions. Effect of temperature, humidity and moisture. Dept. of Forest Products, Swed. Univ. of Agric. Sci., Report No. 222, 55 pp. ISSN 0348-4599.

Viitanen, H., Paajanen, L., Nikkanen, T. & Velling, P. 1998. Decay resistance of Siberian larch wood against brown rot fungi. Part 2. The effect of genetic variation, 6 pp. International Research Group on Wood Preservation, Stockholm. IRG Doc. No. IRG/WP 98-10287.

Williams, E. & Matheson, A. 1994. Experimental design and analysis for use in tree improvement, 174 pp. CSIRO. ISBN 0-643-05555-X.

Zobel, B. J. & Buijtenen, J. P. van 1989. Wood Variation, Its Causes and Control, 363 pp. Springer, Berlin. ISBN 3-540-50298-X.

IV

Holzforschung
56 (2002) 479–486

Differences in Resin Acid Concentration between Brown-Rot Resistant and Susceptible Scots Pine Heartwood

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Keywords

Coniophora puteana
Pinus sylvestris
Moisture content
Hygroscopicity
Increment core
Decay resistance
Resin acids
Extractive content

Summary

The concentration of individual resin acids and the equilibrium moisture content at a relative humidity of 100 % were studied in brown-rot resistant and susceptible Scots pine (*Pinus sylvestris* L.) heartwood. About 90 % of the resin acids in the heartwood were of the abietane type, abietic acid being the most abundant. The concentration of resin acids was higher in the decay-resistant heartwood than in the decay-susceptible heartwood. Resin acids are presumably in part responsible for the decay resistance of Scots pine heartwood. However, no clear relationship was found between the concentration of resin acids and the equilibrium moisture content. The role of resin acids may also be ascribed to mechanisms other than their hydrophobic properties alone. The reasons for the slight differences in moisture content between the decay classes require further study.

Introduction

The level and duration of moisture stress, together with temperature, are the most critical factors affecting the durability of wood. Wooden structures exposed to a risk of moisture and fungal attack should be protected by using construction techniques which ensure that the wood remains dry. However, wood is also frequently impregnated with preservatives that in some cases have harmful effects on the environment and human health. It would, therefore, be both an environmentally and an ecologically sound alternative to use wood that is naturally durable against decay and biodeterioration. In Finland, the heartwood of old-growth Scots pine (*Pinus sylvestris* L.) has traditionally been used as a material in structures exposed to a risk of decay (Löyttyniemi 1986).

Two explanations have been put forward for the traditional experience that heartwood is naturally more durable than sapwood against fungal attack. Firstly, the concentration of extractives in heartwood is higher than that in sapwood. These extractives have been found to have a toxic effect on fungal growth in bioassays (Zabel and Morrell 1992; Bruce 1998). Secondly, the structural and chemical properties of heartwood result in a lower hygroscopicity and permeability to water (Panshin and de Zeeuw 1980). The relative importance of these defence mechanisms may, however, differ among tree species.

Wood extractives are specific to each tree species (Hillis 1987). The members of the *Pinaceae* family produce oleoresin, which is a complex mixture of terpenoids that mainly consist of straight-chain or cyclic compounds: monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$) and diterpenes ($C_{20}H_{32}$) (Kramer and Kozlowski 1979). In Scots pine, oleoresin mainly contains diterpenes, *i.e.* resin acids, with minor amounts of monoterpenes and sesquiterpenes. Mono- and sesquiterpenes are volatile, whereas diterpenes are not.

In sapwood, oleoresin acts both as a chemical and a mechanical barrier that closes the wounds of living trees as the result of an induced process. Oleoresin compounds also act as deterrents to a variety of generalist and specialist insects (Gershenzon and Croteau 1991; Langenheim 1994), and inhibit the growth of fungi (Yamada 1992). In Scots pine heartwood, constitutive resin acids are present in high concentrations and account for 88 % of the total amount of extractives, while in the intact sapwood the amount of non-induced resin acids is substantially lower (Martinez-Inigo *et al.* 1999). The presence of several monoterpenes (Flodin and Fries 1978; Schuck 1982) or resin acids (Henriks *et al.* 1979) has been shown to inhibit the growth of wood-rotting fungi in agar media. However, the extent to which the oleoresin compounds inhibit fungal growth on a natural substrate such as wood is still unclear. Very little is known about the differences in decay resistance associated with the concentration of oleoresin compounds in the wood of forest trees.

During the drying and storing of wood, a high pro-

portion of the monoterpenes are lost through evaporation (Englund and Nussbaum 2000), and the role played by resin acids in the decay resistance of timber is obviously more important. In laboratory experiments (Hart *et al.* 1975; Eberhardt *et al.* 1994), wood blocks impregnated with resin acids suffered less fungal decay than non-impregnated blocks. Resin acids may inherently protect the woody substrate as a result of their toxic effects, or resin impregnation may act as a non-toxic, waterproofing layer that prevents fungal penetration and growth within wood (Yamada 1992; Shain 1995). It has been suggested that resin acids provide resistance against decomposition as a result of their hydrophobic properties rather than their general toxicity (Eberhardt *et al.* 1994).

Wide variation in the durability of heartwood formed in the juvenile core of 30-year-old Scots pine has been found in two independent laboratory tests done with the cellulose-degrading, brown-rot fungus *Coniophora puteana* (Schum.ex Fr) Karst. (Harju *et al.* 2001; and unpublished results). Heartwood of some trees did not degrade at all in the decay test. However, it is not known whether the degrading activity of the fungus was affected by 1) non-volatile toxic or hydrophobic compounds present in the heartwood, 2) mechanical barriers (of either anatomical or chemical origin), 3) the amount of cellulose and lignin present in the heartwood, or 4) the hygroscopic characteristics of the heartwood.

The aim of this study was to investigate whether the concentration of resin acids and the hygroscopic characteristics of wood, represented by the equilibrium moisture content at a relative humidity of 100 %, differ between slowly and rapidly decaying Scots pine heartwood. In this study, the concentration of individual resin acids and the moisture content were determined in the same trees in which, according to earlier tests carried out by Harju *et al.* (2001; and unpublished results), the heartwood decayed either slowly or rapidly in a laboratory test. We were also interested in investigating the relationship between the resin acid concentration and moisture content of the wood.

Materials and Methods

Populations

Scots pine heartwood samples were obtained from 34-year-old progeny tests at Korpilahti and Kerimäki (Table 1). The stems in the test at Korpilahti were straight, while those at Kerimäki were somewhat curved. According to visual assessment, the site type at Kerimäki is more fertile and probably even too nutrient rich for Scots pine. The curved growth habit in the Kerimäki progeny test was most probably due to the planting method and the effects of site fertility. Both straight and somewhat curved trees were included in the sample.

Decay test

In our previous studies (Harju *et al.* 2001 and unpublished results) the wood samples for the decay tests were taken with a 5 mm increment core borer as described in Harju *et al.* (2001). Decay resistance was studied at VTT Building Technology using a malt agar plate decay test, which is a modification of the standardised EN 113 test (Viitanen *et al.* 1998; Venäläinen *et al.* 2001). The dried and weighed, non-extracted increment core sections were placed on a pure culture of a brown-rot fungus, *Coniophora puteana*. The incubation time was six weeks, after which the samples were dried and reweighed. The weight loss was used as an inverse measure of decay resistance.

Quantitative analysis of resin acids

The results of the decay tests were used to take new samples for chemical wood analysis at both locations. Two groups of trees with extreme rates of heartwood decay in the laboratory test were obtained by culling the tails of the weight-loss distribution to give 10 trees with slowly decaying (referred to as resistant) and 10 trees with rapidly decaying (susceptible) heartwood (Table 2). Sampling was made on an individual tree basis, but only one tree from a single half-sib family was accepted in order to avoid a kin structure among the sampled trees. The wood samples were taken with an 8 mm increment core borer in order to obtain a sufficient sample volume (2 or 4 cores per tree). At Kerimäki, where the interval between the two samplings was two growing seasons (Table 1), the samples for chemical analysis were taken at the next inter-whorl (or inter-node) above and below the inter-whorl from where the first sampling was made (Fig. 1). This procedure was followed in order to avoid possible induced changes in the wood chemistry, although unlikely, of the heartwood. At Korpilahti there was a dormant season between the two samplings, and therefore the

Table 1. Basic information about the progeny tests from which the wood samples were taken

	Korpilahti	Kerimäki
Trial no.	310/1	307/1
Location	62 °11'N, 25 °23'E, 135 m asl.	61 °50'N, 29 °23'E, 90 m asl.
Year of establishment	1968	1968
Seedlings	2-year-old, bare-rooted	2-year-old, bare-rooted
Composition	38 half-sib families	38 half-sib families
Design	randomised complete blocks (8) 16 seedlings/square plot, spacing 2×2 m	randomised complete blocks (6) 16 seedlings/square plot, spacing 2×2 m
Mean height at the age of 15 years, m	5.7	5.8
Survival at age of 15 years, %	95	81
First thinning	1989	1992
Sampling for the decay test	August 1999	May 1998
Sampling for resin acid analysis	May 3, 2000	April 25, 2000
Mean sampling height (SD), cm	92 (12)	151 (42)

samples for chemical analysis were taken 10–20 cm above and below the previous sampling point. The heartwood section was marked on the increment core according to visual differences in the moisture content of the sapwood and heartwood immediately after boring. The increment cores were stored in sealed tubes at a temperature of −5 °C for about two weeks.

Fig. 1. A sample tree from the half-sib progeny test at Kerimäki. The white vertical arrows show the section from which the increment cores were taken for the decay tests in April 1998 and the horizontal arrows show the points where the increment cores were bored for chemical analysis in May 2000.

The heartwood sections were separated from the increment cores, and were then oven-dried at a temperature of +60 °C for 48 h, cooled and stored in a desiccator prior to grinding with an analysis mill (Kinematica). The milled sample from each tree was stored in a sealed bottle at −20 °C.

Resin acids were extracted from the milled samples with petroleum ether-diethyl ether following the procedures of Gref and Ericsson (1985). The extracts were analysed on an individual tree basis by gas chromatography – mass spectrometry (Hewlett Packard GC type 6890, MSD 5973) using a 30-meter-long HP-5MS (0.25 mm ID, 0.25 µm film thickness, Hewlett Packard) capillary column as described earlier by Kainulainen *et al.* (1993). For the quantification of individual resin acids (Table 3), calibration curves were prepared using known concentrations of pure compounds relative to known concentrations of the internal standard (heptadecanoic acid).

Moisture content determination

The hygroscopic characteristics of the Scots pine heartwood were studied by determining the moisture content of parallel 5 mm-diameter samples from the Korpilahti progeny test. These core samples were taken in January 2001 from the same 10 resistant and 10 susceptible trees on which the analysis of resin acids had been performed. Samples were taken from the next inter-whorl above the previous samplings. The heartwood was marked, and 40 mm-long sections of heartwood were separated as described above. After oven drying (+60 °C, 48 h) and weighing, the samples were conditioned at a relative humidity (RH) of 100 % at +24 °C. Constant humidity was attained in a closed plastic chamber containing pure distilled water. The relative humidity within the chamber was also measured using an RH meter (Rotronic Hygrometer C94) with a temperature range of −40 to +85 °C, RH 0–100 %, and precision ±3 %. In the chamber, the samples were placed on a plastic net suspended above water (Viitanen and Bjurman 1995). After two weeks of conditioning the samples were weighed, oven dried at +103 °C for 24 h, and reweighed. The equilibrium water content is expressed as mg/cm³, and the equilibrium moisture content as MC = 100 × [weight at 100 % RH − dry weight (+103 °C)] / dry weight (+103 °C) %. The moisture content can be regarded as the water content standardised by the wood density.

Table 2. The distribution of heartwood weight loss in the decay tests (Harju *et al.* 2001 and unpublished data) and the resampling of trees for chemical wood analysis. The symbol *n* refers to the number of individual trees, and the symbol $d_{1.3}$ to the diameter at a height of 1.3 m above the ground

	Trees sampled for the decay test	Trees sampled for chemical analysis	
		Resistant	Susceptible
Korpilahti:			
n	535	10	10
mean weight loss (mg/cm³)	121	12	207
range in weight loss (mg/cm³)	−8–243	−1–19	190–243
heartwood density (mg/cm³)	377	386	378
$d_{1.3}$ (mm)	175	175	186
Kerimäki:			
n	248	10	10
mean weight loss (mg/cm³)	80	−1	161
range in weight loss (mg/cm³)	−16–183	−9–15	152–178
heartwood density (mg/cm³)	337	339	323
$d_{1.3}$ (mm)	185	192	187

Statistical analysis

In order to test whether the difference in the concentration of resin acids or the moisture content of the heartwood originating from the decay-resistant and decay-susceptible trees was statistically significant, a non-parametric Mann-Whitney-Wilcoxon test using ranks of data was employed (Sokal and Rohlf 1995). We used SAS procedure NPAR1WAY (SAS Institute 1996), which gives the exact probabilities for rejecting a true null hypothesis. We applied the one-tailed test for testing the differences in the concentration of resin acids within groups of decay-resistant and susceptible trees. The test was justified since, according to our alternative hypothesis to the null hypothesis, the concentration of resin acids was higher in the heartwood of decay-resistant trees than in the heartwood of decay-susceptible trees. Setting the one-tailed alternative hypothesis was based on prior knowledge concerning the inhibitory effects of resin acids on fungal growth in laboratory tests (Hart *et al.* 1975; Eberhardt *et al.* 1994).

Results

In both progeny tests the total concentration of resin acids was clearly higher in the heartwood of decay-resistant than of decay-susceptible trees (Table 3). At Korpilahti the difference between the two groups was statistically significant, while at Kerimäki the difference was only close to statistical significance (Table 3). When the two progeny tests were compared, the total concentration of resin acids was higher at Korpilahti than at Kerimäki in both decay groups, but the difference was not statistically significant (Mann-Whitney-Wilcoxon test for non-degraded class $p = 0.156$, and for degraded class $p = 0.968$). Due to two evident outliers, which were excluded from the analysis, the sample size at Korpilahti decreased from ten to nine in both decay groups.

The concentrations of the individual resin acids are shown in Table 3. At Korpilahti the concentration of individual resin acids, excluding dehydroabietic acid, differed with statistical significance between the two groups, the decay-resistant heartwood having higher concentrations. The coefficient of variation was considerably lower among the decay-resistant trees for all resin acids except for isopimaric acid. At Kerimäki the trends in the concentration of resin acids were the same as at Korpilahti, but the differences were not statistically

Table 3. Concentration of abietane- and pimarane-type resin acids in decay-resistant and susceptible Scots pine heartwood in the two progeny tests. The probability of rejecting a true H_0 is estimated using a non-parametric Mann-Whitney-Wilcoxon test (exact p-value for one-tailed test). Sample size (number of trees per class) is expressed as n, and the coefficient of variation as CV (%)

	Resistant trees			Susceptible trees			M-W-W test
Compound	n	Mean, mg/g d.wt.	CV (%)	n	Mean, mg/g d.wt.	CV (%)	p
Korpilahti progeny test							
Pimarane-type resin acids:							
pimaric acid	9	4.45	33	9	2.30	65	0.007
sandaracopimaric acid	9	1.09	29	9	0.61	58	0.005
isopimaric acid	9	1.43	70	9	0.67	75	0.025
Total	9	6.98	36	9	3.58	65	0.007
Abietane-type resin acids:							
levopimaric + palustric a.	9	24.23	46	9	11.56	85	0.007
dehydroabietic acid	9	5.55	30	9	4.82	53	0.095
abietic acid	9	41.01	37	9	17.17	64	0.001
neoabietic acid	9	8.27	35	9	3.48	82	0.004
Total	9	79.06	34	9	37.03	69	0.004
Total resin acids	9	86.04	33	9	40.61	69	0.004
Kerimäki progeny test							
Pimarane-type resin acids:							
pimaric acid	10	3.51	66	10	2.75	53	0.241
sandaracopimaric acid	10	0.96	71	10	0.71	65	0.158
isopimaric acid	10	1.01	64	10	0.66	67	0.083
Total	10	5.48	59	10	4.12	55	0.158
Abietane-type resin acids:							
levopimaric + palustric a.	10	18.52	85	10	11.05	107	0.176
dehydroabietic acid	10	5.63	48	10	5.02	58	0.264
abietic acid	10	26.76	73	10	14.50	58	0.072
neoabietic acid	10	5.28	84	10	2.66	89	0.083
Total	10	56.18	74	10	33.24	73	0.072
Total resin acids	10	61.67	72	10	37.36	71	0.072

significant (Table 3). The coefficient of variation was also similar between the two decay groups. At Kerimäki the difference between the decay-resistant and susceptible heartwood was most evident for the concentration of abietic acid, the summed concentration of abietane-type resin acids, and the total concentration of resin acids.

The relative proportion of individual resin acids was similar in both progeny tests (Table 4). The abietane-type resin acids accounted for about 90 % of the total resin acids in both decay groups in both progeny tests. However, the proportion of abietane-type resin acids was slightly higher in the resistant group. The difference between the decay groups for most of the resin acids quantified was from 1 to 2 %, and even the small difference for some resin acids was statistically significant (Table 4). Only dehydroabietic and abietic acid showed slightly higher differences between the groups (4–7 %). The relative proportion of dehydroabietic acid was higher in the heartwood of the susceptible than of the resistant trees, which was an exception compared to the other resin acids of the abietane type.

Heartwood samples from the decay-susceptible trees were more hygroscopic than the ones from the decay-resistant trees (Table 5). The difference in moisture content between the two decay groups was 6.8 %, and the

Table 4. Relative proportion of abietic- and pimaric-type resin acids in decay-resistant and susceptible Scots pine heartwood in the two progeny tests. The probability of rejecting a true H_0 is estimated using a non-parametric Mann-Whitney-Wilcoxon test (exact p-value for two-tailed test). Sample size (number of trees per class) is expressed as n, and the coefficient of variation as CV (%)

	Resistant trees			Susceptible trees			M-W-W test
Compound	n	Mean, %	CV (%)	n	Mean, %	CV (%)	p
Korpilahti progeny test							
Pimarane-type resin acids:							
pimaric acid	9	5.2	17.0	9	5.8	6.9	0.094
sandaracopimaric acid	9	1.3	12.9	9	1.6	14.1	0.002
isopimaric acid	9	1.7	61.7	9	1.7	40.3	0.436
Total	9	8.2	22.3	9	9.1	9.7	0.094
Abietane-type resin acids:							
levopimaric + palustric a.	9	27.6	22.4	9	25.8	21.2	0.386
dehydroabietic acid	9	6.8	22.6	9	13.7	28.6	0.000
abietic acid	9	47.8	13.8	9	43.7	10.3	0.077
neoabietic acid	9	9.6	9.4	9	7.7	24.1	0.019
Total	9	91.8	2.0	9	90.9	0.9	0.094
Kerimäki progeny test							
Pimarane-type resin acids:							
pimaric acid	10	6.2	21.0	10	8.3	31.5	0.029
sandaracopimaric acid	10	1.6	15.0	10	2.0	28.5	0.123
isopimaric acid	10	2.0	67.5	10	1.8	36.3	0.971
Total	10	9.9	22.0	10	12.2	24.4	0.089
Abietane-type resin acids:							
levopimaric + palustric a.	10	26.7	27.1	10	24.9	39.6	0.529
dehydroabietic acid	10	11.1	30.8	10	16.3	60.8	0.143
abietic acid	10	44.6	10.4	10	40.1	24.3	0.280
neoabietic acid	10	7.8	34.7	10	6.6	38.0	0.481
Total	10	90.1	2.4	10	87.8	3.4	0.089

Table 5. Equilibrium moisture content (%) and absolute water content (mg/cm³) in Scots pine heartwood samples after moisture treatment in a saturated atmosphere (RH 100 %). The samples originated from the Korpilahti progeny test. The probability of rejecting a true H_0 is estimated using a non-parametric Mann-Whitney-Wilcoxon test (exact p-value for two-tailed test). Sample size was 10 in each group. Coefficient of variation of the moisture content is expressed as CV (%)

Decay classification of trees	Moisture content, %		Absolute water content, mg/cm³	
	Average	CV (%)	Average	CV (%)
Resistant	36.8	10.9	132.5	6.6
Susceptible	43.6	19.5	160.6	22.7
p-value, M-W-W test	0.089		0.060	

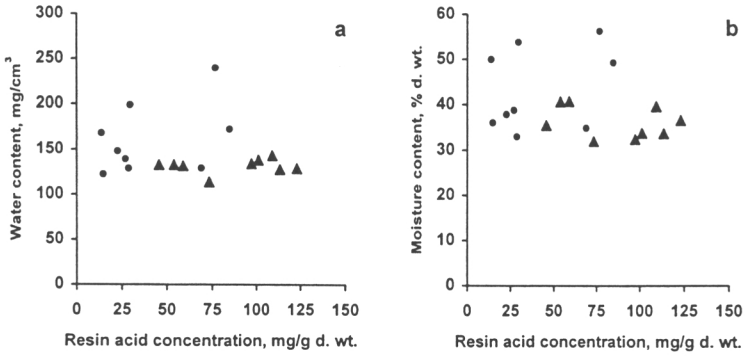

Fig. 2. Water content (a) and moisture content (b) in relation to the concentration of resin acids in decay-resistant and susceptible trees in the Korpilahti half-sib progeny test. ▲ = Resistant, ● = Susceptible.

difference was close to statistical significance. The difference in the absolute water content was 28.1 mg/cm³, which was also close to statistical significance.

Because of the non-continuous distribution of the observations due to arbitrary classification into two groups, we were not able to estimate the correlation between the concentration of resin acids and the moisture content. Visual inspection of the scattergram (Fig. 2) did not reveal any clear-cut relationship between the total concentration of analysed resin acids and the equilibrium moisture content or the absolute water content. A similar result was also obtained for individual resin acids.

Discussion

The concentration of resin acids in the Scots pine heartwood was higher in the group of brown-rot resistant than in the group of susceptible trees, as was expected. In the Korpilahti progeny test, where the trees had a straight growth habit, the concentration of resin acids in the heartwood of decay-resistant trees was about twofold that in the decay-susceptible trees. Although the difference was not as clear between the somewhat curved trees at Kerimäki, the trend was the same as that at Korpilahti. The resistant trees at Korpilahti had a higher concentration of resin acids in their heartwood than the resistant trees at Kerimäki, but this slight difference could be partly due to the sampling height, which was lower at Korpilahti. The concentration of resin acids is known to increase towards the base of the tree (Rissanen and Sirviö 2000). According to our results, there were no marked differences in the relative proportion of individual resin acids between the resistant and susceptible trees (Tables 4).

In our study, abietic and palustric + levopimaric acids clearly dominated over the other resin acids, and thus the relative proportion of abietane-type resin acids was about 90 % in both groups. In the study of Micales *et al.* (1994), the fungitoxic effects of abietane-type resin acids were greater than the fungitoxic effects of pimarane-type resin acids in agar medium cultures at a level of 200 µg resin acid per millilitre. Levopimaric, abietic, and neoabietic acid had the greatest effects on the radial growth of a brown-rot fungus, *Gloeophyllum trabeum* (Micales *et al.* 1994). In our study, levopimaric and palustric acid were quantified together, but the result is in agreement with those of Micales *et al.* (1994). In the study of Micales *et al.* (1994), dehydroabietic acid had a fungitoxic effect when agar medium was used for fungal culture. In our study, however, the concentration of dehydroabietic acid in the heartwood did not differ between the decay-resistant and susceptible trees. The different results obtained in these studies could be explained by the fungal species and experimental conditions used.

The differences in resin acid concentration between the decay groups were clearer in the straight-stem progeny test at Korpilahti, where the heritability of the weight loss due to brown-rot decay was markedly higher than that in the non-straight progeny test at Kerimäki (Harju *et al.* 2001; and unpublished results). The curved growth habit at Kerimäki was induced by a non-specified, experimentally uncontrolled factor of irregular occurrence in the progeny test. We speculated (Harju *et al.* 2001) that the reaction wood present in the curved stems decayed in the laboratory test at a different rate from that of the 'normal' wood. When grouping the trees according to the rate of decay, we did not take into account whether the stems were curved or not. Thus, the two groups at Kerimäki may include trees whose heartwood is resistant or susceptible for diverse reasons (structural or chemical), some of which are probably caused by the growth habit and thus induced by the environment.

The most important factors for the development of brown-rot decay in woody substrate are the moisture content, together with the properties of the wood, temperature conditions and time. The minimum response period required for decay development is strongly dependent on the water potential and temperature (Viitanen 1996). The water potential of wood, which means

the availability of water for the growth and penetration of organisms into the cell walls of the wood, is critical for decay development (Griffin 1977). The water potential depends on the composition and structure of the wood. When water passes into wood, the cell walls swell and more moisture can penetrate. The moisture saturation point, which for pine and spruce is around 30 % of the wood moisture content or an ambient relative humidity of 99.99 %, is often the minimum moisture content for decay development. A slow first stage of decay occurs, however, when the ambient relative humidity is around 96–98 % (Viitanen 1997).

In the present study, the differences in the equilibrium moisture content of the heartwood samples after two weeks' conditioning were rather small between the decay-resistant and susceptible trees. However, even a slight difference in the moisture content can affect the development of decay in wood samples during the early stages of a test (Viitanen 1996). The main wetting factor in the early stages of decay is a high relative humidity. In later stages, the brown-rot fungus transports water and thus increases the moisture content of the wood. Thus, the differences between the rate of decay in wood samples may be caused by the rate of water movement in the early stages of the decay processes, rather than by the equilibrium moisture content attained later. In our study, we did not follow the wetting rate of the heartwood samples.

In the experimental conditions used in our study, the results do not support the hypothesis that resin acids hinder the decay processes of the brown rot fungus via hydrophobic effects. The reasons for the slight differences in moisture content between the decay classes require further study.

The samples for the decay studies and for the analysis of resin acids were not sampled simultaneously, and even the sampling height was different. This has to be taken into account when evaluating the validity of the results. But, in spite of the sampling method, the results do show a difference, especially at Korpilahti, in the concentration of resin acids in the heartwood between the decay-resistant and susceptible trees. Although no definite conclusions can be drawn about the causal relationship between decay resistance and the concentration of resin acids in the heartwood, we suggest that the differences in resin acid concentration between the slowly and rapidly decaying trees were not coincidental. In addition to the components of oleoresin, phenolic compounds are also considered to be of great importance in the resistance of heartwood to fungal decay (Hart and Shrimpton 1979; Hart 1981; Shain 1995).

Acknowledgments

The Academy of Finland (Resource Council for the Environment and Natural Resources, project Nos. 43135, 43140, and 43159) supported this study, which belongs to the Finnish Forest Cluster Research Programme WOOD WISDOM (1998–2001). We thank Arto Haatainen, Heikki Kinnunen, Eija Matikainen and Jussi Tiainen from Punkaharju Research Station for sampling and handling the increment cores, and Terhi Vuorinen from the University of Kuopio for performing the chemical analyses. We also thank Liisa Seppänen at VTT Building and Transport for carrying out the decay tests. We are grateful to John Derome for checking the language.

References

Bruce, A. 1998. Biological control of wood decay. *In*: Forest Products Biotechnology. Eds. A. Bruce, J.W. Palfreyman. Taylor & Francis, London. pp. 251–266.

Eberhardt, T.L., J.S. Han, J.A. Micales and R.A. Young. 1994. Decay resistance in conifer seed cones: Role of resin acids as inhibitors of decomposition by white-rot fungi. Holzforschung *48*, 278–284.

Englund, F. and R.M. Nussbaum. 2000. Monoterpenes in Scots pine and Norway spruce and their emission during kiln drying. Holzforschung *54*, 449–456.

Flodin, K. and N. Fries. 1978. Studies on volatile compounds from *Pinus sylvestris* and their effect on wood-decomposing fungi. II. Effects of some volatile compounds on fungal growth. Eur. J. For. Path. *8*, 300–310.

Gershenzon, J. and R. Croteau. 1991. Terpenoids. *In*: Herbivores. Their Interactions with Secondary Plant Metabolites. Vol. I. The Chemical Participants. Eds. G.A. Rosenthal, M.R. Berenbaum. 2nd edition. Academic Press, San Diego. pp. 165–219.

Gref, R. and A. Ericsson. 1985. Wound-induced changes of resin acid concentrations in living bark of Scots pine seedlings. Can. J. For. Res. *15*, 92–96.

Griffin, D.M. 1977. Water potential and wood-decay fungi. Ann. Rev. Phytopathol. *15*, 19–29.

Harju, A.M., M. Venäläinen, E. Beuker, P. Velling and H. Viitanen. 2001. Genetic variation in the decay resistance of Scots pine wood against brown rot fungus. Can. J. For. Res. *31*, 1244–1249.

Hart, J.H. 1981. Role of phytostilbenes in decay and disease resistance. Ann. Rev. Phytopathol. *19*, 437–458.

Hart, J.H. and D.M. Shrimpton. 1979. Role of stilbenes in resistance of wood to decay. Phytopathology *69*, 1138–1143.

Hart, J.H., J.F. Wardell and R.W. Hemingway. 1975. Formation of oleoresin and lignans in sapwood of white spruce in response to wounding. Phytopathology *65*, 412–417.

Henriks, M.-L., R. Ekman and K. von Weissenberg. 1979. Bioassay of some resin acid and fatty acid with *Fomes annosus*. Acta Academiae Aboensis, Ser. B. *39*, 1–7.

Hillis, W.E. 1987. Heartwood and Tree Exudates. Springer-Verlag, Berlin. 268 p.

Kainulainen, P., H. Satka, A. Mustaniemi, J.K. Holopainen and J. Oksanen. 1993. Conifer aphids in an air-polluted environment. II. Host plant quality. Env. Pollution *80*, 193–200.

Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, Orlando, FL. 811 p.

Langenheim, J.H. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles. J. Chem. Ecol. *20*, 1223–1280.

Löyttyniemi, K. 1986. Männyn sydänpuu – luonnon kestopuuta. Männyn sydänpuun luontaisen lahon- ja hyönteistuhokestävyyden hyväksikäytöstä. Summary: On natural durability of pine heartwood. Metsäntutkimuslaitoksen tiedonantoja *231*, 1–50.

Martinez-Inigo, M.J., P. Immerzeel, A. Gutierrez, J.C. del Rio and R. Sierra-Alvarez. 1999. Biodegradability of extractives in sapwood and heartwood from Scots pine by sapstain and white-rot fungi. Holzforschung *53*(3), 247–252.

Micales, J.A., J.S. Han, J.L. Davis and R.A. Young. 1994. Chemical composition and fungitoxic activities of pine cone extractives. *In*: Biodeterioration Research 4. Mycotoxins,

Wood Decay, Plant Stress, Biocorrosion, and General Biodeterioration. Eds. G.C. Llewellyn, W.V. Dashek, C.E. O'Rear. Plenum Press, New York. 673 p.

Panshin, A.J. and C. de Zeeuw. 1980. Textbook of Wood Technology. Structure, Identification, Properties, and Uses of the Commercial Woods of the United States and Canada. 4th ed. McGraw-Hill, New York. 722 p.

Rissanen, A. and J. Sirviö. 2000. Männyn (*Pinus sylvestris*) ja kuusen (*Picea abies*) puuaineen ja -kuitujen ominaisuuksien vaihtelu. University of Helsinki. Department of Forest Resource Management Publications *23*, 1–77.

SAS Institute Inc. 1996. SAS/STAT® Software: Changes and Enhancements through Release 6.11. SAS Institute Inc. Cary, N.C. 1104 p.

Schuck, H.J. 1982. Monoterpenes and resistance of conifers to fungi. *In*: Resistance to Diseases and Pests in Forest Trees. Eds. H.M. Heybrock, B.M. Stephan, K. Wissenberg. Pudog, Wageningen pp. 169–175.

Shain, L. 1995. Stem defense against pathogens. *In*: Plant Stems: Physiology and Functional Morphology. Ed. B.L. Gartner. Academic Press, San Diego. pp. 383–406.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry. 3rd ed. W.H. Freeman and Company, New York. 887 p.

Venäläinen, M., A. Harju, T. Nikkanen, L. Paajanen, P. Velling and H. Viitanen. 2001. Genetic variation in the decay resistance of Siberian larch (*Larix sibirica* Ledeb.) wood. Holzforschung *55*(1), 1–6.

Viitanen, H. 1996. Factors affecting the development of mould and brown rot decay in wooden material and wooden structures. Effect of humidity, temperature and exposure time. Dissertation Uppsala. The Swedish University of Agricultural Sciences, Department of Forest Products. Thesis. 58 p.

Viitanen, H. 1997. Critical time of different humidity and temperature conditions for the development of brown rot decay in pine and spruce. Holzforschung *51*(2), 99–106.

Viitanen, H. and J. Bjurman. 1995. Mould growth on wood at fluctuating humidity conditions. Mat. und Org. *29*(1), 27–46.

Viitanen, H., L. Paajanen, T. Nikkanen and P. Velling. 1998. Decay resistance of Siberian larch wood against brown rot fungi. Part 2. The effect of genetic variation. The International Research Group on Wood Preservation, Stockholm. IRG Doc. No: IRG/WP 98–10287. 6 p.

Yamada, T. 1992. Biochemistry of gymnosperm xylem responses to fungal invasion. *In*: Defense Mechanisms of Woody Plants against Fungi. Eds. R.A. Blanchette, A.R. Biggs. Springer-Verlag, New York. pp. 146–164.

Zabel, R.A. and J.J. Morrell. 1992. Wood Microbiology: Decay and its Prevention. Academic Press, San Diego. 476 p.

Received April 26th 2001

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V

The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood

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Abstract The concentrations of three individual stilbenes, pinosylvin (PS), pinosylvin monomethyl ether (PSM), and pinosylvin dimethyl ether (PSD), and the total concentration of phenolic compounds were determined in 34-year-old Scots pines which were known to have either decay-resistant or susceptible heartwood. The sample trees were selected from two progeny tests among 783 trees, the decay resistance of which had been screened earlier *in vitro* against a brown-rot fungus *Coniophora puteana*. Ten decay-resistant and ten susceptible trees from each of the progeny tests were analysed. In the heartwood of the resistant trees the average total concentration of the stilbenes was 7.5 and 6.4 mg/g of dry weight, while in the heartwood of the susceptible trees the respective values were 5.0 and 4.7 mg/g. The difference between the decay resistant and susceptible trees was statistically significant in both of the progeny tests. The difference in the concentration of total phenolics, analysed by the Folin-Ciocalteu method, was also significant. A high concentration of phenolics was connected to the low hygroscopicity of wood. The results support the argued hypothesis that the stilbenes make a contribution to the differences in the decay rate of natural wood substrate. On the other hand, the results show that the stilbenes alone do not explain the variation in the decay rate.

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Keywords: *Pinus sylvestris*, *Coniophora puteana*, stilbenes, pinosylvin, hygroscopicity of wood, Folin-Ciocalteu

This study has been supported by the Academy of Finland (Resource Council for the Environment and Natural Resources, projects no. 43140, 43159 and 620012) and belongs to The Finnish Forest Cluster Research Programme WOOD WISDOM. We thank Arto Haatainen, Heikki Kinnunen, Eija Matikainen and Jussi Tiainen from Punkaharju Research Station for looking after the wood sampling, Terhi Vuorinen for the chemical analyses in Kuopio, Tapio Laakso and Veikko Kitunen for the GLC-MS analyses in Vantaa, and John Derome for revising the language.

Introduction

The heartwood of Scots pine (*Pinus sylvestris* L.) contains considerable amounts of two phenolic secondary compounds (called later as phenolics). They belong to the important group of stilbenes (Higuchi 1985, Yamada 1992), and were named as pinosylvin (PS) and pinosylvin monomethyl ether (PSM) by Erdtman as early as 1939. The traditional reputation of Scots pine heartwood durability was connected to the occurrence of PS and PSM soon after they had been discovered and found to be very toxic compounds (Erdtman 1939). The toxicity was demonstrated against several decay fungi by adding PS or PSM to the agar or liquid media and observing the inhibiting effect (Rennerfelt 1943, 1945). The fact that there was a clear difference in the PS and PSM content and decay resistance between the outer and inner part of heartwood (Erdtman and Rennerfelt 1944), confirmed the conclusion of the role of pinosylvins for decades.

However, the actual role played by stilbenes in the natural decay resistance of wood remained unclear, and doubt has even been cast about their importance. Already Erdtman and Rennerfelt (1944) concluded that "no parallelism exists between the inhibitory activity of these substances in agar cultures and in natural heartwood". Furthermore, when wood blocks were impregnated with PS and PSM, the inhibiting effect on the fungi was stronger than that with the same concentration in natural heartwood (Rennerfelt 1947). In their review on the mechanisms of decay resistance, Scheffer and Cowling (1966) presented strong arguments for the toxicity of wood extractives. However, they considered agar media tests to be merely indicative, and emphasised the necessity of wood substrate tests. In the study of Loman (1970), lodgepole pine heartwood meal was added to the agar media and *Coniophora puteana* was found to survive 200 p.p.m of PS, while 50 p.p.m. was lethal on malt agar without wood meal. Hart and Shrimpton (1979) concluded that the stilbenes alone do not provide heartwood with resistance against fungal decay. According to Hart (1981), since the precise location and chemical nature of stilbenes in wood is poorly known, it is difficult to explain the antifungal role of these compounds in nature, even though there is positive correlation between the stilbene concentration and the decay resistance. Schultz et al. (1997) and Schultz and Nicholas (2000) recently proposed a dual functioning mechanism for stilbenes: they have some limited fungicidal activity, but they are primarily excellent antioxidants that interfere with the free-radical degradative mechanism of fungi (see e.g. Ritschkoff 1996).

A wide variation in the durability of juvenile Scots pine heartwood has been found in two independent laboratory tests in which 783 trees in total have been studied (Harju et al. 2001b, Harju and Venäläinen 2001). The testing has been performed against a brown-rot fungus, *Coniophora puteana* (Schum.ex Fr) Karst., common in buildings damaged because of decay (Viitanen and Ritschkoff 1989). It is not known whether the degrading activity of the fungus in the tests has been affected by toxic or antioxidant compounds, by hydrophobic compounds, by me-

chanical barriers (of either anatomical or chemical origin) making the wood less permeable, or by the amount of cellulose and lignin present in the wood. The large number of trees tested and the wide durability variation have given an outstanding opportunity to study in which wood characters there are differences between the slowly and rapidly decaying, i.e resistant and susceptible, Scots pine heartwood.

The aim of this study was to analyse the difference in the concentration of total phenolics, and especially pinosylvin and its derivates, between brown-rot resistant and susceptible Scots pine heartwood. The concentration of pinosylvins was also compared with the hygroscopic properties of the wood. The sample trees were selected among 783 trees, the decay resistance of which had been screened earlier *in vitro* and reported by Harju et al. (2001b) and Harju and Venäläinen (2001).

Materials

The sample trees

The sample trees for the preceding decay tests were obtained from two progeny tests, one at Kerimäki (248 trees) and another at Korpilahti (535 trees), Finland. The heartwood samples, one per each tree, were taken from standing trees with a 5 mm increment core borer as described in Harju et al. (2001b) and Harju and Venäläinen (2001). Decay resistance was studied at VTT Building Technology using a malt agar plate decay test, which is a modification of the standardised EN 113 decay test (Viitanen et al. 1998, Venäläinen et al. 2001). The non-extracted increment core sections were placed on a pure culture of a brown rot fungus, *C. puteana* for 6 (Kerimäki) or 8 (Korpilahti) weeks. The weight loss of the samples, caused by the decay fungus during the incubation, was used as an inverse measure for the decay resistance.

The results of the decay tests were used to perform a new sampling for chemical wood analyses. The tails of the weight loss distributions were culled in order to obtain two extreme groups of trees in respect to wood decay resistance: 10 trees with slowly degrading and 10 trees with rapidly degrading heartwood at both locations. At Kerimäki there was no weight loss in the slowly degrading group while the weight loss in the rapidly degrading group was 161 mg/cm^3. At Korpilahti the respective figures were 12 mg/cm^3 and 207 mg/cm^3. The sample groups are described closer in the study of Harju et al. (2001a) in which exactly the same wood material has been used. At Kerimäki sapwood samples were also included in the decay test. The average weight loss of them was 114 mg/cm^3. At Korpilahti no sapwood samples were included in the decay test.

The wood samples for chemical analyses

The wood samples for the chemical analyses were taken from the standing sample trees at the age of 34 years. An 8 mm increment core borer was used in order to obtain a sufficient sample volume. At Kerimäki, where the interval between the two samplings was two growing seasons, the sampling for chemical analysis was done

in the next inter-whorl above and below the inter-whorl in which the first sampling had been made. This was necessary in order to avoid any induced changes in the wood chemistry. The average height of the sampling was 151 cm. At Korpilahti there was a dormant season between the two samplings and thus the sampling for chemical analysis was done 10 – 20 cm above and below the previous sampling point within the same inter-whorl the average height of the sampling being 91 cm. The heartwood section was marked on the increment core immediately after boring according to the visual difference in the moisture content between sapwood (wet) and heartwood (dry). The increment cores were stored in sealed tubes at a temperature of -5°C for about two weeks.

The heartwood sections were separated from the increment cores, and the sections then oven-dried at a temperature of +60°C for 48 hours, cooled and stored in a desiccator prior to grinding with an analysis mill (Kinematica). The heartwood of the upper and lower sample cores from each tree were mixed in the grinding. The milled samples were stored in sealed bottles in the dark at a temperature of -20°C.

Methods

Chemical analyses

In order to determine the total concentration of all phenolic compounds, 100 mg of milled wood was extracted with 80 % (v/v) aqueous acetone (Kainulainen et al., 1993). The phenols were determined by the Folin-Ciocalteu technique as described by Julkunen-Tiitto (1985). Tannic acid was used as a standard and thus the results are expressed as tannic acid equivalents (mg TAE /g).

In order to determine the concentrations of individual stilbenes, 250 mg of milled wood was extracted with acetone (30 ml) in a mini-Soxhlet apparatus for 6 hrs. Internal standard was added to the solvent (1.0 mg diethylstilbestrol, Sigma, USA). The extracts were evaporated to dryness and stored under nitrogen. Trimethylsilyl (TMS) esters were prepared by adding 0.2 ml bis-(trimethylsilyl)-trifluoroacetamide (Sigma, USA) in 0.2 ml dry pyridine, and heated for 1 hr at 100 °C. The pinosylvin (PS) and its monomethyl (PSM) and dimethyl ethers (PSD) were determined by GLC-MS (HP 6890, mass selective detector 5873) using a wall-coated fused silica column HP-5 (Hewlett-Packart, USA), ID 250 µm, length 30 m, film thickness 0.25 µm; column temperature program: initial 110 °C, 10 °C/min to 300 °C, total run time 34 min; injector temp. 260 °C, transfer line 280, °C MS source 230 °C, split ratio 1:21. Helium was used as carrier gas at a flow rate of 1.2 ml/min (93kPa).

Statistical analyses

In order to test whether the heartwood of decay resistant and susceptible trees was equal as regards the studied variables, the Mann-Whitney U-test using Wilcoxon scores (ranks of data) was employed. The test was carried out by the SAS procedure NPAR1WAY, which provides the exact p-values for the rank statistics (SAS Insti-

tute 1996). A one-sided test was applied for phenolics, since the alternative hypothesis for the null hypothesis was that the concentration of phenolics is higher in the heartwood of decay resistant trees. The one-sided alternative hypothesis was justified since, according to earlier reports, phenolic compounds reduce the fungal growth in laboratory tests.

The relationships between the variables were illustrated by means of scattergrams since the calculation of correlation coefficients was not justified owing to the discontinuous nature of the analysed material.

Results

Each of the studied 40 trees contained measurable concentrations of PS and PSM in their heartwood, while PSD was found only in 33 trees. The average total concentration of the three stilbenes was 0.59 % of dry weight. The mean concentrations and the coefficients of variation (CV %) presented in Table 1 are grouped according to the progeny test location and decay resistance classification. The difference in the concentration of PS between the resistant and susceptible trees was significant at Korpilahti ($p < 0.001$) and at Kerimäki ($p = 0.045$). The difference in the concentration of PSM was significant at an equal risk level in both progeny tests ($0.001 < p < 0.01$). The concentration of PSM was about 15-20 % higher than that of PS, apart from in the group of susceptible trees at Kerimäki where the concentration of PS was slightly higher. The concentration of PSD was low in all the groups, and the difference between the resistant and susceptible trees was not significant. The result of the Folin-Ciocalteu test, reflecting non-specifically the concentration of all phenolic compounds, showed a significant difference between the resistant and susceptible trees ($p < 0.001$ at Korpilahti and $p = 0.002$ at Kerimäki).

According to the scattergrams, the concentration of PS and PSM appeared to correlate positively at both progeny test locations (Fig. 1a, b). The relationship between the total concentration of pinosylvins (PS+PSM+PSD) and the result of the Folin-Ciocalteu test was weak at Korpilahti (Fig. 1c), and at Kerimäki no relationship was detected (Fig. 1d). The grid in Fig. 1c shows that, in the Korpilahti data, the resistant and susceptible trees could be discriminated rather reliably by either the total pinosylvin concentration or the Folin-Ciocalteu test. However, combining these two parameters gave almost perfect discrimination. In the Kerimäki data (Fig. 1d) the same parameters were not as effective in discriminating the resistant and susceptible trees.

Table 1. Comparison of brown-rot decay resistant and susceptible Scots pine heartwood sampled from progeny tests at Korpilahti and Kerimäki, Finland, (10 resistant and 10 susceptible 34-year-old trees from both tests). The classification was based on heartwood weight loss in a laboratory test [1]. The probabilities of rejecting a true H_0 of equality between the groups are presented on the basis of a non-parametric Mann-Whitney-Wilcoxon U-test (exact p-values for one-sided test).

	resistant		susceptible		M-W-W test
	mean	CV(%)	mean	CV(%)	p
Korpilahti					
pinosylvin (PS), mg/g	3.29	16.7	2.29	13.5	**<0.001**
pinosylvin monomethyl ether (PSM), mg/g	4.04	29.4	2.64	37.4	**0.007**
pinosylvin dimethyl ether (PSD), mg/g	0.136	64.3	0.114	43.3	0.241
total pinosylvins, mg/g [2]	7.47	23.1	5.04	23.9	**0.001**
total phenols, mg TAE /g [3]	13.6	28.5	6.7	50.0	**<0.001**
water content, mg/cm^3 of fresh vol., at 100% RH [4][5]	132	6.7	161	22.7	0.060 [6]
Kerimäki					
pinosylvin (PS), mg/g	2.93	23.0	2.41	32.8	**0.045**
pinosylvin monomethyl ether (PSM), mg/g	3.41	29.9	2.22	47.3	**0.009**
pinosylvin dimethyl ether (PSD), mg/g	0.093	83.4	0.086	93.3	0.348
total pinosylvins, mg/g	6.43	24.0	4.72	39.4	**0.022**
total phenols, mg TAE/g	13.2	29.2	8.30	27.0	**0.002**

[1] VTT Building Technology; tested against a brown-rot fungus *Coniophora puteana*
[2] Finnish Forest Research Institute, Vantaa Research Centre
[3] Folin-Ciocalteu –test, University of Kuopio, Department of Ecology and Environmental Science
[4] from Harju *et al.* (manuscript)
[5] University of Kuopio, Department of Applied Physics
[6] two-sided test

There was a connection between the amount of absorbed water, reported by Harju et al. (2001a) and the concentration of pinosylvins: when the total concentration of PS and PSM exceeded 6 mg/g, the amount of water in the conditioned wood samples was low, about 130 mg/cm3, and there was hardly any variation in this property (Fig. 1e). No relationship was found between the PS or PSM concentration and the total concentration of resin acids (Fig. 1f).

Discussion

The results showed that the heartwood of each relatively young Scots pine contained heartwood phenols, i.e. pinosylvin (PS) and pinosylvin monomethyl ether (PSM). Furthermore, the results showed that there was a significant difference in

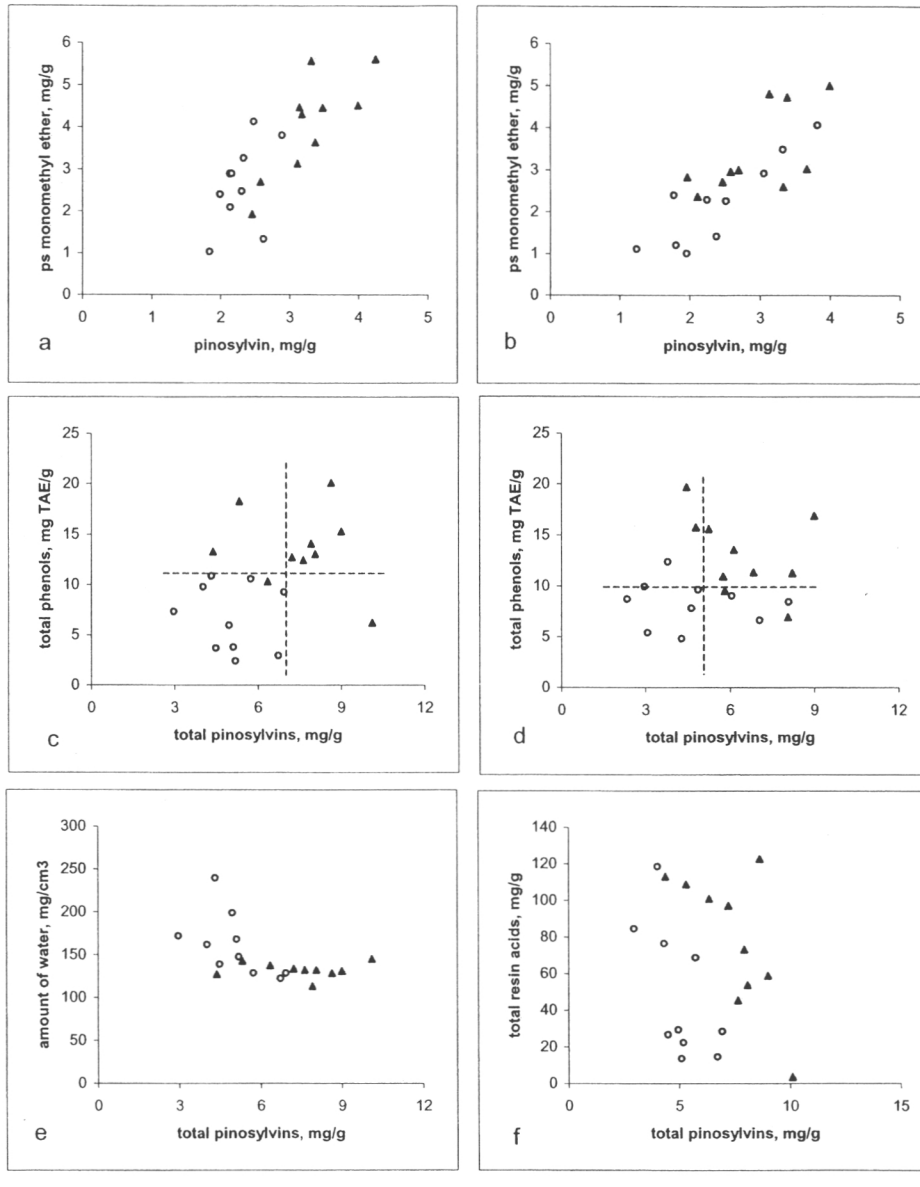

Figure 1. Concentration of heartwood phenolics in brown-rot resistant (▲) and susceptible (○) Scots pines in two progeny tests, Korpilahti (a,c) and Kerimäki (b,d), Finland, and the relationship between the phenolics concentration and the water content (e) and resin acid concentration (f) at Korpilahti (data partly from Harju et al. (2001a) in e and f).

the average concentration of pinosylvins between the decay-resistant and susceptible heartwood. The present study is the first one in which the differences in the decay resistance of natural wood substrate have been compared with the concentration of phenolics using data which is valid for statistical testing.

The stilbenes PS and PSM, which are the major secondary phenolic compounds occurring in Scots pine heartwood but scarce in sound sapwood, have been associated with heartwood durability ever since they were first found to be very toxic to fungi as free compounds (Rennerfelt 1943, 1945). However, several researchers have warned about the dangers of drawing too straightforward conclusions on the basis of agar or liquid media tests because the stilbenes may behave in a totally different way in such tests compared to the situation in a natural wood substrate (Erdtman and Rennerfelt 1944, Rennerfelt and Nacht 1955, Scheffer and Cowling 1966, Loman 1970, Hart and Shrimpton 1979, Hart 1981). Indeed, the mean concentrations of PS and PSM found in the susceptible trees in this study, 0.22-0.26 % of dry weight, were clearly higher than the concentration of 0.005 % known to be inhibitory for *C. puteana* in the agar media (Rennerfelt and Nacht 1955, Loman 1970). Our results do not either indicate any absolute threshold concentration for PS or PSM, which would guarantee a slow decay rate. The concentration ranges of the resistant and susceptible trees overlapped. Furthermore, the results show that the reason for the wide variation in the decay rate of juvenile heartwood can't be the total absence of stilbenes in the rapidly degrading heartwood. At Kerimäki, where the group of rapidly degrading heartwood was less durable than the sapwood in average, the heartwood contained stilbenes. These findings support the doubt concerning the importance of the stilbenes and suggest that the stilbenes alone do not cause the variation in the decay rate between heartwood and sapwood.

PS has been considered to be more toxic to fungi than PSM (e.g. Rennerfelt and Nacht 1955). In the present study the concentrations of these two compounds were positively correlated and it is therefore not possible to state unambiguously which of them has the greater effect on the brown-rot fungus. However, the result for Kerimäki, where the difference in the concentration of PSM between the resistant and susceptible heartwood was higher and more significant compared to that of PS, suggests that PSM may also play a role in the mechanism of decay resistance.

A nearly significant difference in the equilibrium moisture content at 100 % RH between the decay resistant and susceptible heartwood at Korpilahti has been reported by Harju et al. (2001a), but contrary to expectation, the differences in the hygroscopicity of the wood samples were not related to the resin acid content. The present data suggest that the differences in hygroscopicity may instead be related to the concentration of phenolic compounds. In actual fact, the heartwood section of the fresh increment cores was clearly distinguishable according to a visible moisture content boundary! However, the low hygroscopicity alone did not appear to be sufficient to prevent the functioning of *C. puteana*, since the group of samples with the lowest water content also included susceptible trees (Fig. 2e). The indication of a relationship between hygroscopicity and phenolic compounds is in an accordance

with the report of Vologdin et al. (1979), who reported that the radial permeability of pine wood for gases and liquids can be markedly improved by extracting out the phenolic compounds. Also Celimene et al. (1999) recently concluded that "all the stilbenes tested seem to protect the wood by their water repellency properties".

The Folin-Ciocalteu method has been used widely to determine the total concentration of phenols occurring in plants (see e.g. Singleton et al. 1974). In the present study, the difference in the results of the the Folin-Ciocalteu test between the resistant and susceptible heartwood was significant at both locations, and thus this simple test may prove to be useful for screening decay-resistant trees. However, the connection between the Folin-Ciocalteu values and the concentration of PS and PSM, which in reality are the only phenolics in Scots pine heartwood (Steffen et al. 1990), seemed to be weak in this data. The reason for the weak connection may have been the abundance of other substances, present in the aqueous acetone extract, that are not phenolic compounds but may react to some extent with the non-specific Folin-Ciocalteu reagent.

The results of this study support the hypothesis that the stilbenes PS and PSM make a contribution to the differences in the decay rate of Scots pine heartwood. This study does not reveal, however, the kind of mechanism through which stilbenes enhance the decay resistance. Either one of the hypotheses concerning toxic, antioxidant or hydrophobic effects is true, or then all these effects play a joint role. On the other hand, the results show that the stilbenes alone do not explain the variation in the decay rate of natural wood substrate.

References

Celimene CC, Micales JA, Ferge L, Young RA (1999) Efficacy of pinosylvins against white-rot and brown-rot fungi. Holzforschung 53: 491-497

Erdtman VH (1939) Die phenolischen Inhaltsstoffe des Kiefernkernholzes, ihre physiologische Bedeutung und hemmende Einwirkung auf die normale Aufschließbarkeit des Kiefernkernholzes nach dem Sulfitverfahren. Justus Liebigs Annalen der Chemie 539: 116-127

Erdtman VH, Rennerfelt E (1944) Der Gehalt des Kiefernkernholzes an Pinosylvin-Phenolen. Ihre quantitative Bestimmung und ihre hemmende Wirkung gegen Angriff verschiedener Fäulpilze. Svensk Papperstidning 47: 45-56

Hart JH (1981) Role of phytostilbenes in decay and disease resistance. Ann Rev Phytopathol 19: 437-458

Hart JH, Shrimpton DM (1979) Role of stilbenes in resistance of wood to decay. Phytopathology 69: 1138-1143

Harju AM, Kainulainen P, Venäläinen M, Tiitta M, Viitanen H (2001a) Differences in resin acid concentration between brown-rot resistant and susceptible Scots pine heartwood. Holzforschung. In press.

Harju AM, Venäläinen M (2001) Genetic parameters regarding the resistance of *Pinus sylvestris* heartwood to decay caused by *Coniophora puteana.* Scandinavian Journal of Forest Research. In press.

Harju AM, Venäläinen M, Beuker E, Velling P, Viitanen H (2001b) Genetic variation in the decay resistance of Scots pine wood against brown rot fungus. Can. J. Forest Res. 31:1244-1249

Higuchi T (1985) Biosynthesis and biodegradation of wood components. Academic Press, Orlando, 679 p

Julkunen-Tiitto R (1985) Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. Journal of Agriculture and Food Chemistry 33: 213-217

Kainulainen P, Satka H, Mustaniemi A, Holopainen JK, Oksanen J (1993) Conifer aphids in an air-polluted environment. II. Host plant quality. Environ. Pollut. 80: 193-200

Loman A (1970) Bioassays of fungi isolated from *Pinus contorta* var. *latifolia* with pinosylvin, pinosylvinmonomethyl ether, pinobanksin, and pinocembrin. Can. J. Bot. 48: 1303-1308

Rennerfelt E (1943) Die Toxizität der phenolischen Inhaltsstoffe des Kiefernkernholzes gegenüber einigen Fäulnispilzen. Svensk Botanisk Tidskrift 37: 83-93

Rennerfelt E (1945) The influence of the phenolic compounds in the heartwood of Scots pine (*Pinus silvestris* L.) on the growth of some decay fungi in nutrient solution. Svensk Botanisk Tidskrift 39: 311-318

Rennerfelt E (1947) Några undersökningar över olika rötsvampars förmåga att angripa splint- och kärnved hos tall. Meddelanden från statens skogsförsöksanstalt 36: 1-24

Rennerfelt E, Nacht G (1955) The fungicidal activity of some constituents from heartwood of conifers. Svensk Botanisk Tidskrift 49: 419-432

Ritschkoff A-C (1996) Decay mechanisms of brown-rot fungi. Technical Research Centre of Finland, Espoo. VTT Publications 268, 67 p

SAS Institute Inc (1996) SAS/STAT® Software: Changes and Enhancements through Release 6.11. SAS Institute Inc Cary NC, 1104 p

Scheffer TC, Cowling EB (1966) Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4: 147-170

Schultz TP, Nicholas DD (2000) Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry 54: 47-52

Schultz TP, Nicholas DD, Fisher TH (1997) Quantitative structure-activity relationships of stilbenes and related derivatives against wood-destroying fungi. Recent Research Development in Agricultural and Food Chemistry 1: 289-299

Singleton VL, Orthofer R, Lamuela-Raventós RM (1974) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Amer. J. Enol. Viticult. 25: 152-178

Steffen A, Frühwald A, Tamminen Z, Puls J (1990) Biological, chemical, and physical properties of Scots pine from Sweden under special consideration of crown defoliation. The Swedish University of Agricultural Sciences, Department of Forest Products, Report No 217, Uppsala, 312 p

Venäläinen M, Harju AM, Nikkanen T, Paajanen L, Velling P, Viitanen H (2001) Genetic variation in the decay resistance of Siberian larch (*Larix sibirica* Ledeb.) wood. Holzforschung 55(1): 1-6

Viitanen H, Paajanen L, Nikkanen T, Velling P (1998) Decay resistance of Siberian larch wood against brown rot fungi. Part 2. The effect of genetic variation. The

International Research Group on Wood Preservation, Stockholm, IRG Doc. No: IRG/WP 98-10287, 6 p

Viitanen H, Ritschkoff A-C (1989) Ruskolahon kehittyminen ja leviäminen puurakenteissa [Brown-rot decay in wooden construction]. Valtion teknillinen tutkimuskeskus, Technical Research Centre of Finland, Research Reports 637, 85 p

Vologdin AI, Razumova AF, Charuk EV (1979) Die Bedeutung der Extraktstoffe für die Permeabilität von Kiefern- und Fichtenholz. Holztechnologie 20(2): 67-69

Yamada T (1992) Biochemistry of gymnosperm xylem responses to fungal invasion. In: Blanchette, R.A. & Biggs A.R. (eds). Defense mechanisms of woody plants against fungi. Springer Verlag, New York, pp. 146-164

VI

Variation in the decay resistance and its relationship with other wood characteristics in old Scots pines

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Abstract - The importance of factors contributing to the natural decay resistance of Scots pine wood was studied. The decay rate of sapwood and outer and inner heartwood of 16 ca. 170-year-old Scots pines was first measured. A six-week decay test was performed with 5 x 15 x 30 mm wood blocks in dishes containing a brown-rot fungus (Coniophora puteana). The average mass loss in sapwood was 141 mg/cm3, in outer heartwood 57 and in inner heartwood 108. The variation between trees was largest in outer heartwood. The corresponding basic densities were 439, 456 and 411 mg/cm3. The mass loss was then compared with chemical characteristics and the sorption of water by parallel sample blocks in order to determine which factor has the greatest effect on decay resistance. The differences in heartwood mass loss were explained best by the concentration of pinosylvin and its monomethyl ether, which are phenolics belonging to the group of stilbenes, as well as by the concentration of total phenolics determined by the Folin-Ciocalteu method.

decay resistance/ heartwood/ phenolic compound/ pinosylvin/ resin acid/ moisture content

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Résumé - Variation de la résistance à la pourriture et relation avec les autres caractéristiques du bois dans les vieux pins sylvestres.
L'étude a porté sur l'importance relative des facteurs à l'origine de la résistance naturelle à la pourriture du pin sylvestre (*Pinus sylvestris*). Pour commencer, la vitesse de pourriture a été mesurée dans l'aubier et les parties externes et internes du duramen de 16 pins d'environ 170 ans. Un test de pourriture de six semaines a été effectué sur des blocs de 5x15x30 mm dans des boîtes de Petri, dans lesquelles le champignon lignivore de la pourriture brune (*Coniophora puteana*) se développait sur une base d'extrait de malt gélosé. Les pertes de poids de l'aubier, de la partie externe du duramen et de la partie interne du duramen ont été de 141, 57 et 108 mg/cm3, respectivement. La variation entre les arbres était la plus grande dans la partie superficielle du duramen. Les densités du bois correspondantes étaient de 439, 456 et 411 mg/cm3. Ensuite, les pertes de poids, les caractéristiques chimiques des blocs adjacents et la quantité d'eau absorbée par ces derniers ont été comparées, dans le but de déterminer les facteurs affectant le plus la résistance à la pourriture du bois. Ce sont la teneur en composés phénoliques, en pinosylvine et éther mono-méthylique de cette dernière, faisant partie du groupe des stilbènes, et la teneur en phénols totale déterminée par le réactif de Folin-Ciocalteu qui expliquent le mieux les différences de pertes de poids du duramen. Les différences s'expliquent aussi dans une certaine mesure par le taux d'humidité du bois atteint dans une humidité élevée (HR de 100 %). Une corrélation significative existait entre la quantité de stilbènes et la quantité d'eau absorbée par le bois immergé dans l'eau.

résistance à la pourriture/ duramen/ composés phénoliques/ pinosylvine/ acides résiniques/ taux d'humidité

1. INTRODUCTION

Several factors have been postulated to contribute to the variation in the natural durability of wood in different tree species. The same factors may also partly cause the variation between different stem sections and between individuals within durable tree species. These factors are mainly associated with the wood extractives that inhibit the primary metabolism or degradation processes of the fungi, or with the permeability of the wood for water, air and fungal hyphae [23]. Approximately the same factors are involved in the formation of heartwood. The difference in the durability of the sapwood and heartwood in several species is the clearest evidence of within-stem variation, and this difference well demonstrates the potential of natural wood-preservation mechanisms.

The interaction between a rot fungus and construction timber is an attempt by a living organism to colonise dead organic tissue that possesses only passive defence mechanisms. In passive defence the question is whether the wood serves as a suitable living environment for the fungus or not (e.g. [21]). There is no danger of decay as long as the moisture content of the wood remains clearly below the fibre saturation point because easily available water is necessary for several of the metabolic functions of the fungus. If the moisture content remains high for extended periods, then the risk of fungus invasion is high. If desiccation does not take place after colonisation, only the constitutional substances of the wood can interfere with the enzymatic or oxidative reactions caused by the fungus and thus decrease the rate of decomposition.

Scots pine (*Pinus sylvestris*) timber is a widely used softwood in buildings in the Nordic countries. The most common fungus species causing decay damage to buildings in Finland are *Serpula lacrymans*, *Poria/Antrodia sp* and *Coniophora puteana*, all of which cause brown-rot [20]. Untreated Scots pine heartwood is classified as moderately to slightly resistant against decay, while the sapwood is classified as perishable [6]. Several studies have recently dealt with the resistance of the juvenile heartwood of relatively young Scots pines. They have demonstrated the genetic variation in decay resistance [11,13], genetic variation and differences in wood characteristics responsible for the resistance [4,7,8,12,26], as well as the genotypic correlations between these characteristics [5].

This study is a part of a larger project evaluating the possibilities to increase the amount and quality of Scots pine heartwood through tree breeding. The main aim of this study was to investigate the relationships between the decay resistance and chemical and hydrophobic properties of the wood, and thus quantify the importance of the individual factors contributing to the natural durability of Scots pine wood. The variation in the *in vitro* decay rate, and in the extractive content and sorption of water by the sapwood and inner and outer heartwood of mature Scots pine stems were determined.

2. MATERIAL

Twenty Scots pine trees were felled in Kuikonniemi stand (61o 47′ N, 29o 21′ E) in the Punkaharju Nature Conservation Area, Finland, in February 1999. The trees were 20-30 m high, co-dominant or dominant trees in a naturally regenerated, pure Scots pine stand. The age of the trees calculated at stump height was 150 – 190 years, with an average age of 172 years. The trunks were cut into commercial-sized logs, and a 100 mm sample disk was taken from the top of the first and second log. In cases were the wood in the stump appeared to be sound, the target length of the first log was 5 m. As the target length of the second log was also 5 m, the height of the second sampling point was about 10 m (figure 1). In cases were the visual assessment of the stump section surface indicated that the trunk was suffering from rot damage (six trees), the first log was cut to a length of 3 m and the length of the second log varied from 3 to 5 m depending on the soundness of the upper stem. The average number of annual rings in the lower disk was 149 and 131 in the upper one.

The boundary between the sapwood and the heartwood was marked on the disk immediately after cutting according to the clearly visual moisture difference. The average heartwood area of the stump section surface was 51 %. The disks were stored in plastic bags at a temperature of –5oC. In November 2000 the disks were cut into pieces as shown in figure 1. The sampling procedure gave four parallel 5 x 15 x 30 mm (tangential, radial, and longitudinal dimension) sized blocks from six points on each of the 40 disks. The total number of sampling points was 240. The volume of each block was 2.25 cm3. The blocks were stored in plastic boxes at a temperature of –5°C .

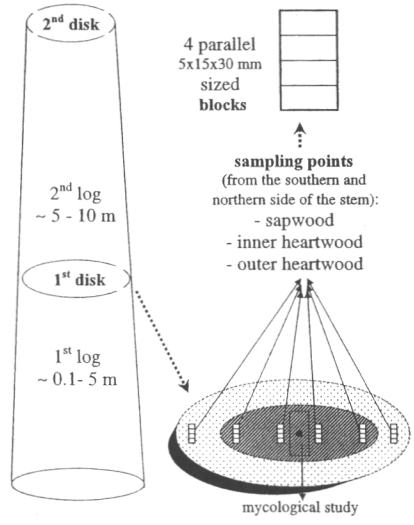

Figure 1. The sampling procedure showing the location of the disks and blocks in the individual trunks.

One additional piece of wood was taken from the centre of each lower disk for mycological studies. Four of the six suspect trees appeared to have heartwood infected by *Phellinus pini* at a height of 3 m. All the other 16 trees were found to be free of rot fungi.

One of the four parallel wood blocks was subjected to an in vitro decay test. Another block was used for determining the water sorption capacity. The remaining two blocks were milled to powder for the chemical analyses. The wood powder was stored in sealed ampoules at a temperature of –20○C. However, in order to reduce costs the chemical analyses were carried out only on samples from the 120 sampling points on the northern side of the stems.

3. METHOD

In vitro decay test

The decay rate was determined at VTT Building and Transport using a malt agar plate test, which is a modification of the standardised EN 113 decay test [25,27]. Three wood blocks per Petri dish were exposed to a pure culture of a brown rot fungus (*C. puteana*) for 6 weeks. The mass loss of the samples during the incubation, expressed per fresh volume of wood, was used as the measure of the decay rate and thus as an inverse measure of the decay resistance of the wood.

Determining the water sorption capacity

Water bound to hygroscopic cell wall constituents and into voids of wood of radius less than 1.5 µm is called *ad*sorbed water. This critical point of sorption is called the fibre saturation point. It represents a water potential of –0.1 MPa and, in theory, a relative humidity of 99.93 % [10]. The water present in the cell lumens and intercellular space is called free or *ab*sorbed water [29]. Adsorption was determined in a tightly closed steel tank that was half-filled with tap water (+25○C). The wood blocks (dried at 60○C for 48 hours) were placed on steel racks immediately above the water surface. The relative humidity of the tank atmosphere varied between 98 and 100 % in the beginning of the experiment, but stabilised to 100 % within about 50 hours (humidity sensor, Davis Instruments). The mass of the blocks was measured at increasing intervals 4, 8, 14, 24, 34, 48, 72, 96, 168, 240 and 336 hours after the start of the test to an accuracy of 1 mg. After the last measurement the blocks were dried at 103○C for 24 hours, and the dry mass measured. The results were presented as the ratio between the mass of adsorbed water and the mass of the dry wood. This ratio was called the moisture content. In the wetting experiment the same blocks were immersed in water. The mass of the

wet blocks was measured 1, 4, 9, 25, 49, 97 and 169 hours after the start of the test, after which the blocks were dried at 103oC for 48 hours. The results were expressed as the gross mass of water (i.e. adsorbed and absorbed) per fresh volume of wood. This variable was called the quantity of water after wetting.

Chemical analyses

Resin acids were extracted from the wood powder with petroleum ether-diethyl ether following the procedures of Gref and Ericsson [9]. The extracts were analysed by gas chromatography - mass spectrometry (Hewlett Packard GC type 6890, MSD 5973) using a 30 meter-long, HP-5MS (0.25 mm ID, 0.25 µm film thickness, Hewlett Packard) capillary column as described earlier by Manninen et al. [18]. For quantification of the individual resin acids, calibrations were made using known amounts of pure resin acids, and the response factors were determined for each substance relative to known amounts of the internal standard (heptadecanoic acid).

For the analysis of the total concentration of all phenolic compounds wood powder was extracted with 80 % (v/v) acetone for 30 minutes. The phenolics were determined by the Folin-Ciocalteu technique using tannic acid as standard [16,24].

For the quantification of individual stilbenes, i.e. pinosylvin (PS) and pinosylvin monomethyl ether (PSM), wood powder was extracted with 80 % (v/v) methanol. The extraction was carried out in tubes with vortex mixing at room temperature for 30 minutes. Vanillin was used as internal standard. The samples were centrifuged and the residue washed two times with 80 % methanol. The supernatants were combined and analysed by HPLC (Hewlett Packard series 1050, 1040 M Series II detection system) using a reversed phase capillary column (HP LiChrospher 100 RP-18, 5 µm, 250 x 4 mm). Analysis was performed by gradient elution with 1 % v/v acetic acid solution in water and methanol/acetonitrile/acetic acid (49.5:49.5:1 v/v/v) as described by Lieutier et al. [17]. The flow rate was 1 ml min-1 and detection wavelength 308 nm. Peak areas were used to quantify the individual substances, and the results (mg/g dry wt) were calculated relative to known amounts of internal standard. The final results of all the chemical analyses were presented as concentration per fresh volume of wood.

Statistical analysis

One-way ANOVA using tree-wise means was applied to test whether the sapwood and the outer and the inner heartwood differed from each other in the studied wood characteristics. The pair-wise comparisons between the stem sections were performed by Tukey's test. Tree-wise means were used in order to smooth out

the random variation between single observations. A simple regression model (response variable = $\beta 0 + \beta 1$ independent variable + ε) was applied to study whether the mass loss was dependant on the chemical or physical wood characteristics. The relationships between the independent characteristics were studied with correlation analysis.

The 16 sound trees were included in the statistical analysis. The distributions of the characteristics were first analysed, and 10 out of 96 full records (i.e. records containing decay test and chemical data) were excluded from the main results because of outliers. Four sapwood records were excluded because of a relatively high concentration of stilbenes (2.7 mg/cm3 on average). Six heartwood records were excluded because of very high concentration of resin acids (65 mg/cm3 on average).

4. RESULTS

The radial variations in basic density, mass loss, quantity of water after wetting and concentration of extractives were significant (table I). The difference between the basic density of the outer and inner heartwood reflected the differences in growth rate and in the properties of juvenile and mature wood. The difference between the basic density of the sapwood and outer heartwood was of the same magnitude as the difference in the mass of the extractives. The decay resistance was clearly best in the outer heartwood. However, according to the coefficient of variation (CV %), the variation among the trees was also clearly the highest in the outer heartwood. The decay resistance of the inner heartwood was approximately halfway between that of the outer heartwood and sapwood. The concentration of extractives clearly differed between the sapwood and heartwood; stilbenes were almost completely absent in the sapwood. The concentration of stilbenes and total phenolics in the outer and inner heartwood also differed significantly. The variation in the concentration of resin acids was still high among the trees even though the outliers were omitted.

Table I. The mass loss and chemical and physical wood characteristics of the 16 mature Scots pines. Each tree was represented by 1-4 samples in each radial section depending on the characteristic and the number of excluded outlying observations. Tree-wise means were used to calculate the overall means and standard deviations (sd) for the sapwood and the outer and the inner heartwood. The coefficients of variation (CV %) were used to describe the variation among the trees. One-way ANOVA was applied to test whether the radial sections differed significantly from each other. The pair-wise comparisons were performed by Tukey's test (– = ignificant difference; ,=non-significant difference; s=sapwood; o=outer heartwood; i=inner heartwood). PS=pinosylvin, PSM=pinosylvin monomethyl ether, TAE=tannic acid equivalent.

	sapwood		heartwood				ANOVA	pair-wise test
			outer		inner			
	mean (sd)	CV %	mean (sd)	CV %	mean (sd)	CV %	p-value	
basic density[1)] (mg/cm^3)	439 (32)	7.3	456 (38)	8.3	411 (37)	9.0	0.004	o,s – s,i
mass loss[1)] (mg/cm^3)	141 (19)	13.5	57 (29)	50.9	108 (23)	21.3	< 0.001	s – i – o
moisture content at RH 100% [1)] (%)	27.4 (1.06)	3.9	26.9 (0.94)	3.5	27.8 (1.21)	4.4	0.075	ns
quantity of water after wetting[1)] (mg/cm^3)	584 (29)	5.0	442 (26)	5.9	447 (32)	7.1	< 0.001	s – i,o
total resin acids[2)] (mg/cm^3)	1.90 (0.49)	25.8	8.01 (6.14)	76.7	7.93 (4.67)	58.9	< 0.001	o,i – s
total pinosylvins[2)] (mg/cm^3)	0.05 (0.09)	not est.	8.93 (2.60)	29.1	4.22 (1.63)	38.6	< 0.001	o – i – s
PS (mg/cm^3)	0.03 0.03	not est.	3.42 (1.20)	35.2	0.93 (0.58)	62.6	< 0.001	o – i – s
PSM (mg/cm^3)	0.03 0.05	not est.	5.51 (1.63)	29.5	3.29 (1.09)	33.1	< 0.001	o – i – s
total phenolics[2)] (mg TAE/cm^3)	0.27 (0.23)	86.1	2.82 (0.73)	25.9	1.79 (0.65)	36.3	< 0.001	o – i – s

[1)] (3-)4 samples per tree [2)] (1-)2 samples per tree

The difference between the quantity of water after wetting in the sapwood and the heartwood was significant. The respective difference in the moisture content at the end of the adsorption test was nearly significant. The variation in both of these characteristics among the trees was low. The adsorption and absorption curves are presented as a function of time in figure 2.

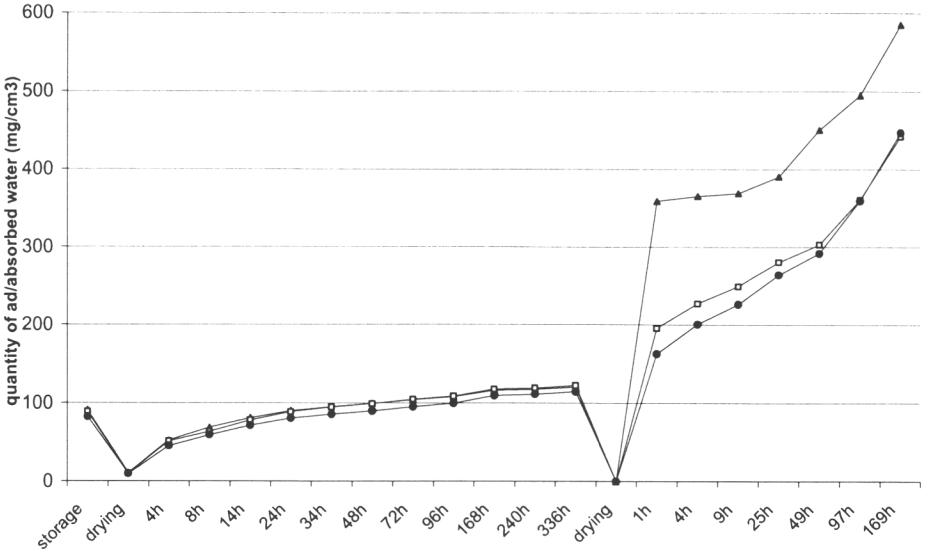

Figure 2. The average sorption of water into 5 x 15 x 30 mm sized wood blocks at high humidity (RH 100%) (on the left) and when immersed in water (on the right) as a function of time at the temperature of about 25°C. The blocks were dried at 60°C for 48 hours after storing, and at 103°C for 24 hours between the determinations. ▲= sapwood, □ = outer heartwood, ● = inner heartwood

The regression model was fitted to the data of tree-wise means separately for the sapwood and for the outer and the inner heartwood (tables IIa, b, c). In the case of the sapwood, the regression analyses were not carried out with the PS or PSM data because of the very low concentrations. According to the R2 values, which show the proportion of variation explained by the fitted model (table IIa), the variation in the sapwood mass loss was not explained considerably by any of the independent variables. The best fit was obtained with the concentration of resin acids. However, the positive regression coefficient, which suggests that the higher the concentration the greater is the mass loss, was not significant. The next best fit was obtained with the quantity of water after wetting, but the negative regression coefficient was not significant.

Table II. Regression analysis with tree wise means (n = 16) with the mass loss as the response variable. The R^2 value shows the proportion of variation explained by the fitted model (*response variable* = $\beta_0 + \beta_1$ *independent variable* + ε), and the *t* statistics tests whether the parameter β_1, the sign of which only is presented, was significantly different from zero.

2a. Sapwood

independent variable	R^2	sign of β_1	*p*-value of *t* test
basic density	0.03	-	0.526
total resin acids	0.15	+	0.151
total phenolics by Folin-Ciocalteu	0.00	-	0.811
moisture content at RH 100%	0.01	-	0.772
quantity of water after wetting	0.13	-	0.171

2b. Outer heartwood

independent variable	R^2	sign of β_1	*p*-value of *t* test
basic density	0.28	+	0.037
total resin acids	0.01	-	0.724
total phenolics by Folin-Ciocalteu	0.23	-	0.059
PS	0.14	-	0.159
PSM	0.25	-	0.048
PS+PSM	0.23	-	0.058
moisture content at RH 100%	0.21	+	0.077
quantity of water after wetting	0.19	+	0.091

2c. Inner heartwood

independent variable	R^2	sign of β_1	*p*-value of *t* test
basic density	0.00	-	0.826
total resin acids	0.16	-	0.123
total phenolics by Folin-Ciocalteu	0.27	-	0.040
PS	0.65	-	< 0.001
PSM	0.41	-	0.011
PS+PSM	0.51	-	0.003
moisture content at RH 100%	0.43	+	0.006
quantity of water after wetting	0.17	+	0.111

In spite of the large variation in the mass loss of the outer heartwood, the R2 values were fairly low for each of the independent variables. Basic density gave the highest R2 but, when one tree with extremely heavy wood was removed from the data, the R2 value was no more than 0.09. The concentration of PSM and total phenolics, measured by the Folin-Ciocalteu method, gave approximately the same R2 value. The negative effect of PSM on the mass loss was significant at the 0.05 risk level, and the effect of total phenolics was nearly significant. The negative effect of PS on the mass loss was less significant although the PS concentration was

relatively high. The moisture content and the quantity of water after wetting seemed to have a positive and nearly significant effect on the mass loss. The concentration of resin acids did not explain any of the variation in the mass loss.

In the inner heartwood, the stilbenes PS and PSM well explained the mass loss variation. PS especially had a very significant effect on the decay resistance, even though the concentration of PS was markedly lower than that in the outer heartwood. Also the concentration of total phenolics explained relatively well the variation in mass loss, while the effect of the resin acids was only indicative. Moisture content had a significant positive effect on the mass loss. However, when one tree with extremely hygroscopic wood was removed from the data, the R2 value was 0.27 and the p value for the t test 0.045. The quantity of water after wetting also had an indicative effect on the mass loss.

The Pearsons' correlation coefficients between the characteristics used as independent variables in the regression analysis are presented in table III. In the sapwood there was no significant correlation between the independent variables. In the outer heartwood, on the other hand, there was significant positive correlation between the concentration of total phenolics determined by the Folin-Ciocalteu method and the concentration of stilbenes, while the correlation between the concentration of total phenolics and the concentration of resin acids was weak. The quantity of water after wetting and the concentrations of stilbenes and total phenolics were significantly negatively correlated. The concentration of resin acids showed no relationship with the variation in the moisture content or the quantity of water after wetting. The moisture content and the quantity of water after wetting did not correlate with each other. The relationships for the inner heartwood resembled those for the outer heartwood, even though the absolute amount of stilbenes was only half of that in the outer heartwood. Differently, the moisture content had nearly significant negative correlation with the concentration of total phenolics and the concentration of PS and resin acids. The correlation between the basic density of the wood and the absolute amount of water adsorbed (mg/cm3) by the wood at high humidity was 0.911 in the sapwood, 0.874 in the outer heartwood, and 0.722 in the inner heartwood (not shown in table III).

Table III. Pearsons' correlation coefficients between Scots pine wood characteristics (n = 15-16). The p-values of the coefficients are given in italics.

3a. Sapwood

	basic density	resin acids	total phenolics	moisture content
resin acids	0.194			
	(0.487)			
total phenolics	0.144	0.171		
	(0.609)	*(0.542)*		
moisture content	0.194	-0.141	-0.235	
	(0.471)	*(0.617)*	*(0.400)*	
quantity of water after wetting	0.179	-0.078	-0.320	-0.398
	(0.508)	*(0.780)*	*(0.245)*	*(0.127)*

3b. Outer heartwood

	basic density	resin acids	total phenolics	PS	PSM	PS+PSM	moisture content
resin acids	-0.185						
	(0.493)						
total phenolics	0.003	0.310					
	(0.992)	*(0.243)*					
PS	0.067	0.118	0.681				
	(0.805)	*(0.664)*	*(0.004)*				
PSM	0.160	0.236	0.778	0.683			
	(0.553)	*(0.378)*	*(0.000)*	*(0.004)*			
PS+PSM	0.131	0.202	0.802				
	(0.628)	*(0.453)*	*(0.000)*				
moisture content	0.304	0.346	-0.297	-0.289	-0.275	-0.305	
	(0.252)	*(0.189)*	*(0.263)*	*(0.278)*	*(0.303)*	*(0.250)*	
quantity of water after wetting	-0.088	0.151	-0.739	-0.636	-0.583	-0.659	0.287
	(0.745)	*(0.577)*	*(0.001)*	*(0.008)*	*(0.018)*	*(0.006)*	*(0.281)*

3c. Inner heartwood

	basic density	resin acids	total phenolics	PS	PSM	PS+PSM	moisture content
resin acids	0.003						
	(0.990)						
total phenolics	-0.095	0.253					
	(0.727)	*(0.345)*					
PS	-0.199	0.297	0.672				
	(0.477)	*(0.282)*	*(0.006)*				
PSM	-0.415	0.183	0.671	0.879			
	(0.124)	*(0.515)*	*(0.006)*	*(0.000)*			
PS+PSM	-0.349	0.229	0.691				
	(0.202)	*(0.412)*	*(0.004)*				
moisture content	0.104	-0.437	-0.482	-0.481	-0.348	-0.406	
	(0.702)	*(0.090)*	*(0.059)*	*(0.070)*	*(0.204)*	*(0.134)*	
quantity of water after wetting	0.099	-0.146	-0.126	-0.603	-0.683	-0.674	0.236
	(0.715)	*(0.590)*	*(0.641)*	*(0.017)*	*(0.005)*	*(0.006)*	*(0.379)*

Scatter plots were used to visualise the radial variation and the variation among the trees, as well as the relationships between the important wood characteristics (figure IIIa,b,c,d,e).

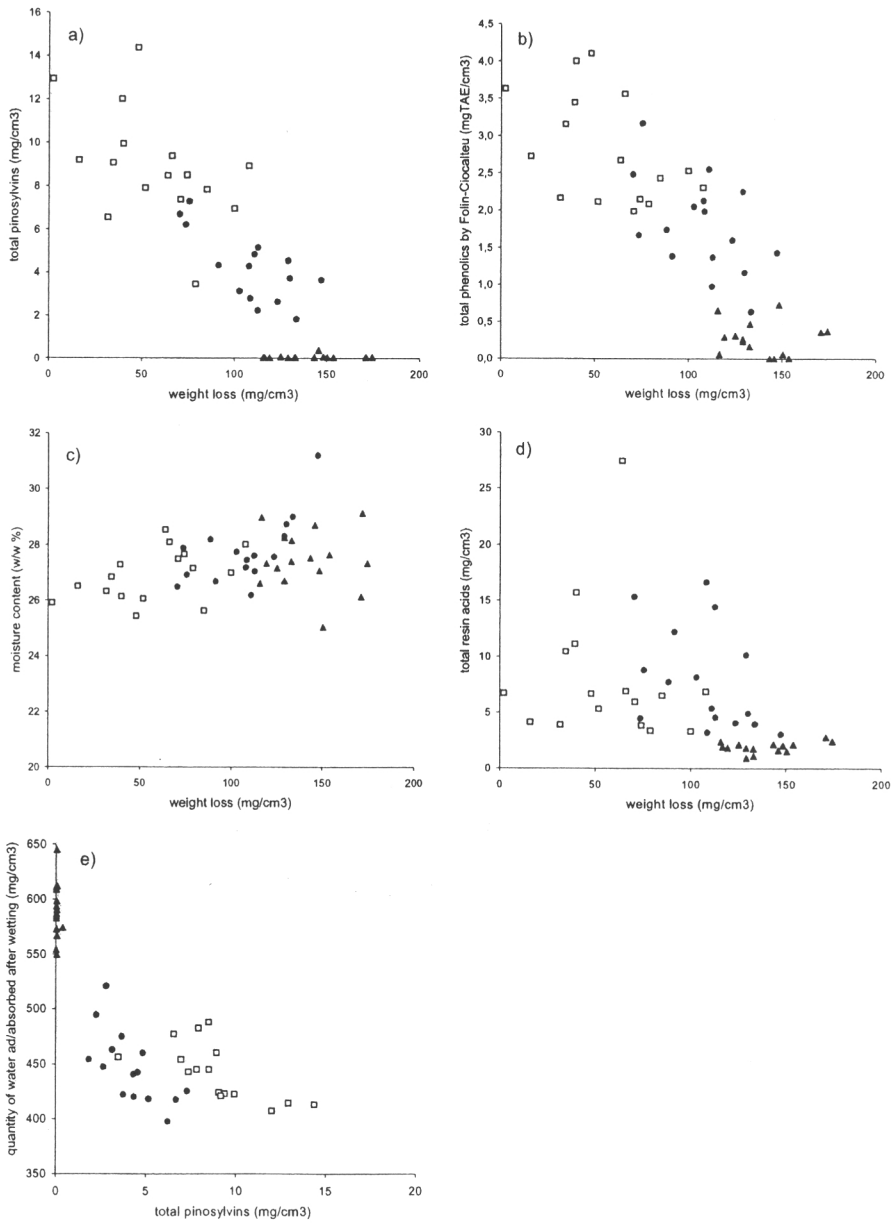

Figure 3. Scatter plots showing the relationships between different wood characteristics. ▲= sapwood, □ = outer heartwood, ● = inner heartwood

The concentration of stilbenes in the excluded sapwood samples was about 75 times that in typical sapwood. The average concentration of resin acids was 7.6 mg/cm3, and the concentration of total phenolics 1.12 mgTAE/cm3 (expressed as tannic acid equivalents).The mass loss was 0.084 mg/cm3, the moisture content 27.9 % and the quantity of water after wetting 0.564 g/cm3. The reason for these outlying observations could have been mistakes in determining the boundary between the sapwood and heartwood. The concentration of resin acids in the excluded heartwood samples was about 8 times that in typical heartwood. The average concentration of stilbenes was 12.5 mg/cm3, and the concentration of total phenolics 8.01 mgTAE/cm3. The mass loss was 0.033 mg/cm3, moisture content 26.6 % and the quantity of water after wetting 0.441 g/cm3. The most important reason for these outlying observations was the vicinity of knots.

5. DISCUSSION AND CONCLUSIONS

The results of this study show that the most durable part of old Scots pine stems is the heartwood located next to the sapwood. The same kind of radial variation has been found in several other tree species ([30] and references therein). Erdtman and Rennerfelt [2] and Rennerfelt [22] carried out decay experiments on 1-7 Scots pine stems using several wood destroying fungi including *C. puteana*, and concluded that the mass loss in the periphery part of the heartwood was lower than that in the centre of the heartwood. The other marked difference between the outer and the inner heartwood found in the present study was in the concentration of pinosylvin (PS) and pinosylvin monomethyl ether (PSM). This has earlier been reported on the basis of colorimetric analyses of total pinosylvins [2,3,22].

The variation in mass loss, caused by the *C. puteana* brown-rot fungus during the relatively short incubation period, was large within the radial sections. The variation within the most durable section, i.e. the outer heartwood, was the largest. However, the variation could not be explained satisfactorily by the other wood characteristics. Only the concentration of PSM had a significant effect at the *0.05* risk level. In the inner heartwood, the role of the stilbenes PS and PSM as decay inhibiting agents was significant at a low risk level, but the proportion of unexplained variation remained high. Furthermore, no independent variable explained the mass loss in the sapwood variation. Together this indicates that either the variation in the *in vitro* decay test had a large random component or that the activity of the fungus is dependent on unknown factors. If incubation with the outer heartwood had been longer and the average mass loss larger, more significant factors might have appeared.

The concentration of the stilbenes PS and PSM appeared to be the most important single factor determining the natural durability of Scots pine heartwood.

This conclusion was supported by the difference in the average mass loss and in the average concentration of heartwood phenolics between the sapwood and the outer and inner heartwood, as well as by the dependence between the mass loss and the PS+PSM concentration, especially within the inner heartwood. The same conclusion was also made by Rennerfelt [22]. However, as shown in figure III a, the decay rates of samples with very different PS+PSM levels can overlap. This supports the suggestion that the activity of the fungus is not regulated only by stilbenes [14,26]. The results of this study do not provide very much information about the mechanism through which PS and PSM slow down the degradation processes.

The role of resin acids in the decay resistance of natural wood substrate seemed to be minor compared to that of stilbenes (figure IIIa,b,d). The concentration of resin acids was approximately the same in the inner and outer heartwood, and thus could not have contributed to the significant variation in the mass loss observed between the inner and outer heartwood. The variation in the concentration of resin acids among the samples was large but, according to the regression analysis, the variation within the normal range had a weak effect on the mass loss, and in this case only in the inner heartwood. The extremely resinous "outlying" samples were relatively durable, but the concentration of phenolics in these samples was also high. This is in accordance with the comparison study of Harju et al. [12], in which the resin acid concentration of decay resistant and susceptible juvenile Scots pine heartwood was significant in one stand ($p = 0.004$), and nearly significant in another ($p = 0.072$). In the significant case the average concentration of resin acids was double in the susceptible heartwood and four-fold in the resistant heartwood compared to the heartwood material of the present study, taken from the upper part of the stems.

The traditional use of pine tar and pitch for ship caulking, i.e. as "naval stores" (see e.g. [15,19]), may be the reason for the speculation that resin acids *in situ* would make the wood hydrophobic. This hypothesis was not supported by the present study, in which the relationship between the total resin acid concentration and the water sorption capacity was analysed in natural wood substrate. Within the radial sections, there was no significant correlation between the resin acid concentration and the moisture content in humid air or the quantity of water after wetting. Even among the eight-fold resinous, "outlier" heartwood samples, the moisture content and the quantity of water after wetting were at almost the same level as in the typical heartwood.

The moisture content was the characteristic that showed the least variation both between and within the radial sections. However, this small degree of variation explained to some extent the large variation in the heartwood mass loss. The uptake of water at the start of the malt agar plate decay test took place via adsorption. In

conditions where the moisture content of the wood surface is near to the lower limit required by the fungus to be active, even small differences in the adsorption rate may cause a delay in decay initiation. The results showed no significant relationship between the moisture content (adsorption) and the quantity of water after wetting (adsorption+absorption), which suggests that adsorption and absorption, both of which depict the interaction between the wood and water, actually reflect completely different wood properties.

The quantity of water after wetting and the concentration of stilbenes showed a significant negative correlation within both the outer and inner heartwood even though there was no difference in the average quantity of water between the outer and the inner heartwood (figure IIIe). There are a few earlier reports on the ability of phenolics to interfere with the penetration of water inside Scots pine wood [1,26,28]. The reason for this relationship does not necessarily have to be related to the chemical nature of phenolic compounds, but it could also be a specific feature in the structure of the wood that is correlated with the concentration of phenolics and the absorption of water. The interesting finding that the quantity of water after wetting to some extent also explained the variation in heartwood mass loss, even though there was no external supply of free water in the decay test, may be a reflection of the correlation between the absorption of water and the amount of stilbenes.

The concentration of phenolics was investigated using two different methods: the non-specific colorimetric Folin-Ciocalteu method, and the specific liquid chromatography analysis (HPLC). In heartwood, where the concentration of phenolics was high, the results of these methods were in good agreement. The Folin-Ciocalteu method also satisfactorily explained the variation in mass loss, which suggests that this simple method could be useful in the screening of durable Scots pine heartwood (figure IIIb).

Acknowledgements: This study has been supported by the Academy of Finland. The authors are also grateful to a number of persons for their assistance during the work. The sample trees were felled and the disks cut by Ari Haapasaari and Pentti Konttinen under the supervision of Hannu Heinonen. The sample disks were handled by Heikki Kinnunen and Sari Lignell. The mycological analysis was carried out by Katriina Lipponen, Anna-Maija Hallaksela and Kerttu Rainio. The sample blocks were prepared by Auvo Silvennoinen and Heikki Kinnunen, and the milling was carried out by Eija Matikainen and Auvo Silvennoinen. The decay test was performed by Liisa Seppänen. Hannele Makkonen, Seija Vatanen and Eija Matikainen performed the determinations with the wood blocks and water. John Derome revised the language of the manuscript.

REFERENCES

[1] Celimene C.C., Micales J.A., Ferge L., Young R.A., Efficacy of pinosylvins against white-rot and brown-rot fungi, Holzforschung 53 (1999) 491-497.

[2] Erdtman V.H., Rennerfelt E., Der Gehalt des Kiefernkernholzes an Pinosylvin-Phenolen. Ihre quantitative Bestimmung und ihre hemmende Wirkung gegen Angriff verschiedener Fäulpilze, Svensk Papperstidning 47 (1944) 45-56.

[3] Erdtman H., Frank A., Lindstedt G., Constituents of pine heartwood XXVII. The content of pinosylvin phenols in Swedish pines, Svensk Papperstidning 8 (1951) 275-279.

[4] Ericsson T., Fries A., High heritability for heartwood in north Swedish Scots pine, Theor. Appl. Genet. 98 (1999) 732–735.

[5] Ericsson T., Fries A., Gref R., Genetic correlations of heartwood extractives in Pinus sylvestris progeny test, Forest Genetics 8 (2001) 73–80.

[6] European standard, EN 350-2, Durability of wood and wood-based products - Natural durability of solid wood - Part 2: Guide to Natural durability and treatability of selected wood species of importance in Europe. European Committee for Standardization, Brussels, 1994.

[7] Fries A., Ericsson T., Genetic parameters in diallel-crossed Scots pine favour heartwood formation breeding objectives, Can. J. For. Res. 28 (1998) 937–941.

[8] Fries A., Ericsson T., Gref R., High heritability of wood extractives in Pinus sylvestris progeny test, Can. J. For. Res. 30 (2000) 1707–1713.

[9] Gref R., Ericsson A., Wound-induced changes of resin acid concentrations in living bark of Scots pine seedlings, Can. J. For. Res. 15 (1985) 92-96.

[10] Griffin D.M., Water potential and wood-decay fungi, Annu. Rev. Phytopathol. 15 (1977) 319-329.

[11] Harju A.M., Venäläinen M., Genetic parameters regarding the resistance of Pinus sylvestris heartwood to decay caused by Coniophora puteana, Scand. J. For. Res. 17 (2002) 199-205. .

[12] Harju A.M., Kainulainen P., Venäläinen M., Tiitta M., Viitanen H., Differences in resin acid concentration between brown-rot resistant and susceptible Scots pine heartwood, Holzforschung 56 (2002) 479-486.

[13] Harju A.M., Venäläinen M., Beuker E., Velling P., Viitanen H., Genetic variation in the decay resistance of Scots pine wood against brown rot fungus, Can. J. For. Res. 31 (2001) 1244–1249.

[14] Hart J.H., Shrimpton D.M., Role of stilbenes in resistance of wood to decay, Phytopathology 69 (1979) 1138-1143.

[15] Hillis, W.E., Heartwood and tree exudates, Springer-Verlag, Berlin Heidelberg, 1987.

[16] Julkunen-Tiitto R., Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics, Journal of Agriculture and Food Chemistry 33 (1985) 213-217.

[17] Lieutier F., Sauvard D., Brignolas F., Picron V., Yart A., Bastien C., Jay-Allemand C.,Changes in phenolic metabolites phloem induced by Ophiostoma brunneo-ciliatum, a bark-beetle-associated fungus, Eur. J. For. Path. 26 (1996) 145-158.

[18] Manninen A.-M., Tarhanen S., Vuorinen M., Kainulainen P.,Comparing the variation of needle and wood terpenoids in Scots pine provenances, J. Chem. Ecol. 28 (2002) 211-228.

[19] Obst J.R , Special (secondary) metabolites from wood, in: Bruce A. and Palfreyman, J.W. (Eds.), Forest products biotechnology, Taylor & Francis Ltd, Padstow, UK, 1998, 151-165.

[20] Paajanen L., Viitanen H., Decay fungi in Finnish houses on the basis of inspected samples from 1978 to 1988, The International Research Group on Wood Preservation, IRG Doc. No: IRG/WP/1401, 1989, 4 p.

[21] Rayner A.D.M., Boddy L., Fungal decomposition of wood. Its biology and ecology, John Wiley et Sons, Chichester, UK, 1988.

[22] Rennerfelt E., Några undersökningar över olika rötsvampars förmåga att angripa splint- och kärnved hos tall. Summary: Some investigations over the capacity of some decay fungi to attack sapwood and heartwood of Scots pine, Meddelanden från statens skogsförsöksanstalt 36 (1947) 1-24.

[23] Scheffer T. C., Cowling E. B., Natural resistance of wood to microbial deterioration, Annu. Rev. Phytopathol. 4 (1966) 147-170.

[24] Turtola S., Manninen A.-M., Holopainen J.K., Levula T., Raitio H., Kainulainen P., Secondary metabolite concentrations and terpene emissions of Scots pine xylem after long-term forest fertilization, J. Env. Qual. 31 (2002) 1694-1701.

[25] Venäläinen M., Harju A., Nikkanen T., Paajanen L., Velling P., Viitanen H., Genetic variation in the decay resistance of Siberian larch (Larix sibirica Ledeb.) wood, Holzforschung 55 (2001) 1–6.

[26] Venäläinen M., Harju A., Saranpää P., Kainulainen P., Tiitta M., Velling P., The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood, Wood Sci. Technol. (2003) (In press)

[27] Viitanen H., Paajanen L., Nikkanen T., Velling P. Decay resistance of Siberian larch wood against brown rot fungi, Part 2, The effect of genetic variation, The International Research Group on Wood Preservation, Stockholm, IRG Doc. No: IRG/WP 98-10287, 1998.

[28] Vologdin A.., Razumova A.F., Charuk E.V., Die Bedeutung der Extraktstoffe für die Permeabilität von Kiefern- und Fichtenholz, Holztechnologie 20 (2) (1979) 67-69.

[29] Walker J.C.F., Primary Wood Processing: pinciples and practice. Chapman, Hall, London, 1993.

[30] Zabel R.A., Morrell J.J., Wood Microbiology: Decay and its Prevention, Academic Press Inc., California, 1992.

ISBN 951-40-1866-4
ISSN 0358-4283
Hakapaino Oy 2002